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Determinación y aplicación de la curva de dilución crítica de nitrógeno para arroz en Uruguay

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TABLA DE CONTENIDO

1. RESUMEN

El ajuste del manejo agronómico de cultivares de alta productividad ha permitido tasas de incremento de rendimiento de 80 kg ha^{-1} $año^{-1}$ del cultivo de arroz en Uruguay en los últimos quince años. Por lo tanto, es importante disponer de herramientas que permitan optimizar objetivamente la fertilización para un manejo sustentable del cultivo a lo largo de su ciclo productivo. Las curvas de dilución críticas de nitrógeno (CCN) cumplen con el propósito de diagnosticar el estado nutricional de nitrógeno (N) de los cultivos en una forma continua durante su ciclo productivo. Los objetivos del trabajo fueron a) construir la CCN para cada subespecie de arroz (INIA Merín y Parao, índica y japónica, respectivamente), b) a partir de la CCN, desarrollar un índice de nutrición nitrogenada (INN), c) relacionar el INN con los requerimientos de nitrógeno del cultivo (RN) y obtener un equivalente de fertilizante y d) estimar en tiempo real, por medio del índice de vegetación de diferencia normalizada (NDVI), las variables de interés en un momento dado. Se generaron ecuaciones de dilución para la concentración crítica de N (Nc) en ambos cultivares ($Nc = 3{,}78*MS^{-0{,}435}$ y $Nc = 6{,}44*MS^{-0{,}690}$ para INIA Merín y Parao). Los parámetros resultantes discriminaron entre condiciones de nutrición nitrogenada subóptima, óptima y de consumo de lujo, y se establecieron ecuaciones que permitieron ajustar la dosis equivalente de fertilizante a partir del INN. Para INIA Merín, el INN óptimo para acumulación de materia seca (MS) fue también el óptimo para el máximo rendimiento relativo en grano (RR). A partir de las relaciones establecidas entre el NDVI y los parámetros en estudio, fue posible estimar la dosis de N a aplicar al cultivo en un momento determinado para alcanzar el estado nutricional óptimo y así maximizar la producción de MS con la mínima concentración de N. Es necesario continuar ajustando y validando dichas curvas para usarlas como herramientas objetivas en el ámbito comercial y, de esa forma, corregir las restricciones nutricionales apuntando a producir la máxima MS, pero con el objetivo final de obtener el máximo rendimiento en grano.

Palabras clave: índice de nutrición nitrogenada, concentración crítica de nitrógeno, subespecie índica, subespecie japónica, rendimiento relativo.

DETERMINATION AND APPLICATION OF THE CRITICAL NITROGEN DILUTION CURVE FOR RICE IN URUGUAY

1.1. SUMMARY

The adjustment of the agronomic management of high productivity cultivars has allowed yield increases of 80 kg ha^{-1} $year^{-1}$ of rice cultivation in Uruguay in the last 15 years. Therefore, it is important to have tools that allow objective optimization of fertilization for sustainable management of the crop throughout its production cycle. Critical nitrogen dilution curves (CNDC) meet the purpose of diagnosing the nutritional status of nitrogen (N) crops in a continuous manner during their production cycle. The objectives of the work were: (a) to build the CNDC for each subspecies of rice (INIA Merin and Parao, Indica and Japonica, respectively), (b) from the CNDC, to develop a nitrogenous nutrition index (NNI), (c) relate the NNI to the nitrogen requirements of the crop (NR) and obtain a fertilizer equivalent and (d) estimate in real time, by means of the normalized difference vegetation index (NDVI), the variables of interest at a given time. Dilution equations were generated for the critical concentration of N (cN) in both cultivars ($cN = 3.78*DM^{-0.435}$ and $cN = 6.44*DM^{-0.690}$ for INIA Merin and Parao). The resulting parameters discriminated between suboptimal, optimal and luxury consumption nitrogen nutrition conditions, and equations were established that allowed adjusting the equivalent dose of fertilizer from the NNI. For INIA Merin, the optimal NNI for dry matter accumulation (DM) was also optimal for the maximum relative grain yield (RR). Based on the relationships established between the NDVI and the parameters under study, it was possible to estimate the dose of N to be applied to the crop at a given time to achieve optimal nutritional status and thus maximize the production of DM with the minimum concentration of N. It is necessary to continue adjusting and validating these curves to use them as tools objectives in the commercial field. In this way, correct the nutritional restrictions aiming to produce maximum DM, but with the goal of obtaining the maximum yield in grain.

Keywords: nitrogen nutrition index, critical nitrogen concentration, indica subspecies, japonica subspecies, relative yield.

1.2. INTRODUCCIÓN

1.2.1. Estrategias de fertilización en arroz

El nitrógeno de origen inorgánico es el principal nutriente del cultivo de arroz en el ámbito mundial. Del total de N agregado globalmente cada año, el arroz utiliza el 21-25 % (Prasad et al., 2017). En promedio se aplican entre 110 kg N ha^{-1} (Ladha et al., 2015) y 150 kg N ha^{-1} (Krupnik et al., 2005), con rangos entre 10 kg N ha^{-1} (Dobermann y Cassman, 2005) y más de 200 kg N ha^{-1} (Zhang et al., 2014).

En Uruguay, el ciclo del cultivo se desarrolla sobre condiciones de humedad del suelo contrastantes. Desde la siembra hasta inicios de macollaje (V4, según Counce et al., 2000), el cultivo se desarrolla en condiciones de secano, mientras que, en la etapa siguiente y hasta cosecha, lo hace bajo inundación. En este sistema de producción, donde el suelo permanece inundado, cubierto con una lámina de agua durante la mayor parte del ciclo del cultivo, se afecta la eficiencia de uso del fertilizante nitrogenado. Esto se debe a que gran proporción del N aplicado como fertilizante no llega a ser absorbido por las plantas.

En el ámbito mundial, el arroz recupera en promedio 35 % del N aplicado (Cassman et al., 1993). La inundación del suelo provoca las mayores pérdidas de N y se recupera menos del 30 % de lo aplicado en situaciones de arroz de trasplante donde se dan pérdidas significativas desde el suelo, volatilización y desnitrificación; según Ladha et al. (2005), 33 % de lo aplicado se pierde solamente por este último proceso. Además de perderse debido a los procesos mencionados, aproximadamente 25 % es retenido por el suelo (arcillas y microorganismos).

En Uruguay, a pesar de que la productividad del cultivo es alta en el ámbito global, las dosis de nitrógeno aplicadas son moderadas. Esto se debe a la rotación de arroz con pasturas, en suelos de textura pesada, capaces de retener N, y al fraccionamiento de la dosis de N en dos momentos del cultivo, al inicio del macollaje con suelo seco y previo al primordio con el suelo inundado. Esto determina que la eficiencia de recuperación de N en Uruguay ronde el 40 % (Castillo, 2018).

1.2.1.1. Fertilización comercial en Uruguay

En Uruguay, aproximadamente 50-70 % del N absorbido hasta el inicio del primordio floral proviene del suelo (Méndez y Deambrosi, 2009), pero no es suficiente para cubrir la demanda de altos rendimientos. La mayor atención de la investigación en el estudio de la nutrición del cultivo de arroz en los últimos 20 años se centró en el manejo del N. Este es sumamente complejo debido a la interacción que tiene con múltiples factores, como la época de siembra, el cultivar, el manejo del riego, las condiciones climáticas y el tipo de suelo.

La información muestra que en 78 % de los casos se encontró respuesta significativa al agregado de N, mientras que en los restantes esta respuesta no fue tan clara e incluso negativa (Deambrosi et al., 2002). Esta disminución en el rendimiento puede deberse a una mayor susceptibilidad del cultivo a las bajas temperaturas en su etapa crítica, la cual es generada por la alta fertilización nitrogenada (Amano, citado por Nakamura et al., 2002). Esto demuestra la variada respuesta del cultivo de arroz al agregado de este nutriente.

El agregado del fertilizante N de forma fraccionada a la siembra, (S0) (Counce et al., 2000), macollaje y primordio floral incrementa el rendimiento en relación con una dosis única a la siembra (Deambrosi et al., 2010; Deambrosi y Méndez, 1998). Esto podría deberse a que el N basal no es capaz de cubrir los requerimientos durante todo el ciclo del cultivo por su mayor riesgo de pérdidas. El N agregado a la siembra tiene efectos en el contenido de N en plantas hasta su macollaje, no así hasta el primordio floral.

Por ese motivo, la fertilización en primordio floral puede ser una herramienta relevante para el aumento de los rendimientos (Deambrosi y Méndez, 1993). Históricamente, el enfoque de la investigación nacional ha priorizado la generación de información sobre las respuestas productivas del cultivo al momento, fraccionamiento y dosis de fertilizante nitrogenado, respecto a establecer parámetros objetivos para el manejo y optimización de este.

Aunque el cultivo de arroz en Uruguay es realizado en ambientes edáficos diversos, los criterios para el manejo de nutrientes y sus dosis en el ámbito comercial no tuvieron cambios significativos hasta iniciada la segunda década del siglo XXI. Sin embargo, en los últimos años se observan algunos cambios de tendencias en el manejo de nutrientes en el cultivo de arroz, particularmente el incremento de las dosis de nitrógeno utilizadas (Molina et al., 2021).

1.2.1.2. Fertilización basada en criterios objetivos

Según Cassman (1999), la seguridad alimentaria mundial en los próximos 30 años dependerá de los rápidos avances científicos en la comprensión de la base fisiológica del potencial de rendimiento de los cultivos. Esto incluye los procesos que rigen la relación entre la calidad del suelo, la ecología de las plantas y sus relaciones con los factores ambientales que interactúan y determinan los rendimientos de los cultivos.

Por lo tanto, es clave desarrollar y adaptar criterios objetivos de fertilización del cultivo de arroz, insumo fundamental para poder implementar prácticas que aumenten la eficiencia de uso de fertilizante y minimicen pérdidas y costos. Los indicadores de disponibilidad de N, niveles críticos, mejoras en el método y momentos de fertilización, fuentes y el uso de inhibidores de ureasa son solo algunos de los caminos a explorar para mejorar la sincronización entre la demanda y el aporte de N para el cultivo (Prasad et al., 2017).

Para aumentar la eficiencia de uso del nitrógeno es necesario conocer la cantidad óptima para aplicar con el fertilizante. Esta cantidad supone cubrir la necesidad del sistema. Para determinar esta cantidad es necesario conocer, en primer lugar, la capacidad de aporte del suelo. Las recomendaciones de fertilización según indicadores del suelo han probado mejorar en 30 % a 40 % la eficiencia de uso del fertilizante y 7 % el rendimiento en el ámbito mundial (Prasad et al., 2017). Otra manera de determinar las necesidades óptimas de N en un momento dado es a partir de indicadores de la planta.

El método más ampliamente difundido de análisis en planta es la determinación de la concentración del nutriente. También se pueden usar criterios como síntomas de deficiencia, pero, para cuando se realiza la corrección, suele ser tarde. Generalmente, los datos obtenidos por este método basan el criterio de fertilización en concentraciones críticas. En Uruguay se utilizan niveles críticos para trigo (4,5 %) y cebada (3 %), ambos a Z-30 con recomendación de dosis (final de V6 según escala Zadocks, Seminario-Taller Análisis de Planta, 2014).

En arroz, el criterio objetivo para la fertilización al inicio del primordio floral (R0) es el nivel crítico de absorción de N (kg ha^{-1}) en planta (Castillo et al., 2014). El problema es que la concentración varía con el ciclo del cultivo lo que reduce el tiempo para realizar el muestreo y verificar su eficiencia. Según Daughtry et al. (2000), el análisis de tejido proporciona la medida más directa; sin embargo, es una técnica que también consume mucho tiempo y los resultados de laboratorio a menudo se reciben más allá del momento en que se deben tomar decisiones sobre fertilización.

En Uruguay, históricamente se fracciona la dosis de N a macollaje y primordio, aportando entre ambas 50-60 kg N ha^{-1}, pero sin tener claro los criterios para decidir la dosis y el fraccionamiento. Debido a que el arroz se siembra sobre una gran variabilidad de suelos y rotaciones (con pasturas o diferente intensidad de arroz, Palmer, 2012), una dosis y fraccionamiento uniforme no sería adecuada para todos los casos. Una meta reciente ha sido generar y adaptar indicadores asociados al rendimiento y la determinación de sus niveles críticos para optimizar la fertilización nitrogenada (Marchesi et al., 2014).

Actualmente, el indicador más robusto para ajustar la fertilización nitrogenada del cultivo de arroz al inicio del macollaje (V4) inmediatamente antes de la inundación es el potencial de mineralización de N (PMN) del suelo en condiciones anaerobias (R^2 = 0,65, Castillo, 2015). Para la etapa posterior a la inundación, al inicio del primordio floral (R0), como ya fue mencionado, no se ha ajustado un indicador efectivo, robusto y práctico. Una de las herramientas que surgen como alternativa para superar estas restricciones es la curva de dilución crítica de N.

1.3. CURVA DE DILUCIÓN DE NITRÓGENO

En general, la curva de absorción de N en el ciclo de la planta es muy similar a la de producción de MS (Lopes et al., 1993). La acumulación de N en la planta en función del tiempo presenta una curva sigmoide donde la acumulación al principio es escasa porque la planta recién se está desarrollando. Luego ocurre una etapa de máxima absorción de N que corresponde al período de activo crecimiento y, finalmente, la tasa de absorción se reduce (Perdomo y Barbazán, 2012). La planta de arroz exhibe dos momentos donde la tasa de consumo de N es elevada, una corresponde al momento de máximo macollaje y otra a la formación de la panoja (Shoji et al., 1986).

Varios estudios han indicado que la concentración de N decrece paulatinamente durante el ciclo de crecimiento para casi cualquier planta (Greenwood et al., 1990), incluso las leguminosas, que obtienen el N mediante vía atmosférica (Lemaire et al., 1985). Durante las etapas tempranas de crecimiento, el fenómeno de dilución del N es poco importante debido a la ausencia de competencia por luz entre plantas (Lemaire y Gastal, 1997).

Diversos factores influyen en el efecto de dilución. Los dos más importantes son, por un lado, la senescencia de hojas (Lemaire et al., 1991) y, por el otro, el cambio en la relación tallo/hoja durante el desarrollo del cultivo, donde la concentración de N en los tejidos del tallo es menor que los de la hoja (Lemaire et al., 1985, 1992). Además, la proporción de elementos constituyentes de la pared celular (celulosa, lignina) se incrementa durante el ciclo del cultivo, mientras que la proteína disminuye (Lemaire et al., 1992). Sin embargo, esto es así considerando el aporte sostenido de N durante todo el ciclo por parte del suelo, pero, cuando las fertilizaciones entran en juego (más aún cuando se trata de cultivos anuales), la respuesta se vuelve irregular debido a las altas dosis de N y tasas de crecimiento (Justes et al., 1994).

Si bien se han determinado curvas de dilución para varias especies, las curvas de dilución de N suelen tener variables relevantes para considerar. Uno de los factores que afectan la determinación de la curva es el metabolismo de las plantas: las curvas de las especies C3 suelen ser similares entre sí y diferentes a las especies C4 (Justes et al., 1994). Otro factor que afecta es el de la especie, por eso mismo se determinan las curvas individuales para distintas especies, más allá de que exista una curva generalizada. Diferentes autores afirman que el clima es otro determinante de la curva de dilución, especialmente en el arroz (Ata-Ul-Karim et al., 2013; Shanyu et al., 2018; Sheehy et al., 1998).

1.3.1. Curva de dilución crítica de nitrógeno

Este concepto, desarrollado inicialmente en la década de los 80 para el N en gramíneas forrajeras, tiene un marco teórico sólido (Briat et al., 2020) y se ha aplicado en más de 30 especies de cultivos herbáceos por científicos de 40 países (Chen et al., 2021). La concentración de N de cada punto de la CCN, llamado nitrógeno crítico (Nc), es la concentración mínima de N observada en un momento dado entre todos los tratamientos de N que produjeron la cantidad máxima de MS hasta ese momento. Corresponde a un nivel de concentración de N en el que la MS aérea no crece significativamente cuando la fertilización con N aumenta, a pesar de una mayor absorción de N del cultivo y la acumulación de N en la planta (Justes et al., 1994).

Se han desarrollado CCN para la mayoría de las especies cultivadas en diferentes lugares del mundo. De todos modos, la pregunta sigue siendo si los parámetros de la CCN para una especie dada se ven afectados por los genotipos, el medioambiente y las condiciones de manejo. Se han informado diferencias entre cultivares de papa (Giletto et al., 2020) y entre sitios para el trigo de invierno (Fontana et al., 2021), pero pueden haberse confundido con otros factores.

Se ha propuesto un nuevo método para determinar esta incertidumbre en las curvas críticas de dilución de N (Makowski et al., 2020). Este método, basado en un enfoque bayesiano, permite una comparación más precisa entre diferentes especies de cultivos y cultivares para analizar las interacciones genotipo-ambiente en la eficiencia del uso de N de cultivos como el maíz (Ciampitti et al., 2021).

1.3.2. Índice de nutrición nitrogenada

La diferencia entre la concentración de N actual (Na) y el correspondiente nivel de Nc para una cantidad dada de biomasa aérea indica la intensidad de la deficiencia o el exceso de N experimentado por el cultivo. Varios autores han demostrado que la reducción en la tasa de crecimiento del cultivo es proporcional a la relación Na/Nc y han propuesto el uso de esta relación como un índice de nutrición nitrogenada (Colnenne et al., 1998; Justes et al., 1994; Lemaire y Gastal, 1997), a partir del cual se puede predecir la respuesta del cultivo al agregado de N vía fertilizante (Tamagno et al., 1999).

De acuerdo con algunos autores, la respuesta del cultivo al agregado de N depende no solamente del estado nutricional, sino también del momento en el ciclo del cultivo en el cual se realice el suministro (Tamagno et al., 1999). Varios autores concuerdan en que es necesario mantener una buena disponibilidad del nutriente en torno a primordio floral (Chamorro et al., 2002; Iriarte y Valetti, 2002), momento de mayor demanda por parte del cultivo (Iriarte y Valetti, 2002) y etapa crítica en la determinación del rendimiento (Tayo y Morgan, como se cita en Tamagno et al., 1999).

Para una situación dada y en cualquier momento del período de crecimiento del cultivo, es posible determinar un INN (Lemaire et al., 2008). Los valores de INN próximos a 1 indican que, en el momento de la determinación del Na, el cultivo está en situación de suministro de N no limitante. Valores mayores a 1 indican un consumo de lujo de N y valores inferiores a 1 indican deficiencia de N. La intensidad de esta deficiencia se puede estimar por el valor del INN (Lemaire et al., 2008). Si bien este índice en su origen es un indicador de estado nutricional del cultivo para el momento en el cual es determinada la biomasa acumulada y su concentración de N, Lemaire et al. (2008) han encontrado que los cambios en INN durante el período vegetativo del cultivo, en el cual se genera el potencial productivo, tienen un gran efecto sobre el número de granos y, por lo tanto, sobre el rendimiento.

Ata-Ul-Karim et al. (2013) sostienen que, cuando el Nc del cultivo se estima simplemente mediante la CCN establecida con MS aérea, se puede calcular el requerimiento de N (RN) del cultivo. Stockle y Debaeke (1997) compararon cuatro enfoques de simulación de RN con diferentes modelos de crecimiento de trigo y encontraron que el modelo CropSyst dio los mejores resultados de simulación de RN porque utilizó la curva de dilución crítica de N. Además, el INN, también se ha utilizado en estudios de diagnóstico de nutrición de N de cultivos (Sylvester-Bradley y Kindred, 2009; Tremblay et al., 2011).

Ata-Ul-Karim et al. (2017) encontraron que había una correlación lineal significativa entre el RN del arroz y el INN, con el coeficiente de determinación por encima de 0,98. Sin embargo, esta relación lineal varió entre las diferentes etapas de crecimiento. El cálculo del INN del arroz es más sencillo que el cálculo del RN, ya que el INN se puede adquirir utilizando un medidor de clorofila portátil (Ziadi et al., 2008), equipo multiespectral (Padilla et al., 2017) o equipo hiperespectral (Chen, 2015). Por lo tanto, si se estableciera una relación alta entre el RN y el INN, el RN se puede calcular directamente mediante el INN medido en el campo.

1.3.3. Índice de vegetación de diferencia normalizada

Para determinar el contenido de N y MS en planta y posteriormente aplicar las medidas de manejo de fertilización necesarias, se requieren técnicas de muestreos destructivos de tejidos vegetales. Esto determina que, en muchos casos, se vuelvan métodos de fertilización ineficientes y poco prácticos para aplicarlos en el campo (Daughtry et al., 2000). Existen tecnologías alternativas disponibles para acelerar la evaluación del contenido de N en el campo, como la carta de colores de hojas (CCH) y el medidor de clorofila para el desarrollo del análisis de plantas en el suelo (SPAD, Balasubramanian et al., 1998; Peng et al., 1996).

La CCH estima el contenido de N con base en el verdor de la hoja, mientras que el medidor de clorofila SPAD mide la diferencia en la transmitancia entre la luz roja e infrarroja cercana que pasa a través de la hoja para estimar el contenido de clorofila (Alam et al., 2005; Uddling et al., 2007). Investigaciones anteriores han demostrado la capacidad de estas tecnologías para evaluar el estado del N del arroz y promover la gestión sostenible del N (Islam et al., 2007; Yadvinder-Singh et al., 2007). Sin embargo, tanto el medidor de clorofila CCH como el SPAD son ineficaces, ya que solo evalúan una hoja a la vez, por lo que se requiere un tiempo y un esfuerzo considerable para evaluar con precisión un campo completo (Daughtry et al., 2000; Xue et al., 2004; Saberioon et al., 2014).

En las últimas décadas, se ajustó y desarrolló la tecnología de teledetección mediante mediciones de reflectancia del dosel para evaluar el contenido de N en los cultivos de una manera rápida y no destructiva. Los datos de reflectancia del dosel se recopilan de forma remota (vía satélite, avión, dron o sensor proximal) y se interpretan a través de un índice vegetativo. El índice de vegetación de diferencia normalizada (NDVI) es el más adoptado (McFarland y Riper, 2013) y es sensible a los compuestos fotosintéticos, lo que lo convierte en un índice potencialmente útil para estimar la productividad de la vegetación en un área definida (Tucker, 1979; Tucker y Choudhury, 1987).

