Genetic variation for rate of establishment in caucasian clover

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Abstract

Slow establishment of caucasian clover (*Trifolium ambiguum* L.) is hindering the use of this legume in pasture mixtures. Improved genetic material is one strategy of correcting the problem. Newly harvested seed of hexaploid caucasian clover germplasm covering a range of origins, together with white and red clover and lucerne, were sown in 1 m rows in a Wakanui soil at Lincoln in November 1995. After 21 days, the caucasian clover material as a group had similar numbers of emerged seedlings as white clover and lucerne, but was inferior to red clover. There was wide variation among caucasian clover lines (48–70% seedling emergence), with the cool-season selection from cv. Monaro ranked the highest. Recurrent selection at low temperatures could be used to select material with improved rates of seedling emergence. Red clover and lucerne seedlings produced significantly greater shoot and root dry weight than caucasian and white clover seedlings. Initially, caucasian clover seedlings partitioned 1:1 shoot to root dry weight compared with 3:1 for white clover. After 2 months, caucasian clover seedlings had similar shoot growth but 3 times the root growth of white clover. Between 2 and 5 months, caucasian clover partitioned more to root and rhizome growth, resulting in a 0.3:1 shoot:root ratio compared with 2:1 for white clover. Both clover species had similar total dry weight after 5 months. Unhindered root/rhizome devel-opment is very important to hasten the establishment phase of caucasian clover. The caucasian clover lines KZ3 and cool-season, both selections from Monaro, developed seedlings with greater shoot and root growth than cv. Monaro. KZ3 continued to produce greater root growth after 5 months, indicating the genetic potential for improvement in seedling growth rate. Different pasture estab-lishment techniques are proposed that take account of the seedling growth characteristics of caucasian clover.

Keywords: establishment, genetic variation, growth, seedling emergence, *Trifolium ambiguum*

Introduction

Caucasian clover has recently shown potential as a persistent perennial pasture legume with high summer herbage production in lowland Canterbury and Bay of Plenty regions of New Zealand (Moss *et al.* 1996; Watson *et al.* 1996). Despite the growth potential, researchers have identified the establishment capability of caucasian clover as a critical factor in the successful use of this new legume in a pasture. The germination and early seedling emergence have been shown to be similar to those of white clover (*Trifolium repens* L.) in cabinet studies (Hill & Luck 1991) and under tussock country conditions (Lowther & Patrick 1992), but studies are required for lowland pastures. Recently, the nodulation and potential growth of seedlings has been improved with the release of an effective new *Rhizobium* strain, ICC148 (Pryor *et al.* 1998). Seedling growth is concentrated in the development of a large tap-root and a number of fleshy rhizomes arising horizontally from the top part of the tap-root (Hill & Mulcahy 1995). This extensive below-ground development appears to be at the expense of above-ground shoot growth which reduces the competitiveness of caucasian clover seedlings with associated grasses during the establishment phase of a pasture.

This study determines the extent of genetic variation for rate of seedling emergence and the subsequent development of those seedlings in a lowland situation using a range of caucasian clover germplasm. The early seedling development sequence was also compared with that of other commonly used pasture legumes.

Materials and methods

Genetic material

The seed lines tested in the experiment originated from a plant screening evaluation containing a range of caucasian clover material at AgResearch, Lincoln (Widdup *et al.* 1996). The hexaploid (6×) cultivars and breeding lines tested in the evaluation (each represented by 30 plants) were from Australia, USA and New Zealand (Table 1). The KZ series of lines was selected from the cultivar Monaro, mainly for improved seed yield (M. Norris pers.comm.). The cool-season selection

for improved early spring growth was also selected from Monaro (Widdup *et al.* 1996). All plants in the screening evaluation were interpollinated with bees in 1994–95 and seed collected from each plant to form a progeny line. A representative group of 14 progeny lines from each of the original parental lines was selected and sown in the establishment experiment (Table 1). Controls included the species Grasslands Huia white clover, Grasslands Pawera red clover (*T. pratense* L.) and Grasslands Kaituna lucerne (*Medicago sativa* L.). The progeny lines and three control species were thoroughly scarified with sandpaper and heavily coated with the appropriate *Rhizobium* inoculum. Commercial inoculum strains were used on the control species and the strain, ICC283b was applied to the caucasian clover progeny lines.

