SELECTION FOR SEEDLING VIGOR AND A QUANTITATIVE GENETIC ANALYSIS OF AGRONOMIC CHARACTERS IN DIALLEL CROSSES WITHIN AND BETWEEN TWO CULTIVARS OF BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS* L.)

by

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DEDICATION

To my beloved family, for their love,
encouragement, and invaluable support,
this work is humbly dedicated.

ABSTRACT

Ph.D. WALID A. KHAYRALLAH Plant Science
Agronomy

SELECTION FOR SEEDLING VIGOR AND A QUANTITATIVE GENETIC ANALYSIS OF AGRONOMIC CHARACTERS IN DIALLEL CROSSES WITHIN AND BETWEEN TWO CULTIVARS OF BIRDSFOOT TREFOIL (LOTUS CORNICULATUS L.)

Two birdsfoot trefoil (Lotus corniculatus L.) cultivars, Mirabel and Leo, were evaluated for seedling vigor and seedling and plant characteristics under growth cabinet, greenhouse, and field conditions. When the advantages of large seeds over small seeds were established, a depth of seeding technique for selection within large-seeded lines was devised and evaluated. Results indicated the possibility of increasing seedling vigor by further selection within large-seeded lines.

The breeding behaviour of field seedling vigor forage productivity and other characters was evaluated in the F_1 and F_2 generations through a 15-clone half-diallel cross. Results indicated that variation due to general combining ability was significant for all characters studied, while variation due to specific combining ability was not. Comparisons of the relative importance of additive, non additive, and environmental variance indicated the general superiority of additive variance for growth habit and pod setting, and the equal importance of other variances for all other characters. Superior clones were selected based on heritability estimates, general and specific combining ability effects and variance components associated with each clone. Phenotypic correlations among characters were mostly positive and for most characters meant the possibility of mutual improvement through selection.

Three selection methods were compared. The greatest efficiency resulted through identification of superior clones for synthetic cultivars, based on diallel crossing and genetic evaluation of these clones.

SOMMAIRE

Ph.D. WALID A. KHAYRALLAH Plant Science
Agronomy

SELECTION DE LA VIGUEUR DE LA PLANTULE ET UNE ANALYSE GENETIQUE QUANTITATIVE DES CARACTERES AGRONOMIQUES CHEZ DEUX CULTIVARS DE LOTIER CORNICULE (LOTUS CORNICULATUS) PAR L'ANALYSE EN DIALLELE ENTRE ET A L'INTERIEUR DES DEUX CULTIVARS

Les deux cultivars de lotier corniculé (Lotus corniculatus L.) Mirabel et Léo ont été évalué en plein champ, en chambre de croissance et en serre pour la vigueur de la plantule ainsi que pour les caractéristiques générales de la plantule et de la plante. La grosseur des graines comme critère de sélection pour la vigueur de la plantule a été évalué et les graines les plus grosses se sont avérées avantagées. De plus, il a été possible de pousser la sélection à l'intérieur d'une lignée à grosses graines en faisant varier la profondeur de semis. Les résultats indiquent que cette dernière méthode permet d'améliorer la vigueur de la plantule par une autre sélection à l'intérieur des lignées à grosse graines.

Les générations F_1 et F_2 obtenues d'un croissement en 1/2 diallèle de 15 clones ont servi à l'évaluation de la transmission par amélioration de la vigueur de la plantule au champ, de la productivité fourragère et de certains caractères agronomiques. Les résultats ont démontré que la variation due à l'aptitude générale à la combinaison était significative pour tous les caractères étudiés alors que l'aptitude spécifique à la combinaison ne l'était pas. L'étude comparative de l'importance relative des variances associées à l'effet additif, non additif et environnemental indique que l'effet additif pour la type de croissance et la formation de gousse prédomine alors que pour les autres caractères il n'y a pas d'effet prédominant. Basée sur les estimations d'héritabilité, les effets d'aptitude générale et spécifique à la combinaison et les composantes des variances associés à chaque clone, une sélection des clones supérieures a été faite. Les corrélations entre les caractères phénotypiques sont positives pour presque tous les caractères ce qui suggère qu'il y a possibilité d'amélioration mutuelle par sélection.

En comparant trois méthodes de sélection il apparaît que pour les cultivars syntétiques les croissements en diallèles et l'évaluation génétique des clones permettent, de façon plus efficace, de sélectionner les clones supérieures.

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CHAPTER I

INTRODUCTION

One of the most important factors limiting the wide acceptance of birdsfoot trefoil (*Lotus corniculatus* L.) as a forage legume is the degree of difficulty in establishing uniform vigorous stands. Other factors are its relatively low forage productivity and slow rate of recovery after cutting compared with other forage legumes such as red clover and alfalfa. Unsuccessful field stand establishment has been mainly attributed to a general lack in rapidity of germination, emergence, and seedling growth compared with competing weeds. One of the main reasons underlying this low vigor is the small size of birdsfoot trefoil seeds. It is generally accepted by forage breeders that an increase in seedling vigor is directly related to increases in seed size. Many breeders have attempted to improve seedling vigor through selection for large seed size. Henson and Tayman (1961) improved seedling vigor by simple selection and intercrossing of plants having large seed. Three cycles of recurrent selection for seed size in Viking and Empire cultivars resulted in 20 and 25% increase in seed size per cycle. Twamley (1967) selected clones with seed size distribution favoring heavy seeds. When their polycross progenies were tested, large variation was noted in their contribution of different seed size and in their seedling vigor performance.

The importance of environmental effects in the control of seed size was demonstrated by Twamley (1970) and Carleton and Cooper (1972). It was demonstrated that both between and within plant differences displayed large variation in seed size. The within-plant variation appeared to be highly correlated with field performance, reflecting the importance of environment.

Thus, while all research reports agree to the advantages of large seeds in increasing seedling vigor, most do not indicate satisfactory estimates of heritability of seedling vigor displayed by increased seed size. Accordingly the selection methods for increasing seedling vigor through increasing seed size did not indicate striking improvement so far.

Another approach to increasing seedling vigor was attempted by Conje and Carlson (1973a, 1973b) through intercrossing genotypes from widely separated gene pools. Intersource crosses displayed an average 15% heterosis over intrasource crosses.

The genetic control of several qualitative characters in birdsfoot trefoil has been established to be mainly due to single genes following tetrasomic inheritance. Genes controlling flower color, leaf size, leaf color, self-incompatability, and cyanogenesis are examples. Miller (1968) studied the combining ability for several characters in a 4-clone diallel cross in birdsfoot trefoil. Significant general combining ability variances were found for some characters, but for most characters specific combining ability variances were also significant. From partial diallel crosses made between Empire and Russian clones of birdsfoot trefoil, Conje and Carlson (1973b) found general combining ability variances to be considerably larger than specific combining ability variances for all characters studied.

The estimation of the relative importance of the different components of phenotypic variance for quantitative characters of crop species has been important in enhancing the efficiency of breeding programs.

The objectives of this investigation were:

1. To increase our knowledge of the relation between seed size and seedling vigor through subjecting the seeds to stress during actual germination and emergence. Accordingly, to devise a stress selection technique that can be used to identify genotypically vigorous seedlings from families having the same size seed in general and large size seeds in particular.
2. To study comprehensively the breeding behavior of seedling vigor and plant performance through the identification of the relative

magnitude and importance of the different components of phenotypic variance associated with agronomic quantitative characters.

3. To evaluate the effectiveness of the diallel mating design and analysis in the identification of superior genotypes to be used in a breeding program aimed at the improvement of birdsfoot trefoil.
4. To study the magnitude and direction of correlations among agronomic traits of birdsfoot trefoil and thus predict some effective ways for improvement through selection for one or more characters.
5. To evaluate and compare selection methods aimed at the improvement of seedling vigor and forage productivity of birdsfoot trefoil.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 General description and agronomic importance

Birdsfoot trefoil (Lotus corniculatus L.) is one of the species belonging to the genus Lotus. It is the most important species of Lotus known for its use as a forage crop in Europe and North America. It is a perennial whose importance as a forage was realized as early as 1774 (Ellis 1774). Most birdsfoot trefoil acreage in North America is found in the north central and north-eastern United States and the southern parts of Ontario and Quebec. Most of this acreage is in the form of pastures.

Birdsfoot trefoil has a taproot system which does not grow as deep as that of alfalfa but rather spreads extensively in the upper soil. Rooting depth and distribution have been used to explain the persistency of birdsfoot trefoil on shallow and poorly drained soils compared with alfalfa. Birdsfoot trefoil ecotypes can vary in their growth habit from prostrate to ascending to erect. The inflorescence is a typical umbel. An average of five to six pods is borne on one peduncle, giving the appearance of a bird's foot. The flowers are self-incompatible, although selfing might occur to a very small degree. Pods usually carry an average of about 20 mature seeds. Seeds are generally very small (375,000-420,000 seeds per pound). Mature seeds generally have hard seed coats that must be scarified for successful germination.

Compared with alfalfa and red clover, birdsfoot trefoil seedlings are generally lacking in vigor. Lack of seedling vigor is the most important factor affecting improper stand establishment. Seedlings are generally slow in their growth rate under field conditions and tend to be taken over by vigorously competing weeds. Once it is established, however, it becomes reasonably competitive and persistent. Establishment of new stands depends greatly on extreme care in seed bed preparation, rate and depth of seeding, grass association, and weed control.

Birdsfoot trefoil can be utilized both for hay, haylage, silage and pastures, but is most commonly used for pasturage. When carefully harvested, its forage is leafy, fine stemmed, nutritious, and of high quality. When pastured it has never been reported to cause bloat in ruminants.

2.2 Seedling vigor

2.2.1 Introduction

The term "seedling vigor" has been used throughout the literature to describe various processes and characters associated with the development of a seed during its life cycle between sowing and plant maturity. Recent literature, however, tries to differentiate among these processes. The scientific terminology now used identifies more clearly the aspects that impart vigor to the seedling and that are themselves directly associated with the seed as an entity.

The term "seed vigor" is now used by the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA). AOSA defines "seed vigor" as "the sum total of all those properties in seeds which, upon planting, result in rapid and uniform production of healthy seedlings under a wide range of environments including both favorable and stress conditions" (Abdelbaki and Anderson 1972).

According to vigor tests, seeds can be differentiated through a range of "quality levels": high, medium, and low vigor seeds. Unlike germination tests, the vigor test evaluates potential seed performance under a wide range of conditions. Vigor tests, to be valid, should be based on reproducible data and on a high correlation with field performance such as seedling emergence or other seedling characteristics.

The level of vigor varies due to the effect of several factors such as genetic constitution, environment, nutrition of the mother plant, stage of maturity at harvest, mechanical integrity of the seed, and deterioration due to aging or pathogenic attack (T. Cuddy 1976, personal communication).

Studies attempting to measure genetic variation of seed vigor should effectively control or account for the last three factors mentioned above.

It is the performance of the seeds that classifies them as being of high or low vigor. Such aspects of performance that have been found as good indicators of seed vigor include biochemical processes and reactions during germination (enzyme reactions, respiratory activity, rate and uniformity of seed germination, seedling emergence and growth, and the ability to emerge under unfavorable and stress environmental conditions). The level of vigor in the seed may persist to affect plant growth, crop uniformity, and yield.

To the seed analyst attempting to establish a vigor rating for a seed lot, all factors affecting seed vigor are equally important: deteriorating seed quality could be due to factors occurring before or after seed maturation on the mother plant. Poor quality before maturation could be due to incomplete biosynthesis or incomplete membranes. After maturation, however, factors such as membrane damage, pathogenic attack, differential humidity and temperature during storage and other factors contribute to deteriorating vigor (Abdel-baki and Anderson 1972).

Our concern in these studies is mainly to identify that part of the variation which is due to the genetic constitution of the seed. The knowledge of other factors simply makes it easy for us to control them or account for them when meaningful comparisons are to be made. Hence, one should not forget that for valid comparisons of genetic variations among different seed classes, the seed should conform to certain basic requirements, such as being viable, not hard, intact and sound, free from pests and diseases, and finally, of similar maturity and age at and after harvest.

What, then, are the different plant characteristics that have been used to measure seedling vigor or those that are very significantly associated with vigor? What are the other factors that affect seedling vigor in crop species, especially legumes, and birdsfoot trefoil in particular? Finally, what are the various breeding techniques that can be used to improve seedling vigor and what are the implications of the breeding behavior to the improvement of seedling vigor in birdsfoot trefoil?

2.2.2 Vigor tests and vigor characteristics

The standard seed germination test and the tetrazolium test have been used to predict seed vigor and seedling establishment. Predictions have had many shortfalls and were not always satisfactory.

Researchers then felt the need to subject seeds to stress tests whereby only viable or vigorous seeds would germinate. This method is now practised by seed analysts in almost all seed vigor evaluations. Throughout the literature one finds references to such stress tests as hot spell or cold spell, where thoroughly soaked seeds are subjected to extreme hot or low temperatures for varying periods of time. Immersion of seeds in concentrated solutions of acids, bases, or salts is another method. In all methods and after the treatment the seeds are germinated under standard conditions. Seeds with inferior viability and quality do not germinate. Those that do germinate are grown further, and measurements on the rate of radicle and epicotyl elongation, dry shoot weight, etc., are taken. Subsequent field planting of germinated seeds can be done and the measurement of plant growth can be used as an indicator of vigor. High correlations between field and laboratory performance indicate the power of the stress test.

Mark and McKee (1968) attempted to find correlations between laboratory stress tests and seed vigor, field performance, and seedling establishment of reed canarygrass (Phalaris arundinacea). They found that germination following hot flooding and cold flooding was a better indicator of field performance than accelerated aging or immersion in concentrated sulfuric acid. Larger and heavier seeds were always correlated with positive results of the stress tests. In sorghum, however, treatment with NH_4Cl prior to germination was found by Abdullahi and Vanderlip (1972) to give much better correlation with field seedling establishment compared with cold or hot flood, standard germination, or artificial aging. Significant variation was also attributed to seed size and source.

The effects of size and age of wheat seeds on seedling vigor, respiration rate, and tetrazolium chloride reduction were studied by Kittock and Law (1968). Significant positive correlations were found between seed weight and tetrazolium reduction and between shoot weight per plot and tetrazolium reduction. This obviously gives the TTC test some advantage in measuring seedling vigor. Nevertheless, if tetrazolium chloride reduction is merely a measure of respiration rates and respiration rates mainly measure the non-genetic factors of seed vigor which are termed seed quality, then the usefulness of this test is restricted to closely related lines or clones. Its advantages lie in its greater speed, economy, and simplicity compared with manometric techniques.

2.2.3 Factors affecting vigor

Seed size, or weight, as it affects seedling vigor, has received consideration in all crop species. The effects of seed size on aspects of seedling vigor such as rapidity of germination, emergence from deep planting, early vegetative growth characteristics, and productivity and yield of forage legumes, was reviewed by Black (1959). He concluded that "within the species examined the proportion of hard seeds seems to increase as seed size decreases, which limits germination greatly. The weight of the seed limits the depth from which a seedling can emerge, and preliminary studies suggest that this is due not so much to depletion of cotyledonary reserves, but to factors controlling hypocotyl elongation." The accumulation of dry matter in the early vegetative phase is directly proportional to seed weight. This is due more to the area of the cotyledons at emergence rather than to food reserves in them. The influence of seed weight on subsequent growth is confounded with the level of competetive stress operative on the individual plants.

More recent reports measured seedling vigor by different attributes such as growth rate, length of nonphotosynthetic seedlings, mitochondrial metabolism, leaf area, rate of leaf appearance, and number of tillers.

Derwyn *et al*. (1966) found that heavy seeds of three *Phalaris* sp. not only had greater growth rates, but kept growing longer than small seeds. McDaniel (1969) found that seedling fresh weight, mitochondrial protein, and biochemical activity of barley were positively correlated with heavier seeds. Such seedlings apparently possessed a greater amount of ATP production and higher respiratory rates, hence a greater growth potential than seedlings coming from small seeds.

Large wheat seeds were found to produce significantly higher number of seed-bearing tillers per plant, and have higher growth rates (Austenson and Walton 1970).

Large Lolium perene seeds were reported by Thomas (1966) to produce plants with increased number of tillers, leaf area, and rate of leaf appearance. Smith (1961) found that seed size of red clover was not useful in screening for seedling vigor. Beveridge and Wilsie (1959), however, found positive correlations between seed size of alfalfa and seedling vigor.

In birdsfoot trefoil, Twamley (1967) found the correlation between seed size and seedling vigor to be high enough to base a breeding program on the selected 20% of the population having large seed. Henson and Tayman (1961), and Stickler and Wassom (1963), also found very high correlations between size and vigor in birdsfoot trefoil seeds.

The size of a legume seed may be a result of genetic or environmental factors or an interaction of both. Kidd (1919) remarked that environmental conditions may (i) directly affect the seed because of its position on the plant, or (ii) indirectly because of its influence on the mother plant. Walter and Jensen (1970) studied the effect of temperature and soil moisture during seed production on seedling vigor of alfalfa. They found that seed produced under cool temperature was heavier and contained a higher percentage of hard seed. Decreased soil moisture had similar effects. The increased seedling vigor was, in both cases, attributed to increased seed size.

Twamley (1970) also found that later-maturing birdsfoot trefoil plants produced more vigorous seeds than earlier maturing ones. Nevertheless, there was an equal chance of selecting lines with good seedling vigor from late-maturing as well as from early or medium-maturing plants.

Genetic differences not related to seed size are also known. Shibbles and Macdonald (1962), and Cooper and Qualls (1968), have shown differences in seedling vigor of birdsfoot trefoil seedlings coming from the same size seed. These differences are believed to be related to more efficient metabolism and to greater ability to partition photosynthates into leaf area.

In birdsfoot trefoil Twamley (1967) found that one could select clones with seed size distribution patterns skewed towards heavy seeds. When put in a polycross, these clones would differ in their contribution of seeds of a given size.

Carleton and Cooper (1972) did some studies to evaluate the effect of environment and genetics on seedling vigor of birdsfoot trefoil, alfalfa and sainfoin. Their data indicated appreciable variability in seed size, both within and between plants.

Black (1959) attributed greater seedling vigor to larger area of cotyledons. Regression of cotyledonary area on seed weight was positive and high (10.1 mm^2/mg) for birdsfoot trefoil. When variation among clones was removed from total variation, the correlation coefficients were reduced. This indicates a confounding effect of genetic and environmental variances for seed size. On the other hand, Carleton and Cooper (1972) found that within-plant differences in seed size were highly correlated with field performance, indicating the importance of environmentally induced variations of seed size. This indicates the degree of difficulty in attempting to separate the environmental and genetic variation imparted to seed size of seeds produced on any plant and especially so in an open-pollinated crop.

2.2.4 Selection and improvement

Several methods have been used to select improved materials for upgrading the potential of birdsfoot trefoil varieties. Phenotypic mass selection followed by random crossing of selected clones has been used by some breeders (Seany and Henson 1970). Other breeders have used various types of progeny testing to evaluate clones for subsequent intercrossing and increase (Twamley 1967, 1969). Recurrent selection combined with progeny testing, phenotypic selection, or both, have also been used (Twamley 1970, 1971, 1972; Draper and Wilsie 1965). Selected clones having high general combining ability have been used to produce synthetic cultivars.

Improvement of seedling vigor has been the most important undertaking by most plant breeders. Of particular interest to this study is the earlier work done by Twamley (1971, 1972) and Conje and Carlson (1973a, 1973b).

Twamley (1971) suggested three selection methods for the improvement of seedling vigor in birdsfoot trefoil. In his experiments, the base material consisted of 72 selected large seeded lines planted in a seedling vigor evaluation experiment. When vigorous seedlings from several lines were selected phenotypically and intercrossed, they constituted a selection-by-phenotype group. The second group (selection-by-genotype) consisted of open-pollinated seed of maternal plants, progenitors, of the most vigorous lines as judged by their mean seedling dry weight. The selection-by-geno-phenotype group consisted of intercross seed of the best phenotypically vigorous seedlings in each of the best lines selected genotypically.

When progeny developed by these three groups were tested for their seedling dry weight in growth rooms, results indicated equal performance of genotypic and geno-phenotypic progeny which were both superior to the phenotypic group alone.

In an evaluation of second generation material of the same three groups, Twamley (1972) found that the geno-phenotypic group outyielded the genotypic group by an average of 11%. The phenotypic group behaved erratically. Heritability for seedling vigor as determined by correlations between parents and offsprings was found to be low ($r = 0.27$).

Conje and Carlson (1973a) evaluated seedling vigor traits of open-pollination seed from F_1 plants of crosses within and between two diverse germplasm sources of birdsfoot trefoil (Empire and

Russian). Both laboratory and field experiments were carried out. In the laboratory, seed size was found to be positively associated with seedling dry weight, hypocotyl length, radicle length, and total seedling length ($r = 0.47$ to 0.87). A 5% heterosis for hypocotyl length was noted in progeny of Empire by Russian crosses, but not for other characters.

Partial diallel and full sib crosses among 18 selected clones of the two diverse sources were evaluated in space-planted field experiments to measure heterosis, combining ability and interrelationships among characters (Conje and Carlson 1973b). It was demonstrated that Empire x Russian F_1 cross progenies yielded 14% more forage than Empire x Empire crosses. Full sib progenies derived from intersource crosses showed superiority over intrasource crosses for spring vigor, winterhardiness, pod production, and forage yield. The study was an attempt to get more genetic diversity in a breeding program and measure the influence of this diversity on the expression of heterosis.

