Genetic variation for seed yield in Caucasian clover

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Abstract

The seed production potential of 34 lines of Caucasian clover (*Trifolium ambiguum* M.Bieb) representing a range of 2x (diploid), 4x (tetraploid) and 6x (hexaploid) material together with ecotypes from the Caucasus region were established in a trial at Lincoln, Canterbury (43°38'S) in October 1993. Measurements were made from individual plants during summer 1994-95. The diploids were earliest to flower followed by the tetraploids and finally the hexaploids, which reached peak flowering in mid December. The hexaploids produced the most florets per inflorescence (115, 101, 96 from 6x, 4x, 2x respectively), the most inflorescences per plant (162, 101, 129 from 6x, 4x, 2x) and the highest seed yield(g) per plant (26, 9, 11 from 6x, 4x, 2x). There was large variation for all the seed production components within the three ploidy levels. For example, the hexaploid cultivar Endura consisted of plants that varied from 25–510 inflorescences per plant. The seed yield components of plants were associated with active spring growth. There was evidence of wide spreading plants producing lower numbers of inflorescences per m^2 and seed yield per unit area. The hexaploid material produced the greatest potential seed yields (1330,685, 1000 kg/ha from 6x, 4x, 2x respectively). There was a 5-fold difference between the lowest-yielding 6x cultivar, Prairie (570 kg/ha), and the highest-yielding KZ2 (2720 kg/ha), a selection from Monaro. Major gains are possible through selection but it is important to select for improved seed yield per plant (not just flowers) and to maintain a wide genetic base. Commercial crops yield approximately half that attained from research plots, which suggests a commercial yield of 900 kg/ha is possible from Endura. This compares favourably with other legumes but requires development of crop management practices that ensure consistently high seed yields.

Keywords: Caucasian clover, genetic variation, Kura clover, seed components, seed yield, *Trifolium ambiguum*

Introduction

Caucasian clover is recognised as a legume with strong perenniality and potential for dry, low fertility environments in the hill and high country regions of the South Island (Lucas *et al. 1981;* Scott 1985; Woodman et *al.* 1992). The ability of Caucasian clover to extend into summer-dry, lowland regions and even into irrigated pastures is currently under evaluation (Moss *et al.* 1996; Widdup, unpubl. data). At present, the critical factor affecting the future uptake and acceptability of this new clover by farmers is the reliable production of quality seed. Current cultivars from Australia, such as Prairie and Monaro, have shown variable and poor seed yields and are not commercially available.

One way of improving seed production is to develop better genetic material and improved cultivars. Caucasian clover contains a ploidy series with chromosome numbers from 2x = 16,4x = 32 and 6x = 48. Kannenberg & Elliot (1962) showed there was very large variation for flowering and seed yield among plants and ploidy levels, and suggested gains could be made through selection. Wrightsons Ltd recently developed KZ1 (released as cv. Endura) through two cycles of natural selection for better seed yield from hexaploid Monaro plants (Michael Norris pers. comm). Further selection from KZ1 for early rhizome production and later flowering has resulted in KZ2, KZ3 and KZ4.

The purpose of this study was to determine the critical plant components linked with seed production in Caucasian clover and to assess the extent of variation between and within the ploidy levels for these seed yield components.

Materials and methods

A range of caucasian clover material was established at AgResearch, Lincoln (latitude 43°38'S) on a Templeton silt loam. The genetic material consisted of diploid, tetraploid and hexaploid cultivars and breeding lines (Table 1) which have been developed in Australia, USA and New Zealand. The KZ series of lines were selected from Monaro (6x) for improved seed yield by Wrightsons Ltd. The cool-season selection and lines with early- to late-flowering patterns were selected from Monaro by AgResearch. The seven ecotypes were part of a collection from the Caucasus region made by the late Dr Margot Forde.

The material was established in a randomised complete block trial with 3 replications. Each line within a replication was represented by 10 plants spaced 1 m apart in a row, resulting in 30 plants per line in the trial. Seedlings were established in trays in theglasshouse, inoculated with recommended *Rhizobium* strains for each ploidy level and transplanted to the field as lo-week-old plants in October 1993. These plants were left to establish with occasional grazing until October 1994, when the trial was closed to assess seed production. The trial site was moderately fertile (pH=5.6, Olsen P=20) and received superphosphate (0-9-O-l 1) at 200 kg/ha annually. The trial was irrigated 2 times during summer 1994-95, with 50 mm at each irrigation. Fertility and moisture conditions were good for legume plant growth.

