Spatial Distribution of Growth and Cell Elongation in the Elongation Zone of Perennial Ryegrass Leaves as Affected by Growth Condition and N Fertilization Sung Seo

# Perennial ryegrass 잎에서 生育條件과 窒素施肥에 따른 伸長部位의 空間的 移動과 細胞伸長

徐 成

# 摘 要

다른 生長條件(growth chamber, outdoor) 과 窒素施肥條件(NO, N60kg ha<sup>-1</sup>)에서 자란 perennial ryegrass 잎에 있어서 葉伸長率(LER)과 伸長部位의 空間的 移動 및 細胞의 伸長등을 알아보고자, 잎 基部내伸長部位의 0~30mm에 pin 처리를 하고 일정시간후 pin hole의 이동거리로서 生長을 조사하였으며 표면복제방법에 의해 細胞의 길이를 측정하였다.

Growth chamber에서 자란 목초의 LER은 25.2mm day<sup>-1</sup> (outdoor 목초에 비해 54.6% 증가), 생육의 공 간분포는 基部로부터 24mm까지, 최대생육은 10~13mm에서, 세포신장은 20mm 까지로 관찰되었다. Outdoor 목초는 LER 16.3mm, 생육분포는 17mm까지, 최대생육은 5~8 mm에서, 세포신장은 14mm까지였다.

질소시비구 목초의 LER은 30.3mm day<sup>-1</sup>(무시비구 목초에 비해 61.2% 증가), 생육분포는 基部로부터 27 mm까지, 최대생육은 13~15mm에서, 세포신장은 21mm까지였으며, 질소무시비구 목초는 LER 21.3mm, 생육분포는 21mm까지, 최대생육은 8~11mm에서, 세포신장은 16mm까지였다.

Ligule 부위의 세포길이는 현저히 짧아졌으며, LER이 빠를때 伸長部位내 生育의 移動速度도 빨랐다.

## I. INTRODUCTION

Forage yield of grasses is primarily determined by the growth of leaves during growing season. Moreover, yield can be divided into two components: the number of tillers per unit area of land and the yield per individual tiller. Characteristics of yield per tiller have been correlated positively with rapid leaf area expansion rate (Nelson et al., 1977), and sward yield once equilibrium between new tiller production and tiller death has been reached (Zarrough et al., 1983).

Also leaf elongation rate (LER) was closely correlated to grass yield in vegetative growth stage (Horst et al., 1978), which suggested LER would be a useful selection criterion for increasing yield. It has been well-documented

that the growth of tiller positively depends on environmental conditions during growing season, ie. light, water deficit, temperature, nitrogen(N) and other nutrient supplying.

Recently Volence and Nelson (1981) reported that LER and elongation of epidermal cells in two genotypes of tall fescue tiller, also LER and growth distribution in the elongation zone of leaves were much influenced by tiller vigor (Seo, 1988), and LER, cell production and cell length of tall fescue were influenced by N fertilization and harvest frequency (Volenec and Nelson, 1983; MacAdam et al., 1985). Up to date, however, little information is available about growth distribution and cell dynamics of grass leaves.

The objective of the present study was to obtain basic understanding on leaf growth

of perennial ryegrass, ie. to determinine LER, spatial distribution of growth, velocity of displacement and cell elongation in the elongation zone of leaves grown at different growth condition and N fertilization.

## II. MATERIALS AND METHODS

## 1. Plant material and growth conditions

Perennial ryegrass (Lolium perenne L.) was planted on September 7, 1986. The plants were propagated vegetatively in greenhouse (Bonn Univ., FRG) to maintain same genotype. On March 24, 1987 vegetative tillers cut at 12cm height were transplanted into small pots (4.2cm diameter x 20.0 cm height). Soil in pot was saturated with enough water, periodically, and saturated one time with 10ml of nutrient solution of N (50), P (33) and K (40 kg ha<sup>-1</sup>), respectively.

