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Fertilisation strategy and ground sensor measurements to optimise rice yield

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Abstract

Nitrogen (N) fertilisation is the main agronomic practice that affects rice yield and quality; similarly, its mismanagement can affect both economic and environmental aspects of crop production. Therefore, it is highly important to direct N fertilisation during the critical growth stages of rice development using vegetation indices (VIs). To this end, a two-year experiment was conducted in 2014 and 2015 in Castello d'Agogna (PV), northwest Italy. The study had three aims: i) establish the best N fertilisation management in temperate rice cropping systems, in terms of total N supply and splitting, to maximise crop yield and N apparent recovery (AR); ii) evaluate the capability of crop N status indicators (CNSIs) measured at panicle initiation stage (PI) to determine grain yield; iii) derive $N_{fertiliser}$_rate_at_PI = f(CNSI) from a field trial to attain specific yield goals.

Results obtained for Centauro variety suggested that to maximise yield while avoiding AR reduction, a low dose of about 50 kg N ha^{-1} should be supplied during early growth, then increased at PI. In addition, the final topdressing fertilisation can compensate for any previous stage supply deficiency and can be determined from VI measurements. Findings also identified the normalised difference red edge (NDRE) index as the best VI to determine rice N status in specific agro-environmental conditions. SPAD and NDVI values measured with Rapid Scan can be used to determine N fertilisation at PI, although such measurements require correction through Sufficiency Indices (SIs) calculated as the ratio between VI measurements and VI values of a well-N fertilised plot. The trial also demonstrated that plots supplied with N amounts of 140 kg N ha^{-1} (pre-sowing and tillering stages combined) can serve as reference plots for SI calculation that allows to consider the effect of weather and soil variability on VI measurements. A notable exception to this finding was NDVI measured with GreenSeeker, which showed limited ability to assess rice N status under study environmental conditions. Indeed, both VI and the derived SI were influenced by seasonal and soil fertility conditions.

Finally, a specific statistical method to derive calibration functions for variable rate application fertiliser spreaders from a suitable experiment was defined. These functions will establish the N amount to be supplied at PI related to the CNSI measure. For each CNSI, a specific slope of the calibration function is determined while the intercept is varied depending on the grain yield goal. The higher the acceptable reduction relative to the maximum obtainable yield, the lower the N supply required at PI.

Keywords: Crop yield estimation; Crop N status; Site-specific N management; Vegetation Indices; Precision Agriculture; Variable rate fertilisation.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, being the staple food for three billion people (Barker *et al*., 2007). On a world scale, rice is grown on an area of 157 million hectares (PROSPERA, 2012). In Italy, rice cultivation is mainly concentrated in the northwest, and it is cropped on about 227 000 ha (Ente Nazionale Risi, 2015).

Nitrogen (N) fertilisation is the main agronomic practice that affects yield and quality of rice crop. Mismanagement of N fertiliser may affect both the economic and environmental aspects of crop production (Tubaña *et al*., 2011a). Nitrogen deficiency results in smaller leaf area, lower chlorophyll content, and biomass production, which lead to stunted crop growth and yield (Lin *et al.,* 2010). Excessive N input on the other hand, results in a dense canopy structure that facilitates pest and disease development, and leads to reduced plant resistance (Wu *et al.*, 2015, Hue *et al.,* 2016). Moreover, it can bring on lodging and extend growth periods and maturity achievement (Dong *et al.,* 2015; Liu *et al.*, 2015). Excessive N fertilisation has also been reported to pollute the environment through N leaching and both N_2O and NH_3 emission (Nguyen *et al*., 2008).

Therefore, tools to calibrate the application of N fertilisers during critical growth stages are needed to improve both grain yield and nitrogen use efficiency (NUE) while avoiding N losses (Sathiya and Ramesh, 2009; Yoseftabar, 2013). In flooded systems, rice requires sufficient N input during the early and mid-tillering stages to maximise panicle number, and during the panicle initiation stage (PI) to optimise the number of spikelets per panicle and percentage of filled spikelets (Biloni and Bocchi, 2003, Bah *et al*., 2009, Xue *et al.,* 2014). Nitrogen also increases sink size during late panicle formation (Manzoor *et al*., 2006; Lee *et al*., 2009; Tayefe *et al.,* 2014), which raises grain yield.

