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Seasonal Changes of Nodule Activity, Carbohydrates and Nitrogen and their Inter-relationships in Alfalfa

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알팔파根瘤의 窒素固定活性과 體內炭水化物 및 窒素含量의 年中變化와 이들의 相互關係

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ABSTRACT

Alfalfa field was established to investigate seasonal changes of nodule activity and contents of carbohydrates, and nitrogen, and also to examine their relationships in alfalfa (*Medicago sativa* L.). Periodical sampling of alfalfa in cutting and uncutting plots was collected to measure growth of plants, development and activity of nodule, and content of carbohydrates and nitrogen in the third year following year of establishment.

Nodule activity of alfalfa root appeared to early April, increased to a maximum in beginning of June (flowering stage), and then decreased and generally remained low from late-July to mid-August, and again increased from early September, and then decreased and generally remained low for the rest of growing season. After flowering, nodule weight tended to decrease slowly throughout the growing season.

Until flowering stage, increase of dry weight was closely related with that of nodule activity. But after flowering the curve pattern of dry weight did not fit to that of nodule activity due to decrease of supply of assimilate to nodule, drought, and high temperature.

Total nonstructural carbohydrates in roots were closely correlated with nodule weight and nodule activity. While, nitrogen contents in leaves were closely correlated with nodule weight and nodule activity. Also cutting on July prevented unnessesary losses of respiration during summer to provide rapid recovery of nodule activity.

INTRODUCTION

Alfalfa, sometimes called the "Queen of the Forages", is one of the most important forage plants. It contains high digestable nutrients and has been used for hay, silage and pasturage. Alfalfa has not been cultivated widely in Korea. It has been perceived that most of field soil in this country was highly acid and this soil condition had bad influence on the growth and nitrogen fixation of alfalfa. Alfalfa researches in Korea have been conducted on growth, development,[9] herbage production,[10] and rhizobium inoculaton effects.[9] There are few data about seasonal changes of nodulation and nodule activity, which are based on the establishment of management practices and selection of breeding objectives.

Nitrogen fixation is known to be related with the availability of photosynthates and environmental conditions. Generally, nodule activity decreases at the time of the flowering and the sub-

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sequent pod filling, and above or below the optimum temperature[4,17]. The seasonal pattern of acetylene reduction activity was clearly correlated with dry weight and photosynthetic area.[17] The increased weight of top and root and nodule mass were associated with increased N_2-fixing activity.[3] It has shown that temperatures above or below the optimum for N_2-fixation had a greater effect on N_2 fixation than on legume growth.[17]

High demand for carbohydrate in symbiotic N_2 fixation process has been documented. Pate[13] explained that the flux or supply of assimilates from the shoot to the root nodules was considered to be one of the primary limiting factors in N_2 fixation. Barta[1] proposed that the degree of nodulation and nitrogen fixation by plant was governed by its internal carbohydrate: nitrogen ratio. Seasonal change in nodule activity can give us data with which we can overcome the disadvantageous conditions and maximizes nitrogen fixation of nodule. The objectives of this experiment were to examine the seasonal changes of nodule activity, contents of carbohydrates and nitrogen and also to evaluate their inter-relationships in Korean environmental condition. Furthermore, we determined the effects of cutting management on the above parameters.

MATERIALS AND METHODS

This experiment was conducted at the Experimental Farm of the Agricultural College, Seoul National University, Suwon in 1982.

(1) Field condition; Alfalfa field was established with cultivar Luna in the fall in 1980. Basal application of P(10kg/10a) and K(10kg/10a) was broadcasted over the plot area. The soil property before experiment was characterized by acid soil with low cation exchange capacity and low organic matter content.

(2) Treatments; (i) Uncutting control (ii) Cutting: Alfalfa plants were cut twice at 10-15cm height from ground level. First cutting was achieved on June 8, flowering stage and second cutting on July 24, seed maturing stage.

