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GRAIN YIELD AND NITROGEN UTILIZATION IN RESPONSE TO REDUCING NITROGEN RATE IN HYBRID RICE TRANSPLANTED AS SINGLE SEEDLINGS

By MIN HUANG†‡§, SHUANGLÜ SHAN†, XIAOBING XIE†, XUEFENG ZHOU†, YINGBIN ZOU† *and* NORMAN UPHOFF‡

†*Southern Regional Collaborative Innovation Center for Grain and Oil Crops (CICGO), Hunan Agricultural University, Changsha 410128, China and* ‡*International Programs-College of Agriculture and Life Sciences (IP-CALS), Cornell University, Ithaca, NY 14853, USA*

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SUMMARY

Transplanting single seedlings rather than seedlings in clumps has been increasingly attractive in hybrid rice production in China due to reduced seed requirements and higher grain yield. This study was conducted to determine grain yield and nitrogen (N) utilization in response to reductions in the N rate in hybrid rice under single-seedling transplanting. Field experiments were done in 2015 and 2016 on a moderate to high fertility soil at the Experimental Farm of Hunan Agricultural University, China. The hybrid rice cultivar Liangyoupeijiu (LYPJ) was used in 2015, and two hybrid cultivars LYPJ and Xiangliangyou 900 were used the next year. In each year, the rice plants transplanted with a single seedling per hill were grown with three N rates, including the usual N rate (150 kg ha^{-1}) and two reduced N rates (120 and 90 kg ha^{-1}). Grain yield, yield attributes, and N uptake and use efficiency were determined for each N rate. Significant reduction in grain yield was observed in only one of three cultivar-year combinations when N rate was reduced by 20% (from 150 to 120 kg ha^{-1}), and the magnitude of yield reduction was only 4%. Although significant reduction in grain yield was observed in two of the three cultivar-year combinations when N rate reduced by 40% (to 90 kg ha^{-1}), the highest yield reduction was only 7%. Yield attributes were generally changed slightly when N rate was reduced by 20%, while compensation among yield attributes and N utilization characteristics could explain why a 40% reduction in N rate did not result in substantial yield loss. Partial factor productivity of applied N (PFP_N) was increased by 21–24% and 56–63% with 20% and 40% reductions in the N rate, respectively. The higher PFP_N with a reduced N rate was attributed to higher recovery efficiency of applied N (RE_N) or to both higher RE_N and internal N use efficiency. Our study suggests that reducing N rate does not necessarily result in yield loss due to compensation among yield components and increased N use efficiency in hybrid rice transplanted as single seedlings under moderate to high soil fertility conditions.

INTRODUCTION

Rice is a staple food crop for about two-thirds of the population in China (Hsiaoping, 2005). Rice yield has more than tripled in the past several decades in China as

§Corresponding author. Email: mhuang@hunau.edu.cn; mh2426@cornell.edu

a result from the development of new cultivars, such as semi-dwarf cultivars in the late 1950s and hybrid rice cultivars in the late 1970s and from improved crop management practices (Peng *et al.*, 2009). Undoubtedly, these technical advances have been important for ensuring food security in China. However, at the same time, fertilizer consumption has increased almost linearly while fertilizer use efficiency has declined sharply in China, especially for nitrogen (N) fertilizer (Fan *et al.*, 2011). At present, the average N application rate for rice production in China is 180 kg ha^{-1}, about 75% higher than the world average (Chen *et al.*, 2014; Peng *et al.*, 2002). Rice yield increases by only 5−10 kg for each 1 kg of N fertilizer input in China, which is very low, if not the lowest among the major rice-producing countries (Peng *et al.*, 2006). Due to the high rate of N application, only 20−30% of N is taken up by the rice plant, and a large proportion of the N applied is lost to the environment (Peng *et al.*, 2009). As a consequence, substantial environmental costs are being observed with the over-use of N fertilizer, including enhanced N deposition, soil acidification and surface water eutrophication (Guo *et al.*, 2010; Le *et al.*, 2010; Liu *et al.*, 2013). In addition, over-application of N fertilizer can cause yield loss from lodging due to wind and rain and from the greater occurrence of diseases and insects as well as poor grain quality in rice (Peng *et al.*, 2009; Yang, 2015). With climate change, biotic and abiotic stresses affecting rice crops are becoming greater. Therefore, reducing rates of N application is of great and growing significance for sustainable rice production in China.

