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Seedling Vigor of Birdsfoot Trefoil Entries Differing in Seed Size

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種子 크기가 다른 두 系統의 벌노랑이 幼植物의 活力 比較

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摘　要

넓은 잎 벌노랑이의 利用에 있어서 初期生育 不振이 가장 심각한 문제점으로 대두되고 있다. 따라서 幼植物 活力增進을 위한 育種의 基礎資料로 이용하기 위하여 種子가 작은 MO-20과 種子가 큰 302921 두 系統을 15℃와 25℃의 생육상에서 栽培하여 유식물 활력을 비교 검토하였다. 二酸化炭素 交換率(CER)은 적외선 가스분석기를 이용하여 closed system과 open system으로 측정하였다. 暗呼吸率은 Gilson 呼吸計로 測定하였으며 日當 純炭素 蓄積率과 유식물 생장분석도 조사하였다.

302921은 MO-20에 비해 種子크기는 약 3.5배 가량이, 最大 伸長 子葉 面積은 약 2배 가량이 더 컸다. 25℃에서 出現 후 3주까지는 302921의 초기 유식물 생장이 더 양호하였으나 3주 이후부터 試驗終了시 까지는 오히려 MO-20이 302921보다 葉面積當 CER이 더 높고 乾物蓄積率도 더 많았다. 반면 15℃에서는 MO-20이 302921보다 生長이 더 억압 받았다. MO-20의 暗呼吸率은 302921보다 약간 높았으나 日當 純二酸化炭素 吸收率은 MO-20이 25℃에서 높았고 15℃에서는 낮았다. MO-20의 相對生長率은 25℃에서는 높은 純同化率로 인하여 302921보다 높았으나 15℃에서는 系統間 差異가 별로 없었다.

I. INTRODUCTION

Early and rapid seedling growth of forage species are important for successful stand establishment. Investigators have shown that early seedling growth of legumes is proportional to seed size (Cooper, 1977). In addition to added food supply, large seeds of epigeal emerging legumes can produce seedlings with large cotyledons and leaf area, thus influencing subsequent plant growth. Seedling vigor of forage legumes can be predicted according to seed size within seed lots of the same cultivar (Black, 1959; Henson and Tayman, 1961; Stickler and Wassom, 1963; Carleton and Cooper, 1972), but not always (McKersie and Tomes, 1982). Little relationship between seed size and seedling vigor may exist among cultivars or species (Cooper and Qualls, 1968; Twamley, 1967). For example, Twamley (1967) found some large-seeded lines of birdsfoot trefoil (*Lotus corniculatus* L.) had poor seedling vigor and some small-seeded lines had excellent seedling vigor, though Stickler and Wassom (1963) reported that regardless of cultivar the dry weights of birdsfoot trefoil seedlings harvested one month after planting were closely related to seed size.

Seed size of introduction 302921, a large-seeded accession of broadleafed trefoil from Spain (Beuselinck, et al., 1985) is about 3.5 times heavier than that of cultivated broadleafed birdsfoot trefoil cultivars (Beuselinck and McGraw, 1983), presumably contributing to its good early seedling vigor. However, its later seedling

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growth has been observed to be inferior to cultivated broadleafed trefoil. Our research was undertaken to evaluate seedling vigor of two broadleafed birdsfoot trefoil entries, MO-20 and 302921, that is differed in seed size. Emphasis was placed in cotyledon area expansion, dry matter accumulation of the seedlings, carbon budget of the seedlings, and relative growth rate (RGR) and its components.

