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Effect of Nitrogen on Cell Dynamics at Leaf Growth Zone in Two Rice Varieties

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ABSTRACT : In plants, nitrogen is the major component for growth and development. Leaf growth is based on the division, elongation and maturation of cells, which are used for making of epidermis, mesophyll, bundle sheath, xylem, phloem and so on. Dynamics of these tissues with respect to nitrogen are required for better understanding. This experiment was conducted to evaluate effect of nitrogen on the elongation of epidermal and guard cell of two rice (*Oryza sativa* L.) varieties, Seoanbyeo and Dasanbyeo on May 2000 at Chungbuk national university in Cheongju. After transplaning the 20-day-old seedlings into a/5000 pots, the main characteristics related with cell elongation were investigated and evaluated. A maximum leaf length reached at 7 or 8 days after emergingfrom the collar, and also the leaf elongation rates were greatly affected by the increase of N application rate. The initial and final cell length were about 17 μm and 130 μm, respectively. Cell divisions occurred within 1.0mm from leaf base. With the higher nitrogen application rate of 22 kg-N 10a^{-1}, cell division per hour was greater 1.5 to 1.9 and 1.2 to 1.3 fold as compared to the N application rate of 0 and 11 kg-N 10a-1, respectively. Cell enlargement of epidermal and guard cell under higher N application rate (22kg-N 10a^{-1}) was finished within about 20 (Seoanbyeo) and 15 hours (Dasanbyeo), while it took much time, about 30 hours.

Keywords: leaf growth, cell dynamic, nitrogen, rice

Plant growth is based on cell division, expansion and maturation. In both experimental (Barow, 1976; Rost *et al.*, 1988) and theoretical (Gardar, 1980; Gardar *et al.*, 1988; Silk, 1984) works, careful delineation of spatial and temporal aspects of growth has permitted a better understanding of functioning of meristem and elongation regions (Silk *et al.*, 1989). Leaf growth responses to environmental change are complex, being completely affected by ontogeny. Part of the rationale for studying leaf growth lies in its importance for determining crop growth and productivity (Thornley, 1991). Vegetative growth of plants consists of shoots and roots which can be considered in terms of the transport and utilization of two major substrates, carbon and nitrogen.

In monocot, the leaf growth zone contains regions of cell division, elongation and maturation which are located at the base of elongating leaves and are enclosed within a whorl of encircling leaf sheaths (Esau, 1965; Volenec *et al.*, 1984a). Leaf growth in grasses is predominantly unidirectional, parallel with the longitudinal axis of the leaf (MacAdam et al., 1989). Epidermal cell divisions occur at approximately 1 to 2 mm above the ligule (MacAdam *et al.*, 1989; Volenec *et al.*, 1981), whereas mesophyll cell divisions occur at about 10 mm distal from the ligule. MacAdam *et al.*, (1989) observed the ratio of mesophyll cells to adjacent epidermal cells was 1 : 1 near the leaf base, while the ratio was 1 : 10 to 15 at the distal portion of leaf growth zone.

Nitrogen is the element required in greatest quantity except for carbon, and usually is a key limiting factor for crop growth. Nitrogen fertilization increased leaf elongation rate due mainly to an increase in cell production. Nitrogen did not affect final size of epidermal cells or relative cell elongation rate (MacAdam *et al.*, 1989; Song, 1993; Volenec *et al.*, 1984b).

Therefore, in this study, we examined the effect of nitrogen supply on cell dynamics in meristem and leaf growth in rice plant.

MATERIALS AND METHODS

Plant material and nitrogen supply

The experiments were carried out in a controlled environment growth chamber of Chungbuk national university, Cheongju, South Korea in 2000. Two rice varieties, Seoanbyeo and Dasanbyeo, were used. Twenty days rice seedlings were transplanted into a/5,000 pots. The treatments consisted of three levels of N (0, 11, 22 kg 10a^{-1}). At each level, urea was split-applied at three times. Phosphorus was applied at 7 kg 10a^{-1} as basal dressing and potassium was split-applied at 8 kg 10a^{-1}.