(3) Sampling; Periodical samples were collected at intervals of 15 to 30 days from April 5 to September 26 to measure accumulation of dry matter and acetylene reduction activity of nodules. At each sampling, whole alfalfa plant was dug out and the leaves washed with water until free of soil. They were oven-dried for 48 hours at 80°C before weighing.

(4) Acetylene reduction assay

N_2-fixing activity of nodule was measured by using the acetylene reduction method by Hardy.[5] Branch test tube included nodules were sealed with rubber stopper and were evacuated and injected with rubber stopper and were evacuated and injected with a syringe containing 0.82% gas mixture (CO_2: 40.3ppm, C_2H_2 1.82%, argon balance) at 0.2 atm. After samples were incubated at 28°C for two hours, one ml mixture gas was injected into a gas chromatograph (Shimadzu Model GC-6A) equipped with a flame inoization detector. C_2H_4 and C_2H_2 were separated from gas mixture in a column filled with porapark®. The column temperature was 45°C and the nigrogen carrier gas was 0.8kg/cm. The retention time of C_2H_4 was four minutes and that of C_2H_2 was three minutes.

Caculations were made as following formulars

(i) Specific nodule activity (SNA)

$$\frac{\mu\text{moles } C_2H_4 \text{ in test tube}}{\text{fresh nodule weight}} \div \text{incubation time}$$

(ii) Total nodule activity (TNA)

$$\frac{\mu\text{moles } C_2H_4 \text{ in test tube}}{\text{No. of plant}} \div \text{incubation time}$$

(5) Total nonstructural carbohydrates (TNC)

TNC were determined by using the Dale Smith method.[2] Samples for determination of TNC were dried for 24 hours at 80°C in dry oven. The 500mg tissue samples were placed in 100ml flask and 15ml distilled water was added, then was heated on a hot plate. After 0.5% Mylase enzyme was added, the solution was filtered through Whatman No. 1 filter paper and 10ml reagent was added, and then was heated for exactly 15 minutes and titrated with 0.02N sodium thiosulfate by using

0.24ml starch solution as the indicator. The end point of titration was determined at color change from blue to light green. The percentage TNC in tissue were calculated as following equation.

% TNC in tissue = Amount of sugar in sample aliquot x dilution factor x 100/sample weight (mg)

(6) Total nitrogen

Total nitrogen was extracted by using Van Schouwenburg Method and measured by Technicon Industrial Method. The 500mg tissue samples were placed in 100ml flask and 5.5ml acid mixture (82% H_2SO_4 solution added 1.4M-salicylic acid) was added and allowed to undergo nitration reaction for about one hour. After nitration reaction, sample solutions were heated on a hot plate at 180°C and added H_2O_2. When final temperature was at 280°C, the soluion color turned white. Extracted solution was filtered and was brought to volume 100ml with adding distilled water and was mixed well to obtain a homogenous mixture. This solution was added sodium nitroprusside and sodium hypochlorite and was measured by Technicon Autoanalizer III at pH 12.8-13.0.

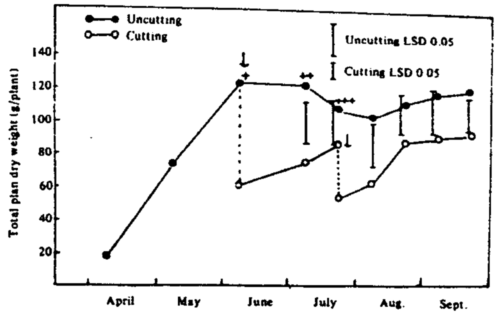

Fig. 1-a. Seasonal changes of total plant dry weight in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

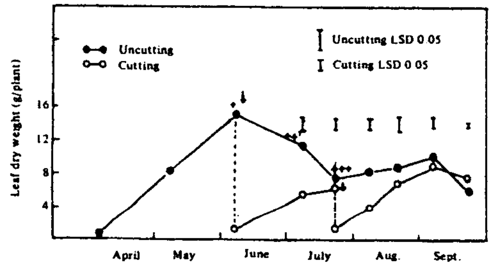

Fig. 1-b. Seasonal changes of leaf dry weight in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ cutting date

