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EFFECT OF TWO INBREEDING GENERATIONS ON AGRONOMIC TRAITS OF SELF-COMPATIBLE RED CLOVER[1]

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Abstract – Open-pollinated progenies (I_0) from self-compatible F_1 red clover (*Trifolium pratense* L.) plants were compared to I_1 and I_2 progenies from the same F_1 plants to evaluate the extent of inbreeding depression.

Eleven agronomic traits were evaluated in the field, and almost all showed progressively poorer performance with inbreeding. Plant dry weight, vigor, height and amount of regrowth decreased from the I_0 to the I_2 generation. Plants of the I_2 generation were later in maturity, with a more upright growth habit, than plants of the I_0 or I_1 generations. However, in both I_1 and I_2 generations, plants could be found that for certain characters were superior to their original I_0 population.

The majority of the characters showed higher genotypic and phenotypic variances in the I_1 generation than in the I_0 and I_2 generations. This might have been caused by segregation of genes for the characters in the I_1 generation. The heritability estimates were quite high for most characters, but showed a decrease with inbreeding.

Estimates of phenotypic and genotypic correlations over all lines suggested that mean dry weight per plant was associated with height, vigor, leafiness and regrowth after cutting. However, the first-year forage yield was not related to the second-year regrowth after over-wintering. Genotypic correlations were generally higher than the phenotypic correlations.

INTRODUCTION

Red clover is a highly heterogeneous species because of obligate cross-fertilization, which results from the gametophytic system of self-incompatibility. Self-compatible plants are rare in red clover. Williams [15] obtained only one self-compatible plant after examining several thousand plants. A few investigators [9, 15] have found self-compatible lines, thought to contain the S_f allele of the incompatibility locus.

To date, no red clover hybrids have been produced commercially. Theoretically,

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inbred lines could be produced by means of the S_f allele, but the problem of eliminating this allele for the synthesis of the hybrids or synthetics would remain. Williams [14] suggested the use of inbreeding for elimination of undesirable recessives, mostly chlorophyll-deficient factors. Because of the large variability in vigor among inbred lines, it would be possible to select those families that retain their vigor when inbred. Such lines could be exceedingly valuable in production of improved strains.

Kirk [5] found that red clover plants within selfed families were much more uniform than those between families. He also demonstrated a great loss of vigor in selfed as compared to open-pollinated progeny of red clover plants. However, some of his selfed lines were as vigorous as the non-inbred material.

Wexelsen [12] observed a decrease in plant height at the flowering stage with increased inbreeding. Out of 126 sib lines, he found one family that exceeded the standard strain "Molsted" in height. He also found a striking uniformity in the inbred material for plant height.

Thomas [11] compared the vigor of 22 S_2 lines, developed by selfing, with that of the open-pollinated seed of the S_1 mother plant for each line. He found that, on average, the vigor of the S_2 lines was only 50% of that of the open-pollinated progenies. In only three instances was the inbred line more vigorous than the outcrossed progeny of the S_1 parent.

Without intentional selection, Wexelsen [13], using sibbing, and Taylor *et al.* [10], using selfing, demonstrated a reduction in the yield of the first inbred generation compared to non-inbred materials. However, the latter investigators were able to find, in all characters, some inbred plants that equalled or exceeded their non-inbred parents.

Inbreeding depression and heterosis, though the reverse of each other, are both expressions of the same phenomenon. Thus the study of one would lead to a better understanding of the other. Also, for a plant breeder to plan efficient programs for the improvement of the species, it is essential to have some estimates of inbreeding depression in different populations. Thus far, such information is limited in red clover.

The present investigation was undertaken to study the effects of inbreeding, by selfing, on several important agronomic traits in red clover and to estimate quantitative genetic parameters of inbred generations.

MATERIALS AND METHODS

Five crosses were made using 4 self-incompatible, adapted clones (67-7, SS18, SS21 and SS22) as female parents and 3 self-fertile inbred lines of unknown generation (W1, W2 and W6) as male parents. The crosses were SS21 X W1, 67-7 X W1, SS18 X W2, SS18 X W6 and SS22 X W6.

