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Estimation of nitrogen fertilizer requirement for rice crop using critical nitrogen dilution curve

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ABSTRACT

Estimating in-season N requirement (NR) is essential for managing N fertilizer application in crop production. Critical N (N_c) dilution curve is an effective and simple-to-use technique for assessment of in-season crop N status, yet its adaptation to make field decisions about dressing N fertilization remains to be determined. This study was endeavored to establish the relations between NR, N nutrition index (NNI) and relative yield (RY) at different crop growth stages in Japonica and Indica rice (*Oryza sativa* L.) eco-types and to estimate time-course NR for recommending supplemental N fertilization on N_c dilution curve basis. Four field experiments of multi-N rates were carried out in east China using three Japonica and two Indica rice hybrids. Growth analysis was carried out at different growth stages from active tillering (AT) to heading (HD). The estimated NR under varied N rates has well differentiated the sub-optimal, optimal and supra optimal growth conditions at different stages in both rice eco-types. The NR-NNI and RY-NR relations for both rice eco-types at different growth stages were highly significant with R^2 values greater than 0.88 and 0.95 for NR-NNI, and 0.83 and 0.91 for RY-NR relations, respectively, the highest R^2 values for both eco-types were 0.98 and 0.99 for NR-NNI and 0.94 and 0.93 for RY-NR relations at panicle initiation (PI) and booting (BT) stages. Validation of the regression models with two independent datasets exhibited a solid model performance at PI and BT stages, with R^2 values greater than 0.96 for NR-NNI while 0.94 for RY-NR relations. Moreover, the root mean square error (RMSE) values lower than 20% for NR prediction from NNI, while 8% for RY prediction from NR also confirmed the robustness of the relationships at PI and BT stages. The kappa (k) coefficients at PI and BT stages for observed and predicted NR and RY were close to 1. Generally, the robust relations at PI and BT stages well elucidated the variation in NR and RY both under deficient and optimum N growing conditions, and gave reliable estimation of NR for quantifying supplemental N fertilization for rice grown in east China. The results of this study will offer a suitable approach for managing N application precisely during the growth period of rice crop.

1. Introduction

Nitrogen (N) fertilizer is the most extensively used agricultural input by rice (*Oryza sativa* L.) growers to enhance rice crop production in China (Chen et al., 2011). China alone contributes to 32% consumption of the world's total N fertilizer application, while 18% of this (1/5th of China's national use) is utilized in rice production (Heffer, 2009). Due to lower costs of N fertilizer in China as compared to that in other countries, it is often considered economically viable option for farmers. The average rate of N application (193 kg ha^{-1}) for rice cultivation in China is 90% higher than that of the world average, while in east China, it is double (387 kg ha^{-1}) of China's national average for rice cultivation (Heffer, 2009). In addition to its over-dosage, application at improper crop growth stages has led to low N use efficiency in China. The N recovery efficiency (NRE, the ratio of plant N to N supply) (30–35%) and agronomic N efficiency (the ratio of yield to N supply) (5–10 kg kg^{-1} N) in China are much lower than those of other rice growing countries (50–60% and 15–18 kg kg^{-1} N, respectively) (Peng et al., 2009). Furthermore, its over-application may actually reduce potential yield of rice by enhancing the risk of lodging, delaying maturity, as well as by increasing susceptibility to insects and diseases (Peng et al., 2010). Moreover, improper time and rate of N fertilizer applica-

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tion, as well as poor N management strategies result in substantial losses of applied and available N in the soil due to inherently low N uptake capacity and absence of extensive root system in rice (Sinclair and Rufty, 2012). Thus, correct in-season assessment of optimal N requirement (NR), the N fertilizer requirement for a crop at any stage of development for reaching the N_c level, i;e. the crop N status corresponding to maximum growth at different crop growth stages is pivotal for precise management of N fertilization rates and timing.

The in-season N management requires development of rapid, effective and simple-to-use, economically feasible and technically rigorous N diagnostic tools. Precision N management can improve crop productivity and overcome adverse effects of N on cropping system (Gastal and Lemaire, 2002). Precision agriculture techniques can annually save up to 11 million tons N globally without any drop in crop yield (Mueller et al., 2012), which rises the need to develop generic, flexible and effective tools for guiding farmers for implementation of in-season N management practices. N fertilizer recommendation and enhancement of N use efficiency greatly depend on accurate appraisal of N status in plant and soil systems (Costa et al., 2001). Crop N status at different growth stages indicates the capacity of soil to supply N, capability of plant to uptake N, as well as interactions between plant and soil N (Wang et al., 2006). N concentration has a close relationship with plant dry matter (DM) and leaf area index (LAI) (Gastal and Lemaire, 2002). Time specific estimates of N concentration, as well as estimation of DM and LAI, can help to monitor plant N status and field diagnosis for N management. Thus, dynamics of DM, LAI and tissue N concentration, together with their time specific relationships should be helpful for developing effective N management and recommendation strategy. Similarly, N concentrations in shoots, leaves and stems of field crops have also been used as diagnostic indicators for assessment of N status for maintaining optimum crop growth rate.

The concept of critical N (N_c), the minimum N concentration required for maximum crop growth has extensively been used for both diagnostic purposes, as well as for modelling plant-N-relations. The N_c derived from DM, LAI and crop growth stages gives insight into development of N_c dilution curve (Gastal and Lemaire, 2002). The N_c dilution curves have been developed on whole plant DM basis for different eco-types of rice (Sheehy et al., 1998; Ata-Ul-Karim et al., 2013) as well as on LAI and specific organ (leaf and stem) DM basis for rice (Ata-Ul-Karim et al., 2014a,b; Yao et al., 2014). This concept can be potentially used for assessing N status, guiding dressing N recommendation and predicting grain yield in crop production (Debaeke et al., 2012; Ata-Ul-Karim et al., 2013, 2016). Numerous attempts have been made to develop a N fertilizer decision support tool using this approach to delay or split N fertilizer application. The relationships derived from N_c based N nutrition index (NNI), the ratio between the actual crop N concentration and critical N concentration, with relative yield (RY), the ratio of the grain yield obtained for a given N rate with the highest grain yield among all N application rates have been previously implicated to assess RY of wheat, corn (Ziadi et al., 2008, 2010) and sunflower (Debaeke et al., 2012), yet no attempt has been made to estimate crop NR for a corrective N fertilization during crop growth period. Moreover, the previous studies used the averaged values of NNI acquired at different crop growth stages to develop these relations. NR and N use efficiency varies across the growth stages, and crop growth stage is a key plant characteristic for estimating the NR and for improving N use efficiency by estimating in-season crop N status. In present study, we hypothesized that, the NR-NNI and RY-NR relations of two rice eco-types at different growth stages can be implemented for reliable estimation on in-season NR, setting yield targets and improving N use efficiency.

