

# Cotyledon Development and Seedling Growth of *Lotus corniculatus*, *Lotus tenuis* and Their Reciprocal Hybrids

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## *Lotus corniculatus*, *Lotus tenuis* 및 이들 相互 交雜種의 子葉과 幼植物의 生長發育

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### 摘 要

*Lotus corniculatus*, *Lotus tenuis* 및 이들 相互 交雜種의 幼植物 活力評價를 위한 방법으로 子葉 面積測定 方法을 開發하고 葉의 展開와 幼植物 生長關係를 調査하였다.

子葉을 植物體에서 떼어내지 않고 부착된 상태로 간단하고 신속하게 연속적으로 子葉面積을 측정할 수 있었다. 子葉은 출현후 첫주에는 매우 빠른 속도로 伸張되다가 그 이후에는 서서히 伸張되어 약 3주경에 최대 면적에 달하였다. 出現 당시의 子葉面積은 種子 크기와 상관관계가 많았으나( $P=0.05$ ), 출현 이후의 子葉 伸張率은 다양하였다. 光合成을 할 수 있는 面積 發生의 신속성은 별노랑이의 유식물 생장에 결정적인 요인이 되었다. 交雜種 중에서  $2 \times m_2$ 와  $1 \times m_2$ 는 別노랑이의 유식물 活力 增進을 위한 育種 가능성을 시사하였다.

### I. INTRODUCTION

The function of cotyledons during early seedling growth is to mobilize and transfer stored food reserves to the developing embryonic axis. Another role of epigeal cotyledons is to provide an early photosynthetic area before production of true leaves (Ashcroft and Murray, 1979; Lovell and Moore, 1970).

The capacity of cotyledons to fix  $CO_2$  varies widely among species. Some epigeal cotyledons with a high expansion factor, have a high photosynthetic rate per unit area, and have a long functional time span, whereas epigeal species with lower cotyledon expansion together with hypogeal species were less well adapted for photosynthesis (Lovell and Moore, 1971; Machado et al., 1974). Photosynthesis by forage legume cotyledons is

critically important for normal seedling development. Williams et al. (1968) and Machado et al. (1974) reported that cotyledons of subclover (*Trifolium subterranean* L.) were decisively important as photosynthetic organs. Lovell and Moore (1970) stressed the importance of cotyledons of red clover (*T. pratense* L.). Cooper and Fransen (1974) reported that first leaf formation and expansion of sainfoin (*Onobrychis viciifolia* Scop.) seedlings depended on cotyledonary photosynthesis. Shibles and MacDonald (1962) presented the importance of photosynthetic area of birdsfoot trefoil (*L. corniculatus* L.) cotyledons for early seedling growth, and reported photosynthetic rates of cotyledons that were equal to leaves.

Factors that affect cotyledon area, other than seed size, have not received much attention. Hill (1925) found that form and size of *Digitalis* spp. cotyledons

Contributions from the Korea Science and Engineering Foundation, Daejeon, Korea, and the Missouri Agricultural Experiment Station, University of Missouri, Columbia, MO 65211 U.S.A. are acknowledged.

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differed among species with  $F_1$  hybrids showing strong material influences. Goodwin (1937) and Lovell and Moore (1970) stated that cotyledon growth subsequent to germination was due chiefly to cell expansion. McKee and Risius (1970) reported that temperature, light intensity, and growing medium significantly affected cotyledon size of crown vetch (*Coronilla varia* L.). They suggested that differences in cotyledon size between seed lots of the same variety were due to environmental conditions during seed development and maturation.

Beuselinck and McGraw (1983) suggested that a *L. tenuis* introduction with large seed size may be a good source for improving seedling vigor of broadleaved birdsfoot trefoil. Earlier we showed that the advantage of *L. tenuis* tended to be expressed in the first 3 weeks of seedling growth. Reciprocal crosses among *L. corniculatus* and the purported *L. tenuis* were made. This paper compares cotyledon and leaf area development with seedling vigor as estimated by a means of evaluating seedling vigor of  $F_1$  interspecific hybrids and their parents. A new simple method is suggested for measuring the size of cotyledons that are attached to the plant.