Four F_1 plants from each of the 5 crosses were chosen at random from a field-spaced nursery and brought to the greenhouse. Open-pollinated seed had been collected from these plants before bringing them to the greenhouse.

Selfing was accomplished by hand-rolling the flower heads in the greenhouse during several flowering periods. Hands were rinsed with 95% alcohol and allowed to dry after selfing each plant. I_1 seed was obtained by selfing F_1 plants, and I_2 seed by selfing I_1 plants. Each F_1 plant was represented by ten I_1 progenies, and each I_1 plant by seven I_2 progenies. The term "family" as used in this paper refers to all open- and self-pollinated progeny tracing to one F_1 plant.

Each family was represented by 6 entries: the open pollinated progeny (I_0), the I_1 selfed progeny, and the I_2 selfed progeny from 4 randomly chosen I_1 plants. All plants were transplanted in the field at the Arlington Experimental Farm near Madison, WI. Plants were spaced 30 cm apart in 4.2-m rows 1 m apart. Fifteen plants representing each population of each family and a check (Lakeland) were established in each of the 4 replications of a split-plot design. Whole plots were families, and each of the 6 entries in a family was a sub-plot.

Measurements were taken on all plants, but because of the loss of some plants, 10 plants of each entry were chosen at random for the final analyses. Rating scales for characters measured on individual plants or rows in the field are listed in Table 1.

Table 1. Rating scales for characters measured on individual plants or rows of red clover

Character*	Date measured	Scale and description
1. Plant height, cm	24 July 1970	Height from the soil surface to highest undisturbed standing head or leaf.
2. Vigor	24 July 1970	1, the most vigorous; 9, poorest vigor.
3. Leafiness vs stemminess	24 July 1970	1, highest leaf-to-stem ratio; 9, lowest leaf-to-stem ratio.
4. Growth habit	25 July 1970	1, upright growth; 9, decumbent growth.
5. Growth stage	26 July 1970	1, vegetative state, mainly leaves; 2, unopened buds; 3, just-opened buds; 4, a few heads opened; 5, all heads opened; 6, base of some heads turned brown; 7, base of all heads matured; 8, large number of mature heads; 9, all heads matured.
6. Dry weight, g	27–28 July 1970	Plants cut at 2 cm above soil surface, dried at 65° C and weighed.
7. Regrowth 1 (vitality)	15 October 1970	1, most regrowth; 8, least regrowth; 9, dead.
8. Regrowth 2 (winter survival)	2 June 1971	Same scoring system as regrowth 1.
9. Days to 50% bloom†	10–27 July 1971	No. of days from transplanting to the time when 50% of plants had bloomed.
10. Uniformity	26 July 1971	1, very uniform in height and size; 9, very heterogeneous.
11. Proportion of flowering heads	26 July 1971	1, very high number of open heads and flowering stems; 9, no flowering stems.

* Characters 1–8 were measured on individual plants, 9–11 on rows.

† A score of 99 was given to rows that had not reached 50% bloom at the time of harvest.

Analyses of variance and covariance were performed for the characters, measured on an individual plant basis. The within-plot variances for each generation were computed and tested for homogeneity by means of the Bartlett test.

Data were then averaged for rows. Phenotypic and genotypic variance and covariance components for the 3 generations were estimated by equating mean squares and cross products to their respective expectations and solving for the required component.

In addition, phenotypic and genotypic correlations between character pairs were obtained by dividing the covariance component by the geometric mean of the respective variance components.