La capacidad del NDVI para evaluar el estado del nitrógeno en los cultivos y desarrollar predicciones de rendimiento se ha estudiado ampliamente en sistemas de producción de trigo (*Tricticum aestivum*) y maíz (*Zea mays*). Muchos han demostrado que el NDVI estima eficazmente el contenido de N en planta en una variedad de etapas de crecimiento y tipos de sensores (Erdle et al., 2011; Li et al., 2008, 2014; Reyniers y Vrindts, 2006). Otros encontraron que el NDVI era útil para desarrollar predicciones de rendimiento durante la estación de crecimiento mediante la estimación de producción de biomasa en trigo y maíz (Inman et al., 2007).

La adopción del manejo del N basado en el NDVI en los sistemas de producción de trigo y maíz mejoró el rendimiento de grano, la eficiencia en el uso del N y los retornos netos (Mullen et al., 2003; Raun et al., 2005; Raun et al., 2002; Tubaña et al., 2008). Comparativamente, se evidencian pocos estudios de este tipo en el arroz. Algunos autores han probado la capacidad del NDVI para evaluar el estado de N del arroz (Gnyp et al., 2014; Lu et al., 2017; Yao et al., 2014; Zhu et al., 2007) y pocos han utilizado el NDVI para desarrollar predicciones de rendimiento en grano (Cao et al., 2016; Harrell et al., 2011; Yao et al., 2012).

Sin embargo, la mayoría de estos estudios han centrado su investigación en sitios únicos, dejando en duda la escalabilidad de sus hallazgos a otros sitios con diferentes suelos y prácticas de manejo. En un estudio realizado en arroz, Rehman et al. (2019) determinaron que el NDVI en primordio floral se correlacionó mejor con la absorción total de N (R^2 = 0,66), seguido de la concentración de N (R^2 = 0,54) y la MS aérea (R^2 = 0,51). Además, la utilidad del NDVI fue mayor en los valores más bajos de contenido de N del cultivo, mientras que, en los valores más altos, el NDVI tendió a saturarse.

También el NDVI en primordio floral se correlacionó positivamente con el rendimiento final de grano (R^2= 0,58), lo que indica su utilidad para desarrollar predicciones de rendimiento. Si bien el NDVI es un indice potencialmente útil para mejorar el manejo de fertilizantes nitrogenados y desarrollar predicciones de rendimiento durante el ciclo productivo del arroz, los índices alternativos que no se saturan probablemente proporcionarán una base para una mejor herramienta. Trabajos presentados por Mistele y Schmidhalter (2008) y Ziadi et al. (2008) afirman que sensores como el medidor de clorofila y los métodos de reflectancia del dosel pueden utilizarse para una estimación rápida y no destructiva del INN.

La mayoría de los estudios sobre teledetección en tiempo real se han centrado en el diagnóstico de N durante el ciclo del cultivo, pero la determinación de los RN sigue siendo difícil de estimar debido a la falta de conocimientos sólidos sobre la relación entre el RN y los índices relacionados con el contenido de N de los cultivos (Samborski et al., 2009). Por tanto, es fundamental profundizar la investigación sobre el empleo de dichas tecnologías para una agricultura de precisión, que contribuyan a elevar las restricciones nutricionales del cultivo de manera objetiva y en tiempo real, aspecto sumamente necesario para el manejo sostenible de los sistemas productivos.

1.4. OBJETIVOS

El propósito del trabajo fue estimar la CCN y el INN durante el ciclo productivo de dos cultivares de arroz (subespecies índica y japónica) de alto potencial de rendimiento para ajustar ecuaciones de referencia que guíen en tiempo real la fertilización nitrogenada en las condiciones de producción de Uruguay.

Los objetivos específicos fueron: (a) construir la CCN para cada cultivar, (b) a partir de la CCN, desarrollar un INN, (c) relacionar el INN con los RN del cultivo y obtener un equivalente de fertilizante, y (d) estimar en tiempo real, por medio de índices como el NDVI, la MS, la concentración de N, la ABSN y el INN del cultivo en un momento dado.

2. DEVELOPMENT OF CRITICAL NITROGEN CURVES FOR RICE CULTIVATION IN URUGUAY

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2.1. ABSTRACT

Rice cultivation in Uruguay has two stages, a rainfed stage from sowing to tillering (V4) and another after flooding from that stage to physiological maturity. The crop is usually integrated into pasture-livestock production systems and is characterized by high yields with relatively low doses of nitrogen (N). The nitrogen recovery efficiency (NRE) of the crop in these systems with a fractional management of two N coverages is approximately 45%, somewhat higher than that reported internationally. Although there are objective criteria for the definition of the first N cover at tillering, there are no robust and practical nitrogen fertilization models for later stages to optimize nitrogen use efficiency. The objective was to determine the critical nitrogen dilution curves (CNDC) in two rice cultivars (INIA Merin: indica and Parao: japonica) under Uruguayan production conditions, to adjust nitrogen fertilization rates according to nitrogen nutrition indices (NNI). The study was carried out in three locations representative of the main production zones, East, North and Center. Biomass and plant nitrogen content were determined from 15 days before to 45 days after panicle initiation. The determined CNDC showed different behaviors from the curves published at international level. At indica, the CNDC presented 12 critical points, a coefficient a = 3.78 and b = -0.435, R^2 = 0.71, and at japonica, 5 critical points were obtained, a coefficient a = 6.44 and b = -0.690 and R^2 = 0.93. A tool was generated to diagnose the nutritional status of the crop and adjust N fertilization during its productive cycle, which needs to be validated at the commercial level.

Keywords: critical point, nitrogen concentration, smoothed models, harvest index.

2.2. INTRODUCTION

Uruguay is a rice exporting nation and one of the highest yielding countries, globally. The cultivated area averages 165,000 ha in the last 10 years, distributed in 3 regions, east (101,349 ha), north (25,167 ha) and center (12,769 ha, MGAP-DIEA, 2021). The main features that differentiate this system from most rice production systems in the world are the integration with livestock systems and with rainfed crops, the harvesting of a single crop per year, and sowing in dry soil with subsequent definitive flooding at the tillering stage (Counce et al., 2000).

Rice-livestock integration based on direct grazing pastures contributes to productive, economic and environmental sustainability (Castillo et al., 2021; Lanfranco, 2009; Pittelkow et al., 2016). Recently, soybean cultivation has been integrated to the rice-pasture rotation favored by good international prices and diversification opportunities for farmers (Terra et al., 2014; Tommasino, 2016). Rice takes approximately 25-40% of the rotation period. The remaining time is occupied by regenerated or sown pastures, mainly a mixture of grasses and legumes (MGAP-DIEA, 2020).

The last 5-years average yield reached 8.5-Mg ha^{-1} (MGAP-DIEA, 2021). In recent decades, the rate of increase in yield was from 50 to 100 kg ha^{-1} $year^{-1}$, depending on the zone (Molina et al., 2021). It was mainly explained by the adoption of modern high-productivity cultivars accompanied by adjustments in the agronomic management. Nevertheless, crop fertilization management had relatively little change until 2015. At that point, basal fertilization was 12 to 18 kg N ha^{-1}, 48-50 kg P_2O_5 ha^{-1}, and 31 kg K_2O ha^{-1}. Meanwhile, N topdressings were carried out at the beginning of tillering immediately before flood, and at panicle initiation, with 25 kg N ha^{-1} in each application. A few producers applied a single topdressing, which could be at tillering, panicle initiation or intermediate at these two moments, with amounts between 35 to 49 kg N ha^{-1} (Molina et al., 2013).

Since the 2012-2013 harvest, there were changes in nutrient management that led to a reduction in the basal P_2O_5 and N doses (10 kg ha^{-1} less each) and an increase in K_2O doses (10 kg K_2O ha^{-1} more). On the other hand, the dose of N applied at topdressing was increased to 70-80 kg N ha^{-1} (Molina et al., 2013). These changes in fertilization, along with improvements in genetic and other agronomic adjustments, contributed to the increase in yield over the past five years.

A sustained yield increase implies more N demanded by the crop. Such demand is not stable throughout the crop cycle but varies according to its needs. Therefore, it is important to match the crop demand with the combined supply of the native N of the soil and that provided by the fertilizer, by monitoring the nutritional status of the crop. There is currently a proposed model for nitrogen fertilization based on the soil nitrogen mineralization potential (NMP, measured as mg NH_4 kg^{-1}) as an objective parameter to define the N cover at tillering, prior to definitive flooding (Castillo et al., 2014). For post-flood stages, the indicator proposed so far is based on plant N uptake (% N), which has not been practical to correct nutritional restrictions at that moment and in real time. For decades, there has been a technique that associates the plant biomass achieved during the crop life cycle to a specific N concentration, which would allow to resolve the limitation mentioned.

The diagnostic technique above mentioned is based on the concept of critical nitrogen concentration or critical nitrogen (cN) introduced by Ulrich (1952), which is defined as the minimum concentration of N required to obtain the maximum accumulation of biomass. The method generally used to determine the cN is that proposed by Justes et al. (1994). The critical point of N concentration is constructed for each moment of crop sampling according to the bilinear model proposed by these authors.

According to Greenwood (2001), the N concentration of each point of the cN is the minimum concentration of N observed among all N treatments that produced the maximum amount of biomass at a given time. It corresponds to an N concentration level at which aboveground biomass does not increase significantly although N fertilization is increased, even though crop N uptake and accumulation continues in the plant.

Plant N concentration decreases with increasing biomass (Greenwood et al., 1990; Lemaire and Gastal, 1997). This reduction can be explained by several reasons associated with the variation of the N content in two conceptual compartments of the plant, the metabolic and the structural (Caloin and Yu, 1984). Various causes include a higher rate of biomass accumulation relative to the rate of N absorption (Perdomo & Barbazán, 2012), leaf senescence (Greenwood et al., 1991), a decrease in the leaf/stem ratio during the crop cycle (Lemaire et al., 1985, 1992) or an increase in the ratio of cellulose to lignin as the protein decreases throughout the crop cycle (Lemaire et al., 1992).

The cN concept based on total aboveground biomass was developed for fescue (*Festuca arundinacea* Schreb.) by Lemaire et al. (1984), and is represented by the following equation:

$$cN=aW^{-b} \tag{1}$$

Where W is the aboveground biomass in Mg DM ha^{-1}, cN is the N critical concentration expressed in percent of DM, whereas a and b are estimated parameters. Parameter a represents the N concentration per Mg ha^{-1} DM and parameter b the dilution coefficient that describes the association between N and biomass accumulation.

The curve defined by this equation differentiates the nitrogen status of the plant into three conditions: N limiting or deficient, N non limiting or luxury consumption, and optimal nitrogen, defined by the CNDC. During the growth early stages (biomass <1 t ha^{-1} DM), Nc takes on a constant value due to the small decrease of cN with increasing biomass and the absence of competition for light in well-distributed plants, resulting in a constant value of N concentration (Lemaire and Gastal, 1997).

Although most of the work in development of CNDC has been focused on determining the minimum concentration of N that maximizes the total accumulation of biomass throughout the plant cycle (Justes et al., 1994; Lemaire et al., 1984, 2008; Lemaire et al., 2007; Shanyu et al., 2018), in others, this development has been oriented towards relating it to the final grain yield (Ata-Ul-Karim et al., 2016; Ata-Ul-Karim et al., 2017; He et al., 2017; Sheehy et al., 1998). This implies the existence of a strong association between the total accumulation of aboveground biomass and that of grain, which would be obtained through stable harvest indices (HI).

Unlike what has been reported for previous rice cultivars in Uruguay (Hernández et al., 2012; Lago et al., 2016), preliminary information from INIA Merin and Parao cultivars, indica and japonica subspecies, respectively, supports that the HI is stable. Therefore, this technology could end the preceding limitations for rice in Uruguay, in terms of defining the target doses of N after panicle initiation with the aim of exploring high yields.

Lemaire and Gastal (1997) suggest that species-specific CNDCs should be developed to obtain a more accurate diagnosis of the nutritional status of the plant, according to the histological characteristics of each species. For rice cultivars, Sheehy et al. (1998) generated two CNDCs for indica subspecies in the Philippines (tropical climate) and China (subtropical climate). The same authors also generated a single general curve for all rice in the tropics using three cultivars and sites. According to Ata-Ul-Karim et al. (2013), the curves generated by Sheehy et al. (1998) were not appropriate for the subspecies japonica, so they generated a new CNDC specifically for japonicas in China (subtropical climate). Shanyu et al. (2018), in northeastern China (temperate climate), also generated a CNDC for subspecies japonica.

Once the CNDC has been constructed, it is possible to determine throughout most of the crop cycle an NNI that allows quantifying the nutritional status of N in the plant (Mills et al., 2009). This index can be used to make decisions about the application of N (Lemaire et al., 2008). NNI values close to one indicate that, at the time of determination of the percentage of N, the crop is in a situation of non-limiting N supply. Values greater than 1 indicate a luxury consumption of N and lower values indicate deficiency of N.

It is crucial to use flexible and practical objective indicators to determine the nutritional status of the crop and to make recommendations for optimal N doses for maximum DM accumulation in real time. This is particularly relevant after flooding when the crop has the greater demand. Thus, the aim is to achieve maximum levels of biomass production as well as grain yield, while reducing the risk of environmental contamination. Based on this, the objectives of the work were: (a) to construct the CNDC for two rice cultivars, one of the indica subspecies and the other of the japonica, and (b) from the CNDCs, to develop an NNI to have an objective tool to trace and quantify the nitrogen status of both cultivars during their production cycle.

The hypotheses were: (a) that in each of the stages of development of INIA Merin and Parao, cultivars of indica and japonica subspecies, respectively, there is an N concentration in the plant from which the accumulation of dry matter becomes maximum and (b) that these modern cultivars make it possible to explore high yields with high accumulations of DM due to their stable HI.

So far, no systematic work has been done in the temperate region of South America, considered to be one of the highest potential yields of rice in the world, to determine the CNDC in the crop, as well as the development of indices from it to guide nitrogen fertilization based on its nutritional status. This research seeks to establish the effectiveness of both parameters (CNDC and NNI) in two cultivars with high yield potential, one japonica and the other indica, covering the three main agroecological rice-producing zones in Uruguay during three harvest seasons.

2.3. MATERIALS AND METHODS

Experimental nitrogen fractionation trials in flooded rice were conducted in a multi-location network across three productive agroecological zones in Uruguay (East, Central, and North) over three years, from the 2016-2017 to the 2018-2019 cropping season (Table 1).

Table 1. Experimental sites and rice cultivars used.

Experimental Unit	Soil unit	Type of soil	OC* (%)	Coordinates	Cultivar
Treinta y Tres (Paso de la Laguna)	La Charqueada	Silty-clay loam Argialboll	1,3	33°16'17.62"S 54°10'24.63"O	INIA Merín (indica subspecies) Parao (japónica subspecies)
Tacuarembó (Pueblo del Barro)	Rio Tacuarembó	Vertic Albaqualf	2,3	31°59'27.62"S 55°24'28.81"O	INIA Merin Parao
Artigas (Paso Farias)	Itapebi Tres Arboles	Typic Hapludert	2,8	30°28'37.74"S 57° 7'11.85"O	INIA Merin

*Note: *OC: organic carbon.*

2.3.1. Rice cultivars and crop management

Two rice cultivars were used in the study, INIA Merin (Pérez, 2016) and Parao (Molina et al., 2011) belonging to the indica and japonica subspecies, respectively. Both high-yielding potential cultivars exhibit robust responses to nitrogen and demonstrate significant stability in the harvest index (HI) as biomass (DM) increases, even under high nitrogen rates. These traits contribute to maintaining optimal nitrogen levels throughout the entire productive cycle for both cultivars.

Rice management was based on conventional practices, towards reaching the highest yield possible. Most of the situations came from established rotations, or at least with no rice or other crops as predecessor. Summer tillage was carried out, along with an appropriate sowing date and plant density. Weeds and other pests were controlled if necessary, and irrigation was established immediately after the second N was broadcast. The harvest was done in good climatic conditions.

2.3.2. Experimental design

A total of 15 trials were conducted, with nine involving INIA Merin (indica) across three locations for three years, and six focusing on Parao (japonica) in the Eastern and Central zones over the same period (Appendix 1). In the initial two years, a randomized complete block split-plot design with three replications was employed across all locations. The primary plot represented nitrogen fertilization at tillering, while the sub-plot represented nitrogen fertilization at panicle initiation. Fertilization rates of 0, 25, 50, and 100 kg N ha^{-1} were randomly applied at both physiological stages, resulting in a factorial combination of 16 treatments (Appendix 2). In the last season (2018-2019), a randomized complete block design with three replications was used to assess five N rates ranging from 0 to 275 kg N ha^{-1}, exclusively at tillering (Appendix 2).

2.3.3. Determinations and statistical analysis

2.3.3.1. Biomass accumulation

Biomass accumulation was assessed by ground-level cuts of two subsamples of rice plants within each plot, covering 0.3 m linear, from 15 days before panicle initiation (15DBPI) to 45 days after panicle initiation (45DAPI) at approximately 15-day intervals.

A total of 2080 rice samples were collected for the indica cultivar, and 1164 for the japonica cultivar. All samples underwent rinsing with tap water, washing with distilled water, and were stored at 5 °C until laboratory processing.

After cleaning, the samples were weighed, then dried in vertical flow ovens at 55 °C for 48 hours to determine dry weight. Subsequently, the samples were ground to 2 mm using a static grinder and stored for later lab analysis. The same procedure was applied to both grain and straw samples collected at harvest.

2.3.3.2. Nitrogen determination in plant

The determination of nitrogen content in rice biomass samples was carried out using the Near-Infrared Spectroscopy (NIRS) technique (Alomar and Fuchslocher, 1998), calibrated with the LECO/DUMAS technique (Ocampo, 2015, $p < 0.01$) at the Integral Analysis Laboratory of INIA Tacuarembó. For NIRS calibration, a subset of samples from the batch was selected, maintaining a proportion of different nitrogen treatments. Initially, these samples were analyzed using the LECO technique, followed by analysis with NIRS. If the correlation coefficient between both methods exceeded 95%, all samples were subsequently analyzed using NIRS.

2.3.3.3. Grain yield and harvest index

Rice grain yield was estimated through manual cutting and a stationary grain thresher or using a self-propelled harvester. The effective harvest area, after a 1 m border overflow from each plot, ranged from 15.3 to 44 m^2 depending on the experiment. Grain was weighted with a field scale to obtain wet yield (kg ha^{-1}) and subsequently cleaned and dried to 13% moisture content to determine dry and clean yield.

Additionally, two rice biomass subsamples of 0.3 m linear per plot were collected to estimate the harvest index (HI) for each cultivar and treatment. This index was calculated as:

$$HI = \text{DM } grain \, / \, (\text{DM } grain + \text{DM straw}) \quad (2)$$

The straw accumulation at the end of the cycle was estimated by taking into account the grain yield (kg ha^{-1}) and the Harvest Index (HI).

2.3.4. Statistical analysis

Various types of analyses were conducted to study the rice plant response and for the construction of the Critical Nitrogen Dilution Curve (CNDC).

2.3.4.1. Plant response

The variables used to describe rice plant responses included biomass (Mg DM ha^{-1}), plant nitrogen content (%), grain yield (Mg ha^{-1}), and HI (%). Analysis of variance was performed using general linear models and mixed-effects models (GLMM).

A significance level of 5% with Fisher's LSD was established. In the model, treatments, years, and locations were defined as fixed effects, while blocks were defined as a random effect. Statistical analysis was carried out using the R statistical software (Garibaldi et al., 2019).

2.3.4.2. Construction of the critical nitrogen dilution curve (CNDC)

The statistical analysis for constructing the CNDC was divided in two steps. First, the critical nitrogen concentrations (cN) were determined at each crop stage point, and then the CNDC was constructed based on these values.

Determination of critical nitrogen concentration points

Locally weighted regression or smoothed models, based on the methodology introduced by Justes et al. (1994), were employed to ascertain the cN for each cultivar. While these models have been prevalent in other disciplines since their development in the 1990s, they represent a novel approach for determining cN in this context. Due to the absence of parametric statistical models that adequately fit data exhibiting an initial oblique behavior followed by a significant change in slope, smoothed models are particularly apt for capturing such trends (Cleveland and Loader, 1996).

Smoothed models serve to minimize and parameterize variance (Cleveland and Loader, 1996). Consequently, the critical point is established at the intersection of x (DM) and y (% N). Utilizing the R statistical software (Garibaldi et al., 2019), these models incorporate a manual slope change explorer, enabling the identification of the critical point using x and y coordinates.

The graphical representation of the model reveals two distinctive zones. The first is an oblique zone with a positive slope, indicating that an increase in plant nitrogen concentration results in a biomass accumulation response. The second zone, represented by a vertical line known as the 'non-response' zone, signifies instances where increases in plant nitrogen concentration do not correspond to observable responses in biomass production. The cN is determined within the model by transitioning the manual explorer from the oblique zone to the vertical zone, and this point is termed the local optimum. Consequently, this model not only confirms mean differences but also estimates the critical point, a procedure unattainable through classical analysis of variance.

Critical points were determined using experiments 1, 2, 3, 4, 7, 8, and 9 for the indica cultivar, and 11, 13, 14, and 15 for the japonica cultivar. Experiments 5, 6, 10, and 12 were excluded for this purpose as no critical points were identified (Table 2), but they were later employed for CNDC validation.

Construction of the critical nitrogen dilution curve (CNDC)

The construction of the CNDC involved power regression analysis, from which the model equation was derived. Subsequently, a statistical significance test was conducted on the model parameters at a significance level of 5%. The statistical software R (Garibaldi et al., 2019) was used with various statistical packages detailed in Appendix 3.

2.3.4.3. Comparison of the critical nitrogen dilution curves (CNDC) obtained for each cultivar

Both CNDCs were compared using an F-test for the comparison of variance between two samples and were graphically represented. In this statistical analysis, if F was greater than the critical F-value, the CNDCs were considered different from each other.

2.3.4.4. Comparison between the critical nitrogen dilution Curves (CNDC) of both cultivars and the nitrogen dilution curves for each rate.

The comparison between the CNDC of each cultivar and the nitrogen dilution curves for each treatment and cultivar was conducted. Once the nitrogen dilution curves for each treatment were established, an F-test for the comparison of variance between two samples was performed, and the results were graphically represented.

2.3.5. <u>Comparison with international reported CNDC</u>

The generated CNDC were evaluated with curves published internationally to assess the degree of similarity or difference between them. An F-test was used for the comparison of variances between two samples.

2.3.6. <u>Validation of critical nitrogen dilution curves</u>

2.3.6.1. Validation based on experimental data

The calculated Critical Nitrogen Dilution Curves (CNDC) for each cultivar were cross-validated independently using information from experiments not used in their calculation (Table 2). Analysis of variance was conducted on these experiments to define nitrogen-limiting (EXPL) and non-limiting (EXPNL) treatments for biomass accumulation according to Fisher's LSD, with a significance level of 5%. A nitrogen dilution curve was determined for the defined response groups, which were then compared with the CNDC of each cultivar.