RESULTS AND DISCUSSION

1. Seasonal changes of dry weight, nodulation and nodule activity, and their interrelationships.

(1) Dry weight

Dry weight of the whole plant increased rapidly from May to June. After flowering stage, there was no significant increase in dry weight of the whole plant. Dry weight in the autumn growing season increased at a fairly steady rate. After first cutting, the dry weight of whole plant in cutting plots increased at a faster rate than that of uncutting plots. A similar pattern was found after the second cutting (Fig. 1-a).

The leaf dry weight of spring growth in June greatly exceeded that of the fall growth in September. Dry weight of leaf showed rapid increase

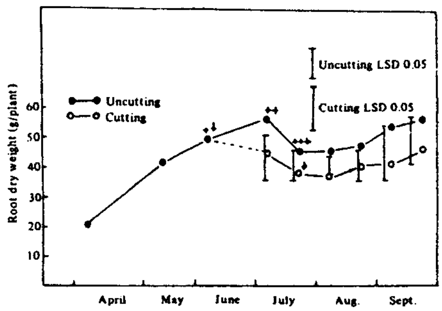

Fig. 1-c. Seasonal changes of root dry weight in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

continually till the beginning of June. But after flowering, dry weight of leaf was decreased. The decrease of leaf weight in uncutting plots appeared to be related mainly to senescence and shedding

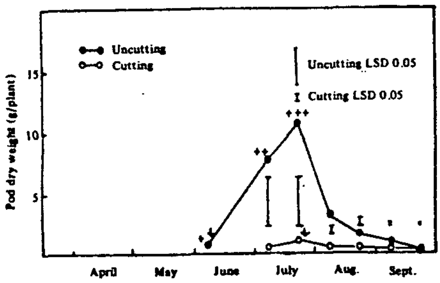

Fig. 1-d. Seasonal changes of pod dry weight in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

of older leaves shaded by the uncutting canopy (Fig 1-b).

The marked seasonal peak in root dry weight got reached at the green seed stage (early July). Root dry weight showed a slight decrease between the end of July and the middle of August. Root dry weight of cutting plots showed similar seasonal pattern to that of uncutting plots. But, root dry weight in cutting plots was reduced more than that of uncutting plots (Fig. 1-c).

Although alfalfa was not a grain legume, the dry weight of pod per plant in uncutting plots increased after flowering stage and reached its maximum at green (early July) and ripe seed stage (late July). However, dry weight of pod in cutting plots was only a small portion in total dry weight throughout the growing season. After flowering, the drop of nodule activity in uncutting plots was coincident with the increase of high pod weight at green and ripe seed stage (Fig 1-d). As the reason, many workers [13,15] suggested that podfilling created such competition for carbon compounds and the nodules were suffered on "carbohydrate deficiency".

(2) Nodule weight and nodule activity

Effective nodule was observed in the beginning of April sampling, but effective nodule weight was very low. Nodule weight increased rapidly and reached a peak in the beginning of June. After

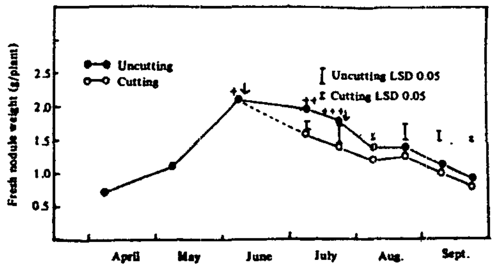

Fig. 2-a. Seasonal changes of fresh nodule weight in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

flowering stage, nodule weight decreased at a fairly steady rate throughout the growing season. Pate[13] found that nodule number and weight decreased severely at the onset of flowering in temperate species.

In this experiment, the onset in decrease of nodule weight was related to the decrease in functional leaf area, or to the onset of flowering and podfilling.