Sample preparation and microscopic observation

To observe growing pattern and length of cell, cell divi-

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sion and elongation zones were carefully excised. The dissected samples were placed in 0.02% Acridine orange (Sigma, USA) in dH_2O for 10min at 20°C, and then immediately rinsed out at three times in dH_2O. Sections were cut 40 mm length with glass knife on slide glass and observed using Confocal Microscope (MRC-1024, Bio-Rad, UK) equiped with an image analyser. Epidermal and guard cells were captured at a constant distance, 1, 5, 10, 20, 25, 30, 35 and 40 mm, from the base. Images of these cells were used for the growth analysis of rice leaf.

Growth analysis

After transplanting, the daily growing rate of new leaves was measured at the fixed time on everyday. Based on cell size and elongation rate, the cell expansion time and cell division rate were calculated at a constant interval. Daily leaf growth rate (LER) was determined through the check every 24 hours. Velocity of displacement (V_x) of epidermis was determined from the following equation (Carmona Cuadrado. 1986; Gandar Hall. 1988; Silk *et al.* 1989):

$V_x = LER\ (L_x/L_f)$

Where:

Vx (mm h^{-1})=displacement velocity at position×mm from the apical initial

LER (mm h^{-1}) = leaf elongation rates

L_x (μm) = cell length at×mm position from the leaf apical initial

Lf (μm) = final length of epidermis.

Time of cell elongation(t_x) was estimated from following equation(MacAdam *et al.* 1989., Pearen. 1991): $t_x = S/V_x$

Where : t_x(h) = time of cell elongation, S(μm) = distance of cell movement in the growth region. The cell flux(F_x) was calculated according to the following equation(Silk *et al.* 1989): $F_x = LER/L_f$

RESULTS AND DISSCUSSION

Analysis of leaf growth

The emerging interval of a new leaf blade in rice (*Oryza sativa* L.) was known as about 7 to 8 days. Leaf elongation rates of two rice cultivars were generally stimulated with increasing N levels (Fig. 1). When the leaf growth rates of both rice cultivars were maintained rapid and linear tendency, the leaf elongation rates of Seoanbyeo, Japonica type, were about 1.6, 2.5 and 3.0cm per day with 0, 11, and 22 kg-N, respectively. In case of Dasanbyeo, Indica type, they were about 2.0, 3.5 and 4.0 cm per day, respectively. A leaf growth was very rapidly increased for 7 to 8 days after the leaf protruded above the collar of previous leaf, gradually slowed down for 2 to 3 days, and stopped. Comparing with