2.3 Genetics

2.3.1 Cytology and cytogenetics

Birdsfoot trefoil is a tetraploid with $2n = 4x = 24$. Pairing of chromosomes is usually bivalent with the occurrence of occasional quadrivalents. The frequency of quadrivalents was found by Wernsman *et al*. (1964) to be about one in every four microspore mother cells.

Dawson (1941) suggested that birdsfoot trefoil is an autotetraploid of Lotus tenuis. Lotus tenuis has a chromosome number of 2n = 12, a diploid. His conclusion was based on morphological characteristics of bivalent pairing, and tetrasomic inheritance of cyanogenetic compounds. Stebbins (1950), however, concluded that tetrasomic inheritance and bivalent pairing were evidence of segmental allopolyploidy. A study of phenolic constituents of Lotus by Harney and Grant (1965) gave indications that Lotus corniculatus is more likely to be an allotetraploid than an autotetraploid. Cyanogenetic studies by Grant and Sidhu (1967) indicated that Lotus tenuis as well as other species of the genus Lotus could be ancestors of the tetraploid Lotus corniculatus. When bivalent pairing was seen in backcross progenies of L. tenuis x L. corniculatus, it indicated a high degree of homology between the chromosomes of the two species. This is considered by Wernsman (1964) as further evidence that L. tenuis is a progenitor of L. corniculatus.

2.3.2 Inheritance of characters

Many attempts have been made to study the mode of inheritance in birdsfoot trefoil. Dawson (1941) studied F_2 progeny of crosses between cyanogenetic and acyanogenetic birdsfoot trefoil plants. He concluded that cyanogenesis is determined by a single dominant gene with tetrasomic inheritance. The concentration of hydrocyanic acid was probably under the effect of a series of modifying genes.

Donovan (1959) worked with crosses between large-leafed L. corniculatus var. vulgaris and small-leafed L. corniculatus var. arvensis. He found ratios very close to those obtained from theoretical tetrasomic inheritance. Observed slight differences in single gene tetraploid ratios were explained on the basis of cytological phenomena in tetraploids, such as pairing, multivalent formation, nondisjunction, chiasmata, and the position of the gene with respect to the centromere. Donovan and McLennan (1964) further studied the same leaf size character and found it to fit tetrasomic type of inheritance. Large leaf was found to be dominant.

Leaf color, or actually chlorophyl content, was studied by Poostchi and MacDonald (1961). They explained their results on the basis of a single dominant gene control of leaf color. This gene showed "random four-chromosome type segregation."

Keel tip color was studied by Bubar and Miri (1965) and Buzzell and Wilsie (1963). Brown and red color were found to be dominant to yellow. Their results indicated that brown color is primarily determined by a single gene, although the existence of suppressor genes was not dismissed. The intensity of the brown keel tip color was explained on a quantitative basis. Self-incompatability, flower color, pubescence, chlorophyl deficiency and corolla streaks were also found by Bubar and Miri (1965) to conform to tetrasomic type of inheritance. Dawson (1941) noted differences in the extent of disomic to tetrasomic inheritance in birdsfoot trefoil, depending on the character studied. Although quadrivalents were observed, they were judged to be of minor importance.

Geneticists and plant breeders have been frustrated in finding good genetic markers to study. Many populations have been examined thoroughly for several easily recognizable morphological characters, and some of them were found. These are: cyanogenesis, leaf size and color, keel tip color, self-incompatability, and pubescence. Almost all such identified characters were found to be controlled by single dominant genes, although some had suppressor or modifying genes. The ratios studied in segregating materials were best explained on the basis of tetrasomic type of inheritance. There has not been any report on the mode of inheritance of such characters as seedling vigor, forage yield, growth habit, and plant height, all of which are quantitative in nature and are extremely difficult to study with normal Mendelian procedures. The study of such characteristics could only be handled through statistical analysis such as that of the diallel cross.

2.3.3 Diallel analysis

The study of quantitative continuous variation of different crop species has been very popular during the last two decades. The most widely used method for assessing components of genetic variance is the diallel crossing method. The application of the technique was fully developed by Jinks and Hayman (1953), Jinks

(1954), Hayman (1954), and Hayman and Jinks (1954a, 1954b). Griffing (1956a, 1956b) outlined various experimental techniques and suggested several methods and models of analysis depending on the experimental material and the assumptions made around it.

Sokol and Baker (1977) recently outlined the assumptions on which a valid genetic interpretation of the data from a diallel experiment depends. These assumptions, specifically related to the original standard procedures, are: (1) homozygous parents, (2) diploid segregation, (3) no reciprocal differences, (4) gene frequencies equal to 0.5 at all segregating loci, (5) genes independently distributed between the parents (no linkage), (6) no epistasis (no non-allelic interactions) and, finally (7) no multiple alleles.

Depending on the assumptions, several approaches have been given for the analysis (Hayman 1954a, 1954b; Jinks 1954; Griffing 1956b; Kempthorne 1956; Matzinger and Kempthorne 1956).

The procedures of diallel analysis have been used mostly on self-pollinated diploids to obtain information pertinent to the inheritance of quantitative traits and predict the degree of genetic relationships among a number of parents. Measures of general combining ability, GCA, and specific combining ability, SCA, as well as prediction of segregation patterns in advanced generations, were made possible by these procedures.

Methodology for the study of quantitative genetics of tetraploids has lagged behind that of diploids. Kempthorne (1955), Dessureaux (1959), and Levings and Dudley (1963), have given some procedures for the analysis of autotetraploids.

Kempthorne (1955) attempted to break up the genetic variance in autotetraploids and laid down some of the basic differences between autotetraploids and diploids relating to the genotypic values of the population. His scheme depended on a basic assumption which the analysis should consider, "Segregation is by chromosomes rather than by chromatids." According to the analysis, a simple autotetraploid population in equilibrium under random mating will have a genotypic array represented by the expression

$$\mu + P^4A^4 + 4\ p^3qA^3a + 6\ p^2q^2A^2a^2 + 4\ pq^3Aa^3 + q^4a^4.$$

The genotypic value of an individual tetraploid is made up additively of the following parts:

a - The population mean μ

b - Four gene effects (corresponding to additive genes in a diploid)

c - Six terms arising from interactions of two genes (analogous to dominance deviations in the diploid case)

d - Four terms arising from interactions of three genes (not existing in the diploid case)

e - One term arising from interaction of four genes, quadrigenic effects (non-existent in diploids).

In the final analysis the genotypic variance would be partitioned by the formula:

$$\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_T + \sigma^2_F$$

where A = additive

D = digenic

T = trigenic

and F = quadrigenic effects.

These evidently can be found by equating expected to observed covariances in the absence of environmental correlations.

Dessereaux (1959) removed the restriction of diploid inheritance from Hayman's (1954) analysis. The method he outlined for autotetraploid analysis also depended on the basic assumption of segregation by chromosomes only. The author realized that the type of existing segregation and increased number of parameters would introduce too many complications to a generalized analysis of the autotetraploid diallel.

For the special case of random mating and equal allele frequency, Dessereaux (1959) presented some formulae to estimate the variances and covariances of an autotetraploid diallel cross. These formulae are quite similar to those of Kempthorne (1955). The author also stated that since the expected array of genotypes is greater in a tetraploid than in a diploid, the number of parents to be used in the diallel as well as the number of progeny from which family means are to be determined, must be larger in a tetraploid than in a diploid diallel. For a 2-gene model there are 25 genotypes in a tetraploid compared with 9 of the diploid. Dessereaux (1959) estimates that 16 parents are the minimum required to express one gene in a random mating population of autotetraploids.

The sib, diallel, and triallel mating designs for the estimation of genetic variance in autotetraploids were evaluated by Levings and Dudley (1963). At the tetraploid level, dominance includes digenic, trigenic, and quadrigenic effects, thus encompassing all effects arising from interactions between alleles. At the diploid level the GCA variance is only due to additive effects and their interactions. In autotetraploids, however, GCA variance is due to additive and digenic effects as well as interactions of additive, digenic, and additive by digenic effects. Specific combining ability, SCA, contains some digenic but mostly trigenic, quadrigenic and resulting interactions. Hence, digenic effects occur both in GCA and SCA variance. Such is not the case in diploids. For autotetraploid alfalfa, Levings and Dudley (1963) provided an estimation procedure based on the use of a partial diallel cross in conjunction with parent-offspring regression and an analysis of variance among parental clones. If epistasis was excluded from the genetic model, estimation of additive, digenic, trigenic, and quadrigenic components can be possible. If the last two components (trigenic and quadrigenic) are excluded as well as higher order epistatic effects, the additive, digenic, additive by additive, and additive by digenic components can be estimated. For these estimations to be representative of the true situation, some restrictions have to be met: (1) parental clones are a random sample from a population in equilibrium, (2) autotetraploid meiosis is regular with non-preferential pairing and chromosomal segregation, (3) equilibrium of linkage phases, (4) additive environmental and genotypic effects, and (5) removal of environment by genotype interactions by space and time.

It is worth noting at this stage that the expected coefficients of additive variance in both the diploid and the autotetraploid inheritance are identical. Thus for GCA estimation the tetraploid material can be handled exactly as a diploid in the analysis.

2.3.4 Combining abilities

The use of diallel crosses for the analysis of genetic variance components in forages was mostly done in autotetraploid alfalfa.

General and specific combining ability in a six-parent diallel cross in alfalfa was studied by Kehr (1960). Diallel analysis was done according to Griffing (1956a). The parental clones were selected on the basis of high GCA for forage yield. The estimated variance components for GCA were slightly larger than for SCA for fall growth habit and rate of recovery. For forage yield and spring growth habit, SCA variance was much larger than that of GCA.

Knowles (1950) studied combining ability relationships in bromegrass and crested wheatgrass by using diallel crosses in which reciprocals were maintained separately. Specific combining ability was of relatively greater importance than GCA among selected bromegrass clones. Among inbred strains of crested wheatgrass previously unselected for combining ability, GCA was of greater importance than SCA.

In a diallel analysis of 16 unrelated clones of alfalfa, Downey (1961) found GCA effects for forage yield to be much greater than SCA effects. Carnahan _et al_. (1960) reported on seedling vigor and fall growth habit of 91 single crosses from 14 alfalfa clones. These clones had not been previously selected for vigor and fall growth habit. They found that estimated GCA components were far larger than SCA components for both characters studied.

Wilcox and Wilsie (1964) analyzed a 9-clonal diallel cross in alfalfa using Griffing's method 3, model I. In this study GCA variance appeared to be of greater importance than SCA variance for fall growth habit, forage yield and spring vigor. Nevertheless, some single cross combinations were noted for their high SCA. These were thought of as being useful for the formation of hybrids.

A study of five clones of alfalfa in a diallel cross analysis was done by Theurer and Elling (1963a, 1963b, 1964). The characters studied were bacterial wilt resistance, forage yield, and winterhardiness. GCA for all characters was found to be more important than SCA. An interaction effect between GCA and years was noted for forage yield.

Dudley et al. (1963) evaluated some 74 clones of Cherokee alfalfa in separate partial diallel mating designs. The estimated components of genetic variance were interpreted on the basis of autotetraploid tetrasomic inheritance with chromosomal segregation. The procedures outlined by Levings and Dudley (1963) were used in the analysis. Estimates of GCA variance and parent-offspring covariance were found to be significant for forage yield, recovery after cutting, spring growth, and fall growth. Estimates of SCA variance, on the other hand, were not significant for any of the characters. The total genetic variance was significantly larger than either twice the estimate of parent-offspring covariance (σ_{po}) or four times the GCA variance (σ_g^2). This was an indication that either trigenic, quadrigenic, or epistatic genetic variance was of importance, since in the absence of such variances the total genetic variance would be equal to $2\ \sigma_{po} = 4\ \sigma_g^2$.

To the author's knowledge the only estimates of GCA and SCA variances in birdsfoot trefoil through the use of diallel crosses were given by Miller (1968). His study was made on 6 single crosses from a 4-clonal diallel. The analysis of variance was done according to Griffing (1956a) method 4 model I. He found significant variances for GCA for flowering date and forage yield. Specific combining ability variances were significant for vigor score and disease score, as well as flowering date and forage yield. The parental clones were selected on the basis of general vigor. From partial diallels made between Empire and Russian clones of birdsfoot trefoil, Conje and Carlson (1973b) found GCA variances to be considerably larger than SCA variances for all characters studied.

CHAPTER III

MATERIALS AND METHODS

3.1 Seed size, depth of seeding, seedling vigor selections and evaluation of selection technique

3.1.1 Genetic material

The genetic material used in these experiments consisted of bulk seed from two open-pollination nurseries of two birdsfoot trefoil cultivars, Leo and Mirabel. Both cultivars were developed at Macdonald College (Bubar 1964; Lawson 1976).[1] Leo was developed by mass selection out of Morshansk 528. It has good winterhardiness and is semi-erect in growth habit. Mirabel was developed through four cycles of mass selection from an introduction from Leningrad, U.S.S.R. Its winterhardiness is equivalent to Leo and it is slightly more erect than Leo in growth habit. The onset of fall dormancy by Mirabel is slightly delayed compared with Leo. Mirabel and Leo are equal in flowering date, seed production, and forage production.

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[1] Personal communication with cultivar originators.

Different seed sizes (large, medium and small) were arrived at by passing bulk seed through different mesh screens. Round, nonshrivelled seed was recovered after passing the different seed size classes on a vibratory separator. Thus,

small size seed (S) was held by a 1/22" or 1.154 mm mesh
medium size seed (M) was held by a 1/20" or 1.27 mm mesh and
large size seed (L) was held by a 1/18" or 1.41 mm mesh.

No seed was held by a 1/15" or 1.69 mm mesh.

3.1.2 Experimental methods

The experiments were conducted in controlled-environment growth chambers, at 21°C day and 16°C night temperatures and a 16-hour photoperiod. Light intensity from florescent and incandescent canopies was in the neighborhood of 2500 ft candles. Cylindrical opaque-white (5 cm x 15 cm) plastic containers filled with a constant amount of an inert medium (turface), were used for germination and growing.

Precise depth of seeding was achieved by placing the seeds on top of the medium and then covering them with an amount of turface premeasured to attain a certain height over the seeds, as required.

Preliminary emergence tests were conducted in the manner described above. Seeds were placed at 3, 2, and 1 cm depths. From the 3 cm depth very few seeds emerged (medium size 2%, large 4%, and small 0%). From the 1 cm depth, emergence was high and uniform for all seed-size classes. Hence, the 1 cm and 3 cm depths were dropped from further experimentation. Further emergence tests from 2, 2.25, 2.5, and 2.75 cm depths were conducted. The percentage emergence for different seed-size classes is shown in Table 1. The percentage from the 2.75 cm depth was too small and undependable for the production of experimental units having equal number of seedlings, thus it was dropped from any further studies. The three seed-size classes of the two cultivars Leo and Mirabel were then evaluated for different seedling characteristics when seeded at 2, 2.25, and 2.5 cm depths. In the growth cabinet experiments, 32 days after emergence, seedlings developed two axillary shoots, and floral buds were being initiated. It was decided to use this stage of growth to indicate the full expression of seedling vigor.[1]

TABLE 1. Average per cent emergence of three seed-size classes of two cultivars of birdsfoot trefoil from 4 depths of seeding in a turface medium

Size	Mirabel				Leo			
	2.00 cm	2.25 cm	2.50 cm	2.75 cm	2.00 cm	2.25 cm	2.50 cm	2.75 cm
Large	95%	68%	32%	10%	89%	59%	28%	8%
Medium	82	62	23	6	79	54	21	5
Small	69	48	16	3	73	43	14	2

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[1] See Appendix 2, page 174.

3.1.3 Experimental design, analysis, and data collection

The experiments were designed in a split-split plot fashion. Each growth chamber represented one replication. The main plot had the depth of seeding, the sub-plot the cultivar, and the sub-sub-plot the seed size class. The sub-sub-plot consisted of nine 5 x 15 cm plastic containers initially seeded with 5 scarified seeds and thinned after emergence to one seedling per pot. A 20-20-20 complete nutrient solution was provided twice weekly throughout the period between emergence and last cut. The seeds placed at 2.5 cm depth took, on the average, two days more to emerge compared with those placed at 2 cm; accordingly, their ultimate seedling measurement was done two days later.

Thirty-two days after emergence the following characteristics were studied:

Branching : number of secondary branches on the main stem.

Thickness : visual score of thickness of the main stem; 1 = very thin and 10 = very thick.

Erectness : visual score of standing ability of the seedlings between 1 and 10; 5 = an angle of around 60° from the horizontal.

Number of stems : number of all stems taller than 3 cm, 32 days after emergence.

Plant height : height in cm of main stem.

Seedling vigor : shoot dry weight in mg, 32 days after emergence.

Aftermath : dry weight (mg) of the aftermath growth 22 days after first cut.

Root weight : dry weight (mg) of the cleaned roots, 54 days after emergence.

3.1.4 Plant selections and evaluation

One thousand seeds from each seed-size class of the two cultivars were seeded in the previous fashion at 2 and 2.5 cm depths in 200 pots at 5 seeds per pot. At emergence these were thinned leaving 1 seedling with the largest cotyledonary area per pot. Shoot dry weights 32 days after emergence were taken and the seedlings having the highest shoot dry matter from each cultivar-size-depth combination were selected.

Six Mirabel seedlings coming from large seeds placed at 2 cm depth and 5 others placed at 2.5 cm depth, herein referred to as MIL-1 to 6-2 and MIL-1 to 6-2.5, were kept. Three Leo seedlings from large seed at 2 cm and 5 others at 2.5 cm depth, LEL-1 to 3-2 and LEL-1 to 5-2.5, were also kept. Other seedlings coming from small seeds of the two cultivars were also kept for further studies.

Several single crosses were made in each cultivar-size-depth combination destined for field evaluation. Polycross seed from each of the four combinations, Mirabel-large-2 cm, Mirabel-large-2.5 cm, Leo-large-2 cm, and Leo-large-2.5 cm, was obtained from mother plants, after each combination was put in isolation for open pollination.

This maternal polycross seed was evaluated by the same procedures followed earlier, but only for seedling vigor (dry shoot weight).

A randomized complete block design with 4 replications (cabinets) was used for this experiment. Large bulk seed of the two cultivars was used as check. Statistical analysis and procedure for all growth chamber experiments were done according to Cochrane and Cox (1957). Simple phenotypic correlation coefficients were computed according to the formula

$$r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \ \Sigma y^2}} \qquad \text{(Steel and Torrie 1960)}$$

3.2 Diallel crosses (F_1 and F_2) and selected materials

3.2.1 F_1 hybrid studies

3.2.1.1 Genetic material

Sixty plants, 30 from Mirabel and 30 from Leo open-pollinated nurseries of 4000 and 1600 plants respectively, were selected in the fall of 1974 for fall vigor and upright growth habit. Clonal propagation of these plants was done and finally 15 clones were kept (8 Mirabel and 7 Leo) to initiate diallel crosses.

One hundred and five F_1 diallel crosses were made among the 15 parental clones excluding reciprocals. No emasculation of female parents was practised because of self-incompatability.

Thirty-three single F_1 hybrids were made earlier among selected materials from seedling vigor evaluation experiments conducted during the fall of 1973 and winter of 1974 and described in Chapter II, section 1.4. Bulk seed (large, small, and non-separated) from the two cultivars was also included in the following investigation to act as check entries. Seedlings from the resulting 144 entries were started in 2" clay pots five weeks before transplanting to the field in June of 1974.

3.2.1.2 Experimental site

The site of the field investigations was at the Emile A. Lods Agronomy Research Centre at Macdonald College. The soil (a Ste. Rosalie clay) was furnished with 200 kg/ha of 5-20-20 commercial fertilizer in the spring of the establishment year. A fall application of 250 kg/ha of 0-15-30 is common practice. No artificial inoculation of seed was done, as the soil is believed to have adequate natural inoculum to start infections.

3.2.1.3 Experimental design, statistical procedures, and data collection

With 144 genotypes, a 12 x 12 triple lattice design was adopted. The experimental plots consisted of six plants spaced 80 cm and rows spaced one meter apart. Individual plants were clipped in late September of 1974 to within 4-5 cm above the crown. Fresh weight of each plant was recorded. Per cent dry matter was recorded using small samples from each plant of the plot. All weights were later converted to dry weights based on plot per cent dry matter.

Several characteristics were measured in the establishment year:

Growth habit : Visual score of 1-5; 1 indicating prostrate, 3 ascending, and 5 erect growth habits.

Podsetting : Visual score of ratio of formed pods to flowers still in bloom. A score of 1 indicates mostly flowers and no pods and that of 10 indicates all pods.

Vigor rating : Visual score of 1-5 describing plant size (volume and density). A score of 5 was given to large dense plants, and 1 to small lax ones.

Seedling vigor : Dry weight of total vegetation produced by the plant during the establishment phase between early June and late September.

Winterhardiness : Number of surviving plants per plot (measured in second growing season).

Spring growth : Dry weight per plant of the first cut in the second growing season.

Forage yield : Calculated as the product of spring growth and the number of surviving plants per plot.

Total yield : Forage yield + plot seedling vigor, expressed in grams of dry matter.