N fertilizer is usually applied to promote tillering to obtain high yield in rice (Peng *et al.*, 2002; Zhong *et al.*, 2002). Because tiller number can be increased by increasing seedling number, increasing the number of seedlings per hill with less N fertilization is considered as a strategy for achieving high yield with less environmental impacts in rice (Zhu *et al.*, 2016). Such a strategy might not be suitable for hybrid rice production because it requires more seeds and those seeds of hybrid rice are expensive. In fact, many Chinese rice farmers are now opting to plant inbred cultivars, which have a lower seed price than hybrid varieties. This consideration accounts at least in part for the current decline in the cultivated area under hybrid rice in China (Peng, 2016). In recent years, seed-saving rice production practices such as transplanting single seedlings, rather than seedlings in clumps of three or more, have been increasingly attractive in China. This can be a desirable change in crop management in its own right because wider spacing between seedlings gives their respective root systems more room to grow and expand (Stoop *et al.*, 2002), especially when other complementary changes are made in crop, soil, water and nutrient management (Thakur *et al.*, 2010; 2016). Higher grain yield can be achieved in hybrid rice transplanted as single than as multiple seedlings due to larger panicle size and greater dry matter per stem (Huang *et al.*, 2018). However, limited information is currently available on the response of hybrid rice to reduced N rates under single-seedling transplanting. To address this gap in knowledge, field experiments were conducted to compare grain yield, yield attributes, and N uptake and use efficiency between the usual and reduced N rates for hybrid rice transplanted with a single seedling per hill.

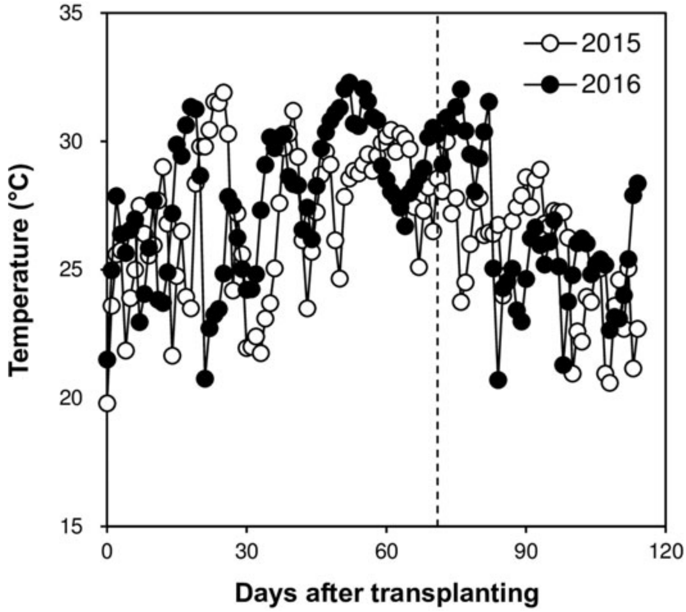

Figure 1. Daily mean air temperature during rice-growing season in 2015 and 2016. The dashed line denotes the date of heading.

MATERIALS AND METHODS

Site and soil

Field experiments were conducted in 2015 and 2016 at the Experimental Farm of Hunan Agricultural University (28°11′N, 113°04′E, 32 m asl), Changsha City, Hunan Province, China. The mean air temperature during the rice-growing seasons was 26.5 and 27.2 °C in 2015 and 2016, respectively (Figure 1). The experiment was done in the same field in 2015 and 2016. The soil of the experimental field was a Fluvisol with pH = 6.35, organic matter = 34.4 g kg^{-1}, total N = 1.49 g kg^{-1}, total P = 1.09 g kg^{-1}, total K = 12.8 g kg^{-1}, available N = 182 mg kg^{-1}, available P = 27.9 mg kg^{-1} and available K = 50.9 mg kg^{-1}. The soil analysis was based on samples taken from the upper 20 cm of the soil before transplanting in 2015.

