韓國잔디에 있어서 Amylase와 Nitrate reductase의 器管別 活性分配에 관한 硏究

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A Study on Allocation of Amylase and Nitrate Reductase Acti ities among the organs *Zoysia japonica* Steud.

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摘 要

*Zoysia japonica*를 부위별로 나누어서 그들간의 amylase와 nitrate reductase의 활성을 조사한 결과는 다음과 같다.

1. Amylase의 활성은 관부에서 8.36~9.46 unit/mg·protein/hr.로 가장 높았고 이삭에서 2.04 unit/mg·protein/hr.로 가장 낮았다. 포복경, 뿌리, 잎에서의 amylase의 활성을 각각 5.42~5.82, 3.76, 2.32~3.16 unit/mg·protein/hr.를 나타내었다.

2. Nitrate reductase의 활성은 빛을 많이 받는 잎에서 0.35~0.66 n mole/mg·protein/hr.로 가장 높았고 관부에서 0.06~0.10 n mole/mg·protein/hr.로 가장 낮았다. 이삭과 포복경에서는 각각 0.31, 0.27~0.63 n mole/mg·protein/hr.를 나타냈다.

이러한 결과로 부터 저장기관인 관부나 관부 절간에서 높은 amylase의 활성을 이용하여, 양분을 이삭으로 이동시키고 있음을 알 수 있었다.

nitrate reductase의 활성은 chloroplast를 갖지 않는 기관보다 광합성기관에서 더 높았다.

잔디밭에서 같이 사는 크로버와 비교해 보면 amylase의 활성이 *Zoysia japonica*보다 2배가량 더 높았다. 이러한 결과로부터 잔디밭에서 크로버가 더 생장력이 큼을 알 수 있었다.

I. Introduction

Zoysia japonica belonging to gramineae family is a perennial herb overwintering as substerranean stem and in next spring makes new shoots. The buds are come out by consuming carbohydrate and other storage material as a source of energy.

There are many kinds of turfgrasses, but they are largely classified to two groups ; cool season types and warm season types.

Among the cool season turfgrasses, kentucky bluegrass(Baker and Jung, 1968), creeping bentgrass (Duff, 1967) and perennial ryegrass(Michell, 1953) are well known. Most korean lawns are warm season turfgrasses except *Poa* spp. and photosynthesize by C−4 mode.

The facts that korean turfgrasses, C4 type, are more affected by intensity of light, day length and temperature is already known(George J., 1974, Yoo et al., 1969). And comparing with turfgrasses of C_3 type, *Zoysia japonica* becomes green color in late spring and as reaching to the fall, it showes discolorization immediately(Yeam et al., 1985).

Although they have shorther green period than C_3 type, they have the advantages of tough, stiff leaves and high quality of uniform and dense

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growing. By this time, though having been morphologically and ecologically about turfgrasses, there have been few approaches through the physiological experiment of enzyme.

The purpose of this study is to investigate the movement of materials produced by photosynthesis and enzyme localization(between orgarns) by estimation enzyme activity of anylase and nitrate reductase.

In addition, we compared with amylase activity of clover living together with turfgrasses.

Ⅱ. Materials and Methods

The experimental period was from 10th. June to 5th. July on which its growth was most active.

1. Preparation of crude extracts

1) Preparation for amylase activity.

The enzyme of each organ was extracted by 0.02M $CaCl_2$ and 1mM Na−Acetate buffer, pH 4.6.(Jun et al., 1983).

After Centrifugaion at 1,000G for 10min., the supernatents was used as enzyme solution.

2) Preparation for nitrate reductase activity.

The components of enzyme extraction solution were as followes ; 3ml of 1M K−phosphate buffer, 0.1ml of EDTA−Na_2, 0.0303 g of L−cysteine, 0.3 g of bovine serum albumin and 0.1 g of poly vinyl pyrrolidinone in 10ml of final volume. The procedure after extracting enzyme is the same as amylase one.

2. Protein assay

The protein concentration of crude extracts was determined according to Lowry et al.(1951).

3. Enzyme assay

1) Amylase activity

The reaction solution was the buffered soluble starch solution which was made by mixing the following components (0.3 g of potato starch, 1.2 g of KH_2PO_4 and 5.88mg of $CaCl_2$ in 200ml of final volume).

The reaction was triggered by adding 1ml of enzyme solution to 1ml of reaction solution and incubated at 30℃ for 30min.

