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Seedling Vigor of Two Lotus Entries and Their Reciprocal F_1 Hybrids

S. N. Hur*, C. J. Nelson, P. R. Beuselinck and J. H. Coutts**

두 Lotus種과 그들 交雜種間의 幼植物 活力 比較

허삼남* · C. J. Nelson · P. R. Beuselinck · J. H. Coutts**

摘　　要

Birdsfoot trefoil(MO-20)과 tetraploid narrowleaf trefoil(PI302921) 및 이들 種間 雜種의 幼植物 活力을 乾物蓄積量, 光合成率, 暗呼吸率, 生長分析 등을 통하여 비교검토하였다.

일반적으로 種間 雜種의 表現型은 그 들 부모의 중간 특성을 나타내었으며, 暗呼吸, 光合成 및 幼植物 生育은 이 들 母系 系統인 MO-20 birdsfoot trefoil과 類似하였다. 중간 잡종은 birdsfoot trefoil 보다 빨리 사랐으나 통계적인 有意性은 없었다. 또한 교잡종은 narrowleaf trefoil보다 유식물 활력이 더 우수하였는데 그것은 낮은 暗呼吸率과 높은 相對生長率에 기인되었다. 앞으로 birdsfoot trefoil의 幼植物 活力의 遺傳的인 改良은 새로운 분야로서 改良의 여지가 많음을 보여 주었다.

I. INTRODUCTION

Improvement of seedling vigor is a desirable objective in a birdsfoot trefoil (*Lotus corniculatus*) breeding program. Seedling vigor has been improved by selection for increases seed size within existing or introduced trefoil cultivars (Twamley, 1971, 1974; Peacock and Wilsie, 1960; Draper and Wilsie, 1965; Hensen and Tayman, 1961; Conje and Carlson, 1973).

A potential method for improving seedling vigor of birdsfoot trefoil may be through crossing with closely related species. MacDonald (1946) and Wernsman et al. (1964) obtained fertile progeny when colchicine-induced 4x *L. tenuis* plants were crossed with *L. corniculatus*. Bent (1962) utilized embryo culture techniques to derive autotetraploids, and obtained three new interspecific hybrids. Somaroo and Grant (1972) reported that transfer of desirable germplasm from diploids to tetraploid *L. corniculatus* by means of experimentally-produced amphidiploids can be accomplished with little difficulty.

Our previous reports (Beuselinck and McGraw, 1983; Hur et al., 1994) suggested the possibility for improving seedling vigor of birdsfoot trefoil through introgressive hybridization, specifically by transferring the superior initial seedling vigor of PI302921 to MO-20 trefoil. This paper presents comparisons of seedling vigor of MO-20, tetraploid accession PI302921, and their reciprocal hybrids. Seedling vigor was evaluated by dry matter accumulation, dark respiration rate, photosynthetic rate, and growth analysis parameters.

II. MATERIALS AND METHODS

Birdsfoot trefoil 'MO-20' (*Lotus corniculatus*), tetraploid narrowleaf trefoil introduction PI302921 (*Lotus tenuis*), and 12 lines of reciprocal F_1 hybrids of these two entries were used to compare seedling vigor. Hybrid seeds were obtained from greenhouse crosses near

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* Department of Animal Science, Chonbuk National University, Chonju 560-756 Korea(전북대학교 농과대학)

** Agronomy Department, University of Missouri, Columbia, MO 65211 U.S.A. (미국 미조리 주립대학)

Columbia, MO, U.S.A., using 4 MO-20 genotypes as female parents crossed to 3 PI302921 genotypes.