This study was aimed to establish the NR-NNI and RY-NR relations at different growth stages of Japonica and Indica rice, and to estimate time-course NR on the basis of N_c dilution curve for quantifying in-season corrective N fertilization and setting the yield targets. The results of this study will offer a useful methodology for managing N application precisely during the growth period of rice crop.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted at two sites, Yizheng (32°16′ N, 119° 10′ E) and Rugao (32°23′ N, 120° 33′ E) situated in east China. This zone is categorized by a subtropical-temperate climate with hot summer and cold winter, and is suitable for planting different eco-types of rice. The region receives 2177 h of sunshine and 1030 mm of rainfall annually. The detailed soil characteristics of the top 20 cm depth and cropping practices of both sites are shown in Table 1.

To establish the relationships between NR, NNI and RY on the basis of N_c dilution curve during vegetative growth, four field experiments were conducted in rice across the sites, as detailed in Table 2. In all experiments, five or six varied N fertilizer rates ranging from 0 to 375 kg N ha^{-1} were imposed as N treatments. The source of N fertilizer was urea in all experiments across the sites. The detailed information about N treatments in four field experiments including N rates, N distribution (%) and N application timings are summarized in Table 2. Over the four experiments, three Japonica rice cultivars, Wuxiangjing-14 (WXJ-14), Lingxiangyou-18 (LXY-18) and Wuyunjing-24 (WYJ-24), as well as two Indica rice cultivars Shanyou-63 (SY-63) and Y-Liangyou-1 (YLY-1) were used. The cultivars/eco-types differences were used to test the reliability of newly developed models in different types of rice for making N management decisions.

All experiments were conducted with randomized complete block design having three replicates. The size of every plot was 4.5 m × 8 m with 15 rows in 2010 and 2011 while it was 5 m × 6 m with 20 rows in 2013 and 2014. The inter-row spacing of 30 cm was used at both sites. The planting density was approximately 22.2×10^4 plants ha^{-1} in all experiments. In each experiment, every plot received 135 kg P_2O_5 ha^{-1} and 190 kg K_2O ha^{-1} before transplantation. All experiments were carried out with optimal crop management according to each site, in order to obtain the potential yield (N fertilizer was the only limiting factor).

2.2. Plant sampling and measurements

Plant samples (five plants from each plot) were collected from 0.23 m^2 area at active tillering (AT), mid tillering (MT), stem elongation (SE), panicle initiation (PI), booting (BT), heading (HD), and maturity for growth analysis. The plant samples were divided into leaf (green leaf blade), stem (culm plus sheath) and panicle. All the samples were oven-dried for 30 min at 105 °C to quickly cease plant metabolic activities and then at 70 °C to constant weight to attain the plant DM (t ha^{-1}). The plant N concentration (%) was determined by using the standard Kjeldahl method. The N accumulation (kg N ha^{-1}) was calculated by multiplying plant DM by plant N concentration. In all experiments, grain yield in each plot was calculated by harvesting plants from three randomly identified areas of 1 m^2. Spikelets were removed from panicle and the final grain yield was adjusted to 14% moisture. The RY was calculated as the ratio of grain yield obtained for a given N rate to the highest grain yield among all N application rates.

Table 1
Soil characteristics of the top 20 cm depth and cropping practices of the four experiments conducted during 2010–14.

Soil and crop information	Experiment 1 (2010) Yizheng	Experiment 2 (2011)	Experiment 3 (2013) Rugao	Experiment 4 (2014)
Soil type	Ultisoles	Ultisoles	Ultisoles	Ultisoles
Soil pH	6.2	6.4	6	6.1
Organic matter	17.5 g kg^{-1}	15.5 g kg^{-1}	13.5 g kg^{-1}	14.9 g kg^{-1}
Total N	1.6 g kg^{-1}	1.3 g kg^{-1}	1.5 g kg^{-1}	1.1 g kg^{-1}
Available P	43 mg g^{-1}	38 mg g^{-1}	30 mg g^{-1}	32 mg g^{-1}
Available K	90 mg g^{-1}	85 mg g^{-1}	84 mg g^{-1}	80 mg g^{-1}
Previous crop	Wheat	Wheat	Wheat	Wheat
Transplanting date	20-Jun	20-Jun	21-Jun	18-Jun
Harvesting date	24-Oct	24-Oct	16-Oct	18-Oct
Rice hybrids	LXY-18, WXJ-14 (Japonica)	LXY-18, WXJ-14 (Japonica)	WXJ-14, SY-63 (Japonica), (Indica)	WYJ-24, YLY-1 (Japonica), (Indica)

Table 2
Nitrogen application rate (kg ha^{-1}), timing and distribution (%) details for the four experiments conducted during 2010-14.