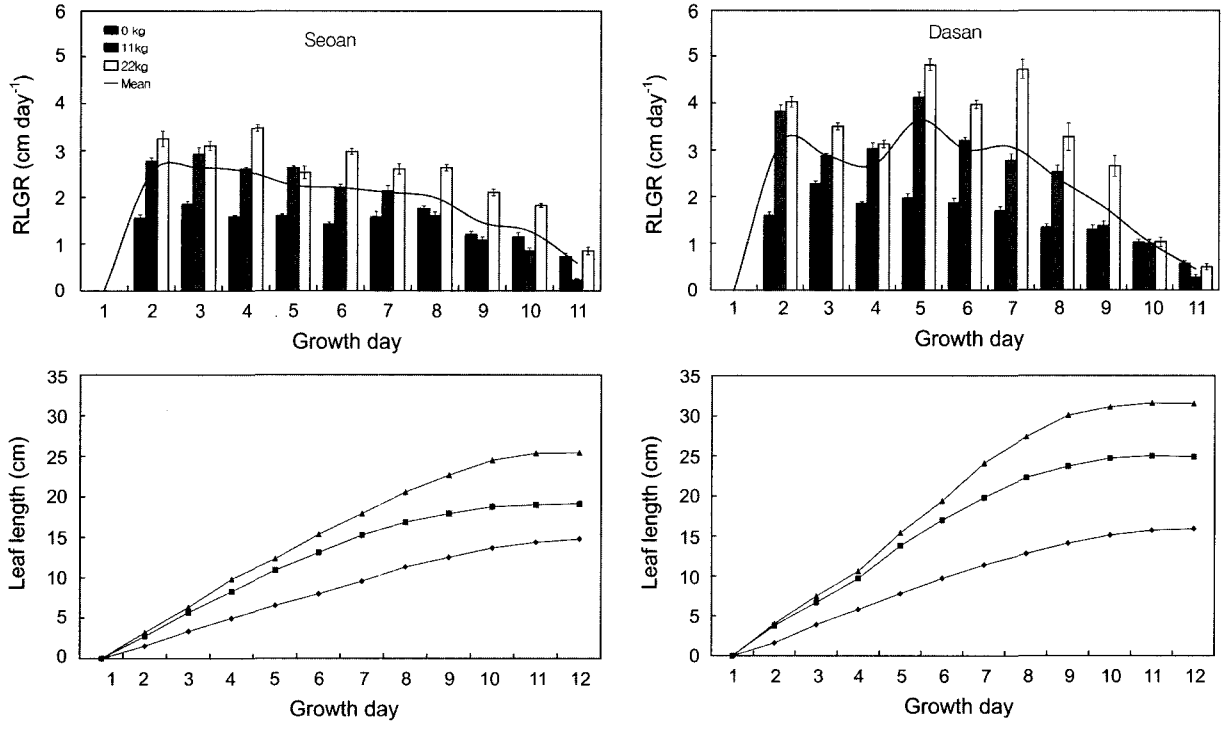

Fig. 1. Daily leaf elongation rate (upper) and accumulated leaf length (lower) of two rice cultivars, Seoanbyeo and Dasanbyeo with different N supply. RLGR is estimated with every 24 hrs interval. All data are means with S.D. (n=9).

previous report (Song, 1995) which a new leaf growth of two rice cultivars, Hwaseongbyeo and Taebackbyeo, was rapidly increased for 3 to 4 days, and then its growth was gradually reduced, the emergence frequency of a new leaf was considered to be strongly dependent on the innate characters of cultivar and variable cutivation conditions. When the leaf growth slowed down, a new leaf come out and this growth patterns have been continued until the end of vegetative growth.

Elongation and anatomy of cell

Epidermal cells were rapidly divided at the leaf meristem, and then elongated toward (Fig. 2). The initial cell length was about 17 μm, the mean both cultivars and N levels, whereas the final cell length was about 130 μm (Fig. 3). The epidermal cell was mainly elongated in about 15 to 20 μm from leaf base. Leaf cells showed similar anatomy and growth patterns for both cultivars and N levels. Epidermal and guard cell elongated sigmoidally by about 130 μm and 34 μm long, respectively. Cell division zones of the epidermal cell could be assumed as being occurred within 1.0 mm from leaf base, and thus active elongation begun at a distance of 1.0 mm. In previous studies (Jensen *et al.*, 1958; Luxova *et al.*, 1973; Webster, 1980) with root tissues, epidermal cells continued division beyond 1.0 mm, although their maximum size reached to about 40 to 50 μm length. It was demonstrated that resultants for this experiment are similar to previous studies which have different cell types. Our data were different in final cell lengths of epidermal and guard cell, although the initial cell lengths of each cell type was quite similar. Further, final cell lengths, which mean the

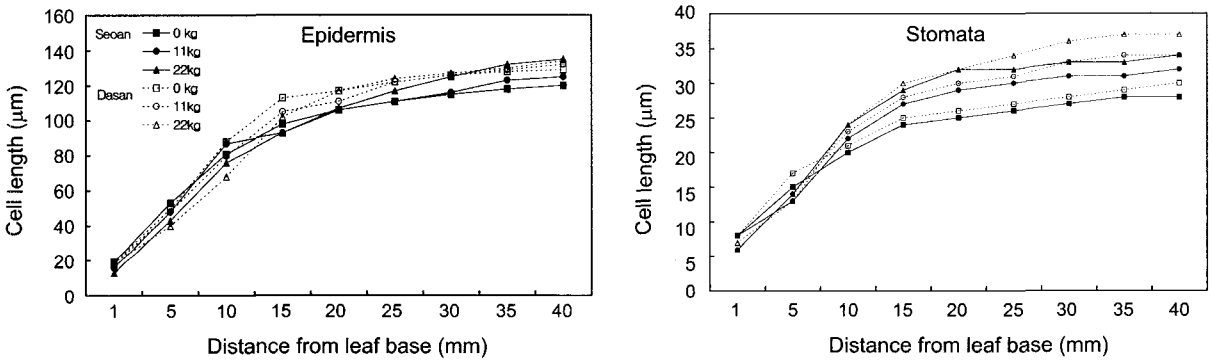

Fig. 2. Length changes in epidermis and stomata with constant distance from base in leaf growth zones of two rice cultivars grown under three N supplies. All data are mean S.D.(n=10).