2.3.6.2. Validation with nitrogen dilution curves of treatments exhibiting maximum yields

The same experiments mentioned in the previous section were used for the validation of the Critical Nitrogen Dilution Curves (CNDC). However, in this analysis, the focus was on the grain yield achieved instead of biomass accumulation. The critical dilution curve was compared with the curve defined between 15 DBPI and 45 DAPI for treatments with maximum yields.

Validation criteria

The validation criteria for both cultivars critical nitrogen dilution curves (CNDC) and the nitrogen dilution curves from the previously mentioned points were two-fold:

1.The relationship coefficients for each of the parameters 'a' and 'b' of the model equation were determined. Each coefficient is expressed as:

$$RCa=ax//ac \quad (3)$$

$$RCb=bx/bc \quad (4)$$

Where RCa and RCb represent the relationship coefficients for parameters 'a' and 'b,' respectively; ac and bc are the critical curve parameters, and ax and bx are the parameters of the curve used for validation.

If RCa and RCb=1, it was assumed that the models were equal.

2. F-test between variances of two samples, with a significance level set at 5%.

2.3.7. Determination of the nitrogen nutrition index

The Nitrogen Nutrition Index (NNI) was calculated based on the Critical Nitrogen Dilution Curve (CNDC) and was used to quantify the intensity of nutritional deficiency or sufficiency of the crop relative to the optimal level of nitrogen concentration in the plant at a specific moment in the cycle.

The equation used was proposed by Lemaire and Maynard (1997):

$$NNI=\mathrm{a}N/cN \quad (5)$$

Where aN represents the actual nitrogen plant concentration, and cN is the critical nitrogen concentration for a given crop stage. Subsequently, after obtaining the NNI for each crop stage and for each N rate treatment, it was related to the relative grain yield (RY) (the relationship between the treatments with maximum yield and the reference treatment). The latter corresponded to the average relative yield for all years and each cultivar. The criteria for selecting the best relationships were the mean square error and the coefficient of determination (R^2).

2.4. RESULTS

2.4.1. Grain yield and harvest index

Significant differences in grain yield were observed among experiments, treatments, and locations for both cultivars. The average yield for indica and japonica cultivars was 11 Mg ha^{-1} (7 to 13.07 Mg ha^{-1}) and 10 Mg ha^{-1} (7.66 to 11.84 Mg ha^{-1}), respectively. The highest productivity was observed in the 2018-2019 season, with experiment 7 in the North yielding 13.1 Mg ha^{-1} and experiment 8 in the Central region reaching 12.9 Mg ha^{-1}.

The highest yields for japonica cultivar were observed in experiments 13, 11, and 15 (11.8, 11.3, and 11.3 Mg ha^{-1}, respectively), all located in the East site during the 2017-2018, 2016-2017, and 2018-2019 cropping seasons, respectively. Across experiments, there were no observed increases in rice yield with nitrogen rates exceeding 75 kg N ha^{-1} for indica and 50 kg N ha^{-1} for japonica.

The rice harvest index (HI) in both cultivars exhibited a consistent trend over the three seasons and locations as the total nitrogen rate increased, explaining 71% and 52% of the variability for indica and japonica, respectively (Figure 1).

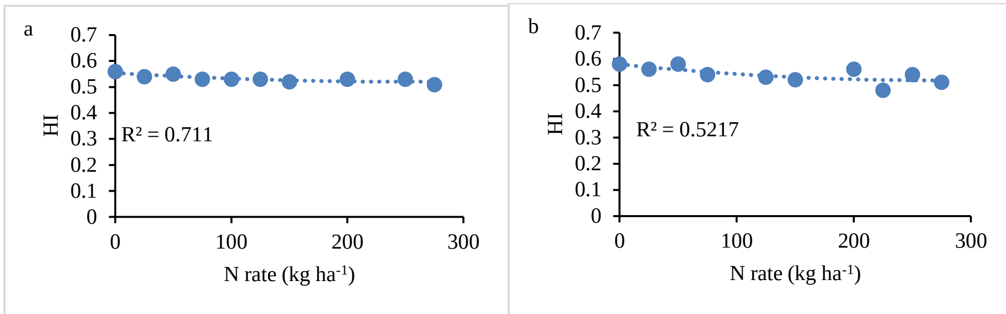


Figure 1. Effect of total N fertilization rate (kg ha^{-1}) between tillering and panicle initiation on the harvest index (HI) in indica (a) and japonica (b) rice cultivars in a multilocation and 3-year experimental network in Uruguay.

2.4.2. Critical nitrogen dilution curves

The smoothed model significantly fitted 12 regressions for indica and 5 for japonica, meeting the conditions established by Justes et al. (1994).

The Critical Nitrogen Dilution Curve (CNDC) showed statistical significance in their parameters for both indica and japonica cultivars. It was determined that both CNDCs were different from each other (F 0.54 > Critical F 0.30). Parameters "a" and "b" in indica were lower than in japonica (coefficient of relation for parameters a and b were 0.59 and 0.63, respectively).

The model became significant from biomass accumulation greater than 1 Mg ha^{-1} for indica, while in japonica, it did so from 2 Mg ha^{-1} onwards (Tables 2 and 3, Figure 2).

Table 2. Characterization of the parameters of the Critical Nitrogen Dilution Curves (CNDCs,) obtained from a three-year experimental database and three locations by combining N rate treatments (kg N^{-1}) applied in tillering and panicle initiation, for the indica cultivar and the japonica cultivar.

Cultivars	Ecuation	Minimum biomass (Mg DM ha^{-1})	Maximum biomass (Mg DM ha^{-1})	Maximum N (%)	Minimum N (%)	Criticals N
Inidca cultivar	$cN = 3,78*DM^{-0,435}$	1,00	18,28	3,66	0,93	12
Japonica cultivar	$cN = 6,44*DM^{-0,690}$	2,00	18,15	3,97	0,93	5

Note: cN: Critical N; DM = dry matter.

Table 3. Characterization of the significance of the parameters of the Critical Nitrogen Dilution Curves (CNDCs,) obtained from a three-year experimental database and three locations by combining N rate treatments (kg N^{-1}) applied in tillering and panicle initiation, for the indica cultivar and the japonica cultivar.

Cultivar	Parameter *a*	Parameter *b*	Probability
Indica cultivar	3,78	-0,435	4,32e-^{06} (a); 1,83e-^{04} (b)
Japonica cultivar	6,44	-0,690	4,79e^{-03} (a); 5,55e^{-03} (b)

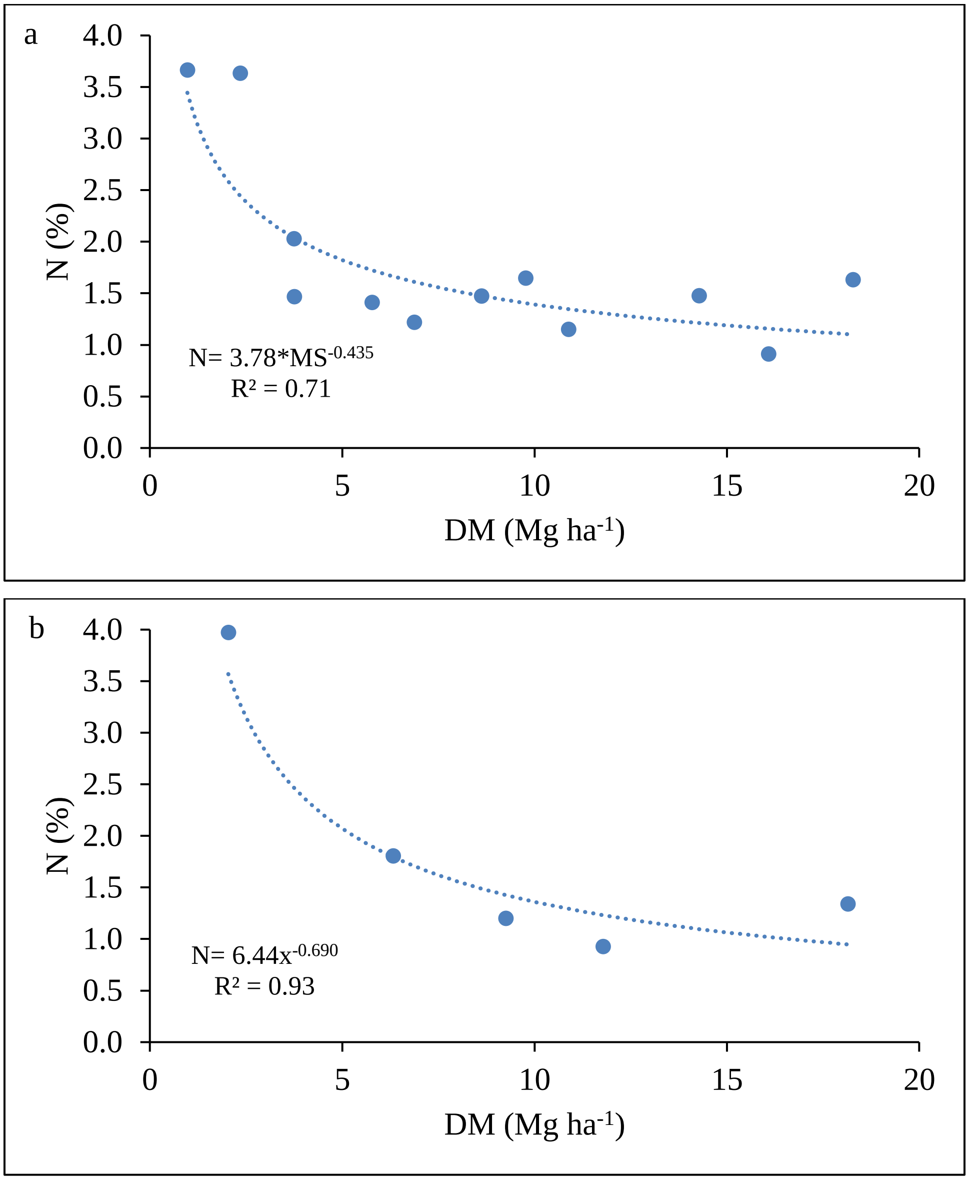


Figure 2. Critical nitrogen dilution curves (CNDCs,) obtained from a three-year experimental database and three locations by combining N rate treatments (kg N^{-1}) applied in tillering and panicle initiation, for the indica cultivar (a) and the japonica cultivar (b).

2.4.3. Comparison between critical nitrogen dilution curves (CNDC) of both cultivars and nitrogen dilution curves for each rate

The CNDC model in indica exhibited differences with the dilution model of the curves for treatments ranging from 0 to 50 N and from 225 to 275 N; but no differences were found with N rates from 75 to 200 kg ha^{-1} (Figure 3a).

However, in japonica, the behavior of the CNDC in relation to the curves of different N treatments was more variable. It did not show differences with doses of 75, 125, 150, 200, 225, and 275 N, but it differed from doses of 0, 25, 50, 100, and 250 N (Figure 3b).

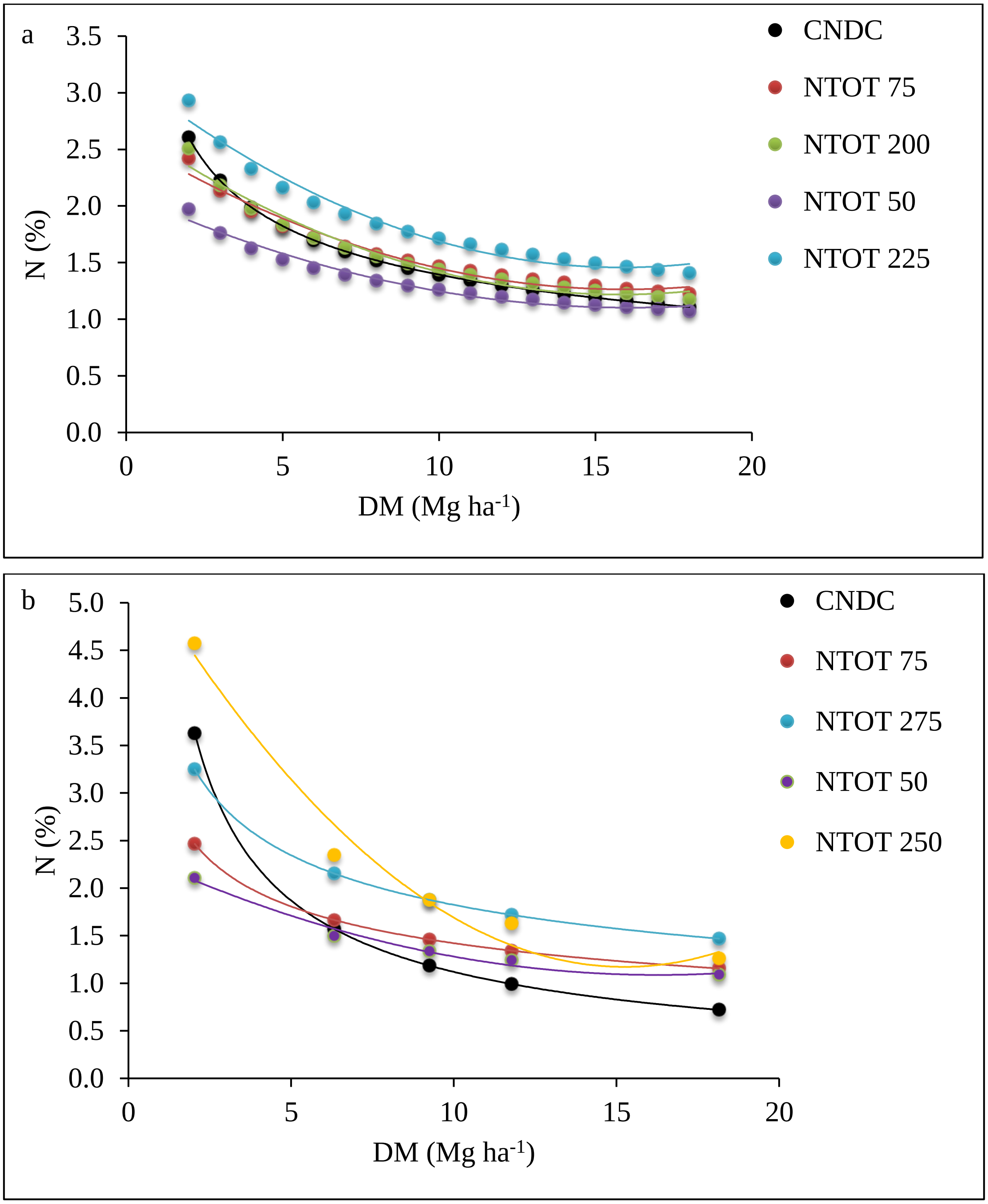


Figure 3. Comparison of the overall critical nitrogen dilution curve (CNDCs) with the dilution curves generated by different rates of nitrogen (N) applied from 15 days before and up to 45 days after panicle initiation in the two rice cultivars indica and japonica for three years in three locations.

2.4.4. Comparison with internationally reported critical curves

The CNDC for indica remained below the internationally reported curves for similar subspecies, whether in tropical or subtropical climates. However, it exhibited a similar trend to those curves after reaching 8 Mg ha^{-1} of dry biomass accumulation (Figure 4a).

On the other hand, the CNDC for japonica was above the curves for other japonica subspecies up to 5 Mg ha^{-1} for both temperate and subtropical climates (Figure 4b). However, between 5 and 8 Mg ha^{-1} of dry biomass, the japonica cultivar was below the curve proposed by Ata-Ul-Karim et al. (2013) for subtropical conditions but above the two curves for temperate climate. Above 8 Mg ha^{-1} of biomass accumulation, its behavior was like the Sheehy tropical curve but below the other curves.

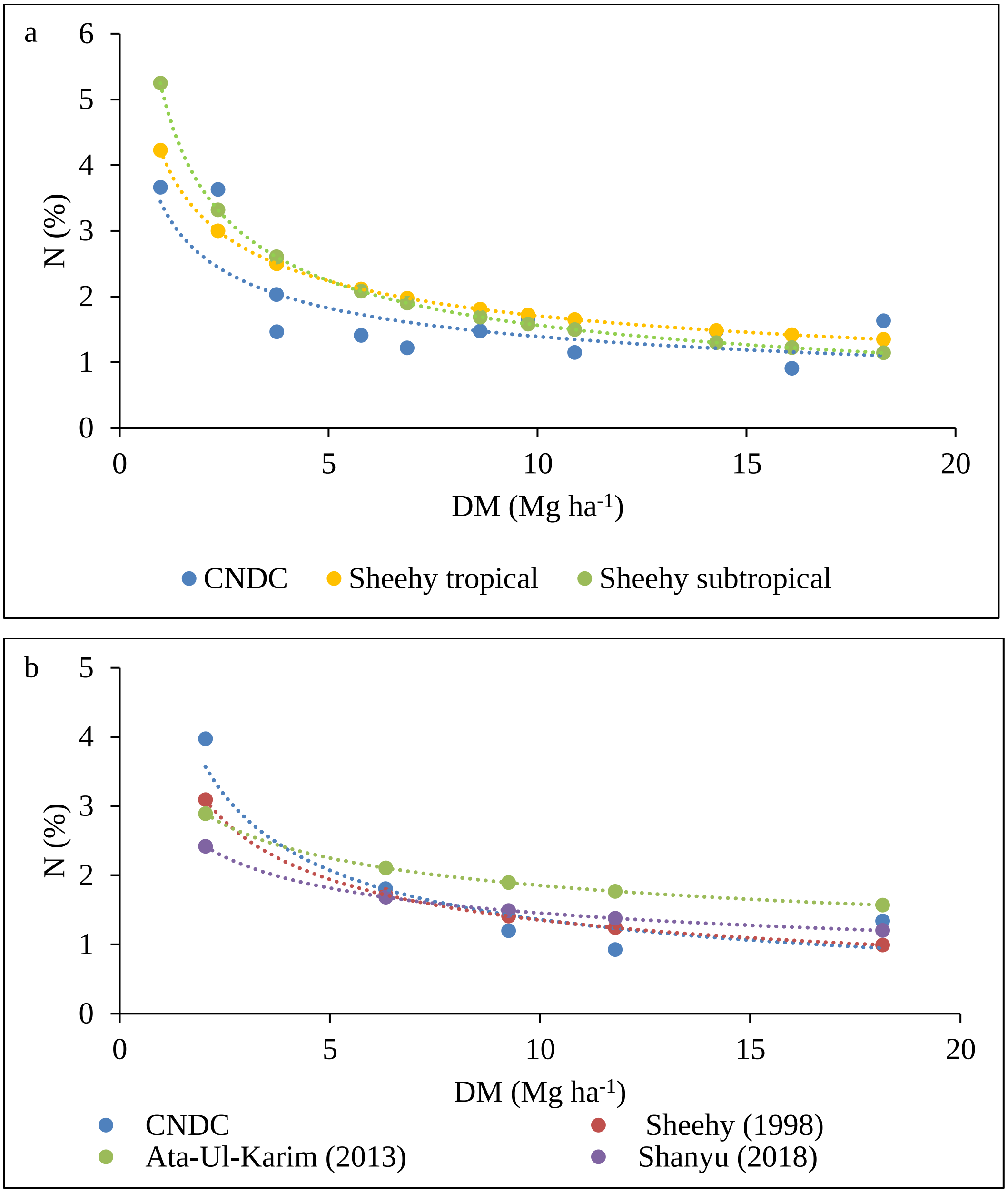


Figure 4. Comparison of the locally generated critical nitrogen dilution curve (CNDC) with international curves for indica cultivars (a) versus Sheehy et al. (1998) in tropical and subtropical climates, and for japonica cultivars (b) versus Sheehy et al. (1998), Ata-Ul-Karim et al. (2013) and Shanyu et al. (2018).

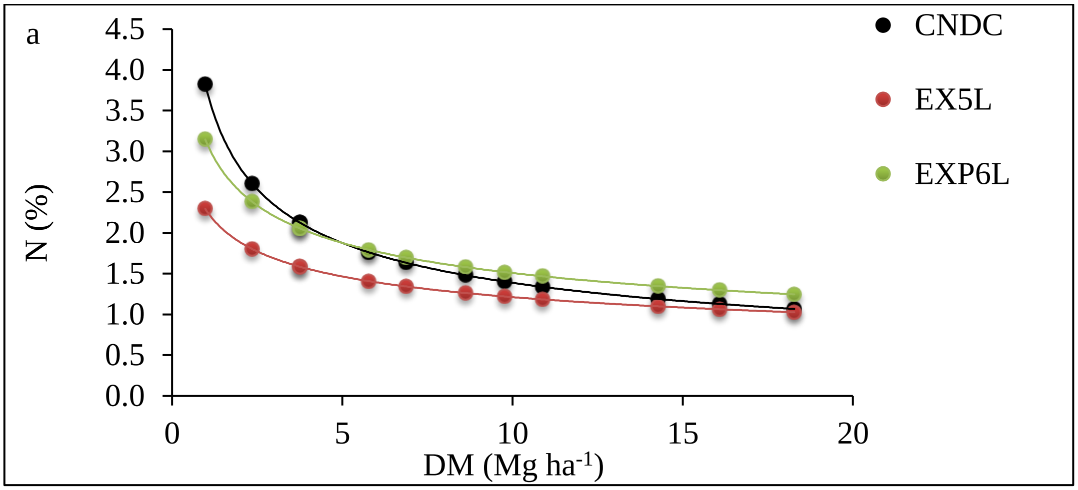
2.4.5. Validation of critical nitrogen dilution curves

2.4.5.1. Validation based on experimental data

The nitrogen dilution curve obtained in indica from the experiment 5 under nitrogen-limiting conditions (EXP5L) behaved differently from the CNDC (F = 4.6; Critical F-value = 2.8; RCa = 1.7; RCb = 1.6), positioning itself below in a region of nutritional deficiency. As expected, no differences were observed in experiments 5 and 6 with non-limiting nitrogen concentration conditions (EXP5NL and EXP6NL) (Figure 5a and b, Table 4).

The maximum biomass accumulation in EXP6L was achieved with the 50 kg N ha^{-1} dose, likely attributed to a high soil nitrogen supply capacity. This factor resulted in the absence of detectable differences between the CNDC and this experiment.

For the japonica cultivar, the preliminary validation was discarded because the experiments intended for this purpose had low grain yields due to issues in their agronomic management.