Experiment No.	N rate (kg ha^{-1})	N distribution (%)	N application timing
Experiment 1 (2010)	N0 (0)		
	N1 (80)	50%	Pre-planting
	N2 (160)	10%	Active tillering
	N3 (240)	20%	Panicle initiation
	N4 (320)	20%	Booting
Experiment 2 (2011)	N0 (0)		
	N1 (90)	50%	Pre-planting
	N2 (180)	10%	Active tillering
	N3 (270)	20%	Panicle initiation
	N4 (360)	20%	Booting
Experiment 3 (2013)	N0 (0)		
	N1 (75)	40%	Pre-planting
	N2 (150)	10%	Active tillering
	N3 (225)	30%	Panicle initiation
	N4 (300)	20%	Booting
	N5 (375)		
Experiment 4 (2014)	N0 (0)		
	N1 (150)	40%	Pre-planting
	N2 (225)	10%	Active tillering
	N3 (300)	30%	Panicle initiation
	N4 (375)	20%	Booting

2.3. Nitrogen parameters

2.3.1. Nitrogen requirement

Nitrogen requirement (kg N ha^{-1}) of rice cultivars before different stages during vegetative growth period was calculated as Eq. (1).

$$NR = \left(\frac{Ncna - Nna}{NRE}\right) \tag{1}$$

where Ncna is the plant N accumulation under the N_c condition, while Nna is the actual plant N accumulation under varied N rates, and NRE is the N recovery use efficiency of in-season N fertilizer application. The NR = zero indicates an optimum N supply while the NR > zero (positive values) or the NR < zero (negative values) represents the N deficit and luxury N uptake, respectively. The detail of N_c determination in rice can be found in previous study by (Ata-Ul-Karim et al., 2013).

The NRE at various crop growth stages was calculated for different rice cultivars/ecotypes, years, and sites as Eq. (2). The N uptake in the fertilized and unfertilized plot as well as △N applied were calculated according to the N distribution at each growth stage and year.

$$NRE = \left(\frac{Nuptfert - Nuptunfert}{\Delta Napplied}\right) \tag{2}$$

where $Nupt_{fert}$ is N uptake in the fertilized plot and $Nupt_{unfert}$ is the N uptake in the corresponding unfertilized plot.

2.3.2. Nitrogen nutrition index

The NNI of rice cultivars at different growth stages during vegetative growth period was calculated as Eq. (3) (Justes et al., 1994).

$$NNI = Na/Nc \tag{3}$$

where Na is the actual plant N concentration while Nc is the critical N concentration. If NNI = 1, N nutrition is considered as optimal, while NNI > 1 and NNI < 1 shows surplus and deficient N nutrition, respectively. The NNI in present study was calculated using the rice N_c dilution curve developed by (Ata-Ul-Karim et al., 2013) and the NNI values are provided in (Supplementary Fig. 1).

2.4. Statistical analysis

Statistical analyses were carried out on the data acquired from experiments conducted in 2010 and 2013 for development of regression models. Data of different growth stages and cultivars/eco-types were subjected to ANOVA using GLM procedures in IBM SPSS Version19.0 (IBM Corporation, Armonk, New York). Simple linear regression was executed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) to examine the NR-NNI and RY-NR relations. Data acquired from two independent experiments conducted in 2011 and 2014 were used for validation of regression models. The relationships between NR, NNI and RY at various crop growth stages during the period of vegetative growth of rice cultivars were performed using simple linear regression.

2.5. Validation of the models

The models developed in the present study were validated with Japonica and Indica rice cultivars to test their reliability for making N management decisions in different rice types. The root mean square error (RMSE), coefficient of determination (R^2), 1:1 plot between observed and predicted values and kappa (k) coefficient, the measure of agreement between an observed accuracy with an expected accuracy were used to assess the fitting goodness of the linear regression models between NR, NNI and RY. The higher values of R^2 and lower values of RMSE represent the better precision and accuracy of the model for estimating NR and quantifying supplemental N fertilization. RMSE was calculated as follows:

$$RMSE\,(\%) = \sqrt{\left(\frac{1}{n}\sum_{i=1}^{n}(yi - \hat{y}i)\right)^2} \times \frac{100}{\bar{y}} \tag{4}$$

where yi, ŷi and ȳ represent the observed, estimated and mean of the observed values, respectively while n represent the number of samples.

2.6. Prediction models

Finally, the prediction models were developed for estimation of NR from NNI and RY from NR before PI and BT stages for three rice groups, using pooled data from four field experiments (Table 3). Among them, six data sets for Japonica rice were from three cultivars, LXY-18, WXJ-14 and WYJ-24; two data sets for Indica rice were from two cultivars, SY-63 and YLY-1; eight data sets for Japonica plus Indica rice were from all five cultivars, LXY-18, WXJ-14, WYJ-24, SY-63 and YLY-1.

3. Results and discussion

3.1. Estimation of in-season crop N requirement based on N accumulation

Estimation of in-season NR (kg N ha^{-1}) during the vegetative growth period is essential for developing an appropriate N management strategy. The concept of N_c dilution curve has been used for in-season estimation of N status of rice crop (Ata-Ul-Karim et al., 2014b), and could also be implemented for in-season estimation of NR before different crop growth stages. In the present study, the in-season NR was estimated on the basis of N_c dilution curve of Japonica rice by using Eq. (1). Fig. 1 shows the ranges and changes in NR from AT to HD stages of vegetative growth period during 2010 and 2013. The NR for Japonica rice under varied N rates ranged from −19.67 to 270.47 kg N ha^{-1} and −28.46 to 383.38 kg N ha^{-1}, while for Indica rice it ranged from −12.77 to 175.67 kg N ha^{-1} and −30.77 to 373.46 kg N ha^{-1} before AT and HD stages, respectively (Fig. 1). The estimated NR successfully differentiated the N deficit and optimal N growing conditions; however, there was an over-estimation of NR under the treatment where N fertilizer was not applied (N_0 treatment) which was in consensus with previous reports on cereals and was attributed to greater N supply from indigenous soil resources and higher N use efficiency under N_0 treatment (Dobermann, 2006). For any given stage, NR showed significant variation due to various levels of N application. The NR appraisal on N_c dilution curve basis increased from AT to HD stages. The NR differed significantly under limiting N growing conditions, although these differences were minor under optimum N growth conditions.