On June 10 plants were cut at 10cm height and transferred to a controlled environment chamber, and grown at continuous light intensity (120µmol.m<sup>-2</sup>.s<sup>-1</sup> PPFD), constant air temperature of 20°C, and constant relative humidity of 80% (Seo, 1988). To acclimatize the plants were grown for two weeks in the growth chamber. Thereafter, experiment was performed.

In experiment 1 plants were grown at growth chamber as described earlier continuously, and grown at outdoor condition (mean temp. was approximately 24°C, max. temp. was 28 to 30°C) after acclimatizing. In experiment 2 plants were grown with N (60kg N ha<sup>-1</sup>) and without N fertilization (N-O) at growth chamber during the experimental period. Before the commence of experiment the tillers were selected with similar length of leaf sheaths.

## 2. Leaf elongation measurement and pinning

The length from leaf tip to top of sheath of next older leaf was measured daily with a ruler. Leaf elongation rate (LER) was determined for five days by the difference in length per day. Fifteen pin holes were made at every 2 mm from leaf attachment to 30 mm along elongation zone of each tiller, equi-distantly (Seo, 1988).

Approximately 6 or 24 hr after pinning the tillers were removed from the plants. Distances between holes were measured with an 0.1 mm calibrated ocular (x8, FRG), first in the outermost nongrowing sheath and then, after expose, in the elongating leaf. The former values were considered as initial distance between holes.

# Surface replicas techniques and measurement of epidermal cell length

The 30 mm of the elongation zone in each tiller was placed on the glass slide. Using a razor the elongation zone was splited into 6 segments with 5 mm length, then Formvar solution (4% in chloroform; Bucken, 1987) was treated with 1 to 2 drops on the each segment for replica the epidermal cells. A few minutes later the solution was coagulated and formulated a thin negative film (Reimer and Schulte, 1973). Lastly the surface film replicated was taken off from the plant tissues carefully with a tweezers and razor and was transferred directly onto the slide for observation and scanning of the epidermal cells on the microscope.

For measuring of cell length the IBAS (Interactive Image (Bild) Analysis System, Kontron Co., FRG, 1982) apparatus was used. Briefly, through the video attached to microscope and videorecorder, the figures of the cells were represented on television and control monitor. Cell length was measured 1 or 2 mm along the replicas film of the elongation zone, also cell length of ligule position and final cell length were observed.

# III. RESULTS AND DISCUSSION

# 1. Effect of growth conditions

Leaf elongation rate (LER), spatial distribution of growth, velocity of displacement and length of epidermal cells in the elongation zone of perennial ryegrass leaves as affected by growth conditions are shown in Fig. 1, Fig. 2 and Fig. 3.

LER of 25.2 mm day<sup>-1</sup> grown in a growth chamber was 54.6% higher than that grown at outdoor condition (Fig. 1). Growth occurred over a great distance in the leaf base grown at growth chamber. The growth was terminated at approximately 24 mm above the ligule attachment. But growth at outdoor condition was terminated at 17 mm above attachment in the elongation zone.

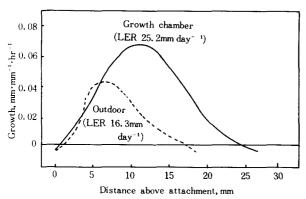


Fig. 1. Spatial distribution of growth in the elongation zone as affected by growth condition

The maximum growth rate was observed at 10 to 13 mm (ca 0.07 mm·mm<sup>-1</sup>·hr<sup>-1</sup>) and 5 to 8 mm (ca 0.045 mm·mm<sup>-1</sup>·hr<sup>-1</sup>) above attachment grown in a growth chamber and outdoor condition, respectively. Length of elongation zone of high LER grass was longer than that of low LER grass as reported by Volenec and Nelson (1981) and Seo (1988).