Table 1 Ploidy level and origin of genetic material in the Lincoln Trial.

Origin	Diploid (2x)	Tetraploid (4x)	Hexaploid (6x)	Ecotypes *
Australia	cv.Summit	cv.Treeline	cv.Prairie	
	cv.Forest	CPI 51139	cv.Monaro (2)	
	CPI 2264			
USA	MS-2X	MS-4X	cv.Rhizo	
			MS-6X	
			MS-Persist	
			ARS 2676	
			Ky-1	
			C-2 Kura	
New Zealand			cv.Endura (KZ1)	
			KZ2	
			KZ3	
			KZ4	
			Cool selection	
			Early type	
			Medium type	
			Late type	
Turkey/USSR		CPI 9949		SPN 6912Armenia
		PI 440697		SPN 6954 Armenia
				SPN 6977 Armenia
				SPN 6966 Armenia
				SPN7071 Dagestan
				SPN 7174 Georgia
				SPN 7601 Armenia

* ploidy level unknown.

Seed production potential was assessed on individual plants over summer 1994-95. A beehive was positioned 50 m from the edge of the trial in November 1994. Reproductive characters measured included rate of inflorescence appearance (counts at weekly intervals during November and December), final number of inflorescences and seed yield per plant. A sample of 5 flowers from each plant was dissected for number of florets per inflorescence and seeds per floret. Plant spread was measured as the most distant daughter plants arising from rhizomes, which enabled an estimate of inflorescences per m^2 and seed yield per ha. Other plant growth characters measured were leaf diameter (mm) and spring growth on a O-9 visual scale. The diploid, tetraploid, hexaploid and ecotype lines were averaged within their groups for the components measured to give an overall effect of ploidy level.

Results

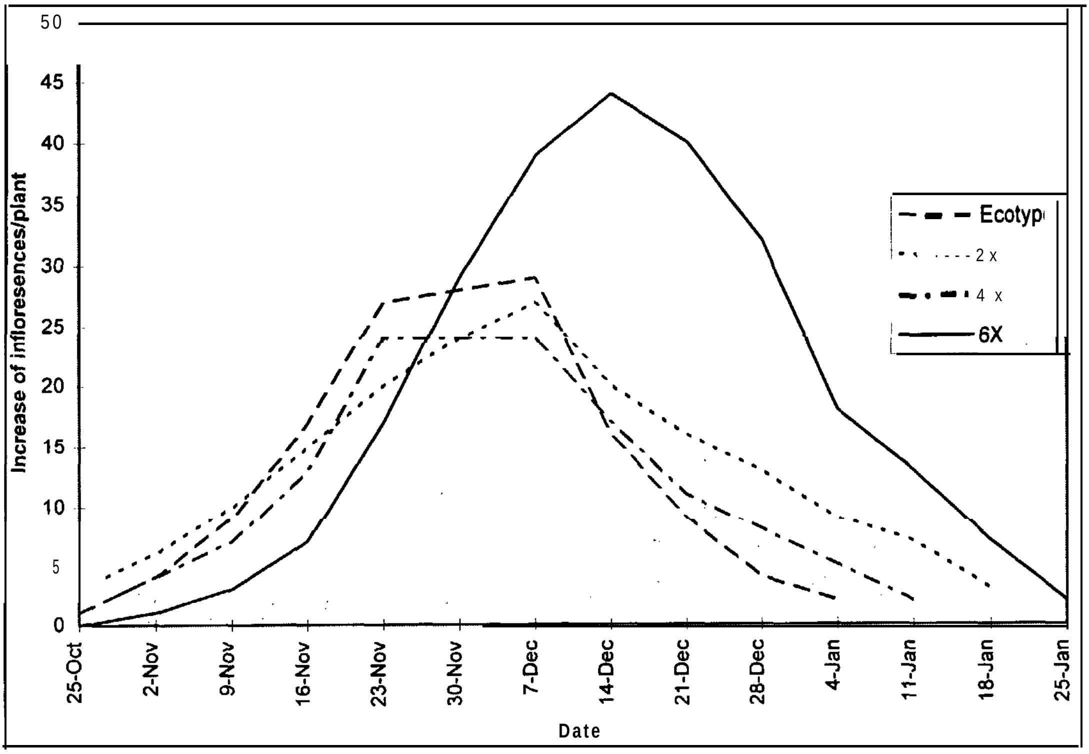
The diploid material started flowering in late October, peaked early December but continued to initiate flowers from rhizome shoots (daughter plants) into February (Figure 1). The tetraploids had a similar curve but more confined. The hexaploids were last to begin flowering but showed a greater peak of flowers by mid December than the other ploidy levels. The hexaploids also continued to flower into February. The ecotypes had a flowering curve similar to that of the diploid and tetraploid material.