3.2. In-season estimation of crop N requirement based on NR-NNI relations

Accurate estimation of NR is imperative for effective N management during crop growth. The N_c dilution curves could be used to develop the relationship between NNI and NR for estimating the in-season NR before different growth stages. In this study, the NR was expressed as a function of NNI at various growth stages during vegetative growth phase and the relationships between NR and NNI indicated that the NR increased with decreasing NNI (Fig. 2). The NR was zero, when NNI was around 1, which indicated an optimum N supply under these conditions. The NR > zero (positive values) when NNI < 1 and the NR < zero (negative values) when NNI > 1 represented the N deficit and luxury N uptake, respectively. The relationships between NNI and NR for AT, MT, SE, PI, BT and HD stages satisfactorily explained the variation in rice NR, with R^2 values greater than 0.88 for Japonica rice and 0.95 for Indica rice. The strongest relationships with R^2 values of 0.99 and 0.98 were observed at PI and BT stages for Japonica and Indica rice, respectively. The robust relationships at PI and BT stages accurately explained the variation in NR and could be implemented for time-course estimation of NR under both limiting and non-limiting N growing conditions. Therefore, the NR-NNI relations on the basis of N_c dilution curve successfully identified the situations of deficient and excess N nutrition in rice and could be used to quantify the rates of supplemental N application for critical growth stages. In addition to destructive sampling method, chlorophyll meter and canopy reflectance methods can be utilized for rapid and non-destructive estimation of NNI (Mistele and Schmidhalter, 2008). Therefore, the present relationships between NR and NNI are of practical meaning for in-season corrective N application as well as for precise adjustment of crop NR for a target yield (Lemaire et al., 2008). The NR-NNI relations of Japonica and Indica rice showed discrepancies during crop growth period. The slope of the relations for AT and MT was greater for Japonica rice than Indica rice, while during BT and HD, the slope for Indica rice is greater than Japonica rice. However, the slope of the relationships for both rice ecotypes was comparable for SE and PI stages. These discrepancies were associated with varied ontogenetic responses due to genetic differences between the rice ecotypes. The differences between the intercept of the models with increasing DM due to increase in NR were attributed to genetic as well as NRE differences between two rice ecotypes. Additionally, the variation in ontogenetic responses of two rice ecotypes was associated with differences in NRE. The differences observed between two rice ecotypes in present study were in agreement with previous report which stated that Japonica rice differ from Indica rice in genetic makeup, crop growth rate, ontogenesis, and N uptake (Weng and Chen, 1987; Sheehy et al., 1998; Yoshida et al., 2006). The differences between the slope and intercept responses of the relationship at different growth stages of two rice ecotypes were because of aforementioned factors, which further resulted in variation of DM partitioning and translocation of photosynthates between two rice ecotypes.

3.3. In-season estimation of grain yield targets based on RY-NR relations

A major challenge to current N recommendation approaches is the difficulty to correctly assess the yield target prior to the season, because yield potential is greatly influenced by year to year deviation in solar radiation and temperature (Yao et al., 2012). Over or under setting the target yield will result in faulty recommendation of N fertilizers. In this study, the RY-NR relations estimated on the basis of N_c dilution curve of Japonica rice at different growth stages are used for estimating the target yield. The RY was articulated as a function of NR at different growth stages (Fig. 3). The RY-NR rela-

Table 3
Prediction models for estimating nitrogen requirement (NR) from nitrogen nutrition index (NNI) and relative yield (RY) from NR at panicle initiation and booting stages using pooled data of four experiments in Japonica, Indica and Japonica plus Indica rice cultivars.

Prediction model	Rice type	Growth stage	Regression equation	R^2
Nitrogen requirement	Japonica	Panicle initiation	NR = – 876.18NNI + 887.44	0.94
		Booting	NR = – 848.69NNI + 855.38	0.97
	Indica	Panicle initiation	NR = – 851.32NNI + 852.2	0.98
		Booting	NR = – 1186.8NNI + 1197.4	0.97
	Japonica+Indica	Panicle initiation	NR = – 871.75NNI + 880.04	0.95
		Booting	NR = – 908.94NNI + 919.55	0.92
Relative yield	Japonica	Panicle initiation	RY = – 0.0011NR + 0.9997	0.91
		Booting	RY = – 0.001NR + 0.997	0.93
	Indica	Panicle initiation	RY = – 0.0016NR + 1.0253	0.93
		Booting	RY = – 0.0011NR + 1.0229	0.93
	Japonica + Indica	Panicle initiation	RY = – 0.0011NR + 1.0034	0.88
		Booting	RY = – 0.001NR + 1.003	0.92