RESULTS AND DISCUSSION

Means of different generations, amount of inbreeding depression, phenotypic and genotypic variances, and heritabilities for different characters are presented in Table 2. Most of the characters, including the more important agronomic traits such as plant height, vigor, yield and regrowth showed highly significant differences among the I_0, I_1 and I_2 generations. The chi-squares (not shown) used to test the homogeneity of variances among plants within different generations (I_0, I_1 or I_2), on a within family, within replicate basis, suggested that the within-plot error variances, although quite small and similar, were highly heterogeneous for all characters except for growth habit. Within-line variances decreased with inbreeding for vigor, dry weight and regrowth scores. Because of inbreeding, there would be an advance towards homozygosity, with a consequential decrease in variability within lines. However, characters such as plant height, growth habit, and growth stage showed a higher within-line variance in the I_1 generation because of segregation, and then a decrease in the I_2 generation because of inbreeding (Table 2).

Mean plant height at harvest time showed a steady and significant decrease from I_0 to I_2 generations. These results agree with those of Aycock and Wilsie [3] for alfalfa and Williams [15], Laczynska-Hulewicz [7] and Wexelsen [12] for red clover.

In general, vitality (regrowth 1) and winter survival (regrowth 2) were reduced considerably in all families as a result of two selfing generations. This result agrees with inbreeding work done in diploid and tetraploid red clover [7, 12, 15]. However, as Laczynska-Hulewicz [7] found in tetraploid red clover, there is great variation between families and inbreds. For example, two I_2 lines were found at regrowth 1 stage and one at regrowth 2 stage to be as good as the check for these measurements.

Koffman and Wilsie [6] and Aycock and Wilsie [3] found a trend towards a more erect growth habit with inbreeding in alfalfa. Similar results were obtained in the present study for red clover. In general, there was a retardation in maturity and a decrease in the proportion of flowering heads with inbreeding. The row uniformity did not seem to be affected much by inbreeding (Table 2).

Plant dry weight was the most variable character and showed the highest phenotypic and genotypic variances in all generations. This is possibly due to the high number of genes affecting directly or indirectly the forage yield. Differences in forage yield (dry weight) among the three generations were highly significant. The greatest drop in this character occurred during the first generation of inbreeding. These results agree closely with those of other investigators [5, 7, 13].

For all traits, the first generation of selfing resulted in the greatest degree of inbreeding depression. The depression between the I_1 and I_2 generation was not as drastic. This result closely parallels the inbreeding curve for a diploid species.

The genotypic variances in some generations were negative, causing negative heritability estimates. This might be caused by a large error term, and if so, the experimental design was not precise enough or population sizes were not large enough to

Table 2. Means, phenotypic and genotypic variances, and heritabilities of different agronomic characters for I_0 progeny and two inbred generations in red clover*

	Generation	Plant height (cm)	Vigor	Leafiness	Growth habit	Growth stage	Dry weight (g)	Regrowth 1	Regrowth 2	Days to 50% bloom	Uniformity	Proportion of flowering heads
Mean	I_0	28.2	4.6	5.3	6.0	6.1	26.4	5.4	6.3	73.2	5.5	2.8
	I_1	23.4	5.8	4.5	5.4	4.8	16.3	6.4	7.2	77.9	6.0	4.3
	I_2	20.4	6.3	4.3	4.9	4.6	11.4	6.9	8.0	81.4	5.6	5.1
	Lakeland	32.9	2.2	4.1	6.6	6.9	53.5	3.8	4.5	71.0	3.0	2.5
Inbreeding depression†	% of I_0	−17.0	26.1	−15.1	−10.0	−21.3	−38.2	18.5	14.3	6.4	9.1	53.6
	% of I_1	−12.8	8.6	−4.4	−9.2	−4.2	−30.1	7.8	11.1	4.5	−6.7	18.6
Phenotypic variance	I_0	5.00	0.33	0.14	0.10	0.14	49.37	0.51	1.63	–	–	–
	I_1	23.09	0.31	0.97	0.47	0.63	15.59	1.39	2.63	–	–	–
	I_2	1.87	0.07	0.27	0.10	0.34	17.68	0.71	0.96	–	–	–
	All	3.21	0.11	0.23	0.09	0.25	12.00	0.21	0.27	–	–	–
Genotypic variance	I_0	2.95	0.23	−0.03	0.00	−0.06	42.64	0.42	1.54	–	–	–
	I_1	21.04	0.20	0.80	0.37	0.42	8.86	1.29	2.55	–	–	–
	I_2	−0.18	0.04	0.10	0.00	0.13	10.95	0.61	0.87	–	–	–
	All	2.87	0.09	0.20	0.07	0.21	10.88	0.19	0.26	–	–	–
Heritability	I_0	59.1	68.0	23.6	−1.3	44.4	86.4	81.4	94.4	–	–	–
	I_1	91.1	65.7	82.3	77.6	67.0	56.8	93.2	96.6	–	–	–
	I_2	−9.4	53.6	37.1	−3.4	39.3	61.9	86.5	90.6	–	–	–
	All	89.4	83.0	87.5	81.0	86.0	90.6	92.4	94.5	–	–	–