Growth condition resulted in different velocity curve of displacement, although the both curves showed sigmoidal forms (Fig. 2). In the basal part of the elongation zone, the velocity was a little slow, then increased re-

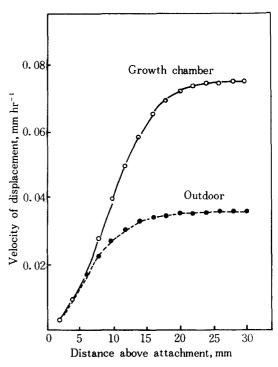


Fig. 2. Velocity of displacement in the elongation zone as affected by growth con dition

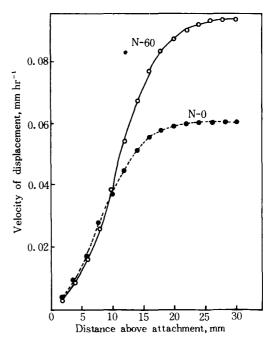


Fig. 5. Velocity of displacement in the elongation zone as affected by N fertilization (kg ha<sup>-1</sup>)

markably along the elongation zone under both growth conditions, and then the velocity was nearly stopped at about 23 to 24 mm and 17 to 18 mm above attachment grown at growth chamber and outdoor, respectively. These results compares closely with the data of Schnyder and Nelson (1987).

Length of epidermal cells was almost linearly increased along the elongation zone, regardless of growth condition (Fig. 3). The length of final epidermal cell, in this experiment, was 500 to 550 $\mu$ m, and the length of 500 $\mu$ m could be considered as the length of adulted cell in perennial ryegrass leaves. Therefore, cessation of cell elongation occurred at 20 mm and 14 mm above attachment in the elongation zone in growth chamber and outdoor, respectively. Cessation positions of cell elongation were a little shorter than position of growth cessation is growth distribution.

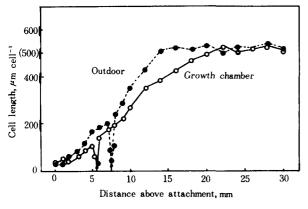


Fig. 3. Length of epidermal cells in the elongation zone as affected by growth condition (†; Position of ligule)

High LER grown at growth chamber showed longer cell elongation zone than low LER leaves. Similar result has been reported by Volenec and Nelson (1981). In both growth condition, cell length was rapidly decreased at the position of ligule, and then was significantly increased immediately. The length of ligule cell was only a little longer than that of attachment cell.

#### 2. Effect of N fertilization

Leaf elongation rate, spatial distribution of growth, velocity of displacement and length of epidermal cells in the elongation zone of perennial ryegrass leaves as affected by N fertilization are shown in Table 1, Fig. 4, Fig. 5 and Fig. 6.

LER of 30.3 mm day<sup>-1</sup> with N (60 kg ha<sup>-1</sup>) was 61.2% higher than that of non-N treatment, and the LER was changed by the length of elongating leaves (Table 1), ie. LER in 10 to 20 cm of elongating leaves was the fastest, then in 0 to 10cm length, but LER over 20 cm of length was the lowest (Seo, 1988). It was been reported that high N doubled LER (MacAdam et al., 1985) and LER was increased by 89% with 336 kg N ha<sup>-1</sup> compared to grasses receiving 22 kg N (Volenec and Nelson, 1983).

Table 1. Leaf elongation rate (LER) of perennial ryegrass as affected by N fertilization and length of elongating leaf

N, kg ha <sup>-1</sup>	Length of a elongating leaf, cm			Mean
	0-10	10 - 20	over 20	Mean
		— LER, m	m day-1	
N-60	31.4ª	34.3ª	25.2ª	30.3ª
N-0	21.3b	21.3 <sup>b</sup>	13.8 <sup>b</sup>	18.8 <sup>b</sup>

<sup>\*</sup>Different superscripts represent significant difference at the 0.05 level, with same column