The rate of N fertilisation and the growth stages critical to optimising N application vary with rice cultivar (Bah *et al*., 2009). Several destructive and non-destructive methods have been developed to establish optimum N fertilisation by monitoring crop N status (Tubaña *et al*., 2011a). An ideal method to monitor N status has the following characteristics: non-destructive, fast, cost-effective, reliable, and obtains a value representative of the entire field (Xue *et al*., 2004; Bajwa *et al*., 2010). Optical properties of some leaf pigments, and in particular chlorophyll, have been shown to be reliable crop N status indicators that can be determined through vegetation indices (VIs) (Muñoz-Huerta *et al*., 2013).

Different instruments are able to measure light transmission through leaf (chlorophyll meters, *e.g.* SPAD-502) or canopy reflectance (*e.g.* GreenSeeker and Rapid Scan). These optical measurements are normally affected by growth stage, cultivar, soil water availability, and non-N nutrient deficiencies (Muñoz-Huerta *et al*., 2013), as well as by sun angle, soil roughness, and soil colour.

Vegetation indices are calculated from sensor data, based on certain waveband combinations (Bajwa *et al*., 2010). The most frequently used are NDVI (Normalized Difference Vegetation Index) and NDRE (Normalized Difference Red Edge) indices (Rouse *et al.*, 1974; Barnes *et al.,* 2000). NDVI has been reported to have low sensitivity at high chlorophyll content or abundant aboveground biomass, that induce saturation (Li *et al.,* 2010; Kanke *et al*., 2012; Shi *et al*., 2015). In rice, the index becomes saturated when aboveground biomass is about 4000 kg ha^{-1} and total N uptake reaches about 100kg ha^{-1} (Yao *et al*., 2014). Therefore, the anticipation of VI measurements must consider that, in flooded rice systems, reflectance is influenced by the presence of water, especially during the early growth stages when canopy cover is limited (Yao *et al*., 2014). Measurements taken at PI to guide the last topdressing N application fit with the optimal time for data acquisition.

Under dense green biomass, NDRE is a more suitable N status measure, as it is less susceptible than NDVI to saturation (Barnes *et al.,* 2000*;* Kanke *et al.,* 2016). Red edge wavelength (730 nm), corresponding to the inflection point of the crop reflectance curve; combined with the NIR band has shown to be the most effective measure for estimating rice plant N uptake before the heading stage (Yu *et al*., 2013)

Previous studies have shown that these VIs can be used to estimate rice yield based on the relation between crop spectral measurements and N status that allows to quantify N requirements (Chang *et al.,* 2005; Tubaña *et al.,* 2011b). Needed now is a quantitative estimate of the VIs and grain yield relationship to develop a precise rice N fertilisation management that is part of today's move to precision agriculture. Such a tool has the potential to improve crop N management and to mitigate negative environmental impacts of intensive rice production (Zhang *et al*., 2011).

This study pursued the following goals:

i) establishment of the best N fertilisation management, in terms of total N supply and splitting, to maximise crop yield and N apparent recovery (AR);
ii) evaluation of the capability of crop N status indicators (CNSIs) measured at PI to determine grain yield;
iii) derivation of $N_{fertiliser}$_rate_at_PI = f(CNSI) from a field trial to attain specific yield goals.

These tools will allow the development of a site-specific crop management strategy that can be adapted to different agro-environments reducing the effect of spatial variability, avoiding the negative impacts of N imbalances.

2. Materials and methods

2.1 Site description and soil properties

An experiment was designed to test a wide range of crop N statuses through different fertilisation managements expected to correlate with crop yield.

The study was carried out during two growing seasons (2014 and 2015) in an experimental field of the Rice Research Centre of Ente Nazionale Risi, located in Castello d'Agogna (PV) in northwest Italy (8° 41' 52'' E; 45° 14' 48'' N).

Climate of the area is temperate, with hot summers and two main rainy periods in spring and autumn. In Figure 1, the mean temperature and rainfall recorded during the experimental periods of March-October in 2014 and 2015 in Castello d'Agogna have been compared against 30-year (1984 to 2013) values.

