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Non-additive Gene Effects for Creeping-root in Lucerne (*Medicago sativa* L.)

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Summary. Both general and specific combining abilities for creeping-rootedness of lucerne were found to be highly significant although there were substantial differences among genotypes for both parameters. These results indicate that both "additive" and "non-additive" gene effects are involved in the genetic substrate of creeping-rootedness; hence utilization of heterosis would seem to be the most appropriate procedure for further improvement in this trait.

Introduction

Breeding for creeping-rootedness in *Medicago sativa* L. was initiated by Heinrichs (1954) in Canada and similar breeding programmes are in progress at present in Australia, U.K., USSR, Hungary etc. In spite of the extensive breeding activities the published results of genetic analyses of creeping-rootedness are still inconsistent.

The choice of breeding technique to maximize the rate of genetic advance for creeping-rootedness in lucerne depends interalia upon the relative proportions of the components of genetic variance, e.g. if the additive component accounts for a large proportion, straightforward mass selection is preferable; on the other hand, if the non-additive component constitutes a substantial proportion of the genetic variance, then additional appropriate artifices (e.g. family selection) to utilize heterosis must be applied.

Morley and Heinrichs (1960) analysed data on the frequency of creeping-rootedness within an F_3 population derived from crosses between *Medicago falcata* L. and *Medicago media* Pers. They concluded that genotypic variation was predominantly additive, with heritability of 20 per cent, and predicted that mass selection without additional artifices is the most expedient breeding technique. In contrast, an F_3 population of *M. sativa* (Daday 1962), derived from crosses among the Rambler, Hunter River, Hairy Peruvian and African cultivars, showed a substantial amount of non-additive genetic effects when analysed by the half-sib and full-sib method, and hence family selection was recommended. Furthermore, Heinrichs and Morley (1962) analysed an additional moiety of their F_3 *M. falcata* × *M. media* breeding material by the half-sib and full-sib method, and found that, with one exception, all groups of data had a ratio of [cov(FS) - cov(HS)]/cov(HS) greater than 1.0 thus demonstrating the presence of non-additive components of genetic variation. Hence they also recommended family selection procedures.

In both the Canadian Rambler and the Australian Cancreep cultivars, which were developed from the respective breeding populations, 65 per cent of the plants are creeping-rooted, and further improvement appears to be practicable.

The aims of the present series of investigations are to reexamine the genetic variance of creeping-rootedness in an advanced breeding population and to investigate the utilization of general and specific combining ability.

Materials and Methods

Combining ability experiments

Six randomly selected F_5 creeping-rooted plants descended from the Australian breeding population (Daday 1962) were hand crossed in reciprocal diallel combinations under glasshouse conditions. The total number of plants in the 28 families (6 × 5; two families missing) was 2518. Each family consisted of approximately 90 full-sib plants, distributed over four replications. The seedlings were grown in glasshouses and transplanted to the field on 12 June 1966. The first adventitious shoots appeared from creeping-roots within six months after transplantation.

The plants were scored for creeping-rootedness versus non-creeping; the final scoring was carried out on the 1st November 1967 (Daday et al. 1974).

Biometrical methodology

The combining ability analyses were carried out according to Griffing (1956a,b), Models I and II. The main use of Model I was in the assessment of general and specific combining abilities of each of the six parental lines. Model II was used to obtain some indirect estimates of cov(HS) and cov(FS) for estimating the proportion of the non-additive component of the Total Hereditary Variance for comparison with previous findings on creeping-rootedness (Morley and Heinrichs 1960; Daday 1962; Heinrichs and Morley 1962). Although lucerne behaves as an autotetraploid (Twamley 1955) prior experience (Daday 1962; Griffing 1956a) has shown that the covariances extricated from the combining ability analysis may be interpreted as follows (Kempthorne 1955):

$$\mathrm{Cov(HS)} = \frac{1}{4}\hat{\sigma}^2_A \qquad (1)$$

$$\mathrm{Cov(FS)} = \frac{1}{2}\left(\hat{\sigma}^2_A + \frac{1}{2}\hat{\sigma}^2_{NA}\right) \qquad (2)$$