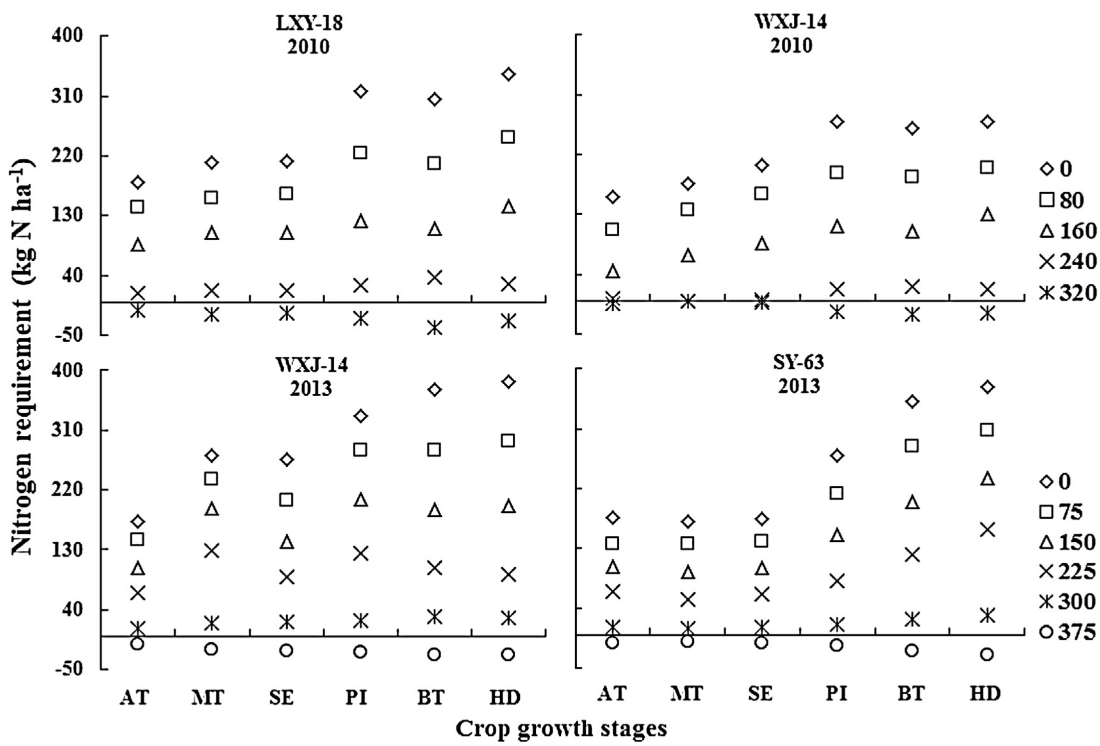

Fig. 1. Changes of nitrogen requirement (NR, kg N ha^{-1}) during the vegetative growth period in Japonica and Indica rice cultivars under varied N rates (0–375 kg N ha^{-1}) obtained from plant DM based critical nitrogen (N_c) dilution curve in experiments conducted during 2010 and 2013. For X-axis, AT represents active tillering, MT mid tillering, SE stem elongation, PI panicle initiation, BT booting, and HD heading.

tions during the period of vegetative growth of rice crop accurately explained the variation in RY both under deficient and optimum N growing conditions, with R^2 values greater than 0.83 for Japonica rice and 0.91 for Indica rice, and the strongest relationships for both eco-types with R^2 values of 0.91 and 0.93 were observed at PI and BT stages. The robustness of the relationships between NR and RY at PI and BT stages was attributed to physiological reasons due to dependency of N uptake on plant growth rate. The amount of N absorbed during later vegetative growth (particularly at PI and BT stages) effectively contributed to accelerated spikelet production and grain filling in rice (Chen et al., 2012). Thus, the relationships between NR and RY at PI and BT stages in this study could be implemented to adjust the N dressing application during the mid-growth stages of rice crop without any negative impact on grain yield.

The commercial and mechanized farming often requires an application of 70–80% of the total N fertilizer at transplanting or shortly after it (Peng et al., 2006; Sinclair and Rufty, 2012). Since rice inherently uptakes low N at early growth stages, mainly due to lack of extensive root system and lower biomass accumulation (Sinclair and Rufty, 2012), the rice plants can utilize only up to 20–30% of applied N at the early growth stages, while the substantial amount of the applied N dissipates into the atmosphere (Peng et al., 2006). This implies that the relationships between NR and RY at early vegetative growth could not delimit the yield potential at maturity, hence are less accurate for scheduling corrective N fertilizer application. In addition, previous studies in cereal crops have shown the negative effect of N application at HD on grain yield. This limited effect of N application at HD stage on grain yield in rice was due to start of reproductive growth period that further attributed to lower N uptake from soil by rice plants (Gislum and Boelt, 2009). Thus, the relationship between NR and RY at HD stage is not recommended for scheduling supplemental N fertilization because of the negative effects of N fertilizer during this period on grain yield.

The relationships between NR-NNI and RY-NR indicated that at early growth stages (AT and MT), Japonica rice showed higher NR and RY as compared to Indica rice, while at later growth stages (BT and HD) both ecotypes showed similar pattern for RY-NR relationships. However, both eco-types showed differences for the NR-NNI

