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Determination of Critical Nitrogen Concentration and Dilution Curve for Rice Growth

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ABSTRACT : Critical nitrogen concentration (Nc), which is defined as the minimum % N in shoots required to maintain the maximum growth rate of top dry weight (W) at any time, was determined for rice plant. Using two rice varietal groups, japonica varieties and an indica x japonica "Dasanbyeo", 18 data points fulfilling the statistical criteria for determining Nc were obtained through eight N-fertilization experiments over two years at Suwon (37°16'N), Korea. Nc dilution curve for each variety was obtained by fitting the Nc-W relationship to power function. However, The critical nitrogen curves for the two variety groups were not different statistically. Thus, a Nc dilution curve was fitted for the Nc data points pooled over the two variety groups and proposed in rice as: Nc=4.08, where W<1.73 t ha^{-1}, $Nc = 5.197W^{-0.4253}$ (R^2=0.964), where 1.73 t ha^{-1}<W<12 t ha^{-1}. The Nc for W<1.73 t ha^{-1} were estimated as a constant value of 4.08%, the mean value of the maximum N concentration for N-limiting condition and the minimum N concentration for N non-limiting condition. The model for Nc is applicable to diagnosing the nitrogen nutrition status during the rice growth period from emergence to heading stage. The Nc curve well discriminated the 144 data points between the N limiting and the N non-limiting groups regardless of varieties, cultural methods, and years.

Keyword : rice, critical nitrogen concentration, critical nitrogen dilution curve

Plant nitrogen concentration has been reported to decrease during the growth cycle by many researchers (Justes *et al.*, 1994; Plénet and Lemaire, 1999; Colnenne *et al.*, 1998; Greenwood *et al.* 1990; Lemaire & Salette, 1984; Duru *et al.*, 1997). With the progress of plant growth, the self-shading of leaves occurs and induces a non-uniform leaf N content from the top canopy layers with high N concentration to the shaded leaf layers with low N concentration (Pons & Pearcy, 1994; Sinclair & Horie, 1989). Even when crops grow with non-limiting N supply, an increase in the proportion of plant structural and storage tissues with a lower nitrogen concentration leads to the decrease of plant N concentration (Caloin & Yu, 1984).

Nc in shoot dry matter is defined as the minimum N concentration required for the maximum plant growth and can be found at any time of plant growth cycle (Ulrich, 1952; Greenwood *et al.*, 1990). Nc decreases with the progress of the plant growth and can be related to W by the following equation (Lemaire & Salette, 1984) :

$$Nc = \alpha W^{-\beta} \quad (1)$$

where W is expressed in t ha^{-1} and Nc in % N. Below this curve N availability becomes a limiting factor for plant growth. Nc dilution curves have been proposed for many crops (Colnenne *et al.*, 1998; Greenwood *et al.*, 1990; Justes *et al.*, 1994; Plénet & Lemaire, 1999), but to our knowledge none for rice. Since plant canopy is not closed, and mutual shading of leaves and competition for light among plants are low during the early stage of growth, the value of in equation (1) remains very low as 0.12~0.15 and during this period Nc takes a constant value (Lemaire and Gastal, 1997). The W below which N_C takes a constant value were reported as about 1.0 t ha^{-1} (Plénet & Lemaire, 1999) 1.5 t ha^{-1} (Justes *et al.*, 1994). This constant value of Nc differs among crops and was reported as 4.4% (Justes *et al.*, 1994; Justes *et al.*, 1997) for wheat, 4.8% for grasslands (Lemaire and Salelte, 1984; Duru *et al.*, 1997), and 3.4% for maize (Plénet & Lemaire, 1999).

The Nc dilution curves for crop could be used to determine the N nutrition status of plant and calculate nitrogen nutrition index (NNI), which quantifies the nitrogen status of the plant (Lemaire *et al.*, 1989). NNI would be used as an intermediate variable in dynamic models to take into account of the effects of nitrogen nutrition status on crop growth and yield (Justes *et al.*, 1997). Jeuffroy and Bouchard (1999) characterized some nitrogen deficiency parameters based on the changes in time course of NNI and estimated the grain number per unit area using the relationship between this parameter and grain number.

