Inheritance of multifoliolate leaves in white clover

J.L. FORD and R.B. CLAYDON
AgResearch Grasslands, Private Bag 11008, Palmerston North

Abstract

During 1988/89 selected white clover (*Trifolium repens* L.) genotypes were studied to determine their inheritance of multifoliolate (mf) leaves in relation to the multifoliolate percentage of the parent material. Genotypes differing in multifoliolate frequency were crossed in an incomplete diallel with plants from three Grasslands white clover cultivars, Huia, Kopu and Tahora. Each cultivar was used in three separate pair crosses, with genotypes expressing multifoliolate leaf percentages of 25%, 50% and 75% respectively. These mf plants were also pair crossed and plants from within each cultivar were pair crossed 3 times. All plants in the total of 21 pair crosses were bee pollinated. Harvested seed from each genotype was sown and raised under glasshouse conditions, and after ninety days each young plant was evaluated for mf leaf production. Leaf count results showed that 31% of cultivar x mf genotypes expressed mf leaves, while in the mf x mf programme 90% of genotypes displayed mf leaves. A distinct increase in the percentage of multifoliolate leaves occurred in crosses between mf plants and the trifoliolate cultivars when the mf genotype was the maternal parent. It is apparent that the multifoliolate character is heritable and that the percentage of mf leaves can be increased through breeding and selection.

Keywords: expression levels, inheritance, multifoliolate, white clover

Introduction

Multifoliolate (mf) leaves have been reported in a number of legume species, including alfalfa at both the diploid (Bingham 1964; Bingham & Murphy 1965; Brick et al. 1976) and tetraploid level (Bingham 1964), crimson clover (*Trifolium incarnatum* L.) (Knight 1969), soybean (*Glycine max* L.) (Fehr 1972), and red clover (*Trifolium pratense* L.) (Jaranowski & Broda 1978). The only reference to multifoliolate leaves in white clover (*Trifolium repens* L.) is in the registration of FL-ML white clover germplasm Baltensperger et al. (1989).

Since white clover is an outcrossing species, there can be considerable variation within breeding lines for the mf character.

The objective in this study was to determine the inheritance of the mf leaf characteristic in white clover. This was established by the frequency of plants displaying the mf character and the leaf number expression of these plants.

Materials and methods

Three selected genotypes with different percentages of multifoliolate leaf expression, 25%, 50% and 75%, were vegetatively propagated by stolon cuttings to produce five clones of each. In addition, genotypes representing the three different cultivars, Grasslands Huia, Grasslands Kopu, and Grasslands Tahora were cloned to produce 9 plants of each. These cultivar genotypes were free of any multifoliolate expression. All clones were taken in July 1988, and grown in nursery trays in a glasshouse situation (15–18°C).

An incomplete diallel crossing programme, was established, between the three cultivars, and the three mf genotypes averaging 25%, 50% and 75% leaf expression. These mf genotypes were selected following four generations of breeding and selection to increase plant mf percentage. The pair crossing programme occurred in the summer of 1988/89. Each plant representing the three different multifoliolate percentage levels were paired with each cultivar, (F1 generation) and the remaining mf plants were paired with each other. In addition, plants from the three cultivars were crossed with plants from within the same cultivar (e.g. Kopu × Kopu) by three replications. This gave a total of 21 pair crosses.

At flowering the plants were crossed in isolation within small insect proof breeding cages. Wild bumble bees were used for the cross-pollinating. The bees had been washed to ensure that any stray white clover pollen did not contaminate the pollinating process.

Upon the ripening and the drying of the flowers, all plants were harvested and all seed collected. In April 1989, seed from the maternal parent of each pair cross, were germinated, then sown in propagating trays. One hundred germinated seedlings were raised, of each harvesting, i.e. 42 different seedlines representing both sides of each pair cross. The seedlings were grown in the glasshouse for approximately ninety days, and then each plant was evaluated for multifoliolate and trifoliolate leaf numbers and percentages.

Table 1: Inheritance of multifoliolate (mf) character in pair crossing programme.