Denoting the Total Hereditary Variance by $\hat{\sigma}^2_H$ and its additive component by $\hat{\sigma}^2_A$, it is obvious that $\hat{\sigma}^2_{NA} = \hat{\sigma}^2_H - \hat{\sigma}^2_A$ is the observed non-additive component. It is noted that $\hat{\sigma}^2_{NA} = \hat{\sigma}^2_D$ *only if* attention is restricted to a single locus, e.g. Binet and Morris (1962); Binet (1963), where full biometrical exposition is found, *or if* inter-locus-interaction of the alleles is negligible. This later assumption is rarely justified hence, in operative conditions,

$$\hat{\sigma}^2_{NA} > \hat{\sigma}^2_D$$

Collating and rearranging (1) and (2):

$$\frac{\hat{\sigma}^2_{NA}}{\hat{\sigma}^2_H} = \frac{\mathrm{Cov(FS)} - 2\,\mathrm{Cov(HS)}}{\mathrm{Cov(FS)} - \mathrm{Cov(HS)}} = q$$

This statistic measures the non-additive proportion of total genetic variability.

In diallel crosses Griffing (1956b) showed that $\hat{\sigma}^2_A = 2\,\hat{\sigma}^2_{gca}$ and $\hat{\sigma}^2_{NA} = \hat{\sigma}^2_{sca}$

$$\text{Thus } \mathrm{Cov(HS)} = \frac{1}{2}\hat{\sigma}^2_{gca}$$

$$\text{and } \mathrm{Cov(FS)} = \hat{\sigma}^2_{gca} + \frac{1}{4}\hat{\sigma}^2_{sca}$$

$$\text{Then } q = \frac{\hat{\sigma}^2_{sca}}{2\hat{\sigma}^2_{gca} + \hat{\sigma}^2_{sca}}$$

It is submitted that this statistic is a more expedient indicator than the conventional:

$$\frac{\mathrm{Cov(FS)} - \mathrm{Cov(HS)}}{\mathrm{Cov(HS)}} = (1 - q)^{-1}$$

because it expresses explicitly the non-additive component of the Total Hereditary Variance.

Results

Combining ability experiment

The contribution of the six parental plants to the mean percentage of creeping-rootedness in full-sib families from a reciprocal diallel crossing system is presented in Table 1. There were considerable differences between some parental genotypes in their contribution to this incidence, genotype 93 being the best contributor of high frequencies of creeping-rootedness to its half-sib progeny. In addition, certain particular crosses (e.g. 97 × 15) produced full-sib families with higher frequencies of creeping-rooted plants than the other crosses. The general and specific combining abilities, and reciprocal differences, as components of observable total hereditary variance (Griffing 1956a,b), all had highly significant mean squares (Table 2), indicating the presence of considerable amounts of both additive and non-additive gene effects on creeping-rootedness.

Estimates of the general and specific combining ability effects are displayed in Table 3.

It is conspicuous that plants 93, 8, 15 and 79, in that order, are those which show the highest positive general combining abilities, the corresponding estimates for plants 13 and 97 being negative.

Specific combining ability effects were also significantly better in some crosses (e.g. 97 × 15) than in others.

Table 1. Mean percentage of creeping-rooted plants in the F_6 generation

Parents	97	8	13	15	93	79	Mean
97		74.1	80.0	94.9	77.8	76.1	80.6
8	76.0*		84.6	94.0	96.4	88.6	87.9
13	57.8	77.7		67.3	69.9	72.4*	69.0
15	87.7	93.9	70.0		89.7	83.9	85.0
93	98.7	94.8	82.6	87.0		90.8	90.8
79	91.8	94.2	74.3	88.4	94.0		88.5
Mean	82.4	86.8	78.3	86.3	85.6	82.4	83.6

* Estimate of missing values equal reciprocal plus maternal effect

Table 2. General and specific combining ability analysis for creeping-rooted percentages