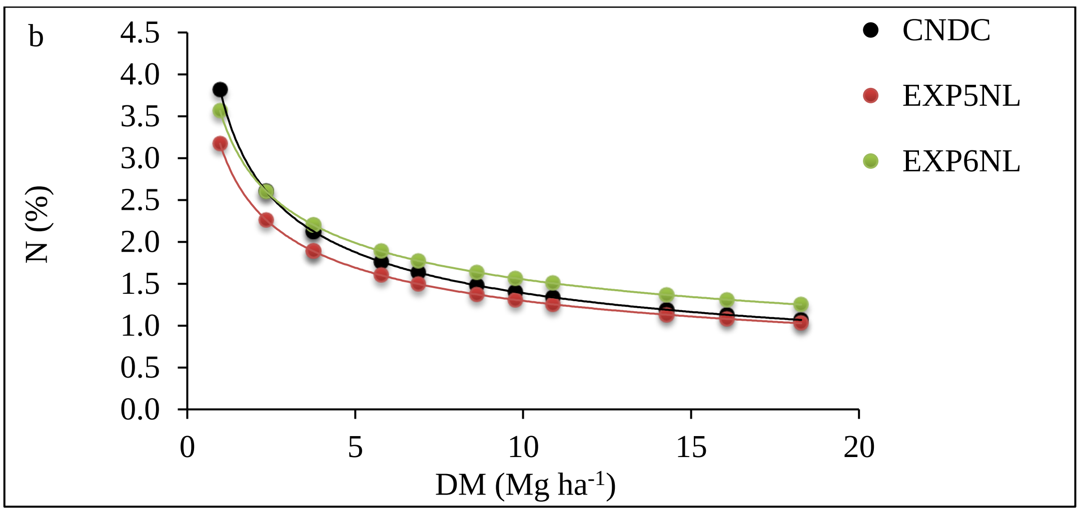


Figure 5. Critical nitrogen dilution curves (CNDC) in indica cultivar from experimental dry matter (DM) and nitrogen (N) dataset during vegetative stage, compared with (a) limiting nutritional condition of N treatments from experiments 5 (EXP5L) and 6 (EXP6L), and (b) non-limiting nutritional condition of N treatments from experiments 5 (EXP5NL) and 6 (EXP6NL).

Table 4. Adjusted relationship coefficients for parameters a and b of the models used for the validation of the critical nitrogen dilution curve (CNDC) in the indica cultivar.

MODEL	a	b	Coefficient of relationship *a*	Coefficient of relationship *b*
CNDC	3,78	0,44		
EXP5L	2,28	0,27	1,7	1,6
EXP6L	3,12	0,32	1,2	1,4
EXP5NL	3,14	0,39	1,2	1,1
EXP6NL	3,53	0,36	1,1	1,2

Note: EXP5L and EXP6L: N dilution curves from the N rate treatment group of experiment 5 and 6 limiting nutritional condition; EXP5NL and EXP6NL: N dilution curves from the N rate treatment group of experiment 5 and 6 non-limiting nutritional condition.

2.4.5.2. Validation with nitrogen dilution curves of treatments exhibiting maximum yields

The dilution curve for maximum yields did not show differences from the CNDC developed for indica. The nitrogen concentration required for maximum biomass accumulation was the same as that needed for maximum grain yield (Figure 6, Table 5).

For the case of japonica, it was decided to discard the preliminary validation for the same reason as presented for the validation based on limiting and non-limiting treatments for nitrogen concentration and maximum biomass accumulation during the vegetative stage.

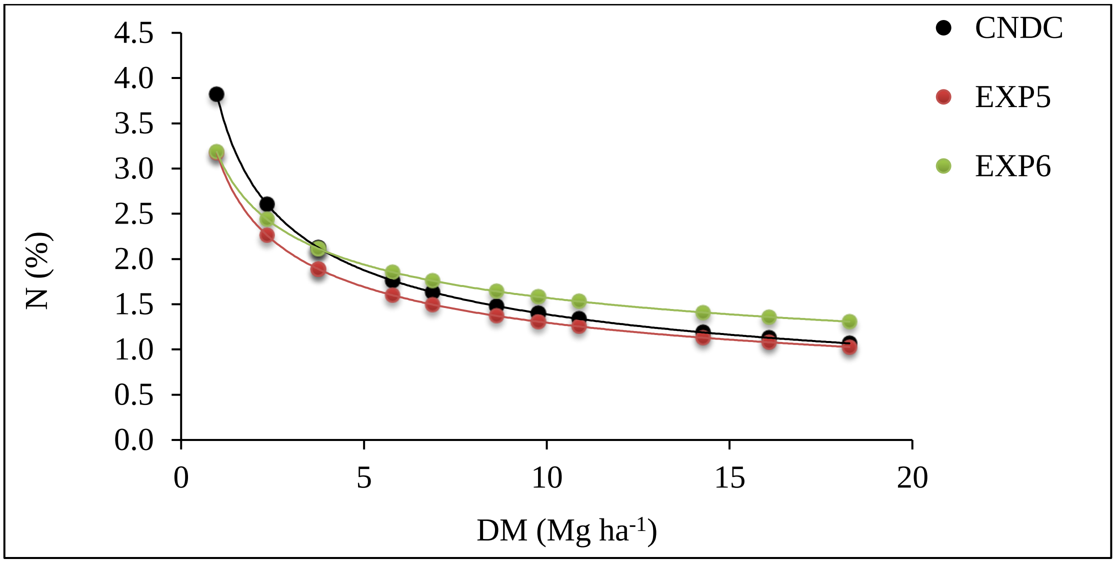


Figure 6. Comparison of the critical nitrogen dilution curve (CNDC) of the indica cultivar with the N dilution curves of nitrogen (N) treatments that obtained maximum grain yields in experiments 5 (EXP5) and 6 (EXP6).

Table 5. Relationship coefficients of parameters a and b of models used for the validation of the critical nitrogen dilution curve (CNDC) in the indica cultivar, from the N dilution curves of the N treatments that presented the maximum grain yields in experiments 5 (EXP5) and 6 (EXP6).

Model	a	b	Coefficient of relationship *a*	Coefficient of relationship *b*
Exp5	3,14	0,39	1,2	1,1
Exp6	3,16	0,30	1,2	1,4
CNDC	3,78	0,44		

2.4.6. Nitrogen nutrition index and relative yield

The relationship between Relative Yield (RY) and Nitrogen Nutrition Index (NNI) for indica was fitted using a second-degree polynomial equation. The maximum of each equation was reached when the relative yield was about 95%. The maximum RY for the three years was achieved with an NNI close to 1 at each sampling time (Figure 7, Table 6).

For the japonica cultivar, there was not a good fit in the relationship between NNI and RY at all sampling times. Only at 15 DBPI, 30 DAPI, and 45 DAPI were the moments that showed a statistically significant fit. At these moments, the maximum RY was achieved with an NNI of 1.1. These maximum RY values were 102%, 90%, and 79% for 15 DBPI, 30 DAPI, and 45 DAPI, respectively (Figure 8, Table 7).

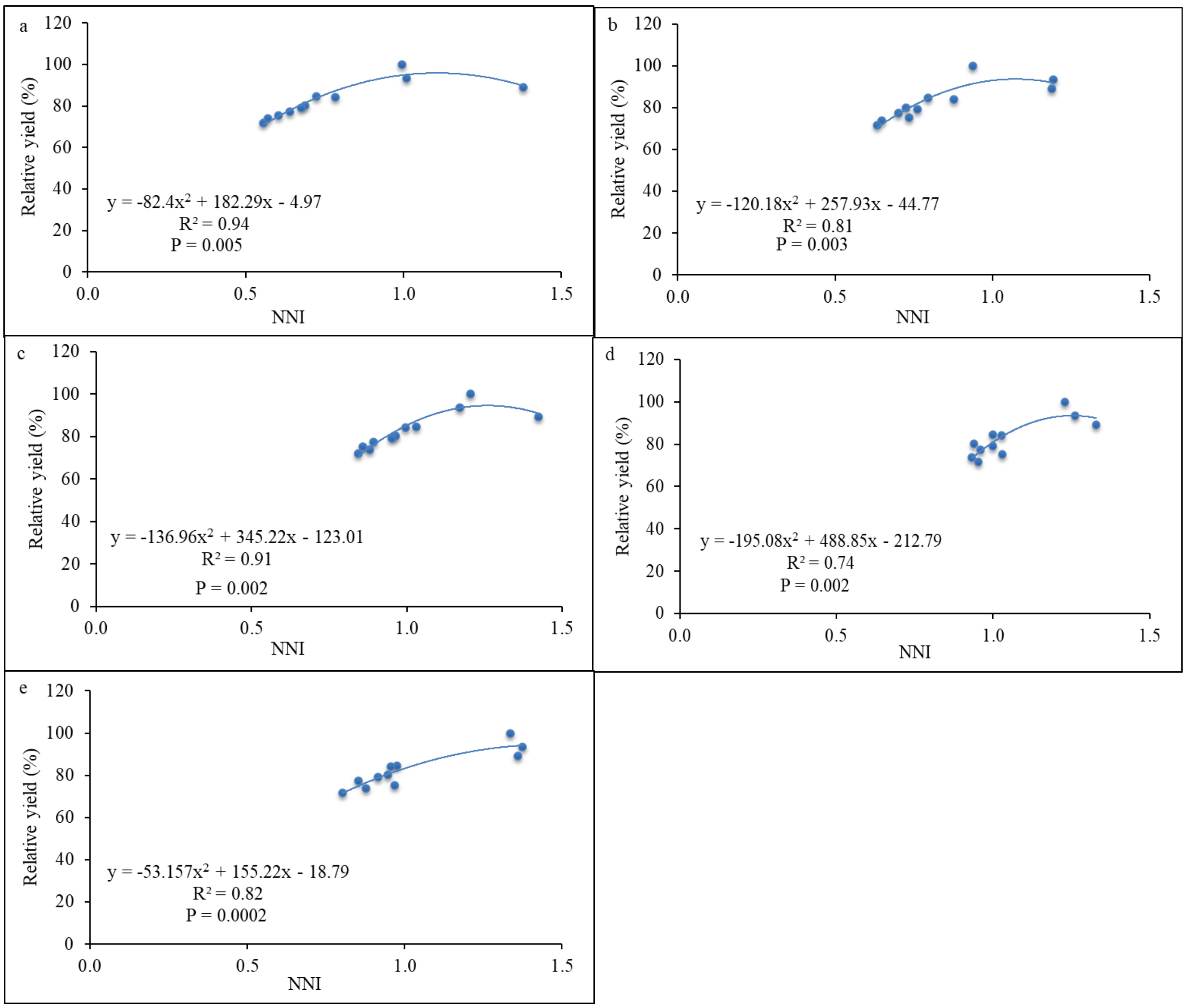


Figure 7. Regression between the nitrogen nutrition index (NNI) and the relative yield (RY) in the indica cultivar for the stage a) 15 days before panicle initiation (15DBPI), b) panicle initiation, c) 15 days after panicle initiation (15DAPI), d) 30 days after panicle initiation (30DAPI) and e) 45 days after panicle initiation (45DAPI).

Table 6. Characterization of each regression model between the nitrogen nutrition index (NNI) and the relative yield (RY) in grain for the indica cultivar, at the moments 15 days before Panicle initiation (15DBPI, a), Panicle initiation (b), 15 days after Panicle initiation (15DAPI, c), 30 days after Panicle initiation (30DAPI, d) and 45 days after Panicle initiation (45DAPI, e).

MODEL	Relative Yield	Nitrogen Nutrition Index	Sampling times	N uptake range (kg DM/ha^{-1})	DM range (Mg DM/ha^{-1})	Probability (P)
a	96	1,1	15DBP1	27 -32	1,1-1,3	0,005
b	94	1,1	Panicle initiation	47-52	2,5-2,7	0,003
c	95	1,3	15DAPI	91-99	5,2-5,6	0,002
d	94	1,2	30DAPI	131-143	9,4-10,1	0,002
e	95	1,4	45DAPI	147-162	12,6-13,4	0,0002

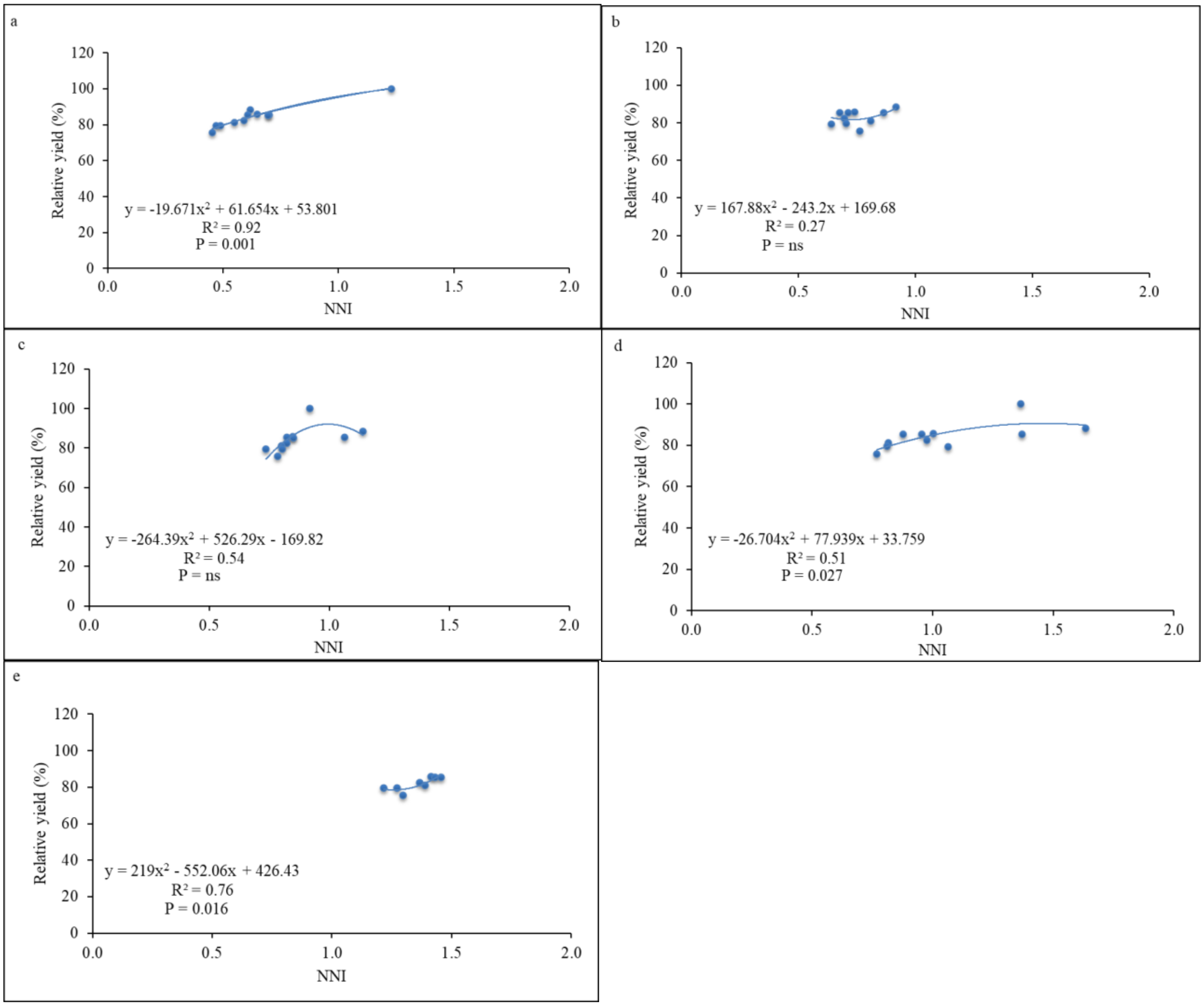


Figure 8. Regression between the nitrogen nutrition index (NNI) and the relative yield (RY) in the japonica cultivar for the stage a) 15 days before panicle initiation (15DBPI), b) panicle initiation, c) 15 days after panicle initiation (15DAP), d) 30 days after panicle initiation (30DAP) and e) 45 days after panicle initiation (45DAP).

Table 7. Characterization of each regression model between the nitrogen nutrition index (NNI) and the relative yield (RY) in grain for the japonica cultivar, at the moments 15 days before Panicle initiation (15DBPI, a), Panicle initiation (b), 15 days after Panicle initiation (15DAPI, c), 30 days after Panicle initiation (30DAPI, d) and 45 days after Panicle initiation (45DAPI, e).

MODEL	Relative Yield	Nitrogen Nutrition Index	Sampling times	N uptake range (kg DM/ha^{-1})	DM range (Mg DM/ha^{-1})	Probability (P)
a	102	1	15DBPI	42-47	1,7-1,9	<0,001
b	82	1,1	Panicle initiation	71-77	2,1-4,8	ns
c	92	1,1	15DAPI	92-98	6,6-7,1	ns
d	90	1,1	30DAPI	114-129	9,0-9,7	0,027
e	79	1,1	45DAPI	190-209	13-15	0,016

2.5. DISCUSSION

2.5.1. Critical nitrogen dilution curve

The critical nitrogen dilution curve (CNDC) was first proposed in 1952 (Ulrich, 1952). Until now, most publications have focused on the development of nitrogen dilution curves considering aboveground biomass (Justes et al., 1994; Lemaire et al., 1984; Lemaire et al., 2007; Plénet & Lemaire, 2000; Zhang et al., 2021), while only a few studies have focused on cash crop grain yield, such as beet, barley, and rice (Chakwizira et al., 2016; Zhao et al., 2016; Ata-Ul-Karim et al., 2017; Ata-Ul-Karim et al., 2016; He et al., 2017; Sheehy et al., 1998). Additionally, some of these studies assessed rice crops, but all focused on transplanted rice, without studies recorded on high-yielding (>10 Mg ha^{-1}) direct-seeded rice integrated into a pasture rotation for direct livestock grazing. It is also known that high-yielding systems frequently rely on large amounts of nitrogen applied as fertilizer, making the achievement of an adequate nitrogen use efficiency (NUE) highly relevant. In such cases, the availability of easy-to-use tools could be useful. The CNDC could be an example of such a tool, combining both productive and efficiency objectives. It is known that the indica and japonica subspecies vary in several physiological characteristics (e.g., temperature requirements, photosynthesis, respiration rates, nitrogen efficiency), which explain their ecological adaptation to tropical and subtropical regions (Ata-Ul-Karim et al., 2013; Islam et al., 1996). In this study, differences were found in the CNDC between subspecies across the crop cycle, showing greater values for the CNDC of the japonica subspecies than for indica below 10 Mg ha^{-1} of aboveground dry matter. The opposite trend was reported by Makowski et al. (2020), showing that the indica subspecies demonstrated a higher CNDC compared to japonica, particularly at lower biomass levels. These findings could be attributed to the superior performance of the indica subspecies under subtropical conditions in their study compared to the local temperate climate. Other authors have affirmed the importance of genotype, management, and environmental differences (Lemaire et al., 2007). According to Justes et al. (1994), dilution curves vary both between and within species. Greenwood et al. (1990) posit that variations may occur between experimental sites for the same species and even among growth stages when there is overlap. The CNDC generated in this study exhibited disparities with other internationally generated curves, highlighting the considerable variability attributed to various interacting factors (Ata-Ul-Karim et al., 2013; Huang et al., 2018; Sheehy et al., 1998).

2.5.2. Critical nitrogen dilution curves validation

In well-managed crops and for a specific cultivar, a close relationship between maximizing vegetative dry matter and grain yield was found when nitrogen nutrition was based on the CNDC approach (Hay, 1995). In present study, were found the same relationship due to the stable HI of the selected varieties. Although this may seem basic, that relationship does not hold in ancient rice varieties where maximizing vegetative dry matter does not ensure an increase in grain yield due to a preferred dry matter partitioning towards the vegetative biomass component (Sinclair, 1998), also linked to other crop issues such as plant lodging and pest incidence. However, genetic improvement in the last decades has developed rice varieties that can achieve greater total biomass and higher HI, which are directly linked to crop grain yield (Meng et al., 2022), as well as pest tolerance or resistance (Houi, 2018). The latter could partly explain why the adjusted CNDCs in this study showed a lower shape compared to previous international reports that included older rice varieties. Despite that lower shape, when both CNDCs were compared to the dilution curves of maximum grain yield treatments, no differences were found, indicating that the adequate plant nitrogen concentration for maximum vegetative biomass was also the same as for maximum grain yield. During the validation stage and for the indica subspecies, the CNDC could also discriminate between limiting and non-limiting treatments except for one situation where the soil's native nitrogen contribution ensured the maximum dry matter accumulation with very low applied nitrogen. But despite the apparent sensitivity of the CNDC shown thus far, how reliable could it be when exploring maximum yields? The answer to this question arises when linking the NNI to the achieved RY. As in several cash crops (Ata-Ul-Karim et al., 2017; Chakwizira et al., 2016; Zhao et al., 2016), for the indica subspecies and for all the defined stages during the crop cycle, both variables were correlated, reaching RY values higher than 90% with NNIs around 1. A similar trend was found for the japonica subspecies, which achieved a good agreement between both variables for 60% of the selected crop stages. For rice, Ata-Ul-Karim et al. (2016) found that the highest correlation between NNI and RY was at panicle initiation and 30 DAPI, which was similar to present results, ranging from 15 DBPI to 45 DAPI for the indica subspecies, while for the japonica subspecies it was at 15 DBPI, 30 DAPI, and 45 DAPI. Based on these results, it seems that the adjusted data could be a useful tool when defining nitrogen fertilization strategies, aiming at the crop's NNI and ensuring at least 90% of the maximum attainable yield. For example, one key crop stage for nitrogen fertilization is at panicle initiation, and based on this work data, an adequate NNI for the indica subspecies corresponds to an uptake of around 50 kg N ha^{-1}, like what was reported by Castillo et al. (2014) for that rice stage. For the japonica subspecies at panicle initiation, the nitrogen uptake corresponding to an adequate NNI was 30% higher than the reported threshold, which is likely because the entire database for that sufficiency level calculation at panicle initiation (56 kg N ha^{-1} uptaken) was primarily composed of indica subspecies varieties. In addition to the previously mentioned good agreement, nitrogen fertilization based on CNDC could provide flexibility when a specific crop stage is missed and the nitrogen concentration for each dry matter unit is known. Despite the good results for the indica subspecies, were acknowledge the limitations of this study for the japonica subspecies. Firstly, the available database for the latter was limited compared to indica, resulting in fewer CNDCs for japonica compared to indica subspecies (5 vs. 12). Secondly, were lacked a consistent set of independent experiments to conduct the validations. Nevertheless, the good match between the CNDC and grain yield in both subspecies seems clear, indicating that developing models for nitrogen fertilization recommendations could be the next step. Although the NNI is considered adequate for quantifying the crop's nitrogen nutrition state, its determination is time-consuming because it involves direct biomass measurement to account for dry matter and nitrogen concentration for calculations (Lemaire et al., 2008). Several indirect and non-destructive alternatives have been proposed to address this issue. For corn, some studies showed a good match between the NNI and readings with a SPAD chlorophyll meter (Ziadi et al., 2008). For rice, Cao et al. (2015) and Foster et al. (2017) used a handheld NDVI device, while RGB or hyperspectral images were employed with the same goal (Shi et al., 2021; Yu et al., 2023). The next step toward assessing the crop's nitrogen status throughout the crop cycle should involve quick and real-time measurements and devices to achieve precision nutrition at the right time, which will be addressed in the next chapter of this thesis.