A		B	Mean plant (mf) frequency A	B	Mean leaf mf expression A	B	Mean mf frequency of crossing groups (%)	Mean mf leaf expression of crossing groups (%)
Kopu	x	Kopu	0	0	0	0	0	0
Huia	x	Huia	0		0			
Tahora	x	Tahora	0	0	0	0		
			5		14			
Kopu	x	mf25	6	9	11	13	a	10
Huia	x	mf25		12		11		
Tahora	x	mf25	0	17	0	11		
					17	13		
Kopu	x	mf50	15	28	14	14	39	14
Huia	x	mf50	41	53				
Tahora	x	mf50	37	60	13	11		
				27	11	26		
Kopu	x	mf75	15	75	13	33	45	21
Huia	x	mf75	52					
Tahora	x	mf75	34	67	10	34		
				92				
mf25	x	mf50	95	89	43	45	90	44
mf25	x	mf75	81		40	37		
mf50	x	mf75	92	95	50	51		

Results

The plants were evaluated approximately 90 days after sowing. The inheritance of the mf character varied across the three crossing groups (cultivar x cultivar, multifoliolate x cultivar and multifoliolate x multifoliolate). There was 0% expression for the cultivar x cultivar crosses, 31% for the cultivar x mf crosses, and 90% for the mf x mf crosses (Table 1).

Expression of the mf character in the Huia x mf and Tahora x mf crossing groups was between 20% and 24% higher than with the Kopu x mf crosses. Results gave only a 16% mf expression in the Kopu crosses, with 40% in the Huia crosses and 36% in the Tahora crossing group.

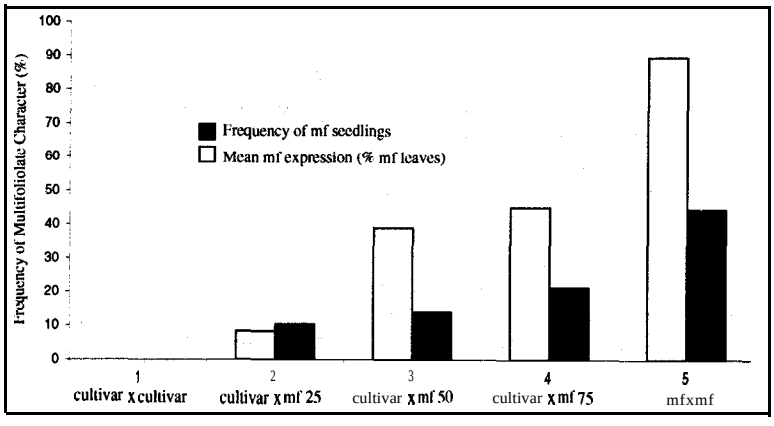
Higher mf seedling percentages were gained from the high mf genotypes used in the crossing programme (Figure 1).

With the crossing groups involving the 25% mf plants, the mean mf seedling frequency was 8% (range 0–17%) with a mean mf leaf expression of 10%. In comparison the 50% mf plants had a mean frequency of 39% (range 15-60) with a 14% mf leaf expression. The 75% mf group had 45% mf seedlings (range 1.5-67) with a mean leaf expression of 21%. In the mf x mf crossing group, the mean frequency was 90% (range 81-95) with a 44% leaf mf expression (Figure 1 and Table 1).

The increase in parental mf percentage from 25% to 50% produced a 31% increase in the number of new seedlings with the mf character, while the further increase from 50% to 75% mf produced only another 6% increase. Results showed a distinct advantage when the maternal parent was the multifoliolate plant. This trend was similar over the three mf x cultivar crossing groups, but was not evident (as expected) with the mf x mf or the cultivar x cultivar groups (Figure 2).

The mean frequency for the mf maternal parents in the mf x cultivarcrossing group was 39%, compared with 23% when thecultivar genotypes were used as the maternal parents. This maternal effect did not hold for the seedling mf leaf expression figures in the 25% mf and 50% mf groups but there were large maternal differences in the 75% mf crosses. The mf maternal mean was 31%, while the cultivar in paternal expression was 11% (Figure 3).

Figure 1: Multifoliolate character inheritance relating to frequency of mf plants and mf expression (plant leaf numbers).



Discussion

Results clearly demonstrated that the multifoliolate character is heritable in the F_1 generation at varying frequencies. These findings are similar to results obtained in diploid alfalfa (a frequency of 33%) (Brick et al. 1976), in crimson clover where Knight (1969) reported a 3:1 inheritance ratio, and in soybeans (Fehr 1972).

Also evident was a defined maternal relationship in particular when the mf genotypes were crossed with the trifoliolate cultivar material. In most cases the mf maternal parent of the cross produced higher mf percentages than the maternal trifoliolate parent. This is a significant result and warrants further investigation as there are few references to any white clover genetic traits influenced by maternal inheritance. When high frequency mf genotypes were crossed with either a trifoliolate cultivar or with other high frequency mf plants, the high expression of the mf character was maintained. This is similar to results reported for alfalfa (Bingham & Murphy 1965).