Ⅱ. MATERIALS AND METHODS

Seeds of MO-20 and introduction 302921 were stored in sealed containers at about 4℃ until experimentation. Seeds were processed using a seed separator (Blower) to improve uniformity of seed size within each lot. Uninoculated seeds were germinated on moistened filter paper in covered Petri- disher at 20℃. Two days after imbibition, seven germinated seeds were transferred from Petri-dishes and planted about 1.0 cm deep in plastic pots, 10 cm dia by 15 cm deep, filled with a soil mix consisting of Mexico silt loam topsoil (Udollic Ochraqualf), peat, and sand in a 2:1:1 ratio. Each pot had six 3-mm holes in the bottom to permit drainage. The pots were placed in growth chambers as below at 20℃, where the seedlings emerged (cotyledons folded, but visible at soil level) in 2 days.

At cotyledon emergence six pots of each species were sampled, and remaining plants were transferred to controlled growth chambers at constant 15° and 25℃. Photosynthetic photon flux density (PPFD) of 500 μmol $\cdot m^{-2} \cdot S^{-1}$ at the canopy level was provided by cool-white fluorescent and incandescent bulbs. Photoperiod was 14 hours and relative humidity was maintained about 70%. Pots were watered with distilled water as needed, and weekly with 50 ml of Hoagland's complete nutrient solution. Subsequently, six pots of each entry were sampled from each temperature each week, three pots for measuring photosynthetic rate and three pots for measuring dark respiration rate. Following gas exchange and dark respiration measurements, tissue from all six pots was dried at 70℃ in a forced-draft oven and weighed. Seedlings in remaining pots were thinned from 7 to 4 per pot 2 weeks after emergence, and to one per pot 4 weeks after emergence to minimize competition in the pots.

Dark respiration.

Plant roots were carefully washed free of soil, then separated from shoots by cutting just below the cotyledonary node. About 20 to 30 mg of tissue of a dry-matter basis were placed in the outer well of a 17-ml single sidearm Gilson respirometer flask containing 2 ml of 0.2 M phosphate buffer solution (pH 6.7). For a CO_2-free atmosphere 0.2 ml of 20% KOH (w/v) was placed on a filter paper wick in the center well. The respirometer flask was shielded from room light with aluminum foil. Tissue was equilibrated at 15° or 25℃ for 30 minutes before rate of O_2 uptake was measured at 30 -min intervals over a 2-hour period.

CO_2 exchange rate

The CO_2 exchange rate (CER) of all plants grown at 15℃ and plants grown for 1 to 3 weeks at 25℃ were measured using an infrared CO_2 analyzer in a closed system (Lister et al., 1961; Wiedenroth and Poskuta, 1978).

The CER of plants grown for 4 or 5 weeks at 25℃ was measured using an open system (Wolf et al., 1969; Nelson et al., 1974) with air-sealed chambers developed especially to measure photosynthetic rate of the shoot. A PPFD of 500 μmol $\cdot m^{-2} \cdot S^{-1}$ was provided during measurement using both systems.

For measurements of CER in the closed system seedlings were washed free of soil and the hypocotyls placed in a split rubber stopper. The stopper was used to seal an 18 ml bottle such that the roots were immersed in distilled water. Then the top growth as enclosed in a 442 ml plexiglass chamber that was closed on the top and sealed to the stopper on the bottom. Shoots were illuminated from both sides with a total PPFD of 500 μmol $\cdot m^{-2} \cdot S^{-1}$ at the leaf surface by 600 W incandescent bulbs (General Electric model DYH). Water was circulated around the plexiglass photosynthetic chamber to keep the plants at 15° or 25℃. Volume of the closed system including the infrared analyzer, pump, and flow meter was 497 ml. Flow rate was 1 L · min $^{-1}$. After a 30-min adaptation period, measurements of CO_2 exchange rates of shoots were determined. The time required for CO_2 concentration in the chamber to decrease from 340 to 300 μl · L $^{-1}$ was used to calculate CER.