Source	D.F.	Mean Squares	Expected Mean Squares	
			Model I	Model II
General combining ability (g.c.a.)	5	366.36***	$^{\ddagger}0.052\,\sigma_e^2 + \sigma_i^2 + \frac{8}{5}\sum_h g_h^2$	$^{\ddagger}0.052\,\sigma_e^2 + \sigma_i^2 + 2\sigma_r^2 + 12\sigma_g^2$
Specific combining ability (s.c.a.)	9	58.08**	$0.052\,\sigma_e^2 + \sigma_i^2 + \frac{2}{9}\sum\sum_{h<j} s_{hj}^2$	$0.052\,\sigma_e^2 + \sigma_i^2 + 2\sigma_r^2$
Reciprocal effect	13	58.88**	$0.052\,\sigma_e^2 + \sigma_i^2 + \frac{2}{15}\sum\sum_{h<j} r_{hj}^2$	$0.052\,\sigma_e^2 + \sigma_i^2 + 2\sigma_r^2$
Block × Family	81	17.14	$0.052\,\sigma_e^2 + \sigma_i^2$	$0.052\,\sigma_e^2 + \sigma_i^2$
Error	2407	12.14	σ_e^2	σ_e^2

Components of variance	
g.c.a.	38.54
s.c.a.	20.47
Reciprocal	20.87
Block × family	16.51

*** $P < 0.001$; ** $P < 0.01$;

‡0.052 is the inverse of the harmonic mean of the number of plants per plot

Reverting our attention to Table 2 it seems informative to partition the "Sums of Squares" on which those variance components are based. All the three genetic variance components are partitioned to ascertain each parental plant's contribution. Table 4 summarises the operatively effective conclusion to be drawn from the data, viz. the individual worth of the parents and the favourable and unfavourable matings.

The low average specific combining ability variance associated with genotype 93 indicates that genotype 93 uniformly transmits its high creeping-rooted ability to all of its progenies, whereas the high specific combining ability variance associated with geno-

Table 3. Estimates of general ($\hat{g}_i$) and specific ($\hat{s}_{ij}$) combining ability effects

Parents	$\hat{s}_{ij}$ 97	8	13	15	93	79	$\hat{g}_i$	Order of $\hat{g}_i$
97		-10.6300	0.4325	7.8075	1.6450	0.7450	-2.6950	5
8			5.2575	3.0325	1.5700	0.7700	4.7290	2
13				-5.0550	-0.5675	-0.0675	-12.4830	6
15					-3.4925	-2.2925	2.5420	3
93						0.8450	5.6540	1
79							2.2540	4

Least Significant Differences		
$\hat{s}_{ij} - \hat{s}_{ik}$	7.12	$i \neq j,k;\ j \neq k$
$\hat{s}_{ij} - \hat{s}_{kl}$	5.87	$i \neq j,k,l;\ j \neq k,l;\ k \neq l$
$\hat{g}_i - \hat{g}_j$	4.12	

Table 4. Estimates of general and specific combining ability and reciprocal variances associated with each parent and estimates of the environmental variances on individual ($\hat{\sigma}_e^2$) and mean bases ($\hat{\sigma}^2$) for creeping-root

Parent No.	$\hat{\sigma}^2_{g_i}$	$\hat{\sigma}^2_{s_i}$	$\hat{\sigma}^2_{r_i}$	± Indv. Basis $\hat{\sigma}^2_e$	Mean Masis $\hat{\sigma}^2$
97	5.914	39.494	94.24	1132.20	12.14
8	21.476	33.367	-1.30*		
13	154.478	8.571	51.042		
15	5.113	23.434	-2.17*		
93	30.619	-0.255*	31.68		
79	3.732	-3.076*	17.60		

* A negative value implies a zero or small variance

type 15 indicates that there are specific combinations of genotype 15 with other genotypes with higher frequencies than would be expected (e.g. 97 × 15) and other combinations with much lower frequencies (e.g. 13 × 15) than expected. For these reasons genotype 93 is probably superior to 15 for inclusion in the production of a synthetic cultivar, and genotype 15 is probably superior to 93 if specific high creeping-rooted combinations are required.

