Selection and evaluation of high-nitrogen efficiency of early rice cultivars in red soil agro-ecosystem in South China

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***Abstract*—It is one of the effective ways to reduce the amount of nitrogen fertilizer by using rice varieties with adaptation to low nitrogen environment or high efficient utilization of soil nitrogen. Red soil agro-ecosystem of South China is an important production base of double-cropped paddy rice. However, the rice yield and N use rate in this area have been at a low level especially for early rice, due to the unreasonable use of nitrogen fertilizer and the acid characteristic of red soil. To screen early rice cultivars with high nitrogen efficiency and reveal their physiological characterizations, twenty one rice cultivars widely grown in red soil area of South China were tested in randomized block field experiments under normal and low nitrogen supplies. The biological characteristics of early rice varieties with high nitrogen efficiency were identified by analyzing parameters related to yield and its components, the aboveground nitrogen accumulation, the leaf SPAD value and photosynthetic rate, and nitrate Reductase activity. Based on rice yield and nitrogen efficiency analysis, Zhongxuan972, Zhongjiazao17, Zhongzu 7 and zhongyouzao13 were selected as high nitrogen efficient cultivars due to their more than 6000 kg ha^{-1} yields and more than 0.81 nitrogen efficiency, and Zhongjiazao2, Xiangzaoxian32, Jiayu948 and Zhongxuan 181 as low nitrogen efficient cultivars due to their lower yield and less than 0.70 nitrogen efficiency. Comparison of yield components revealed that the significant difference between two type cultivars was effective grain numbers of per unit area (panicle number ×spikelet number per panicle).The aboveground nitrogen accumulation and net photosynthetic rate in high nitrogen efficient cultivars were significantly higher than those in low ones at the heading stage under nitrogen-free condition, and there was not a significantly difference in leaf SPAD between the high and low nitrogen efficient cultivars. However, leaf SPAD values of high nitrogen efficient cultivars declined more slowly than that of the other four low nitrogen efficient cultivars under nitrogen-free condition. Nitrate Reductase activities of high nitrogen efficiency varieties under nitrogen-free condition were 38% to 53% activities under normal nitrogen condition, and nitrate Reductase activities of low nitrogen efficiency varieties were 22% to 35% activities. It suggested that the high nitrogen efficient rice cultivars had more grain numbers of per unit area under low nitrogen supply condition, their functional leaves maintained a higher level of photosynthetic rate and nitrate Reductase activity and lower chlorophyll decomposition rate especially during heading stage, which might be the reason for enhancing nitrogen assimilation and acquiring the higher yields.**

Keywords—red soil agro-ecosystem, cultivars screening and evaluation, early rice, nitrogen efficiency

I. INTRODUCTION

Rice is the most important food crop in China. It played an key role in ensuring the national food security. In order to maintain high yield and super high yield of rice, nitrogen (N) fertilizer was usually overused in intensive farmland. Excessive application of N fertilizer, for rice itself, would lead to lodging, exacerbating pests and diseases, and even affecting eating quality [1]. As for the environment, overuse of N fertilizer would have caused environmental problems such as soil, rivers, groundwater and air pollution, because N in soil not absorbed by crops entered into the environment through the way of ammonia volatilization, denitrification and leaching [2-3]. Considering the production cost, the N fertilizer cost accounted for about 35% of the total production costs in rice in China [4]. Moreover, the rate of N fertilizer utilization decreased and the yield didn't increase accordingly with the increase of N fertilizer, and the economic benefit of applying N fertilizer was decreasing [5].

Development of rice varieties with improved N use efficiency (NUE) is essential for sustainable agriculture. Present studies have shown that there is a significant genotypic difference in N use efficiency of rice[6-8], which provides a theoretical basis for screening and breeding rice varieties with high N efficiency. Under the same N level, nitrogen uptake and use efficiency from different genotypic rice varieties were significantly different. Difference of dry matter weight formed by unit N absorption was 13.4% among 16 rice varieties and difference of grain yield formed by unit N absorption was 34.2%[6].The study on N use efficiency from 48 rice varieties revealed that the agronomic utilization rate of N varied from

This article is supported by the national key basic research and development program (2013CB127405).

-22.65 to 17.51kg kg^{-1}, and the recovery rate of N varied from -53.91% to 55.14% [7]. With the increase of N application, the decrease of N use efficiency in different genotypic rice was not the same [8].