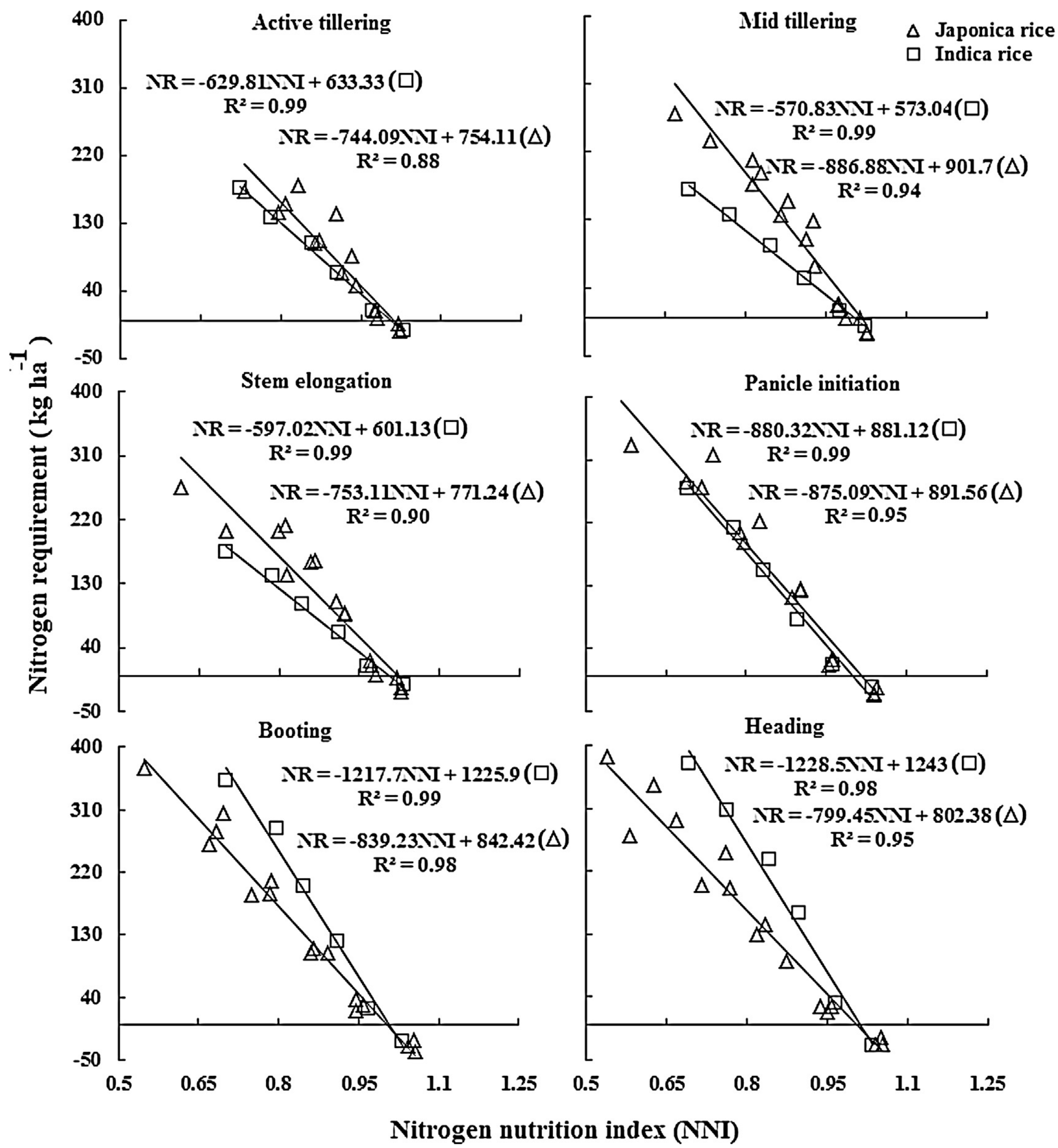

Fig. 2. Relationships between nitrogen nutrition index (NNI) and nitrogen requirement (NR) during vegetative growth period in Japonica and Indica rice cultivars under varied N rates with plant DM based critical nitrogen (N_c) dilution curve in experiments conducted during 2010 and 2013.

relations at these stages. The differences between NR-NNI relations were associated with the genetic differences between two rice ecotypes, due to which Indica rice showed higher DM accumulation, partitioning and NR than Japonica rice. The similar RY-NR relaions at later stages might possibly be associated with use of relative values instead of absolute values for grain yield. The relationships between absolute grain yield (GY) and NR are provided in (Supplementary Fig. 2) to give the comparison of the relationships developed using relative grain yield (RY) and absolute grain yield (GY-NR).

3.4. Validation of the models

The models developed with the dataset in 2011 and 2013 on the basis of N_c dilution curve for Japonica and Indica rice in the present study (Figs. 2 and 3) were validated with data set obtained from two independent experiments conducted in 2010 and 2014. The performances of the models were estimated by comparing the RMSE and R^2 at different growth stages. The RMSE values for NR-NNI relations during the vegetative growth phase ranged from 18% to 35% for Japonica rice and 18% to 35% for Indica rice, and for RY-NR relations they ranged from 4% to 7% for Japonica rice and 5% to 10% for Indica rice (Fig. 4). The R^2 values for the NR-NNI and RY-NR relations ranged from 0.93 to 0.97 and 0.86 to 0.96 for Japonica rice, while 0.96 to 0.98 and 0.88 to 0.95 for Indica rice (Fig. 5). The comparatively higher values of RMSE, 35% and 29% for the NR-NNI relations, as well as 7% and 10% for the NR-RY relations, were observed for AT and MT stages. Similarly, comparatively lower R^2 values for the NR-NNI and RY-NR relations were also observed for AT and MT stages. The comparatively higher RMSE and lower R^2 values for AT and MT stages, might be due to inherently low N uptake, lower biomass accumulation and lack of extensive root system at early growth stages (Sinclair and Rufty, 2012). The lower values of RMSE and higher values of R^2 for the NR-NNI and RY-NR relations at PI and BT stages for both Japonica and Indica rice exhibited a good agreement between the observed and predicted values. Moreover, the linear regression forced through the origin (y = kx) was fitted between the predicted (y) and the observed (x) NR and RY. The