The aims of the present study were to determine the Nc along with plant growth and the Nc dilution curve for rice.

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MATERIALS AND METHODS

Field experiments

Eight N fertilization experiments including dry direct-seeding and transplanting rice culture were conducted over two years on sandy loam soil of paddy fields at the university farm of Seoul National University located at Suwon (37° 16'N), Korea. The cultivars used and the application rate and method of fertilizer N were shown in Table 1 and Table 2. In experiments 7 and 8, N fertilizers were split into five times at an interval of 14 days from May 18 to avoid the nitrogen nutrition deficiency, which is different from the conventional fertilizer application method in Korea. In the other experiments, the fertilizers were applied according to the conventional method for rice culture.

In the transplanting experiments the rice seedlings were transplanted at a hill spacing of 30 cm × 15 cm with three seedlings per hill. In the dry direct-seeding experiments, the presoaked seeds (40 kg dry seeds per ha) were seeded on dry soil in rows spaced 30 cm. All the plots were laid out in randomized block design with three replications.

Shoot dry matter was weighed with three hills per each plot sampled at an interval of 10 to 14 days until heading stage and dried at convective oven of 80°C for 48 hours. The dried samples were ground through 40 meshes and determined for total nitrogen concentration with automatic Kjeldahl analysis system.

Data analysis

Nc was determined by the method proposed by Justes *et al.* (1994). The shoot dry matter and shoot N concentration at each measurement date were compared among the N treatment plots with a Student's two-tailed t-test at 10% level of probability in order to separate into two groups of data points: the first group is the non N-limiting group, in which a supplement of N application does not lead to an increase in W, but induces an increase in shoot N concentration, and the second group is the N-limiting group, in which a supplement of N application leads to a significant increase in W with increase in shoot N concentration. For each measurement date, the relationship of shoot N concentration with W was described using two linear lines of (a) a vertical line showing an increase of plant N concentration without any increase in W which corresponds to the first group and (b) a oblique regression line relating the W increase with shoot N concentration increase. Nc was determined as the ordinate of the intersection of the two lines. Most of the Nc data points were obtained from the data sets of experiment 7 and 8. The obtained data points for Nc was fitted to a power regression

Table 2. Summary of nitrogen split application treatments in dry direct-seeding rice culture in 1999.

Treatment	Basal Fertilizer ($kg\ ha^{-1}$)	Tillering Fertilizer ($kg\ ha^{-1}$)	Panicle Fertilizer ($kg\ ha^{-1}$)	Total ($kg\ ha^{-1}$)
1-1	0	0	0	0
1-2	0	0	75	75
1-3	0	75	0	75
1-4	0	75	75	150
1-5	0	75	150	225
1-6	0	150	0	150
1-7	0	150	75	225
1-8	0	150	150	300
1-9	50	75	75	200
1-10†	0			200
1-11‡	0			300

†2.5 kg N/10a was appled eight times at an interval of 10 days from 4-leaf stage to heading
‡3.75 kg N/10a was appled eight times at an interval of 10 days from 4-leaf stage to heading

Table 1. Summary of N fertilization field experiments.

No.	Year	Cultivar	N fertilizer treatments $kg\ ha^{-1}$	Transplanting or seeding date	Sampling times (before heading)
1	1999	Hwasung (Japonica)	0, 75, 150, 200, 225, 300†	5/9(seeding date)	11
2	1999	Hwasung (Japonica)	60, 120, 180, 240, 360‡	5/16(Transplanting date)	9
3	1999	Dasan (Tongil type)	60, 120, 180, 240, 360‡	5/16(〃)	9
4	2000	Hwasung (Japonica)	0, 140, 220‡	5/27(〃)	9
5	2000	Dongjin (Japonica)	0, 140, 220‡	5/27(〃)	9
6	2000	Odae (Japonica)	0, 140, 220‡	5/27(〃)	9
7	2000	Hwasung (Japonica)	120, 240, 360, 480§	5/19(〃)	7
8	2000	Dasan (Tongil type)	120, 240, 360, 480§	5/19(〃)	7