When plants were too large to fit the closed system, an open system and a square plexiglas chamber was used for measuring CER. A plexiglas plate with an open slit was slid under the cotyledons of the intact seedling such that hypocotyl was nearly centered. A square chamber was placed on the plate forming an air seal of positive pressure exiting the slit. Chamber dimensions were adjusted to fit the plant size without disturbing the natural canopy geometry. Flow rate was 3.0 to 3.5 litters min $^{-1}$ in the chamber, depending on plant size, to give a maximum decrease of 40 μl · L $^{-1}$ CO_2 from the 340 μl · L ^{1} source. Air temperature was 15° or 25℃ and PPFD was 500 μmol · m $^{-2}$ · S $^{-1}$.

Growth analysis.

Plants measured for photosynthetic rate and dark respiration were also used for growth analysis. Plants were seperated into cotyledons, leaf blades and petioles, stems, and roots. Cotyledon and leaf area were measured by a LI-COR model LI-300 leaf area meter. Plant parts were dried for 48 hours at 70℃, then weighed.

The daily carbon budget was constructed by cumulating CER over 14 h, then subtracting dark respiration of shoots cumulated over 10 h and roots cumulated over 24 h. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were computered from formulas following Thorne (1960).

Ⅲ. RESULTS AND DISCUSSION

Accession 302921, which has a larger seed mass and larger cotyledons, grew faster than MO-20 during the first 3 weeks after emergence at 25℃ (Fig. 1). There after, MO-20 had more rapid increase in shoot and root weight. Both entries grew better at 25℃ than at 15℃, but MO-20 differed more between temperatures than did 302921. Gist and Mott (1957) reported best shoot and root growth of broadleafed trefoil occurred at 21℃, and better shoot growth at 27℃ than at 16℃.

Cotyledon area increased over time for both entries (Fig. 2) and was affected by growth temperature. At 15 ℃, cotyledons expanded continuously until 3 weeks after emergence, while cotyledons at 25℃ expanded rapidly during only the first week after emergence, and then senescenced quickly. At high temperature cotyledons were relatively short-lived, and they had withered and fallen off by 14 days after emergence. Cotyledons were decisively important directly after emergence as photosynthetic organs (Fig. 3) and contributed to the early seedling growth.

Though seed weight and cotyledon area of 302921 (Fig. 2) was about 3.5 times greater than those of MO-20, fully expanded cotyledons weighed not more than twice than those of MO-20. Fully expanded cotyledonary area per mg of seed weight were 0.7 and 0.14 cm^2 for MO-20 and 302921 respectively. MO-20 seeds produced wider cotyledons per unit weight and had more rapid relative expansion rates than did 302921 (Fig. 2).

The CER of seedlings increased rapidly, with similar photosynthetic rates per unit leaf area until 3 weeks after emergence at 25℃ (Fig. 3). Afterwards MO-20 increased CER slowly, while 302921 decreased in CER indicating a major difference between entries. At 15℃ CER of 302921 was much higher than that of MO-20 until 3 weeks after emergence.

When seedlings emerged, dark respiration of shoots was very high (Fig. 4), but as cotyledons expanded the rate decreased rapidly and reached almost a stable level by the first week after emergence (Fig. 4). Dark respiration was higher at 25° than at 15℃. MO-20 seedlings showed a higher respiration rate than did 302921 at 25℃.

Net carbon accumulated in the plant tissue was calculated by deducting carbon lost daily by respiration from the carbon acquired daily by photosynthesis. At 15℃ net carbon accumulated in MO-20 was much lower than that of 302921, but at 25℃ net carbon accumulated was about double than that of 302921 (Table 1).