3.2.1.4 Diallel analysis

Ideally the diallel analysis should have been carried out as outlined by Kempthorne (1957), Levings and Dudley (1963) or Desseureaux (1959), all of which deal with diallel analysis for autotetraploids with tetrasomic inheritance. Levings and Dudley suggested the use of partial diallels in conjunction with parent-offspring regression and an analysis of variance among parental clones. Having these parameters, the general and specific combining ability variances would be estimated according to the formulae

$$\sigma_T^2 = 48\ \sigma_g^2 + 18\ \sigma_s^2 - 23\ \sigma_{po} - 0.5\ \sigma_c^2$$

$$\sigma_F^2 = 36\ \sigma_g^2 - 18\ \sigma_s^2 + 15\ \sigma_{po} + 1.5\ \sigma_c^2$$

where σ_g^2 = GCA variance,
σ_s^2 = SCA variance
σ_{po} = parent-offspring covariance, and
σ_c^2 = variance of parental clones.
σ_T^2 = trigenic variance and
σ_F^2 = quadrigenic variance.

An insurmountable difficulty is caused by the virtual impossibility of making vegetative cuttings or propagules that grow and develop at the same rate as progeny derived from seed. Hanson (1959) found large coefficients of variation among clonal cuttings and seedling progenies in the year of establishment. This variation due to method of propagation was greatly narrowed down after the year of establishment. Kehr and Gardner (1960) discussed the presence of an interaction between method of propagation and genotype. It was, however, very difficult to account for such interaction.

In alfalfa, Dudley et al. (1963) found that the importance of digenic, trigenic, and quadrigenic variance and their interactions was very small compared with the additive genetic variance for the variables, forage yield recovery and spring growth. This undermines the extra effort spent in trying to estimate these components.

In birdsfoot trefoil, only one report has appeared on estimation of GCA and SCA - that of Miller (1968). The diallel analysis used was that outlined by Griffing (1956b). On the other hand, whether birdsfoot trefoil, Lotus corniculatus L., is an autotetraploid or an allotetraploid has not been resolved.

Accounting for all the above considerations, the diallel analysis was done as if birdsfoot trefoil was a diploid, using Griffing's (1956b) method 4 model I, where only the F_1 crosses are evaluated, and conclusions would apply only to that particular set of 15 selected clones of the two cultivars Leo and Mirabel. The computer program of Littlewood et al. (1964) was utilized for the diallel analysis.

The model for the combining ability analysis is represented by

$$X_{ij} = \mu + g_i + g_j + S_{ij} + \frac{1}{bc} \sum_k \sum_l e_{ijkl}$$

where μ is the population mean, g_i and g_j are the GCA effects, S_{ij} is the SCA effect such that $S_{ij} = S_{ji}$, and e_{ijkl} is the error effect peculiar to the ijklth observation. Several restrictions are made for this model: e_{ijkl} are normally and independently distributed with mean 0 and variance σ_e^2, and $\sum_i g_i = 0$, and $\sum_{i \neq j} S_{ij} = 0$ (for each j) are imposed on combining ability effects. The expected mean squares of the analysis are shown in Table 2.

Three diallel analyses were conducted on the 105 F_1 crosses:

1 - Considering all the 15 clones (105 crosses)
2 - Considering only the 8 Mirabel clones (28 crosses)
3 - Considering only the 7 Leo clones (21 crosses)

The objective behind the breakdown is the comparison of GCA and SCA components as they occur within and between two germplasm sources.

As there was no advantage of the lattice analysis over the randomized complete block analysis, the uncorrected RCB variances were used to test the differences between the F_1 hybrids. The results of spring vigor are extracted from two replications, as one replication was lost during the second growing season due to severe weed infestation.

TABLE 2. Analysis of variance for Griffing's method 4, model I, giving expectations of mean squares

Source	D.F.	Sum of squares	MS	Expectation of mean squares
GCA	p-1	S_g	M_g	$\sigma^2 + (p-2)(\frac{1}{p-1}) \sum_i gi^2$
SCA	p(p-3)/2	S_s	M_s	$\sigma^2 + (\frac{2}{p(p-3)}) \sum_{i<j} \sum S_{ij}{}^2$
Error	m	S_e	M_e'	σ^2

$$S_g = \frac{1}{p-2} \sum_i X_{i\cdot}^2 - \frac{4}{p(p-2)} X_{\cdot\cdot}{}^2$$

$$S_s = \sum_{i<j} \sum X_{ij} - \frac{1}{p-2} \sum X_{i\cdot}^2 + \frac{2}{(p-1)(p-2)} X_{\cdot\cdot}{}^2$$

GCA effects are tested by $F_{[(p-1),m]} = M_g/M_e'$

SCA effects are tested by $F_{[p(p-3)/2,m]} = M_s/M_e'$

3.2.2 Maternal F_2 studies

3.2.2.1 Experimental material

Three separate isolated open-pollinated nurseries corresponding to the three distinct groups (Leo x Leo F_1), (Mirabel x Mirabel F_1), and (Mirabel x Leo F_1), were established in the summer of 1974 to obtain maternal F_2 seed. A fourth nursery having the original 15 clones was also established to get maternal seed of the original clones. The requirement for isolation forced the use of an unsatisfactory nursery location which resulted in failure to produce (Leo x Leo) maternal seed.

3.2.2.2 F_2 greenhouse winter studies

Fifty-six Mirabel x Leo F_2's, 28 Mirabel x Mirabel F_2's, 15 Mirabel and Leo mothers, and one Mirabel large selection, making 100 entries, were seeded in greenhouse beds for seedling vigor evaluation and selection of superior material. Beds were filled with a 2:1:1 sterilized mixture of soil to a 25-cm depth. Beds were made up of wood and were elevated to about 60 cm above the ground level. No commercial fertilizer was mixed with the soil mix.

Microplot rows having 13 seedlings per entry, spaced 5 cm apart, were used. Microplots were spaced 12 cm apart. Data were collected on the inner 9 seedlings per plot. The 100 entries were established in a RCBD with two replications.

Seedlings grew in soil beds at temperatures ranging between 21°C day and 16°C night, and received natural sunlight supplemented with incadescent light for a 16-hour photoperiod. A 20-20-20 soluble fertilizer was supplied with sprinkler irrigation once every week.

Four characteristics were studied:

Stems per plant = number of stems taller than 2 cm.

Erectness = visual score of 1-5.

Vigor index = visual score of 1-5. Scores of 1 indicated prostrate, non vigorous, and 5 erect and vigorous. The three characters above were measured 36 days after planting.

Seedling vigor = Fresh weight in grams for shoots per seedling cut 2 cm above the crown, 66 days after planting.

Aftermath yield = Dry weight in grams per plot (9 seedlings) 48 days after first cut.

Statistical analyses and correlations were done according to Cochrane and Cox (1957) and Steel and Torrie (1960).

3.2.2.3 F_2 field studies

The same 100 entries mentioned above were started in Jiffy-7 pots (compact cylindrical sphagnum moss pellets) in the greenhouse and transplanted to the field in June 1975.

A 10 x 10 triple lattice design was used. A plot consisted of six plants spaced 1 m apart. The rows were also spaced 1 m apart.

In 1975 (the year of establishment), only vigor ratings were taken on the material. Nevertheless, plants were cut back in September 1975. In early June of 1976 winterhardiness was measured by the number of living plants per plot. Plants were harvested in September and dry weight per plant was taken as a measure of forage productivity. The following characteristics were measured:

Growth habit = visual rating of 1-10.
Pod setting = visual rating of 1-10.
Vigor rating = visual rating of 1-5.

This agrees with the scoring system used for F_1 hybrids (Section 3.2.1.3).

Spring growth, winterhardiness, and forage yield were also evaluated as in Section 3.2.1.3, described for the F_1 hybrids.

3.3 <u>F_2 plant and line selections</u>

3.3.1 Experimental material

Out of the 100 entries studied in the greenhouse during the winter of 1975, 29 maternal F_2 lines were identified whose greenhouse progeny performance, both in seedling vigor and aftermath growth, was consistently superior to the overall mean. Since they were identified due to the superior performance of their progeny, we can say that they were genotypically selected and will accordingly be referred to as the genotypic lines group. Furthermore, the 4 most superior plants in seedling vigor and aftermath growth within each of the 29 genotypic lines group were selected. They will be referred to as the geno-phenotypic group (Twamley 1972).

Maternal F_2 seedlings from the 29-line genotypic group, propagated in Jiffy pots, were transferred to an isolated polycross nursery in the field for the production of maternal F_3 seed. Similarly, the maternal F_2 plants belonging to the geno-phenotypic group were transferred into a polycross isolated nursery.

No seed was collected from the genotypic lines nursery due to contamination by pollen from unsuspected birdsfoot trefoil plants near the nursery site. Maternal F_3 seed, however, was recovered from the geno-phenotypic group on each plant. Seed from each plant corresponding to one line was bulked. The end result was 29 geno-phenotypically selected maternal F_3 families.

3.3.2 Evaluation of selected families

The 29 geno-phenotypically selected F_3 families were evaluated together with F_1 polycross seed of the 15 Mirabel and Leo parental clones in a microplot technique in controlled environment growth cabinets.

Microplots were 37 cm rows spaced 5 cm apart. Each plot contained 14 seedlings spaced 2.5 cm apart. The 44 entries were randomized in a complete block design in two replications consisting of two growth chambers running at 20°C day and 15°C night temperature with 16 hours photoperiod.

Seedlings were harvested 36 days after planting. Seedling vigor was measured as the plot dry matter in grams.

The possibility exists that genotypic differences between the 44 entries might be confounded with the environmental effect of the microplot. To test this possibility, genotypically and phenotypically different birdsfoot trefoil cultivars were tested in the same microplot technique under the same conditions. The results (Appendix 1, Tables 1, 2, 3) indicated the nonexistence of such confounding.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Seedling vigor studies

4.1.1 The effect of size, cultivar, and depth of seeding on nine seedling characters

An analysis of variance was conducted on the nine seedling characters measured in growth cabinets from a replicated seedling vigor evaluation experiment. Variances for these characters pertaining to depth, cultivar, and seed size differences, as well as the appropriate error variances are presented in Table 3.

The depth of seeding did not seem to cause any significant variation in branching, number of stems, stem thickness and erectness, seedling vigor, aftermath growth, or total yield.

The two cultivars, Mirabel and Leo, were significantly different in stem thickness and seedling height, but not so in all other characters. Mirabel always produced seedlings whose main stems were thicker than Leo, whereas Leo produced significantly

TABLE 3. Pertinent portion of ANOVA for 9 characters of two varieties of birdsfoot trefoil planted at 3 depths

Source of variation	d.f.	Variances for				
		Branching	Thickness	Erectness	No.of stems	Plant height (cm)
Depth (D)	2	9.91	0.95	13.70	4.66	587.36*
Cultivar (C)	1	27.21	26.27**	0.91	2.67	143.41*
Depth x cultivar	2	1.05	1.48	4.19	0.72	8.72
Size (S)	2	64.10**	9.91**	0.62	1.45	927.07**
Depth x size	4	7.93	0.66	1.04	0.08	1.35
Cultivar x size	2	14.56	2.25	0.67	2.45	1.20
D x C x S	4	5.93	0.95	5.70	0.44	11.46
C.V.		47%	25%	33%	28%	22%

Source of variation	d.f.	Variances for			
		Seedling vigor (mg)	Aftermath cut (mg)	Root weight (mg)	Total yield (mg)
Depth (D)	2	141424	378159	353248**	949961
Cultivar (C)	1	790	2683	44013	560
Depth x Cultivar	2	21508	563658**	66485*	761646*
Size (S)	2	1733458**	707393**	203546**	4636848**
Depth x size	4	28945	18323	14123	90151
Cultivar x Size	2	27983	46729	12013	130796
D x C x S	4	29017	39713	7903	114743
C.V.		27%	39%	43%	33%

*, ** Statistically significant at the .05 and .01 levels of probability respectively.

taller seedlings than Mirabel by an average of about 2 cm. The differences in plant height and stem thickness were not, however, translated into differences in erectness, as the two cultivars were not significantly different for this character. Different seed sizes, however, produced significantly different stem thicknesses equally within the two cultivars. Large seeds tended to produce thicker stems.

The different cultivar x depth combinations, pooled over all seed sizes, were significantly different for aftermath growth and root weight but not for all other characters. Leo at 2.5 cm had higher values for aftermath growth, root weight, and total yield, followed by Mirabel at 2.5, Mirabel at 2.25, and Leo at 2.25. Depth x size, cultivar x size, and depth x cultivar x size interactions were not significant for any of the measured characters.

For all characters studied except number of stems per seedling, highly significant differences were found among the three seed size classes. There was a direct relationship between seed size and other characters. Large seeds, for example, imparted a higher number of branches, thicker stems, more erect and taller seedlings, more shoot dry matter in the first cut (vigor) and second cut (aftermath growth), and more developed heavier root systems. This was true irrespective of cultivar or depth of seeding differences.

This kind of direct relationship between seed size and seedling characters in this study agrees with other findings for other characters as well, like ability to emerge from deep planting and early vegetative characteristics (Black 1959; Twamley 1967; Stickler and Wassom 1963).

Such an advantage of large seeds of birdsfoot trefoil over small seeds has been attributed to large size cotyledons or more reserve material in them that could help produce longer hypocotyls and support their growth for a longer period of time (Black 1959; Derwyn _et al_. 1966). McDaniel (1969) found that heavier seeds had more mitochondrial protein and increased biochemical activity compared with small seeds.

The heterotrophic stage of seedling development was defined by Derwyn _et al_. (1966) as the stage from water imbibition until emergence and commencement of photosynthesis. Within this stage the amount and rate of hypocotyl elongation of birdsfoot trefoil seeds was found by Cooper and Qualls (1968) to be directly correlated to seedling vigor as measured by net assimilation and relative growth rates.

The transitional stage of seedling development starts when all cotyledonary reserves are exhausted and the seedling is entirely dependent on its photosynthesis. The earlier photosynthesis starts the more advantage will the seedling have in accumulating dry matter and producing heavier and taller shoots.

It can now be stated that the ability of a seedling to emerge from deep planting is directly associated with its inherent (seed) potential to produce a long hypocotyl and produce it fast enough to emerge the cotyledons and place them in a photosynthetic status for earlier commencement of vegetative production and dry matter accumulation.

The rate of seedling emergence of large and small size seeds of birdsfoot trefoil from depths between one and two centimeters was found to be very uniform, although large seeds emerged faster than small ones (Stickler and Wassom 1963; Hanson and Tayman 1961). Our preliminary studies on emergence have also shown the same results (Table 1). From deep seeding (>2 cm), however, large, medium, and small seeds did not emerge at the same rates and, moreover, not all seeds within any seed size class emerged. This, we think, is proof that deep seeding produces some selection pressure against seeds with inferior potential in producing long hypocotyls (Table 1).

Frequency distribution of seedling vigor for all cultivar-size-depth combinations is shown in Table 4. Large size seeds of the two cultivars seeded at 2.5 cm depth produced a majority of seedlings having shoots heavier than 604 mg and practically no seedlings producing shoots whose weights were less than 350 mg. Medium and small seeds, however, emerging from different depths, produced seedlings with seedling vigor ranging between 112 and 800 mg, with the majority being less than 604 mg.

When such a distribution is pooled for depth x size, irrespective of cultivar, and the seedling vigor means are plotted as frequency

TABLE 4. Frequency distribution of seedling vigor (dry shoot weight/seedling) of two cultivars of birdsfoot trefoil at 3 seeding depths

	51-173 112	174-296 235	297-419 358	420-542 481	543-665 604	666-788 727	789-911 850	913-1034 973	Mean
LeL 2.5[1]			1	9	10	3	1	3	610
MiL 2.5			2	8	5	4	3	5	628
LeM 2.5		3	5	6	6	6	1		526
MiM 2.5	1	3	6	8	4	3	2		486
LeS 2.5		4	10	5	7	1			444
MiS 2.5	4	8	4	5	4	2			385
LeL 2.25			3	8	9	3	3	1	588
MiL 2.25		3	5	5	5	6	3		521
LeM 2.25	1	2	7	7	8	1	1		476
MiM 2.25		2	5	8	10	2			503
LeS 2.25	4	5	7	7	3	1			332
MiS 2.25		9	10	5	2	1			366
LeL 2.0	1	2	3	7	8	5	1		535
MiL 2.0			5	5	11	4	2		576
LeM 2.0		3	12	6	5	1			431
MiM 2.0		1	6	13	5	2			485
LeS 2.0	2	2	13	8	2				393
MiS 2.0	2	7	10	7	1				366

[1] Le = Leo, Mi = Mirabel, L = large size, M = medium size, S = small size; 2.0, 2.25 and 2.5 are depths of seeding.

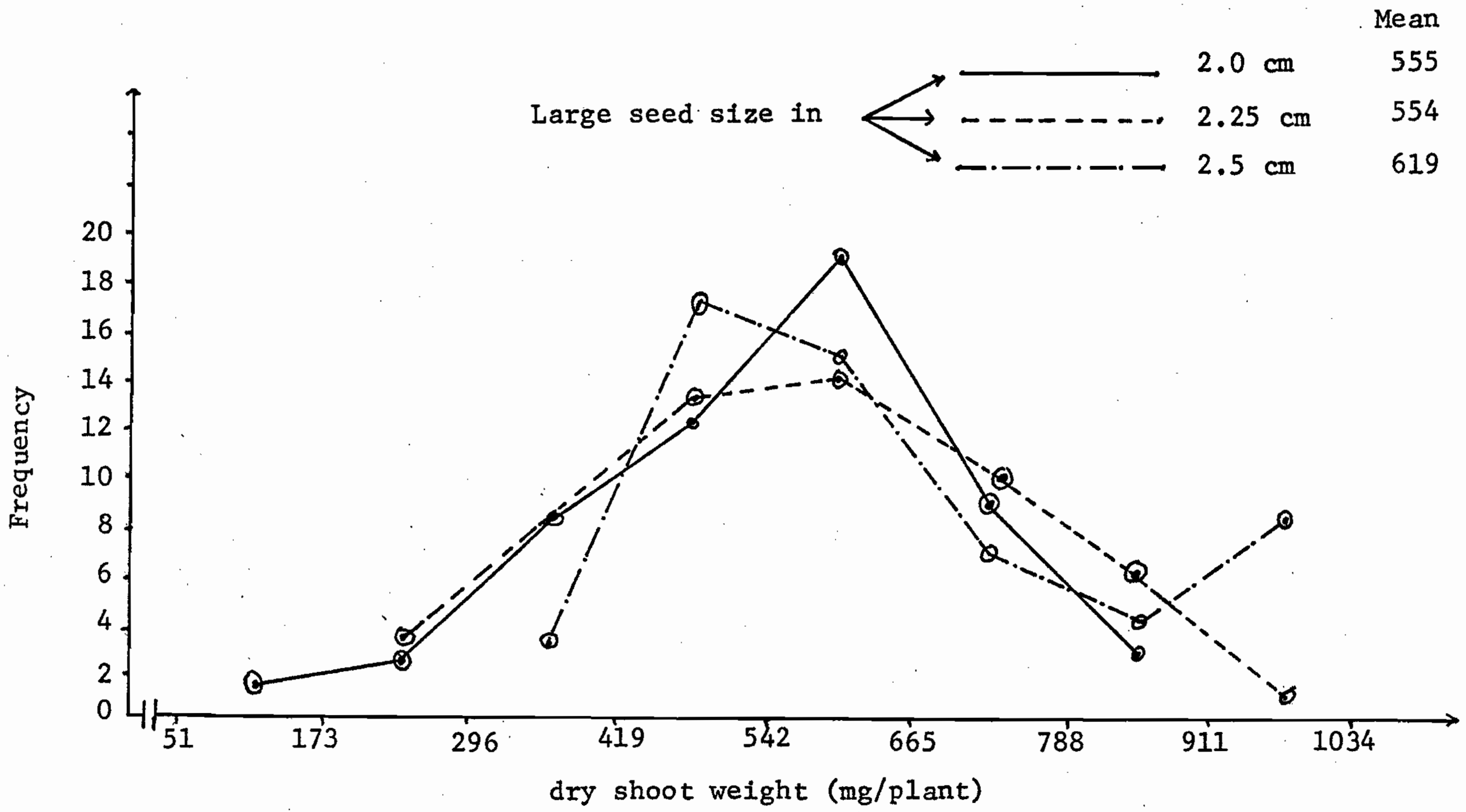

Figure 1 (a). Frequency distribution of dry shoot weights from large seed size class of pooled Mirabel and Leo birdsfoot trefoil, as emerged from three depths of seeding.