Site soil properties are summarised in Table 1. The soil texture was silty loam. Other soil characteristics included low organic matter content, slight acidity, and low available N. The C/N ratio was well balanced with normal organic matter mineralisation. The Cation Exchange Capacity (CEC) was low as expected with low organic matter and clay contents. P Olsen content was high, while exchangeable K availability was very low. Further details of the soil at the site can be found in Miniotti *et al.,* 2016.

2.2 Experiment design and agronomic management

The experiment compared four rates of N supplied as the sum of N amount supplied at the pre-sowing and tillering stages ($N_{PRE+TILL}$, 0-60-100-140 kg N ha^{-1}) combined with four N rates supplied at PI ($N_{PI,}$ 0-30-60-100 kg N ha^{-1}). A plot supplied with a total N amount of 300 kg ha^{-1} (200 + 100 kg N ha^{-1}) was added as an over-fertilised plot. In all plots, dry granular urea was used as fertiliser. Treatments were laid out using a split plot design with $N_{PRE+TILL}$ in the main plots and N_{PI} in the subplots. Each main plot measured 4.5*26.4 m and was divided into subplots measuring 4.5*6.6 m. Four replications for each treatment were established.

Both in 2014 and 2015 cultivar Centauro was planted, a round grain variety. The trial was ploughed in spring with conventional tillage equipment, after which it was laser levelled, and then harrowed. Phosphorus (56 kg ha^{-1} of P_2O_5) and potassium (112 kg ha^{-1} of K_2O) were uniformly applied in all plots before harrowing, using a 0-14-28 fertiliser. Water seeding was carried out on May 19, 2014 and May 18, 2015. Water was managed with continuous flooding for most of the growing season. The only exceptions were a “pin-point” period of 4-6 days to allow for root extension, and two other 4-6 day periods of drainage for mid-season fertiliser and herbicide application during the second half of June and July, as is the traditional management of the area. Final draining occurred on September 8, 2014 and August 29, 2015. Adequate measures to control diseases were taken throughout plant growth. Weed control was performed with *oxadiazon, cyhalofop butyl, propanil, MCPA,* and *halosulfuron methyl*. The crop was harvested between October, 21 and 29 and between October 12 and 21 in 2014 and 2015, respectively. The date difference merely reflected when the crop matured, as determined by N supply.

2.3 Field measurements

SPAD, NDVI, and NDRE were determined using suitable instruments to establish crop N status at PI (July 15, 2014 and July 13, 2015). The Soil-Plant Analysis Development (SPAD) index was determined using SPAD-502 (Konica Minolta, Japan). Readings were acquired from the fully expanded top leaf of plants, approximately one-third of the distance away from the tip, as described in Lin *et al*., 2010. To obtain a representative value for the entire plot, 20 readings for each plot were taken from 20 leaves belonging to twenty different plants. For NDVI calculations, both GreenSeeker (Trimble©, Sunnyvale, California, USA) or Rapid Scan (Rapid Scan CS-45, Holland Scientific, USA) handheld active optical sensors were used. The first device detects canopy reflectance in the red (660 nm) and NIR spectral regions (770 nm), whereas the second incorporates three optical measurement channels (670, 730, and 780 nm), of which the first and third were used for NDVI calculations. The wavebands used to determine NDVI are different for the two instruments, so the two VIs are indicated as GS NDVI and RS NDVI for GreenSeeker- and Rapid Scan-read measurements, respectively. Finally, NDRE was measured and calculated using only Rapid Scan, considering canopy reflectance at 730 and 780 nm wavebands.

The measurements were collected by holding the instruments approximately 0.5 m above the rice canopy and walking at a constant speed along the entire length of the plot, as suggested in Xue *et al.*, 2014. Due to the technical characteristics of the instruments, sensor measurements width is approximately 0.3 m. Two measurements were taken from each of the length-wise sides of the plot in each treatment. The two values were then averaged to determine the mean value for each plot.

Biometric measures at PI are usually well correlated to final grain yield (Bajwa *et al.,* 2010). To confirm this relationship, aboveground biomass, total N concentration, and total N uptake were also determined. Aboveground biomass was collected from three 0.25 m^2 areas, oven-dried at 40°C to a constant weight, and then analysed by the Dumas method to establish N concentration. Then, total N uptake at PI was also calculated by multiplying plant N concentration and the sampled biomass (Zavattaro *et al.,* 2012).