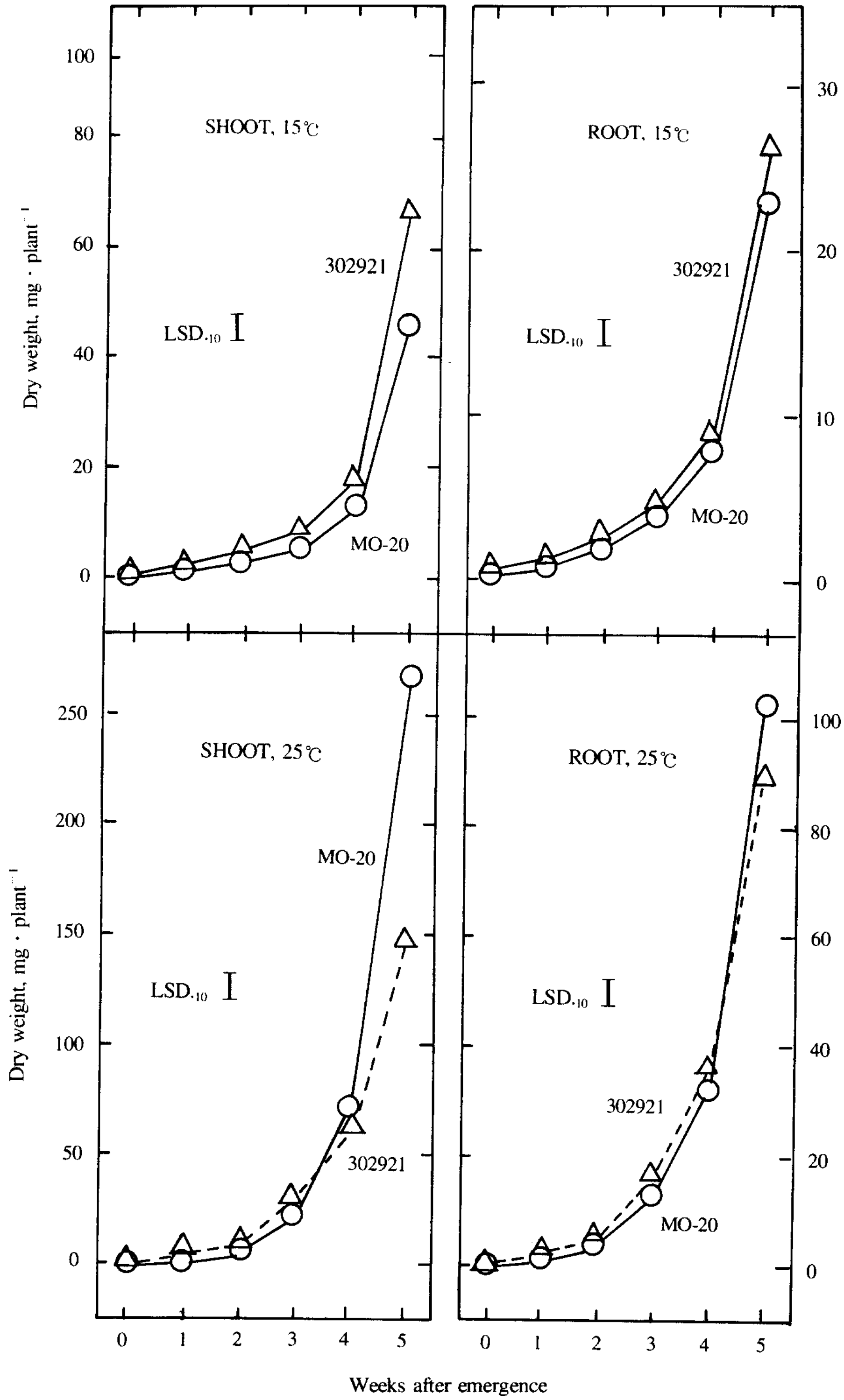

Fig. 1. Root and shoot seedling growth of MO-20(—○—) and 302921 (—△—) broadleafed birdsfoot trefoil entries grown at 15° and 25℃.