Plants and treatments

The hybrid rice cultivar Liangyoupeijiu (LYPJ) was used in 2015, and two hybrid rice cultivars LYPJ and Xiangliangyou 900 (XLY900) were planted in 2016. LYPJ is an *indica-japonica* hybrid cultivar released in 1999 and widely commercialized, being planted in a wide range of climatic areas in southern China and southeastern Asia, from 12 °N to 35 °N (Lü and Zou, 2003). This cultivar has a cumulative planting area of about 5.95 million hectares (China Rice Data Center, http://www.ricedata.cn/variety/varis/600132.htm). XLY900 is a newly developed *indica* hybrid cultivar with high yield potential. This cultivar was named as Chaoyou 1000 before being released in 2017. In the regional rice trials, XLY900 produced an average grain yield of 9.93 and 9.50 Mg ha^{-1} in 2015 and 2016, respectively, (China Rice Data Center, http://www.ricedata.cn/variety/varis/616642.htm).

Three N rates, including the usual N rate in the study region (N_{150}: 150 kg ha^{-1}) (Huang *et al.*, 2011) and two reduced N rates (N_{120}: 120 kg ha^{-1}, N_{90}: 90 kg ha^{-1}), were applied in both 2015 and 2016. Urea was used as N fertilizer. In 2015, the treatments (N rates) were arranged in a randomized complete block design with three replications and a plot size of 48 m^2 (6 m × 8 m). In 2016, the treatments (N rates and cultivars) were laid out in a split-plot design with N rates as the main plots and cultivars as sub-plots; the experiment was replicated three times with sub-plot sizes of 24 m^2 (6 m × 4 m). To quantify N uptake from fertilizer and soil, two ^{15}N tracing micro-plots were established in each plot or sub-plot by inserting PVC cylinders (40-cm long, 40-cm inner diameter) into the soil at a depth of 20 cm with a collar of 20 cm aboveground. Except for the N fertilizer using ^{15}N-labeled urea (abundance 5.18%, produced by the Shanghai Institute of Chemical Industry, China), the micro-plots were managed in the same way as the main plots or subplots.

Pre-germinated seeds were sown in seedbeds to raise the seedlings. Twenty-five-day-old seedlings were manually transplanted at a hill spacing of 20 cm × 20 cm with a single seedling per hill. N was applied in three splits: 50% as basal, 30% at mid-tillering and 20% at panicle initiation. Phosphorus (90 kg P_2O_5 ha^{-1}) was applied basally, and potassium (180 kg K_2O ha^{-1}) was split equally at basal and panicle initiation. The water management regime was a sequence of flooding (about 3 cm depth) after transplanting, midseason drainage (a period of 10 days just before panicle initiation), re-flooding (about 3 cm), moist intermittent irrigation (during the grain-filling period), and draining 1 week before harvest. Weeds, insects and diseases were controlled in all plots as needed using chemicals to avoid yield loss.

Sampling and measurements

Ten hills (0.40 m^2) were sampled diagonally from a 5-m^2 harvest area for each plot. Panicle number per hill was counted to calculate panicle number per m^2. The plant samples were separated into straw (including rachis) and grains by hand threshing. Filled spikelets were separated from unfilled spikelets by submergence in tap water. Three subsamples of 30 g filled spikelets and all unfilled spikelets were counted to calculate spikelet number per panicle, spikelet number per m^2, spikelet filling percentage and grain weight. Dry weights of straw and of filled and unfilled spikelets were determined after oven-drying at 70 °C to a constant weight. Total aboveground biomass was the total dry matter of straw plus filled and unfilled spikelets. Harvest index (100 × filled grain dry weight/total aboveground biomass) and sink–source ratio (spikelet number per m^2/total aboveground biomass per m^2) were calculated. Grain yield was determined from a 5-m^2 area in each plot and adjusted to the standard moisture content of 0.14 g H_2O g^{-1} fresh weight.