Table 1 Number of seedlings emerged after 7, 14, 21 days and seedling vigour at 54 days.

	Seedlings			Vigour**
	7 days	14 days	21 days	(1–9)
Parental Line*				
ARS-2678, USA	11	41	52	4.9
I-2 Kura, USA	14	49	58	5.6
Ky-1, USA	11	36	48	5.0
MS-6X, USA	14	47	58	5.6
MS-Persist, USA	19	54	66	6.0
cv. Rhizo, USA	14	44	55	5.2
KZ1, NZ	16	49	61	5.6
KZ2, NZ	16	50	60	5.7
KZ3, NZ	16	46	60	5.6
KZ4, NZ	18	53	65	5.7
Cool-season, NZ	22	61	70	6.0
cv. Monaro, Australia	15	46	57	5.4
cv. Prairie, Australia	15	47	61	5.5
Control species				
Huia white clover	18	48	62	5.0
Pawera red clover	22	67	75	8.8
Kaituna lucerne	36	51	57	6.3
LSD (5%)	7	12	12	1.3

* Each parental line is the mean of 14 progeny lines
** 1=low to 9=high

Trial design

The seed lines were sown in a randomised complete block experiment with 4 replications, at the AgResearch Lincoln Farm on a fertile Wakanui silt loam on 15 November 1995. Each line was sown with 100 seeds in 1 m rows at 5–10 mm depth using a Hege91 precision seeder with rows separated by 1 m. The mean maximum and minimum air temperatures during the Nov–Dec period were 19.5°C and 8.5°C respectively. The trial was irrigated with 50 mm on 5 December 1995 and 11 January 1996 to prevent any moisture stress on the seedlings. Dicotyledon weeds were intially hand weeded in the rows then controlled with Preside (a.i. flumetsulam) at 65 g/ha on 27 December 1995.

Measurements

The number of seedlings emerged was recorded from each row 7, 14 and 21 days after sowing. Seedling vigour was visually scored after 54 days on a 1–9 scale based on the herbage growth and degree of development of each row. After 65 days, one progeny line from each parental line together with the control species were selected for a partial destruction of the row. A group of seedlings were removed from each row across the four replications, and five seedlings/rep were randomly selected and dissected for shoot, root and rhizome dry weight (DW). A second destuctive sampling was done after 165 days and the same plant characters assessed.

Results

Seedling emergence

After 7 days, lucerne seedlings emerged significantly faster than all other material (Table 1). The caucasian clover lines had similar seedling emergence to red clover and white clover, although the US material tended to have slower emergence. After 21 days, red clover had the greatest number of seedlings followed by the caucasian clover cool-season selection. The caucasian clover lines (with the exception of Ky-1) produced similar seedling numbers as white clover.