Grain (normalised to a moisture content of 14%) and straw yield were determined at harvest. In order to have three sub-samples, three 0.25 m^2 areas were harvested by hand in each plot. Only a bulk of the three sub-samples of both grain and straw was analysed for N concentration using the above-mentioned method. Finally, N AR was determined according to Zavattaro *et al.* (2012):

$$AR = \frac{N\ removal - N\ removal_{0N}}{N\ fertilizer} \tag{1}$$

where *N removal* is the amount of N removed as yield, $N\ removal_{0N}$ represents the amount of N removed by the unfertilised plot, and *N fertiliser* is the total amount of N supplied with fertiliser application.

2.4 Data analysis

Statistical analysis was performed using R software, version 3.3.0 (R Development Core Team, 2016).

General Linear Model (GLM) was used to explain yield and AR as a function of different N rates and splitting, year, block, and their interactions as follows:

$$x = \beta_1 * N_{PRE+TILL} + \beta_2 * {N_{PRE+TILL}}^2 + \beta_3 * N_{PI} + \beta_4 * {N_{PI}}^2 + \beta_5 * N_{PRE+TILL} * N_{PI} + \beta_6 * N_{PRE+TILL} * YEAR + \beta_7 * N_{PI} * YEAR + BLOCK + YEAR \tag{2}$$

where *x* represents yield and AR, while β_1 to β_7 represent the slopes of the covariates. YEAR is the year effect related to the agro-climatic conditions and BLOCK is the block effect.

With an aim to determine the N_{PI} that maximises grain yield and AR for each N rate supplied at pre-sowing and tillering stage, the first order partial derivative was calculated with respect to N_{PI} and then set to zero.

The resulting equation is:

$$N_{PI} = \frac{-\beta_3 - \beta_5 * N_{PRE+TILL}}{2 * \beta_4} \tag{3}$$

Equation 3 can be expressed as $N_{PI} = f(N_{PRE+TILL})$ only when N_{PI} and $N_{PRE+TILL}$ show significant interaction. Otherwise, N_{PI} and $N_{PRE+TILL}$ contribute both to increases in yield and AR. In such an instance, no compensative effect exists and the equation cannot be calculated.

A correlation analysis was also applied to investigate the capability of different indicators to determine crop N status. The different indicators of crop N status (CNSI) here considered were both VIs (SPAD, GS NDVI, RS NDVI, NDRE), and biometric measures (aboveground biomass, its N concentration and total N uptake) detected at PI.

Next, grain yield was determined through the same GLM as mentioned above, only CNSIs took the place of $N_{PRE+TILL}$. The goal was to determine the relations between N_{PI} and CNSIs, under the larger aim of describing an equation to establish the best N_{PI} based on CNSIs. This statistical model was built as:

$$Yield = \gamma_1 * N_{PI} + \gamma_2 * {N_{PI}}^2 + \gamma_3 * CNSI + \gamma_4 * CNSI^2 + \gamma_5 * CNSI * N_{PI} + \gamma_6 * CNSI * YEAR + \gamma_7 * YEAR * N_{PI} + BLOCK + YEAR \quad (4)$$

where γ_1 to γ_7 represent the slopes of the covariates.

To obtain good indicators of crop N status in a given season and location, Sufficiency Indices (SI) and Response Indices (RI) were also calculated and used as CNSIs. Several studies on different cropping systems (Holland and Shepers, 2013; Muñoz-Huerta *et al.,* 2013; Xue *et al.,* 2014) suggested that a well-fertilised plot serve as a reference plot to calculate SIs, defined as the ratio of vegetation index obtained from a to-be-evaluated crop to VI of a well-N fertilised plot, integrating the confounding effect provoked by factors other than crop N status (Hussain *et al.*, 2000; Holland and Shepers, 2013). Hussain *et al.* (2000) state that reference plot establishment is suitable in irrigated rice conditions. Indeed, continuous flooding, common in temperate rice cropping systems, avoids water stress onset and consequent influence on rice spectral response. Moreover, Tremblay and Belec (2006) put forth that the reference plot might be considered as an internal standard against which measurements taken in other plots can be compared. Consequently, in order to standardise VIs measurements considering site-specific conditions, a reference plot has to be established in each location. RI is defined as the ratio of the vegetation index measured on the to-be-evaluated crop to the vegetation index measured in an unfertilised plot (Mullen *et al.,* 2003). Last, an equation to optimise fertiliser application, as a function of a measured CNSI value was determined for CNSIs that have shown a negligible effect of year and soil variability, originally or after transformation in SI or RI.