Plants in micro-plots were sampled to determine their N content (VAP50 Kjeldahl meter, Gerhardt, Königswinter, Germany) and ^{15}N abundance (Delta V Advantage isotope mass spectrometer, Thermo Fisher, Waltham, MA, USA). Total N uptake and N uptake from fertilizer and soil were calculated according to Huang *et al.* (2014). Recovery efficiency of applied N (RE_N), internal N-use efficiency (IE_N) and partial factor productivity of applied N (PFP_N) were calculated according to the following equations:

$$RE_N = 100 \times \frac{\text{N uptake from fertilizer}}{\text{Applied N rate}} \tag{1}$$

$$IE_N = \frac{\text{Grain yield}}{\text{Total N uptake}} \tag{2}$$

$$PFP_N = \frac{\text{Grain yield}}{\text{Applied N rate}} \tag{3}$$

Statistical analysis

Statistical analyses were performed in Statistix 8 (Analytical software, Tallahassee, FL, USA). The data for each cultivar-year were separately analyzed by analysis of variance (ANOVA) to determine the effects of N rate. The statistical model included replication and N rate. Means of N rates were compared using the least significant difference test (LSD) at the 0.05 probability level. All data were analyzed by ANOVA to determine the effects of cultivar-year. The statistical model included replication, cultivar-year, N rate and the interaction between cultivar-year combination and N rate. Means of cultivar-year combinations were compared according to LSD (0.05). The relationships between total N uptake with N uptake from fertilizer and soil, and between IE_N and total N uptake were analyzed by linear regression with significance set at the 0.05 probability level.

RESULTS

Grain yield and yield attributes

There was no significant difference in grain yield among three N rates for LYPJ in 2015 (Table 1). For LYPJ, the difference in grain yield between N_{120} and N_{150} was not significant in 2016, while grain yield was 7% lower under N_{90} than under N_{150}. For XLY900, grain yields under N_{120} and N_{90} were 4% lower than that under N_{150} in 2016. Averaged across the three N rates, LYPJ produced 19% higher grain yield in 2015 than in 2016. XLY900 had 14% higher grain yield than LYPJ in 2016.

Panicle number per m^2 diminished with decreasing N rate for all cultivars and years (Table 1). The difference in spikelet number per panicle among the three N rates was not consistent across cultivars and years. Spikelet number per m^2 was lower under N_{120} and N_{90} than under N_{150}. Conversely, spikelet filling percentage and grain weight under N_{120} and N_{90} were generally higher than those under N_{150}. On average for the three N rates, LYPJ had higher panicle number per m^2, spikelet number per m^2, spikelet filling percentage and grain weight, and similar spikelet number per

Table 1. Grain yield and yield components in hybrid rice cultivars Liangyoupeijiu (LYPJ) and Xiangliangyou 900 (XLY900) transplanted as single seedlings under three N rates in 2015 and 2016.

N rate*	Grain yield (Mg ha^{-1})	Panicles m^{-2}	Spikelets $panicle^{-1}$	Spikelets m^{-2} ($\times 10^3$)	Spikelet filling (%)	Grain weight (mg)
			LYPJ-2015			
N_{150}	11.27 (0.70)	253 (10)	237 (14)	59.9 (1.3)	74.4 (1.5)	25.5 (0.2)
N_{120}	11.11 (0.88)	248 (11)	237 (17)	58.7 (2.5)	74.3 (1.0)	25.7 (0.1)
N_{90}	11.01 (0.26)	222 (10)	249 (6)	55.1 (2.4)	77.2 (0.8)	25.6 (0.0)
Mean[†]	11.13 (0.59) a	241 (17) a	241 (13) b	57.9 (2.8) a	75.3 (1.7) b	25.6 (0.2) a
LSD (0.05)[‡]	1.41	14	19	4.3	2.7	0.3
			LYPJ-2016			
N_{150}	9.66 (0.08)	229 (4)	251 (9)	57.3 (1.8)	67.7 (1.7)	23.4 (0.1)
N_{120}	9.47 (0.06)	216 (4)	241 (9)	52.0 (1.2)	70.4 (0.6)	23.4 (0.1)
N_{90}	8.99 (0.14)	204 (5)	226 (2)	46.2 (1.0)	74.3 (0.5)	23.6 (0.1)
Mean	9.37 (0.31) c	216 (11) b	239 (12) b	51.8 (5.0) c	70.8 (3.1) c	23.5 (0.1) b
LSD (0.05)	0.19	9	21	3.7	2.8	0.2
			XLY900-2016			
N_{150}	11.00 (0.04)	177 (9)	370 (8)	65.3 (2.3)	75.1 (2.8)	21.0 (0.0)
N_{120}	10.56 (0.04)	164 (4)	307 (11)	50.5 (2.1)	81.1 (2.3)	21.6 (0.3)
N_{90}	10.54 (0.08)	159 (5)	327 (13)	51.8 (1.3)	81.8 (1.5)	22.0 (0.5)
Mean	10.70 (0.23) b	167 (10) c	335 (29) a	55.9 (7.3) b	79.3 (3.8) a	21.5 (0.5) c
LSD (0.05)	0.15	13	13	5.4	6.0	0.9