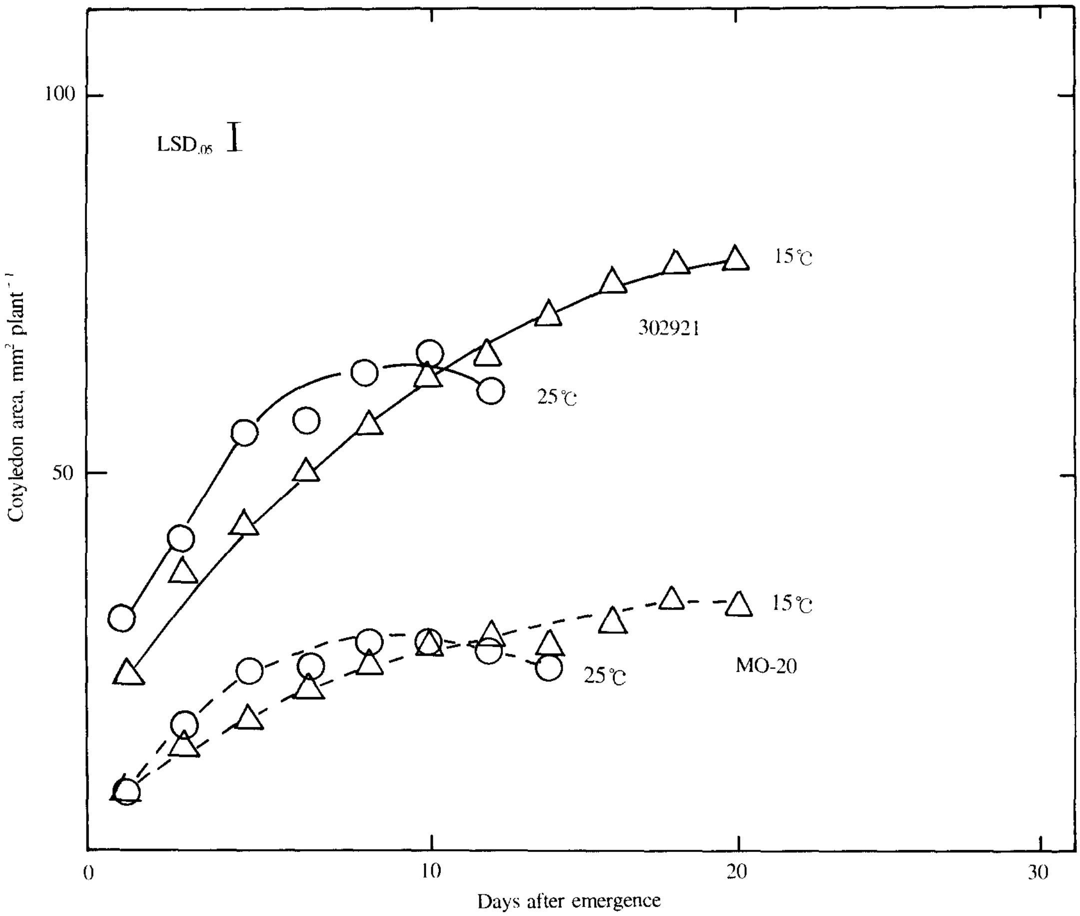

Fig. 2. Changes in cotyledon area of MO-20(—○—) and 302921 (—△--) broadleafed birdsfoot trefoil entries during seedling development at two temperatures.

Table 1. Net carbon accumulated in plants of two broadleafed birdsfoot trefoil entries at two temperatures

Entries	Temp.	Net carbon accumulated (mg. CO_2 / day)					
		Week 0	Weeks 1	Week 2	Week 3	Week 4	Week 5
Mo-20	15℃	−0.058	0.285	0.457	1.885	5.801	13.601
	25℃	−0.070	0.769	2.503	9.785	27.246	104.445
302921	15℃	−0.098	0.489	1.590	2.299	4.083	19.190
	25℃	−0.212	1.078	3.232	11.652	21.407	40.422

MO-20 trefoil had a greater RGR than the other entry at 25℃ with a marked difference at the later test periods. The greater RGR of MO-20 compared with 302921 was due to a greater NAR. The RGR of both entries decreased with age at 25℃, while little difference occurred through the growth period at 15℃. Both RGR and NAR at 25℃ were higher than at 15℃ and LAR of both increased with time (Table 2).