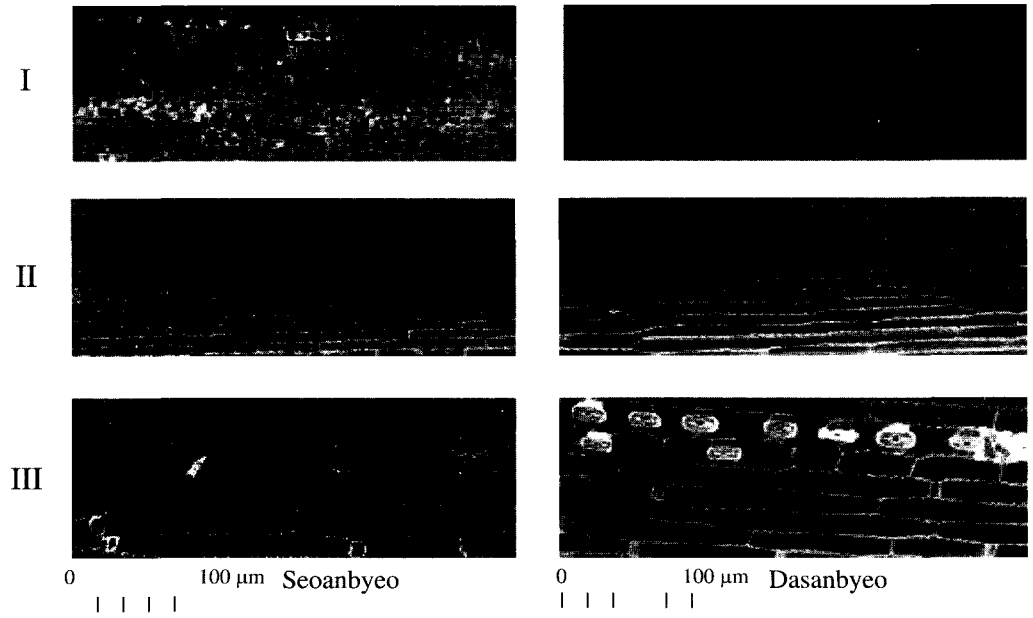

Fig. 3. Longitudinal section of cell division zone (I), cell elongation (II) and cell maturation region (III) of two rice cultivars. Photograph is 200X. The sections are leaf surface grown with 11kg $10a^{-1}$ N.

expanding potential and energy metabolism, for each cell type were affected greatly by N application. Also, leaf growth was dependent on cell flux and rates of cell elongation rather than initial or final cell size.

Cell dynamics

Initial length of epidermal and guard cell was slightly longer at Non-N application than at 11- and 22 kg-N (Table 1). Final cell length, however, showed a different elongation pattern that enhanced by increasing N application. Growth patterns of these cells are positively correlated with N application. Due mainly to the reflection in the relative cell elongation rates, the fastest leaf elongation rate occurred at 22 kg-N. With the higher nitrogen application rate of 22kg-N $10a^{-1}$, cell division per hour was greater 1.5 to 1.9 and 1.2 to 1.3 fold as compared to the N application rate of 0 and 11 kg-N 10a-1, respectively. In each cultivar, the number of cell division of Dasanbyeo with N application was superior to Seoanbyeo. High N application caused a persisting cell division and a stimulation in cell elongation, whereas cell growth at the distal portion of the growth zone and final cell

Table 1. Parameters of cell dynamics on the leaf growth of two rice cultivars with three N levels.