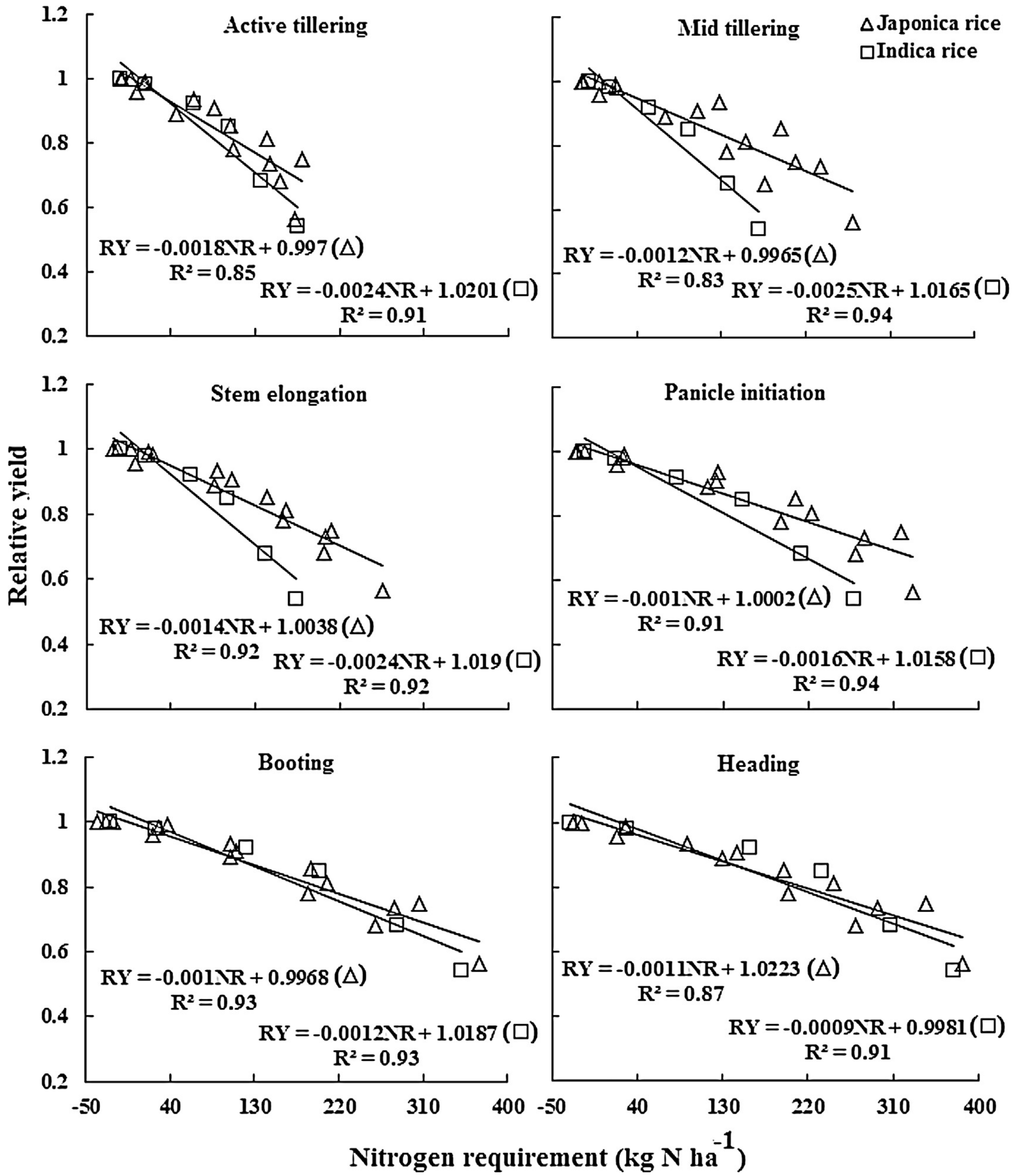

Fig. 3. Relationships between nitrogen requirement (NR) and relative yield (RY) during vegetative growth period in Japonica and Indica rice cultivars under varied N rates with plant DM based critical nitrogen (N_c) dilution curve in experiments conducted during 2010 and 2013.

prediction was considered accurate if the k coefficient was close to 1. As seen in Fig. 6, the k coefficients for the relationships between NR and NNI and between NR and RY at PI and BT stages were close to 1. The validation of the results obtained in present study with and independent data set well supported our hypothesis that Nc dilution curve based NR-NNI and RY-NR relations can be utilized for improving N use efficiency without effecting the rice crop yield by estimating time-course NR and providing guidance for corrective N fertilization and setting the yield targets.

3.5. Prediction models

Individual prediction models may be significant for a specific location, cultivar and year, but would have poor predictive performance under different production conditions. A model intended to be used for prediction purposes should be more robust and widely applicable. To make prediction models acceptible for a wide-ranging production conditions, a model should be developed using the data that is replicated in time and space. Therefore, the final prediction models in present study were developed using the data collected from four experiments (4 years, 2 locations and 5 cultivars). With the different cultivars used in this study, the relationships were consistently robust for PI and BT stages, thus the models with pooled data of PI and BT stages were further applied for accurate estimation of NR and RY in different eco-types of rice (Table 3). The R^2 values for the final NR prediction models ranged from 0.92–0.98, while for final RY prediction models, it ranged from 0.88–0.93 for Japonica, Indica and Japonica + Indica rice groups. The results showed that three rice groups having R^2 values all greater than 0.92 exhibited significantly stronger NR-NNI relations than the RY-NR relations with the R^2 greater than 0.88. The unified prediction models for estimating NR from NNI and RY from NR at PI

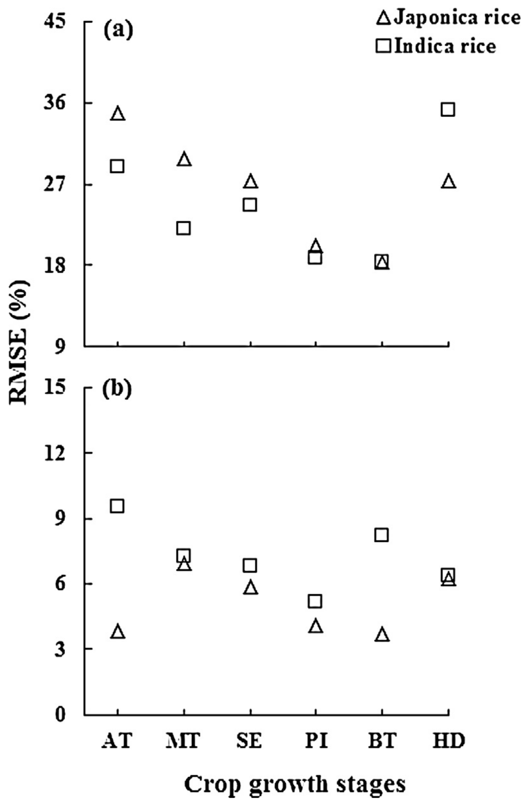

Fig. 4. Root mean square error (RMSE, %) for validation of relationships between nitrogen requirement (NR), nitrogen nutrition index (NNI) and relative yield (RY) during vegetative growth period in Japonica and Indica rice (a: validation of NR-NNI relations; b: validation of RY-NR relations). For X-axis, AT represents active tillering, MT mid tillering, SE stem elongation, PI panicle initiation, BT booting, and HD heading.