Seasonal changes of nodule activity were known to have "initiation time", "exponential increase phase", and loss of exponential phase" proposed by Hardy.[5] Seasonal changes of nodule activity in this experiment was somewhat variable with having the three phases. The critical inactivation of nodules would closely associate with the gain of pod weight at green and ripe seed stage in uncutting plots (Fig. 1-d). Pate[13] postulated that the low nodule activity after flowering stage resulted from decreased supply of assimilate to the nodules. Besides, the decrease in nodule activity might be accerelated by the high temperature and the drought during July when plants were at the period of late flowering and green seed stage in present experiment. Huang et al.[8] reported that moisture stress affected nodule activity directly and indirectly through reduced photosynthesis.

Nodule activity was low during summer months when average temperature was about 25°C-26°C and maximum air temperature was about 29°C-31°C. During this period the high air temperature might have influenced on nodule activity directly

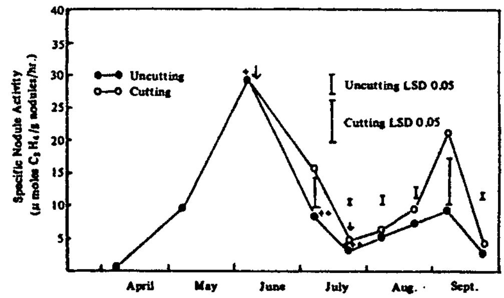

Fig. 2-b. Seasonal changes of Specific Nodule Activity (SNA) in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

Table 1. Correlation coefficients between nodule weight, nodule activity and dry weights of each part.

Characteristics		Correlation coefficient		
		Nodule w.t	Specific nodule activity	Total nodule activity
Total plant	D.W	0.34**	0.22	0.19
Shoot	D.W	0.42**	0.21	0.18
Root	D.W	0.24	0.14	0.33*
Stem	D.W	0.27	0.09	0.08
Leaf	D.W.	0.43**	0.43**	0.36**

* Significant at 0.05 ** Significant at 0.01
+ Observation No. = 45

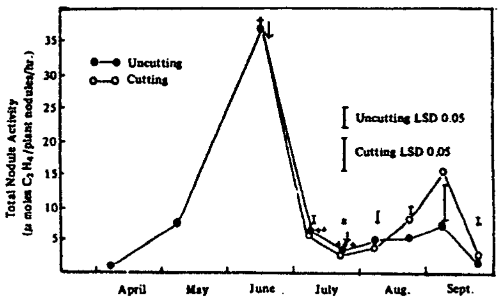

Fig. 2-c. Seasonal changes of Total Nodule Activity (TNA) in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

or indirectly through the decreased legume growth. Some short term studies showed that temperature above or below optimum for N_2 fixation (about 22°C) had greater effect on N_2 fixation than on legume growth[17]. It has been suggested that the summer yield reduction on "summer slump" of alfalfa was due to high air temperature causing reduction of N_2 fixation.

(3) Relationships between dry weight, nodulation and nodule activity

Dry weight of whole plant or shoot, and top root ratio were significantly correlated with nodule weight, but were not correlated with nodule activity. Until flowering stage, the increase of dry weight was clearly correlated with that of nodule activity. After flowering stage, the curve of dry weight showed no change, but the curve of nodule activity showed a sharp decrease. However, dry weight of leaf was significantly correlated with nodule weight and nodule activity (Table 1). Rüegg[16] said that the seasonal pattern of acetylene reduction activity was closely correlated with dry weight and photosynthetic area.

2. Seasonal changes of total nonstructural carbohydrates and total nitrogen content, and their relationships with nodule activity.

(1) Total nonstructural carbohydrates (TNC)

The TNC percentage of whole plant increased markedly from the beginning of May to the end of June and peaked in the beginning of June. Thereafter, the TNC percentage of the whole plant in uncutting plots decreased gradually from the beginning of June (green seed stage) to the end of July (ripe seed stage). The TNC percentage of the whole plant was low during the period of the end of July to the middle of August. A high level of carbohydrates was eventually stored again during the autumn months.