The reaction was terminated and developed color by adding 1ml of acidified I_2KI solution with 0.05N HCl.(Stoddart, 1971)

After the solution developed color was diluted to 7ml, it was measured at 710nm. The specific enzyme was expressed in unit / mg protein / hr. One unit was defined as activity of changing optical density, 0.1, form undigested control at spectrophotneter(Spectronn 70. Bausch & Lomb).

2) Nitrate reductase activity.

Reaction mixture contained 200μmole K−phosphate buffer(pH 7.6), 20μmole NADH. The reaction was triggered by the addition of 400ul of enzyme solution and final volume was 1.6ml.

After incubation at 30℃ for 1hr, the reaction was stopped by adding 0.2ml of 0.5M Zn−acetate and 0.2ml of 46ml / L phenazine methosulfate.

4. Illustration of organs

The order of leaf and stolon was determined by its age, That is, the older it is, the higher the number is. The case for clover was the same, too.

Ⅲ. Results and Discussion

1. Amylase activity and nitrate reductase activity of *Zoysia japonica*.

The ability of regeneration of *Zoysia japonica* is related to crown which is organ connecting an errect stem with stoniferous rhizome.

So, if it is cutted below crown, critical result irrecoverable will be cause.(Joo, 1983).

The significant fact obtained form the result of amylase activity was that activities of crown and internode were higher than any other organ.

We could suggest that the product of photosyn-

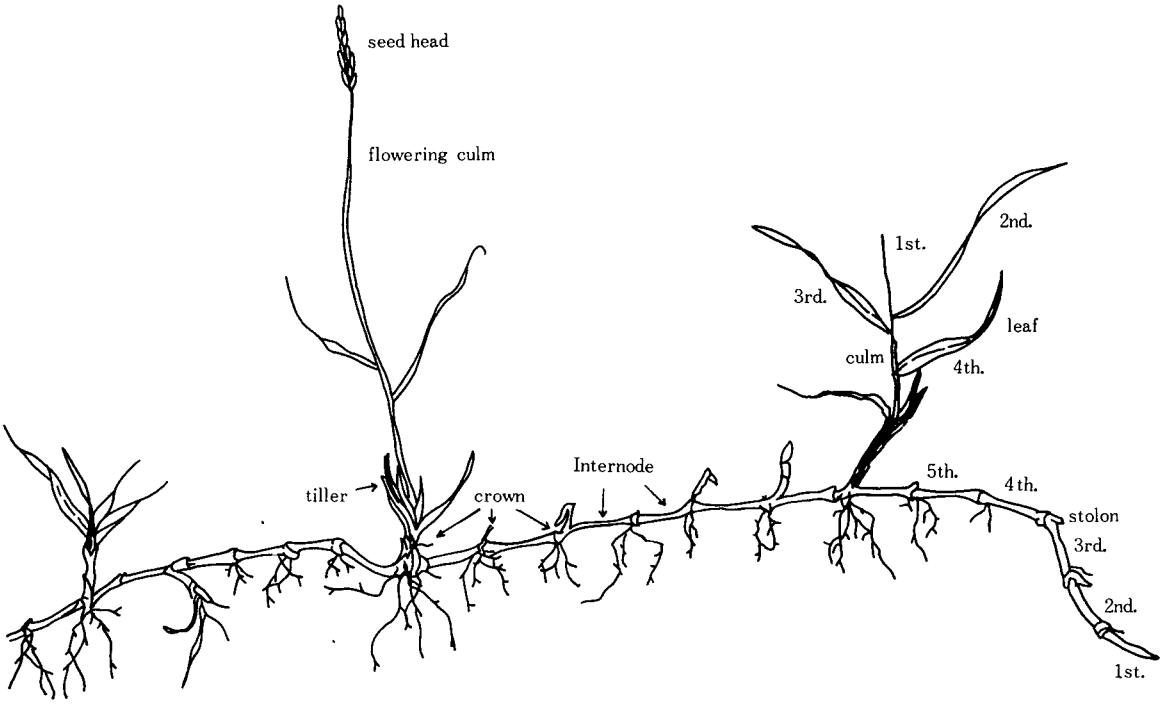

Fig 1. Morphology and terminology of organ of ***Zoysia japonica***

thesis go down to subterranean stem such as crown and internode, and accumulate in the forms of starch and transport to seed head having the lowest amylase activity, if necessary.(Table 1.)

