Effects of water and nitrogen coupling on growth, physiology and yield of rice

Yuanyuan Li^{1*}, Xiaohou Shao², Daoxi Li¹, Menghua Xiao³, Xiujun Hu⁴, Jing He⁵

(1. School of Water Conservancy, North China University of Water Resource and Electric Power, Zhengzhou 450045, China;

2. College of Water Conservancy and Hydropower, Hohai University, Nanjing 210098, China;

3. Zhejiang Institute of Hydraulics and Estuary, Hangzhou 310020, China;

4. Institute of Hydraulic and Environmental Engineering, Zhejiang University of Water Resources and Electric Power, Hangzhou 310018, China;

5. Nanjing Water Conservancy Construction Engineering Testing Center Co., Ltd., Nanjing 210036, China)

Abstract: Water and nitrogen fertilizer are two essential factors for quality and yield formation of rice. Experimental study was carried out to investigate the effects of water and nitrogen fertilizer coupling on yield-related factors, such as growth (height), physiological indicators (chlorophyll and leaf area index (LAI)) and yield composition indicators (productive panicles, thousand grain weight and total grains per panicle). Results showed that, the height difference under two irrigation regimes was not significant, and it showed no difference until the tillering stage (p > 0.05). The water control method for controlled and mid-gathering irrigation (CMI) was favorable for nutrients converting to rice grain. Meanwhile the height difference for CMI and conventional irrigation (CVI) was the biggest at 80 d after rice transplantation. Variance analysis showed the effect of fertilization on height was significant (p < 0.05). With organic fertilizer application, it could control plant growth and promote the nutrients converting to the panicle. The change curve of LAI was similar to chlorophyll content. Organic fertilizer application could not only promote chlorophyll content and LAI, but also delay leaf fading and promote yield. Nitrogen fertilizer factors showed significant difference on rice yield, compared to irrigation regimes showing no significance. Considering the irrigation and fertilizer factors together, the interaction was significant. The descending orders for the effects of water and nitrogen on rice yield were fertilizer, water and fertilizer, water. Regression analysis showed that the productive panicles and total grains per panicle of rice were extremely significant on rice yield, and the direct effect of total grains per panicle on yield was greater than that of productive panicle. This study results could provide theoretical basis for water and nitrogen management to improve rice production.

Keywords: water and nitrogen coupling, controlled and mid-gathering irrigation (CMI), organic fertilizer, growth and physiology, regression and path analysis, rice yield

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1 Introduction

Water and nitrogen are two essential factors during rice growth and development, and they interact and constrain each other in rice growth stages. The coordination of water and nitrogen fertilizer could guarantee the rice growth and promote the water and fertilizer use efficiency^[1,2]. Reasonable water and fertilizer management could effectively promote rice yield^[3]. Previous studies by scholars have found that water-saving irrigation techniques could improve nitrogen fertilizer absorption, and is favorable for nitrogen migration to the growth center, which could promote the rice yield^[4-7]. Liu^[8] found that total grains per panicle, productive panicles and thousand grain weight was not decreased obviously compared to conventional irrigation. When inorganic fertilizer combined with organic fertilizer application, rice yield had increased 14.2%-20.1% compared to inorganic fertilizer application only, and the similar conclusion was drawn by Tian and Shi^[9].

Rice leaf area index (LAI) and leaf chlorophyll content are considered as two important factors for getting high yield of paddy rice. Different water and fertilizer treatments also had large impact on physiology and growth characteristics of rice. Yuan et al.^[10] found that with the combination of organic and inorganic fertilizer application, the chlorophyll content was increasing. Wang et al.^[11] found that controlled irrigation could improve rice tiller, and increase dry matter accumulation of root, which could increase total grains, panicles and thousand grain weight finally. However, Liu^[8] found that under controlled irrigation, rice height and dry matter accumulation was decreased compared to conventional irrigation. Wei et al.^[12] drew the conclusion that, LAI of rice was promoted by appropriate water supply. The effect