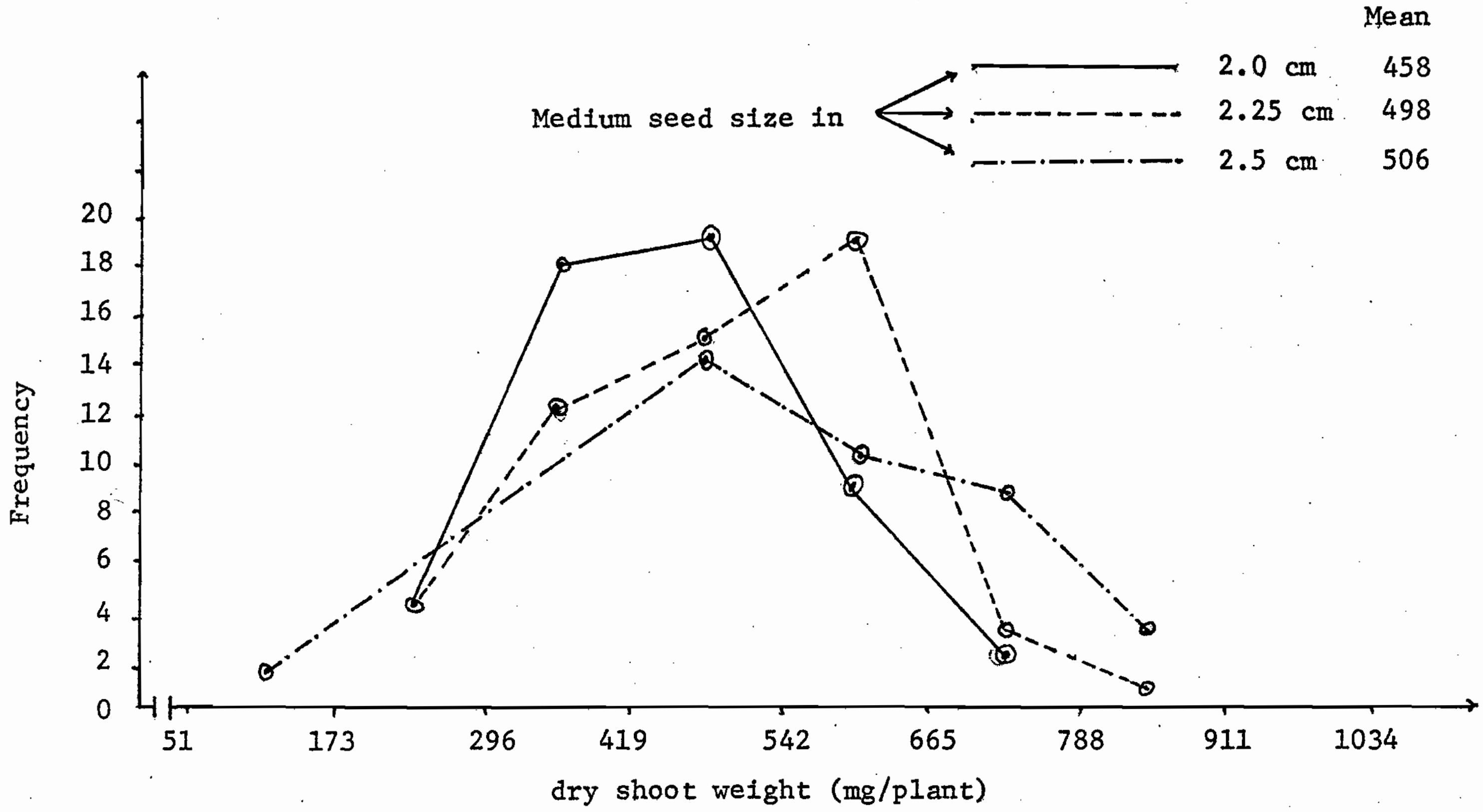

Figure 1 (b). Frequency distribution of dry shoot weights from medium seed size class of pooled Mirabel and Leo birdsfoot trefoil, as emerged from three depths of seeding.

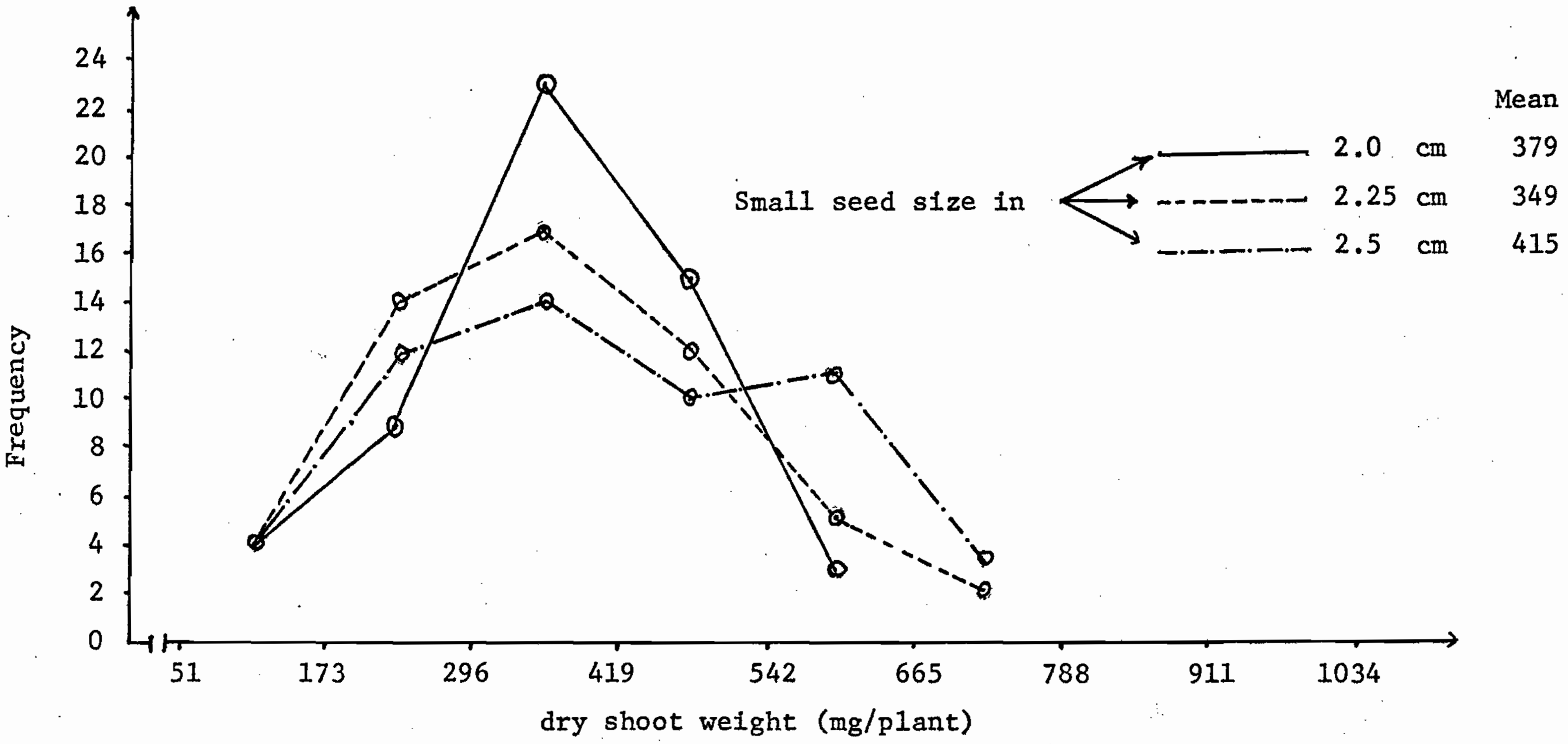

Figure 1 (c). Frequency distribution of dry shoot weights from small seed size class of pooled Mirabel and Leo birdsfoot trefoil, as emerged from three depths of seeding.

polygons (Figure 1, a, b, c) one would learn more about the effect of depth of seeding in minimizing the emergence of inferior seedlings. The curves, in general, look much like normal distributions with different degrees of skewness to the right or left depending on the size x depth combination. One thing is clear, however; regardless of which seed size curve we examine, the seedlings coming from the 2.5 cm depth are more frequently heavier than those coming from the 2.25 or 2.0 cm depths.

Since large seeds are superior to either medium or small seeds in seedling vigor, and the indication that a large proportion of their seedlings coming from 2.5 cm depths have shoot weights ranging between 911 and 1034 mg, then a certain kind of pressure must be operative against large seeds with inferior vigor potential. This pressure must be related to the rate and amount of hypocotyl growth.

If we examine the percentage emergence of the large seed size class (Table 1) from different depths, it becomes obvious that deep seeding is exerting a certain pressure against seeds with inferior vigor.

If we assume that X, the percentage of seed of similar seedling vigor potential within any seed size class is the same in every pot seeded, and knowing that 5 seeds were placed in every pot, and assuming 100% seed viability, the following calculations can be made:

Depth	% Emergence	Selection pressure	Number of potential emergers/pot
2.0 cm	95	5%	4.75
2.25 cm	70-80	20-30%	3.5-4
2.5 cm	30-40	60-70%	1.5-2

Twenty-seven pots were studied in each depth, each having one seedling (others were thinned out). The chances that the kept seedling in each pot is a vigorous seedling are

$$27X \times \frac{1}{4.75} \times \frac{1}{5} = (\frac{27X}{23.75} = 1.14X) \text{ for the 2.0 cm depth}$$

$$27X \times \frac{1}{3.75} \times \frac{1}{5} = (\frac{27X}{18.75} = 1.45X) \text{ for the 2.25 cm depth, and}$$

$$27X \times \frac{1}{1.75} \times \frac{1}{5} = (\frac{27X}{8.75} = 3.1X) \text{ for the 2.5 cm depth.}$$

The observed ratios of the frequency of the seedlings with the highest seedling vigor (789-1034 mg) are 12 from 2.5, 7 from 2.25, and 3 from 2.0 cm depths (Table 4). Although the observed ratios are not equal to the expected ones we must point out that they are highly proportional. The discrepancy in the ratios could be explained on the occurrence of different frequencies of potentially vigorous seedlings among pots as well as the actual per cent emergence among pots within the same size-depth combination.

If the same sort of calculations are made for the medium size and small size classes, the same trend is observed: more seedlings of higher seedling vigor are detected from the 2.5 cm depth compared with the 2.25 or 2.0 cm depths (Figure 1, b, c). The occurrence of the same trend for all seed size classes goes only to establish with more confidence that the depth of seeding does indeed have selective pressure for vigorous seedlings and against inferior ones, and thus could be used as a selection technique for seedling vigor in birdsfoot trefoil.

4.1.2 Evaluation of selected materials and their progeny from depth of seeding selection technique

Figure 1,a shows the frequency polygons of seedling vigor for seedlings coming from large seed planted at 3 depths. Of particular interest is the curve representing those seedlings coming from the 2.5 cm depth since they have a mean seedling vigor (619 mg) significantly higher than the other two depths. The hypothesis to be tested here is whether the selection of the seedlings with highest seedling vigor would result in any genetic advance in the progeny, and whether the 2.5 cm depth has any advantages in identifying superior genotypes within the large seed size class as opposed to the other two depths.

The same seedlings studied here could not be used since they were destroyed when root weights were measured. Nevertheless the same technique was followed to obtain certain selections that would help test our hypothesis (Materials and Methods, Section 3.1.4).

Nineteen individual plant selections were made from large seed size seedlings emerging from 2.0 and 2.5 cm depths. The mean seedling vigor of such seedlings was at least one standard deviation superior to the corresponding overall mean.

Within the large seed size class seeds were very uniform, leading one to expect that they have the same size cotyledons and, hence, the same amount of food reserve to produce hypocotyls of similar lengths. The rate of hypocotyl formation and elongation for different seeds were noted to be variable at emergence. The selected seedlings were generally those that have emerged the first and were best looking 3-4 days after emergence. They are then at an advantage in starting their photosynthetic activity and production of dry matter in their shoots, a character later used as a measure of seedling vigor.

The four resulting categories of seedling selections, i.e., Mirabel large 2.0 cm, Mirabel large 2.5 cm, Leo large 2.0 cm, and Leo large 2.5 cm, were put in isolated polycross nurseries and maternal seed from each of the 19 seedlings was collected, scarified and tested for seedling vigor by the same procedures used before. Mean seedling vigor performance and analysis of variance are shown in Table 5.

Several individual crosses were made among the selected seedlings in each category and these were tested in the field, along with other single crosses made among selections from medium size seed and small size seed planted at two depths. The mean field performance of single crosses corresponding to the 19 large seed selections is presented in Table 6.

TABLE 5. Mean seedling vigor and analysis of variance of maternal polycross progeny from 19 seedling selections of Leo and Mirabel chosen by the depth of seeding technique

Entry	Rep.I	Rep.II	Rep.III	Rep.IV	Mean	Rank
MiL-1-2[a]	595[b]	533	495	613	559	
MiL-2-2	632	650	453	440	544	
MiL-3-2	702	517	410	526	538	
MiL-4-2	562	648	556	571	584	
MiL-5-2	650	610	539	526	581	
MiL-6-2	659	617	656	585	626	
Mean MiL-2	633	596	518	543	573	(4)
LeL-1-2	730	658	556	420	591	
LeL-2-2	786	746	578	437	637	
LeL-3-2	621	669	434	366	523	
Mean LeL-2	712	691	522	407	583	(3)
MiL-1-2.5	620	707	730	556	653	
MiL-2-2.5	798	589	691	486	641	
MiL-3-2.5	801	629	661	426	629	
MiL-4-2.5	720	645	692	437	649	
MiL-5-2.5	661	568	665	593	622	
Mean MiL-2.5	720	627	687	519	638	(1)
LeL-1-2.5	592	764	476	502	583	
LeL-2-2.5	904	811	541	492	687	
LeL-3-2.5	809	793	546	458	651	
LeL-4-2.5	723	711	494	398	581	
LeL-5-2.5	641	685	694	499	630	
Mean LeL-2.5	734	753	550	469	627	(2)
MiL (check)	760	531	572	420	571	
LeL (check)	685	532	671	455	586	

Analysis of variance

Source of variation	d.f.	SS	MS	F
Blocks	3	51.03	17.01	
Entries	20	15.30	0.7648	1.06 NS
Error	60	43.30	0.7216	

[a] Depth of seeding from which seedlings were selected.

[b] Mean fresh weight in mg/seedling from 9 observed seedlings.

TABLE 6. Mean field seedling vigor and spring growth for 19 F_1 single crosses made among 19 seedling selections chosen by depth of seeding technique

Cross	Depth[1] (cm)	Seedling vigor (gm)	Spring growth (gm)	Growth cabinet[2] parental average (mg)
LeL-1 x LeL-3	2	100.8	153.9	557
LeL-2 x LeL-3	2	134.0	127.9	580
MiL-1 x LeL-2	2	127.0	153.3	598
LeL-1 x MiL-2	2	104.1	156.7	568
MiL-1 x MiL-2	2	102.2	194.9	552
MiL-2 x MiL-3	2	123.3	163.8	541
MiL-3 x MiL-4	2	104.8	168.4	561
MiL-4 x MiL-5	2	119.4	170.9	583
MiL-5 x MiL-6	2	119.8	193.9	604
MiL-6 x MiL-1	2	100.2	186.3	593
LeL-1 x LeL-2	2.5	152.3*	202.4	635
LeL-1 x LeL-3	2.5	114.6	166.4	617
LeL-2 x LeL-3	2.5	138.9	170.3	669
LeL-3 x LeL-4	2.5	140.1	158.3	616
LeL-5 x LeL-1	2.5	110.1	174.0	607
LeL-1 x MiL-2	2.5	136.3	235.4*	612
MiL-5 x LeL-5	2.5	152.6	200.0	626
MiL-2 x MiL-3	2.5	114.0	180.0	635
MiL-4 x MiL-5	2.5	118.0	212.3	636
MiL		121.1	156.3	571
LeL		103.7	145.0	586

*Significantly different at the .05 level of probability as determined by an L.S.D. value.

[1] Depth of seeding from which the two parental seedlings were selected.

[2] Growth cabinet mean seedling vigor averaged from the progeny of the two parental seedlings involved in the cross.

Results from growth cabinet seedling evaluation (Table 5) indicate that the mean seedling vigor of progeny corresponding to the seedlings selected from the 2.5 cm depth was higher than those coming from the 2.0 cm depth as well as the two check non selected entries by an average of 50 mg. The mean performance of the two non selected check entries and the progeny of those selected from the 2.0 cm depth were almost equal. Progeny from MiL-2.5 selections averaged higher than progeny from LeL-2.5 selections in seedling vigor by 11 mg. On individual basis, however, maternal LeL-2-2.5 progeny produced the highest mean seedling vigor (687 mg).

The overall experiment, however, did not show any significant differences among the progeny from all selections as well as the two check entries. In other words, the average performance of maternal progeny corresponding to selections made from the 2.5 cm depth was not significantly higher than progeny corresponding to selections from 2.0 cm depth, nor from the two check entries. Hence, the transmittance of superior seedling vigor from parent to progeny was not done effectively. This can be an indication that the type of seedling vigor selected for by deep planting was not completely controlled genetically. The variance due to replications was rather larger than usually expected to happen in controlled environment growth cabinets.

The mean field performance for seedling vigor and spring growth for 19 F_1 single crosses and two check entries is presented in Table 6. Except for one single cross (LeL-1 x LeL-2 from 2.5 cm depth), differences between all single crosses were not significant for field seedling vigor. The parental progeny of this cross were also high in growth cabinet seedling vigor. The same non significant differences occurred for spring growth except for the single cross between the two cultivars (LeL-1 x MiL-2 from 2.5 cm depth) whose spring growth was significantly higher than all other single crosses.

The results of field seedling vigor evaluation correspond to the growth cabinet evaluation and further establish the unreliability of the type of seedling vigor selected for by the depth of seeding technique.

It can be argued that such results were not improbable considering the possibility of existing variation of environmental nature imparted to the seeds studied at the time of physiological seed filling, maturity, and storage. The fact that differential genetic vigor exists in this kind of open-pollinated seed from nurseries having high variability, should not, however be overlooked.

Carleton and Cooper (1972) found different seed sizes from different pods on the same plant, although they were not very much separated in time of maturity. The same authors have also noted a relation between seed size and seedling vigor, yet both among plant and within plant effects on seed size were present. Such effects might have helped in masking the potential differences that might have been found in our studies. The nature and origin of the studied seeds do not permit us to account for these effects.

If seed size within a clone is independent of the genotype of the seed, since seeds of different sizes have been found within a clone, then the striking within-plant effects on seedling vigor result from direct effects of environmentally induced changes in seed size. And if the major differences among clones is genetic, then genetic differences in seed size have less relationship to seedling vigor than do the within-plant environmental effects (Carleton and Cooper (1972).

If, then, one is to be more definite about the type of seedling vigor one is selecting for in a deep seeding technique, such effects should be taken into consideration. If the same size seed is taken from the same pod, and different pods from different clones are tested, the amount of genetic and environmentally induced variation governing seed size-seedling vigor association would be better established.

It should not be overlooked also that since variability in rate of emergence and seedling growth could be caused by physical barriers in the germination medium (large particles), we would suggest other types of more suitable media which would not pose such difficulties. We would recommend quartz, but then the critical depths of seeding that would impose some pressure on the seeds might have to be varied. With sand, our experience shows that 3 cm depths are quite adequate. The medium, we feel, should have adequate water holding capacity and not easily disturbed by watering or movement of containers. It should also be quite inert, so that nutrient levels could be controlled easily in different treatments.

The experimental material was seeded in four complete blocks. One growth cabinet represented one complete block. The environment in the four growth cabinets was standardized as far as possible. In spite of that, large variation was recorded among blocks (variance = 23.57**, Table 5). This leaves us with a note of caution concerning the use of controlled environment growth cabinets. We cannot tell easily the cause of such variation, but we can advise a periodic check on growth cabinets regarding all possible environmental adjustments.

Although the non existence of statistically significant differences among the progenies of seedling selections made from the 2.0 and 2.5 cm depths challenges the merit of the deep seeding technique for selection of seedling vigor, we have to admit the following: From the 2.5 cm depth seedling selections having superior seedling vigor produced progeny whose seedling vigor was indeed superior to the progeny of those selections chosen from the 2.0 cm depth, although not significantly so. The same thing was true for field seedling vigor and spring growth of the single crosses made among seedling selections chosen from the 2.5 cm depth as opposed to the 2.0 cm depth. This consistency of superiority of selections chosen by this technique cannot but leave us optimistic about its merits. If used judiciously for the screening of some birdsfoot trefoil clones with known genetic diversity, or even for the study of within-clone variation, it could be very useful, as it has a large capacity for screening appreciable amounts of seed within any one clone, and with rapidity enough to allow its use several times during the non growing season.

4.1.3 Correlations among characters

Simple phenotypic correlations were carried out among all characters studied to learn how these characters are associated, and to gain some insight into the association between seedling vigor and other characters as well as to find out which character best represents seedling vigor in the event of using a deep seeding technique in a breeding program.

Correlation coefficients (Table 7) were all positive except for that between erectness and plant height, and significantly negative between stem thickness and number of stems per seedling (r = -.15*). All other correlations were positive and significantly so in most cases at the 5% and 1% levels of probability.

Although correlations were significant, the magnitudes of the coefficients were below 0.5 for most correlations. Seedling vigor was positively and significantly correlated with all other characters of the seedling, although coefficients of such correlations were

TABLE 7. Simple phenotypic correlation coefficients among 9 seedling characters in birdsfoot trefoil[1]

Characteristic	Branching	Thickness	Erectness	Plant height	Number of stems	Aftermath	Root weight	Total weight
Seedling vigor	.40**	.41**	.24**	.59**	.12*	.52**	.45**	.85**
Branching		.29**	.15**	.37**	.40**	.06	.15	.25*
Thickness			.21**	.53*	-.15*	.15	.17**	.31*
Erectness				-.09	.001	.11	.22**	.19*
Plant height					.02	.26**	.26**	.47**
Number of stems						.14**	.13**	.15**
Aftermath							.67**	.89**
Root weight								.65**

*, **, significant at the .05 and .01 levels of probability, respectively.