Discussion

Genetic aspects

Having established the presence of significant additive and non-additive gene effects from the analysis of general and specific combining ability variances (q.v. Table 2), we can collate the findings of Morley and Heinrichs (1960); Heinrichs and Morley (1962); Daday (1962) and our present findings in Table 5.

This table shows, in contrast to the finding of Morley and Heinrichs (1960), that results reported by Daday (1962), Heinrichs and Morley (1962), and also our current investigation indicate the presence of substantial genetic variance component, due to non-additive gene effects, accounting for the important specific combining abilities (q.v. Tables 2 and 3).

Husbandry and breeding aspects

The present investigation was conducted under spaced plant conditions but previous results (Daday et al. 1974) confirmed that creeping-rootedness was also expressed under row sward conditions. Creeping-

Table 5. Summary of genetic analyses of creeping-rootedness

Source	M&H;'60	D;'62	H&M;'62	H&M;'62	H&M;'62	H&M;'62	H&M;'62	H&M;'62	D.G.B.'71
Year of Exp.	1950	1961	1951	1952	1952	1951	1952	1952	1967*
Feature	Score	Score	Score	Score	Class	Score	Class	Score	Class
Cov(FS)	0.153	0.206	0.074	0.276	0.032	0.196	0.039	0.405	43.63
Cov(HS)	0.091	0.038	0.047	0.131	0.014	0.081	0.019	0.169	19.27
$(1-q)^{-1}$	0.6813	4.4211	0.5745	1.1069	1.2857	1.4198	1.0526	1.3969	1.2644
q	<0	0.7738	<0	0.0966	0.2222	0.2957	0.0499	0.1839	0.2089

$$\sigma^2_{NA} = \sigma^2_H - \sigma^2_A = \sigma^2_{Dominance} + \sigma^2_{Epistasis}$$

$$(1-q)^{-1} = \frac{\hat{\sigma}^2_H}{\hat{\sigma}^2_A} = \frac{Cov(FS) - Cov(HS)}{Cov(HS)}$$

$$q = \frac{\hat{\sigma}^2_{NA}}{\hat{\sigma}^2_H} = \frac{Cov(FS) - 2\ Cov(HS)}{Cov(FS) - Cov(HS)}$$

M&H = Morley and Heinrichs; H&M = Heinrichs and Morley; D = Daday; D.G.B. = Daday, Grassia and Binet

* Cov(FS) and Cov(HS) were calculated from mean cross-products in each analysis except the present one, where they were calculated from the components of variance for specific and general combining ability.

rootedness was significantly correlated with survival in lucerne (Daday 1967) under spaced plant conditions. Plant husbandry practices for optimal utilization of the genetic substrate were discussed, and might warrant further agronomical investigations.

Attention was given to forage production of creeping-rooted lucerne and Lutz (1970) found that the forage production of Hunter River (a local cultivar) is superior to that of Cancreep (creeping-rooted cultivar). In contrast, Daday et al. (1974) demonstrated that no significant difference exists between forage production of Cancreep and Hunter River.

It is concluded that conventional mass selection (by "truncation" on individual performance only) is not adequately efficient as it can not fully utilize the available genetic variation. As we have presented conclusive proof of the presence of substantial non-additive component, we assert that to avoid undesirable "plateauing", judicieous combinations of family selection with appropriate refinements (e.g. reciprocal recurrent selection) seems to be advisable. Substantial hybrid vigor exists (at least potentially) in the cultivar we investigated. Although utilization of heterosis for forage production (Tysdal et al. 1942) in lucerne was considered, there were several problems such as clonal propagation, self fertilization (Hanson et al. 1964), effect of environmental factors on self-compatibility (Dane and Malton 1973), which may be further investigated before a heterosis programme is undertaken.

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