2.6. CONCLUSIONS

The CNDCs for the indica and japonica rice cultivars (INIA Merin and Parao, respectively), were determined from a set of experiments spanning several years and sites. These results confirm the work hypothesis, since the cN was obtained for the maximum accumulation of DM in a large part of its production cycle. Due to the variability observed in the comparison with the international curves and the differences between the two cultivars, the importance of adjusting CNDC for each subspecies and region is demonstrated, which would allow to manage more accurately the crop nutritional status.

The second objective of the study was achieved by determining the NNI for each moment of assessment in both cultivars. In the case of indica, validation based on both DM and grain yield made it possible to differentiate suboptimal from optimal or supra-optimal nutrition situations. More than 90% of the maximum relative yields were achieved with an NNI about 1 at each moment in the crop cycle, so the optimal NNI for maximum DM was also optimal for maximum grain yield. In japonica, the limitation of a narrow database meant that fewer cN were determined. For this reason, the magnitude of the results for this cultivar was less robust than in indica. The relevance of having a vast database for the generation of CNDCs is evident.

Once the CNDCs have been generated and their direct relationship with grain yields has been demonstrated, it is necessary to advance in the generation of the association between this tool and models that allow to adjust N fertilization recommendations in an accurate, practical, and real-time system.

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2.8. APPENDIXES

Appendix 1. Description of the experiments used during the three years, according to cultivar used, location, treatments, N dose, sowing date and experimental objective.

Experiment and year	Cultivar	Location	Treatments (n.º)	Total N doses (kg ha^{-1})	Sowing date	PMN (mg kg^{-1})	Experiment objective
1 (2016-2017)	INIA Merín	Artigas	16	0-200	04/10/2016	32	OCU
2 (2016-2017)	INIA Merín	Tacuarembó	16	0-200	04/10/2016	10	OCU
3 (2016-2017)	INIA Merín	Treinta y Tres	16	0-200	18/10/2016	24	OCU
4 (2017-2018)	INIA Merín	Artigas	16	0-200	06/10/2017	19	OCU
5 (2017-2018)	INIA Merín	Tacuarembó	16	0-200	16/10/2017	16	VAL
6 (2017-2018)	INIA Merín	Treinta y Tres	16	0-200	11/10/2017	11	VAL
7 (2018-2019)	INIA Merín	Artigas	5	0-275	08/10/2018	12	OCU
8 (2018-2019)	INIA Merín	Tacuarembó	5	0-275	16/10/2018	7	OCU
9 (2018-2019)	INIA Merín	Treinta y Tres	5	0-275	23/10/2018	22	OCU
10 (2016-2017)	Parao	Tacuarembó	16	0-200	04/10/2016	10	VAL
11 (2016-2017)	Parao	Treinta y Tres	16	0-200	18/10/2020	24	OCU
12 (2017-2018)	Parao	Tacuarembó	16	0-200	16/10/2017	16	VAL
13 (2017-2018)	Parao	Treinta y Tres	16	0-200	11/10/2017	11	OCU
14 (2018-2019)	Parao	Tacuarembó	5	0-275	16/10/2018	7	OCU
15 (2018-2019)	Parao	Treinta y Tres	5	0-275	23/10/2018	22	OCU

Appendix 2. Description of nitrogen (N) treatments, N fractionation at tillering and panicle initiation and total N rates (kg N ha^{-1}) of the treatments evaluated from harvests 2016-2017 to 2018-2019.

Treatments	Tillering rate (kg N ha^{-1})	Panicle initiation rate (kg N ha^{-1})	Total rate (kg N ha^{-1})
Treatments harvest 2016-2018*			
1	0	0	0
2	25	0	25
3	50	0	50
4	100	0	100
5	0	25	25
6	25	25	50
7	50	25	75
8	100	25	125
9	0	50	50
10	25	50	75
11	50	50	100
12	100	50	150
13	0	100	100
14	25	100	125
15	50	100	150
16	100	100	200
Treatments harvest 2018-2019			
1	0	0	0
2	50	25	75
3	125	100	225
4	150	100	250
5	150	125	275

*Note: *harvest 2016-2018 includes harvest 2016-2017 y 2017-2018.*

Appendix 3. The statistical software used was R (Garibaldi et al., 2019) with the following packages:

GGREPEL, LATTICE, NLME, GGPLOT2, MINPACK.LM, DPLYR, AGRICOLAE, DATA.TABLE, FUNMODELING, EMMEANS, TIBBLE, PLOTLY, GAPMINDER, MBLM y BROOM.

3. AGRONOMIC USE OF THE NITROGEN CRITICAL DILUTION CURVE FOR RICE IN URUGUAY

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3.1. SUMMARY

Nitrogen fertilization of rice (*Oryza sativa*) based on objective indicators allows to optimize productivity, efficiency of this nutrient and minimizing environmental externalities. Soil nitrogen mineralization potential (NMP) is a robust indicator to define the nitrogen (N) requirements of the crop at the beginning of tillering (V4). However, plant N uptake, a parameter adjusted to define fertilization at the beginning of the reproductive stage (R0), is impractical to apply. Critical N dilution curves (CNDC) would allow a more flexible adjustment of N requirements (NR) during cultivation. In this work, based on CNDC obtained in two local rice varieties, one japonica subspecies and the other indica, at different growth stages, relationships were established between NR and the nitrogen nutrition index (NNI), as well as between the normalized difference vegetation index (NDVI), the NNI and the agronomic variables, dry matter (DM), plant N uptake (PNU) and plant N concentration. The determinations were made at five points in time, from 15 days before panicle initiation (15DBPI) to 45 days after panicle initiation (45DAPI) with intervals of approximately 15 days. At the indica subspecies the R^2 between NR-NNI was 0.65, 0.95, 0.99 and 0.99, while at the japonica it was 0.84, 0.88, 0.98 and 0.99 at 15DBPI, panicle initiation (PI), 15DAPI and 30DAPI, respectively. N requirements were calculated locally, and nitrogen fertilization recommendation equations were established based on the nutritional status of the crop as reflected by the NNI at a given time. Significant adjustments between NDVI and the studied parameters were obtained to estimate in real time the nutritional status of the crop. These results would allow the implementation of CNDC as an objective tool for real-time decision making in production systems.

Keywords: fertilization, nitrogen nutrition index, NDVI, N uptake.

3.2. INTRODUCTION

In rice and other cereals, N is demanded throughout the growing season, but the greatest requirement occurs between the onset of tillering (V4) and panicle initiation (PI) (Dobermann and Fairhurst, 2005). Nitrogen is an essential nutrient that promotes rapid growth (increase in plant size and tillering) and increases leaf size, number of spikelets per panicle, percentage of filled spikelets and protein content in the grain (Dobermann and Fairhurst, 2005). High productivities are generally associated with N additions at rates that sometimes equal or even exceed the total crop requirements (Jin et al., 2002).

In Uruguay, N fertilization of the rice crop has been gradually increased since 2005 to reach 80 kg N ha^{-1}, which, concurrently with other factors, explains the current productivity levels of 9.1 Mg ha^{-1} (MGAP-DIEA, 2022). In comparison, in other rice systems globally, between 120 and 180 kg N ha^{-1} are added (Giller et al., 2004) to obtain yields ranging between 5-6 Mg ha^{-1}. In China, fertilizer use is even higher with N ha^{-1} rates ranging from 193 to 387 kg N ha^{-1} (Heffer, 2013). One of the reasons that explains this difference in productivity and N addition between the dominant systems in Uruguay and that of other regions, is the rotation of rice with pastures for direct livestock grazing. Such systems have significant N inputs from cattle feces and urine complementary to that of fertilizer, and a high recycling of this nutrient in the system (Castillo 2018, Castillo et al., 2021).

This relative increase in the use of N fertilizers can be associated with considerable externalities leading to potential environmental problems (Peng et al., 2006), affecting surface and subsurface waters and the atmosphere, which also leads to a decrease in N use efficiency and effectiveness (Ju et al., 2009; Liu and Diamond, 2005). Therefore, the correct assessment of N requirement during the crop cycle is fundamental for the precise management of N fertilization rates and timing.

Wang et al. (2006) mention that N concentration in rice crop at different growth stages indicates the ability of the soil to supply N, the ability of the plant to uptake it, as well as the interactions between the plant and soil N. Objective N management during the crop cycle requires the development of effective and practical, economically feasible and technically rigorous diagnostic tools (Ata-Ul-Karim et al., 2017). According to Mueller et al. (2012), precision farming techniques can annually save up to 11 million Mg of N worldwide without affecting crop yields.

Targeted management and supply of N fertilizer can improve crop productivity, with less adverse effects of N fertilizer on the environment (Gastal and Lemaire, 2002). Costa et al. (2001) stated that in order to make nitrogen fertilization recommendations and improve its use efficiency, an accurate assessment of N status in plants and soils of the production systems must be made.

N accumulation in the plant as a function of time presents a sigmoid curve that is initially low because the plant is just developing. Then, there is a stage of maximum N uptake corresponding to the period of active growth and, finally, the rate of uptake decreases at the end of the growth period (Perdomo and Barbazan, 2012). Several studies have indicated that N concentration decreases during the growth cycle for almost any plant (Greenwood et al., 1990), including legumes that obtain N via the atmospheric route (Lemaire et al., 1985).

It is from this knowledge that the study of critical N dilution curves (CNDC) arises. This concept, initially developed in the 1980s for N in forage grasses, has a solid theoretical framework (Briat et al., 2020) and has been applied in more than 30 herbaceous crop species by scientists from 40 countries (Chen et al., 2021).

The CNDC is formed by the critical N concentration or critical N (cN) that the crop presents throughout its productive cycle. The cN is the minimum plant N concentration necessary to achieve the maximum dry matter (DM) accumulation of the crop (Greenwood et al., 1986). Schröder et al. (2000) and Benincasa et al. (2011) state that generally this concentration is the same as the economically optimal one. Other authors studied the CNDC behavior of several species and claim that the variation in cN did not show differences among the same subspecies, such as japonica rice, indica rice or winter wheat (Justes et al., 1994; Ziadi et al., 2010).

On the other hand, Sheehy et al. (1998) generated a general curve for rice in the tropics using three cultivars and sites. However, Ata-Ul-Karim et al. (2013) state that the curves generated by Sheehy et al. (1998) are not appropriate for the japonica subspecies, so they generated a new curve for subtropical climates, specifically for japonica. Another curve generated for the japonica subspecies is that of Shanyu et al. (2018) in northeastern China under temperate climate conditions. In the present work, the curves proposed by Moreira et al. (n.d.), generated in temperate climate, for one cultivar of indica subspecies and another of japonica subspecies, were used as a basis. This evidence clearly shows the variability of CNDC at the international level and the different factors that determine them.

With the CNDC it is possible to know the optimum N content in the plant throughout its productive cycle, but not what N rate to apply in situations of nutritional deficiency at a given time. Taking into account that maximum DM accumulation in modern rice cultivars, with stable harvest indexes (HI), is associated with maximum grain yields, several authors (Ata-Ul-Karim et al., 2017; Wang et al., 2020) affirm the possibility of establishing relationships from the CNDC between NR, relative grain yield (RY) and NNI, in order to make fertilization recommendations throughout the crop cycle, both for japonica and indica subspecies.

NNI quantifies crop N sufficiency or deficiency, calculated as the ratio of actual plant N concentration (aN) to cN. It has also been used in diagnostic studies of crop nitrogen nutrition (Tremblay et al., 2011). An NNI < 1 quantitatively indicates the degree of N deficiency of the crop, the optimum level takes the value of 1 and excess N greater than 1. However, only with this index it is also not possible to adjust an N rate according to the nutritional situation of the crop.

The NR (kg N ha^{-1}) represents the N rate to be added to the crop to raise the nutritional constraint and move from a zone of DM production response to N addition, to an optimum zone where DM accumulation is maximal. NR considers cN and apparent N recovery efficiency (NRE, Ata-Ul-Karim et al., 2017; Wang et al., 2020). N use efficiency and NRE vary according to crop growth stages. The latter is a key plant characteristic for determining NR and improving N use efficiency by estimating crop nutritional status (Ata-

Ul-Karim et al., 2017).

To estimate in real time the N requirements, associations between NR and indices that quantify the nutritional status of the crop have been performed. Ata-Ul-Karim et al. (2017) found that there was a significant linear correlation between rice NR and NNI, with an R^2 above 0.98, and presented variations among different growth stages. Unlike NR, the calculation of NNI can be estimated using different tools or sensors. These allow for the qualification and quantification of the radiant energy flux.

The four processes (emission, uptake, reflectance and transmission of radiation) occur simultaneously and their relative intensities at different wavelengths of the electromagnetic spectrum characterize the chlorophyll, nitrogen or water contents in vegetation, to obtain spectral responses representing these contents (Sá et al., 1999). Reports made with a portable chlorophyll meter (Ziadi et al., 2008), some multispectral (Padilla et al., 2017) or hyperspectral (Chen, 2015) equipment are found in the literature. Therefore, if a robust relationship between rice NR and NNI were to be established, the former could be calculated directly using field-measured NNI.

Different types of instruments can be used for N nutrition diagnostics during the rice cycle. Some examples are the GreenSeeker sensor that estimate NDVI (Cao et al., 2015; Foster et al., 2017), portable chlorophyll (SPAD) meters, hyperspectral equipment, and commercial digital cameras (Ata-Ul-Karim et al., 2017; Li et al., 2014; Lin et al., 2010; Wang et al., 2014). These have been used to estimate aerial biomass, N concentration or other nutrition-related N indices (Feng et al., 2015; Zhang et al., 2021).

Although these indices can reflect rice N nutritional status to some extent, they cannot be used to directly recommend rates needed to raise N nutritional restrictions, as relationships between spectral indices and nitrogen nutrition indices are affected by cultivar, sampling stages, climatic conditions, and field management practices (Tremblay et al., 2011).

The NDVI (normalized difference vegetation index) or green index allows integrating and analyzing light spectrum measurements in the red and far-red zones, made with remote sensors or close to the plants, and identifying the presence of green and living vegetation based on their reflection in the frequency ranges of light corresponding to these wavelengths. There is a strong relationship between green index and some vegetation characteristics such as biomass, leaf area index (LAI) or productivity, among others (Sellers, 1985).

The equation that defines it is the following:

$$NDVI = \frac{(NIR - RED)}{(NIR + RED)} \quad (1)$$

Where RED (mmoles m^2 s) is the red radiation incident on the plant surface (570-680nm) and NIR (mmoles m^2 s) is the infrared radiation reflected by it (725-1020 nm, Jones et al., 2007).

Due to its normalization, the ranges of NDVI measurements are between -1 and 1, with higher values (0.7-0.8) being indicators of plants in good condition. A value of 1 would represent the highest possible plant density of green and healthy leaves (Gutiérrez et al., 2011). Bare soils generate low positive values (0.1-0.2) and free water values ranging from -0.1 to 0.1 or 0.2 (Huete, 1988). This may have an influence when making measurements in rice cultivation, given the conditions under which rice cultivation is practiced. A commonly used sensor to estimate NDVI is the GreenSeeker (Trimble Inc.). Its interpretation can contribute to rapid and targeted diagnosis of nutritional (especially nitrogen) conditions, physiological status, stress incidence, and crop yield potential (Inman et al., 2005).

It is essential to have objective data at the local level that allow the use of models to estimate the N requirements of the crop during its commercial production cycle, with prior diagnosis of its current state and without the need to use destructive manual techniques for this purpose. The use of remote sensors or satellite images that allow reflecting the nutritional situation of the crop is key to advance in the development of precision agriculture, which is essential for the sustainable management of agroecosystems.

The first hypothesis of this work is that there is an NNI for each situation and moment of the rice crop and with this it is possible to establish an equation that allows relating the NR with an equivalent fertilizer rate. The second hypothesis is that it is possible to establish a relationship between NDVI with DM accumulation, N concentration, PNU and NNI to adjust equivalent fertilizer rates in a practical way and in real time.

The objectives of this work were to relate, from a three-year data set, the NNI determined based on the CNDC of the indica and japonica subspecies, to the crop's NR and to obtain a fertilizer equivalent. In addition, to estimate in real time by means of a GreenSeeker NDVI sensor, DM, N concentration, PNU and NNI during the different stages of rice growth, providing a simple and objective method for NR estimation.

3.3. MATERIALS AND METHODS

3.3.1. Description and management of experiments

Information from a network of nitrogen fertilization trials conducted between the 2016-2017 and 2018-2019 harvests (three years), in three locations corresponding to the main rice producing areas (north, center and east) was used (Table 1, Annex 1).

Table 1. Experimental sites and rice cultivars used.

Experimental Unit	Soil unit	Type of soil	OC* (%)	Coordinates	Cultivar
Treinta y Tres (Paso de la Laguna)	La Charqueada	Silty-clay loam Argialboll	1,3	33°16'17.62" S 54°10'24.63" O	INIA Merin (indica subspecies) Parao (japonica subspecies)
Tacuarembó (Pueblo del Barro)	Río Tacuaremb ó	Vertic Albaqualf	2,3	31°59'27.62" S 55°24'28.81" O	INIA Merin Parao
Artigas (Paso Farias)	Itapebi Tres Arboles	Typic Hapludert	2,8	30°28'37.74" S 57° 7'11.85"O	INIA Merin

*Note: *OC: organic carbon.*

Of a total of fifteen trials, nine corresponded to the INIA Merin indica subspecies cultivar (indica), the one with the largest cultivated area in the country; the remaining six trials were carried out with Parao (japonica), of the japonica subspecies (Annex 2).

3.3.2. Experimental design

In the 2016-2017 and 2017-2018 harvests, randomized complete block experiments with split-plot arrangement were set up at the three sites. The large plot corresponded to nitrogen fertilization at tillering, while the smaller plot was fertilization at panicle initiation. The rates used at each phenological stage were 0, 25, 50 and 100 kg N ha^{-1} in randomized treatments. A total of 16 treatments were achieved (Table 2). In the 2018-2019 harvest, a randomized complete block design with five treatments and three replications was used; N rates were 0, 75, 225, 250 and 275 kg N ha^{-1} total (Table 2).

Table 2. Description of nitrogen (N) treatments, N fractionation at tillering and panicle initiation and total N rates (kg N ha^{-1}) of the treatments evaluated from harvests 2016-2017 to 2018-2019.

Treatments	Tillering rate (kg N ha^{-1})	Panicle initiation rate (kg N ha^{-1})	Total rate (kg N ha^{-1})
Treatments harvest 2016-2018*			
1	0	0	0
2	25	0	25
3	50	0	50
4	100	0	100
5	0	25	25
6	25	25	50
7	50	25	75
8	100	25	125
9	0	50	50
10	25	50	75
11	50	50	100
12	100	50	150
13	0	100	100
14	25	100	125
15	50	100	150
16	100	100	200
Treatments harvest 2018-2019			
1	0	0	0
2	50	25	75
3	125	100	225
4	150	100	250
5	150	125	275

*Note: *harvest 2016-2018 includes harvest 2016-2017 y 2017-2018.*

3.3.3. Determinations and statistical analysis

3.3.3.1. Dry matter accumulation

Plant sampling was carried out approximately every 15 days, starting 15 days before panicle initiation (15DBPI) until 45 days after panicle initiation (45DAPI). They were carried out by flush cuts of two subsamples of 0.3 m linear. A total of 2080 samples were taken from the indica cultivar and 1164 from the japonica cultivar. The samples were washed and kept cold at 5 °C until processing. In the laboratory they were rinsed with potable water and washed with distilled water. After weighing, they were dried in vertical flow ovens at 60 °C for 48 hours. Subsequently, the dry weight was determined, they were ground in a static grinder to 2 mm and stored under optimal preservation conditions. The same procedure was performed separately for the straw and grain fractions of the harvest sampling.

3.3.3.2. Determination of nitrogen in plants

The N content of each DM sample was determined. The determination was carried out at the Integral Analysis Laboratory of INIA Tacuarembó. In the vegetative stage, N analyses were carried out with NIRS equipment, after calibration with the LECO/DUMAS technique ($p < 0.01$; R^2 0.99). For this calibration, a group of samples was taken at random from the same lot and analyzed by LECO technique and then by NIRS. When the correlation coefficient between both determinations was greater than or equal to 95 %, the remaining samples were analyzed by NIRS.

3.3.3.3. Determination of nitrogen uptake in plants

Nitrogen uptake was determined at each sampling time of the crop. For this calculation, the DM production, and the percentage of N at each moment were considered. As a result, the N content in kg ha^{-1} on a dry basis was obtained.

$$PNU = \frac{\% \, N \times DM}{100} \quad (2)$$

Where PNU is plant N uptake and % N is plant N concentration and DM is dry matter.

3.3.3.4. Estimation of the normalized difference vegetation index.

NDVI estimates were made with the GreenSeeker hand-held remote sensor (Trimble Inc.) in each of the sampling stages. This sensor works by emitting radiation in the red (570-680nm) and near-infrared (725-1020 nm) range of the electromagnetic spectrum and estimates the proportion of this radiation reflected by the crop. One integrated measurement per plot was made by passing the hand-held sensor at a height of 60 cm above the leaf cover with a constant speed from the central row and in a longitudinal direction in each plot. This allowed obtaining an average NDVI value for each plot for each crop time and for each cultivar.

3.3.4. Determination of apparent nitrogen recovery efficiency

The following equation was used to calculate the NRE:

$$NRE = \frac{(pNfert - pNnofert)}{difNaplicado} \quad (5)$$

Where pNfert is the N uptake of the fertilized plot (kg ha^{-1}), pNnofert is the N uptake in the unfertilized plot (kg ha^{-1}) and difNapplied is the N rate applied (kg ha^{-1}).

The NRE was calculated for the 15DBPI, PI, 15DAPI and 30DAPI moments. For the calculation of the NRE at 15DBPI and panicle initiation, the N rates at tillering were taken, leaving constant the rates at panicle initiation, and for the NRE at 15DAPI and 30DAPI, the rates at panicle initiation were taken, leaving constant the rates at tillering.