A statistical model was then built to determine yield from PI N supply and CNSI values as follow:

$$Yield = \gamma_8 * N_{PI} + \gamma_9 * {N_{PI}}^2 + \gamma_{10} * CNSI + \gamma_{11} * CNSI^2 + \gamma_{12} * N_{PI} * CNSI \quad (5)$$

where γ_8 to γ_{12} represent the slopes of the covariates.

Year, block, and their interactions were not included in the statistical model, as after the results analysis, these parameters were shown not to be significant in determining yield.

To derive the most appropriate N fertilisation as a function of measured CNSI, the Maximum Grain Yield Approach was followed. Therefore, a first order partial derivative with respect to N_{PI} was calculated for *Equation 5* and then set to zero to determine the N amount that has to be supplied at PI to maximise rice grain yield.

First order partial derivative with respect to N_{PI} can be expressed as:

$$Yield' = \gamma_8 + 2\gamma_9 * N_{PI} + \gamma_{12} * CNSI \qquad (6)$$

After rearranging the equation and setting the partial derivative to zero, N supply at PI can be determined as:

$$N_{PI} = \frac{-\gamma_8 - \gamma_{12} * CNSI}{2\gamma_9} \qquad (7)$$

Again, *Equation 7* can be expressed as N_{PI} = f(CNSI) only when N_{PI} and CNSI show significant interaction. Otherwise, N_{PI} and CNSI both contribute to increase yield and AR. In such an instance, no compensative effect exists and the equation cannot be calculated.

Results analysis highlighted that a high N amount has to be supplied at PI to achieve the highest grain yield, as the function that describes maximum grain yield shows a smooth curvature close to the peak. Worthy of note is the considerable reduction in N rates that can be obtained with just a slight reduction in maximum grain yield. So, a method to determine N_{PI} to achieve a reduced yield was studied, with the assumption that the reduced yield could be considered as a percentage of maximum grain yield (*Equation 8*).

$$Reduced\ yield = R * Maximum\ Yield \qquad (8)$$

where R is the reduction coefficient, and assumed to be 0.90, 0.95, 0.99, 0.995, and 0.999 to analyse different reductions in maximum grain yield and the consequent reductions in N fertiliser applied.

Moreover, this approach allows identification of the CNSI threshold over which no further N fertiliser must be added, depending on the grain yield goals.

3 Results

3.1 Climate

The two cropping seasons exhibited different climatic conditions. Rainfall was plentiful in the summer of 2014. Conversely, the 2015 summer saw reduced rainfall (704 mm) and higher temperature (13°C) compared to the 30-year means.

3.2 Response of rice yield to different N rates

Rice grain yield showed a parabolic increase with rising total N supply ($N_{PRE+TILL}$ plus N_{PI}) in both years (Figure 2). Maximum grain yield (11.1 Mg ha^{-1}) was achieved in 2014 when a total of 200 kg N ha^{-1} was applied, while in 2015 the maximum grain yield (11.2 Mg ha^{-1}) was reached with 120 kg N ha^{-1}. Additional N increases resulted in either a constant yield or yield reduction in both years.

Total N uptake (grain + straw) behaved differently than did grain yield. The trend was almost linear across all explored N fertilisation levels in both years (Figure 3), despite different N uptake value ranges in each year (2014: 127 to 325 kg ha^{-1} and 2015: 129 to 223 kg ha^{-1}). Consequently, AR values were different between the two growing seasons, even though N uptake in the unfertilised treatments was quite similar in both years (Figure 4). In both years AR decreased when N supplied increased, but with a slope more pronounced in 2014. In 2014, at lower N levels, mineral fertilisation promoted higher crop exploitation of soil N resources than observed in the unfertilised plot, and produced AR values above 1.0. On the contrary in 2015, AR values were lower and remained almost constant with rising N amount.

The GLM based on *Equation 2* was applied. Results are reported in Table 2.