[1] Coefficients of variations for all characters ranged between 22 and 47% (Table 3).

greater than 0.5 only with plant height (r = .59**), aftermath growth (r = .52**), and total weight (r = .85**). It appears that plant height could be the best representative of seedling vigor if no quantitative measurements are to be taken. Moreover, plant height is significantly positively correlated with stem thickness (r = .53**), branching (r = .37**), aftermath (r = .26**), root weight (r = .26**), and total weight (r = .47**). Root weight was positively and significantly correlated with all other characters. Its correlation with seedling vigor (r = .45**) and aftermath growth (r = .67**) were, however, the highest. This is an indirect indication that vigorous seedlings not only produce a high amount of dry matter in the shoots but also in the roots. The high correlation between root weight and aftermath growth indicates the importance of producing a vigorous and large root system for the establishment of a birdsfoot trefoil seedling. The high correlation between seedling vigor and total seedling weight (r = .85**) indicates the importance of the initial seedling growth in the production of well established and highly productive plants.

The generally low coefficients of correlation among most characters might have been caused by the existence of high coefficients of variability in the materials studied for most characters (C.V. = .22%-47%).

4.2 F_1 diallel crosses and combining ability

The knowledge of the breeding behaviour of crop characters is an important prerequisite for the effective manipulation of these characters in the process of upgrading of the crop. It is generally accepted that most of the characters of economic value in crops are quantitative in nature. The mode of inheritance of such characters is generally difficult to account for by methods described in simple Mendelian genetics. One of the most widely used systems of mating for the genetic study of quantitative characters is the diallel mating design. Another advantage of this design is its ability to identify parents which can be used in making single cross hybrids or synthetic cultivars depending on the nature of their combining abilities for any character under study. The diallel mating design and analysis have been advantageously used for acquiring such information. In this mating design several inbred lines are crossed among each other in all possible ways to produce n(n-1) single crosses, where n is the number of inbreds used. When reciprocal differences are of no interest, one-way crosses would be made resulting in $\frac{n(n-1)}{2}$ single crosses. When a diallel analysis is performed on the F_1 hybrids, the combining abilities of the parents can be determined. If the F_2 generation is analyzed in the same way the ability of the parents to transmit their characters to their progeny as well as the breeding behaviour of these characters in advanced generations would become known.

Many diallel mating designs and analyses have been reported (Chapter II). Although birdsfoot trefoil is a tetraploid, and diallel analysis for autotetraploids has been outlined (Kempthorne 1955, Dessereaux 1959, Levings and Dudley 1963), the nature of the material under study, the complexity of the autotetraploid analysis, the doubtful nature of tetraploidy in birdsfoot trefoil, and the comparative efficiency and ease of the diploid diallel analysis, made us choose Griffing's (1956a) method 4 model 1 to conduct the combining ability analysis of the material under study. This method and model consider only one set of F_1 hybrids with no consideration to the parents. The combining ability expected variances are presented in Table 2.

4.2.1 Mean performance and analysis of variance

Before carrying out a genetic analysis of the type mentioned above, the experimental material under study must be statistically analyzed to determine the existence of genetic differences among F_1 crosses and identify statistically the magnitude of such differences. The mean performance of the 138 F_1 hybrids studied for seven agronomic characters is presented in a concise way (Table 8), where different groups are reported separately. The presentation of data for every individual F_1 hybrid is not of special interest in this context, and interesting data will be pointed out later. Analysis of variance for different groups of interest (Table 9) indicates highly significant

TABLE 8. Mean performance of 138 F_1 hybrids (105 diallel crosses, and 33 single crosses from growth cabinet seedling selections), and 6 check entries of birdsfoot trefoil for seven agronomic characters

F_1 hybrid groups	Growth habit	Pod setting	Winter-hardiness	Seedling vigor(g)	Spring growth(g)	Forage yield(g)	Total yield(g)
Mirabel x Mirabel (28)[a]	4.2*	1.7	5.1	105	154	785	1415
Leo x Leo (21)	3.1	2.6	4.7	117	148	695	1397
Mirabel x Leo (56)	3.6*	2.2	5.0	123	165	829	1525
Selected (33)[b]	2.4	3.0	4.7	118	166	780	1488
LeL x LeL, 2.5 (5)	1.6	3.8	4.8	131	174	840	1626
LeL x LeL, 2.0 (2)	2.5	2.2	5.0	117	141	697	1399
MiL x LeL, 2.5 (2)	1.6	4.9*	4.9	144*	218*	1055	1922*
MiL x LeL, 2.0 (2)	2.2	3.2	4.3	115	155	658	1353
MiL x MiL, 2.5 (4)	3.3	2.9	5.1	118	179	919	1627
MiL x MiL, 2.0 (6)	3.2	2.1	5.2	112	179	927	1599
Leo large (check) (1)	1.7	2.1	4.1	103	145	595	1213
Mirabel large (check) (1)	2.3	2.8	4.0	121	156	624	1350
Leo small (check) (1)	1.5	3.1	3.5	115	139	486	1176
Mirabel small (check) (1)	2.8	2.3	3.5	106	136	476	1112
Leo (check) (1)	1.7	2.1	4.0	104	152	608	1232
Mirabel (check) (1)	2.5	2.8	3.5	106	148	518	1154
Mean of checks (6)	2.1	2.5	3.8	109	146	551	1206
L.S.D. .05	1.3	2.0	1.8	35.0	62	448	555
C.V.	20.1	42.8	19.7	16.5	20.3	30.2	19.6

*Statistically significant at the 5% level of probability as determined by an L.S.D. test.

[a] Numbers within parantheses are numbers of hybrids or entries from which the mean was calculated.

[b] The selected crosses include 12 single crosses among seedlings selected from small and medium size seeds in addition to 21 single F_1 crosses from large size seeds.

TABLE 9. Analysis of variance for the inter-cultivar F_1 diallel crosses (105), intra-cultivar F_1 diallel crosses (28 and 21), inter-cultivar F_1 single crosses (56), and 33 crosses among seedling selections for seven agronomic characters in birdsfoot trefoil

Source	d.f.	Growth habit	Pod setting	Winter-hardiness	Seedling vigor	Spring growth	Forage yield	Total yield
Inter-clonal diallel Mirabel x Leo diallel	104	1.28**	2.46**	1.24*	925**	1548**	73411*	133369**
Intra-clonal								
8 Mirabel clone diallel	27	1.42**	1.47*	0.80	799**	1564**	55656	109202*
7 Leo clone diallel	20	0.69	3.84**	1.47	657	1656*	77405	129988
Error								
15 clone diallel	208	0.45	0.76	0.87	368	958	51520	79832
8 Mirabel diallel	54	0.43	0.74	0.76	273	673	37680	58270
7 Leo diallel	40	0.35	1.18	1.26	444	730	55267	86546
Inter-clonal (Mi x Le) single crosses	55	0.90**	2.22**	1.30*	1022**	5128	80712	150644*
Selected F_1 crosses + 6 checks	38	2.22**	4.45**	1.64*	954	1936*	112342**	175385**

*,** Statistically significant at the .05 and .01 levels of probability, respectively.

differences among the 105 F_1 diallel crosses among the 15 clones of Mirabel and Leo (interclonal diallel) for all the characters studied. When only the 28 F_1 diallel crosses among the 8 Mirabel clones (Mirabel intraclonal hybrids) are analyzed, significant differences occur for all characters except winterhardiness and forage yield. Similarly, when only the 21 F_1 diallel crosses among the 7 Leo clones (Leo intraclonal) are considered, differences are not significant for all characters except pod setting and spring growth. The 56 single cross hybrids (interclonal single crosses) having always one Mirabel and one Leo parent, however, showed significant differences for all characters except spring growth and forage yield.

The failure to detect significant differences among the Leo x Leo F_1 hybrids for most characters indicates the stability and uniformity of the Leo germplasm for those characters, and that within a Leo open-pollinated nursery the degree of variation among plants is not large (Leo was licenced in 1961). Variation was large, however, among the Mirabel x Mirabel F_1 crosses for most of the characters, indicating that genetic advance can still be achieved by selection within the Mirabel germplasm. The highly significant variation observed among the Mirabel x Leo F_1 crosses indicates the advantage of combining the two germplasm sources to increase genetic recombination and increase the likelihood of crop improvement by selection. Inter-cultivar crossing advantages are especially observed in the MiL x LeL F_1 hybrids (Table 8) made among seedlings selected by the

2.5 cm depth of seeding selection technique discussed earlier. Significant differences for all characters except seedling vigor were also observed among the F_1 hybrids of seedling selections and 6 check entries (Table 9).

Significant differences among mean performances of the different groups outlined in Table 8 were only observed when these groups were compared with the MiL x LeL (2.5) group. When the L.S.D. values, however, are used to compare individual F_1 crosses, many such crosses would be superior to the check entries. F_1 hybrids were generally superior to all check entries for most of the characters studied (Table 8). Of particular interest is the significant superior erectness of growth of the Mirabel x Mirabel F_1 hybrids, followed by Mirabel x Leo F_1 hybrids, compared with other groups.

4.2.2 Combining ability analysis

Having found out that significant phenotypic differences occur among the F_1 diallel crosses, we can now proceed to do a combining ability analysis to shed some light on the component of such variance corresponding to different characters.

Combining ability analyses were performed separately for the inter-cultivar diallel crosses and the intra-cultivar diallel crosses to gain some information on the gene action in each of the 8 Mirabel and 7 Leo clones when their genes are combined together or recombined within the same cultivar. Those characters mainly governed by additive genes would most likely behave the same way in the inter-cultivar or intra-cultivar F_1 crosses. The epistatically affected characters, however, would behave differently, depending on specific combination of the clones under investigation.

Mean squares (MS) for GCA and SCA, as well as GCA and SCA variance components ($\hat{\sigma}^2_{gca}$, $\hat{\sigma}^2_{sca}$) and other statistics are shown in Table 10. Highly significant differences among the 15 clones were observed for general combining ability in all the characters studied. No significant differences were noted for specific combining ability. Hence, when crossed together in all one-way combinations, the 8 Mirabel and 7 Leo clones would show variation for characters the type of which is mostly governed by additive genetic variance. Among the 8 Mirabel clones, however, significant differences were observed for growth habit, seedling vigor, and total yield, but not for pod setting and spring growth. Among the 7 Leo clones, significant differences in GCA were observed for pod setting and spring growth in addition to growth habit. No significant differences in SCA were observed within the two intra-clonal groups of Mirabel and Leo.

The observed significant variation among F_1 diallel crosses (Table 9) must then be explained more on the basis of additive genetic variance than on non-additive variance. A comparison of the magnitude of mean squares (MS) of GCA and SCA substantiates this

TABLE 10. Combining ability analysis, mean squares (MS), general ($\hat{\sigma}^2_{gca}$) and specific ($\hat{\sigma}^2_{sca}$) combining ability variance components, the ratio of SCA to GCA sums of squares, and the coefficient of determination (R^2), of inter-cultivar and intra-cultivar diallel crosses of birdsfoot trefoil for 7 agronomic characters

	Growth habit			Pod setting			Winterhardiness[1]			Seedling vigor		
	ML (a)[2]	MM (b)[3]	LL (c)[4]	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)
GCA[5]												
d.f.	14	7	6	14	7	6	14	7	6	14	7	6
MS	3.32**	1.98**	0.91**	5.22**	1.70	3.51**	1.48**			1323**	835**	
$\hat{\sigma}^2_{gca}$	0.24	0.29	0.15	0.37	0.22	0.59	0.08			87.6	116.4	
SCA[6]												
d.f.	90	20	14	90	20	14	90	20	14	90	20	14
MS	0.22	0.26	0.11	0.61	0.40	1.27	0.49			329.8	255.0	
$\hat{\sigma}^2_{sca}$	0.004	0.05	-0.07	0.22	0.03	0.68	0.05			145.7	118.8	
Error												
d.f.	104	27	20	104	27	20	104	27	20	104	27	20
MS	0.23	0.21	0.18	0.38	0.37	0.58	0.43			184.1	136.6	
$\hat{\sigma}^2_e$	0.23	0.21	0.175	0.38	0.37	0.58	0.43			184.1	136.6	
$\frac{SCA(SS)}{GCA(SS)}$	0.43	0.38	0.28	0.75	0.68	0.85	2.10			1.6	0.87	
R^2 [7]	70	73	78	57	60	54	32			38	54	

(table continued)

TABLE 10 (continued)

	Spring growth			Forage yield[1]			Total yield		
	ML (a)[2]	MM (b)[3]	LL (c)[4]	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)
GCA[5]									
d.f.	14	7	6	14	7	6	14	7	6
MS	2204**	1171	1389**	108735**			188630**	91425*	
$\hat{\sigma}^2_{gca}$	132.7	139.0	204.7	6382.7			11439	10381.6	
SCA[6]									
d.f.	90	20	14	90	20	14	90	20	14
MS	553	658	581	25460			48247	41766	
$\hat{\sigma}^2_{sca}$	74.9	321.7	216.4	-299.9			8331	12631.1	
Error									
d.f.	104	27	20	104	27	20	104	27	20
MS	478.9	336.5	365	25760			39916	29135	
$\hat{\sigma}^2_e$	478.9	336.5	365.1	25760			39916	29135.3	
$\frac{SCA(SS)}{GCA(SS)}$	1.60	1.61	0.98	1.51			1.64	1.31	
R^2[7]	38	38	50	40			38	44	

*,** Significant at the .05 and .01 levels of probability, respectively.

1. General and specific combining ability was not performed for MM and LL since their crosses were not significantly different as determined by analysis of variance.

2,3,4. (a) 15 interclone diallel; (b) 8 Mirabel diallel; (c) 7 Leo diallel.

5. GCA is the mean performance of a clone in hybrid combination.

6. SCA is the performance of a particular cross in comparison with the average performance of the parental clones.

7. $R^2 = \frac{\text{GCA(SS)}}{\text{total genetic (SS)}}$ = ratio of the GCA sum of squares to the total sum of squares for genetic variation among the progeny in a diallel cross, given as a percentage.

conclusion, as GCA mean squares appear to be larger than SCA mean squares. The relative importance of specific combining ability might be underestimated in such a comparison of mean squares. Griffing (1956b) suggested the use of the ratio of SCA sum of squares to the GCA sum of squares. Baker (1977) recommended the use of the GCA and SCA variance components to compare their relative importance. Sokol and Baker (1977) used the ratio of SCA sum of squares to the GCA sum of squares to assess the importance of specific combining ability. An estimate of how well GCA will predict hybrid performance can be given by taking the GCA sum of squares as a per cent of the total sum of squares for genetic variation (additive plus non additive). This per cent has been called by Baker (1977) the coefficient of determination R^2.

In the present investigation, the GCA and SCA variance components ($\hat{\sigma}^2_{gca}$ and $\hat{\sigma}^2_{sca}$), the ratio of SCA to GCA sum of squares, and the ratio of the GCA sum of squares to the total genetic sum of squares (R^2) were calculated to determine the relative importance of both GCA and SCA (Table 10). The GCA variance components were higher than those of SCA variance components for growth habit, pod setting, winterhardiness, spring growth, forage yield, and total yield, when the 15 clone diallel is considered. When the 8 Mirabel clone diallel is considered alone, SCA variance components were larger than GCA variance components for seedling vigor, spring growth, and total yield, but not for growth habit and pod setting. The same is true for pod setting and spring growth among the F_1 Leo diallel crosses. Such comparisons become more evident when we examine the ratios of SCA to GCA sum of squares. The contribution of non additive genetic variance becomes almost equal to additive genetic variance for pod setting and spring growth in the Leo intra-clonal crosses (.85 and .98 respectively), and for seedling vigor in the Mirabel intra-clonal crosses (.87). The ratio of SCA sum of squares to GCA sum of squares for seedling vigor, spring growth, forage yield, and total yield, becomes more than 1.5 no matter which F_1 diallel cross is considered. This indicates that the contribution of non additive gene action is at least 1 1/2 times that of the additive gene action in the materials under investigation and for the characters mentioned above. This contribution becomes twofold for the winterhardiness character. The coefficients of determination (R^2) which measure the relative contribution of GCA or additive genetic effects to the total genetic variation are high (70-80%) in all F_1 groups for growth habit, slightly higher than 50% for pod setting, and lower than 50% for all other characters, especially winterhardiness. A low SCA/GCA sum of squares ratio, together with a high coefficient of determination, indicates the ability of the parental clones to perform well in all hybrid combinations. This is mainly due to additive gene action, so the progeny performance can be measured by simply evaluating the performance of the parents themselves. A high SCA/GCA ratio coupled with a low R^2 indicates the importance of non additive gene action in the control of these characters in hybrid combinations and, hence, the parental performance cannot be taken as a measure of average performance of the hybrids. Specific hybrid combinations between mediocre or week parents might produce hybrids greatly exceeding their parents in performance. The examination of the data on these two ratios, SCA/GCA(SS) and R^2 in Table 10, indicates the importance of specific combinations in all F_1 groups studied for the characters, winterhardiness, seedling vigor, spring growth, forage yield and total yield. Such non-additive effects might partly be due to trigenic and quadrigenic effects and their interactions. The nature of the analysis does not permit us to reflect on that with any precision.

While selection in a certain population is mostly based on the phenotypic value of the selected individuals, only a portion of the phenotype is transmitted to the following generation. The phenotype results from a developmental pattern predetermined by the genotype and conditioned by the environment. The relative importance and magnitudes of the components of phenotypic variance are important in a breeding program and very much determine how selection should be directed.

Estimates of additive, non additive, genetic, environmental, and phenotypic variances for all characters in the inter-cultivar and intra-cultivar F_1 hybrids are presented in Table 11. These estimates were calculated from Griffing's (1956b) diallel analysis as described

ABLE 11. Estimates of different variance components of inter-cultivar and intra-cultivar F_1 diallel crosses of birdsfoot trefoil for seven agronomic characters

haracter	σ^2_A [1]			σ^2_{NA}			σ^2_G			σ^2_E			σ^2_P		
	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)	ML (a)	MM (b)	LL (c)
rowth abit	.48	.58	.30	.004	.05	.07	.484	.63	.37	.23	.21	.175	.714	.84	.545
od etting	.74	.44	1.18	.22	.03	.68	.96	.47	1.86	.38	.37	.58	1.34	.84	2.44
inter- ardiness	.16			.05			.21			.43			.64		
eedling igor	175.2	232.8		145.7	118.8		320.9	351.6		184.1	136.6		505.0	488.2	
pring rowth	265.4	278.0	409.4	74.9	321.7	216.4	340.3	699.0	625.8	478.9	336.5	365.0	819.2	1035.5	990.0
orage ield	12765.4			299.9			13065.3			25760.0			38825.3		
otal eld	22878	20763		8331	12631		31209	33394		39916	29135		71125	62529	

[1] A = additive; NA = non additive; G = genotypic; E = Error; P = phenotypic; variance components.
(a) = 15 interclone diallel; (b) = 8 Mirabel diallel; (c) = 7 Leo diallel.

by Kempthorne and Curnow (1961). These estimates and their ratios also help shed more light on relative importance of additive and non additive genetic control of the different characters in the clonal samples investigated. Additive genetic variance was appreciably larger than non additive variance for growth habit and pod setting, both in the inter-cultivar 15 clonal F_1 hybrids and the intra-Mirabel F_1 hybrids, but less than twice as important in the intra-Leo F_1 hybrids. This further establishes the importance of additive effects in the control of these two characters as determined by previous analysis, and agrees with the findings of Miller (1968).

Contribution of additive variance in the phenotypic outcome of growth habit and pod setting was more than twice as important as environmental variance. This suggests with some certainty that selection for these characters can be started in early generations. It should be mentioned at this stage that those estimates are calculated on the basis of no epistasis. Such an assumption cannot be made with any certainty, since we cannot test for its validity. With this assumption, however, the non-additive genetic variance will be totally attributed to SCA variance component (σ^2_{sca}) and does not consider the contribution of interactions of digenic, trigenic, and quadrigenic effects reported to be of some importance in the overall genetic variance of autotetraploid alfalfa (Levings and Dudley 1963).

For the character, winterhardiness, we have established (Table 10) that non additive gene action was twice as important as additive gene action since for this character the ratio of SCA to GCA sum of squares was 2.1 and the coefficient of determination (R^2) was a low 32%. The estimates in Table 11 contradict this finding and establish importance of additive effects over non additive effects for this character (A/NA = .16/.05 = 3.2). The apparent conflict can be resolved when we consider the magnitude of environmental variance (.43) for winterhardiness. This magnitude is related to highly variable winter conditions associated with environments and years. Selection for this character (in the material studied) should then be done depending on results accumulated over environments and years.