Nitrate reductase activity showed different aspects from anylase one. That is to say, leaves or stolon absorbing full light showed the highest activity, while the crown and internode having the highest amylase activity showed the lowest one.(Tale 2.)

Because the experimental period was flowering season and most energy source might be trans-

Table 1. Amylase activity of ***Zoysia japonica***.

organ / Activity	leaf 1st, 2nd, 3rd, 4th	Stolon 1st, 2nd, 3rd, 4th, 5th	Seed head	flowering culm	Crown	Internode	culm	root
Amylase Activity (Unit/g/hr.)	9.22 12.48 10.78 10.20	10.52 10.24 10.24 8.86 8.46	3.40	4.30	11.60	9.80	9.80	2.80
	mean : 10.67	9.66						
Total Solube protein (mg/ml)	2.92 4.27 4.27 4.39	1.94 1.94 1.76 1.14 1.59	1.67	1.28	1.39	1.04	2.26	0.75
Amylase Activity (Unit/ mg·protein/hr.)	3.16 2.92 2.52 2.32	5.42 5.28 5.82 7.76 5.32	2.04	3.36	8.36	9.46	4.34	3.76
	mean : 2.73	6.32						

Table 2. Nitrate reductase activity(NRA) of ***Zoysia japonica***

organ / Activity	leaf 1st, 2nd, 3rd, 4th,	Stolon 1st, 2nd, 3rd, 4th, 5th	Seed head	flowering culm -	crown	Internode	culm	root
NRA (nmole/g/hr.)	2.30 4.23 2.36 2.14	1.81 1.00 1.71 2.25 1.49	1.60	2.24	0.24	0.21	0.92	*ND
	mean : 2.76	1.65						
Total soluble protein (mg/ml)	5.06 6.36 6.06 6.19	3.73 3.64 3.13 3.58 3.67	5.12	3.35	2.35	3.21	4.63	2.73
NRA (nmole/ mg·protein/hr.)	0.45 0.66 0.39 0.35	0.49 0.27 0.55 0.63 0.41	0.31	0.67	0.10	0.06	2.20	*ND
	mean : 0.46	0.47						

*N.D : Non-detected.

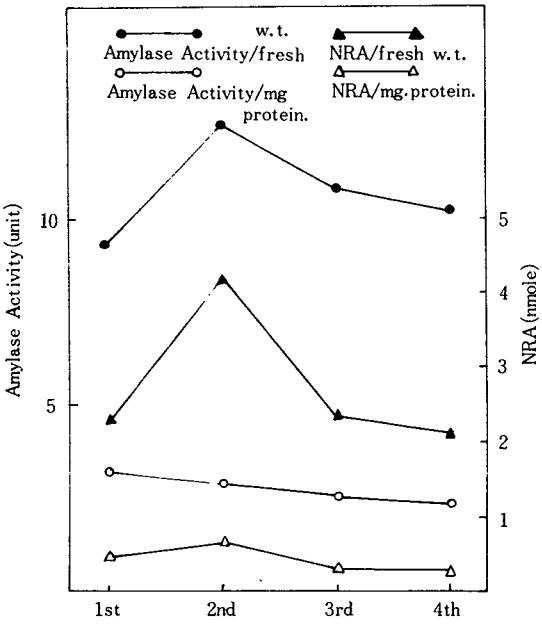

Fig.2. Amylase activity and nitrate reductase activity (NRA) of ***Zoysia japonica*** according to age of leaf.

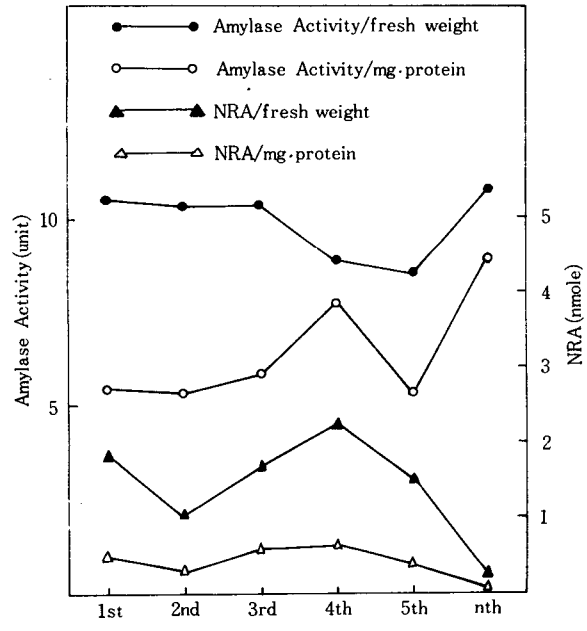

Fig.3. Amylase activity and nitrate reductase activity(NRA) of ***Zoysia japonica*** according to age of stolon.