Grain yield and total biomass production were influenced by N applied at the pre-sowing plus tillering stages, N supplied at PI, and their interaction. Total N concentration and total N uptake were instead influenced by N fertilisation at the sum of pre-sowing and tillering, and PI stages, but not by their interaction. Differences among years were significant for yield, total N concentration, and AR. Interaction between year and N supply at pre-sowing and tillering stages or at PI was significant for most of the variables considered.

3.3 Crop yield maximisation and consequences on N apparent recovery

The N amount that must be supplied at PI to maximise yield as a function of $N_{PRE+TILL}$, can be calculated using *Equation 3* (represented in Figure 5). Maximum grain yield (11.1 Mg ha^{-1}) was achieved when about 42 kg N ha^{-1} was applied during pre-sowing and tillering; maximum AR (59.8%) was reached with a $N_{PRE+TILL}$ of about 53 kg N ha^{-1}. When 42 kg N ha^{-1} was applied at the pre-sowing and tillering stages, AR fell slightly (59.7%). As Figure 5 shows, 150 kg N ha^{-1} must be supplied at PI to maximise yield, while just 140 kg N ha^{-1} is enough to maximise AR. Nonetheless, some uncertainties are associated with these early stage and PI fertilisation values.

3.4 Capability of different VIs to determine N status at PI stage

The capability of different VIs to determine rice N status at PI was verified by correlation analysis. Results are reported in Table 3.

Analysed VIs correlated highly with one another as their coefficients were above 0.8. They also correlated highly with biometric measures, with very high coefficients for NDRE and falling progressively for SPAD, RS NDVI, and GS NDVI. Moreover, VIs correlated better with crop aboveground biomass than with N uptake, except for SPAD. Alternatively, N concentration determination was the poorest. Differences recorded between GS and RS NDVI in the various correlations related to the differing wavelengths used by GreenSeeker and Rapid Scan.

3.5 Capability of different CNSIs to determine grain yield

The capability of different CNSIs, including both biometric measures and VIs measured at PI, to determine yield was investigated using GLM according to *Equation 4.* Results are shown in Table 4. All CNSIs and most of their squares detected at PI were significant on determining crop yield. Interaction of the different CNSIs with N supply at PI was also found to be significant for all considered CNSIs.

According to Table 4, the biometric measures evaluated at PI were good at determining grain yield as demonstrated by high R^2 values; biomass was best, followed by N uptake, and N concentration alone led to a poorer estimate. All VIs performed better than N concentration. The NDRE R^2 was even better than that obtained by biomass. The two NDVIs were shown to be a little less able to determine grain yield; SPAD was the poorest. Moreover, VIs are essentially proxy measures of biometric variables as the correlation analysis demonstrated and can be extensively measured.

Except for NDRE, year or block effect was significant for all VIs. This makes quantification of N fertiliser needs based on VI measurements difficult because of the wide variation in agro-climatic and soil conditions. Consequently, Sufficiency Indices (SIs) and Response Indices (RIs) were calculated. Plots that received 60, 100, 140, or 200 kg N ha^{-1} as $N_{PRE+TILL}$, or 0 kg N ha^{-1} were considered reference plots to determine the SIs or RIs, respectively. Then, the *Equation 4* was applied to determine rice grain yield at the best using SIs and RIs as CNSIs.

SIs and RIs calculated for each VI were also found to determine yield significantly (Table 5). Interaction between SI or RI and N supplied at PI was always significant too. Maximum R^2 values were obtained for all SIs except GS NDVI using as a reference plot those receiving 60 kg N ha^{-1} at pre-sowing and tillering. With these SIs, block effect was negligible, while year effect was significant for SIs calculated from SPAD and GS NDVI. If plots considered as reference received 140 kg N ha^{-1} at the pre-sowing and tillering stages, R^2 values were slightly lower (except for GS NDVI), but year and its interaction effects were not significant (except again for GS NDVI). As previously shown, year and block effects were originally negligible only for NDRE. SI calculation considering reference plots as those receiving $N_{PRE+TILL}$ = 140 kg N ha^{-1} allowed their effect to be eliminated from SPAD and RS NDVI measurements. Consequently, calibration functions were calculated for NDRE, SPAD SI, and RS NDVI SI only, as they were more suitable to assess rice N status in specific agro-environmental conditions.