†Nitrogen fertilizer application treatments were shown in table 2.
‡The basal, tillering and panicle fertilizer were applied in the ratio of 40%, 30% and 30%.
§N fertilizer were applied five times at an interval of 14 days from May 18.

equation(1) to get the Nc dilution curve for rice

The Nc-W relationship was validated with the data sets of the remaining experiments according to the method of Plénet and Lemaire (1999).

RESULTS

The Nc points were determined by the intercept between the vertical and oblique lines fitted through the data points for each sampling date until heading stage as shown in Fig. 1 and Fig. 2 for japonica varieties and an indicajaponica variety "Dasanbyeo", respectively,. Among the 70 sampling dates (Table 1), only 18 dates fulfilled the statistical criteria for the determination of Nc. For young stage with W<1.73 t ha^{-1}, only two sets of data were satisfied for the statistical criteria and the Nc were determined as 4.25% for japonicas and 4.14% for an indica × japonica. Generally it is regarded difficult to determine Nc due to the steep slope of the oblique line during the young stage of plant as there is no competition for light and the decrease of Nc with biomass accumulation is small (Justes *et al.*, 1994; Plénet & Lemaire, 1999). The Nc could be considered as a constant value during young stage and positioned between the two nitrogen concentrations: the minimum nitrogen concentration growing under non N-limiting condition and the maximum nitrogen concentration growing under N limiting condition. Thus, the mean value of the two nitrogen concentrations could be taken as the Nc (Justes *et al.*, 1994; Colnenne *et al.*, 1998). In our study the minimum N concentration was 4.27% under N non-limiting condition and the maximum N concentration was 3.89% under N limiting condition, The mean value of 4.08% was very close to the values of 4.25% and 4.14%, which were determined based on the statistical criteria.

Excluding the two data points at young growing stage with W<1.73t ha^{-1}, the eight data points of Nc for each varietal group were fitted to the equation (1) to obtain the Nc dilution curve for each varietal group. The two curves were fitted well with the determination coefficients of 0.9526 and 0.9527, respectively, for japonica and indica × japonica. The two curves were tested for the statistical differences according to Hahn (1997). The obtained F-value of 0.2491 was less than the critical value of $F_{(1,12)}$=4.75 at 5% probability level, indicating that there was no significant difference between the two curves statistically. Thus, the data for the two varietal groups were pooled over to obtain a Nc dilution curve: $Nc=5.19W^{-0.4253}$. This curve showed the good fitting with determination coefficient of R^2=0.964 (Fig. 3). The confi-