Environmental factors should not be neglected in the selection and evaluation of high N efficient of rice varieties, especially for the soil physical and chemical properties. Red soil agro-ecosystem in South China is an important production base of double-cropped paddy rice. However, the rice yield and N use rate in this area have been at a low level, especially for early rice, due to the unreasonable use of N fertilizer and the acid characteristic of red soil. To screen high N efficient early rice cultivars and reveal their physiological characterizations, twenty one rice cultivars widely grown in red soil agro-ecosystem were tested in randomized block field experiments under normal and low N supplies conditins. The biological characteristics of early rice varieties with high N efficiency were identified by analyzing parameters related to yield and its components, the aboveground N accumulation, the leaf SPAD value and photosynthetic rate, and nitrate Reductase activity.

II. MATERIALS AND METHODS

Experimental site and Rice cultivars

The field experiments were conducted in Jinxian County, Jiangxi Province, China（28°38´N, 116°24´E）in 2014 and 2015. This research area is a red soil agro-ecosystem and belongs to typical subtropical climate. Its annual average temperature is 21^{o}C, and effective accumulated temperature is about 5250^{o}C. The annual average precipitation is about 1200mm. The cropping system is double-cropped paddy rice. Its organic C from 0～20cm deep soil profile is 28.10 g kg^{-1}, total N is 1.72 gkg^{-1}, Olsen P is 14.79 $mgkg^{-1}$, available K is 83.86 mg kg^{-1}, soil bulk density is 1.40$gcm^{-3}$and pH is 5.13.

Twenty one *early* rice cultivars are Zhongjiazao2, Jiayu948, Xiangzaoxian32, Zhongxuan181,Zhongzu4,Jaiyu253, Jinzao47, Zhongzao1, Zhongzao27, Zhongzao39, Zhongzu3,Zhongzao16, Zhongjiazao66, Zhongzao31, Zongyouzao81, Xiangzaoxian45, Zhongzao35, Zhongxuan972, Zhongjiazao17, Zhongzu7 and Zhongyouzao13(Table1).

The experimental design and fertilizer treatments

The experimental design included two N treatments: 1) N0 treatment, as an unfertilized control, 2) N180 treatment, 180 kg N ha^{-1} was applied. The plots, each 12m×16m in size, were arranged in a randomized complete block experimental design with three replicates. Twenty one early rice varieties were planted in the same plot. Each cultivar has the area with 3 m×3 m, and the sow and plant spacing was 20cm×20cm. N, phosphorus (P) and potassium (K) fertilizers were urea (46.4% N), calcium magnesium phosphate (l6%P_2O_5) and potassium chloride (60%K_2O), respectively. 180 kg N ha^{-1} were applied with 33% base fertilizer, 33% jointing fertilizer and 33% booting fertilizer. 90 kg ha^{-1} P fertilizers and 120 kg ha^{-1} K fertilizers were applied into the red soil agro-ecosystem as basal fertilizer together.

Rice yield and its components

The rice plants on the area with 3 m×3 m were all collected and then yields were measured directly at harvest time. Five plants were randomly selected from each plot to investigate the panicle number, spikelet number per panicle, filled grain percentage and 1000 grain weight.

The leaf SPAD and photosynthetic rate

At the tillering stage and heading stage, three plants were randomly selected from each cultivar, and ten leave per plant were determined during 9:00-11:00 am. The leaf SPAD was determined by SPAD-502Plus.The photosynthetic rate from the top fully expanded leave of different rice varieties was measured by LICOR-6400XT Portable Photosynthesis System. The leaf SPAD and photosynthetic rate were measured for three times.

The leaf nitrate Reductase activity

At tillering stage and heading stage, three plants were randomly selected for each cultivar. The top fully expanded leave were taken to measure nitrate Reductase activity. Take 0.5 g Leave and put it into test tube (10ml), and put 9ml KNO_3, isopropanol and phosphate buffer mixture (3.03g KNO_3 is dissolved in 300ml 0.1mol L^{-1} phosphate buffer with pH7.4 and then added 3ml isopropyl alcohol). Then the test tube with leave was connected to the vacuum pump and was pumped up for several times until the leave in the test tube were sunk at the bottom of the tube. Place the tube with the leave and KNO_3, isopropanol and phosphate buffer mixture at the dark place at room temperature for 30min. Add 1ml trichloroacetic acid into the tube and shake it for a while to terminate nitrate Reductase activity. 2ml supernatant was taken from the above tube and put in new test tube (10ml). Then add 4ml p-aminobenzene sulfonic acid (1%) and 4ml 1- naphthylamine (0.2%) in the tube. After 30 min at room temperature, the leaf nitrate Reductase activity was measured by SHIMADZU UV-2600 spectrophotometer under 540nm wavelength. The standard curve of sodium nitrite was made by the same method.