ported to seed head for maturing it, maybe nitrate reductase activity of leaves did not show so high value than other plants' one. Therefore, it is suggested that how much the flowering of seed head effect on the quality of lawn grasses(Jeon, 1985).

The enzyme activity of leaf according to its age was the highest in the 2nd. leaf which grew rigorously. In the case of stolon, we examined that the stolon differentiated into rhizome as aging to the nth stolon. Also, we concluded that the rhizome displayed the role of complete storage organ.

Table 3. Amylase activity of cover clover

organ / Activity	leaf					Stem						crown	root
	1st.	2nd.	3rd.	4th.	5th.	1st.	2nd.	3rd.	4th.	5th.	nth.		
Amylase Activity (unit/g/hr)	5.15	5.28	7.23	6.05	6.51	6.66	9.63	11.64	12.36	12.54	15.86	19.44	12.96
	mean : 6.05					mean : 11.44							
Total Soluble Protein (mg/ml)	4.45	4.66	5.93	5.98	5.15	1.68	1.90	2.16	1.82	1.81	1.92	1.27	1.41
Amylase Acitvity (unit/mg protein/hr)	0.95	1.13	0.82	1.01	1.26	3.96	5.07	5.39	6.79	6.93	8.26	15.31	9.19
	mean : 5.00					mean : 6.05							

2. Amylase activity of clover

The clover living together with lawns, which are perennial herb passing winter as rhizome like *Zoysia japonica*, showed the similar results, i.e. as stems grew older to the nth stolon, its activity was higher.

3. A section view of leaf and stolon of *Zoysia japonica*.

Z · japonica, the typical C_4 plant was the representing Kranz type.

As shown in Fig.3. we examined that many granules were packed in and they could be identitied as starch by staining with I_2KI solution.

As shown in Fig.2., the place of photosynthesis in leaves was bundle sheath cell and starch was accumulated in there.

Considering with previous results of enzyme assay, we concluded that the function of stolon was being transformed into the storage one.

In clovers, amylase activity was two times hig-

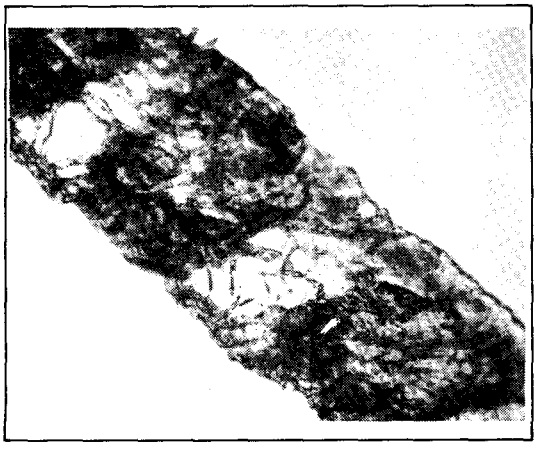

Fig 3. A : A section view of leaf before staining starch · granules. (×400)
B : A section view of leaf with staining starch · granules. (×400)