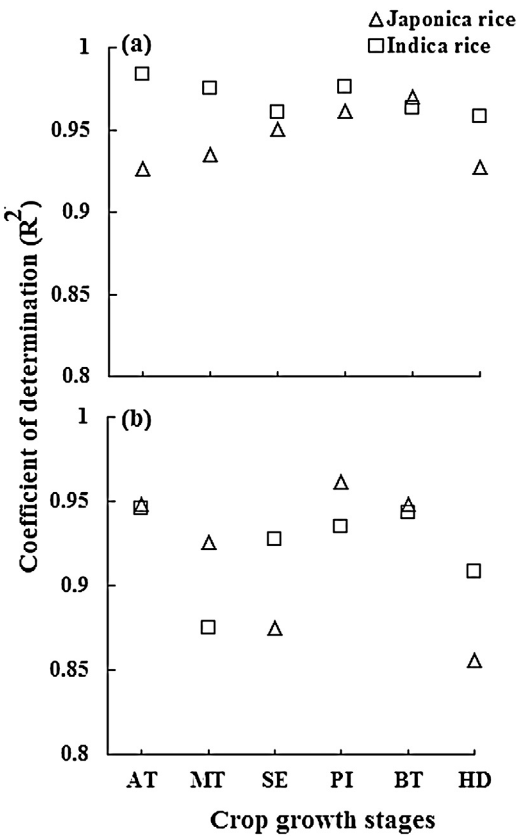

Fig. 5. Coefficient of determination (R^2) for validation of relationships between nitrogen requirement (NR), nitrogen nutrition index (NNI) and relative yield (RY) during vegetative growth period in Japonica and Indica rice (a: validation of NR-NNI relations; b: validation of RY-NR relations). For X-axis, AT represents active tillering, MT mid tillering, SE stem elongation, PI panicle initiation, BT booting, and HD heading.

and BT stages for three rice groups seems very close to each other except NR-NNI relations at BT stage for Indica rice. This exception was attributed to the difference in DM accumulation and partioniong between Japonica and Indica rice types, which resulted in NR with increasing DM. In contrast, a single prediction model for RY-NR relations across the rice eco-types and growth stages was associated with the relative values of yield instead of absolute values.

3.6. Application of N_c dilution curve for N management and modelling

Accurate estimation of supplemental N fertilization for development of a fertilizer decision support method requires consideration of NR at most critical crop growth stages (Thind et al., 2012). The NR-NNI and RY-NR relations on the basis of N_c dilution curve in the present study can help in decision making about appropriate amount of N application in rice. The robust NR-NNI and RY-NR relations for PI and BT stages in this study are in agreement with the report on ryegrass by Gislum and Boelt (2009). The amount of N absorbed during PI and BT stages can effectively contribute to spikelet production and grain filling in rice (Gislum and Boelt, 2009; Chen et al., 2012). Therefore, it is prudent to ensure sufficient N uptake prior to onset of reproductive phase for grain filling and development. This explains why the N_c dilution curve based relationships at PI and BT stages in the present study can be utilized to relate RY to the NNI and NR. Moreover, the algorithms obtained by using this concept can be coupled with other crop growth and management dynamic models. These will help to offer precise estimation of crop growth status, grain yield and quantification of time-course NR as per N management decisions. The issues of over or under-N nutrition could be avoided effectively using this N management strategy to adjust N rates for a particular field situation. Thus, the relationships established in this study can be widely used for precision N management and corrective decision making during rice growth. Application of this approach could further help to set yield targets according to different N application rates (Ata-Ul-Karim et al., 2016). It should be noted that the destructive sampling required for determination of DM, NNI and NR may limit the practical implementation of this approach, but new tools such as the GreenSeeker and Crop Circle ACS-470 sensor are available for reliable in-season estimation of DM and NNI during different crop growth stages (Cao et al., 2013). This integrated approach will be further investigated in the future studies for smart rice production.

4. Conclusion

This study indicated that the NR-NNI and RY-NR relations for critical crop growth stages on the basis of N_c dilution curve can be implemented for making in-season decisions about appropriate N fertilizer application in rice. In particular, the robust relationships at PI and BT stages accurately explained the variation in NR and RY both under deficient and optimum N growing conditions and could be reliably applied for predicting in-season NR and thus quantifying N fertilizer application during mid-growth stages. The implementation of the newly developed models can expect to improve N management and enhance N use efficiency, thus contributing to the sustainable rice production in east China. Investigations under

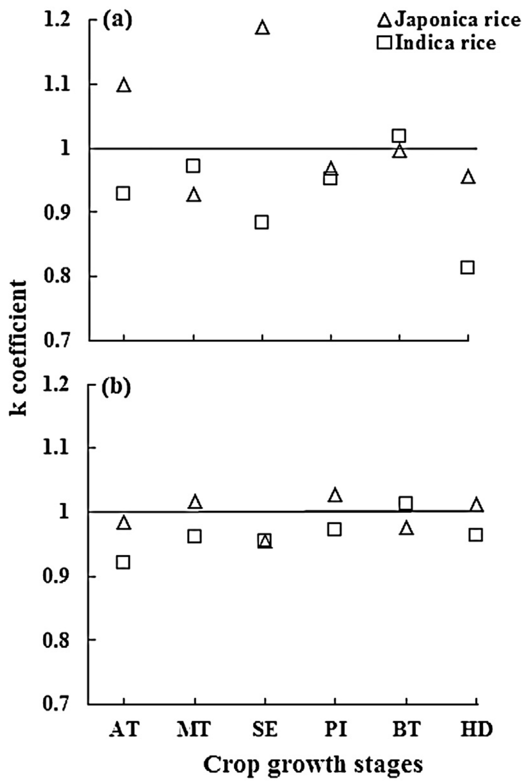

Fig. 6. The agreement between observed and predicted nitrogen requirement NR with nitrogen nutrition index (a) and relative yield (b) during vegetative growth period in Japonica and Indica rice. The symbols represent slope (k coefficient) of the linear regression forced through the origin (y = kx). For X-axis, AT represents active tillering, MT mid tillering, SE stem elongation, PI panicle initiation, BT booting, and HD heading.

different environmental conditions and production systems would extend the validity of this approach in broader aspects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2016.10.009.

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