Statistical analysis

Statistical analysis was accomplished by Microsoft Excel and variance and mean values were compared at the 5% level by the SPSS software package. The formulas are as follows:

Nitrogen efficiency (%) = yields on Nitrogen free treatment / yields on 180kg N ha^{-1} treatment

Nitrogen accumulation (kg N ha^{-1}) = dry matter weight * nitrogen content * 100%

Nitrate Reductase activity (N$\mu gg^{-1}h^{-1}$) = the corresponding value of the sodium nitrite standard curve * the volume of the extracted buffer/crude enzyme volume * leaf weight * reaction time

III. RESULTS

Yields and N efficiency of rice varieties under different nitrogen treatments

Field evaluations over twenty one varieties revealed that grain yield of Zhongzuo7, Zhongjiazao17, Zhongyouzao13,

Table 1 The yield and nitrogen efficiency of rice cultivars under two different levels of nitrogen treatments

Cultivars(2015)	Yield of N0 treatment（kg Ha^{-1}）	Yield of N180 treatment（kg Ha^{-1}）	N efficiency
Zhongjiazao2	3599.7 d	5891.5d	0.62
Xiangzaoxian32	3775.0 d	6520.0c	0.58
Jiayu948	4403.9 c	7079.4 b	0.62
Zhongxuan181	3816.0 d	6077.5c	0.63
Zhongzao39	4553.8 c	6670.0c	0.68
Zhongzao35	6073.7 b	7280.0 b	0.83
Zhongxuan972	6213.3 ab	7093.1b	0.87
Zhongyouzao13	6376.4 ab	7435.8 b	0.85
Zhongjiazao17	6576.9 a	7853.2 a	0.84
Zhongzu7	6751.1 a	7855.4 a	0.86
Cultivars(2014)	**Yield of N0 treatment（kg Ha^{-1}）**	**Yield of N180 treatment（kg Ha^{-1}）**	**N efficiency**
Zhongjiazao2	3760.0 c	6520.0 bc	0.57
Xiangzaoxian32	4663.3 b	6930.0 bc	0.67
Jiayu948	4753.3 b	6770.0 bc	0.70
Zhoangxuan181	4800.0 b	7666.7 ab	0.63
Zhongzuo4	4956.7 ab	6533.3 bc	0.76
Zhongzao1	5153.3 ab	6126.7 c	0.84
Zhongzao27	5193.3 ab	7233.3 ab	0.72
Jinzao47	5323.3 ab	7463.3 ab	0.71
Zhongzao39	5400.0 ab	8083.3 a	0.67
Zhongzu3	5403.3 ab	6950.0 bc	0.78
Zhongzao16	5446.7 ab	6910.0 bc	0.79
Jiayu253	5513.3 ab	7343.3 ab	0.75
Zhongjiazao66	5723.3 ab	8226.7 a	0.70
Zhongzao31	5810.0 ab	7646.7 ab	0.76
Zhongyouzao81	5823.3 ab	7253.3 ab	0.80
Xiangzaoxian45	5910.0 a	6836.7 bc	0.86
Zhongzao35	6036.7 a	6983.3 bc	0.86
Zhongxuan972	6113.3 a	7170.0 ab	0.85
Zhongjiazao17	6446.7 a	7683.3 ab	0.84
Zhongzu7	6546.7 a	7743.3 ab	0.85
Zhongyouzao13	6553.3 a	7833.3 ab	0.84

Note: No significant difference among the mean values with the same letter in the column（$p < 0.05$）, N efficiency= yield of N0 treatment/ yield of N180 treatment, N0-Nitrogen free treatment, N180-180kg N Ha^{-1}treatment.