The 15 experiments carried out during the three years were used. Regression models were performed in Microsoft Excel (Carey et al., 2010). The models were fitted by minimizing the mean square of the error and the coefficient of determination (R^2). For the definition of each model, DM and PNU intervals were established, with confidence interval analysis (95 %) with the Infostat program (Di Rienzo et al., 2008).

3.3.5. Determination of the nitrogen nutrition index

The NNI is used to quantify the nutritional deficiency or sufficiency of a crop, relative to the optimum level of N concentration in the plant. It is calculated from the CNDC and can be performed for different moments of the crop cycle. The equation used was that proposed by Lemaire and Meynard (1997).

$$NNI = \frac{aN}{cN} \quad (3)$$

Where aN represents the actual N concentration of the plant and cN is the critical N concentration for a given time.

3.3.6. Determination of the nitrogen (N) requirement model

In order to make an N fertilization recommendation, a regression was fitted between NR and NNI for four cycle moments: 15DBPI, PI, 15DAPI and 30DAPI. To evaluate the goodness of fit of the regression models, the coefficient of determination (R^2) and mean square of the error were calculated. Those regressions with the highest magnitude of the first and lowest magnitude of the second selection parameter were selected. The equation generated by Ata-Ul-Karim et al. (2017) was used to calculate the N requirements:

$$NR = \frac{(Ncna - Nna)}{NRE} \quad (4)$$

Where NR is the N requirement (kg ha^{-1}), Ncna the N uptake under critical level condition, Nna the actual N uptake and NRE the apparent N recovery efficiency.

3.3.7. Evaluation of the normalized difference vegetation index as an estimator of agronomic parameters and indicators of interest.

To evaluate NDVI as an estimator of variables of interest, regressions were performed between this parameter and DM, N concentration, NNI and PNU at each moment of the crop cycle. From these regressions, equations were determined that allowed estimating in real time and in a practical way the NNI, PNU, DM and N at specific moments of the crop cycle. In this way, fertilization recommendations could be made, taking the critical N dilution curve as a criterion for decision making. The goodness-of-fit criteria for the selection of each model were the coefficient of determination (R^2) and the mean square of the error. Those regressions with the highest magnitude of the first and lowest magnitude of the second selection parameter were selected.

3.4. RESULTS

3.4.1. Apparent nitrogen recovery efficiency, plant N uptake range and associated dry matter range

The NRE was different between both cultivars and between stages for each cultivar (Tables 3 and 4). In indica, the NRE was lower at 15DBPI and 30DAPI than at the PI and

15DAPI stages. In the japonica cultivar, the panicle initiation and 30DAPI stages presented the highest NRE values.

PNU also behaved differently among cultivars. In absolute terms, japonica absorbed 15 and 24.5 kg ha^{-1} more N than indica at 15DBPI and PI, respectively. On the other hand, indica absorbed 15.5 kg ha^{-1} more N than japonica at 30DAPI and no differences in PNU were observed among cultivars at 15DAPI. The amplitude of the average range of PNU was similar among cultivars: in indica it was 5, 5, 8 and 12 kg ha^{-1} of N and in japonica 5, 6, 6 and 15 kg ha^{-1} of N for the 15DBPI, PI, 15DAPI and 30DAPI moments, respectively (Tables 3 and 4).

DM accumulation ranges showed differences among cultivars at the different evaluation times. Except for 30DAPI, japonica presented higher DM accumulation than indica at the previous moments (Tables 3 and 4).

Table 3. Measured values of apparent nitrogen recovery efficiency (NRE), N uptake rate (PNU) expressed in kg ha^{-1} and dry matter (DM) accumulation rate expressed in Mg ha^{-1} in the cultivar indica at 15 days before panicle initiation (15DBPI), panicle initiation (PI), 15 days after panicle initiation (15DAPI) and 30 days after panicle initiation (30DAPI).

Stage of cultivation	Apparent N recovery efficiency (%)	Range of N uptake (kg ha^{-1})	DM range (Mg ha^{-1})
15DBPI	22	27-32	1.1-1.3
PI	36	47-52	2.5-2.7
15DAPI	37	91-99	5.2-5.6
30DAPI	14	131-143	9.4-10.1

Table 4. Measured values of apparent nitrogen recovery efficiency (NRE), N uptake rate (PNU) expressed in kg ha^{-1} and dry matter (DM) accumulation rate expressed in Mg ha^{-1} in the cultivar japonica at 15 days before panicle initiation (15DBPI), panicle initiation (PI), 15 days after panicle initiation (15DAPI) and 30 days after panicle initiation (30DAPI).

Stage of cultivation	Apparent N recovery efficiency (%)	Range of N uptake (kg ha^{-1})	DM range (Mg ha^{-1})
15DBPI	25	42-47	1.7-1.9
PI	32	71-77	2.1-4.8
15DAPI	18	92-98	6.6-7.1
30DAPI	40	114-129	9.0-9.7

3.4.2. <u>Determination of nitrogen fertilization requirement from the nitrogen nutrition index</u>

Early in the crop cycle (15DBPI and PI), there was less variability in the NNI and the rates of N to be added to reach the nutritional optimum. When going from optimum NNI to 0.5 NNI, the rates to be added increased to 140 kg ha^{-1} and 74 kg ha^{-1} of N at japonica and indica, respectively.

However, as the crop cycle progressed (15DAPI and 30DAPI), the N fertilization requirement increased as the NNI moved away from the optimum plant N concentration level (Figure 1).

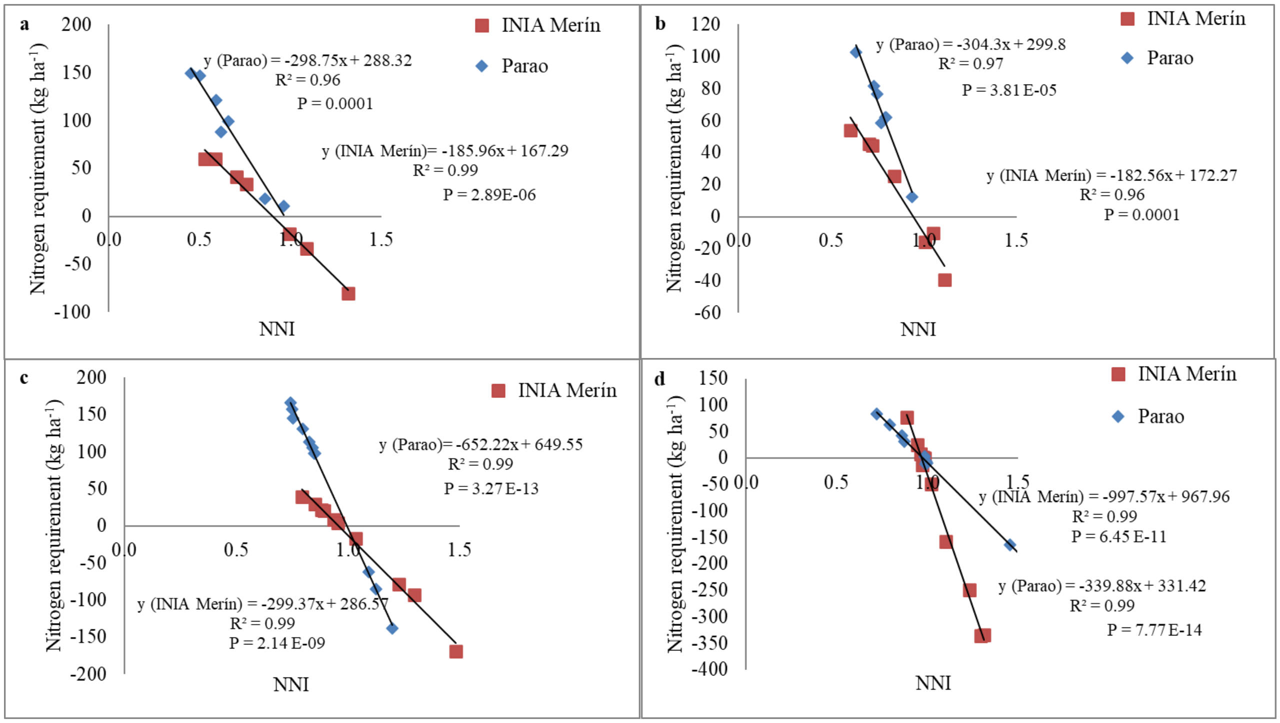

Figure 1. Relationship between the rate of nitrogen (N) to be added and the nitrogen nutrition index (NNI) in indica (INIA Merin) and japonica (Parao) rice cultivars at four moments of the crop cycle: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI) and (d) 30 days after panicle initiation (30DAPI).

3.4.3. <u>Association of the normalized difference vegetation index with agronomic variables and indices.</u>

A good fit between PNU and NDVI was observed at indica at the five stages of the cycle evaluated. The coefficient increased steadily from 15DBPI to 45DAPI with R^2 values of 0.73, 0.86, 0.91, 0.95 and 0.96, respectively. The amplitude of the NDVI range decreased as the cycle progressed, 0.47-0.80 at 15DBPI, 0.46-0.76 at PI, 0.51-0.79 at 15DAPI, 0.58-0.78 at 30DAPI and 0.59-0.76 at 45DAPI (Figure 2, Table 5).

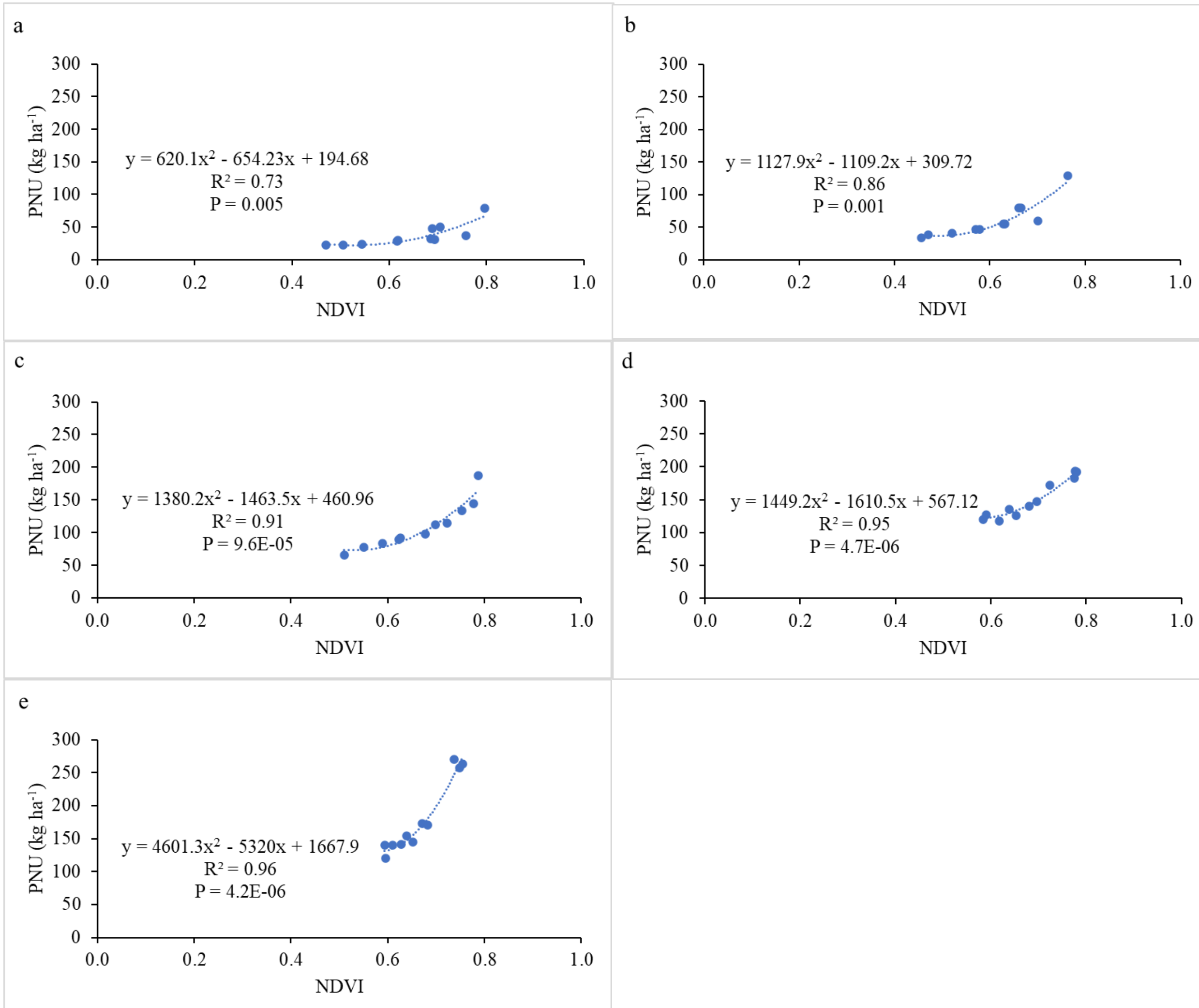

Figure 2. Regression between normalized difference vegetation index (NDVI) and nitrogen uptake in rice plants (PNU) during five moments of the indica cultivar cycle: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

Table 5. Measured values of dry matter (DM) range expressed in Mg ha^{-1} used in each regression model between normalized difference vegetation index (NDVI) and plant nitrogen uptake (PNU) expressed in kg ha^{-1} in the indica cultivar. Moments (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI),

Variable	Growth stage	DM Range (Mg ha^{-1})	Model equation	R^2
PNU (a)	15DBPI	1.09-1.27	$y = 620.1x^2 - 654.23x + 194.68$	0.73
PNU(b)	PI	2.46-2.69	$y = 1127.9x^2 - 1109.2x + 309.72$	0.86
PNU (c)	15DAPI	5.20-5.57	$y = 1380.2x^2 - 1463.5 + 460.96$	0.91
PNU (d)	30DAPI	9.36-10.08	$y = 1449.2x^2 - 1610.5 + 567.12$	0.95
PNU (e)	45DAPI	12.61-13.43	$y = 4601.3x^2 - 5320x + 1667.9$	0.96

In japonica, the relationship between these variables had a good fit at 15DBPI, 15DAPI and 30DAPI, with an R^2 of 0.92, 0.89, and 0.91, respectively. The amplitude of the NDVI range was greater at the 15DBPI stage with a range of 0.42-0.80, being narrower at the 15DAPI stage with 0.62-0.81 and 0.66-0.80 in 30DAPI (Figure 3, Table 6).

For the same stage of the cycle, japonica produced 0.6, 1.5 and 1.45 Mg ha^{-1} of DM more than indica at 15DBPI, PI and 15DAPI, respectively (Tables 5 and 6).

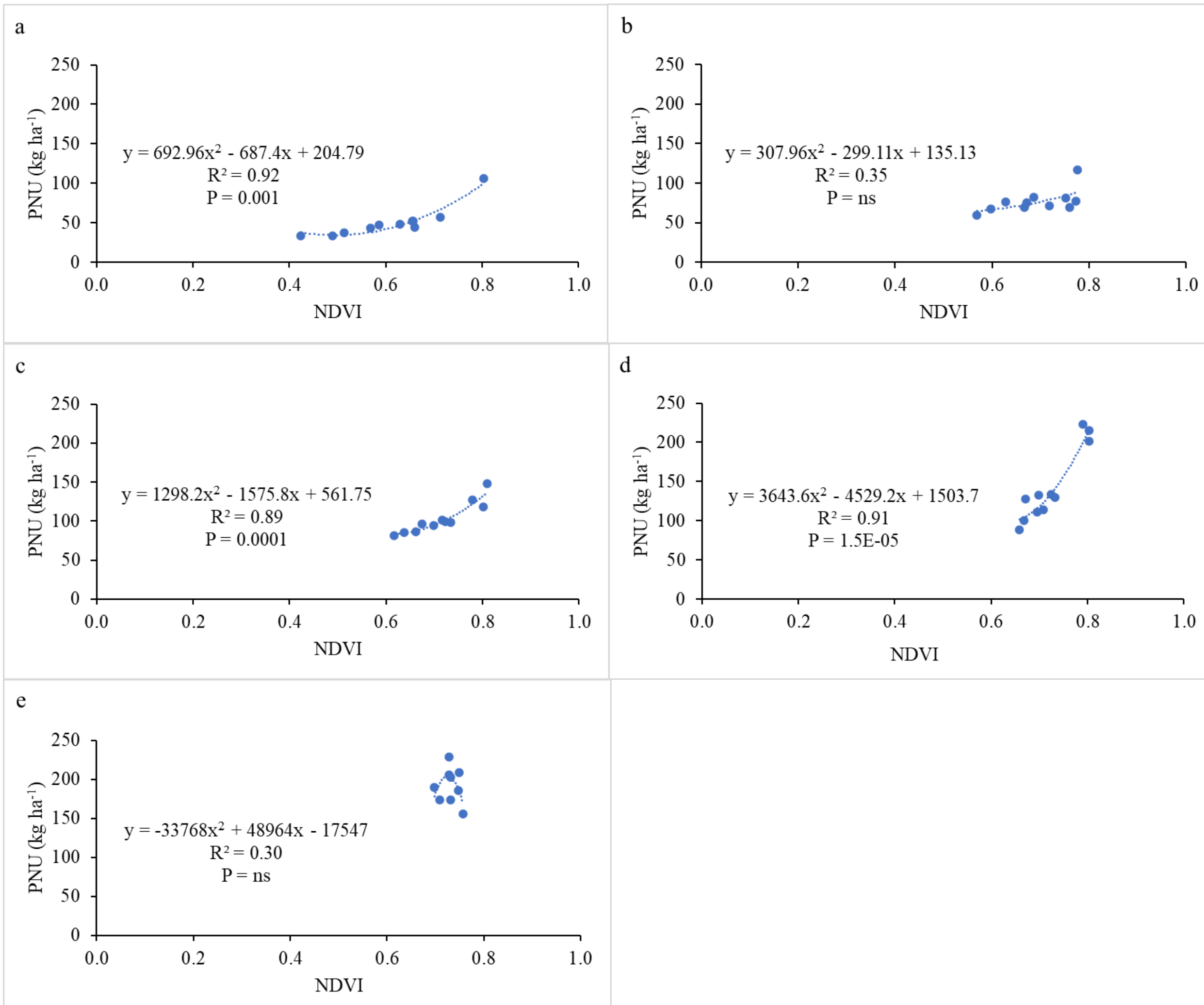

Figure 3. Regression between normalized difference vegetation index (NDVI) and nitrogen uptake in rice plants (PNU) at five stages of the cycle of the japonica cultivar: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

Table 6. Measured values of dry matter (DM) rank expressed in Mg ha^{-1} used in each regression model between normalized difference vegetation index (NDVI) and plant nitrogen uptake (PNU) expressed in kg ha^{-1} in the cultivar japonica for the stages (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

Variable	Growth stage	DM range (Mg ha^{-1})	Model equation	R^2
PNU (a)	15DBPI	1.66-1.85	$y = 692.96x^2 - 687.4 + 204.79$	0.92
PNU (b)	PI	3.95-4.28	$y = 307.96x^2 - 299.11x + 135.13$	0.35
PNU (c)	15DAPI	6.57-7.07	$y = 1298.2x^2 - 1575.8x + 561.75$	0.89
PNU(d)	30DAPI	9.03-9.67	$y = 3643.6x^2 - 4529.2x + 1503.7$	0.91
PNU (e)	45DAPI	13.55-14.75	$y = -33768x^2 + 48964x - 17547$	0.30

The regressions between NDVI and NNI at indica (Figure 4) always presented a good fit, with coefficients of determination increasing from 15DBPI to 30DAPI. NDVI ranges were higher at 15DBPI and PI, showing a subsequent decrease at 15DAPI, 30DAPI and 45DAPI. At all times evaluated, an NNI value of around 1 was reached with NDVI values of 0.75; 0.7; 0.7; 0.7 and 0.69 at 15DBP, PNU, 15DAP, 30DAP and 45DAP respectively.

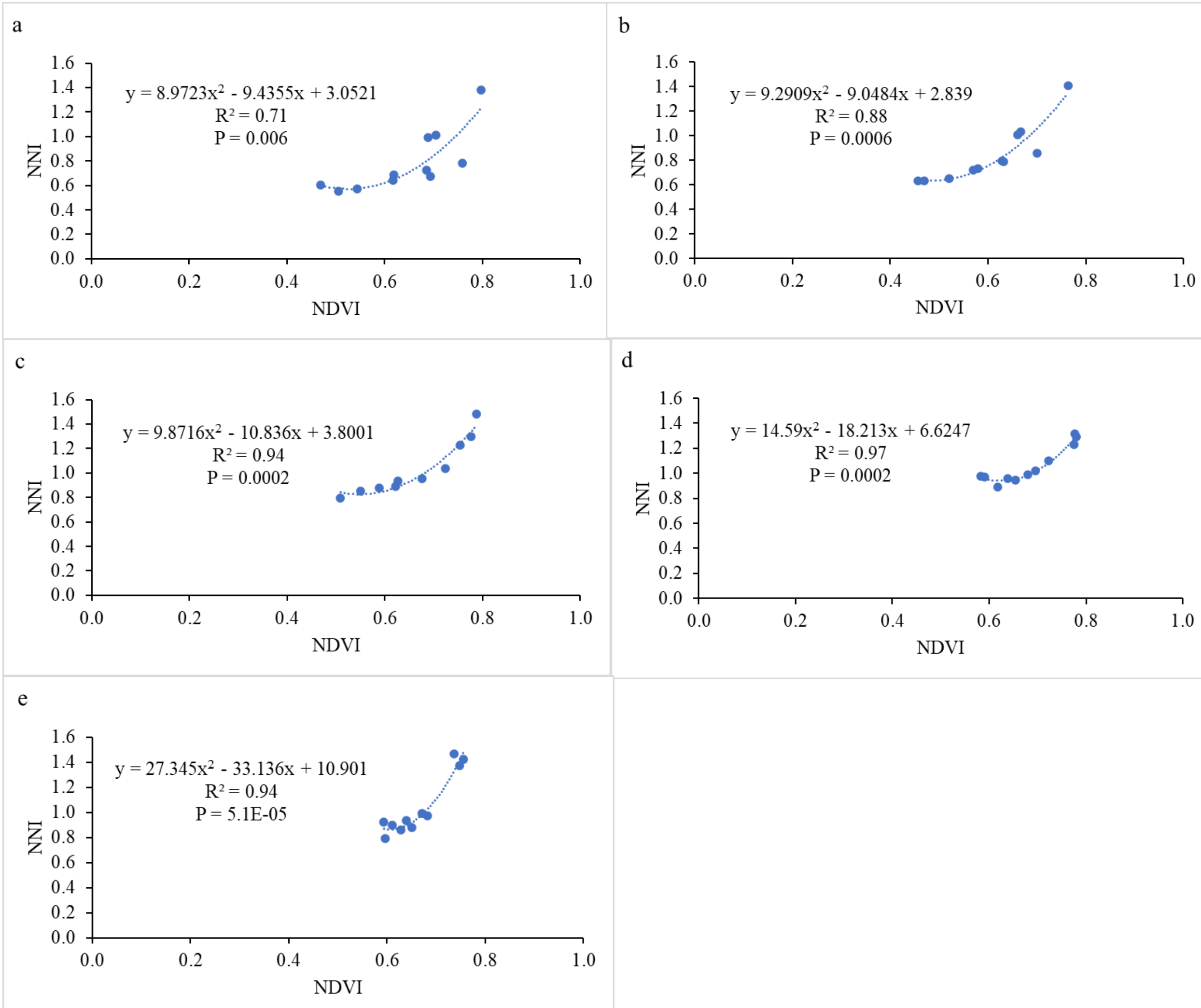

Figure 4. Regression between the normalized difference vegetation index (NDVI), independent variable, and the nitrogen nutrition index (NNI), dependent variable, for five growth stages of the crop cycle in the cultivar indica (a), 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

A good fit between NDVI-N and NDVI-DM was also observed at all stages of the indica cycle (Figure 5 and 6). In both cases, the fit was lower at 15DBPI and increased in the following stages. In the NDVI-N relationship, the R^2 was 0.60; 0.87; 0.92; 0.92 and

0.90 from 15DBPI to 45DAPI, respectively.