Values in parentheses are standard deviations.
*N_{150}, N_{120} and N_{90} are 150, 120 and 90 kg ha^{-1}, respectively.
†Means with the same letters for each parameter are not significantly different according to LSD (0.05).
‡LSD (0.05) values are for comparison among N rates for each parameter within each cultivar-year.

panicle in 2015 compared to 2016. XLY900 had lower panicle number per m^2 and grain weight, but higher spikelet number per panicle, spikelet number per m^2 and spikelet filling percentage than LYPJ in 2016.

A decrease in total aboveground biomass with decreasing N rate was observed for all cultivars and years (Table 2). There was no significant difference in harvest index between N_{120} and N_{150}, while N_{90} generally had higher harvest index than N_{150}. There was no consistent difference in sink–source ratio among the three N rates across cultivars and years. Averaged across the three N rates, LYPJ had higher total aboveground biomass and harvest index and comparable sink–source ratio in 2015 than in 2016. XLY900 had similar total aboveground biomass and higher harvest index and sink–source ratio compared to LYPJ in 2016.

N uptake and use efficiency

Uptake of N from fertilizer and soil sources and total N uptake varied slightly with the rate of N application for LYPJ in 2015, while these measures decreased obviously with decreasing N rate for LYPJ and XLY900 in 2016 (Table 3). On average for the three N rates, LYPJ had a lower N uptake from fertilizer but higher N uptake from soil, and higher total N uptake in 2015 than in 2016. There were no significant differences in N uptake from fertilizer and soil and in total N uptake between XLY900

Table 2. Total aboveground biomass, harvest index and sink–source ratio in in hybrid rice cultivars Liangyoupeijiu (LYPJ) and Xiangliangyou 900 (XLY900) transplanted as single seedlings under three N rates in 2015 and 2016.

N rate*	Total aboveground biomass (g m^{-2})	Harvest index (%)	Sink–source ratio (spikelets g^{-1})
	LYPJ-2015		
N_{150}	2113 (38)	53.6 (1.2)	28.3 (0.5)
N_{120}	2098 (64)	53.4 (0.5)	28.0 (0.4)
N_{90}	1947 (45)	55.8 (0.6)	28.3 (0.6)
Mean†	2053 (91) a	54.3 (1.35) a	28.2 (0.5) b
LSD (0.05) ‡	76	1.7	1.3
	LYPJ-2016		
N_{150}	1981 (34)	45.8 (0.5)	28.9 (0.5)
N_{120}	1874 (22)	45.7 (1.1)	27.8 (0.8)
N_{90}	1766 (19)	46.0 (0.4)	26.2 (0.3)
Mean	1874 (96) b	45.8 (0.7) c	27.6 (1.3) b
LSD (0.05)	61	1.0	1.3
	XLY900-2016		
N_{150}	2067 (45)	49.9 (0.6)	31.6 (0.9)
N_{120}	1802 (60)	49.0 (1.0)	28.0 (0.2)
N_{90}	1795 (40)	51.9 (1.0)	28.9 (0.8)
Mean	1888 (141) b	50.3 (1.5) b	29.5 (1.7) a
LSD (0.05)	134	2.0	1.8

Values in parentheses are standard deviations.
*N_{150}, N_{120} and N_{90} are 150, 120 and 90 kg ha^{-1}, respectively.
†Means with the same letters for each parameter are not significantly different according to LSD (0.05).
‡LSD (0.05) values are for comparison among N rates for each parameter within each cultivar-year.

and LYPJ in 2016. Total N uptake was not significantly related to N uptake from fertilizer but was closely related to N uptake from the soil (Figure 2). About 90% of the variation in total N uptake was explainable by N uptake from the soil (Figure 2b).