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Biographies: Xiaohou Shao, Professor, research interest: agricultural soil and water environment theory, Email: shaoxiaohou@163.com; **Daoxi Li**, Associate Professor, research interest: water-saving irrigation technology, Email: ldx97042@163.com; **Menghua Xiao**, Deputy Senior Engineer, research interest: controlled irrigation and drainage theory, Email: menghuaxiao@aliyun.com; **Xiujun Hu**, Lecturer, research interest: water environment governance, Email: huxiujun86@sina.com; **Jie He**, Engineer, research interest: project consultation, Email: 672727129@qq.com.

^{*}Corresponding author: Yuanyuan Li, Lecturer, research interest: agricultural soil and water environment protection. 136 Jinshuidong Road, Zhengzhou 450045, China. Tel: +86-15903662916, Email: liya66720@126.com.

of nitrogen fertilizer on LAI played a leading role in rice production. It also drew the conclusion that rice production was promoted with LAI increasing. Therefore, carrying out research on the effects of water and nitrogen coupling on growth and physiological indicators was essential for improving rice yield. The purpose of this study was to evaluate contribution of growth indicators (height, chlorophyll and LAI) and yield composition indicators (productive panicles, thousand grain weight and total grains per panicle) to rice production, which could provide guidance to agricultural water and fertilizer management.

2 Materials and methods

2.1 Experimental site

This study was carried out at Vegetables (Flowers) Scientific Institute (latitude 31°43'N, longitude 118°46'E), Jiangning District of Nanjing, Jiangsu Province in China from June to October of 2013 and 2015. The field site was located at subtropical humid region. The average annual evaporation and precipitation amount was 1472.5 mm and 1106.5 mm, respectively. The average annual sunshine hours were 2017.2 and average annual temperature was 15.7°C. Maximum average humidity in this area was 81% and average wind speed was 19.8 m/s.

Before the experiment, the paddy field soil of 0-60 cm soil layer was took to test the soil physical and chemical properties. It was as follows: clayey loam, field capacity 28%, bulk density 1.35 g/cm³, pH 5.87, hydrolyzed nitrogen 86.5 mg/kg, available phosphorus 25.3 mg/kg, and organic matter 21.7 g/kg.

2.2 Experimental design

Paddy rice (*Oriza sativa* L. cv. Kaohsiung 139) was grown in test plot with the size of 10 m² (2 m×5 m). There were two irrigation treatments: controlled and mid-gathering irrigation (CMI) and conventional irrigation (CVI). Three levels of fertilizer application were adopted: combination of organic and inorganic fertilizer treatment (CF), inorganic fertilizer treatment (FO) only and unfertilized treatment (NF). All the treatments were replicated three times and they were set with completely randomized blocks. Each test plot received separate irrigation and drainage. There were also water meter and lysimeter in each test plot for the record of related water data. All the test plots shared one rain gauge installed in experimental site. The field experiment layout was shown in Figure 1.



Figure 1 Experimental layout in paddy field

CMI is developed from controlled irrigation^[6]. The ratio of organic and inorganic fertilizer was set as 4:6 (according to former local experience), and the total nitrogen amount in fertilizer was the same for each plot. When the rice seedling was transplanted, basal compound fertilizer was applied, and then tillering compound fertilizer was applied at about 30 d after transplanting, while urea was applied as panicle fertilizer at about 60 d after transplanting. The irrigation, rainfall and fertilizer application amount was shown in Table 1.