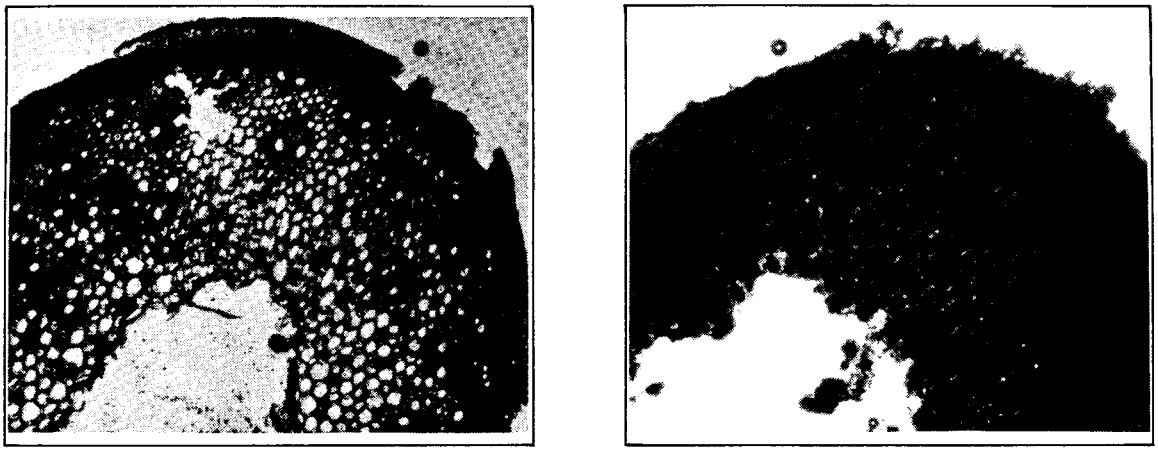

Fig.4. A : A section view of stolon before staining starch granules. (×400)
B : A section view of stolon with staining starch granules. (×400)

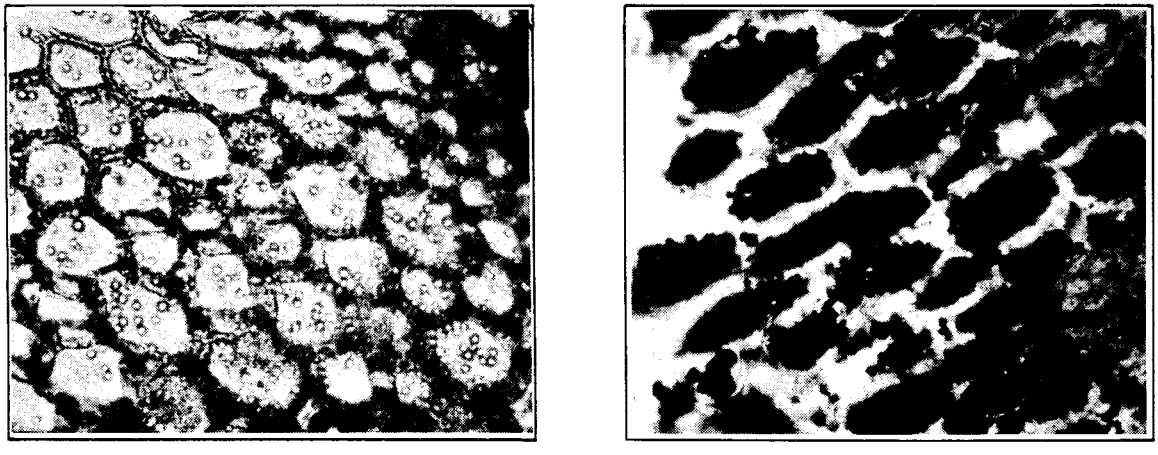

Fig. 5. Section views between stolon and crown. As showen in Fig. 5. as young stolon grew older to the nth. stolon, rhizome, the more granules were packed in.

her than *Z · japonica.*

From the results, we could predict that clover would be more competent in lawn ground.

Ⅳ. Summarry

Z · japonica was studied to investigate enzyme activity such as amylase activity and nitrate reductase activity between various organs.

The results were as follows.

1. Amylase activity was the highest as 8.36~9.46 unit / mg protein / hr in crown and the lowest as 2.04 unit / mg protein / hr in seed head.

Amylase activities in stolon, root and leaves were 5.42~5.82 unit / mg protein / hr, 3.76 unit / mg protein / hr, 2.32~3.16 unit / mg protein / hr, respectively.

2. Nitrate reductase activity was the highest

as 0.35~0.66 nmde / mg protein / hr in leaves absorbing full light and the lowest as 0.06~0.10 nmole / mg protein / hr in crown.

In seed head and stolons nitrate reductase activities were 0.31 nmole / mg protein / hr, 0.27~0.63 nmole / mg protein / hr, respectively.

From the results, it is suggested that the organs such as crown and internode, which is essential in accumulating materials, transport organic nutrients to the seed head to mature fruits by high amylase activity.

And nitrate reductase activity was higher in photosynthetic organs than ones not having chloroplast. Comparing with clover, living together with lawn grasses, amylase activity of clover was higher as about two times than *Zoysia japonica*. We concluded that clover was more competent in lawn ground.

V. References

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