MO-20 grew very slowly during the first 3 weeks after emergence, due partially to the small seed size and

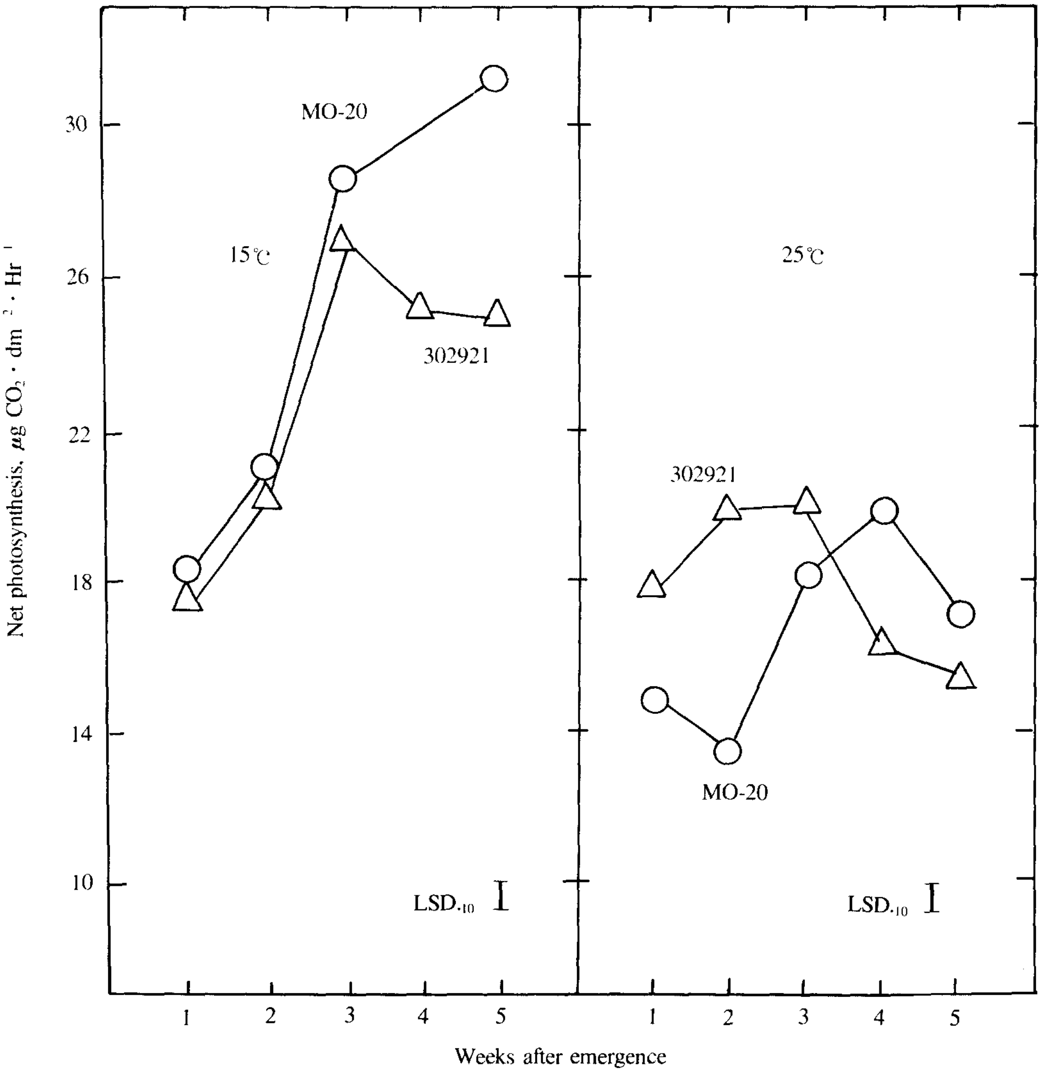

Fig. 3. Changes in net photosynthetic rate of MO-20(—○—) and 302921 (—△—) broadleafed birdsfoot trefoil entries at 15° and 25℃.