Table 2 Rice shoots nitrogen accumulation and yield components under two different levels of nitrogen treatments

Rice with	PN（No.m^{-2}）		SPP（No.）		FP（%）		GW（g）		N A（kgN Ha^{-1}）	
Low NUE	N0	N180	N0	N180	N0	N180	N0	N180	N0	N180
Zhongjiazao2	147.0b	268.8b	71.3c	79.5d	95.1a	94.2a	26.5b	28.8a	56.7d	131.1d
Xiangzaoxian32	109.2c	239.4c	84.5c	179.0a	74.1c	78.4b	31.4a	30.8a	47.8d	153.0c
Jiayu948	155.4b	294.0a	87.4c	124.3c	84.5b	75.5bc	30.0a	29.4a	60.2c	159.0c
Zhongxuan181	121.8c	264.6b	129.2a	145.2b	89.6ab	75.8bc	31.2a	32.2a	53.8d	196.0b
Zhongzao39	151.2b	331.8a	105.5b	132.4c	88.6ab	72.6bc	31.5a	30.0a	61.5c	182.2b

Table 2 continues

Rice with high NUE	PN（$No.m^{-2}$）		SPP（No.）		FP（%）		GW（g）		N A（$kgN\ Ha^{-1}$）	
	N0	N180	N0	N180	N0	N180	N0	N180	N0	N180
Zhongzao35	126.0c	235.2b	132.8a	133.7c	89.3ab	80.8b	31.4a	30.7a	63.9bc	191.8b
Zhongxuan972	180.6a	323.4a	91.4bc	129.8c	94.5a	79.7b	31.8a	30.7a	64.8b	157.9c
Zhongjiazao17	121.8c	231.0b	147.1a	177.8a	90.1ab	70.9c	31.4a	29.8a	88.3a	224.3a
Zhongzu7	184.8a	252.0b	126.3a	150.9b	80.3b	74.5bc	32.2a	31.4a	68.8b	186.7b
Zhongyouzao13	155.4b	294.0a	138.0a	154.4b	80.7b	75.9bc	31.2a	30.0a	67.9b	190.3b

Note: No significant difference among the mean values with the same letter in the column（$p < 0.05$）,NUE-Nitrogen Use Efficiency, PN-Panicle Number, SPP-Spikelet Number per Panicle, FP-Filled Grain Percentage, GW-1000 grain weight, NA-Nitrogen Accumulation at Rice Shoots, N0-Nitrogen free treatment, N180-180kg N Ha^{-1} treatment

Table 3 The leaf SPAD and Photosynthetic Rate of rice cultivars under two different levels of nitrogen treatments

Rice with Low NUE	Tillering Stage SPAD			Heading Stage SPAD			PR（$\mu mol\ CO_2 m^{-2} s^{-2}$）		
	N0	N180	N0/N180	N0	N180	N0/N180	N0	N180	N0/N180
Zhongjiazao2	27.8b	40.1ab	0.69	34.5a	41.9a	0.82	14.69b	18.67ab	0.79
Xiangzaoxian32	32.2a	43.7a	0.74	33.2a	38.4ab	0.86	12.42c	15.93c	0.78
Jiayu948	30.6ab	40.7ab	0.75	32.2a	37.9b	0.85	15.52b	18.26ab	0.85
Zhongxuan181	31.5a	41.4a	0.76	32.3a	39.3ab	0.82	12.52c	18.34ab	0.68
Zhongzao39	31.6a	36.8b	0.78	32.3a	37.1b	0.87	13.80bc	17.62bc	0.78
Rice with high NUE	**Tillering Stage SPAD**			**Heading Stage SPAD**			**PR（$\mu mol\ CO_2 m^{-2} s^{-2}$）**		
	N0	N180	N0/N180	N0	N180	N0/N180	N0	N180	N0/N180
Zhongzao35	32.2a	39.4b	0.82	34.1a	38.6ab	0.90	17.35a	18.66ab	0.93
Zhongxuan972	30.3ab	36.5b	0.83	34.1a	38.2ab	0.89	17.85a	20.97a	0.85
Zhongjiazao17	30.7ab	37.7b	0.81	34.2a	37.9b	0.90	16.61ab	18.41ab	0.90
Zhongzu7	33.0a	42.3a	0.78	34.8a	38.6ab	0.90	17.25a	18.42ab	0.94
Zhongyouzao13	28.6b	39.7b	0.80	35.4a	38.7ab	0.91	17.14a	20.03a	0.86