Seeds used in this study were screened to remove very large and very small seeds from each seed lot. One hundred seed weights of PI302921, MO-20, and the hybrid were 368, 103, and 107 mg, respectively. Seeds of each entry germinated 2 days after imbibition at 20℃. Germinated seeds were carefully transplanted into 10-cm wide by 15-cm deep plastic pots, filled with a soil mixture consisting of Mexico silt loam topsoil (Udollic Ochraqualf), peat and sand in a 2:1:1 ratio. Seedlings of the twelve seperate F_1 hybrids were planted at random to measure mean seedling vigor of the F_1 hybrid. Six seedlings were planted per pot for the samplings of week 0 (cotyledons emerged above soil), 1, and 2, four seedlings per pot for week 3 and 4, and two seedlings per pot for the sampling of week 5 and 6. Pots of all entries were placed at random within a growth chamber, then changed in location weekly.

Most seedlings emerged 2 days after transplanting. Subsequent to emergence the plants were grown at 25℃ with the photosynthetic photon flux density of 500 umol $m^{-2} \cdot S^{-1}$ at the canopy level by cool-white fluorescent and incandescent bulbs. Photoperiod was 14 hours and relative humidity was maintained near 70%. Pots were watered weekly during the growth period with 50 ml of complete nutrient solution and with distilled water when needed.

Every week 6 pots of each entries were removed from the growth chamber. Three pots were used for measuring apparent photosynthesis and the other 3 pots for measuring dark respiration. Dark respiration was estimated manometrically and apparent photosynthesis was measured using infrared analysis at 25℃ as described earlier (Hur et al., 1994). After measurement of dark respiration and apparent photosynthesis, plants were separated into cotyledons, leaves, stems, and roots. Areas of cotyledons and leaf blades were measured with