The diploid, tetraploid and ecotype material had smaller flowers with significantly fewer florets per inflorescence than the hexaploids (Table 2). The tetraploids and ecotypes had significantly fewer seeds per floret than the diploid and hexaploid material, which contained about 0.9 seed per floret (Table 2). The combined effect was that hexaploids had more seeds per inflorescence than the other ploidy levels. Tetraploids and ecotypes produced fewer inflorescences per plant than the diploids, which in turn had significantly fewer than the hexaploids. Seed yield per plant is a function of the number of inflorescences and the number of seeds per inflorescence. The result was that hexaploids produced significantly greater seed yield per plant than the other ploidy levels.

When plant spread was taken into account, the diploids, with their narrow plants, produced potentially more inflorescences per m^2 than higher ploidy levels (Table 2). However, the highest potential seed yield of 1330 kg/ha was from the hexaploid material. The seed yields of the diploids were not significantly different to that of the hexaploids, whereas the tetraploids and ecotypes had significantly poorer yields. The hexaploids produced larger leaves and the most active spring growth compared with lower ploidy levels, and this was associated with better seed yield per plant and total seed yield.

The hexaploid material was examined more closely to determine the extent of genetic variability of plant

Figure I Rate of inflorescence appearance on the diploid, tetraploid, hexaploid and ccotype Caucasian clover populations.



charaters that impact on seed production. Seven lines representative of the range of material from Australia, USA and New Zealand were chosen (Figure 2). The KZ series of lines had significantly greater seed yields per plant than Monaro, the cultivar from which they were selected for improved seed yield (Figure 2a). The other USA and Australian cultivars were inferior to the KZ series for seed yield per plant. The KZ series showed more variation for this character than other material and offers wide scope for further selection. The USA line ARS 2678 and KZ2 had the most inflorescences per m² (Figure 2b) and the least plant spread (Figure 2c). In contrast Prairie, Monaro and KZ3 had fewer inflorescences per m² but the greatest plant spread. Again, there was considerable variation for these two characters and the possibility for further gains through selection depending on the heritability of the two traits. The final potental seed yield (kg/ha) was greatest from the KZ series and ARS 2678 (Figure 2d), KZ2 producing the highest seed yields.

Table 2 Affect of ploidy level on the seed production components in Caucasian clover.