An increase in RE_N with decreasing N rate was observed for all cultivars and years (Table 3). The difference in IE_N among the three N rates was small for LYPJ in 2015, while IE_N increased with a decrease in N rate for XLY900 and LYPJ in 2016. A remarkable increase in PFP_N with decreasing N rate was observed for all cultivars and years. N_{120} and N_{90} had 21–24% and 56–63% higher PFP_N than N_{150}, respectively. Averaged across the three N rates, LYPJ had lower RE_N, similar IE_N, and higher PFP_N in 2015 compared to 2016. XLY900 had comparable RE_N and higher IE_N and PFP_N than LYPJ in 2016. There was a significant negative relationship between IE_N and total N uptake (Figure 3).

DISCUSSION

In this study, significant reduction in grain yield was observed in only one of the three cultivar-year combinations when N rate was reduced by 20% (from 150 to 120 kg ha^{-1}), and the magnitude of this yield reduction was only 4% (Table 1). Although significant reduction in grain yield was observed in two of the three cultivar-year combinations when N rate reduced by 40% (from 150 to 90 kg ha^{-1}), the highest

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Table 3. N uptake and use efficiency in hybrid rice cultivars Liangyoupeijiu (LYPJ) and Xiangliangyou 900 (XLY900) transplanted as single seedlings under three N rates in 2015 and 2016.

	N uptake (kg ha^{-1})			N use efficiency†		
N rate*	From fertilizer	From soil	Total	RE_N (%)	IE_N (kg kg^{-1})	PFP_N (kg kg^{-1})
			LYPJ-2015			
N_{150}	35 (3)	156 (7)	191 (9)	23.3 (2.3)	59.0 (1.0)	75.1 (4.7)
N_{120}	34 (1)	155 (4)	189 (3)	28.3 (0.9)	58.8 (3.8)	93.6 (7.3)
N_{90}	32 (5)	156 (25)	188 (30)	35.6 (5.4)	58.6 (7.9)	122.3 (2.9)
Mean‡	34 (3) b	156 (13) a	190 (16) a	29.1 (6.0) b	58.8 (4.4) b	97.0 (21.2) a
LSD (0.05)§	9	38	45	8.8	10.8	10.9
			LYPJ-2016			
N_{150}	46 (5)	140 (10)	186 (12)	30.7 (3.0)	51.9 (3.2)	64.4 (0.5)
N_{120}	41 (3)	130 (10)	171 (10)	34.2 (2.2)	55.4 (3.1)	79.0 (0.5)
N_{90}	34 (2)	113 (4)	147 (3)	37.8 (2.1)	61.2 (0.4)	100.0 (1.6)
Mean	40 (6) a	128 (14) b	168 (19) b	34.2 (3.9) a	56.2 (4.5) b	81.1 (15.5) c
LSD (0.05)	8	8	13	6.4	4.6	2.0
			XLY900-2016			
N_{150}	47 (4)	137 (2)	184 (3)	31.3 (2.6)	59.8 (0.7)	73.3 (0.3)
N_{120}	39 (2)	116 (5)	155 (5)	32.5 (1.4)	68.1 (2.5)	88.0 (0.3)
N_{90}	32 (1)	113 (4)	145 (4)	35.6 (1.3)	72.7 (2.0)	117.1 (0.9)
Mean	39 (7) a	122 (12) b	161 (18) b	33.1 (2.6) a	66.9 (5.9) a	92.8 (19.3) b
LSD (0.05)	3	7	10	2.6	4.2	1.5

Values in parentheses are standard deviations.

*N_{150}, N_{120} and N_{90} are 150, 120 and 90 kg ha^{-1}, respectively.

†RE_N, recovery efficiency of fertilizer N; IE_N, internal N use efficiency; PFP_N, partial factor productivity of fertilizer N.

‡Means with the same letters for each parameter are not significantly different according to LSD (0.05).

§LSD (0.05) values are for comparison among N rates for each parameter within each cultivar-year.