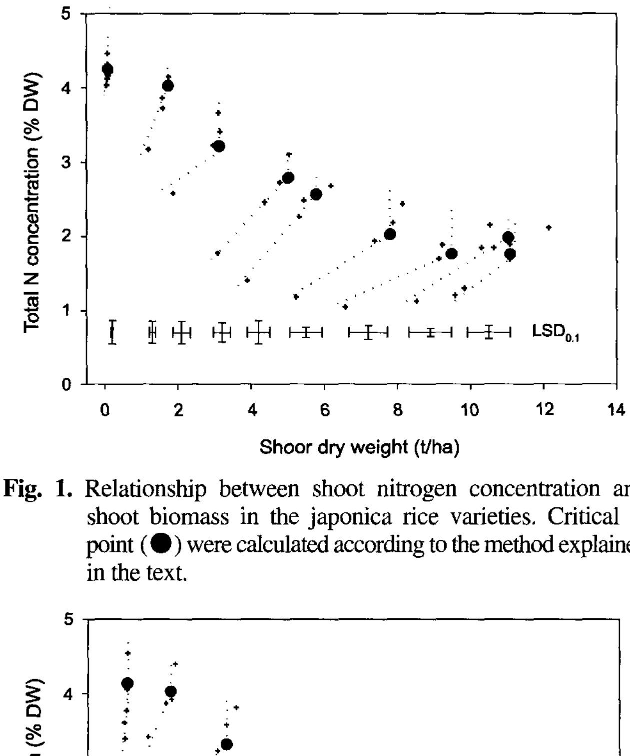

Fig. 1. Relationship between shoot nitrogen concentration and shoot biomass in the japonica rice varieties. Critical N point (●) were calculated according to the method explained in the text.

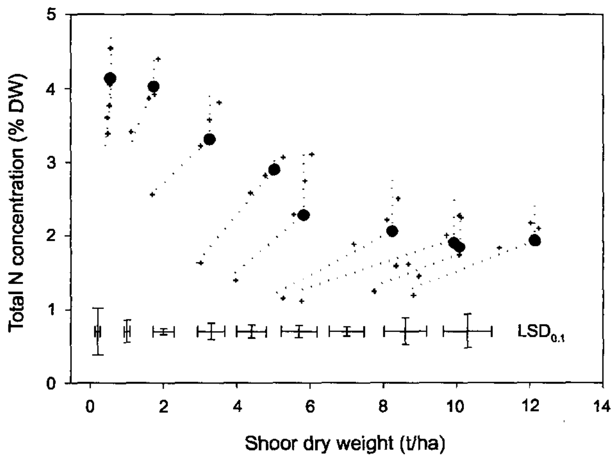

Fig. 2. Relationship between shoot nitrogen concentration and shoot biomass in an indicaxjaponica variety "Dasanbyeo". Critical N point (●) were calculated according to explained in the text.

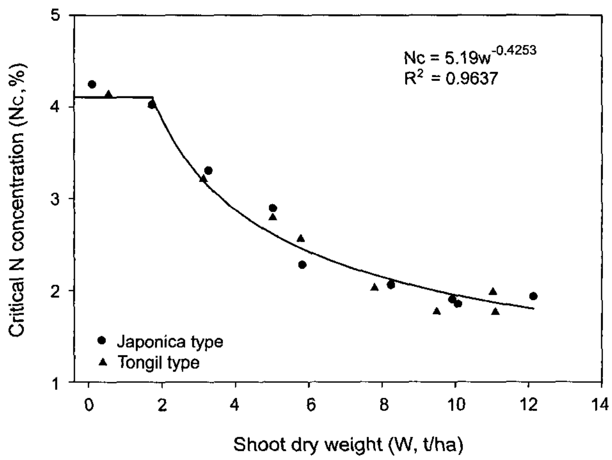

Fig. 3. Relationship between critical nitrogen concentration and shoot dry weight. Data were pooled over japonic and indicaxjaponica varieties of rice.

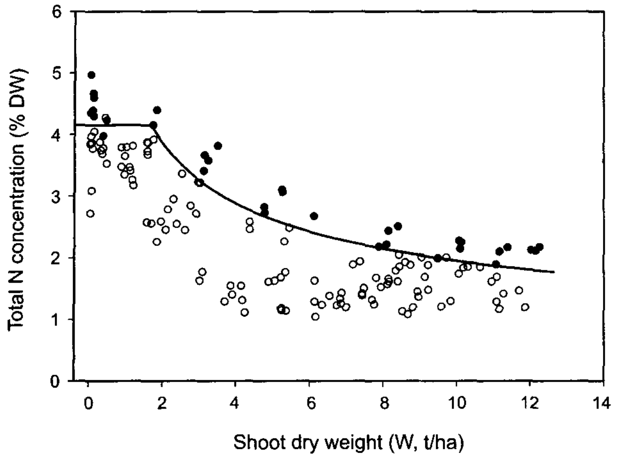

Fig. 4. Validation of the critical N dilution curve. ○ ; N limiting growth condition, ● ; N non-limiting growth condition, — ; critical N dilution curve.