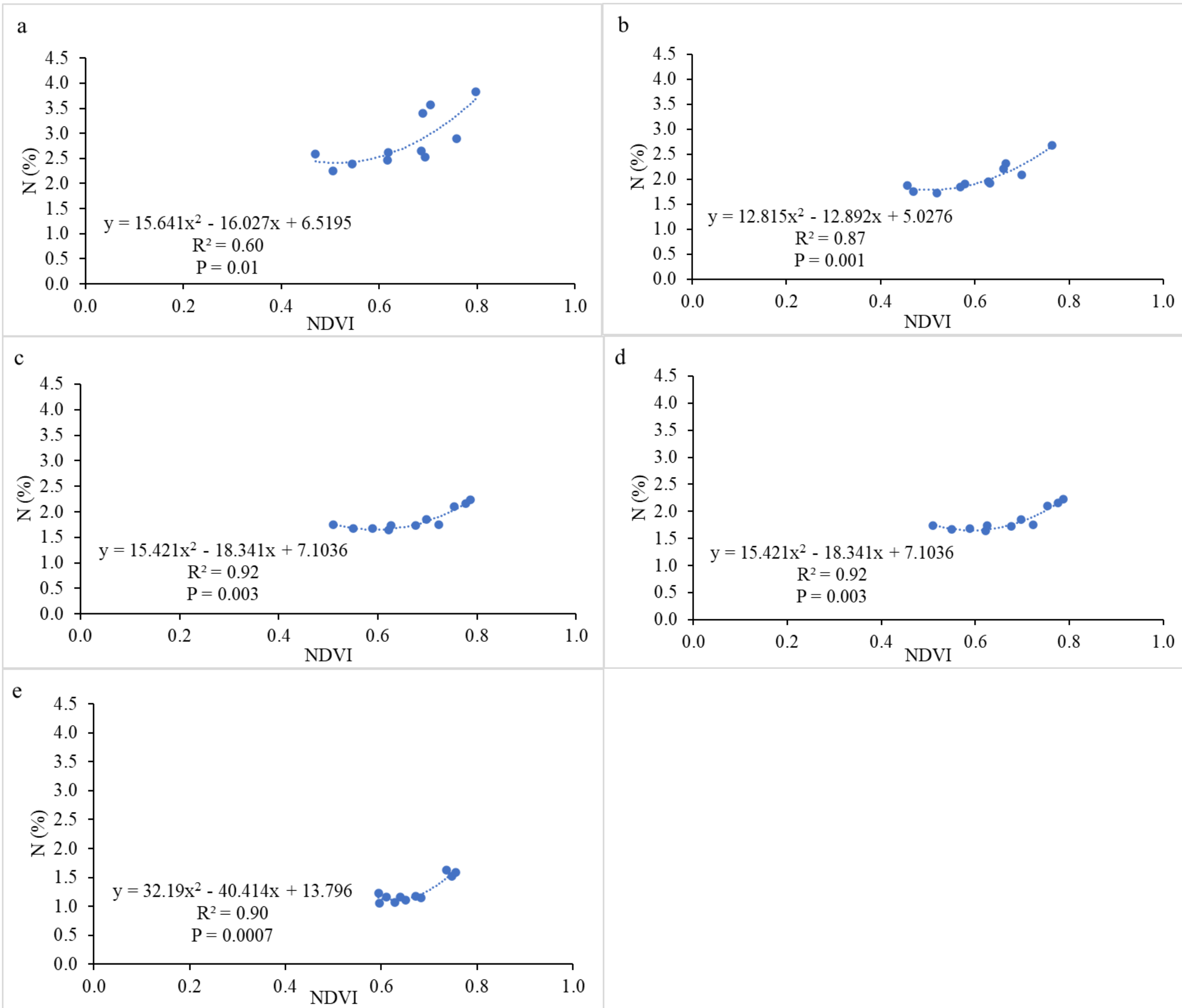

Figure 5. Regression between normalized difference vegetation index (NDVI), independent variable, and nitrogen (N), dependent variable, for five growth stages of the crop cycle in the indica cultivar: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI) (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

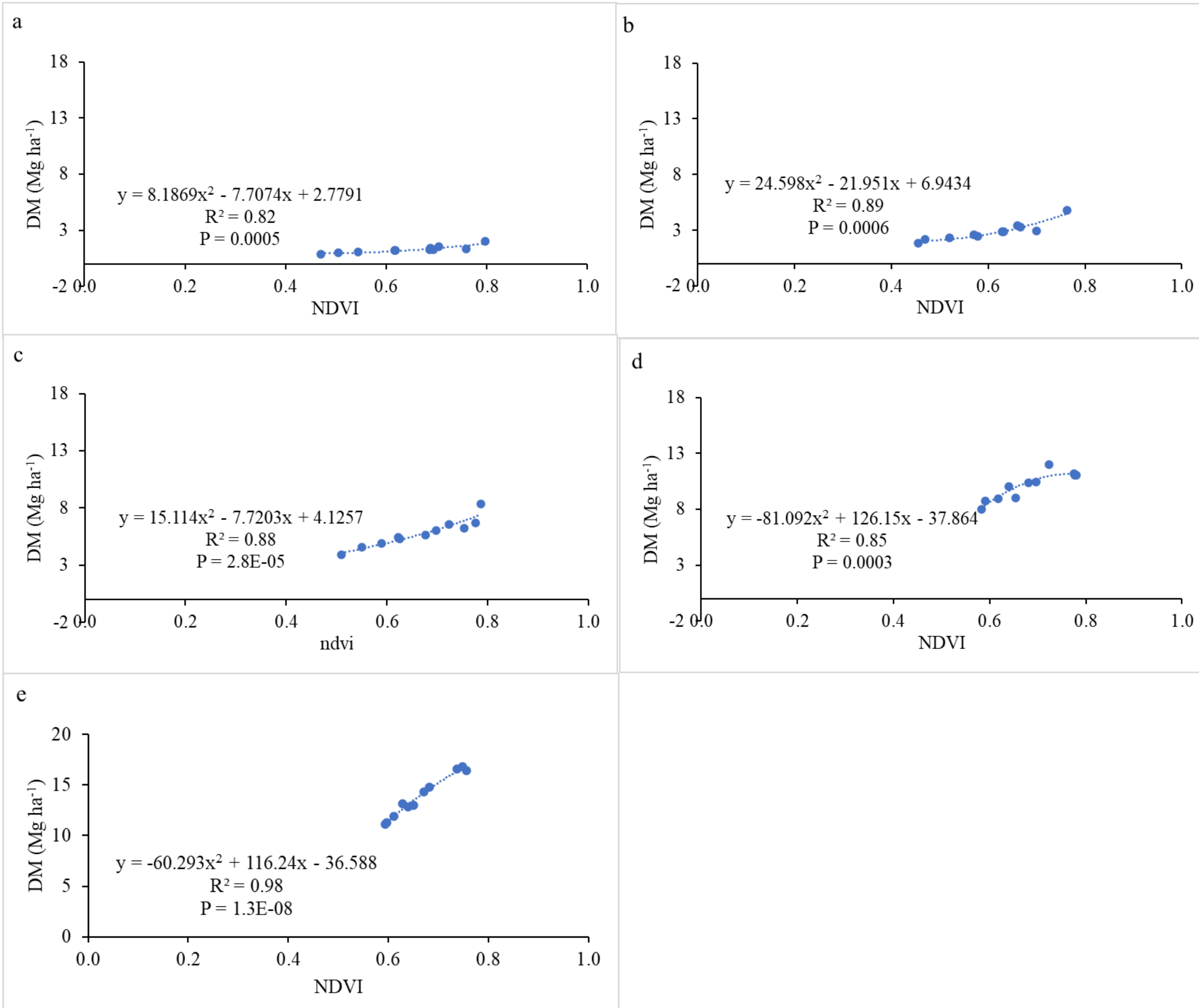

Figure 6. Regression between the normalized difference vegetation index (NDVI), independent variable, and dry matter (DM), dependent variable, for five growth stages of the crop cycle in the indica cultivar: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

The cultivar japonica presented a good fit between NNI and NDVI with an R^2 of 0.93, 0.54, 0.93 and 0.92 at 15DBPI, PI, 15DAPI and 30DAPI, respectively (Figure 7). For the time of 45DAPI, the fit was not significant. At all times evaluated (except 45DAP), an NNI value of around 1 was reached with NDVI values of 0.77; 0.8; 0.77 and 0.74 at 15DBP, PNU, 15DAP, 30DAP respectively.

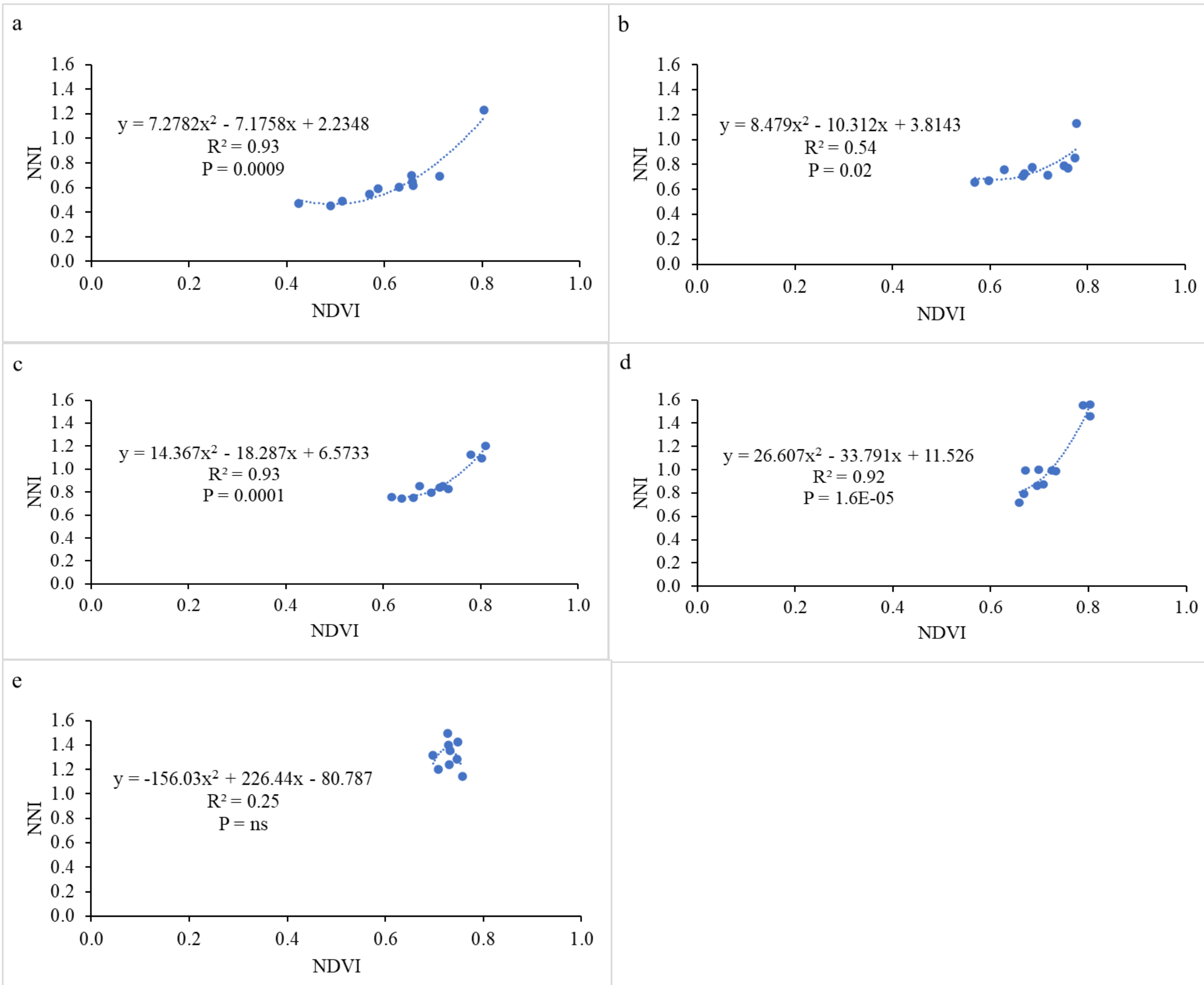

Figure 7. Regression between the normalized difference vegetation index (NDVI), independent variable, and the nitrogen nutrition index (NNI), dependent variable, for five growth stages of the crop cycle in the japonica cultivar: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

In japonica, robust and significant adjustments were observed between NDVI and

N at 15DBPI, PI, 15DAPI and 30DAPI, with an R^2 of 0.64, 0.74, 0.68 and 0.90, respectively. The time 45DAPI did not present significant adjustment (Figure 8).

A good fit between NDVI and DM was also observed at 15DBPI and 30DAPI, while they were not significant in the other cycle growth stages. The time that presented the best performance was 30DAPI, with an R^2 of 0.89 (Figure 9).

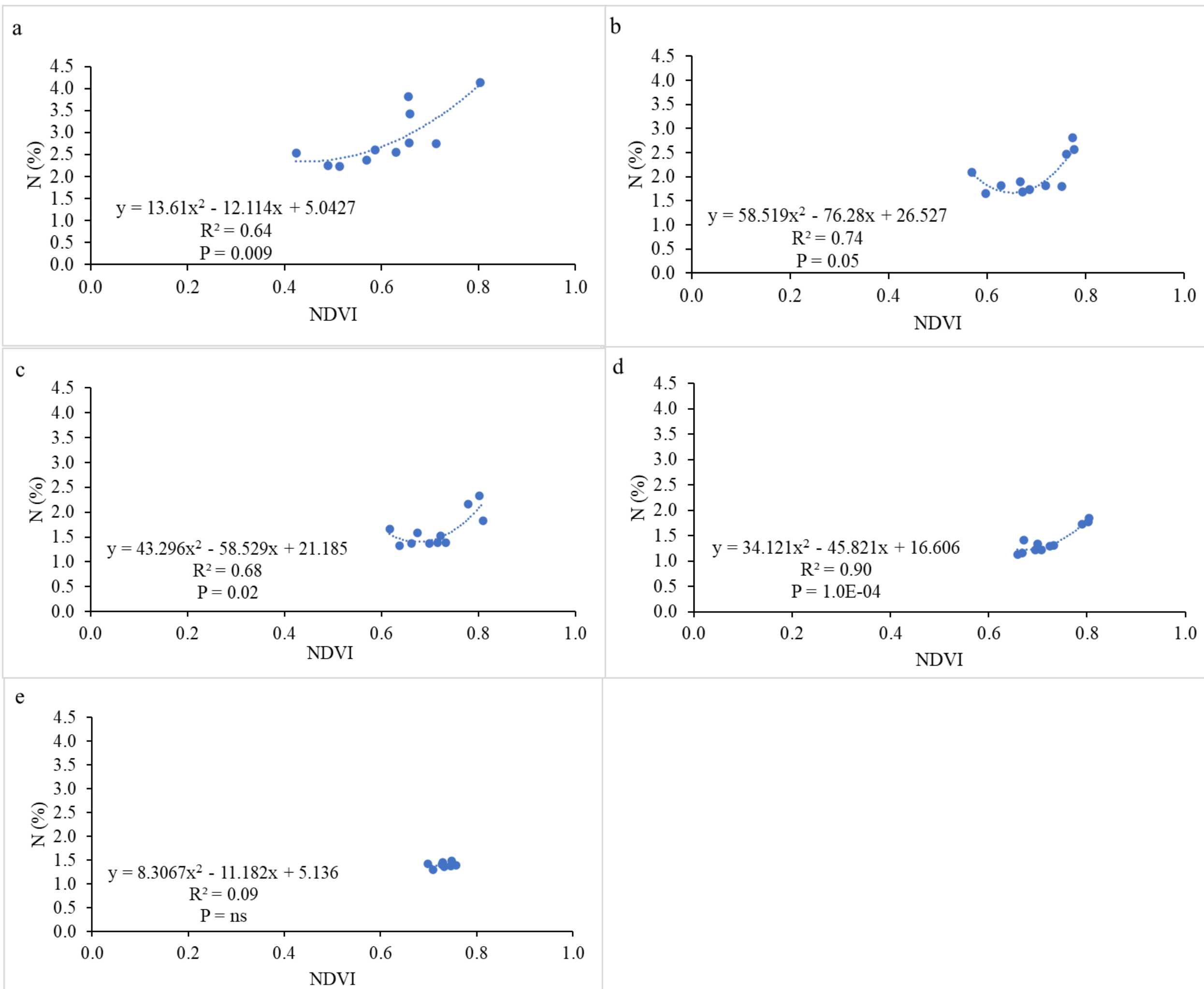

Figure 8. Regression between the normalized difference vegetation index (NDVI), independent variable, and nitrogen (N), dependent variable, for five growth stages of the crop cycle in the japonica cultivar: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

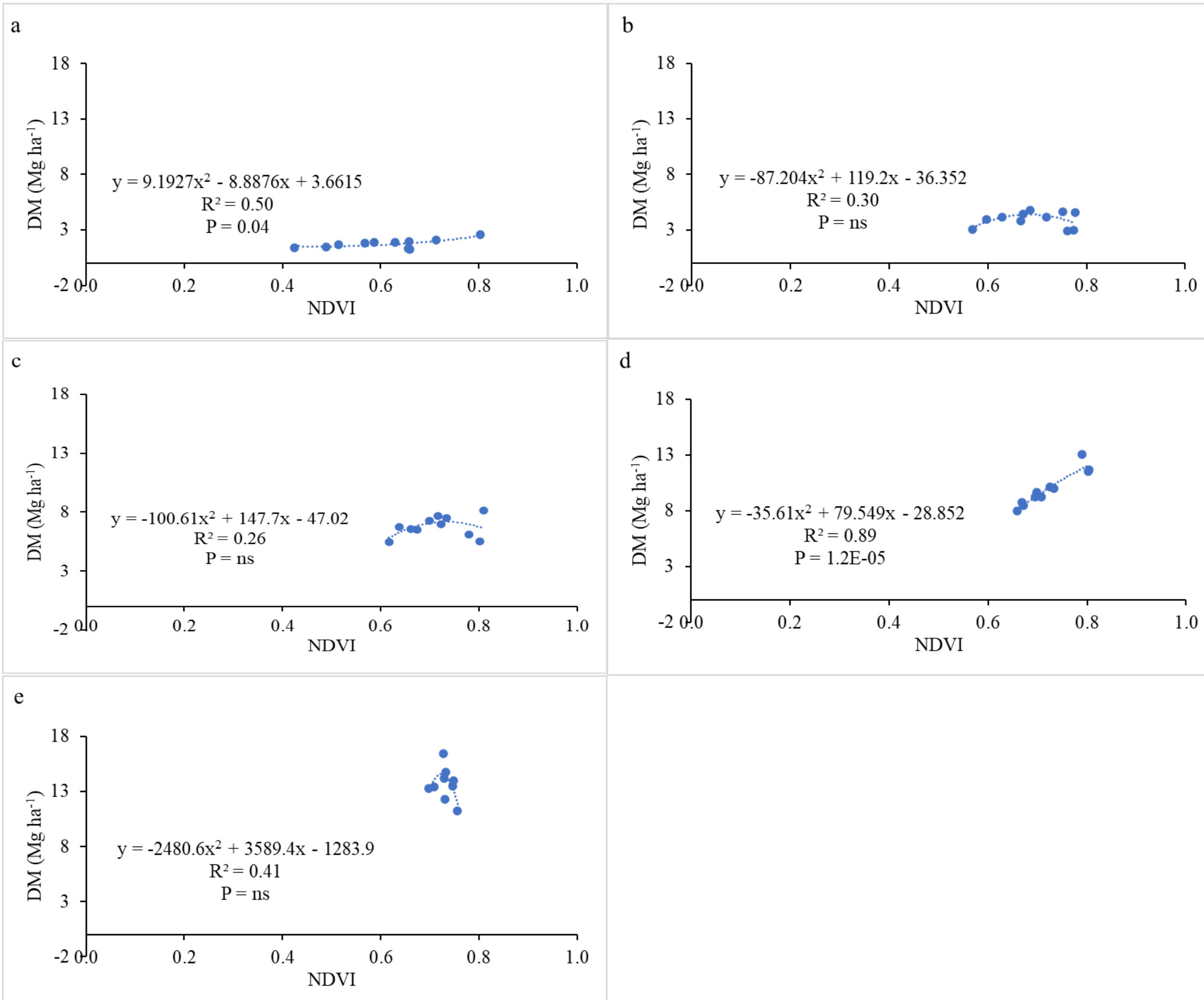

Figure 9. Regression between the normalized difference vegetation index (NDVI), independent variable, and dry matter (DM), dependent variable, for five growth stages of the crop cycle in the japonica cultivar: (a) 15 days before panicle initiation (15DBPI), (b) panicle initiation (PI), (c) 15 days after panicle initiation (15DAPI), (d) 30 days after panicle initiation (30DAPI) and (e) 45 days after panicle initiation (45DAPI).