High summer temperature generally resulted in the poor accumulation of root carbohydrates.[14] It seemed that high temperature reduced net photosynthesis of forage legume and assimilates available in transporting into the root nodule. It was reported that root TNC of alfalfa was not markedly affected by high temperature, but plant mortality was found to increase at the root temperature of 30°C, and the mean acetylene reduction of alfalfa showed a significant reduction due to high temperature. Nodule senescence and disintegration might have occurred in the high temperature.[1)]

Many workers[1,12)] explained that late summer decline in alfalfa productivity was related to the effects of high temperature on root reserves, shoot number and net photosynthetic rates. Since alfalfa stored the largest proportion of its carbohydrates in the roots, the percentage and amount of root carbohydrate may have relationship between nodule activity and nodule weight. The TNC percentage in roots was positively correlated with nodule weight and nodule activity, but each TNC percentage in the whole plant, stems and crown was

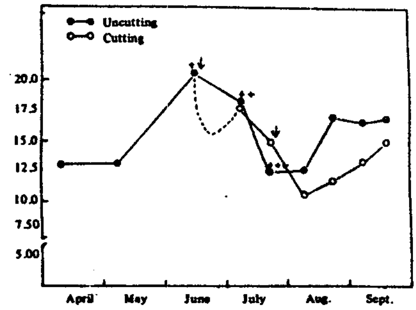

Fig. 3-a. Seasonal changes in percentage of Total Nonstructural Carbohydrates (TNC) of the whole plant in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

Table 2. Correlation coefficients between total nonstructural carbohydrates content and nodule activity.

Characteristics		Correlation coefficients		
		Nodule w.t	Specific nodule activity	Total nodule activity
Carbohydrates (%)	Stem	0.13	0.26	0.36
	Crown	0.05	0.16	0.36
	Root	0.59*	0.45*	0.45*
	Whole plant	0.18	0.26	0.13
Carbohydrates (g/plant)	Stem	0.66**	0.29	0.44
	Root	0.77**	0.45*	0.34
	Whole plant	0.78**	0.41	0.41

* Significant at 0.05 ** Significant at 0.01
\+ Observation No. = 16

not correlated with nodule activity. Each TNC amount of the whole plant, stems and roots was positively correlated with nodule weight (r=0.78**, 0.66**, 0.77** respectively), but was not correlated with nodule activity. The amount of TNC content in roots was positively correlated with specific nodule activity (Table 2). Therefore, carbohydrate storage in root can be considered on energy source which is available for nitrogen fixation in root nodule.

Cutting on July provided rapid recovery of specific nodule activity during autumn growth. Removing top might reduce unnecessary losses from respiration throughout hot summer months. Futhermore, the higher content of non-structural carbohydrate in cutting plants could used for both of rapid shoot regrowth and active nitrogen fixation.

(2) Total nitrogen content

The total nitrogen of the whole plant markedly

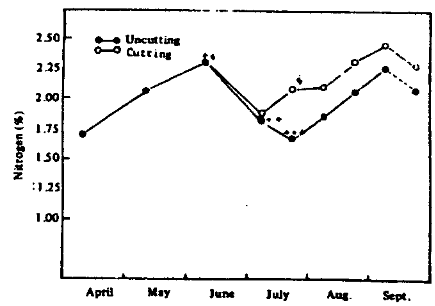

Fig. 3-b. Seasonal changes in percentage of total nitrogen of the whole plant in cutting and uncutting
+ Flowering stage, ++ Green seed stage
+++ Ripe seed stage, ↓ Cutting date

Table 3. Correlation coefficients between total nitrogen content and nodule activity.