The contributions of additive, non additive, and environmental effects in the phenotypic expression of seedling vigor were of almost equal magnitude when the inter-cultivar F_1 hybrids are considered. Environmental effects were slightly more responsible than the additive effects and had slightly more than half the effects of the genotype on the expression of seedling vigor. In the intra-Mirabel F_1 crosses, however, additive effects were twice as large as non additive ones and contributed most to the phenotypic outcome of the F_1 hybrids in seedling vigor. In this group, then, early generation selection for seedling vigor might be feasible and a phenotypic recurrent selection program for seedling vigor could be fruitful in the production of new synthetic cultivars. Such a program depends on the identification of high general combiners that would transmit their additive effects to their offsprings. As determined from the 105 F_1 inter-cultivar crosses, additive genetic variance was more than three times as large as non additive variance but only slightly more than one-half as important as the environmental effects in the phenotypic expression of spring growth. The genetic variance was around two-thirds the environmental variance. Obviously, then, and although additive effects are more important than non additive ones, in the control of spring growth, the phenotypic selection for this character is highly dependent on the environment. This necessitates the use of progeny testing and selection of superior clones depending on their progeny performance in a breeding program for the improvement of spring growth.

The same analysis given for spring growth seems to explain differences in forage yield and total yield. This is not unexpected since the latter two characters are calculated from winterhardiness, spring growth, and seedling vigor, all of which showed comparative trends in estimates of additive, non additive, and environmental variances.

4.2.3 Heritability and genetic advance due to selection

The progress due to selection in a breeding program is mainly dependent on the amount of genetic variation and the extent of contribution of this variation to the phenotype. The amount of genetic variation can be estimated from σ_G^2 (Table 11), while the contribution of genetic variance to the phenotypic variance can be estimated by a broad sense heritability estimate, $H = \sigma_G^2/\sigma_p^2$. The expected genetic advance due to selection G_s depends on the per cent of the population to be selected and can be estimated by the formula $G_s = K\sigma_p H$, where K is a constant that assumes different values depending on the severity of selection. When the top 5% of the population is selected, K = 2.06 (Allard 1960). A genetic coefficient of variation (GCV) was estimated from the σ_G^2 as

$$GCV = \frac{\sqrt{\sigma_G^2}}{\bar{x}} \times 100$$

This was necessary for more consistant comparisons, as the σ_G^2 does not give reliable information, considering the existence of different means and scales of measurement for the different characters studied (Johnson _et_ _al_. 1955).

Heritability estimates, genetic advance due to selection of the top 5% of the population, and the genetic coefficient of variability estimates for the inter-cultivar and intra-cultivar F_1 hybrids are presented in Table 12. Broad sense (B.S.) and narrow sense (N.S.) heritability estimates were, in general, equally high for the two characters, growth habit and pod setting in both inter-cultivar and intra-cultivar F_1 crosses. For the remaining characters, however, both types of heritability estimates were low, and B.S. estimates were always higher than N.S. estimates. The genetic coefficients of

TABLE 12. Estimates of narrow sense (NS) and broad sense (BS) heritabilities, expected genetic advance from selection (G_s) and genetic coefficient of variation (GCV) for seven agronomic characters of birdsfoot trefoil inter-cultivar and intra-cultivar F_1 hybrids[1]

Character	NS heritability[2]			BS heritability[2]			G_s (5%)			GCV		
	ML diallel	MM diallel	LL diallel	ML diallel	MM diallel	LL diallel	ML diallel	MM diallel	LL diallel	ML diallel	MM diallel	LL diallel
Growth habit	.67	.69	.55	.68	.75	.68	1.18	1.42	1.03	19.4	18.9	19.6
Pod setting	.55	.52	.48	.72	.56	.76	1.72	1.06	2.45	45.2	40.3	52.5
Winter-hardiness	.25			.33			.54			9.4		
Seedling vigor	.35	.48		.64	.72		29.6	32.8		15.9	17.9	
Spring growth	.32	.27	.41	.42	.68	.63	24.8	45.1	40.8	12.1	17.2	16.9
Forage yield	.33			.34			138.0			15.5		
Total yield	.32	.33		.44	.53		241.7	273.0		12.5	12.9	

[1] NS = σ^2_A/σ^2_P; BS = σ^2_G/σ^2_P.

G_s (5%) = $K\sigma_P$ BS, and if the top 5% are selected, then K = 2.06 (Allard 1960).

$$GCV = \frac{\sqrt{\sigma^2_G}}{\bar{X}} \times 100$$

[2] NS and BS heritabilities are calculated following Griffing's analysis (1956b) as outlined by Kempthorne and Curnow (1961).

variation were highest for pod setting (40-50%) and lowest for winter hardiness (9.4%). If the top 5% of F_1 crosses are selected within both inter-cultivar and intra-cultivar diallel crosses, the resulting genetic advance would be comparatively equal for all characters except pod setting. For this character, genetic advance is much more likely to occur by selection within the Leo x Leo F_1 crosses.

High estimates of genetic advance (G_s) and genetic coefficient of variability indicate that pronounced genetic improvement of pod setting in birdsfoot trefoil can be achieved with little difficulty by simple breeding programs, such as choosing the superior plants phenotypically and mating them together in a polycross nursery. If the genetic coefficients of variation were a little higher than observed, the same type of selection would also improve the growth habit in those clones of birdsfoot trefoil.

For the characters, seedling vigor, spring growth, forage yield, total yield and winterhardiness, simple phenotypic selection programs would not likely result in appreciable genetic advance. This is so because, for these characters, genetic coefficients of variation were generally low, and the narrow sense (N.S.) heritabilities due to additive genetic variance were not high (.27-.33). Therefore, more complex breeding programs for the improvement of these characters should be adopted. The existence of high epistatic and environmental variance in the phenotypic expression of these characters, as evident from Table 11, makes their improvement possible only when genetic materials are selected depending on progeny tests over years and environments. This is especially true of the character winter-hardiness.

4.2.4 Correlations among characters

The knowledge of the kind and degrees of associations between plant characters is of considerable value in plant breeding programs. When two characters are linearly and significantly correlated, their mutual improvement can be made possible by the selection for one of these characters (usually the more easily measured). A linear association usually indicates that the underlying genetic system controlling these characters shows some kind of linkage or pleiotropy associating them. A phenotypic correlation is a linear combination of genetic and environmental correlations. Although the relative proportions of genetic and environmental correlations to phenotypic correlations might be of considerable interest to a plant breeder, their estimation has not been done in this study. The phenotypic correlations will be discussed in conjunction with the degree of genetic and environmental variances controlling the two characters. Broad sense heritability estimates (Table 12) should help us determine the relative importance of these phenotypic correlations.

The relative difficulty of quantitative measurements of forage species compared with grain crops makes selection and improvement rather difficult. The identification of visual estimates of plant or stand performance that would be linearly associated with quantitative measurements are very helpful in simplifying and speeding up selection procedures. In the present investigation one such visual estimate, a vigor index, was attempted. It was taken as a score of 1-5. Plants rated at 5 were considerably bigger in vegetative mass (diameter and fullness) and of increased erectness than plants scored at 1.

Significant but low phenotypic correlation coefficients were found between vigor index and all other characters except winterhardiness (Table 13). The correlation between vigor index and seedling vigor (a quantitative measure of dry matter per plant) was the highest (r = .39**). This is a direct indication that vigor index can be used as a representative estimate of seedling vigor. Its significant and positive correlations with spring growth, forage yield, and total yield add to its merits as a possible assessing index for overall plant performance. The association of vigor index with growth habit and pod setting is of special interest. It was positively associated with growth habit (r = .30**) but negatively so with pod setting (r = -.33**). This implies that even though plants selected for high vigor index will tend to be more erect in growth habit, they will be inferior in production of seed pods. This is mainly due to a negative correlation between growth habit and pod setting (r = -.32**). In essence then, the selection of vigorous,

TABLE 13. Simple phenotypic correlations among eight agronomic characters in 105 F_1 diallel crosses of birdsfoot trefoil

Character	Pod setting	Winter-hardiness	Seedling vigor	Spring growth	Forage yield	Total yield[1]	Vigor index
Growth habit	-.32**	.16**	-.13*	-.03	.07	-.01	.30**
Pod setting		-.16**	-.01	-.08	-.13*	-.11	-.33**
Winter-hardiness			-.05	0.24**	.78**	.58**	.11
Seedling vigor				.37**	.20**	.64**	.39**
Spring growth					.77**	.78**	.22**
Forage yield						.88**	.20**
Total yield							.35**

*,** Significant at the .05 and .01 levels of probability that such estimates are greater than 0.

[1] Total yield = the summation of seedling vigor in one year and forage yield the following year.

hay type, high dry matter producing cultivars in birdsfoot trefoil, is most likely going to result in lower seed production of these cultivars. Such a decrease in seed production can be avoided if new cultivars can be synthesized by the inclusion of specific clones having high GCA effects for both vigor and seed production, a topic which will be discussed later. The relatively low phenotypic correlation coefficients can be attributed to the low genetic variances and heritabilities associated with seedling vigor, spring growth, forage yield, and total yield, combined with high environmental effects. Thus, selection for vigor index, to be effective, must be done in several environments and for a number of years.

Pod setting was negatively correlated with all other characters, but only significantly so with winterhardiness, forage yield and growth habit. Nevertheless, the correlation coefficients were extremely low (-.13 to -.16).

The genetic variance and heritabilities associated with pod setting (Tables 11 and 12) were relatively high in addition to a high genetic coefficient of variability and low environmental variance. All these combined should have resulted in higher estimates of correlations with pod setting. The counter argument, however, would be the low and high genetic and environmental variances respectively associated with characters other than pod setting. Where high σ_G^2 and low σ_E^2 occurred, growth habit for instance, the correlation coefficient with pod setting was high ($r = .32^{**}$). The practically nil association between pod setting and seedling vigor, spring growth, and total yield reasonably indicates that this character is not likely to be linked to any of the quantitative characters mentioned. Hence, in a breeding program, selection and improvement of these characters can be done independently. The same thing can be said about the association of winterhardiness and seedling vigor. Many birdsfoot trefoil breeders have established that generally, the more vigorous introductions of birdsfoot trefoil have frequently poor winter survival. This is often attributed to a lack of early fall dormancy in the more vigorous types.

The complexity of the matter can be seen from the relative importance of environmental variance to genetic variance controlling the expression of this character. The high association between winterhardiness and forage yield (r_p = .78**) was not unexpected since forage yield was calculated from spring vigor and winterhardiness. Its association with total yield, a measurement dependent on seedling vigor and forage yield, must mainly be related to its high association with forage yield. Positive correlations between seedling vigor and spring growth, but not winterhardiness, indicate that superior seedling vigor in the year of establishment is a good indicator of ability of the plants to produce more forage during the following season, but not necessarily to impart greater potential to avoid winterkilling. It also implies that one measurement of seedling vigor can be representative of overall plant productivity. In the growth cabinet studies this was also found to be true, since seedling vigor was highly and positively correlated with aftermath growth.

The correlations between spring growth, forage yield, and total yield, as well as between forage yield and total yield, were positive and high as expected (Table 13).

4.2.5 Prediction of superior genotypes and crosses in the production of synthetic cultivars or hybrids

One of the most reliable methods of improving the performance of cross pollinated crops in general and forage crops in particular is the identification of several superior genotypes or clones having superior GCA and combining them together in what is known as a synthetic cultivar. One of the advantages of synthetic cultivars is the relative simplicity of producing elite seed from them compared with hybrid seed production. Another advantage is the increased life span of a synthetic (3-10 generations) before it starts undergoing a process of increased homozygosity due to self fertilization. The degree of self compatability in birdsfoot trefoil is very small and will not likely cause any appreciable deterioration of genetic potential in short periods of time. This will be especially true if the synthetic cultivar has a wide genetic base, and if the component clones not only have superior GCA but also are able to transmit this character to their progeny (Twamley 1972).

The production of hybrid cultivars in forage species has not been adopted yet because the process of seed production is neither simple nor economic. A good and reliable source of male sterility has not yet been identified in birdsfoot trefoil. Additional problems are associated with the nature of pollination in alfalfa and seed shattering in birdsfoot trefoil. Thus, even though good indications of heterosis have been noted for single crosses in alfalfa, birdsfoot trefoil and other forages, in general this advantage has not been utilized for the improvement of forage crop cultivars. Heterotic single crosses for some characters will be cited later in this discussion. Most birdsfoot trefoil cultivars in use today were developed by simple breeding procedures such as mass selection or phenotypic recurrent selection. The two cultivars used for this investigation were developed by several cycles of mass selection.

The diallel analysis can be profitably used to study the breeding behaviour of several characters of interest in the crop, and provides various estimations and procedures for the identification of superior genotypes having either high GCA or SCA. It remains in the hands of the plant breeder to utilize this information towards improving his crops.

In the present investigation, information about the 15 clones studied has been compiled in several tables. Array[1] mean performance and rank for seven agronomic characters are shown in Table 14.

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[1] Array means of each clone are calculated as the average of the mean performance of the 14 F_1 crosses in which the clone appears as a parent.

TABLE 14. Array means (parents not included) and the parental order of means for seven characters in 105 F_1 inter-cultivar diallel crosses in birdsfoot trefoil*

Parental clone	Growth habit	Pod setting	Winter hardiness	Seedling vigor	Spring growth	Forage yield	Total yield
Mirabel 1	3.45	2.58	4.92	118.5	142.6	716	1428
Mirabel 2	4.26	1.47	5.28	116.6	158.8	836	1530
Mirabel 3	4.11	2.03	5.25	103.5	147.2	772	1397
Mirabel 4	4.07	1.74	4.53	115.7	151.0	673	1405
Mirabel 5	3.67	1.92	4.64	93.0	139.2	668	1226
Mirabel 6	4.37	1.63	5.03	125.3	176.6	889	1642
Mirabel 7	3.37	2.75	4.46	113.8	157.9	706	1389
Mirabel 8	3.81	1.69	4.89	98.0	165.7	817	1404
Leo 1	3.30	3.13	4.53	113.1	143.8	664	1341
Leo 2	3.67	1.30	4.60	115.7	138.8	646	1341
Leo 3	3.10	2.09	5.10	119.0	174.5	912	1627
Leo 4	3.80	2.52	4.17	107.1	154.6	670	1312
Leo 5	2.66	2.36	4.62	125.2	157.1	733	1487
Leo 6	3.20	1.79	4.96	111.8	141.6	709	1383
Leo 7	3.79	3.22	4.71	122.2	153.0	732	1467
Grand mean	3.64	2.15	4.78	113.2	153.5	743	1425
Parental order first 6 means	M6,M2,M3 M4,M8,L4	L7,L1,M7 M1,L4,L5	M2,M3,L3 M6,L6,M1	M6,L5,L7 L3,M1,M2	M6,L3,M8 M2,M7,L5	L3,M6,M2 M8,M3,L5	M6,L3,M2 L5,L7,M1

*Each and every array mean is calculated from the 14 F_1 crosses in which the clone was a parent.

Estimates of GCA effects and GCA and SCA components of variance associated with each of the 15 clones are presented in Table 15 for each of the seven characters studied. The estimates of GCA and GCA and SCA variance components for the two intra-cultivar diallels are presented in Tables 16 and 17. A preferential classification of clones according to high GCA effects is presented in Table 18.

From Table 14 we observe that the highest array means belong to Mirabel clones: M_6, M_2, M_3, M_4, and M_8. This is an indication that the Mirabel clones impart more erectness to their crosses, while Leo clones generally impart prostrate growth habit. An examination of the GCA effects (Table 15) associated with these clones shows their superiority in general combining ability. The components of variance associated with these clones (Table 15) are indicative of a very high degree of additive gene action, since the SCA variance component is practically zero. Additive variance was also noted to be high for growth habit (Table 11). Combined with a high estimate of N.S. heritability for this character, the above considerations positively point out the degree of genetic advance for erect type birdsfoot trefoil through the use of the above identified clones. The preferential classification of the 15 clones according to their high GCA effects (Table 18) also points out the superiority of these clones in providing a good base for a synthetic cultivar with a more erect growth habit.

TABLE 15. Estimates of GCA effects (g_i), GCA and SCA variances associated with each of 15 birdsfoot trefoil clones (8 Mirabel and 7 Leo) crossed in a diallel fashion, for 7 agronomic characters[1]

Clone	Growth habit			Pod setting			Winterhardiness			Seedling vigor		
	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}
Mirabel 1	-0.21	0.0	0.1	0.46	0.2	0.2	0.15	0.0	0.1	5.8	20.9	214.3
Mirabel 2	0.66	0.4	-0.0	-0.73	0.5	-0.1	0.54	0.3	0.2	2.4	-.75	108.5
Mirabel 3	0.50	0.2	0.0	-0.13	-0.0	-0.1	0.50	0.2	0.3	-9.9	85.3	250.3
Mirabel 4	0.46	0.2	-0.0	-0.44	0.2	0.0	-0.27	0.0	0.1	2.6	-6.4	18.3
Mirabel 5	0.03	0.0	0.0	-0.24	0.0	0.1	-0.15	0.0	0.1	-21.9	464.0	-9.5
Mirabel 6	0.81	0.6	0.1	-0.56	0.3	-0.2	0.27	0.0	1.0	13.2	161.8	226.0
Mirabel 7	-0.30	0.1	0.1	0.65	0.4	0.4	-0.35	0.1	0.6	0.7	-12.7	153.2
Mirabel 8	0.18	0.0	-0.0	-0.49	0.2	0.3	0.12	0.0	0.1	-16.4	255.2	127.5
Leo 1	-0.38	0.1	-0.1	1.05	1.1	0.4	-0.27	0.0	0.0	-0.2	-13.2	149.9
Leo 2	0.03	-0.0	-0.1	-0.91	0.8	-0.2	-0.19	0.0	0.1	2.7	-6.0	119.6
Leo 3	-0.57	0.3	-0.1	-0.06	-0.0	0.2	0.35	0.1	0.0	6.2	24.7	10.9
Leo 4	0.17	0.0	-0.0	0.40	0.1	0.6	-0.65	0.4	0.1	-6.6	30.5	277.2
Leo 5	-1.06	1.1	-0.1	0.22	0.0	0.4	-0.15	0.0	0.4	13.2	159.8	180.8
Leo 6	-0.48	0.2	0.1	-0.39	0.1	0.0	0.19	0.0	0.1	-1.5	-10.9	255.4
Leo 7	0.16	0.0	0.0	1.16	1.3	1.0	-0.08	0.0	0.0	9.7	80.7	-65.0
S.E.($\hat{g}_i$-$\hat{g}_j$)	0.19			0.24			0.26			5.3		

(table continued)

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[1] Estimates as per the combining ability analysis when all the 105 F_1 crosses of the complete (15 clonal) diallel are considered, i.e., inter-cultivar diallel.

TABLE 15 (continued)

Clone	Spring growth			Forage yield			Total yield		
	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}
Mirabel 1	-12.02	110	-42	-29.58	-974	914	5.57	-2834	22869
Mirabel 2	3.91	-19	-34	99.11	7973	-5859	113.27	9963	-5598
Mirabel 3	-6.63	9	39	30.73	-905	-2190	-16.27	-2600	32727
Mirabel 4	-2.48	28	280	-68.96	2906	-9156	-62.89	1089	1173
Mirabel 5	-16.40	234	-115	-81.66	4818	1190	-211.50	41867	-2726
Mirabel 6	25.06	593	-87	164.27	25134	-9484	233.80	51799	1328
Mirabel 7	4.91	-10	724	-41.04	-165	18922	-38.35	-1395	22621
Mirabel 8	13.29	142	-15	78.27	4276	-47	-21.35	-2409	6193
Leo 1	-10.25	70	-224	-85.73	5500	-12604	-89.89	5213	-4265
Leo 2	-15.63	210	-19	-105.04	9184	-5595	-76.04	2916	6169
Leo 3	22.83	487	506	181.03	30924	15087	218.20	44741	4911
Leo 4	1.37	-32	297	-79.81	4520	10129	-121.43	11878	31344
Leo 5	5.75	-1	-62	11.81	-1709	364	66.72	1586	-13409
Leo 6	-12.63	125	-41	-37.81	-419	4675	-45.04	-837	33152
Leo 7	-1.09	-33	-165	-11.96	-1706	-10498	45.19	-823	-21129
S.E.(g_i-g_j)	8.58			62.95			78.36		

$\hat{g}_i$ = GCA effect.

σ^2_{gi} = GCA variance component associated with each parental clone.

σ^2_{si} = SCA variance component associated with each parental clone.

In a breeding program aimed at the increase of winterhardiness in the two cultivars of birdsfoot trefoil, the clones M_2, M_3, L_3, M_6 and L_6 are the most profitable candidates (Table 14). A look at the GCA effects associated with these clones (Table 15) also ascertains this observation. When the GCA and SCA components of variance are, however, examined, we find that non additive genetic components associated with most of these clones are higher than additive components. This indicates that although the average performance of F_1 crosses having these clones as parents is high, it owes this increase more to some superior specific combinations with other clones rather than the merits of the clones themselves. Going back to the tables of means of F_1 crosses (not shown here) we can identify the following specific combinations as being responsible for the increase in average performance in winterhardiness of the identified clones ($M_2 \times M_3 = 6.0$, $M_2 \times M_5 = 6.0$, $M_3 \times L_3 = 6.0$, $M_8 \times L_3 = 6.0$, $L_6 \times L_3 = 6.0$). A close look at the specific combining ability effects associated with these combinations (Table 22) substantiates this observation. These later observations do not negate the fact that the superior array performance of the identified clones is mainly due to GCA effects. Yet the presence of a high non additive variance component associated with the expression of superior winterhardiness in the F_1's makes it rather hard for us to predict the performance of the progeny of related crosses in advanced generations. Probably the transmission of high GCA from parent to progeny would not be complete. In as much as these clones are destined to the formation of a synthetic cultivar, such manifestations would make the stability of winterhardiness in future years rather improbable.