In 10 to 20 cm length of elongating leaf, LER of 34.3 mm day<sup>-1</sup> with N-60 had a great distance of growth up to 27 mm above attachment in the elongation zone. The maximum growth rates with and without N were observed at 13 to 15 mm (ca 0.09 mm·mm<sup>-1</sup>·hr<sup>-1</sup>) and 8 to 11 mm (ca 0.07 mm·mm<sup>-1</sup>·hr<sup>-1</sup>) above attachment, respectively. Similar results have been obtained in tall fescue by Volenec and Nelson (1983). The velocity of displacement with N-60 was very fast, and nearly stopped at 25 to 26 mm (Fig. 5), while that of non-N treatment was stopped at 19 to 20 mm above

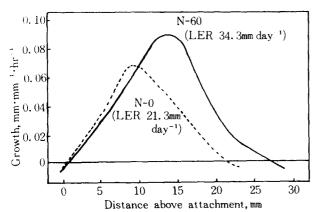


Fig. 4. Spatial distribution of growth in the elongation zone as affected by N fertilization (kg ha<sup>-1</sup>)

attachment in the elongation zone.

Length of epidermal cells declined a little at between 1 to 3 mm above attachment, near the meristem, where cell division was presumably occurring and cell elongation had not commenced (Volenec and Nelson, 1981). Thereafter, cell length was linearly increased along the elongation zone, regardless of N fertilization (Fig. 6).

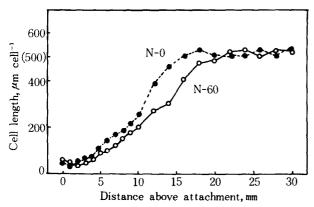


Fig. 6. Length of epidermal cells in the elongation zone as affected by N fertilization (kg  $ha^{-1}$ )

As shown in Fig. 3 the length of final cells was 500 to 550 µm. In this experiment the cessation of cell elongation with and without N occurred at 21 mm and 16 mm above attach-

ment in the elongation zone, respectively. Lengths of mature epidermal cells were similar for both growth condition and N treatment (MacAdam et al., 1985; see Fig. 3 and Fig. 6).

High LER grown with N showed longer cell elongation zone above attachment when compared to low LER grass receiving no N. Volenec and Nelson (1983) have also reported that length of leaf intercanary meristems receiving low level of N tended to be shorter than that receiving high level of N. However, MacAdam et al. (1985) have reported that N had little influence on length of the cell elongation zone.

High LER plants could be obtained when grown at optimum growth condition and with N, which may be due to longer length of the cell elongation zone, vigorous cell production (Volenec and Nelson, 1983), and long cell division zone (MacAdam et al., 1985) under better condition. In this experiment, LER of grass leaves such as perennial ryegrass is easily modified by management practice and/or environmental conditions. Modifications in LER are related to cell division and cell elongation is the elongation zone of grass leaves.

## IV. SUMMARY

Investigations were performed to understand leaf growth better. The purpose of this experiment was to determine leaf elongation rate (LER), spatial distribution of growth and cell elongation in the elongation zone above attachment of perennial ryegrass leaves grown at different growth condition (growth chamber and outdoor) and N fertilization (N-O and N-60 kg ha<sup>-1</sup>).

LER of 25.2 mm day<sup>-1</sup> grown in growth chamber was 54.6% higher than that of grown at outdoor. Growth was terminated at about 24 mm and 17 mm, maximum growth rate was observed at 10 to 13 mm and 5 to 8 mm, and cessation of cell elongation occurred at

20 mm and 14 mm above attachment in the elongation zone at growth chamber and outdoor, respectively.

LER of 30.3 mm day-1 with N was 61.2% higher than that of non-N fertilization, and LER responded differently by length of elongating leaves. Growth was terminated at about 27 mm and 21 mm, maximum growth rate was observed at 13 to 15 mm and 8 to 11 mm and cessation of cell elongation occurred at 21 mm and 16 mm above attachment in the elongation zone grown with and without N, respectively.

Length of epidermal cells was linearly increased along the elongation zone. The length of epidermal cells, however, was very short in the position of leaf ligule, and the growth velocity of displacement was fast when LER was high.

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