Varieties	N levels	RLER[a)]	ICL		FCL		CF	TCE
			EC	GC	EC	GC	EC	EC
	kg $10a^{-1}$	mm h^{-1}	---------- μm ----------				cells h^{-1}	h
Seoanbyeo	0	0.66	19	8	122	28	5.4	61.5
	11	0.82	16	8	125	32	6.6	48.9
	22	1.07	13	6	135	34	7.9	37.6
Dasanbyeo	0	0.73	19	8	130	30	5.6	56.0
	11	1.10	18	6	133	34	8.3	36.9
	22	1.43	19	7	134	37	10.8	28.5
LSD (0.05)	V	**	*	ns	*	**	*	*
	N	**	ns	ns	*	**	**	**
	V×N	ns	ns	ns	ns	ns	ns	ns

[a)]RLER :Relative leaf elongation rate; ICL:Initial cell length; FCL:Final cell length; CF:Cell flux; TCE:Time of cell elongation; EC:Epidermal cell; GC:Guard cell

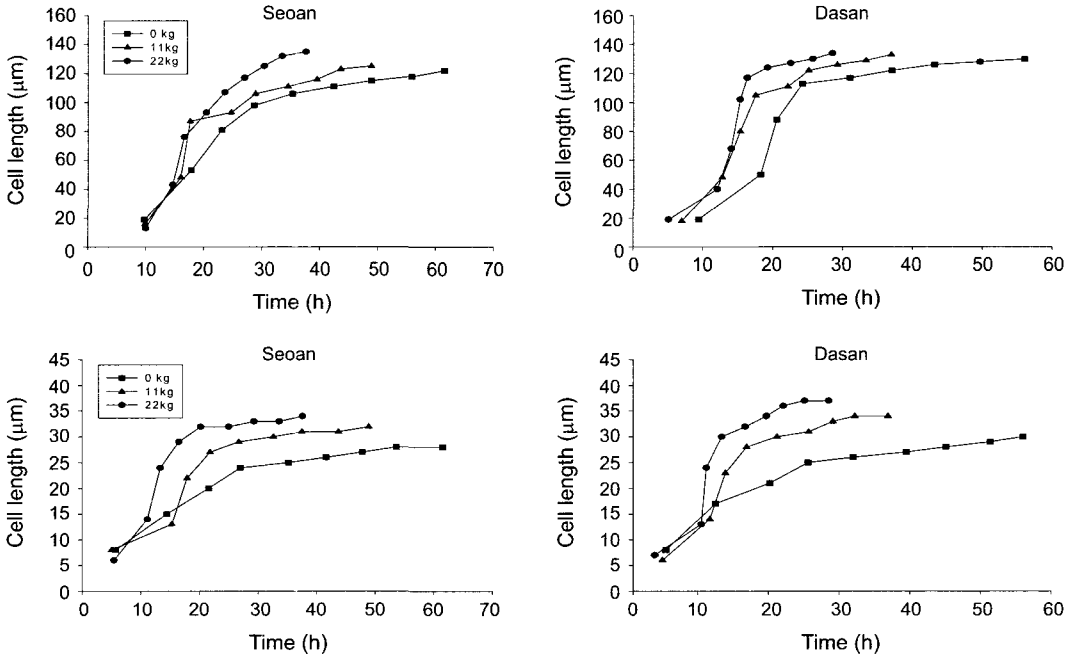

Fig 4. Relative cell elongation rates for epidermal (upper) and guard (lower) cell of two rice cultivars, Seoanbyeo (left) and Dasanbyeo (right), subjected to three N application rate. Cell length data were fitted with an exponential function to estimate cell elongation rate.

length were not greatly affected by N levels. Cell elongation rate was calculated by plotting the natural logarithms for cell length vs. duration of cell elongation. Expansion of epidermal and guard cell was much slower until cell length reached about 40 μm and 15 μm, respectively, expansion rate was suddenly increased and then cells quickly reached final length. At 22 kg-N, cell enlargement of epidermal and guard cell completed within about 20 (Seoanbyeo) and 15 hours (Dasanbyeo), whereas it took much time, about 30 hours, to complete cell maturation at non-N and 11 kg-N applications. Our data that leaf elongation and cell division were promoted by increasing N application is similar to some previous reports, but the effect of nitrogen on the root elongation was a negative. The leaf growth rate is stimulated by increasing of N application, while the root growth rate was reduced. In the vegetative growth stages of plants, plant organs could be separated tinto source and sink tissues. The sink tissues are the growth zones of leaf and root tips, whereas the source tissues are the mature leaf blades and stems. Different plant organs have their capacities to store and utilize the carbohydrates and the N compounds (Volenec & Nelson, 1984; Spollen & Nelson, 1988).

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