* See Table 1 for system of scoring.

† $(I_1 - I_0/I_0) \times 100$ and $(I_2 - I_1/I_1) \times 100$. Negative values indicate the amount of inbreeding depression.

Table 3. The number and percentage of plants of different generations superior to the mean of the I_0 population or Lakeland

	Mean of I_0 as base			Mean of Lakeland as base			
Character	I_1 *	I_2 †	Total	I_0 *	I_1 †	I_2 †	Total
Plant height	156(32)‡	244(15)	400(19)	141(29)	85(18)	105(6)	331(16)
Vegetative vigor	139(29)	249(15)	388(18)	80(17)	23(5)	22(1)	125(6)
Growth stage	178(37)	561(35)	739(35)	254(53)	168(35)	546(34)	968(46)
Plant dry weight	97(20)	144(9)	241(11)	44(9)	18(4)	43(3)	105(5)

* Total number of plants in this generation = 480.
† Total number of plants in this generation = 1620.
‡ Percentages are shown in parentheses.

measure small genetic differences.

Heritability estimates were quite high for most characters. Dry weight was a highly heritable character in this population. Also, heritability estimates decreased for most characters with inbreeding beyond the I_1 generation. The overall estimates in Table 2 are measures of variability and heritability of characters considering the three generations collectively.

Based on the mean performance of different generations, one could conclude that inbreeding is quite deleterious in red clover. However, this is only a generalization, because it was possible to find plants that were inbred but performed much better than their parents and the check variety for any single character. Table 3 presents the number and percentage of such plants which exceeded the mean of their respective I_0 parents and the mean of Lakeland for several characters.

Twenty-two I_1 and 12 I_2 plants were found that were taller, more vigorous, earlier in maturity and higher in dry weight than the mean of their respective I_0 parents. However, when the mean of Lakeland was used as a check, only 8 I_0, 2 I_1 and no I_2 plants were found to perform better in all of the above 4 characters simultaneously.

Table 4. Phenotypic* (lower diagonal) and genotypic (upper diagonal) correlations between characters over all three generations

Character	HT	V	L	GH	GS	DW	R1	R2
Plant ht. (HT)		−0.92	0.52	0.67	0.44	0.70	−0.71	−0.70
Vigor (V)	−0.89		−0.45	−0.78	−0.53	−0.93	0.70	0.58
Leafiness (L)	0.54	−0.46		0.70	0.89	0.41	−0.17	−0.24
Growth habit (GH)	0.60	−0.67	0.62		0.68	−0.61	−0.52	−0.44
Growth stage (GS)	0.48	−0.54	0.88	0.62		0.56	−0.11	−0.11
Dry weight (DW)	0.67	−0.88	0.41	0.58	0.55		−0.44	−0.21
Regrowth 1 (R1)	−0.68	0.65	−0.19	−0.47	−0.13	−0.42		0.93
Regrowth 2 (R2)	−0.67	0.52	−0.25	−0.39	−0.11	−0.21	0.89	

* Phenotypic *r* values required for significance at 5 and 1% probability levels are 0.36 and 0.46, respectively. These values are not appropriate for genotypic correlations.