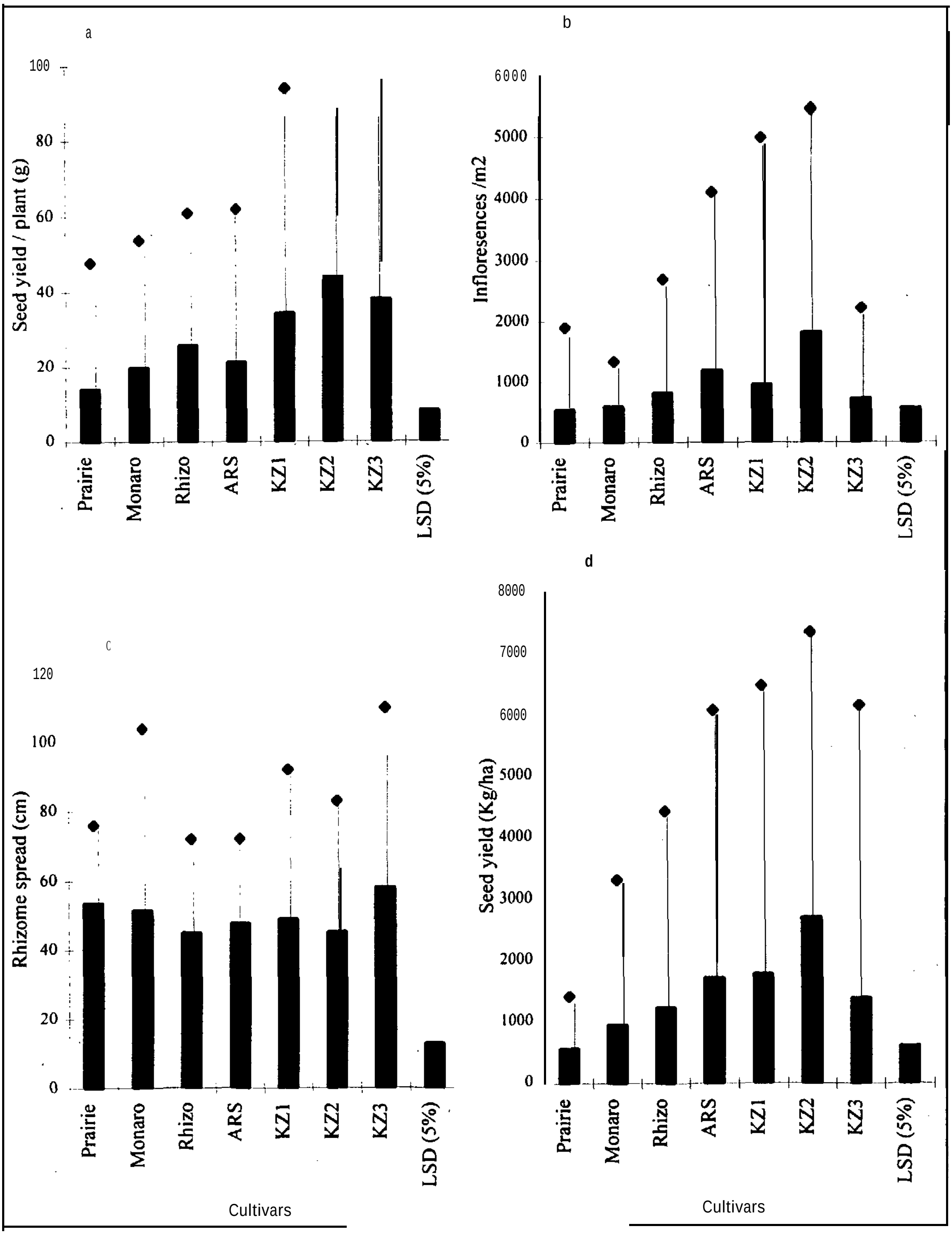
Character	Ecotype	2x	4x	6x	Endura
Number lines	7	4	5	18	1
Seed yield components					
Seed yield/plant (g)	6.3 d*	10.9 c	9.3 c	25.8 b	34.4 a
Inflorescences/plant	104 c	129 b	101 c	162 a	179 a
Florets/inflorescence	93 b	96 b	101 b	115 a	123 a
Seeds/floret	0.53 b	0.82 a	0.59 b	0.88 a	0.92 a
Plant Morphology					
Leaf diameter (mm)	12.3 c	12.4 c	16.1 b	18.7 a	19.2 a
Plant spread (cm)	36 b	37 b	39 b	51 a	49 a
Spring growth **	4.8 c	4.8 c	5.3 b	6.0 a	7.1 a
Estimated data					
Inflorescences/m²	960 b	1290 a	900 b	900 b	955 b
Seed yield (kg/ha)***	645 c	1000 b	685 c	1330 b	1780 a

* Different letters indicate significant differences for $P<0.05$.
** Spring growth on 0-9 scale. 0=no growth, 9=excellent growth.
*** Potential yield of research plots are approximately twice those achieved from field crops.