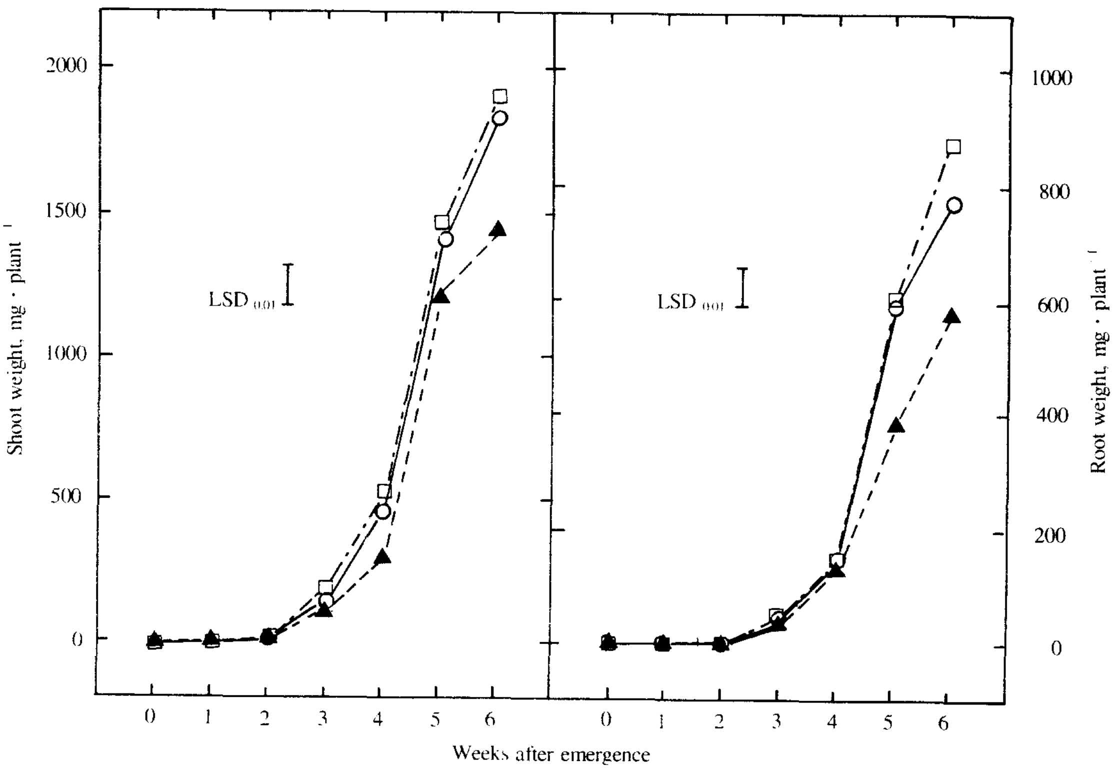

Fig. 1. Dry matter yield of shoot and root of *Lotus corniculatus*(—○—), *Lotus tenuis*(---▲---), and their F_1 hybrids(---□---) grown at 25℃.

a LI-COR model 3000 leaf area meter. All plant parts were dried in a forced-draft oven for 48 hours at 80℃ and weighed. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were computed from formulas following Cooper(1966).

Ⅲ. RESULTS AND DISCUSSION

Introgressive F_1 hybrids taken as the mean of 12 crosses exhibited better shoot and root growth than did their parents (Fig. 1). But there was no significant difference between the hybrids and birdsfoot trefoil(MO-20) in seedling growth. Narrowleaf trefoil(PI302921) showed better initial seedling growth than MO-20 or the hybrid, but 2 weeks after emergence both MO-20 and the hybrid grew much faster than 302921. The differences in plant weight among entries increased with ages of seedlings.

The seedling growth of parents showed growth patterns similar to those in our previous study (Hur et al., 1994). The hybrids were slightly more vigorous than their parents, perhaps due to heterosis, an exact reason cannot be determined because the seed lots tested were produced in different years and locations. However, Wernsman et al. (1965) reported vigorous interspecific hybrids between *L. tenuis* and *L. corniculatus*, and Somaroo and Grant (1971) obtained very vigorous interspecific hybrids between diploid species of *Lotus*, suggesting that heterosis may account for the expreseed vigor of our hybrids.

With increasing age, less of the dry matter was partitioned into leaves than into stems for all entries (Fig. 2).