Based on the phenotypic and genotypic variances and covariances, the phenotypic and genotypic associations between character pairs were computed for data combined over all 3 generations (Table 4). The three characters – plant height, vigor and growth habit – were phenotypically and genotypically associated with all other characters measured in this study. Plant dry weight was found to be phenotypically and genotypically related to all characters measured in the first-year growing season. However, it was not correlated with regrowth 2. This would indicate that, in space-planted nurseries, first-year plant yield is a poor predicter of plant performance in the second-year harvest. On the basis of phenotype, if one were to select for dry weight in red clover, the chances for success would be greater if the chosen plants had high vigor, fewer leaves, and were taller.

It is found from this study and a previous one [10] that inbreeding depression and its reverse phenomenon, heterosis, play major roles when red clover is inbred and when inbreds are crossed, respectively. Leffel and Muntjan [8] have suggested the use of pseudo-self-compatibility system and Anderson *et al.* [1, 2] and Cornelius *et al.* [4] have used the gametophytic self-incompatibility system to obtain inbred lines for synthesis of hybrid red clover. It is proposed that incorporation of S_f allele, as used in the present study, simplifies the steps of hybrid production in red clover if a method of elimination of the self-fertility allele is found prior to crossing of inbreds by bees.

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LITERATURE CITED

1. Anderson M.K., Taylor N.L. & Hill R.R., Jr. 1974. Combining ability in I_0 single crosses of red clover. *Crop Sci.* **14**, 417–419.
2. Anderson M.K., Taylor N.L. & Kirthavip R. 1972. Development and performance of double-cross hybrid red clover. *Crop Sci.* **12**, 240–242.
3. Aycock M.K., Jr. & Wilsie C.P. 1968. Inbreeding *Medicago sativa* L. by sib-mating. II. Agronomic traits. *Crop Sci.* **8**, 481–485.
4. Cornelius P.L., Taylor N.L. & Anderson M.K. 1977. Combining ability in I_1 single crosses of red clover. *Crop Sci.* **17**, 709–713.
5. Kirk L.E. 1927. Self-fertilization in relation to forage crop improvement. *Sci. Agric.* **8**, 1-35.
6. Koffman A.J. & Wilsie C.P. 1961. Effects of inbreeding on various agronomic traits in Du Puits alfalfa. *Crop Sci.* **1**, 239–240.
7. Laczynska-Hulewicz T. 1963. Self-fertility and inbreeding in tetraploid red clover. *Genet. Pol.* **4**, 97–119.
8. Leffel R.C. & Muntjan A.I. 1970. Pseudo-self-compatibility in red clover (*Trifolium pratense* L.). *Crop Sci.* **10**, 655–658.
9. Rinke E.H. & Johnson I.J. 1941. Self-fertility in red clover in Minnesota. *J. Am. Soc. Agron.* **33**, 512–521.
10. Taylor N.L., Johnston K., Anderson M.K. & Williams J.C. 1970. Inbreeding and heterosis in red clover. *Crop Sci.* **10**, 522–525.
11. Thomas H.L. 1955. Inbreeding and selection of self-fertilized lines of red clover, *Trifolium pratense* L. *Agron. J.* **47**, 487–489.
12. Wexelsen H. 1945. Studies on fertility, inbreeding, and heterosis in red clover (*Trifolium pratense* L.). *Skr. norske Vidensk-Akad.*, I. Mat.-naturv. Kl. 1.
13. Wexelsen H. 1965. Studies in tetrapolid red clover. Fertility and seed yield. Inbreeding and heterosis effects. *Sci. Rep. Col. gen. Educ. Osaka Univ.* **44**, 1–23.
14. Williams R.D. 1937. Genetics of red clover and its bearing on practical breeding. *4th Int. Grassl. Cong.*, Gt. Brit., pp. 238–251.
15. Williams R.D. 1937. Heterosis in red clover. Effect of inbreeding in F_2 and F_3 population. *Welsh J. Agric.* **13**, 172–190.