3.6 Calibration functions aimed at reaching specific yield goals

Figure 6 shows the calibration functions obtained when considering different grain yield goals. The solid black line represents the first estimation of the calibration function that has to be used to achieve the maximum grain yield for the three different CNSIs values. The dashed black lines represent the calibration functions obtained when a reduction coefficient (R) is used with respect to maximum grain yield. R was assumed equal to 0.999, 0.995, 0.99, 0.95, and 0.90 in the various scenarios, respectively. As expected, all calibration functions recommended lower N amounts when vigour increases. When NDRE, SPAD SI, and RS NDVI SI reached 0.5, 124, and 110, respectively, no additional N supply at PI was required to maximise yield. For each CNSI, the slope of the function remains almost constant, while the intercept varies depending on the R value being proportional to it. Therefore, yield reduction limits the N amount that has to be supplied at PI.

4 Discussion

4.1 Crop yield maximisation and consequences on N apparent recovery

In each year of the experiment, a similar (about 11.2 Mg ha^{-1}) maximum grain yield was reached, but it was accomplished with very different total N amounts associated with different crop densities in the two years. Maximum grain yield was reached with about 200 kg N ha^{-1} in 2014 and 120 kg N ha^{-1} in 2015. Rice grain yield stayed constant or decreased with additional N fertiliser, showing a parabolic trend. The absent or negative effect of further N doses after the peak yield confirmed the results of previous studies, in which yield declines were linked to lodging or disease (Dong *et al.,* 2015; Liu *et al.*, 2015).

Grain yield was affected by N fertilisation at the pre-sowing + tillering and at PI stages and by their interaction, which revealed the potential to compensate with an N topdressing fertilisation at PI for deficient N supplies during the initial stages. These results align with reports of previous studies by Manzoor *et al*. (2006) and Lee *et al*. (2009). Rice responded well to increased N supply at PI, especially when low N amounts had been applied during the early growth stages.

In general, increases in N supply reduced apparent recovery (AR) (Xue and Yang, 2008; Yesuf and Balcha, 2014). Differences between the two cropping seasons were evident. Even though N uptake of the unfertilised plots was similar in both seasons, 2015 AR values were lower than 2014. In 2014, reaching maximum grain yield resulted in an AR of nearly 0.8 *versus* the 2015 value of just below 0.5. The lower 2015 AR value is a consequence of reduced N uptake mainly from a lower N concentration in the aboveground biomass of rice at harvest (2.26 % and 0.95 % in 2014 and 2015, respectively). Indeed, the 2014 crop was denser than in 2015. In 2015, rice compensated for a low panicle density with an increase in spikelets per panicle and in 1000-grain weight, which produced the same grain yield. Consequently, AR values were very different, despite similar N uptake in the unfertilised treatment in both years. *Equation 3* established the N supply required at PI for each

$N_{PRE+TILL}$ maximising grain yield or AR. The relationship between grain yield or AR and $N_{PRE+TILL}$ showed a clear parabolic trend. Maximum grain yield and maximum AR were obtained separately and they approximated the same total N amount (about 195 kg ha^{-1}), with about 50 kg N ha^{-1} supplied at the first two applications and the remaining dose at PI. The amount of N at the pre-sowing and tillering stages needed to maximise AR was slightly lower than that required to maximise grain yield.

4.2 Capability of different CNSIs to determine grain yield

Correlation analysis showed that VIs (SPAD, GS NDVI, RS NDVI, and NDRE) were highly correlated with biometric measures at PI. In particular, NDRE had the highest correlation coefficients. All VIs correlated most with total aboveground biomass, except as expected for SPAD, which correlated better with total N uptake. NDVI, measured with both GreenSeeker and Rapid Scan instruments at PI, correlated less well with crop N status than other Vis did.

This result may arise from the presence of an abundant biomass at which saturation starts to reduce index sensitivity (Kanke *et al.,* 2012; Muñoz-Huerta *et al.,* 2013; Novotna *et al.,* 2013; Cao *et al*., 2016).

In this study, NDVI saturated at a biomass production of about 7600 kg ha^{-1}, or an N uptake of 180 kg ha^{-1}.