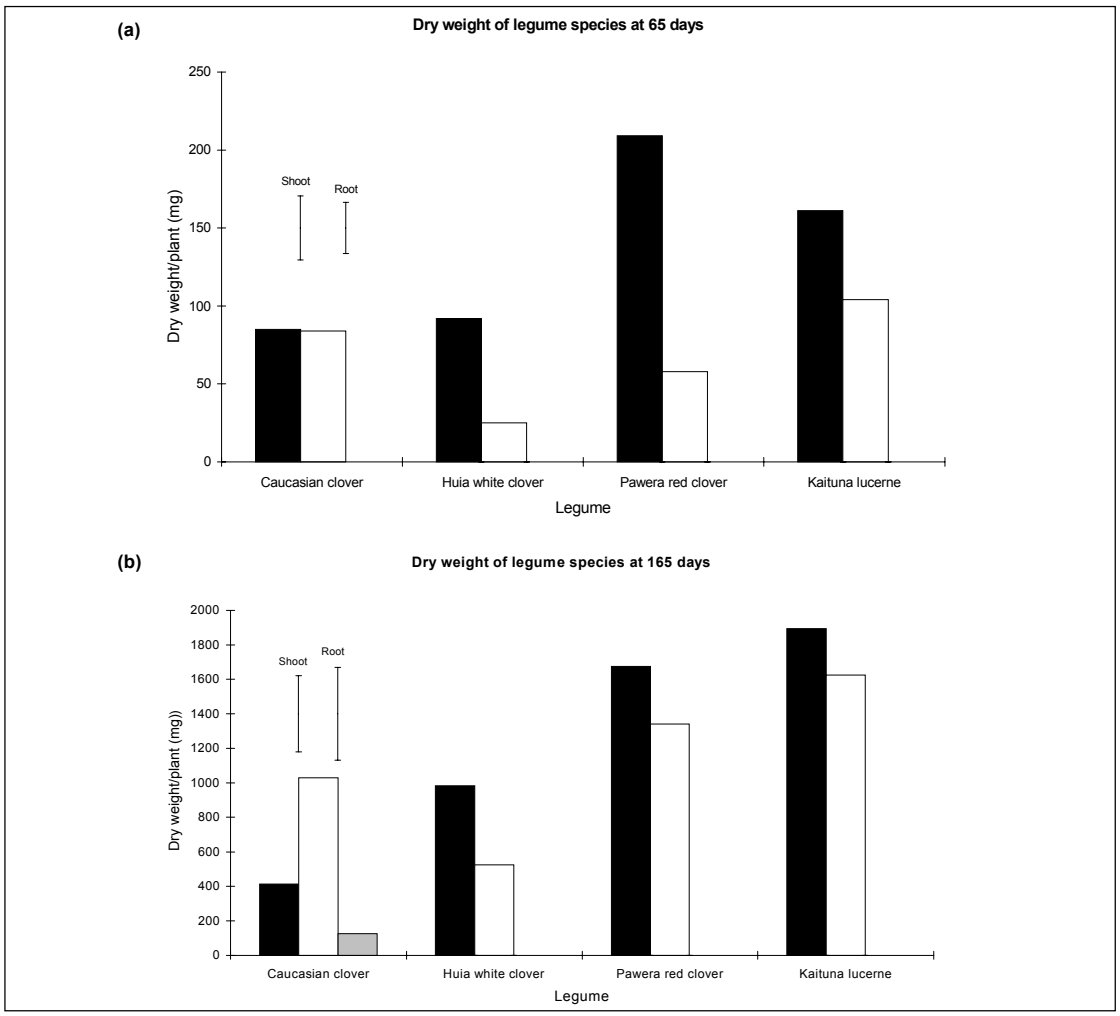
The vigour of seedlings after 54 days followed a similar pattern to the number of seedlings emerged after 21 days (Table 1). Red clover seedlings were the most vigorous followed by lucerne, the caucasian clover lines and white clover which were not significantly different. Comparing caucasian clover lines, the cool-season selection and MS-Persist were the most vigorous and ARS-2678 the least.

Seedling growth

After 65 days, red clover and lucerne produced significantly greater shoot DW than white and caucasian clover (Figure 1a). The caucasian clover (mean of all lines) produced similar shoot yields but significantly higher root yields than white clover. Rhizomes were just beginning to lengthen from the crown. The shoot:root ratio for lucerne, red clover, white clover and caucasian clover were 1.5:1, 3:1, 3:1 and 1:1 respectively.

Growth of seedlings increased 10-fold from 65 to 165 days after sowing (Figure 1b). The red clover and lucerne continued to produce the greatest shoot and root yields. At this stage, caucasian clover had half the shoot yield but twice the root yield of white clover, resulting in similar total DW. The rhizome yield made up 10% of the underground biomass. The caucasian clover shoot:root ratio now measured 0.3:1 compared with 2:1 for white clover.