Table 1 Irrigation, rainfall and fertilizer application amount

Vaar	Growth store	Rainfall	Irrigation a	Nitrogen	
rear	Growin stage	/mm	CMI	CVI	/kg·hm ⁻²
	Green-up	75.2	60	60	135
	Tillering	232.8	90	210	40.6
2013	Jointing-booting	155.6	60	150	
	Heading-flowering	24.6	60	90	58
	Milking	0	30	60	
	Total	488.2	300	570	233.6
	Green-up	28.3	40	40	149.6
	Tillering	241.4	80	180	44.9
2015	Jointing-booting	27.5	100	150	
	Heading-flowering	96.2	60	90	
	Milking	74.4	0	30	
	Total	462.8	280	490	194.5

2.3 Indicators and measurements

Chlorophyll content and LAI of rice was measured during sunny day, between 10:00 am to 14:00 pm. Chlorophyll content was determined by chlorophyll meter (CCM200, USA), and LAI was determined by canopy analyzer (SunScan, UK). Plant height before heading stage was measured from soil surface to the highest blade tip by a measuring tape, and the height after heading stage was from soil surface to the top of the highest spike.

Theoretical yield of rice was calculated by yield components. Firstly, 10-30 rice plants in a sample area of 1 m^2 were randomly selected in each test plot after harvesting, then the plant leaves, stems and roots were cleaned separately before being killed out in the drying cabinet at 105°C for 10-15 min, until the rice plants were dried to constant weight in oven at 85°C. At the end, the rice plant biomass was weighed by a balance. The yield components viz. productive panicle number, kernel weight and kernel number per panicle were determined by experimenter's counting.

2.4 Statistical analysis

Simple data calculation and diagramming was completed by Excel 2013. Correlation analysis and regression analysis was carried out by SPSS Statistics 19. The variance homogeneity of the ANOVA was tested before ANOVA analysis.

3 Results and discussion

3.1 Plant height

As is shown in Figure 2, the rice height increased slowly at the beginning, then it increased quickly at jointing-booting stage, finally it turned out to be rather stable. Totally speaking, the height difference under two irrigation methods was not obvious, and it showed no difference until the tillering stage (p>0.05). The final plant height under CMI was a little bit lower than that under CVI.

At the beginning, plant heights for CMI were 52.4 cm and 34.7 cm in 2013 and 2015, respectively, while those for CVI were 51.1 cm and 34.2 m, respectively. At the milking stage, there were 87.9 cm and 82.3 cm for CMI in 2013 and 2015, while those for CVI were 88.9 cm and 83.7 cm, respectively. In the same year, plant height for CVI was a little bit lower than that for CMI, however, it surpassed at 40 d and 45 d after the rice transplantation. Then the height for CVI kept a higher level than that for CMI. That was because of the long-term soil water saturation under CVI treatment, which restrained root respiration and rice growth. However, the water condition under CMI method was favorable for

vegetative growth of rice plant at earlier stage, and no water layer state in field surface at the later stage promoted vegetative growth converting to reproductive growth, which restrained excessive plant height increase, thus it was favorable for lodging resistance of paddy plants, leading to a higher production finally.





D-value for both CMI and CVI firstly increased and then decreased. It was negative at the beginning, and turned to be positive at the later tillering stage. The peak *D*-value was at 80 d after rice transplantation, and there were 6.6 cm and 2.7 cm respectively. That was mainly due to the nutrients converting to

rice grain both in paddy soil and rice plant when the rice changed from vegetative growth to reproductive growth at this stage. The water control for CMI was favorable for nutrients converting to rice grain. Thus the height difference for CMI and CVI was the biggest.

From Figure 3, it was clear that rice height at turning-green stage increased rather obviously, and from tillering stage, it increased slowly then rapidly till stable finally. The plant height increased obviously at jointing-booting stage for both 2013 and 2015. Generally speaking, plant height under fertilized treatment was higher than that under no fertilized treatment. Variance analysis showed the effect of fertilization on height was significant (p < 0.05). The plant height change curve for CF was similar to FO in 2013, while plant height for FO was higher than that for CF in 2015. It illustrated at the same nitrogen level, inorganic fertilizer was favorable for vegetative growth, while organic fertilizer could control plant growth and promote the nutrients converting to the panicle, and this effect was noticeable in 2015. With no fertilizer application treatment, the rice height was rather lower, and the slow growth in all stages showed the serious inhibition of vegetative growth. In 2013, final height for CF, FO, and NF were 97.5 cm, 98.9 cm and 71.5 cm, with the increasing rate of 40.6%, 41.3% and 45.1%, respectively. In 2015, there were 86.1 cm, 92.0 cm, and 70.9 cm, with the increasing rate of 61.8%, 61.8% and 50.2%, respectively.