The correlation between NDRE and biometric measures at PI was also strong under dense biomass. Indeed, the red-edge wavelengths utilised by Rapid Scan are more sensitive at higher levels of chlorophyll content, as is illustrated by its strongest correlation with crop N concentration at PI, and consequently with rice N uptake. In this study, NDRE saturation effects were first noted at biomass production levels of about 8000 kg ha^{-1} or 190 kg N ha^{-1} of total N uptake.

When CNSIs replaced $N_{PRE+TILL}$ in the statistical model (*i.e.*, using *Equation 4* instead of *Equation 2*), all CNSIs and most of their squares resulted significant on determining grain yield. Moreover, interaction with N rate supplied at PI was also significant on determining yield for all CNSIs considered. This confirms that topdressing N fertilisation at PI can compensate for low CNSI values, showing again that N supplied at PI can balance low $N_{PRE+TILL}$ amounts. All CNSIs demonstrated themselves to be good at determining grain yield, with R^2 values near or above 0.60. NDRE was the best.

Nonetheless, as year or block differences were detected for all VIs except NDRE, SIs were calculated from other corresponding VIs to overcome the influence of year and block on VIs values. Year and block can be assumed to represent the effect due to climate and soil variability in the direction of blocks. Sufficiency Indices calculated using reference plots that received 140 kg N ha^{-1} supplied during pre-sowing and tillering combined removed agro-climatic and soil variability effects best. In addition, statistical models applied to SIs improved yield determination, as R^2 reached values higher than 70%. Consequently, the results of this study not only confirmed the benefit of establishing a well-fertilised reference plot to obtain better indicators of in-season rice N status (Hussain *et al.,* 2000), but also estimated that plots receiving 140 kg N ha^{-1} as the sum of the pre-sowing and tillering stage N supplies, can serve as a reference plot for Centauro variety. Of course, the reference plot must be relocated each year to avoid long term effects of differentiated N fertilisation (Holland and Shepers, 2013).

4.3 Calibration functions aimed at reaching specific yield goals

Calibration functions are improved when obtained from CNSIs not influenced by agro-climatic conditions and soil variability. In this study, NDRE alone demonstrated these features. However, through SI calculations, block and year effects were also made negligible for SPAD and RS NDVI, which made it possible to determine the calibration functions for these CNSIs in addition. It is recommended that NDRE be determined at PI, but SPAD and RS NDVI can work, so long as SIs referred to these VIs are calculated from a well-N fertilised reference plot.

Obtained calibrations associate required N supplies at PI to field-measured CNSIs, and the calibration equation relates to a specified grain yield goal. The highest yield goal requires the highest supply of N at PI. Alternatively, a lower yield goal permits a reduced N_{PI} amount, as the slope of the function remains almost constant while the intercept is proportional to the grain yield goal.

One method to determine an acceptable maximum yield reduction is selection of a CNSI threshold over which N fertilisation at PI is ineffective. Suitability of the threshold should be based on the field and potential yield in specific situations.

5 Conclusions

Results reported in this study suggest that yield and apparent recovery maximisation are not conflicting goals. The statistical models developed here indicate that Centauro variety grain yield is optimised most effectively when N fertiliser supply is reduced in the early growth stages and concentrated at PI (about 70% of total N). Moreover, a topdressing N fertilisation amount can be determined from measured CNSI values to avoid N imbalances. This study confirmed that VIs measured at PI act as biometric proxy measures, and help avoiding time-consuming and destructive analyses. NDRE was demonstrated to be the best at determining grain yield variability specific agro-environmental situations. It can be used to determine Centauro variety calibration functions that improve N fertilisation.

Sufficiency index (SI) calculations that consider as reference those plots receiving 140 kg N ha^{-1} N supply (sum of the pre-sowing and tillering stages) can correct SPAD and RS NDVI measurements, making possible to calculate the calibration function for these CNSIs as well. NDVI measured with GreenSeeker was less suitable for making N fertilisation determinations at PI for Centauro variety in the environmental conditions presented in this study, as both the index and derived SI were influenced by ago-climatic conditions and soil variability.

The determined calibration functions allow a site-specific rice N fertilisation management that accounts for year and spatial variability, and avoids consequent negative environmental impacts. It must be noted that the calibration functions were derived only for Centauro variety under the specific environmental conditions presented. Therefore, extension to other rice varieties and environments can be obtained following the same method presented in this work.

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