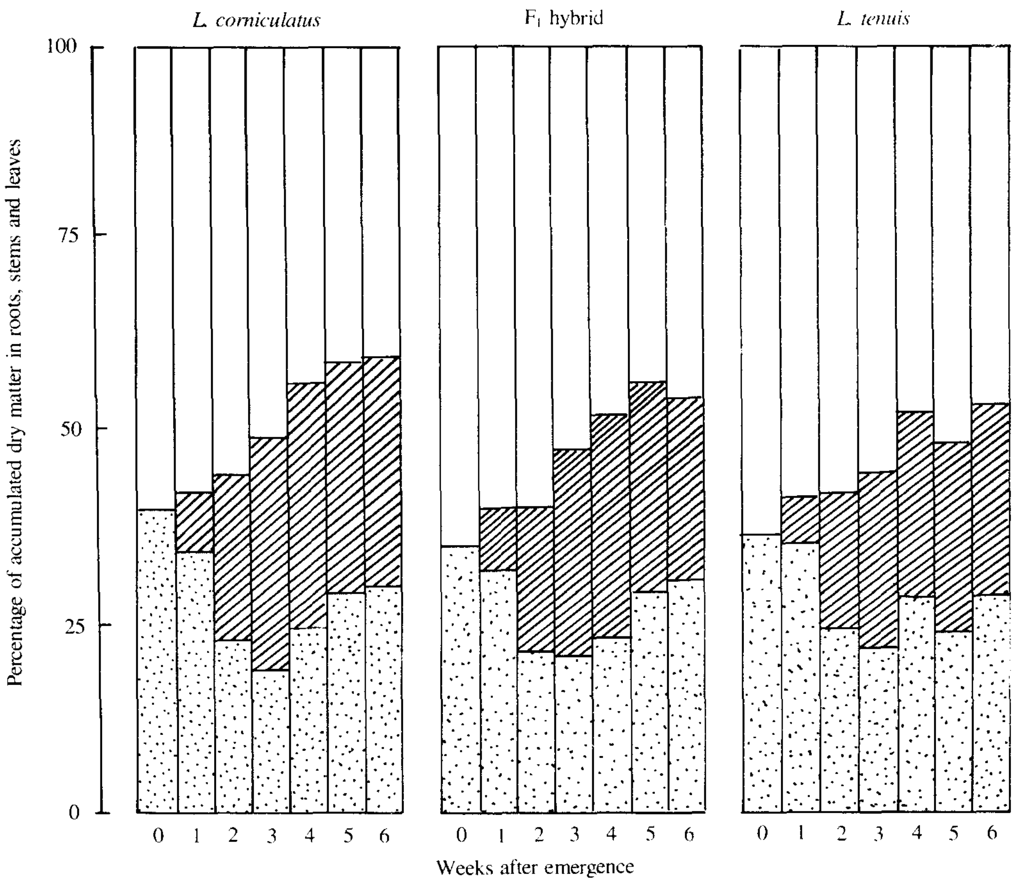

Fig. 2. Changes in partition of accumulated dry matter into leaves(□), stems(▨), and root(▩) of *Lotus corniculatus, Lotus tenuis*, and their F_1 hybrid with time.

Proportion of dry matter accumulated in roots decreased for the first 3 weeks then increased to 6 weeks of age in all entries. PI302921 and the hybrids tended to have more leaves than MO-20. Cooper (1967) studied the distribution of the dry matter of alfalfa and birdsfoot trefoil at 2, 4, and 6 weeks of age under different light intensities and showed that with increased seedling age more dry matter was partitioned into roots and then into stems.

Apparent photosynthesis of parents and their F_1 hybrid is shown in Fig. 3. During week 1 and 2 photosynthesis of all entries was predominately from the cotyledons. Following expansion of true leaves 3 weeks after emergence the rate of photosynthesis was higher, but dropped sharply with cotyledon senescence, leaf age and shading until 6 weeks of age. Lane and Hesketh (1977) reported that apparent photosynthetic rate per cotyledon of cotton increassed until expansion was completed, and then decreased linearly and steeply with age. Elmore et al. (1967) also reported that young, but fully expanded leaves of 13 different species of plants had the highest photosynthetic rate, followed by a steady decrease until the leaves became photosynthetically inactive. There was no difference in photosynthetic rate among entries in early seedling growth until about 3 weeks of age. The higher photosynthetic rate of 302921 at week 5 and 6 was attributed to its slower growth and less leaf shading than for MO-20 or the hybrid.

The dark respiration rate of the seedlings was initially very high at emergence (Fig. 4), then decreased rapidly, stabilized for weeks 1 through 3, and then dropped again to the end of the test period. Dark respiration rate of the shoot was higher but quite similar to that of the root. In the early weeks of seedling growth, the dark respiration of MO-20 was higher than that of 302921 or the hybrid, while 302921 had the highest respiration rate in later weeks. Dark respiration and photosynthetic rate of plants older than 3 weeks decreased as cotyledons senescenced and leaves aged. Qualls and Cooper (1968) showed that maximum respiration rate of birdsfoot trefoil occurred from 2 to 4 days after germination at 21.1 and 26.7℃, then decreased linearly. Opik and Simon (1963), and Lane and Hesketh (1977) studied respiration rates of bean and cotton cotyledons and reported that respiration rates were high on 1-day-old cotyledons, but decreased linearly with age until the onset of cotyledon senescence.