Figure 4 Rice height change under different fertilizer applications

3.2 Chlorophyll content and LAI

The chlorophyll content increased rapidly in returning-green stage, while it increased slowly and decreased slowly from tillering stage, with the peak value at 65 d and 60 d after transplantation. The peak value for CMI and CVI were 19.1 and 19.6 in 2013, while there were 25.9 and 26.3 in 2015. As for CF, FO, NF treatments, the peak value were 25.1, 21.9, 10.9 in 2013 and there were 32.4, 32.7, 13.4 in 2015. Variance analysis showed the effect of irrigation method on chlorophyll content was not

significant (p>0.05), while fertilizer method showed extremely significance on chlorophyll content.

The chlorophyll content under fertilized treatment was obviously higher than that under no fertilized treatment. It indicated that nitrogen application could promote chlorophyll content. Compared to inorganic fertilizer application, chlorophyll content under organic fertilizer increased rapidly at the earlier stage, and decreased slowly later, which showed the organic fertilizer could delay leaf fading. As was shown in Figure 6, the change curve of LAI was similar to chlorophyll content, nitrogen application was also favorable for improving LAI. Water had no significance on LAI, while fertilizer effect was significant. In conclusion, LAI showed the trend of increasing then decreasing. At tillering stage, with adequate nutrient in the soil, water and fertilizer absorption ability

of root system was enhanced, resulted in fast rice plant growing and LAI increasing. The difference between all treatments was not obvious, and LAI reached the peak at 65 d and 60 d after transplantation in 2013 and 2015, respectively. With combination of CMI and CF, LAI increased rapidly and reduced slowly, which was favorable for yield promotion.



Figure 6 Leaf area index change under different water and fertilizer treatments

3.3 Yield composition

According to Table 2, the main component indicators of rice yield are thousand-grain weight, total grains per panicle and productive panicle number per m². As shown in Table 2, under FO treatment, panicle number for CMI was higher, but total grains per panicle and thousand-grain weight was lower, and the yield was lower than CVI. Under CF treatment, productive panicle number

for CMI was relatively lower compared to CVI, while total grains per panicle and thousand-grain weight was higher, resulting in no significant difference on final yield. Under NF treatment, panicle number and thousand-grain weight was higher for CMI, and the total grains per panicle was much the same compared to CVI, resulting in higher yield.

Fable 2	Rice	yield	compositions
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Year	Traatmant	Theoretical yield	Actual yield	Yield composition			
	Treatment	/kg·hm ⁻²	/kg·hm ⁻²	Productive panicles/m ⁻²	Total grains per panicle	Thousand-grain weight/g·1000 ⁻¹	
	CMICF	9160.6	8876.5 Aa	222	153	27.0	
	CVICF	9148.6	8823.8 Ba	255	136	26.4	
2013	CMIFO	6207.8	6024.5 Ab	212	121	24.2	
	CVIFO	6912.7	6535.0 Bab	182	140	27.1	
	CMINF	3721.4	3566.7 Ac	176	77	27.5	
	CVINF	3062.5	3024.9 Bc	153	78	24.9	
	CMICF	7680.5	7237.4 Aa	235	115	28.4	
	CVICF	7309.0	7145.5 Bab	261	97	28.9	
2015	CMIFO	6545.9	6415.3 Ab	255	85	30.2	
2015	CVIFO	8099.1	6738.2 Bab	269	101	29.8	
	CMINF	3637.8	3371.5 Ac	201	69	26.2	
	CVINF	3526.3	3044.8 Bc	213	65	25.5	

Notes: According to ANOVA, there was no significance (ns) at p>0.05 and significant differences (*) at p<0.05, while there was extremely significant difference (**) at p<0.01 at a=0.05 level. For same fertilizer, the effects of different irrigation conditions on yield were shown by capital letters (A or B), while for the same irrigation, the effects of different fertilizer conditions on yield were shown by lowercase letters (a, b, or c).