## II. MATERIALS AND METHODS

Seeds of MO-20 birdsfoot trefoil (*L. corniculatus* L.), narrowleaf trefoil (*L. tenuis* Wald. et Kit.), and their reciprocal hybrids were used to measure the changes in cotyledon area and seedling development. Hybrid I and II refers to the  $F_1$  obtained from using the *L. corniculatus* and *L. tenuis* as maternal or paternal parents. Seedlings germinated two days after imbibition at 20°C were planted to plastic pots, 10 cm wide by 15 cm deep, filled with a soil mixture consisting of Mexico silt loam, peat, and sand in a 2:1:1 ratio. Two seedlings were planted per pot, and the pots were placed at random distribution in a growth chamber with a 14 hours photoperiod at 20°C. Relative humidity was maintained around 70% with light intensity of  $500 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$  by cool-white fluorescent and incandescent bulbs. Most seedlings emerged in two days. Subsequent to

emergence the temperature was changed to 25°C. Seedlings were watered during the growth period with 50 ml of a Hoagland's complete nutrient solution weekly and distilled water as needed.

Cotyledon size was measured with a compass and read with an ocular magnifier to nearest 0.1 mm from seedling emergence to cotyledon senescence at a 2 day interval (12 replications of each entries). The ratio of cotyledon length to cotyledon width was near constant according to the entries. Thus it was found that cotyledon length could be estimated with relative accuracy ( $R^2=0.889$ ) by multiplying 1.47, 1.28, 1.41, and 1.37 to the cotyledon widths of *L. corniculatus*, *L. tenuis*, hybrid I, and hybrid II, respectively. Then cotyledon area was calculated with the following formula:  $A = 1/4 \pi ab$  where A, a, and b represent area, width and length of the cotyledon. Cotyledon expansion rate was measured by the area increase per unit of area per unit of time ( $\text{cm}^2/\text{cm}^2/\text{day}$ ).

Three weeks after emergence, seedlings were harvested to measure net photosynthetic rate, dark respiration rate, and seedling dry weight. Net photosynthetic rate was measured with three replications at 25°C with  $500 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{S}^{-1}$  at the canopy level using open system and flow rate of 3 L of  $\text{CO}_2$  per min. CER was measured using a Beckman Model 215, a differential IR gas analyzer. Gilson respirometer was used for measurement of dark respiration at 25°C with the same method as described earlier (Hur et al., 1994a). After measurement of photosynthesis and dark respiration, leaf area was measured by a LI-COR model 300 leaf area meter and seedlings were dried in a convection oven for 48 hours at 80°C and weighed to nearest 0.1 mg.

## III. RESULTS AND DISCUSSION

### Cotyledon Area Measurement.

As the cotyledons of most forage legumes are oblong to broadly oval in shape, it was relatively easy to estimate the size of attached cotyledons with a simple, rapid method. Estimated cotyledon length was similar to

the actually measured length ( $R^2=0.889$ ). Correlation coefficients and multiple  $R^2$  values between estimated and actual measured cotyledon area by leaf area meter are presented in Table 1. There was not much difference between the estimated area calculated by the compass method and that actually measured by the leaf area meter. However, the ratios of cotyledon length to width were different among the entries and this ratio could change according to the growing environment of the seedlings. The measurement of cotyledon width alone was much easier than the measurement of cotyledon length or both.

Table 1. Correlation coefficients<sup>1)</sup> and multiple  $R^2$  values from regression showing the relation between estimated and actual cotyledon area of *L. corniculatus*, *L. tenuis*, and their reciprocal hybrids<sup>2)</sup>

Species	r	$R^2$
<i>L. corniculatus</i>	0.913	0.834
<i>L. tenuis</i>	0.993	0.987
Hybrid I	0.916	0.839
Hybrid II	0.846	0.715

<sup>1)</sup>  $r > 0.468$ ,  $> 0.590$  is significant at  $p < 0.05$ ,  $0.01$ .

<sup>2)</sup> Hybrid I is means of 4 lines crossed between *L. corniculatus* and *L. tenuis* using *L. corniculatus* as the female parent. Hybrid II is means of 4 lines crossed between *L. corniculatus* and *L. tenuis* using *L. tenuis* as the female parent.

Most previous investigators have measured cotyledon area detached from the plant, as it was difficult to measure changes in cotyledon area attached to the plant. Cotyledon areas have been measured with planimeter or by weight after their outlines were drawn on paper (Brown, 1941; Lovell and Moore, 1970; Machado et al., 1974; Shibles and MacDonald, 1962), or leaf area meter (Cooper and Fransen, 1974). McKee and Risius (1970) measured cotyledon length and width or crown-vech with a plastic rule. The method suggested here was more simple and correct. It was possible to measure sequential development of intact cotyledon area of the plants.