The most important characters bearing a direct impact on total forage yield of birdsfoot trefoil in this investigation are winterhardiness, seedling vigor, and spring growth. An analysis of the array means and parental order of means (Table 14) identifies three clones superior in all three characters. These are M_2, M_6, and L_3. In the character total yield the exact order of superiority is $M_6 > L_3 > M_2$, as evident from their respective GCA effects, GCA and SCA components of variance (Table 15). We have already seen that these clones owe their superior general winterhardiness in F_1 crosses to several specific combinations. For seedling vigor, however, M_6 owed its performance almost equally to general and specific combinations, although SCA variance was higher than GCA variance. L_3, however, had a higher GCA variance component than SCA, but M_2 had all its advantage due to specific combinations (σ^2_{si} = 214.3, σ^2_{gi} = -7.5).

It becomes evident from the previous discussion that it is not simple to find clones that are high in general combining ability for several traits, and at the same time have most of their genetic variance attributed to additive effects. In the present investigation 15 clones were studied in a diallel fashion. If the number of clones studied is increased, the probability of recovering superior general combiners with high additive variance components is increased. There is, however, a practical and economic limit for the number of clones that can be studied in a diallel fashion. For a 20-clone diallel, the number of F_1 crosses becomes 190. If these are to be evaluated under spaced conditions, the magnitude of work and the cost to carry it becomes unrealistic. If the number of desired clones in the synthetic is around 6, then the number of clones to be evaluated might have to be more than 30. There are no indications in the literature that the evaluation of a 30-clone half diallel has been attempted. The reason, in addition to economics and hard work, is that quite similar results can be achieved by increasing the number of clones to be studied and mating them in several smaller "partial diallels." Thus, 30 clones would be evaluated as 5 six-clonal diallels. The total number of crosses to be made is $5[\frac{6(6-5)}{2}]= 75$, whereas in a complete 30-clone one-way diallel $\frac{30 \text{ x } 29}{2} = 435$ crosses would have to be made. Partial diallels have been described by Fyfe and Gilbert (1963) and Kempthorne and Curnow (1961). Conje and Carlson (1973a, 1973b) and Dudley and Moll (1969) have used partial diallels to estimate combining abilities and genetic variance in birdsfoot trefoil and alfalfa respectively.

The actual comparison of the potentials of both partial and complete diallel mating designs with respect to identification of clones of superior combining abilities has not, to the author's knowledge, been done. The nature of the material in this investigation allows us to attempt such a comparison. Tables 16 and 17 compile GCA effects as well as GCA and SCA variance components associated with each individual clone for the two intra-cultivar diallels (8 Mirabel and 7 Leo), which make up the whole 15-clone diallel. When these estimates are used in the same fashion described earlier, it can be observed that clones M_6 and M_2 have superior GCA effects for growth habit, spring vigor, and total yield. M_6 and M_2 were also identified in the 15-clone diallels. Table 17 shows that where L_7 was superior in growth habit and pod setting, no one clone was superior in all three characters, and L_3 was superior only in spring growth. In the 15-clone diallel L_3 was identified to be superior in more than spring growth. When we compare Tables 16 and 17 with Table 18 we can identify the concurrence of clones selected as good general combiners in the complete (15-clone) diallel or the two partial diallels.

According to these observations the use of partial diallels can be recommended for the study of genetic variance and especially for the identification of clones having superior combining abilities. Probably 30-50 clones could have been studied by partial diallels for the same amount of effort and money used on a 15-clone diallel. These 50 clones can be studied in 10 partial diallels of 5 clones each. The total number of resulting crosses would be $10(\frac{5 \times 4)}{2} =$ 100.

TABLE 16. Estimates of GCA effects ($\hat{g}_i$), GCA and SCA variances associated with clones, for 4 agronomic characters, when only the diallel crosses among 8 Mirabel clones are considered

Clone	Growth habit			Seedling vigor			Spring vigor			Total yield		
	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}
Mirabel 1	-0.96	0.89	-0.12	12.3	131.2	29.7	-15.5	192.4	71.5	41.6	-2520	1134
Mirabel 2	0.56	0.29	0.19	4.9	4.7	32.4	11.9	93.9	183.3	177.1	27109	-9319
Mirabel 3	0.44	0.16	-0.00	-17.0	270.5	288.0	-20.4	366.1	64.2	-202.6	36791	29966
Mirabel 4	0.20	0.01	-0.01	15.6	224.2	4.4	6.9	-0.7	773.7	33.8	-3109	10503
Mirabel 5	-0.02	-0.03	0.05	-11.5	113.3	-87.6	-10.3	58.6	-111.7	-102.6	6274	-15780
Mirabel 6	0.60	0.33	0.09	3.5	-7.9	91.7	19.3	323.1	-44.5	132.4	13285	3380
Mirabel 7	-0.67	0.42	0.20	2.8	-12.1	375.8	2.5	43.0	1091.3	-30.8	-3303	48375
Mirabel 8	-0.16	-0.00	-0.06	-10.5	91.2	57.7	5.6	-17.4	117.0	-48.9	-1856	15948
S.E.	0.27			6.7			10.6			98.5		
Highest general combiners	M6, M2, M3			M4, M1, M2			M6, M2, M4			M2, M6, M1		

$\hat{g}_i$ = GCA effect.

σ^2_{gi} = GCA variance component.

σ^2_{si} = SCA variance component.

TABLE 17. Estimates of GCA effects ($\hat{g}_i$), GCA and SCA variances associated with clones, for 4 agronomic characters, when only the diallel crosses among the 7 Leo clones are considered

Clone	Growth habit			Pod setting			Spring vigor		
	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}	$\hat{g}_i$	σ^2_{gi}	σ^2_{si}
Leo 1	-0.0	-0.0	-0.02	0.71	0.41	0.18	-11.5	69.9	-186.4
Leo 2	0.37	0.11	0.00	-1.20	1.32	-0.15	-28.9	773.4	33.3
Leo 3	-0.41	0.14	-0.09	-0.02	-0.10	1.05	22.1	425.2	277.3
Leo 4	0.61	0.34	-0.01	0.09	-0.09	0.65	12.7	98.3	616.9
Leo 5	-0.57	0.29	-0.10	0.14	-0.08	0.29	6.1	-25.6	63.7
Leo 6	-0.21	0.01	-0.09	-0.92	0.75	-0.05	-2.7	-55.2	-140.4
Leo 7	0.21	0.02	-0.08	1.19	1.31	1.86	2.3	-57.3	547.2
S.E.($\hat{g}_i$-g_j)	0.26			0.48			12.1		
Highest general combiners	L4, L2, L7			L7, L1, L5			L3, L4, L5		

$\hat{g}_i$ = GCA effect.

σ^2_{gi} = GCA variance component.

σ^2_{si} = SCA variance component.

TABLE 18. Preferential classification of clones according to high GCA effects ($\hat{g}_i$), and their associated GCA (σ^2_{gi}) and SCA (σ^2_{si}) variances. I. Selection of hay-type vigorous clones for the production of a synthetic cultivar of birdsfoot trefoil

		Class 1 (a)	Class 2	Class 3
Growth habit		M6 > M2	M2 > M3 > M4	M5 > L4 > L7 > L2 > M5
	σ^2_{gi}	0.6 0.4	0.4 0.2 0.2	0.0 0.0 0.0 -0.0 0.0
	σ^2_{si}	0.1 -0.0	-0.0 0.0 -0.0	0.0 -0.0 0.0 -0.1 0.0
Pod setting		L7 L1	M7 M1 L4	L4 L5
	σ^2_{gi}	1.3 1.1	0.4 0.2 0.1	0.1 0.0
	σ^2_{si}	1.0 0.4	0.4 0.2 0.6	0.6 0.4
Winterhardiness		M2 M3 L3 M6	L3 M6 L6 M1 M8	L6 M1 M8 L7
	σ^2_{gi}	0.3 0.2 0.1 0.0	0.1 0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0
	σ^2_{si}	0.2 0.3 0.0 1.0	0.0 1.0 0.1 0.1 0.1	0.1 0.1 0.1 0.0
Seedling vigor		M6 L5 L7	L7 L3 M1	L3 M1 L2 M4 M2
	σ^2_{gi}	161.8 159.8 80.7	80.7 24.7 20.9	24.7 20.9 -6.0 -6.4 -7.5
	σ^2_{si}	226.0 180.8 -65.0	-65.0 10.9 214.3	10.9 214.3 119.6 18.3 108.5
Spring vigor		M6 L3	M8 L5 M7	L5 M7 M2 L4 L7
	σ^2_{gi}	593 487	142 -1 -10	-1 -10 -19 -32 -33
	σ^2_{si}	-87 506	-15 -62 724	-62 724 -34 297 -165
Forage yield		L3 M6 M2	M2 M8	M8 M3 L5 L7
	σ^2_{gi}	30924 25134 7973	7973 4276	4276 -905 -1709 -1706
	σ^2_{si}	15087 -9484 -5859	-5859 -47	-47 -2190 364 -10498
Total yield		M6 L3	M2 L5 L7	L5 L7 M1
	σ^2_{gi}	51799 44741	9963 1586 -823	1586 -823 -2834
	σ^2_{si}	1328 4911	-5598 -13409 -21129	-13409 -21129 22869

(a) GCA effects are higher in Class 1 than in Class 2 than in Class 3.
GCA effects in any one class are not significantly different according to the standard error test (S.E.).

With the production of an improved synthetic cultivar of birdsfoot trefoil in mind, the 15 clones have been classified in three groups (regions) according to a decreasing magnitude in their positive GCA effects (Table 18). This has been done for all seven characters. The GCA and SCA variance components (σ^2_{gi} and σ^2_{si} respectively) are presented for each clone to reflect the importance of additive to non additive genetic effects occurring in each.

The results of this classification are in accordance with the classification done previously according to the array means (Table 14). Different clones in any one class have GCA effects that do not differ from each other by more than one standard error of the effects. Clones in Class 1 have GCA effects significantly superior to effects of clones in Classes 1 and 2. The natural choice will then be in Class 1. Compiling the most common clones in Class 1 over all measured characters, we end up choosing clones M_2, M_6, and L_3 to form a hay-type birdsfoot trefoil synthetic cultivar of superior forage producing capacity. To have an idea about the actual performance of such a potential cultivar, we take a look at the actual mean performance of the F_1 three single crosses in comparison with all F_1 single crosses having one of the three clones M_6, M_2 and L_3 as a parent. Maternal[1] F_2 performance of the crosses is also presented in

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[1] Maternal F_2 seed refers to the polycross seed produced on an F_1 hybrid plant where paternal pollen is also from other unknown F_1 plants in the nursery.

Table 19. The average performance ($\overline{X}_2$) of the three single crosses involving M_6, M_2, and L_3 clones, is higher than the average performance ($\overline{X}_1$) of all the crosses combined, both in the F_1 and maternal F_2 for all characters measured except growth habit in the F_2, and pod setting in the F_1. This is an indication of the potential superiority of such a synthetic cultivar over the average performance of the remaining clones. The fact that this superiority was even maintained in the maternal F_2 generation substantiates the ability of these three clonal hybrids to transmit their additive gene action to advanced progeny even though the contribution of the paternal parent was not controlled in the production of F_2 seed. It is highly probable that if the original three clones (M_2, M_6, and L_3) or their F_1 hybrids (M_2 x M_6, M_2 x L_3, and M_6 x L_3) were polycrossed in isolation for the production of the following generation seed, then the performance of the synthetic seed would be even higher than $\overline{X}_2$.

The production of hybrid cultivars in birdsfoot trefoil is still a matter which is not feasible nor practical. No efficient way has been devised to control pollination between any two clones. The heterotic effects in hybrids between initially heterozygous parents are generally poor compared with superior heterosis resulting from crossing two homozygous parents. The heterozygosity itself in a crop like birdsfoot trefoil is a way of maintaining general vigor in any one cultivar. The composition and size of the gene pool base of one cultivar, in a self-incompatible crop, determines the amount and

TABLE 19. Mean performance, in 7 agronomic characters, of all F_1 and F_2 and maternal F_2 crosses involving birdsfoot trefoil clones selected for high GCA in the F_1 generation

Cross	Growth habit		Pod setting		Winter-hardiness		Seedling vigor*	Spring growth		Forage yield		Total yield*
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_1	F_2	F_1	F_2	F_1
M1M2	3.8	3.5	1.3	2.9	5.5	4.8	125	163	244	888	1087	1640
M2M3	5.0	3.6	1.3	4.4	6.0	3.5	78	120	243	717	867	1183
M2M4	4.6	3.5	1.2	3.9	5.5	3.5	124	137	280	744	1053	1485
M2M5	3.8	2.9	1.3	4.5	6.0	2.8	95	173	186	1037	557	1608
M2M6	5.6	3.8	1.0	5.0	5.5	4.1	124	193	268	1057	1245	1798
M2M7	5.2	4.3	2.6	2.2	5.5	3.5	101	185	182	1018	728	1625
M2M8	4.8	3.0	1.0	5.3	5.0	3.0	119	178	256	891	854	1605
M2L1	3.8	3.6	2.6	3.9	4.5	4.4	126	163	245	799	1058	1534
M2L2	4.1	3.4	1.3	3.5	4.5	3.0	118	159	221	735	733	1443
M2L3	3.7	3.1	1.0	5.0	5.5	3.8	117	179	233	970	820	1672
M2L4	4.2	4.1	1.0	3.2	5.0	4.5	147	139	228	725	1074	1605
M2L5	2.9	2.9	2.0	4.2	5.5	4.0	123	149	303	829	1294	1567
M2L6	3.7	3.0	1.4	4.5	5.0	3.5	91	120	271	560	1078	1145
M2L7	4.4	2.9	1.8	7.5	5.0	4.3	129	140	253	733	1130	1507
M1M6	3.7	3.6	2.5	4.3	5.5	4.1	128	171	249	953	1182	1723
M3M6	5.9	3.1	1.0	7.0	5.0	3.4	95	146	332	702	890	1271
M4M6	5.0	2.8	1.1	5.2	3.5	4.0	125	173	326	617	1353	1365
M5M6	5.2	3.7	1.1	4.5	5.5	4.8	101	163	247	888	1145	1496
M6M7	3.2	2.8	2.2	5.4	5.5	4.2	80	149	213	831	904	1311
M6M8	4.4	3.1	1.3	7.5	5.5	3.9	101	198	287	1105	1115	1712
M6L1	4.1	3.5	3.7	4.1	4.5	4.2	141	166	236	733	1021	1581
M6L2	4.1	4.2	1.6	4.4	5.5	2.0	119	160	166	887	416	1602
M6L3	4.1	2.7	1.8	5.6	5.5	4.4	141	230	261	1268	1393	2116
M6L4	4.5	3.1	1.9	6.4	4.5	3.9	150	219	280	957	1082	1855
M6L5	3.3	3.1	1.2	5.7	5.0	3.0	168	181	225	904	720	1914
M6L6	3.4	3.8	1.2	2.9	5.0	4.0	135	154	247	769	1002	1579
M6L7	5.0	4.0	2.6	6.0	4.5	3.4	147	170	293	779	1016	1661
$\overline{X}_1$(a)	4.3	3.4	1.6	4.8	5.1	3.8	120	166	251	852	993	1578
$\overline{X}_2$(b)	4.5	3.2	1.6	5.2	5.5	4.1	127	201	254	1098	1153	1862

*Seedling vigor and total yield were not measured for maternal F_2 crosses.

(a) $\overline{X}_1$ is the overall average of all the crosses in any one generation and for any one character.

(b) $\overline{X}_2$ is the average of the three single crosses M2M6, M2L3, and M6L3.

continuity of vigor in this cultivar. Crosses among clones extracted from two widely separated cultivars of birdsfoot trefoil were found by Conje and Carleson (1973) to have increased heterosis over those crosses within the same cultivar. The mean performance of 56 F_1 inter-cultivar hybrids (Table 8) was also observed to be higher than the mean performance of the intra-cultivar hybrids in all characters except pod setting. Although the heterotic effects due to high SCA for specific crosses cannot be shown in an average way, examples of such single crosses can be seen for any of the characters in Tables 20 to 26. These tables were extracted from the diallel analysis and they represent the estimates of SCA constants for any F_1 cross. High negative constants indicate poor performance whereas high positive constants indicate superior performance.

Low negative or positive estimates can be practically accepted as zero effects and signify the non-occurrence of specific combining ability or non additive genetic effects for that cross. The observed mean performance would be an average of the overall mean of the two array means representing the two clones of the hybrid.

The information demonstrated by specific combining ability effects can be used profitably to determine the effectiveness of selecting clones of high GCA effects. The choice of clones of superior GCA can be enhanced if the SCA estimates of crosses among these clones is either positive or zero. When high negative estimates are encountered, it usually means a deterioration of general performance

TABLE 20. Estimates of SCA constants $(\hat{\sigma}_{ij})$ for the character growth habit in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	-0.3	-0.4	-0.4	0.1	-0.5	-0.4	-0.9 2.8	0.4	-0.1	-0.0	0.2	0.2	1.5 5.4	0.6
M_2		0.2	-0.2	-0.5	0.5 5.6	1.2 5.2	0.3	-0.1	-0.2	-0.1	-0.3	-0.3	-0.1	-0.0
M_3			-0.4	0.8 5.1	1.0 5.9	0.1	0.0	-0.1	-0.1	-0.6 2.9	-0.6 3.7	0.4	-0.1	-0.4
M_4				0.3	0.1	0.1	0.6	0.4	0.5	0.1	-0.4	0.0	-0.3	-0.8 3.5
M_5					0.7 5.2	-0.2	0.2	-0.5	-0.5	-0.2	-0.6	-0.4	-0.3	0.8
M_6						-1.0 3.2	-0.2	0.0	-0.4	0.2	-0.1	-0.1	-0.6	0.4
M_7							-0.4	-0.4	0.4	0.4	0.3	-0.6 1.8	-0.4	0.6
M_8								-0.4	-0.0	0.7	0.5	-0.5	0.2	-0.1
L_1									-0.3	-0.1	0.8	0.0	0.1	-0.0
L_2										0.3	-0.3	0.2	0.4	0.0
L_3											-0.1	-0.1	-0.0	-0.6 2.7
L_4												0.5	0.0	-0.1
L_5													0.2	0.3
L_6														-0.6

$S.E._1 = 0.64$ = standard error between effects of crosses having one parent in common.

$S.E._2 = 0.61$ = standard error between effects of crosses having no parent in common.

TABLE 21. Estimates of SCA constants $(\hat{\sigma}_{ij})$ for the character pod setting in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	-0.6 1.3	0.5	-0.8 1.3	-0.8 1.6	0.5	0.1	0.0	-0.2	-0.4	-0.3	1.2	1.5 4.4	0.5	-1.1
M_2		0.0	0.2	0.1	-0.0	0.5	0.1	0.1	0.8 2.4	-0.4	-0.8 1.0	0.4	0.4	-0.7
M_3			-0.2	-0.2	-0.5 1.0	-0.3	-0.5	0.0	0.5	-0.7 1.3	0.5	-0.6	0.2	1.2 4.4
M_4				0.4	-0.1	-0.7	-0.1	-0.3	0.0	-0.2	-0.9 1.2	0.7	0.9 2.5	1.1 3.9
M_5					-0.3	-0.8 1.6	-0.2	1.7 4.7	-0.2	-0.2	1.1	-0.4	-0.3	0.0
M_6						-0.0	0.2	1.0 3.7	-0.0	0.3	-0.1	-0.6	-0.0	-0.2
M_7							2.3 4.7	-0.3	0.0	0.9 3.7	-0.9 2.3	-0.3	0.4	-0.9 3.0
M_8								-1.0	0.0	-0.3	0.7 2.8	-1.0 1.0	0.1	-0.1
L_1									-0.6 1.7	1.6 4.8	-0.8 2.7	-0.7	-0.6	0.1
L_2										0.5	0.1	0.3	-0.1	-0.9 1.5
L_3											-0.6	-0.1	0.9	-1.4 1.8
L_4												-0.8	-0.9 1.2	2.1 5.8
L_5													-0.3	1.9 5.4
L_6														-1.0 1.9

$S.E._1$ = 0.84 = standard error between effects of two crosses having one parent in common.

$S.E._2$ = 0.81 = standard error between effects of two crosses having no parent in common.