Generally speaking, the rice yield in 2015 was lower than that in 2013, especially for combined fertilizer treatment. The inter-annual difference was might due to lower rainfall and irrigation amount in 2015, and the organic fertilizer effect could not be fully played, leading to lower nitrogen use efficiency and yield formation. Taking the data of 2013 for example, according to analysis of variance, panicle numbers increased significantly under the combination of organic and inorganic fertilizer application. The productive panicle numbers per m² were 238 (CF)>197 (FO)> 164 (NF). For different water treatment, the productive panicle numbers per m² were 203 (for CMI)>196(CVI). It was also clear that there was no significance on total grains per panicle and thousand-grain weight for water treatments. Water and nitrogen interaction played no significance on productive panicles. The difference of nitrogen fertilizer on total grains per panicle was significant while on thousand-grain weight was not significant. Thus, fertilizer conditions showed extremely difference on rice yield, while irrigation showed no significance since all the yield components under different water condition had no significance. Also, the interaction between irrigation and nitrogen fertilizer was significant.



Figure 7 Measurement of yield components

From the above results, it was clear that nitrogen fertilizer played a rather more important role than water factors in rice yield formation. With organic fertilizer addition to inorganic fertilizers, rice yield increased 2570.4 kg/hm² and 614.7 kg/hm² compared to inorganic fertilizer application only, with the increasing rate up to 40.9% and 9.4%, respectively. That was mainly because micro-elements in organic fertilizer application could regulate release intensity and velocity of nutrients.

Theoretical yield was calculated according to Equation (1), and actual yield was weighed directly from each experiment plot. After the correlation analysis on theoretical and actual yield, it showed the significant positive correlation both in 2013 and 2015, and the coefficient was 0.99918 and 0.95091.

$$Y = M \times N \times P/100 \tag{1}$$

where, Y is theoretical yield, kg/hm²; M is productive panicles, m⁻²; N is total grains per panicle; P is thousand grain weight, g/1000.

3.4 Water and nitrogen coupling equation construction and analysis

According to Tables 1 and 2, considering water (total irrigation and rainfall amount) and nitrogen coupling effect on rice yield, the equation was constructed as following, basing on regression simulation of quadratic function mathematical model. $Y = 0.0000256X_1^2 - 0.241X_2^2 + 0.00115X_1X_2 - 0.617X_1 + 58.566X_2 + 6663.178 (R^2 = 0.8579)$ (2)

where, *Y* was rice yield, kg/hm²; X_1 was the tall amount of rainfall and irrigation, m³/hm²; X_2 was nitrogen fertilizer amount, kg/hm².

After regression test on the above model, water and nitrogen coupling effect on yield was significant (F=7.2477 Significance F=0.0159). Therefore this model could reflect the relation between water and nitrogen on yield with a higher reliability.

From the model obtained above, it was clear that the coefficient of water was negative, while the coefficient of nitrogen and water and nitrogen interaction was both positive, which illustrated the yield increasing effect of nitrogen fertilizer application on rice, and the water and nitrogen fertilizer coupling effect on yield conformation was positive. The coefficient of X_2^2 was negative, which explained much more nitrogen could reduce yield. However, the coefficient of X_1X_2 was positive, which illustrated rice yield could be promoted with the water and fertilizer regulation. Thus water and nitrogen input during rice growth should be reasonable, not the more the better.