dence interval varied between 3.91~4.32% for W=1.73 t ha^{-1} and 1.76~1.96% for W=11.1 t ha^{-1}. However, this Nc dilution curve is not valid for W<1.73 t ha^{-1} since its extrapolation for the lower W gives much higher Nc than the predetermined Nc, 4.08% for the young growth stage. Thus, the Nc concentration curve can be described as the following two equations:

$$Nc=4.08, \text{ where } W < 1.73 \text{ t ha}^{-1},$$
$$Nc=5.197W^{-0.4253}, \text{ where } 1.73 \text{ t ha}^{-1} < W < 12 \text{ t ha}^{-1} \quad (2)$$

This critical nitrogen dilution curve was validated using the data (Table 1). According to the method of Plénet and Lemaire (1999), the data were classified as either N limiting or N non-limiting groups as in Fig. 4. All the 144 data points were well discriminated between the N limiting and the N non-limiting groups by the Nc dilution curve, confirming the validity of this curve in years, cultural methods, and varieties.

DISCUSSION

Nc dilution curve could be used as a useful tool for the diagnosis of plant N status (Lemaire *et al.*, 1989). The Nc dilution curve for rice was determined and validated with eight series of N fertilization experiments over two years.

During early growth stage with little mutual shading among leaves, plant growth is approximately exponential as the relative growth rate (RGR) is almost constant. Under these conditions RGR is linearly proportional to plant nitrogen concentration and the exponential growth occurs only if plant nitrogen is constant (Ingestad, 1982). In our study, Nc was estimated as a constant value of 4.08% at W<1.73 t ha^{-1}. Nc and the limit values of W below which Nc is constant were reported as 4.4% and 1.55 t ha^{-1} in winter wheat (Justes *et al.,* 1994) and as 4.63% and 1.18 t ha^{-1} for winter oilseed rape (Colnenne *et al.,* 1998). The Nc for rice was estimated a little lower than the reported values for other C_3 crops. During vegetative stage, plant was found to accumulate N in shoot in proportion to green leaf area and take a constant value of about 3 g N m^{-2} of leaf area in wheat and lucerne crops under non-limiting N supply (Grindlay *et al.,* 1993; Sylvester-Bradley *et al.,* 1990; Lemaire & Gastal, 1997). In our study, this value corresponding to the estimated Nc of 4.08% was 3.1 g N m^{-2} of leaf area, conforming the validity of the estimated Nc for rice at W<1.7 t ha^{-1}. The limit value of W for rice was estimated a little higher than the reported values. The leaf area index (LAI) was reported to be about 2.5 when W reached the limit values (Justes *et al.,* 1994; Lemaire & Gastal, 1997). In our study, the LAI corresponding to the estimated limit value of W=1.73 t ha^{-1} was about 2.3 which is very close to the reported values for the other crops.

In dense canopy above the limit value of W, Nc decreases with plant growth and can be related to W by the power equation (1). In our study, the two Nc dilution curves were obtained for japonica varieties and an indica × japonica variety and tested for their statistical difference. No significant difference was found between the two curves statistically. Thus, data were pooled over varieties to obtain a common Nc dilution curve: rm $Nc=5.19W^{-0.4253}$ (Fig. 3). The Nc dilution curve well discriminated the N limiting and non-limiting groups of data regardless of varieties, cultural methods, and experimental years (Fig. 4). Other researchers (Plénet & Lemaire, 1999; Colenne *et al.*, 1998) also reported that a common Nc curve to a crop could be applied under contrasting pedoclimates and cultivars.

In conclusion, a Nc dilution curve for rice was determined and validated through statistical procedures. The curve could be used as a tool for diagnosing the N nutrition status of rice plant from seedling emergence to heading stage. However, the Nc curve cannot be used during the grain filling period as the nitrogen redistribution among organs occurs and should be determined newly.

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