## Cotyledon Development

Fig. 1 shows the development of cotyledons from emergence to senescence. At emergence, cotyledon area of hybrid I and II were similar to open-pollinated(OP), non-hybrid seedlings of their maternal parents, however the maximum areas were smaller. In general, cotyledon expansion rates of hybrids I and II were lower than that of the non-hybrid, OP seedlings of their maternal parents. Cotyledons of all entries expanded rapidly during the first week, then the rate of expansion declined until maximum size was reached at about 3 weeks after emergence. After 3 weeks the cotyledons entered senescence and began to drop. Maximum cotyledon area of *L. corniculatus*, *L. tenuis*, and hybrid I and II were respectively 6.1, 5.6, 4.9 and 4.7 times larger compared to those at emergence. One mg of seed of each entry produced cotyledon areas of 0.52, 0.29, 0.31 and 0.22  $\text{mm}^2$ , respectively. Lovell and Moore(1970) reported that cotyledons with a high expansion factor and photosynthetic ability increased in weight during seedling development in contrast to the non-expansion cotyledon type. Machado et al. (1974) also showed that the species in which the cotyledons are important as photosynthetic

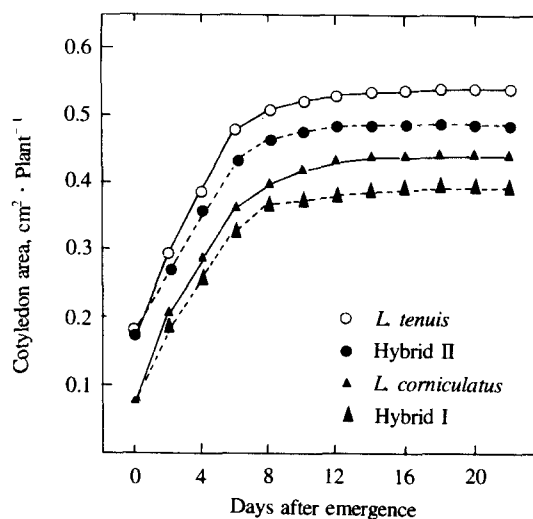


Fig. 1. Changes in cotyledon area of *L. corniculatus* (—▲—), *L. tenuis* (—○—), hybrid I (---▲---), and hybrid II (---●---) during early seedling development.

organs had the function of increasing cotyledon area with a corresponding increase in weight. Our results indicate that cotyledons of trefoil species appear to be decisively important as photosynthetic organs.

### Factors Governing Seedling Growth

Table 2 indicates that larger seeds produced a greater initial cotyledon area, however cotyledon expansion rate was not correlated with seed size. Although cotyledon area upon emergence was correlated with seed size, after emergence there was a divergence in expansion rates for

cotyledons among the entries not attributable to seed size. Shibles and MacDonald (1962) also indicated that there were varietal divergences in growth and photosynthetic area expansion which were independent of initial seedling weight and cotyledon area of birdsfoot trefoil. McKersie and Tomes (1982) reported that seed size of trefoil alone was not a reliable indicator of seedling vigor and Twamley (1972) found that the seed size of trefoil parents could not be used to predict seedling vigor of the progeny. The most striking feature in Table 4 was the early onset of leaf production of the hybrids

Table 2. Correlation coefficients among 100-seed weight, cotyledon area, cotyledon expansion rate, leaf area, seedling weight, and net photosynthetic rate

	Cotyledon area	Cotyledon expansion rate	Leaf area	Seedling weight	Net photosynthetic rate
100-seed weight	0.609	-0.101	0.545	0.657*	0.426
Cotyledon area		0.409	0.607	0.659*	0.493
Cotyledon expansion rate			0.354	0.394	0.082
Leaf area				0.978**	0.697*
Seedling weight					0.701*

\*\*\*: Statistically significant at 0.05 and 0.01 probability levels, respectively.