The correlation coefficients and path coefficients were shown in Tables 3 and 4. After analysis, the impact of nitrogen application per unit on yield promotion was significant than water input per unit. The effect orders of water and nitrogen factors on rice yield was as follows, $X_2 > X_1 X_2 > X_1$.

 Table 3
 Correlation coefficients among factors in yield effect model

	Y	X_1^2	X_{2}^{2}	X_1X_2	X_1	X_2
Y	1.0000					
X_1^2	0.0296	1.0000				
X_{2}^{2}	0.8766**	-0.0437	1.0000			
X_1X_2	0.8957**	0.1729	0.9612**	1.0000		
X_1	0.0282	0.9992**	-0.0421	0.1742	1.0000	
X_2	0.8984**	-0.0221	0.9963**	0.9710**	-0.0213	1.0000

Note: ****** means extremely significant difference (p<0.01), ***** means significant difference (p<0.05).

	Table 4	Path c	oefficients	;	
	X_{1}^{2}	X_{2}^{2}	X_1X_2	X_1	X_2
Partial regression coefficient	0.0000256	-0.241	0.00115	-0.617	58.566
Standard deviation	23786897	20725.92	908729.5	1323.066	100.511
Path coefficient	0.285	-2.333	0.488	-0.381	2.750

3.5 Multiple regression and Path analysis on rice yield

After statistical analysis on growth indicators (height, chlorophyll and LAI), yield composition indicators (productive panicles, thousand grain weight and total grains per panicle) and the theoretical yield, results showed in Table 5. It was clear that the skewness was small, which provided reliability for correlation, regression and path analysis.

Taking rice yield as dependent variable, plant height, chlorophyll content, LAI, productive panicles, thousand grain weight and total grains per panicle were considered as independent variable, then multiple regression equation was constructed as Equation (3). It showed that the six independent variables could cover 99.5% of the yield change, of which the contribution on yield of X_2 , X_4 , X_5 and X_6 factors were positive, while X_1 and X_3 factors were negative. Thus during the rice growth period, rice height and LAI should be coordinated and controlled to promote their contributions to final yield. After significance test on this model, it was extremely significant (*F*=164.3972 Significance

F=1.41E-05). The significance test on coefficients of partial regression in Table 6 showed the productive panicles and total grains per panicle of rice were extremely significant, while rice height, chlorophyll, LAI and thousand grains weight on rice yield was not significant.

 $Y = -41.7908X_1 + 36.5377X_2 - 570.94X_3 + 30.7775X_4 + 73.6360X_5 + 150.1255X_6 - 8494.38 (R^2 = 0.9950)$ (3)

where, Y is theoretical yield, kg/hm²; X_1 is plant height, cm; X_2 is chlorophyll content; X_3 is LAI; X_4 is productive panicles; X_5 is total grains per panicle; X_6 is thousand grain weight, g/1000.

Table 5	Statistical analysis on growth and yield factors

	Height	Chlorophyll	LAI	Productive panicles	Total grains per panicle	Thousand-grain weight	Yield
Mean	86.1517	14.5750	1.0708	219.5000	103.0833	27.1658	6251.0170
Standard error	3.4559	2.1225	0.1516	10.6959	8.5683	0.5458	644.2347
Standard deviation	11.9716	7.3527	0.5250	37.0516	29.6815	1.8909	2231.6940
Variance	143.3194	54.0621	0.2757	1372.8180	880.9924	3.5754	4980460
Kurtosis	-1.5781	-1.6081	-0.6785	-0.9029	-1.2351	-0.8271	-1.4573
Skewness	-0.3161	0.4875	-0.0017	-0.3221	0.3475	0.1399	-0.2649