subsequent initial cotyledon area. Especially at low temperature, cotyledons of MO-20 expanded slowly and new leaves were produced slowly. The photosynthetic role of cotyledons overtime at low temperature appeared more important than at high temperature as the plants in low temperature produced leaves more slowly. The seedling growth of MO-20 during the first week after emergence could be the critical stage for successful stand establishment and subsequent growth. The seed reserves were being utilized rapidly as dark respiration rate was very high, while photosynthetic rate was low at this stage.

In agreement with Black (1956), initial plant growth in our study was dependent on seed size and subsequent plant yield was determined by RGR and photosynthetic ability. Black (1957, 1959) revealed in his later studies that the initial advantage in growth possessed by large seeds ceased to be of significance when plants had developed to a leaf area index of 4. The accession 302921 with large seeds and large cotyledon area had faster growth at 25℃ than MO-20 during the 3 weeks after emergence, but afterwards grew comparatively slowly,

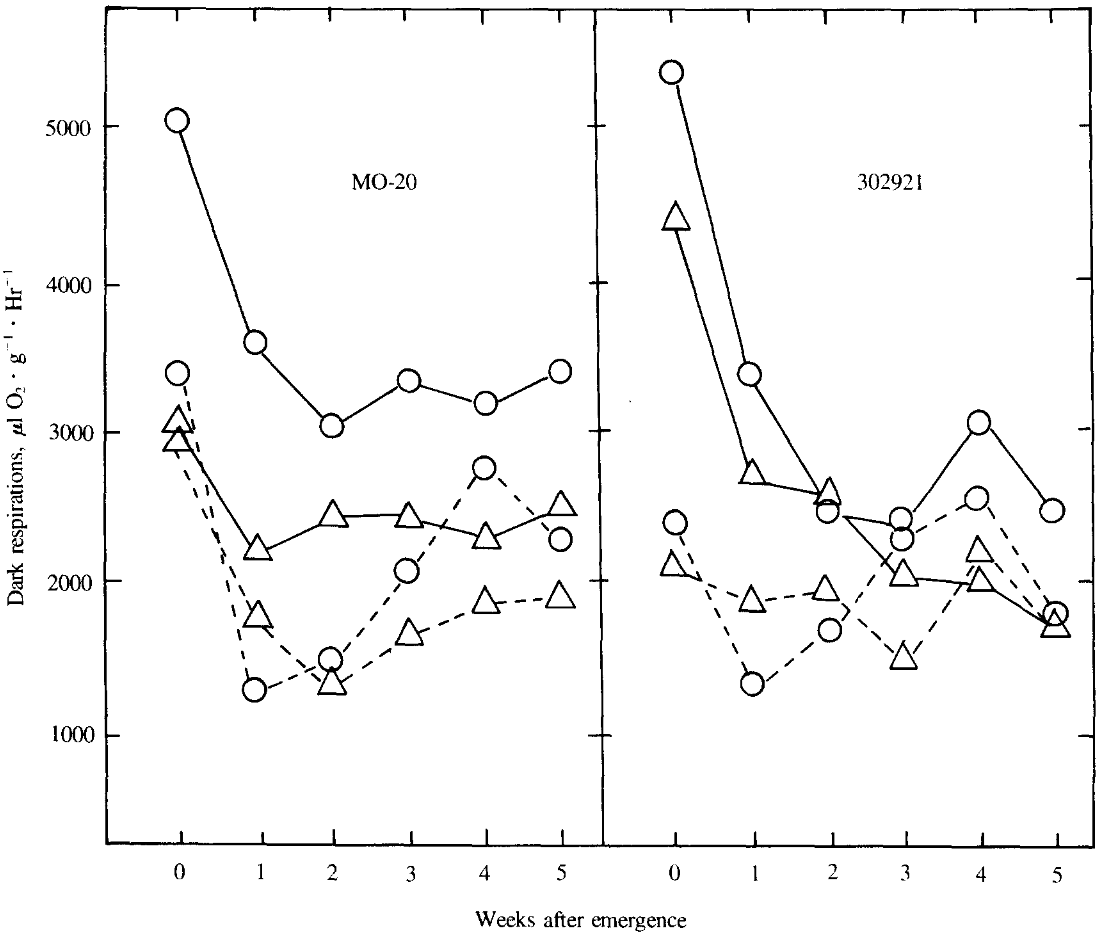

Fig. 4. Changes in dark respiration of two broadleafed birdsfoot trefoil entries during seedling development at two temperatures, shoot at 15℃(…○…); root at 15℃(…△…); shoot at 25℃ (—○—); root at 25℃(—△—).

probably due to the higher photosynthetic rate and NAR of MO-20.

These results show the possibility of improving seedling vigor of broadleafed trefoil by introgressive crossings with 302921 for large seed size. Based on our data attention should be directed toward increasing cotyledon expansion rate in addition to seed size in breeding trefoil for better seedling vigor. The response to large seed size would occur largely in the first 3 weeks after which growth would depend more on photosynthetic activity of the true leaves. Reciprocal crosses have been made between MO-20 and 302921 for evaluations of progeny for seedling vigor.

Ⅳ. SUMMARY