Characteristics		Correlation coefficients		
		Nodule w.t	Specific nodule activity	Total nodule activity
Nitrogen (%)	Stem	0.20	0.19	0.16
	Leaf	0.40	0.45*	0.49*
	Crown	0.15	0.15	0.17
	Root	0.25	0.19	0.15
	Whole plant	0.18	0.46*	0.41
Nitrogen (g/plant)	Stem	0.84**	0.38	0.33
	Leaf	0.74**	0.71**	0.79**
	Root	0.09	0.29	0.34
	Whole plant	0.79**	0.29	0.34

* Significant at 0.05 ** Significant at 0.01

+ Observation No. = 16

increased during May and peaked in the beginning of June. A marked decrease of total nitrogen of the whole plant in uncutting plots occurred between in the beginning of July and in the end of July. The percentage of total nitrogen in cutting plots was higher than that in uncutting plots over the growing season (Fig. 3).

The stronger nodule activity in plant was, the higher was nitrogen content. The percentage of total nitrogen in leaves was positively correlated with specific nodule activity and total nodule activity (r=0.45*, r=0.49*, respectively), but the percentage of nitrogen in other parts was not consistently correlated with nodule weight and nodule activity. The amount of total nitrogen in leaves was positively correlated with nodule weight, specific nodule activity, and total nodule activity (r=0.74**, r=0.71**, r=0.79**), while there was no significant correlation between the amount of total nitrogen of other parts and nodule activity (Table 3). Therefore, nitrogen fixed by root nodules must transport to the leaves to provide enough nitrogenous substances for carboxylase enzyme.

The total nigrogen content changed seasonally in the same pattern as TNC content. At early growth stage, seasonal changes in nitrogen content showed a similar pattern to those of nodule activity of plant. After flowering, nitrogen content did not still change but nodule activity decreased.

摘 要

알팔파 根瘤의 窒素固定活性의 年中變化를 규명하고 乾物重과 炭水化物 및 窒素含量의 年中變化를 推蹟하여 이들의 關係를 考察하기 爲하여 造成後 3年째인 알팔파 圃場에서 非刈取區와 刈取區로 區分하고 時期別로 根瘤의 무게와 Acetylene還元法에 依하여 根瘤의 窒素固定活性을 測定하였으며 各部位別 非構造的 全炭水化物 및 全窒素含量을 分析하였다.

1. 根瘤의 무게는 6月初에 最大로 높았으며 開花期부터는 全生育期間을 걸쳐 느린 速度로 減少되었다. 根瘤의 窒素固定活性의 年中變化는 4月初에 나타나기 始作하여 5月에는 急激히 增加하여 6月初에 最大로 높았고, 그리고 開花期로부터 낮아지기 始作하여 7月末부터 8月中旬까지는 낮은 活性을 維持하다가 8月末과 9月初에 다시 높은 活性을 보였다.

2. 各部位의 乾物重의 變化曲線과 根瘤의 무게 및 活性의 變化曲線은 開花期까지는 같은 傾向의 增加曲線을 보였다. 그러나 開花後期에 乾物重은 變化가 없었으나 莢의 形成에 따른 根瘤가 利用할 수 있는 光合成物質의 減少, 旱魃, 高溫 等에 依하여 根瘤의 窒素固定活性이 急激히 낮아졌기 때문에 이들의 相互關係는 開花後부터 맞지 않았다.

3. 炭水化物含量과 窒素含量은 開花期인 6月初에 가장 높고 7月의 結莢期부터 낮아지기 始作하였으며 여름기간동안 높은 溫度의 影響을 받아 7月末과 8月中旬동안 낮은 含量을 보이다가 가을에 다시 增加하였다. 各部位의 炭水化物含量 및 窒素含量과 根

瘤의 窒素固定活性과의 相互關係는 뿌리의 炭水化物含量과 잎의 窒素含量은 根瘤의 窒素固定活性과 높은 正의 相關關係를 나타내었다.

4. 7月中의 刈取는 高溫期의 炭水化物의 과도한 消耗를 防止하여 가을철에 再生에 使用되었고 根瘤活性 회복에 寄與하였다.

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