White clover genotypes containing the multifoiiolate character have aesthetic value in the market place as good luck tokens, both as plants or individual leaves. However greater value may lie in improving the proportion of high quality leaf dry matter in grazed swards. The effect of the mf trait on white clover productivity and forage quality needs to be determined. A future cultivar(s) could result once the mf character is stabilised and eventually increased through breeding and selection.

Figure 2: Comparison of maternal mf seedling frequency among different crossing groups.

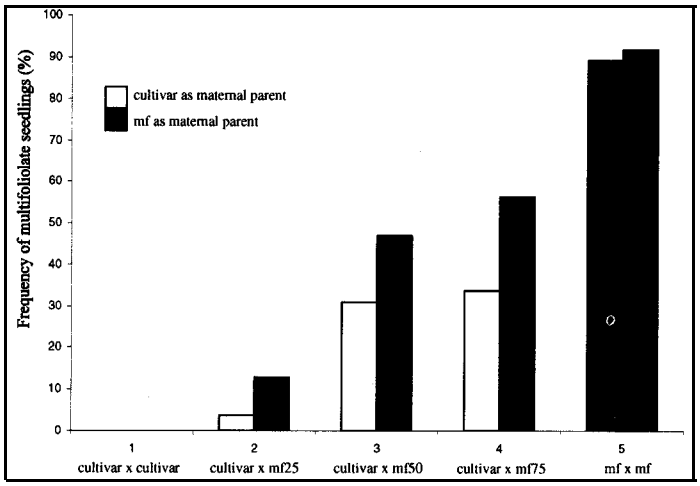
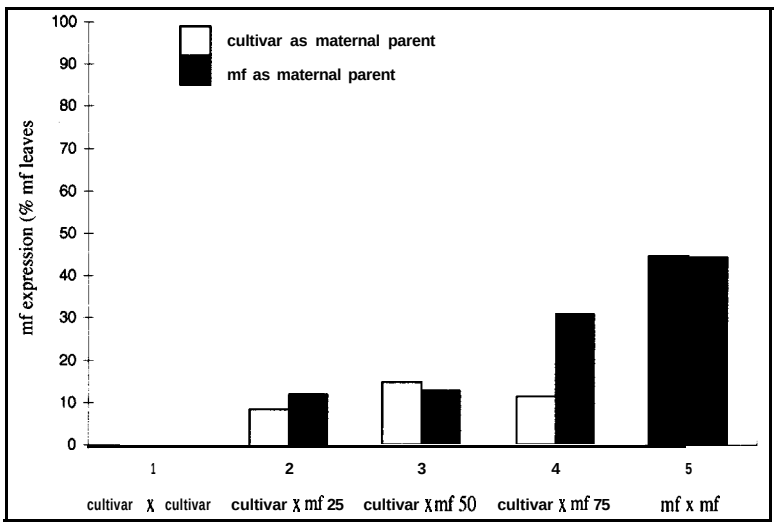


Figure 3: Maternal differences in plant mf leaf expression (% mf leaves).



References

Baltensperger, D.D.; Wofford, D.S.; Anderson, W.H. 1991. Registration of FL-ML White Clover Germplasm. *Crop Science 31: 853.*

Bingham, E.T. 1964. A genetical and morphological investigation of multifoliolate leaves of alfalfa, *Medicago sativa* L. PhD Dissertation. *Cornell*

University (Library Congress Card No. Mic 64–13,770. Dissertation Abstract 25:3217).

Bingham, E.T.; Murphy, R.P. 1965. Breeding and morphological studies on multifoliolate selections of alfalfa, *Medicago sativa* L. *Crop Science 5*: 233–235.

Brick, M.A.; Dobrenz, A.K.; Schonohost, M.H. 1976. Transmittance of the multifoliolate leaf characteristic into non-dormant alfalfa. *Agronomy Journal 68*: 134–136.

Fehr, W.R. 1972. Genetic control of leaflet number in soybeans (*Glycine max* L.). *Crop Science 9*: 221–224.

Jaranowski, J.K.; Broda, Z. 1978. Leaf mutants in diploid red clover (*Trifolium pratense* L.). *Theoretical Applied Genetics 53*: 97–103.

Knight, W.E. 1969. Inheritance of multifoliolate leaves, glabrous leaves and petiolulate leaflet attachment in crimson clover (*Trifolium incarnatum* L). *Crop Science 9*: 232–235.

■