Table 6	Significance test	on coefficients	of partial	regression
	0			

	Standard Error	t Stat	p-value	Lower limit 95%	Upper limit 95%
Height	18.1233	-2.3059	0.0693	-88.3781	4.7965
Chlorophyll	29.8554	1.2238	0.2755	-40.2081	113.2834
LAI	403.2849	-1.4157	0.2160	-1607.6166	465.7373
Productive panicles	3.6924	8.3355	0.0004	21.2860	40.2690
Total grains per panicle	8.7900	8.3772	0.0004	51.0406	96.2314
Thousand-grain weight	70.4843	2.1299	0.0864	-31.0601	331.3112

From the above results, taking consideration of the main factors of yield, they were productive panicles and total grains per panicle. Then the optimal regression equation was constructed as Equation (4), and it was extremely significant (F=191.0461 Significance F=4.25E-08). The significance test on partial regression coefficients of productive panicles and total grains per panicle both showed extremely significant (P_1 -value=8.29E-06, P_2 -value=2.15E-06, P_3 -value=2.49E-07). Table 7 illustrated the correlation coefficients of productive panicles and total grains per panicle with yield both reached extremely significant, while the correlation coefficient between productive panicles and total grains per panicle was not significant.

$Y = 33.1675X_1 + 53.3316X_2 - 6526.84$	$(R^2=0.9770)$	(4)
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 Table 7
 Correlation coefficients between main factors of yield

	Productive panicles	Total grains per panicle	Yield
Productive panicles	1.0000		
Total grains per panicle	0.2184	1.0000	
Yield	0.7056**	0.8296**	1.0000

Note: ** means extremely significant difference (p<0.01), * means significant difference (p<0.05).

The direct path coefficients of productive panicles and total grains per panicle on yield (P_{y1}, P_{y2}) were calculated according to partial regression coefficient of independent variable multiply by standard deviation of independent variable, then divide standard deviation of dependent variable, thus as follows^[13,14]:

P_{v1}=33.1675×37.0516/2231.6940=0.5507

Py2=53.3316×29.6815/2231.6940=0.7093

While indirect path coefficients of productive panicles and total grains per panicle on yield (P_{x1}, P_{x2}) were calculated according to direct path coefficient multiply by correlation coefficient between them, thus as follows:

 $P_{x1} = P_{y1} \times r_{12} = 0.5507 \times 0.2184 = 0.1203$ $P_{x2} = P_{y2} \times r_{21} = 0.7093 \times 0.2184 = 0.1549$ In conclusion, the direct effect on yield for total grains per panicle was greater than that of productive panicle, and the indirect effect for productive panicle according to total grains per panicle was a little bit less than that of total grains per panicle on yield according to productive panicle.

4 Conclusions

(1) The water condition under CMI promoted vegetative growth converting to reproductive growth, which restrained excessive plant height increase, thus the final rice height under CMI was a little bit lower than that under CVI. Rice height under fertilized treatment was higher than that under no fertilized treatment. At the same nitrogen level, inorganic fertilizer was favorable for vegetative growth, while organic fertilizer could control plant growth and promote the nutrients converting to the panicle.

(2) The change curve of LAI was similar to chlorophyll content. They increased and then decreased, with the peak value both at 65 d and 60 d after transplantation. The effect of irrigation regime on them was not significant (p>0.05), while fertilizer method showed extremely significance. Nitrogen application was favorable for improving chlorophyll content and LAI.

(3) With organic fertilizer application, productive panicle numbers can increase significantly. Irrigation regimes played no significance on productive panicles, thousand-grain weight and total grains per panicle. Nitrogen fertilizer showed extremely difference on paddy rice yield. The interaction between irrigation and nitrogen was significant. Rice yield increased 40.9% and 9.4% in 2013 and 2015 with organic and inorganic fertilizers application, compared with inorganic fertilizer application only.

(4) The six variances (plant height, chlorophyll content, LAI, productive panicles, thousand grain weight and total grains per panicle) could cover 99.5% of the yield change, of which the productive panicles and total grains per panicle of rice were extremely significant. Path analysis showed that the direct effect on yield for total grains per panicle was greater than that of productive panicle.

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