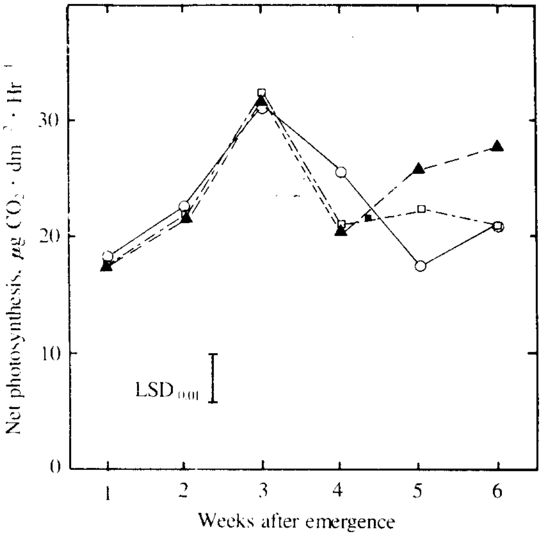

Fig. 3. Changes in net photosynthetic rate of *Lotus corniculatus*(—○—), *Lotus tenuis*(---▲---), and their F_1 hybrid(-·-□-·-) with time.

Relative growth rate (RGR) and its components are shown in table 1. During their first 3 weeks, the hybrids and MO-20 showed significantly higher RGR than 302921. The 302921 showed lower NAR than MO-20 or the hybrids. The RGR and NAR of all entries were higher during the first 3 weeks of growth than during the later 3 weeks. It was assumed the decreased RGR in later growth period occurred due to increased leaf age, competition among plants within the pot, and self-shading. The LAR increased with time except during the last week in all entries.

Seed size of the hybrids using MO-20 as the female parent was almost the same as that of MO-20 parent, and both were much smaller than seeds of 302921. Growth characteristics of the hybrid were similar to that of MO-20, as initial seedling growth of the hybrid was inferior to that of 302921, but in later showed higher