Note: No significant difference among the mean values with the same letter in the column（$p < 0.05$）, NUE-Nitrogen Use Efficiency, PN-Panicle Number, PR-Photosynthetic Rate at Heading Stage, N0-Nitrogen free treatment,N180-180kg N Ha^{-1}

Table4 The leaf Nitrate Reductase activity of rice cultivars under two different levels of nitrogen treatments

Rice with Low NUE	Tillering Stage NR（$N\mu g g^{-1} h^{-1}$）			Heading Stage NR（$N\mu g g^{-1} h^{-1}$）		
	N0	N180	N0/N180	N0	N180	N0/N180
Zhongjiazao2	10.25b	37.53a	0.27	11.34b	39.06a	0.29
Xiangzaoxian32	10.25b	32.90b	0.31	7.38d	32.94bc	0.22
Jiayu948	8.95c	31.42b	0.28	9.72cd	34.84b	0.28
Zhongxuan181	7.85c	26.44c	0.30	8.46cd	31.50bc	0.27
Zhongzao39	9.82bc	30.46b	0.32	10.62c	30.68 c	0.35
Rice with High NUE	**Tillering Stage NR（$N\mu g g^{-1} h^{-1}$）**			**Heading Stage NR（$N\mu g g^{-1} h^{-1}$）**		
	N0	N180	N0/N180	N0	N180	N0/N180
Zhongzao35	10.03bc	30.98b	0.32	12.519 b	35.02b	0.36
Zhongxuan972	12.02a	32.81b	0.37	13.338a	29.70c	0.45
Zhongjiazao17	12.00a	31.51b	0.38	16.029a	33.30bc	0.48
Zhongzu7	9.16bc	27.05c	0.34	13.104ab	31.32bc	0.42
Zhongyouzao13	12.87a	34.18b	0.38	15.561a	30.16c	0.52

Note: No significant difference among the mean values with the same letter in the column（$p < 0.05$）, NUE-Nitrogen Use Efficiency, NR-Nitrate Reductase,N0-Nitrogen free treatment, N180-180kg N Ha^{-1} treatment

Zhongxuan972 and Zhongzao35 were significantly higher than other early rice varieties under different N application treatments. Their yields were more than 6000 kg ha^{-1} and their N efficiency were between 0.83 and 0.87. So these five varieties were selected as high N efficient varieties for further study. Among them, Zhongzuo7 and Zhongyouzao13 showed excellent character in terms of yield and N efficiency during two consecutive years. Our field results also demonstrated that grain yield of Zhongjiazao2, Jiayu948, Xiangzaoxian32, and Zhongxuan181 were significantly lower than other early rice varieties under different N application conditions. These 4 varieties were selected as low nitrogen efficient cultivars due to their lower yield and less than 0.70 nitrogen efficiency. Although Zhongzao39 were not one of the lowest yield of rice varieties, it was also selected as a low N efficient cultivar due to its the nitrogen efficiency between 0.67 and 0.68 in two years.

Yield components and aboveground N accumulation of rice varieties with different N efficiency

Comparison of yield components revealed that there was no significant difference in 1000 grain weight (GW) between nitrogen efficient and low efficiency varieties, and the filled grain percentage (FGP) was not clearly regular. However, the significant difference between two type rice cultivars was effective grain number of per unit area (panicle number × spikelet number per panicle). This means that high N efficiency rice varieties obtained more grain numbers per unit area under deficiency and normal N supply conditions (Table 2).

The aboveground N accumulation in high N efficiency cultivars was significantly higher than those in low ones at the heading stage under nitrogen-free condition except Zhongzao35. Among them, there was the highest aboveground N accumulation in Jiazao17 with high N efficiency under nitrogen-free and normal N supply conditions(Table 2).

Leaf SPAD value and photosynthetic rate of rice varieties with different N efficiency

Applying N fertilizer could increase leaf SPAD value and photosynthetic rate. There was no significant difference in leaf SPAD value between rice Cultivars with high and low N efficiency. However, leaf SPAD values of high N efficient cultivars declined more slowly than that of the other low N efficient cultivars. Leaf SPAD value ratio between nitrogen-free condition and nitrogen-normal condition were 0.69-0.78 in low N efficient cultivars, while the ratio were 0.78-0.83 in high N efficient cultivars at tillering stage. Compared with leaf SPAD at tillering stage, leaf SPAD value difference between N-free and N-normal condition decreased at heading stage. Leaf SPAD value difference on high N efficient cultivars was less that on low N efficient cultivars (Table 3).