Figure 1 Seedling dry weight (mg) of caucasian clover, white clover, red clover and lucerne after (a) 65 days and (b) 165 days. ■ Shoot □ Root ▒ Rhizome I LSD (5%)

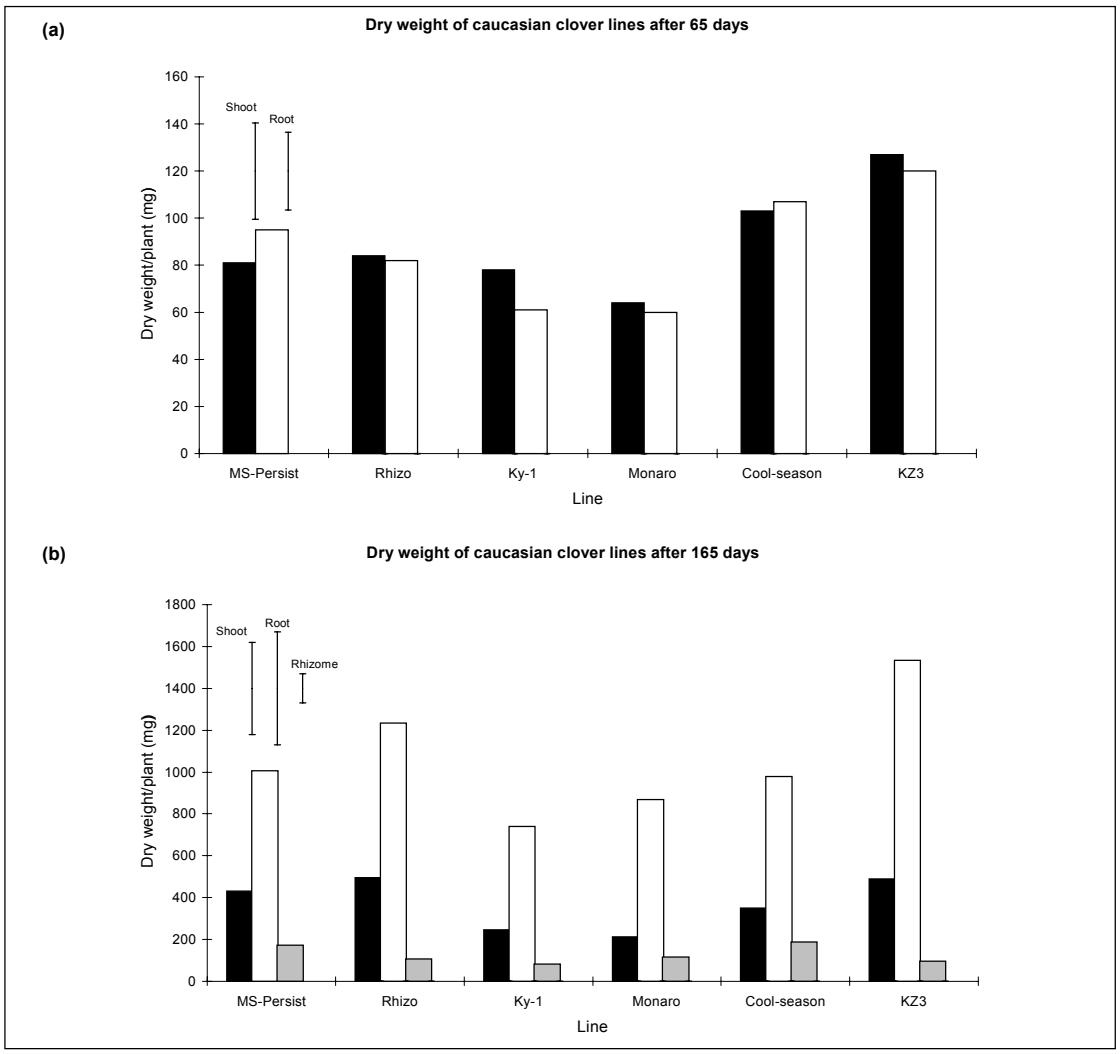


After 65 days the cool-season and KZ3 lines, both selections from Monaro, had significantly better shoot and root yields than the standard cultivar Monaro (Figure 2a). MS-Persist was the best of the US material. The shoot:root ratio varied from 0.85:1 for MS-Persist to 1.3:1 for Ky-1. After 165 days, the shoot and rhizome yields were not significantly different among lines, but KZ3 had significantly higher root yield than Monaro (Figure 2b). The shoot:root ratio was less variable after 5 months, ranging from 0.28:1 to 0.37:1.