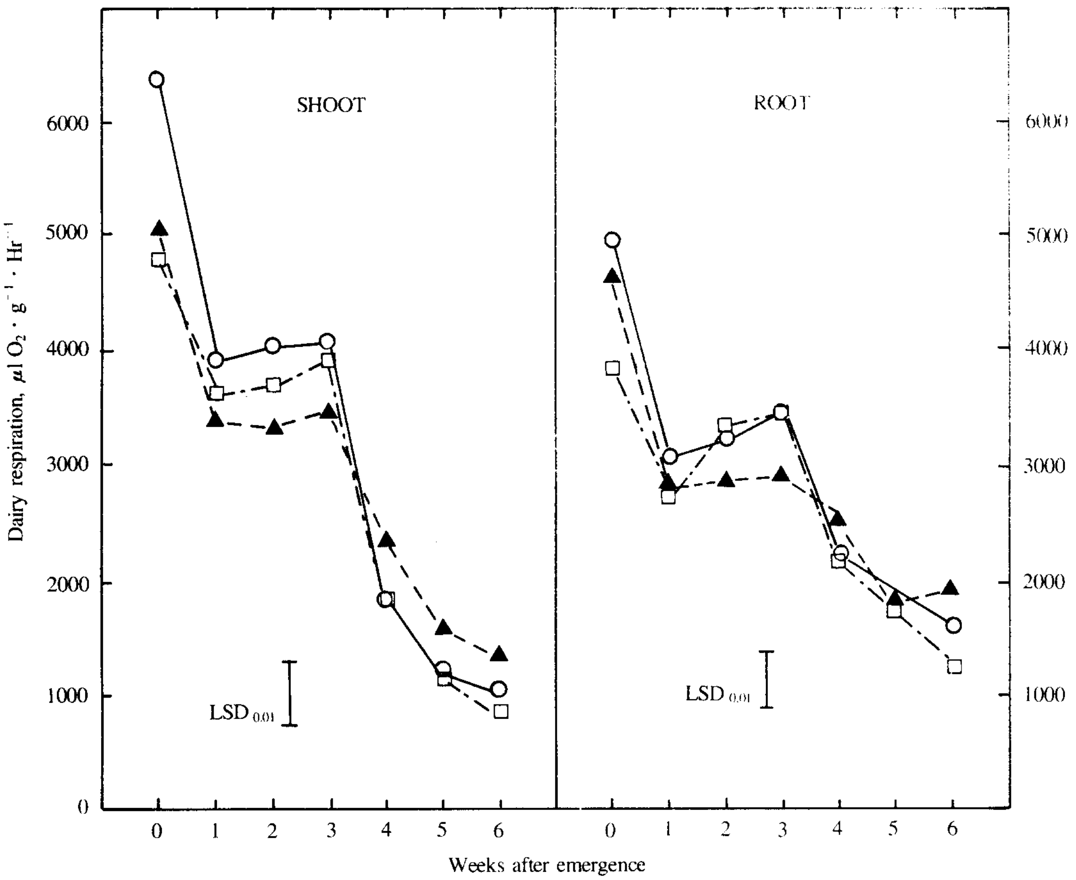

Fig. 4. Trends of dark respiration of *Lotus corniculatus*(—○—), *Lotus tenuis*(---▲---), and their F_1 hybris(-·-□-·-).

RGR and better seedling growth. These results compare well with earlier reports that initial photosynthetic area and seedling vigor are determined by seed size (Black, 1956; Stickler and Wassom, 1963), and subsequent plant yield is determined by relative growth rate (Cooper, 1966). Cooplr (1967) suggested RGR as a useful tool in studying differences in seedling vigor. Reciprocal hybrids have now been produced and are under evaluation. As wide genetic diversity exists within *L. corniculatus* (Bent, 1962) and trait differences occur within and between *Lotus* species (Smith, 1956), there is a possibility for improving seedling vigor of *Lotus corniculatus* using introgressive crossings.

Ⅳ. SUMMARY

Seedling vigor of introgressive hybrids between birdsfoot trefoil 'MO-20'(*Lotus corniculatus*) used as the maternal parent and tetraploid (2n=4x=24) accession PI302921 narrowleaf trefoil (*Lotus corniculatus* L.), and parents were compared in terms of dry matter accumulation, net photosynthetic rate, dark respiration rate, and growth analysis. The hybrids showed better seedling vigor than PI302921 as they had lower dark respiration rate and higher relative growth rate (RGR) than PI302921. Hybrids grew faster than MO-20, but there was not significant difference between them. Net photosynthesis per unit area increased during three weeks after emergence without difference among the entries, then decreased sharply with leaf age and shading marking much differences among entries. Dark respiration

rate was very high just after emergence, after which it decreased rapidly until it reached a stable level at 1 week through 3 weeks of age, and then dropped again. In general, dark respiration, photosynthesis, and seedling growth of the hybrid was similar to those of MO-20. Although there are some reports about interspecific hybrids of *Lotus* species, it still remains as a new area for genetic improvement of seedling vigor of *L. corniculatus*.

Table 1. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) of *L. corniculatus* (MO-20), *L. tenuis* (PI3-2921), and their F_1 hybrids.

Species	Week	RGR	NAR	LAR
L. corniculatus	1	0.29	2.39	0.06
	2	0.24	1.39	0.42
	3	0.31	2.12	3.38
	4	0.18	1.75	6.39
	5	0.17	2.45	13.94
	6	0.04	0.66	4.82
	mean	0.20	1.79	4.82
L. tenuis	1	0.19	2.20	0.06
	2	0.23	1.59	0.56
	3	0.22	1.69	2.31
	4	0.16	1.72	4.33
	5	0.17	2.37	11.89
	6	0.03	0.49	4.09
	mean	0.17	1.68	3.89
F_1 hybrid	1	0.28	2.13	0.07
	2	0.29	1.59	0.71
	3	0.29	2.03	4.43
	4	0.15	1.56	5.88
	5	0.16	2.35	13.08
	6	0.05	0.88	6.66
	mean	0.20	1.76	5.14
$LSD_{0.05}$		0.04	0.42	2.14

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