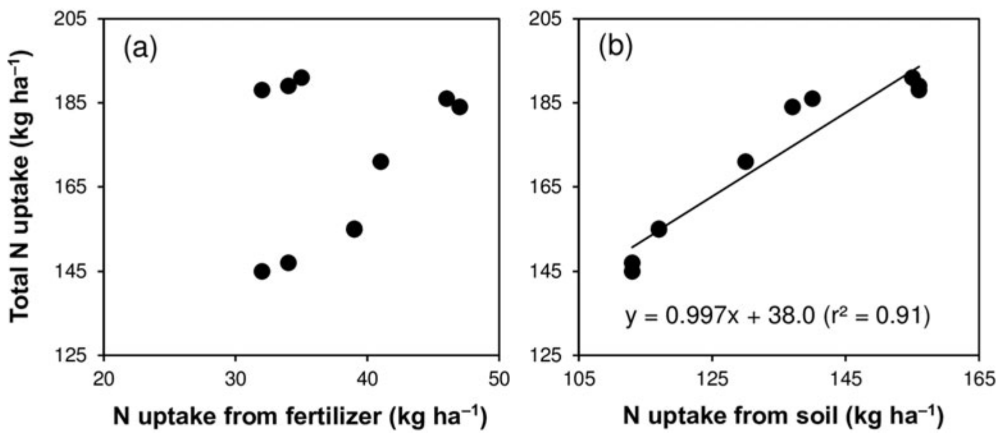

Figure 2. Relationships between total N uptake with N uptake from fertilizer (a), and N uptake from soil (b) in hybrid rice transplanted as single seedlings. Each data point is the mean of three replications for each N rate within each cultivar-year.

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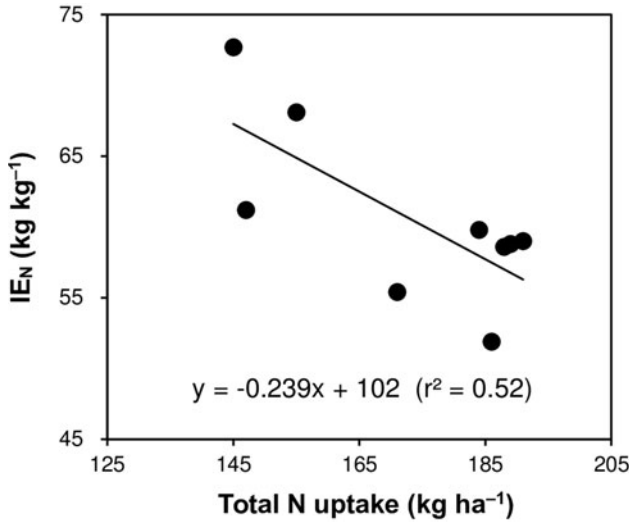

Figure 3. Relationship between internal N use efficiency (IE_N) and total N uptake in hybrid rice transplanted as single seedlings. Each data point is the mean of three replications for each N rate within each cultivar-year.

yield reduction was only 7%. These results are similar to those observed in hybrid rice transplanted as double seedlings (Huang *et al.*, 2008; Huang *et al.*, 2016; Yuan *et al.*, 2017). Most important in agronomic and environmental terms, they suggest that hybrid rice transplanted as single seedlings does not benefit from applying more N fertilizer to produce high grain yield. However, because this study was conducted on a moderate to high fertility soil, the results might not be applicable to low soil fertility conditions. Therefore, more experimentation should be done to determine how, and how much, changes in grain yield there would be in response to reduced N rates in hybrid rice transplanted as single seedlings under low soil fertility conditions.

Yield attributes were generally changed little when the N rate was reduced from 150 to 120 kg ha^{-1} (Tables 1 and 2). This is consistent with what is reported for hybrid rice transplanted as double seedlings by Huang *et al.* (2008), who observed that the N response of LYPJ and another hybrid cultivar Shanyou 63 was not evident at N rates ranging 120–150 kg ha^{-1}. By contrast, when N rate was reduced from 150 to 90 kg ha^{-1}, most of the yield attributes were changed significantly (Tables 1 and 2). More interestingly, compensation among yield attributes was observed when the N rate was reduced from 150 to 90 kg ha^{-1}. First, reducing the N rate from 150 to 90 kg ha^{-1} resulted in decreased panicle number and spikelet number per m^2, but it increased spikelet filling percentage and grain weight. Second, reducing the N rate from 150 to 90 kg ha^{-1} caused a decrease in total biomass production, but an increase in harvest index. These findings are in agreement with those observed in hybrid rice under double-seedling transplanting (Huang *et al.*, 2016; Yuan *et al.*, 2017). It could explain why the large reduction in N rate (40%) did not result in a substantial yield loss in hybrid rice transplanted as single seedlings. Moreover, our results showed that the small response of grain yield to the large reduction in N rate in hybrid rice transplanted with a single seedling per hill also could be explained by N utilization characteristics: (i) RE_N was significantly increased with a decreasing N rate (Table 3); (ii) total N uptake mostly depended on N uptake from soil (Figure 2); and (iii) total N uptake was negatively related to IE_N (Figure 3). However, the second characteristic raises a concern about the long-term sustainability of soil N supply. This could be confirmed by the results that uptake of N from soil and total N uptake varied slightly with the rate of N application in 2015, while these measures decreased obviously with decreasing N rate in 2016 (Table 3). These facts highlight that improving and maintaining soil fertility should be a focus for sustainable production of hybrid rice transplanted as single seedlings under reduced N conditions.