Lack of seedling vigor is considered a serious deterrent to use of broadleafed birdsfoot trefoil (*Lotus corniculatus* L.). Our objectives were to compare early seedling growth of broadleafed birdsfoot trefoil cultivar "MO-20" and the large-seeded accession 302921 at 15° and 25℃ in controlled-environment chambers. Carbon dioxide exchange rate (CER) was measured in a closed or open system using infrared gas analysis. Dark respiration rate was measured manometrically. Net carbon accumulated per day and growth analysis of the seedlings were calculated. Initial seed mass of 302921 was 3.5

Table 2. Components of relative growth rate of two broadleafed birdsfoot trefoil entries at two temperatures

Entries	Weeks after emergence	RGR		NAR		LAR	
		15℃	25℃	15℃	25℃	15℃	25℃
MO-20	Week 1	0.202	0.210	2.081	1.627	0.025	0.041
	Week 2	0.088	0.188	1.063	1.646	0.024	0.122
	Week 3	0.107	0.177	1.183	1.618	0.065	0.397
	Week 4	0.112	0.154	0.956	1.392	0.193	1.118
	Week 5	0.170	0.182	1.344	1.805	0.880	3.849
302921	Week 1	0.129	0.167	1.573	1.508	0.024	0.049
	Week 2	0.103	0.175	1.261	1.528	0.046	0.183
	Week 3	0.056	0.166	0.563	1.645	0.049	0.490
	Week 4	0.081	0.097	0.675	1.097	0.139	0.642
	Week 5	0.211	0.117	1.695	1.503	1.255	1.537

RGR; Relative growth rate (mg/mg/day).
NAR; Net assimilation rate ($mg/cm^2/day$).
LAR; Leaf area ratio (cm^2/mg plant).

times larger and final cotyledon area was 2 times larger than those of MO-20, and early seedling growth was better. But, from 3 weeks after emergence until the end of the test period at 25℃, MO-20 showed higher CER per unit leaf area and faster dry weight accumulation than did 302921. Compared with 25℃, growth of MO-20 at 15℃ was suppressed more than that of 302921. Dark respiration rate of MO-20 was slightly higher than that for 302921, but not CO_2 uptake per day for MO-20 was highest at 25℃ and lowest at 15℃. The relative growth rate (RGR) of MO-20 was higher than 302921 at 25℃ due to high net assimilation rate, but there was little difference in RGR between entries at 15℃.

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