Table 3. Cotyledon area, leaf area, total seedling weight, net photosynthetic rate, and dark respiration rate of *L. corniculatus*, *L. tenuis* and their reciprocal hybrids measured 3 weeks after emergence

Species	Cotyledon area (cm <sup>2</sup> /plant)	Leaf area (cm <sup>2</sup> /plant)	Total seedling weight (mg/plant)	Net photosynthetic rate (μg CO <sub>2</sub> dm <sup>-2</sup> Hr <sup>-1</sup> )	Dark respiration rate (μl O <sub>2</sub> g <sup>-1</sup> Hr <sup>-1</sup> )	
					Shoot	Root
<i>L. corniculatus</i>	0.47 <sup>bcd</sup>	23.8 <sup>bc</sup>	258.3 <sup>cd</sup>	35.6	3727	3113
<i>L. tenuis</i>	0.55 <sup>b</sup>	17.1 <sup>cd</sup>	189.3 <sup>de</sup>	33.9	3540	2723
M2 × 9	0.40 <sup>de</sup>	21.6 <sup>bc</sup>	223.2 <sup>cd</sup>	39.8	3537	3432
M2 × 1	0.42 <sup>de</sup>	24.1 <sup>bc</sup>	223.8 <sup>cd</sup>	29.9	3819	2928
M2 × 4	0.30 <sup>f</sup>	10.6 <sup>d</sup>	126.0 <sup>ef</sup>	26.9	4026	3093
M2 × 2	0.45 <sup>cd</sup>	24.2 <sup>bc</sup>	224.5 <sup>cd</sup>	34.9	3945	3359
4 × m2	0.34 <sup>ef</sup>	9.1 <sup>d</sup>	100.3 <sup>f</sup>	34.1	3374	3159
2 × m2	0.53 <sup>bc</sup>	40.4 <sup>a</sup>	417.5 <sup>ab</sup>	41.1	3188	2921
1 × m2	0.52 <sup>bc</sup>	39.1 <sup>a</sup>	454.7 <sup>a</sup>	39.2	3969	3312
9 × m2	0.66 <sup>a</sup>	26.9 <sup>b</sup>	320.7 <sup>bc</sup>	36.1	4217	3094
LSD .05	0.09	9.3	99.4	11.3	555	523

of  $2 \times m_2$  and  $1 \times m_2$  compared to the others, end earlier leaf development contributed to their vigorous seedling growth. Most previous studies stressed the importance of seed size for the seedling vigor of birds-foot trefoil, but Table 3 and 4 show that photosynthetic area which was not attributed to seed size was a major determinant factor for initial seedling growth. These results confirm our previous reports. Our previous reports concluded that initial seedling growth was dependent on cotyledon size and subsequent plant yield was determined by photosynthetic ability (Hur et al., 1994a). Moreover, no differences in photosynthetic rate among trefoil species were observed during early seedling growth, while there was some difference among species during the later seedling growth (Hur et al., 1994b).

Hybrids  $2 \times m_2$  and  $1 \times m_2$  showed initial rapid photosynthetic area development, higher rates of photosynthesis and the most vigorous seedling growth. It was supposed that seedlings with larger cotyledons produced true leaves earlier than those with smaller

cotyledons. Seedlings with larger initial cotyledons and low cotyledon expansion rate appear to have used photosynthate in the production of true leaves, while those with smaller cotyledons and higher cotyledon expansion rate concentrated in cotyledon development which delayed leaf development. Data of Machado et al., (1974) revealed that species with large cotyledons showed decreased cotyledon area and produced leaves earlier, while species with small cotyledons showed increased cotyledon area and poor leaf area production in early seedling growth. Hybrids  $2 \times m_2$  and  $1 \times m_2$  rapidly produced true leaves having a high photosynthetic rate. There was a promising possibility for these two  $F_1$  hybrids to contribute to the increase of seedling vigor of birdsfoot trefoil. Breeding for improved seedling vigor of birdsfoot trefoil should emphasize selection for the rapid development of photosynthetic area combined with high rate of photosynthesis as observed in hybrids  $1 \times m_2$  and  $2 \times m_2$ .