Discussion

The progressively later flowering with increasing ploidy was similar to results from Kannenberg & Elliott (1962) and Stewart (1979). In late October, stems appeared

Figure 2 The mean (■) and maximium (◆) values for the seed yield components of seven hexaploid populations.
(a) Seed yield/plant (g)
(b) Number of inflorescences/m^2
(c) Rhizome spread (cm)
(d) Seed yield (kg/ha).



from the crown or rhizomes and continued to develop flower-bearing branches from each leaf axil, so that the youngest flowers were at the tip and the older flowers located further down the stem. This growth habit resulted in a wide span of flowering, with inflorescences appearing on stems into February. However, peak flowering had occurred by mid December. The ecotypes collected from the Armenian region had flowering patterns and flower structure similar to those of the diploids and tetraploids, but cytological examination would be required to verify their ploidy level. There is a lack of knowledge on the photoperiod and temperature effects on flower induction in Caucasian clover, although low temperature appears to be required for flower induction (Taylor 1994).

The hexaploids produced the largest inflorescences with more florets than other ploidy levels. Pollination was effective by honey bees, with the hexaploids and diploids producing approximately one seed per floret. Caucasian clover can develop two ovules per ovary in the floret (Gamtsemlidze 1995), which is similar to red clover (*Trifolium pratense* L.). Some hexaploid plants produced an average of 1.6 seeds per floret, indicating the possibility of selection for more seeds per inflorescence. The hexaploids also produced the most inflorescences per plant, and together with more seeds per inflorescence resulted in significantly greater seed yield per plant than lower ploidy levels. High seed yield per inflorescence and per plant was strongly associated with actively growing plants. Full development of each reproductive component depends on the partitioning of adequate resources, as has been shown with white clover (Clifford 1987). Seed yields from Caucasian clover appear to be maximised where plants have abundant space, nutients and moisture to fulfil their reproductive potential.

There was evidence of wide spreading plants producing fewer inflorescences per m^2, with reduced total seed yield. Care should be taken in breeding programmes, when selecting plants for their ability to spread in a grass sward, not to penalise the associated seed production components. Optimium seed yield per ha will be achieved from cultivars with moderate rhizome development together with appropriate-row spacing. Recent studies have shown 45 cm as the optimal spacing with Endura (Guy 1996). The hexaploid plants in this study averaged 50 cm in width after 18 months, which would have resulted in full canopy cover. Diploid and tetraploid plants averaged 35 cm in width, suggesting a 30 cm row spacing as more appropriate to maximise seed yield with lower ploidy cultivars.

There was considerable variation for seed yield between and within the hexaploid populations. Prairie had the lowest estimated yields (570 kg/ha) whereas KZ2 had a potential yield of 2720 kg/ha, a S-fold advantage. Two cycles of selection for improved seed yield per plant in Monaro have doubled the seed yield to 1780 kg/ha for Endura (KZ1). KZ2 represents another cycle of selection which provided a further 50% increase in seed yield. There is still sufficient variation in these lines to enable further selection gains. The major components on which to concentrate efforts are seed yield per plant and number of inflorescences per m^2. However, selection for higher seed yields has not always been successful. A recurrent selection programme for improved flower numbers in Rhizo did increase flowers for the first season but seed yield was not improved (Taylor 1994). There was an associated decline in plant vigour, probably owing to inbreeding depression. This has not been the experience with the KZ series, where selection was for improved seed yield per plant (not just flowers), and a broad genetic base was maintained by allowing elite plants to open pollinate with the whole population.

A small group of lines was covered with animal exclusion cages in October 1995 to determine reproductive potential for the third season. Endura was typical of the lines tested, with a decline from 1000 to 250 inflorescences per m^2 and from 34 g to 6 g seed per plant, a 5-fold decrease, contrary to results from Dalyet *al.* (1993) where seed yields were maintained into a third season. Further research is required on the factors involved with producing multiple seed crops over a number of years.

Commercial crops yield approximately half the potential indicated from research plots owing to losses in harvesting and errors in extrapolating to larger areas, which would be equivalent to 500 kg/ha from Monaro and 900 kg/ha from Endura. Hexaploid Caucasian clover has potential to produce high seed yields which compare favourably with those of other legumes. The challenge now is to further develop the management requirements for a field crop to achieve consistently high crop yields.

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