Discussion

The establishment process first involves the germination and emergence of seedlings. Controlled environment studies have shown that white clover and caucasian clover germination characteristics are similar (Hill & Luck 1991). In the Lincoln field trial, the temperatures were favourable for clover germination, and after 21 days the number of seedlings emerged and early vigour of the caucasian clover lines were similar to those of white clover. When examining the variation within the caucasian clover material, the cool-season selection from Monaro and MS-Persist produced the highest number and vigour of seedlings. Selection for improved growth at cool temperatures has possibly resulted in caucasian clover material with faster seedling emergence. Use of recurrent selection at low temperatures for improving the rate of seedling emergence requires further examination.

Figure 2 Seedling dry weight (mg) of a range of caucasian clover lines after (a) 65 days and (b) 165 days.
Shoot Root Rhizome I LSD (5%)



The second stage in the establishment process involves the growth of the seedlings. Red clover and lucerne clearly showed superior shoot and root growth compared with white and caucasian clover (Figure 1). In the first 2 months, caucasian clover seedlings partitioned 1:1 proportion of dry weight to shoot and root compared with 3:1 ratio for white clover. Caucasian clover seedlings showed active growth in the early stages, producing similar shoot growth but 3 times the root growth of white clover. Between 2 and 5 months, the caucasian clover partitioned more dry weight to root and rhizome, resulting in a 0.3:1 shoot:root ratio. Previous studies have shown that the development of a large root and rhizome stucture is the most important requirement during the establishment phase of caucasian clover (Hill & Mulcahy 1995). Any situation that hinders the root and rhizome development of caucasian clover, such as competition from associated grass roots, dry conditions or frequent defoliation, will slow down establishment. Unfortunately, in a new pasture sward containing associated grasses, the combination of root competition limiting root and rhizome growth, and the natural tendency toward a low shoot:root ratio in caucasian clover, results in low competitiveness for light, and low yields in above-ground shoots.

One approach to improve the establishment phase may be to develop caucasian clover material with a greater rate of seedling growth and/or modified

shoot:root ratio. The KZ3 line selected from Monaro showed significantly better shoot and root growth than Monaro after 2 and 5 months. The advantage was owing to superior seedling growth rate per se, as the initial 1:1 and final 0.3:1 shoot:root ratios were consistent with other caucasian clover. Ky-1 from the US had a larger initial shoot:root ratio (1.3:1) but this did not result in larger seedlings after 5 months. The response shown by KZ3 indicates establishment can be improved by selecting material with a faster rate of seedling growth.

Moorhead *et al.* (1994) and Lowther *et al.* (1998) have emphasised the management strategeies required for successful establishment of caucasian clover in tussock grasslands. Factors include the superiority of direct drilling over oversowing, removal of existing vegetation, fertiliser application at sowing, coating seed with 5 times the commercial rate of *Rhizobium* inoculum and sowing into moist soil conditions. While many of these factors have wider relevance, modified strategies will be required for successful establishment of caucasian clover in pasture mixtures for productive lowland situations. Hill & Mulcahy (1995) suggested establishing caucasian clover initially as a pure stand to maximise root and rhizome development in the first year. Caucasian clover could be sown alone early in spring when soil moisture and temperatures are suitable, allowing several months of active growth to achieve well-developed plants. Following this inital clover establishment period, perennial grasses would be direct drilled in autumn at low to moderate sowing rates, thereby keeping the competitive impact low. More research is required into caucasian clover and grass seeding rates for pasture mixtures, sowing times and methods and early grazing management in productive lowland regions of New Zealand.

Conclusions

1. Caucasian clover seedlings emerge at a similar rate to white clover in lowland conditions.
2. Recurrent selection for seedling emergence at low temperatures is suggested as a method of developing material with improved rate of emergence.
3. Caucasian clover seedlings grew at a similar rate to white clover but slower than red clover and lucerne. However, caucasian clover partitioned three-quarters of the growth to root and rhizome resulting in low shoot yields and above-ground competitiveness.
4. KZ3 (a selection from Monaro) seedlings grew faster than seedlings from Monaro indicating the potential for genetic improvement in seedling growth.
5. Sowing caucasian clover as a pure stand in spring to maximise root and rhizome development followed by grass drilling in autumn is suggested as a strategy for successful establishment of caucasian clover in lowland pastures.

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