Leaf photosynthetic rate of high N efficiency varieties under nitrogen free condition was significantly higher than that in low N efficiency varieties at heading stage (Table 3).

Leaf nitrate Reductase activity of rice varieties with different N efficiency

At tillering stage, no application of N fertilizer resulted in decreasing the leaf Nitrate Reductase activity of rice varieties. But there was no significant difference in the leaf nitrate Reductase activity between rice cultivars with high and low N efficiency

At heading stage, the nitrate Reductase activity of the high N efficient varieties was significantly higher than that of the low N efficient varieties under nitrogen deficiency, except Zhongjiazao2 and Zhongzao35. Nitrate Reductase activities of high N efficient varieties under nitrogen-free condition were 38% to 53% activities under N180 treatment, and nitrate Reductase activities of low nitrogen efficient varieties were 22% to 35% activities. The variation of nitrate Reductase activity in high N efficient varieties at heading stage (Table 4) was basically consistent with that of N accumulation of rice varieties (Table 2)

IV. Discussion

Accurate definition and evaluation of high N efficient rice varieties is a relatively complex problem. On the one hand, rice yield was not consistent with N efficiency under different N levels conditions [9]. On the other hand, the N uptake efficiency among rice varieties was not the same at different growth stages [10]. In order to obtain typical materials with high N efficiency, a great deal of time consuming work relating cultivars screening and evaluating have to be done. Therefore, simple, fast, accurate and practical are the factors that should be considered in determining the selection criteria of rice varieties. In this study, only nitrogen efficiency and rice yield on N free treatment have been considered to select and evaluate high N efficient cultivars. Zhongxuan972, Zhongjiazao17, Zhongyouzao13, Zhongzu7, and zhongzao35 were selected as high N efficient cultivars. The aboveground N accumulation of high N efficient varieties were significantly higher than that of the low N efficiency varieties under N free treatment, except zhongzao35, which indicated the method based on the two factors of nitrogen efficiency and yield to screen rice varieties with high N efficiency was right and effective.Zhongzao35 was finally not taken as a high N efficient variety because their yield, aboveground N accumulation and leaf nitrate Reductase activity were not significantly higher than those of other early rice cultivars.

Yield components could be used as a screening index for high N efficient varieties because the more N absorption by rice will contribute to promote grain filling percentage and panicle Number [11]. Our results showed that there was no significant difference in 1000 grain weight and the filled grain percentage between N high and low efficiency varieties. Comparison of yield components revealed that the significant difference between two type cultivars was effective grain numbers of per unit area, which seemed one of the physiological characterizations of high N efficient varieties.

Nitrate Reductase is a key limiting enzyme in plant assimilation of nitrate, and plays am important role in the N uptake and utilization by plants. During the late growth stage of rice, the nitrate Reductase activity in shoots is significantly positive correlation to N transport, which is one of the important indexes to select and evaluate rice varieties with high N efficiency [12]. Our researches found that the nitrate Reductase activity of the high N efficient varieties was significantly higher than that of the low N efficient varieties under N deficiency at heading stage, except Zhongjiazao2 and Zhongzao35 (Table4). The variation of nitrate Reductase activity in high N efficient varieties (Table 4) was basically consistent with that of aboveground N accumulation of rice varieties (Table 2). This suggested that high N efficient plants had higher nitrogen accumulation in plant by maintaining higher nitrate Reductase activity under N deficiency condition.

In summary, based on rice yield and nitrogen efficiency analysis, Zhongxuan972, Zhongjiazao17, Zhongzu7 and zhongyouzao13 were selected as high nitrogen efficient cultivars grown in the red soil agro-ecosystem of South China. It suggested that the high nitrogen efficient rice cultivars had higher effective grain number of per unit area under low nitrogen supply condition, their functional leave maintained a higher level of photosynthetic rate and nitrate Reductase activity and lower chlorophyll decomposition rate especially during heading stage, which might be the reason for enhancing nitrogen assimilation and acquiring the higher yields.

ACKNOWLEDGMENT

This article is supported by the national key basic research and development program (2013CB127405) and special funds for the construction of modern agricultural technology system(CARS-01-31).

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