3.5. DISCUSSION

3.5.1. Determination of nitrogen fertilization requirements

In high production rice systems, it is important to determine accurately and in real time the NR of the crop for efficient fertilization management. There are works that have focused on N diagnosis during the crop cycle by remote sensing or other real-time analysis methods. However, it is necessary to advance in the knowledge of the relationship between NR and the indices that estimate crop N content (Samborski et al., 2009). The NR estimated in many studies may be insufficient to meet actual crop demands at the optimum level of N. Moreover, if N stress is too severe or the crop has advanced in its cycle, full recovery may not be possible (Prasertsak and Fukai, 1997).

Therefore, in the eventuality that the applied N may be insufficient to cover the NR, other factors, including crop development and the degree of N deficiency, should be considered for proper N management in the field (Li et al., 2014). In this work, relationships between NNI and NR were generated from 15 days before and up to 45 days after panicle initiation based on the CNDCs of two rice cultivars, one indica and one japonica subspecies. Equations were obtained to adjust nitrogen fertilization in real time using normalized difference vegetation indices that allow covering the nutritional requirements during the cycle and obtaining the maximum DM accumulation with the minimum N concentration in the plant, which optimizes N use efficiency.

The differences observed between the nitrogen fertilization recommendation models during the cycle of both cultivars were associated with the genetic particularities of each rice subspecies that determine their growth and N uptake rates, as well as differences in NRE (Sheehy et al., 1998; Weng and Chen, 1987; Yoshida et al., 2006). The relationship between NNI and NR in both cultivars was able to explain the variation in N requirements during the period between 15DBPI and 30DAPI of the cycle and to identify situations of limiting nutrition, as well as optimal and luxury intake (Figure 1).

Similar results were reported by Ata-Ul-Karim et al. (2017), who found that there was a significant linear correlation between rice NR and NNI, particularly during panicle initiation and 30DAPI with coefficients of determination greater than 0.98. In this work, the coefficients of determination were also higher from the PI onwards. Thus, the first hypothesis was confirmed, since it was possible to determine an NNI for each situation and time of the crop, and with this it was possible to establish an equation that allowed relating the NR to an equivalent rate of fertilizer.

NRE varied both at the different times evaluated per cultivar and among cultivars, with an average for indica and japonica of 27 % and 29 % respectively (Tables 3 and 4), values like those reported by Wang et al. (2020) of 26 % to 36 % and lower than those presented by Dobermann and Cassman (2004) of 30 % to 50 %. The variation in NRE may have been due in part to the fact that the calculation method depended primarily on the unfertilized treatment; therefore, any change in aN (actual plant nitrogen concentration) in the unfertilized treatment would directly affect the NRE value in the other treatments (Chen et al., 2013; Zhang et al., 2012). In addition, aN is affected by many factors, such as climate, soil fertility, soil water content, and pest and disease management. Even in a long-term experiment, variations in light and temperature between years are likely to lead to different aN levels (Wang et al., 2020). Although there are other methods to measure NRE, such as ^{15}N isotopic techniques, these same authors point out that the apparent recovery method more closely reflects what happens under field conditions, due to the processes that occur to N in the soil in interaction with microorganisms. In this work, it was not possible to calculate the NRE at the end of the cycle because the N in grain and straw was not quantified at that time, so these values were calculated as the average of the NRE of each time studied.

In local works, Lago et al. (2016) obtained NRE values of 21 % to 40 %, like the range of this study. On the other hand, Castillo (2018) obtained an NRE at the end of the cycle of 37 % and 42%, within the range reported by Dobermann and Cassman (2004), and whose explanation may be due, among other factors, to the contribution in recovery efficiency to the N applied to tillering, in dry soil and with subsequent flooding (Norman et al., 2009).

According to Ladha et al. (2005), in commercial field trials, NREs of 31% can be expected, while in more controlled trials in experimental fields, NREs of 46% can be expected. Due to the high variability of this parameter among genotypes, sites, agronomic management, production systems and climatic conditions, it is advisable to evaluate and validate it in a wide range of production situations for its use.

Once the mathematical models are established to adjust a nitrogen fertilization recommendation to cover the nutritional requirements of the crop based on its current state, it is necessary to use sensors to estimate the value of the variable required by the model at the different stages of the crop.

3.5.2. Normalized difference vegetation index for estimation of variables of interest

In this work, NDVI was an adequate index to estimate the relevant variables in both cultivars, including PNU, NNI, DM and N (Figure 2 to 9). At indica, NDVI and the variables had a very good fit at all times of the cycle, particularly after PI. Meanwhile, at japonica, no significant adjustments were observed at 45DAPI, mainly because the observed data presented low variability, probably due to a smaller database and some restrictions of the experiments conducted.

Similar results were reported by Rehman et al. (2019), the NDVI explained much of the variation in PNU ($R^2 = 0.66$), N concentration ($R^2 = 0.54$) and DM ($R^2 = 0.51$), with a useful index at low levels of N concentration and saturating values at high levels (0.76-0.78). In the present work, NDVI saturated at maximum values close to 0.8. NDVI saturation is the result of the crop reaching 100% leaf cover, but DM accumulation and leaf area index continue to increase (Gitelson, 2004).

Yao et al. (2014) reported that NDVI in relation to DM or PNU saturated at approximately 0.80 and 0.78, respectively. Gnyp et al. (2014) determined that the NDVI related to DM saturated at approximately 0.90. In this work, the indica cultivar reached optimal NNI levels with an NDVI value around 0.7 at all times evaluated, indicating that optimal N values in the crop can be estimated below the saturation line. The same result was found in the japonica cultivar for the times from 15 DAP to 30 DAP, with an average NDVI value of 0.77. The good fit of NDVI observed in this work suggests that it may be a valuable tool for adjusting nitrogen fertilization of rice, thus requiring validation in the commercial setting. Recent studies suggest that indices incorporating a red-edge band (690 nm to 730 nm) can improve the assessment of rice nitrogen nutritional status by overcoming the saturation problem (Cao et al., 2013; Dunn et al., 2016; Wang et al., 2012).

Cao et al. (2013) found several red-edge-based indices that explain much of the variability in rice N content when described by linear regression models. Wang et al. (2012) developed a three-band red-edge-based index that estimated N concentration with high accuracy while reducing saturation. Dunn et al. (2016) confirmed a strong correlation of red-edge bands with PNU in rice based on their analysis of high-resolution hyperspectral data.

The body of information suggests that it is necessary to deepen the knowledge between red edge-based indices and the nutritional status of the rice crop to adjust fertilization, considering the strong association between these variables and NDVI saturation in early stages of the cycle. Some authors have shown that the variation of the correlation between NDVI and crop N concentration can vary significantly from very high (R^2 = 0.81) to weak (R^2 = 0.08) depending on the phenological stage of the crop or rice cultivars (Lu et al., 2017; Zhu et al., 2007). Li et al. (2018) reported a good correlation between NDVI and leaf PNU (R^2 = 0.70), similar to the values found in this study using whole-plant PNU.

This work indicates that, based on prior knowledge of the range of DM accumulation that defines the growth stages in which the crop is found and determining the NDVI with a sensor such as the GreenSeeker, it is possible to estimate in real time the PNU, DM, N or NNI of the crop. Work presented by Mistele and Schmidhalter (2008) and Ziadi et al. (2008) report that sensors such as the chlorophyll meter and canopy reflectance methods can be used for rapid and non-destructive estimation of NNI. Moreover, NNI can be estimated with multispectral (Padilla et al., 2017) or hyperspectral (Chen, 2015) equipment.

Therefore, based on a solid relationship between NR and NNI, and as observed in the results of the present work, rice NR can be directly calculated using the NNI measured in the field.

A limitation of this work was not having experiments for the validation of the models built in this work. It is relevant to validate these models so that they can be used as objective tools to cover nutritional requirements in real time and in the commercial field, to optimize N use efficiency and to obtain maximum DM values, with the main objective of achieving maximum grain yield.

The development of statistically robust nitrogen fertilization models and the possibility of estimating NNI from NDVI models expand the possibility of using these tools. It is necessary to validate this or similar tools at the farm scale in commercial farms to make real-time and objective crop nutrition decisions.

3.6. CONCLUSIONS

The NR were determined based on the local CNDC established by Moreira et al. (n.d.) and equations were developed to adjust an N fertilization rate according to the nutritional status of the crop, based on the knowledge of the NNI at a given time.

The NDVI measured with a manual sensor allowed estimating in real time the NNI, PNU, DM and N which, entered in the requirement equations, allowed determining the optimum N rate to be applied to the crop at a given moment of its cycle.

These results make it possible for future research to deepen these and other methodologies for objective determination of NR in a flexible manner throughout the crop cycle, in real time and adjusting to the demands of the crop, to improve the efficiency of N use and reduce the risk of environmental contamination, leaving aside the use of manual and destructive methods that make such management complex to implement.

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3.8. APPENDIXES

Appendix 1. Basic information on experimental sites.

Experiment	Basal fertilization NPK	Plot size (m^2)	Farm history and management
1	5-25-25	16.0	4-year return, no artificial pastures; summer T and systematization; DS on levees.
2	5-25-25	16.0	Intermediate year (rice in 14/15 and sorghum in 15/16); T with disks, 2 landplane
3	9-25/25-25	15.3	3-year return, no artificial pastures, minimum T. on a previous summer T, DS
4	5-30-15	16.0	4-year return, with pastures; summer T and systematization; DS
5	5-30-15	16.0	Rice in 15/16
6	9-25/25-25	15.3	3-year return, no pastures, minimum T on a previous summer T, DS
7	5-25-25	44.0	3-year return, no pastures, disks (2) in June, landplane (2) in August-September
8	5-25-25	44.0	4-years return, with pastures, tillage: disks (2) in January, landplane (2) in October
9	9-25/25-25	15.3	3-year return, no pastures, minimum T on a previous summer T, DS
10	5-25-25	16.0	Rice in 14/15 and sorghum in 15/16; T with disks, 2 landplane
11	9-25/25-25	15.3	Return 3 years, no meadow, minimum T. on a previous summer T., DS
12	5-30-15	16.0	Rice in 15/16
13	9-25/25-25	30.0	3-year return, no pastures, minimum T on a previous summer T, DS
14	5-25-25	44.0	4-years return, with pastures, tillage: disks (2) in January, landplane (2) in October
15	9-25/25-25	30.0	3-year return, no pastures, minimum T on a previous summer T, DS

Note. T: Tillage; DS: direct seeding.

Appendix 2. Basic information about the experiments

Experiment	Cultivar	Localidad	Treatments	Total N rate (kg ha^{-1})	Sowing date
1 (year 2016-2017)	INIA Merin	Artigas	16	0-200	04/10/2016
2 (year 2016-2017)	INIA Merin	Tacuarembó	16	0-200	04/10/2016
3 (year 2016-2017)	INIA Merin	Treinta y Tres	16	0-200	18/10/2020
4 (year 2017-2018)	INIA Merin	Artigas	16	0-200	06/10/2017
5 (year 2017-2018)	INIA Merin	Tacuarembó	16	0-200	16/10/2017
6 (year 2017-2018)	INIA Merin	Treinta y Tres	16	0-200	11/10/2020
7 (year 2018-2019)	INIA Merin	Artigas	5	0-275	08/10/2018
8 (year 2018-2019)	INIA Merin	Tacuarembó	5	0-275	16/10/2018
9 (year 2018-2019)	INIA Merin	Treinta y Tres	5	0-275	23/10/2018
10 (year 2016-2017)	Parao	Tacuarembó	16	0-200	04/10/2016
11 (year 2016-2017)	Parao	Treinta y Tres	16	0-200	18/10/2020
12 (year 2017-2018)	Parao	Tacuarembó	16	0-200	16/10/2017
13 (year 2017-2018)	Parao	Treinta y Tres	16	0-200	11/10/2020
14 (year 2018-2019)	Parao	Tacuarembó	5	0-275	16/10/2018
15 (year 2018-2019)	Parao	Treinta y Tres	5	0-275	23/10/2018

4. RESULTADOS Y DISCUSIÓN

El uso de indicadores objetivos para la toma de decisiones de fertilización nitrogenada en el cultivo de arroz es una necesidad y el presente trabajo es una contribución en este sentido. Se generó una base de datos amplia, de tres zafras, en tres sitios y con dos cultivares (subespecie japónica e índica). Se evaluó la MS aérea y la concentración de N en tejido vegetal en diferentes momentos del ciclo, así como rendimiento de grano. Se utilizó una metodología innovadora con modelos suavizados (Cleveland y Loader, 1996) para obtener los Nc que son la base para determinar las CCN.

Las CCN se determinaron a partir de la acumulación de biomasa aérea de cultivos de arroz de alta productividad (11 y 10 Mg ha^{-1} para INIA Merín y Parao, respectivamente) en rotación con pasturas, sembrados en suelo seco con inundación en macollaje, y que se relacionaron posteriormente con acumulación de biomasa en grano. En este contexto, el incremento de la eficiencia de uso del N en el cultivo es clave desde el punto de vista económico y ambiental. Este trabajo contribuye con nuevas herramientas objetivas y prácticas para optimizar la fertilización nitrogenada de cultivo en etapas posteriores al macollaje luego de la inundación.

Las CCN de ambos cultivares presentaron diferencias entre sí. La CCN de INIA Merín (subespecie indica) presentó mayor número de Nc que la de Parao (subespecie japónica, 12 vs. 5 respectivamente), probablemente debido a que contó con una base de datos más amplia. Esto reafirma la importancia del estudio de ajustar la CCN a las características de cada cultivar considerando la interacción con factores de clima, genotipo, idiotipo y ubicación geográfica, entre otros (Ata-Ul-Karim et al., 2013; Justes et al., 1994; Shanyu et al., 2018; Sheehy et al., 1998). Estos conceptos se reafirmaron al comparar las CCN locales con las determinadas en otras regiones del mundo.

El comportamiento de los cultivares utilizados en este trabajo confirmó la estabilidad de su índice de cosecha (Moreira, s. f.). Por tanto, el desarrollo de curvas para maximizar la biomasa aérea se relaciona directamente con la obtención de altos rendimientos.

Los resultados de la validación en la etapa vegetativa y de las curvas de dilución de

N de los tratamientos de mayor rendimiento en INIA Merín confirman la utilidad de la CCN como herramienta para diagnosticar el estatus nutricional del cultivo y estimar sus rendimientos máximos.

Este resultado cobró mayor solidez al relacionar los RR en grano con el INN para cada momento del ciclo del cultivo. En INIA Merín, el INN y el RR en grano aumentaron gradualmente bajo el estado óptimo de N del cultivo. Con base en la correlación positiva entre ambas variables en diferentes etapas de crecimiento, se explicaron las variaciones en el rendimiento en grano bajo diferentes condiciones nutricionales. Se han reportado correlaciones similares a las presentadas en este trabajo (Ata-Ul-Karim et al., 2017), previamente. En el cultivar Parao no existió un buen ajuste entre el INN y RR en la etapa de primordio y 15DDP. Solo en 15DAP, 30 y 45 DDP se observaron ajustes estadísticamente significativos. Los RR máximos se alcanzaron con un INN de 1,1. El RR fue 102 %, 90 % y 79 % para 15DAP, 30 y 45DDP, respectivamente.

Cuando se relacionan las CCN con los indicadores de fertilización objetivos utilizados en el país hasta el momento (PMN en macollaje y absorción de N en planta a primordio floral, Castillo et al., 2014), se observa que ambas estrategias se complementan. Para etapas previas a inundación, donde la CCN no tiene buen ajuste debido a que está determinada para valores de MS mayor a 1 y 2 Mg ha^{-1} para INIA Merín y Parao, respectivamente, la recomendación basada en PMN a macollaje ha demostrado ser robusta. Para la etapa de primordio floral, el indicador absorción de N en planta presenta un nivel crítico para máxima respuesta en rendimiento de 56 kg ha^{-1}. Desafortunadamente, este indicador es poco práctico para ser aplicado en tiempo y forma. En este trabajo se demostró que el intervalo de absorción de N de la regresión para INIA Merín en dicho momento fue de 47-52 kg ha^{-1}, valor similar al nivel crítico empleado en el indicador hasta ahora utilizado. Además, la CCN permite trazar el estatus nitrogenado durante gran parte del ciclo del cultivo para ajustar el suministro con base en la demanda en una ventana de acción más amplia, práctica y en tiempo real.

En Parao, el intervalo de absorción de N de la regresión fue 71-77 kg ha^{-1}, valor que excede el nivel crítico empleado en el indicador hasta ahora utilizado, lo que muestra la variabilidad entre subespecies en cuanto a las necesidades nutricionales durante el ciclo productivo. Esto sugiere que es necesario continuar el ajuste y validación de la CCN como herramienta objetiva para la toma de decisiones de fertilización nitrogenada. El buen ajuste obtenido entre la CCN y el rendimiento del grano indicaría la necesidad de generar modelos para establecer recomendaciones de fertilización nitrogenada precisas y en tiempo real durante el ciclo del cultivo. En este trabajo, se generaron ecuaciones para definir las dosis de N en tiempo real sin utilizar métodos destructivos.

Las diferencias observadas entre los modelos de recomendación de fertilización nitrogenada durante el ciclo de ambos cultivares pueden estar relacionadas a diferencias genéticas entre cada subespecie de arroz que determinan, entre otras, sus tasas de crecimiento y de absorción de N, así como también diferencias en ERAN (Sheehy et al., 1998; Weng y Chen, 1987; Yoshida et al., 2006). En ambos cultivares, las relaciones entre el INN y el RN para 15DAP, primordio floral, 15DDP y 30DDP explicaron satisfactoriamente la variación de requerimientos, ya que permitieron identificar situaciones de nutrición limitante, así como de consumo óptimo y de lujo, resultados similares a los presentados en los trabajos realizados por Ata-Ul-Karim et al. (2017).

Una vez establecidos los modelos matemáticos que permiten ajustar la dosis de fertilización nitrogenada para satisfacer las necesidades del cultivo en función de su estado nutricional actual, es necesario usar sensores para estimar en tiempo real los requerimientos durante el ciclo del cultivo. En este trabajo, al igual que en otros (Gnyp et al., 2014; Lu et al., 2017; Yao et al., 2014; Zhu et al., 2007), el NDVI resultó ser un índice confiable para estimar las variables de interés de ambos cultivares, incluyendo ABSN, INN, MS y N. Las relaciones entre este índice y las demás variables mostraron ajustes satisfactorios durante el ciclo de INIA Merín, con los mejores valores a partir de primordio floral.

En Parao, mientras tanto, se observó mayor variabilidad: las variables no tuvieron ajuste significativo hasta 45DDP. Los resultados menos consistentes en Parao podrían deberse a que la base de datos empleada fue menor al cultivar de subespecie índica y a fallas de algunos de los ensayos experimentales. Resultados similares presentaron

Rehman et al. (2019): la correlación más alta fue observada con ABSN (R^2 = 0,66), seguida por la concentración de N (R^2 = 0,54) y MS (R^2 = 0,51), con un índice útil a bajos niveles de concentración de N, mientras que el NDVI presentó saturación a valores altos (0,76-0,78). En este trabajo, el cultivar indica alcanzó niveles de NNI óptimo con un valor de NDVI en el entorno de 0,7 en todos los momentos evaluados, lo que indica que se pueden estimar valores óptimos de N en el cultivo por debajo de la línea de saturación. El mismo resultado se encontró en el cultivar japónico para los momentos de 15DBP a 30DAP, con un valor promedio de NDVI de 0.77. El buen ajuste del índice observado en este trabajo sugiere que es una herramienta útil para el manejo objetivo de la fertilización nitrogenada del arroz y que necesita validación en el ámbito comercial.

Este trabajo tuvo algunas limitaciones, principalmente en el cultivar Parao. Se utilizó una base de datos más acotada que en INIA Merín, que generaron menos Nc (5 vs. 12). Esto provocó que los resultados para dicho cultivar fueran menos robustos respecto a los de INIA Merín. De aquí la importancia de contar con una base de datos amplia para generar CCN con mayor solidez estadística. Otra limitante fue no contar desde el inicio con un grupo de experimentos independientes para realizar la validación de dichas curvas. Resulta fundamental la investigación para validar dichos modelos en el ámbito comercial para usarse como herramientas objetivas que permitan suplir las restricciones nutricionales en tiempo real, optimizar la eficiencia en el uso del N y maximizar la producción de MS y el rendimiento de grano.

La obtención de modelos de fertilización nitrogenada robustos estadísticamente y la posibilidad de estimar el INN a partir de modelos que utilizan el NDVI abre un nuevo camino de ajuste de la fertilización. Es necesario profundizar en la validación de estos en predios comerciales, así como la implementación de otras tecnologías que permitan tomar la información que brindan las imágenes multiespectrales captadas por satélites o drones para ser empleadas por los productores y actuar en el cultivo en tiempo real.

5. CONCLUSIONES

A partir de una base de datos extensa, se determinaron por primera vez en Uruguay las CCN de dos subespecies de arroz de alto potencial de rendimiento, uno de ellos es el cultivar más sembrado desde el año 2020. Se confirmó la hipótesis del trabajo con la obtención de los Nc para la máxima acumulación de MS en momentos claves del ciclo productivo. Debido a la variabilidad observada en la comparación con las curvas internacionales y las diferencias entre ambos cultivares, queda demostrada la importancia de ajustar curvas de dilución críticas para cada subespecie y región, lo que permitiría manejar con mayor precisión el estado nutricional del cultivo en cada una de las etapas de su ciclo productivo.

En INIA Merín, la validación tanto a partir de MS como del rendimiento en grano permitió discriminar situaciones de nutrición subóptima de óptimas o supraóptimas. Se demostró que la concentración de N óptimo para máxima MS también lo fue para máximo rendimiento en grano. En Parao, la magnitud de los resultados para dicho cultivar fueron menos robustos que en INIA Merín, consecuencia de una estrecha base de datos que hizo que se determinaran menos Nc y tampoco fuera posible validar la CCN.

Se determinaron los RN con base en las CCN y se desarrollaron las ecuaciones que permiten ajustar una dosis de fertilización de N en función del estado nutricional del cultivo, partiendo del conocimiento del INN en un momento del ciclo determinado.

El NDVI determinado por un sensor manual fue un índice robusto para estimar en tiempo real las variables INN, ABSN, MS y % N que, ingresadas en las ecuaciones de RN, permitieron determinar la dosis óptima de N a aplicar al cultivo durante el ciclo.

Es necesario evaluar y validar esta herramienta en el ámbito comercial. La determinación objetiva del RN en tiempo real durante el ciclo del cultivo ajustando la fertilización a la necesidad de las plantas contribuirá a mejorar la eficiencia de uso del N, reducir el riesgo de contaminación ambiental y aumentar los rendimientos del cultivo de una forma práctica y económica de diagnóstico.

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