Table 4. Leaf development of *L. corniculatus*, *L. tenuis*, and their reciprocal hybrids

Species	Days after emergence							
	4	6	8	10	12	14	16	20
<i>L. corniculatus</i>	0.05	0.7	1.5	2.4	3.2	4.3	5.7	11.7
<i>L. tenuis</i>	0.14	1.1	1.9	3.5	4.1	5.4	6.7	12.4
$M_2 \times 9$	0.00	0.8	1.2	2.1	3.1	3.7	5.4	9.8
$M_2 \times 1$	0.14	0.9	1.7	2.7	3.1	4.1	5.4	10.0
$M_2 \times 4$	0.00	0.4	1.4	2.2	2.8	3.7	4.4	7.1
$M_2 \times 2$	0.00	0.7	1.3	2.3	3.5	4.5	5.7	10.5
$4 \times m_2$	0.00	0.3	0.8	1.9	2.8	3.8	4.4	7.0
$2 \times m_2$	0.50	1.4	2.1	3.2	4.5	5.9	7.8	17.7
$1 \times m_2$	0.50	1.4	2.0	3.0	4.6	6.1	8.8	18.8
$9 \times m_2$	0.20	0.8	1.5	2.6	4.2	5.9	7.4	13.6

#### IV. SUMMARY

Seedling development among reciprocal  $F_1$  hybrids and their parents, *Lotus corniculatus* and *L. tenuis*, were studied using a simple method of measuring cotyledon

area.

Cotyledon area attached to the plant could be measured with simple rapid method. Cotyledons expanded very rapidly during the first week, then slightly expanded until maximum size was reached around 3 weeks after

emergence. Cotyledon area upon emergence was correlated with seed size ( $P=0.05$ ), but after emergence there was a divergence in cotyledon expansion rate. Rapidity of photosynthetic area development was determinant factor for seedling growth of trefoil species.  $2 \times m^2$  and  $1 \times m^2$  among the hybrids promised the possibility increasing seedling vigor of birdsfoot trefoil.

## V. LITERATURE CITED

1. Ashcroft, W.J. and David R. Murray. 1979. The dual functions of the cotyledons of *Acacia iteaphylla* F. Muell. (Mimosoidead). Aust. J. Bot., 27:343-352.
2. Beuselinck, P.R. and R.L. McGraw. 1983. Seedling vigor of three *Lotus* species. Crop Sci. 23:390-391.
3. Brown, R. 1941. The growth of isolated cotyledons of Cucurbita Pepo. Ann. Bot. 18:175-191.
4. Cooper, C.S. and S.C. Fransn. 1974. Contribution of cotyledons to growth of the sainfoin seedling. Crop Sci. 14:732-735.
5. Goodwin, R.H. 1937. Studies on the seedling development of *Solidago rugosa* Hill., *S. senoer-vurebs* L. and the reciprocal hybrids between them. Amer. J. Bot. 24:627-640.
6. Hill, J.B. 1925. Cotyledon form and sile in reciprocal hybrids between species of *Digitalis*. Bot. Gaz. 80:84-92.
7. Hur, S.N., C.J. Nelson, P.R. Beuselinck and J.H. Coutts. 1994a. Seedling vigor of birdsfoot trefoil entries differing in seed size. J. Korean Grassl. Sci. 14(3):186-194.
8. Hur, S.N., C.J. Nelson, P.R. Beuselinck and J.H. Coutts. 1994b. Seedling vigor of two *Lotus* entries and their reciprocal  $F_1$  hybrids. J. Korean Grassl. Sci. 14(4):288-294.
9. Lovell, P.H. and K.G. Moore. 1970. A comparative study of cotyledons as assimilatory organs. J. Exp. Bot. 21:1017-1030.
10. Lovell, P.H. and K.G. Moore. 1971. A comparative study of the role of the cotyledon in seedling development. J. Exp. Bot. 22:153-162.
11. Machado, A.D., W.A. Williams and C.L. Tucker. 1974. Dry matter contribution by cotyledons of lima beans and other epigeal legumes. Crop Sci. 14:90-93.
12. McKee, G.W. and M.L. Risius. 1970. Varietal differences in crownvetch cotyledon size. Agron. J. 62:711-714.
13. McKersie, B.D. and D.T. Tomes. 1982. A Comparison of seed quality and seedling vigor in birdsfoot trefoil. Crop Sci. 22:1239-1241.
14. Shibles, R.M. and H.A. Mac Donald. 1962. Photosynthetic area and rate in relation to seedling vigor of birdsfoot trefoil (*Lotus corniculatus* L.). Crop Sci. 2:299-302.
15. Twamley, B.E. 1972. Heritability for combining ability of seedling vigor in birdsfoot trefoil. Can. J. Plant Sci. 5:1059-1065.
16. Williams, W.A., J.N. Black and C.M. Donald. 1968. Effect of seed weight on the vegetative growth of competing annual Trifoliums. Crop Sci. 8:660-663.