It has been well documented that an increase in fertilizer-N use efficiency can be achieved by reducing the N rate in rice (Peng *et al.*, 2002; 2006). PFP_N is an aggregate efficiency index that includes contributions to grain yield derived from the uptake of indigenous soil N and from changes in RE_N and IE_N (Cassman *et al.*, 2003). In this study, average PFP_N across three cultivar-year combinations was increased by 23% and 60% with reductions in N rate of 20% and 40%, respectively (Table 3). The higher PFP_N under a reduced N rate was attributable to higher RE_N or to both higher RE_N and IE_N. Unexpectedly, the RE_N values for LYPJ in this study (23–38%) were even slightly lower than RE_N values obtained from this same cultivar (26–39%) under higher N rates (161–225 kg ha^{-1}) in the same location (Jiang *et al.*, 2016). This might be related to the different methods used for determining RE_N. In this study, RE_N was determined by the ^{15}N tracer method, while Jiang *et al.* (2016) calculated this by a subtraction method, i.e., 100 × (total N uptake in a plot with N application − total N uptake in a plot without N application)/N application rate. It is suggested that N mineralization can be increased by N fertilization (Yan *et al.*, 2006), which can result in an over-estimate of the RE_N when using the subtraction method of calculation.

There was a significant difference between years in grain yield for LYPJ, with yield being lower in 2016 than in 2015 (Table 1). Analysis of yield components indicated that the lower grain yield in 2016 was attributed to lower panicle number per m^2, lower spikelet filling percentage, and lower grain weight than in 2015. The poorer performance in yield components in 2016 could be explained by variation in air temperature (Figure 1), namely there was (i) lower panicle number per m^2 related to the lower temperature at mid-tillering stage, 21–25 days after transplanting; (ii) lower spikelet filling percentage attributable to higher temperature at flowering stage, 1–6 days after heading; and (iii) lower grain weight due to higher average temperature (about 1 °C) during ripening. On the other hand, poor grain filling is also related to source limitation in rice (Huang *et al.*, 2012; Lu *et al.*, 1994; Yuan, 1994). In this study, although total aboveground biomass was lower in 2016 than in 2015, source limitation was not responsible for the poor grain filling in 2016 than in 2015 because sink–source ratio was comparable between the two years (Table 2).

Significant difference in grain yield was also observed between cultivars in 2016 (Table 1) and XLY900 produced 14% higher grain yield than LYPJ. The magnitude of this yield difference between XLY900 and LYPJ is close to that recorded between these two cultivars (16%) under double-seedling transplanting (Huang *et al.*, 2017).

The results of yield-component analysis showed that larger panicle size (more spikelet number per panicle) was one of the factors contributing to the higher grain yield in XLY900 than LYPJ (Table 1). This suggests that cultivars with large panicle size should be selected to achieve the highest yield from hybrid rice transplanted as single seedlings per hill. Higher spikelet filling percentage was another factor responsible for the higher grain yield in XLY900 than LYPJ (Table 1). In addition and as mentioned above, the lower spikelet filling in LYPJ in 2016 was attributable to higher temperature at the flowering stage. Therefore, XLY900 might have higher tolerance to high temperature stress than LYPJ.

CONCLUSIONS

Reducing N rate does not necessarily result in yield loss due to compensation among yield components and increased N use efficiency in hybrid rice transplanted as single seedlings under moderate to high soil fertility conditions. Further investigations are required to determine the effect of reducing N rate on the long-term sustainability of soil N supply, especially under low soil fertility conditions.

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