TABLE 22. Estimates of SCA constants ($\hat{\sigma}_{ij}$) for the character winterhardiness in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	0.02	$\underline{-0.9}$ 4.5	0.3	0.2	0.3	$\underline{0.9}$ 5.5	0.4	-0.2	0.3	-0.8	-0.3	0.7	$\underline{-1.6}$ 3.6	0.6
M_2		0.2	0.4	$\underline{0.8}$ 6.0	-0.1	0.5	-0.4	-0.6	-0.6	-0.2	0.3	0.3	-0.5	-0.2
M_3			-0.0	-0.1	-0.6	0.0	0.1	0.5	-0.1	0.4	-0.1	0.4	0.0	0.3
M_4				0.6	$\underline{-1.3}$ 3.5	-0.2	0.4	-0.3	0.2	-0.4	0.1	$\underline{-0.9}$ 3.5	0.3	0.6
M_5					0.6	0.2	-0.2	-0.4	0.6	0.0	$\underline{-1.0}$ 3.0	$\underline{-1.5}$ 3.0	-0.8	$\underline{0.9}$ 5.5
M_6						$\underline{0.8}$ 5.5	0.3	-0.3	$\underline{0.6}$ 5.5	0.1	0.1	0.1	-0.2	-0.5
M_7							$\underline{-1.1}$ 3.5	$\underline{1.3}$ 5.5	0.3	$\underline{-1.3}$ 3.5	$\underline{1.2}$ 5.0	$\underline{-1.8}$ 2.5	0.4	$\underline{-1.4}$ 3.0
M_8								$\underline{0.9}$ 5.5	-0.7	$\underline{0.8}$ 6.0	-0.2	-0.7	0.4	0.2
L_1									$\underline{-0.8}$ 3.5	0.1	-0.4	0.6	-0.7	0.1
L_2										0.1	$\underline{-1.4}$ 2.5	$\underline{1.1}$ 5.5	0.7	-0.0
L_3											$\underline{1.0}$ 5.5	0.0	0.7	-0.6
L_4												0.5	0.7	-0.6
L_5													0.7	0.4
L_6														0.1

$S.E._1$ = 0.89 = standard error between SCA effects of two crosses having one parent in common.

$S.E._2$ = 0.85 = standard error between SCA effects of two crosses having no parent in common.

TABLE 23. Estimates of SCA constants ($\hat{\partial}_{ij}$) for the character seedling vigor in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	3.5	-21.2 88	-6.7	17.7 115	-1.4	-3.8	17.3 120	-2.0	17.2 139	34.7 160	-6.5	-12.3	-45.6 72	9.2
M_2		-27.7 78	5.7	1.2	-4.9	-15.4 101	19.7 119	10.5	-0.4	-4.8	37.9 147	-5.8	-23.1 91	3.6
M_3			7.0	-9.5	-21.6 95	25.9 130	-28.9 58	4.8	12.9	-0.5	19.3	24.5 141	28.2 130	-13.1
M_4				16.9	-4.1	22.4 139	3.5	7.3	-8.6	-4.1	-19.3 90	-29.1 100	11.6	-2.6
M_5					-3.7	1.9	3.9	-3.3	9.9	-23.6 74	-20.8 64	13.4	4.1	-8.1
M_6						-47.2 80	-9.1	14.6	-10.2	8.3 141	30.1 150	28.3 168	10.0	10.8
M_7							-4.6	5.2	12.3	-3.1	-4.4	16.9	-5.4	-0.7
M_8								29.3 126	-11.6	-8.1	-15.3	25.9 136	-9.4	-12.6
L_1									-43.8 72	-12.3	-16.5 90	-13.3	18.4 130	1.2
L_2										13.9	-7.4	-4.1	-3.4	23.3 149
L_3											2.2	1.4	-6.9	2.8
L_4												-21.8 98	32.9 138	-10.4
L_5													-15.9	-8.1
L_6														4.6

$S.E._1$ = 18.4 = standard error between effects of two crosses having one parent in common.

$S.E._2$ = 17.7 = standard error between effects of two crosses having no parent in common.

TABLE 24. Estimates of SCA constants ($\hat{\sigma}_{ij}$) for the character spring growth in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	17.7	-18.7	-13.9	8.0	4.6	-35.3 111	10.4	-2.1	-1.7	17.8 182	6.3	29.9 177	-37.7 91	14.7
M_2		-30.6 120	-17.8	32.1 173	10.7 193	22.8 185	7.4	16.0	17.4	-1.1	-19.6	-14.0	-24.6 120	-16.2
M_3			10.7	1.7	-25.8 146	12.4	-38.0 122	-4.5	19.9	28.4 198	3.9	- 2.5	35.9 170	7.4
M_4				-1.5	-2.9	71.2 227	4.8	34.4 175	-18.3	-40.7 133	-20.3 132	-5.6	4.7	-4.8
M_5					0.9	-12.9	1.7	-19.7 107	21.7 143	21.2 181	-33.3 105	-22.7 120	-2.3	5.1
M_6						-34.3 149	6.3	-2.2	-2.8	28.7 230	39.2 219	-3.2	-11.8	-7.3
M_7							-44.6 127	4.9	26.4 169	-57.1 124	2.4	24.9 189	14.4	4.8
M_8								1.5	26.0	28.5 218	-13.0	13.6	-6.0	1.4
L_1									-7.5	-22.0	14.5	-13.9	1.5	-1.0
L_2										-33.6 127	-37.1 102	-6.5	-3.1	-0.6
L_3											15.4	-25.9 156	-0.6	40.9 216
L_4												38.5 199	37.9 180	-34.6 119
L_5													-5.5	-7.0
L_6														-2.6

$S.E._1$ = 29.7 = standard error between effects of two crosses having one parent in common.

$S.E._2$ = 28.5 = standard error between effects of two crosses having no parent in common.

ABLE 25. Estimates of SCA constants ($\hat{s}_{ij}$) for the character forage yield in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
	74.6	-219.0 526	-3.4	78.3 953	74.4	-54.3	122.4 915	-43.6 581	11.7	-60.4	-43.5	281.5 984	-361.5 315	142.6
		-156.7	-30.0	275.6 1037	49.7 1057	216.0 1018	-30.3	41.7	-2.9	-54.0 970	-38.2	-2.2	-245.2 560	-98.0
			64.3	32.9	-236.9 702	85.4	-181.9 671	35.1	79.4	233.3 1189	3.2	65.2	185.2	76.3
				63.7	-122.2	225.1 859	75.8	82.8	-51.9	-251.9 604	-68.1	-140.1 523	86.9	69.0
					61.5	-36.2	-45.5	-113.5 463	159.8 906	62.7	-248.4 334	-291.4 359	-106.4	172.7 823
						-36.1	-118.6	-89.4	83.9	178.8 1268	128.6	7.6	-101.4	-117.2
							-334.1 447	227.9 845	162.2	-360.9 523	185.9 809	-212.0 479	111.9	-180.9 510
								120.6 857	-8.1	301.8 1305	-121.3	-66.3	21.6	26.8
									-140.1	-121.2	32.6	30.6	-94.4	30.8
										-185.9 634	-305.0 254	122.9	76.9	-2.9
											221.9 1067	-115.1 798	89.9 977	61.0
												198.5	272.7 900	-219.1 433
													72.7	47.9
														-9.1

E.$_1$ = 218 = standard error between SCA effects of two crosses having one parent in common.

E.$_2$ = 209 = standard error between SCA effects of two crosses having no parent in common.

TABLE 26. Estimates of SCA constants ($\hat{\sigma}_{ij}$) for the character total yield in 105 diallel crosses among 15 (8 Mirabel and 7 Leo) clones of birdsfoot trefoil

	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	97	-358 1056	-36	214	59	-78	225 1634	-55	100	146	-83	209	-638 747	197
M_2		-339 1183	10	282 1608	26	125	88	86	-19	-84 1672	188 1605	-38	-348 1145	-76
M_3			99	-104 1093	-371 1271	232	-371 1015	51	310 1462	220 1847	107	195	338 1702	-12
M_4				171	-231	368 1691	106	138	-108 1177	-267 1313	-171 1069	-303 1125	164	61
M_5					49	-28	-24	-132	202	-80	-376 716	-215	-84	125
M_6						-309 1311	75	12	20	239 2116	318 1855	189 1914	-34	-43
M_7							-358 1007	262	224	-377 1227	159	-111	75	-183
M_8								298 1611	-91	-254 1875	-212 1070	92	-36	-45
L_1									-416 843	-191	-61	-45	16	38
L_2										-117	-360 867	89.7	41.5	125.2
L_3											233 1754	-105	47	81
L_4												69	668.8 1727	-280.0 1068
L_5													-25.3	-2.5
L_6														14.2

$S.E._1$ = 271 = standard error between SCA effects of crosses having one parent in common.

$S.E._2$ = 259 = standard error between SCA effects of crosses having no parent in common.

of a clonal synthetic corresponding to the probability of occurrence of the single crosses having this high negative effect. Within the F_1 crosses (M_2M_6, M_2L_3, and M_6L_3), and for all characters studied, SCA effects were either high and positive or practically zero. Thus, in this synthetic, one does not have to worry about deterioration of performance due to high and negative specific combining ability. Nevertheless, enough information has not been obtained to say confidently that such a narrow based synthetic (3 clones) will not deteriorate in performance with time. The following facts are clear, however. Firstly, the clones initially have been selected from the two cultivar nurseries according to fall vigor as estimated visually; secondly, the performance of F_1 hybrids in the establishment and first production years has been superior to the overall average of the 105 F_1 crosses; thirdly, the performance in maternal F_2 has also been superior to the overall average of the F_2 crosses and the average of the mothers. This we consider is enough proof of the potential superiority of the projected 3-clone synthetic. Some doubt, however, remains due to the lack of knowledge on disease resistance and environmental effects on winterhardiness, which are two basic factors in forage yield determination. If the suggested synthetic was to be actually produced and tested over several environments, its performance relative to the two initial cultivars could be established.

In addition to using SCA effects in Tables 20 to 26 as warning signals in the identification of some hybrid combinations, especially those corresponding to highly negative effects, some very high positive effects can be used to identify some superior crosses. These can be profitably used in the introduction of specific gene combinations into materials lacking them. In growth habit for example, the combination M_3M_6 has a high positive effect and its actual mean is around 6, an indication of upright growth. If prostrate types are required, then a combination like M_7L_5 might help. The observed mean performance for crosses showing high and either positive or negative SCA effects is added in each cell illustrating the SCA effects in Tables 20 to 26. There is no need to describe these combinations in detail, but it should be mentioned that, on the average, high positive effects indicate significant superior performance to the overall average of the 105 F_1 crosses, and high negative effects indicate significant inferior performance to the overall average.

4.3 Maternal F_2 studies and comparison of selection methods

4.3.1 Maternal F_2 greenhouse and field performance

Maternal F_2 polycross seed was produced on F_1 mother plants in one inter-cultivar (Mirabel x Leo) and one intra-cultivar (Mirabel x Mirabel) nursery. The resulting (56 Mirabel x Leo, and 28 Mirabel x Mirabel) maternal F_2 lines were tested in the greenhouse and field, together with 15 polycross parental lines produced on the original clones (see sections 3.2.2.2 and 3.2.2.3).

Mean performance of the 99 entries for several agronomic characters is presented in Tables 27 to 31. The mean performance of the different maternal F_2 progeny varied from one cross to another in all studied characters. The analysis of variance for both greenhouse and field experiments is presented in Table 32.

The analysis of variance for the greenhouse experiment was conducted on the basis of the randomized complete block design. The variances for the different characters studied shown in Table 32 indicate significant differences among the 99 entries (treatments) for erectness, vigor index, seedling vigor, and aftermath growth. A significant difference between the two replications (greenhouse beds) was noted for vigor index and aftermath growth. Such a variation leads us to contemplate the uniformity of the environment under which the trial was conducted. It was not expected, considering the efforts spent to control the greenhouse environment (temperature-light, and soil-water-nutrition). The possibility of a high temperature effect from an adjoining greenhouse room cannot be dismissed.

In the field, the experiment was set up in a triple lattice design with three basic replications. When, for some characters, the analysis of variance for this design did not show any efficiency compared with the randomized complete block design, the error variance of the latter was taken to test the treatment variance, and the treatment means were unadjusted. This was the case for the two characters, growth habit and pod setting. For the remaining

TABLE 27. Greenhouse mean performance of 84 maternal F_2 and 15 parental polycross lines of birdsfoot trefoil, for erectness and vigor index*

	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	4.7* 1.2*	4.4 2.8	2.9 2.1	3.7 1.8	3.6 2.5	3.5 1.5	4.2 1.2	3.8 2.3	2.9 2.6	3.8 1.8	3.1 2.3	3.9 2.7	3.1 1.9	3.7 2.4	4.3 1.5
M_2		4.1 2.6	3.6 2.1	4.6 2.2	4.3 2.2	4.4 1.5	4.6 2.2	4.4 1.9	2.8 1.9	4.5 1.3	3.9 3.1	3.0 2.4	4.4 2.3	4.4 2.7	4.7 2.1
M_3			4.8 1.6	2.9 2.0	4.4 2.0	4.8 2.5	3.8 1.8	4.3 1.8	2.9 2.7	4.0 2.7	3.3 1.4	4.4 2.6	4.3 2.1	2.6 2.7	3.9 2.2
M_4				3.0 2.3	4.3 2.2	4.3 1.5	4.4 1.8	3.9 2.0	3.0 1.9	4.3 2.8	4.6 1.4	4.2 2.1	3.1 3.2	3.4 2.2	3.1 2.5
M_5					4.9 2.1	4.3 2.3	3.5 1.6	3.8 2.5	4.6 1.9	4.7 1.6	4.3 2.9	3.4 2.5	3.8 2.4	3.1 1.6	4.3 2.1
M_6						4.3 2.3	3.4 2.7	4.0 2.0	4.2 2.1	3.4 2.2	4.2 3.0	4.3 2.4	4.7 1.6	4.3 3.6	4.8 2.6
M_7							4.3 1.8	3.8 2.1	3.6 2.4	3.4 2.3	4.4 1.9	4.3 1.8	4.8 2.2	4.4 2.2	3.3 2.3
M_8								3.8 2.9	4.5 2.7	4.1 2.2	4.1 2.6	4.1 3.3	4.2 1.7	4.2 2.1	4.3 2.9
Leo parental means									3.3 2.2	3.3 3.1	4.9 1.4	4.7 1.6	4.6 1.8	2.9 1.7	4.6 2.8

*Erectness in upper row, and vigor index in lower row.

TABLE 28. Greenhouse mean performance of 84 maternal F_2 and 15 parental polycross lines of birdsfoot trefoil for seedling vigor and aftermath dry plot yield*

	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	4.8* 14.3*	5.2 14.5	6.3 15.5	5.6 13.9	5.5 10.9	4.1 11.2	4.0 10.6	4.8 11.8	6.6 13.5	4.9 10.8	5.8 14.9	5.8 14.6	5.6 11.6	4.5 11.5	3.7 12.0
M_2		4.1 12.4	4.7 11.4	6.1 15.5	5.0 14.2	5.9 17.3	4.9 12.9	5.7 15.5	5.4 11.4	4.6 10.6	6.0 16.9	5.7 16.3	6.2 14.0	5.3 13.7	4.6 13.0
M_3			4.1 13.7	5.4 12.6	5.7 12.0	5.9 11.4	6.4 15.9	5.6 16.0	4.0 16.3	5.3 14.4	3.8 10.0	5.5 13.3	6.2 16.3	5.8 14.9	4.7 14.0
M_4				4.9 13.4	5.5 12.1	5.9 15.5	4.6 13.8	4.8 13.0	3.2 9.1	5.6 14.8	5.6 15.6	6.1 15.7	5.9 16.5	4.6 10.8	5.7 15.0
M_5					5.7 12.7	4.8 11.1	5.3 13.5	4.0 13.1	3.7 12.1	5.2 14.6	5.8 16.6	4.6 13.3	4.6 11.9	4.7 12.8	5.5 13.6
M_6						4.9 13.6	5.1 12.7	6.8 16.2	3.5 10.7	4.8 11.4	5.8 14.8	4.0 11.7	6.6 15.0	4.4 12.4	6.1 15.3
M_7							4.6 11.8	6.4 15.1	4.6 12.8	4.4 11.9	4.0 10.8	4.0 10.5	5.8 15.8	5.8 16.5	4.0 14.0
M_8								5.3 14.8	5.7 14.8	5.2 14.1	4.6 11.2	5.0 14.4	6.5 19.8	4.4 13.5	6.6 15.1
Leo parental means									6.1 14.2	5.9 13.7	4.2 11.5	4.5 13.5	4.1 10.6	5.4 11.6	4.9 11.1

*Seedling vigor (fresh weight per seedling) in upper row, and aftermath yield in lower row.

TABLE 29. Field mean performance of 84 maternal F_2 and 15 parental polycross lines of birdsfoot trefoil for growth habit and pod setting*

	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	3.3 5.5	3.4 2.9	3.0 6.8	3.9 5.4	3.2 4.7	3.6 4.3	2.9 6.8	3.5 4.0	4.5 4.3	3.5 5.5	3.1 5.3	3.2 6.1	3.0 6.5	3.3 3.0	3.7 6.0
M_2		3.4 4.0	3.6 4.4	3.5 3.9	2.9 4.5	3.8 5.0	4.3 2.2	3.0 5.3	3.6 3.9	3.4 3.5	3.1 5.0	4.1 3.2	2.9 4.2	3.0 4.5	2.9 7.5
M_3			3.7 1.8	4.0 2.9	3.6 3.5	3.1 7.0	3.7 4.6	3.9 4.1	3.0 5.5	3.3 1.5	3.6 6.0	2.9 5.6	2.9 5.5	2.9 7.0	3.8 4.4
M_4				4.0 2.0	2.3 7.5	2.8 5.2	3.7 4.3	2.9 6.2	4.3 4.9	3.6 3.4	2.9 6.3	4.3 6.3	3.2 6.9	4.0 3.7	3.6 2.9
M_5					3.6 1.9	3.7 4.5	3.7 5.0	3.0 5.0	3.7 3.8	3.8 5.0	2.9 4.8	3.8 5.4	4.2 3.7	4.1 5.0	4.0 4.2
M_6						3.2 3.0	2.8 5.4	3.1 7.5	3.5 4.1	4.2 4.4	2.7 5.6	3.1 6.4	3.1 5.7	3.8 2.9	4.0 6.0
M_7							3.7 4.5	3.1 5.0	3.0 5.3	3.7 3.6	3.5 6.0	3.9 3.6	3.6 7.5	3.2 5.4	3.7 6.7
M_8								3.6 1.6	3.4 4.6	3.5 4.4	4.0 3.3	4.1 4.5	3.1 5.3	3.5 3.1	3.8 4.1
Leo parental means									3.6 4.7	3.3 3.2	3.3 5.6	3.4 6.0	3.5 4.5	2.8 2.0	2.9 2.9

*Growth habit in first row, and pod setting in lower row.

TABLE 30. Field mean performance of 84 maternal F_2 and 15 parental polycross lines of birdsfoot trefoil for vigor index and winterhardiness*

	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	2.8* 4.3*	2.9 4.8	2.0 3.5	2.5 4.1	2.1 4.7	2.0 4.1	2.1 4.1	2.2 5.0	3.6 2.9	3.0 3.1	3.3 5.1	2.9 3.1	2.6 2.5	2.4 4.6	2.7 3.5
M_2		3.0 4.4	1.9 3.5	2.5 3.5	1.9 2.8	2.9 5.6	2.0 3.5	2.5 3.0	2.9 4.3	2.6 3.0	3.0 3.8	2.9 4.5	2.8 4.1	2.8 3.5	2.9 4.3
M_3			2.5 4.1	2.7 4.6	2.3 3.4	2.8 3.3	2.4 3.6	2.3 4.2	2.3 2.9	2.5 3.7	2.4 4.1	2.4 3.9	3.4 4.0	3.1 4.6	2.1 3.7
M_4				2.8 2.8	2.3 4.7	2.9 4.0	2.2 3.5	2.4 3.9	3.0 4.2	2.9 4.3	2.8 4.9	2.8 3.1	2.4 4.7	3.0 3.4	2.7 3.1
M_5					2.3 2.9	2.0 4.8	3.2 4.1	1.4 5.0	2.9 4.7	2.8 3.0	2.9 4.3	2.4 4.2	3.0 3.9	2.5 3.5	2.6 3.4
M_6						2.3 3.9	2.1 4.1	2.1 3.9	2.9 4.2	2.3 2.0	3.0 4.4	2.4 3.9	2.9 3.0	3.2 3.4	2.8 4.6
M_7							2.6 6.1	3.0 3.9	3.2 4.3	2.3 4.1	2.3 4.7	2.6 2.2	3.0 3.0	2.6 3.8	2.4 3.8
M_8								2.4 4.8	3.0 3.9	2.3 2.9	2.6 4.3	2.2 4.1	2.6 3.6	2.5 3.8	2.5 4.3
Leo parental means									3.2 4.5	2.5 4.9	2.7 4.3	2.8 3.5	3.5 3.7	2.5 4.1	2.9 3.0

*Vigor index in first row, and winterhardiness in lower row.

TABLE 31. Field mean performance of 84 maternal F_2 and maternal polycross seed of 15 birdsfoot trefoil clones for spring growth and forage yield*

	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_1	247* 1106*	244 1086	286 982	198 772	238 1071	249 1182	220 861	253 1214	240 939	152 621	221 1160	277 848	287 722	227 1098	232 869
M_2		244 1176	243 867	280 1053	186 557	268 1245	182 728	256 854	245 1058	221 733	284 1331	298 1074	303 1294	271 1078	253 1130
M_3			173 754	236 1107	205 735	332 890	270 920	252 1413	233 671	201 913	267 1022	269 1092	301 1302	291 1323	231 869
M_4				178 682	233 820	326 1353	215 873	260 1036	255 1068	272 1192	271 1316	277 958	311 1417	263 919	182 472
M_5					293 917	247 1145	241 1021	171 983	204 959	176 662	294 1334	302 1422	195 730	193 717	225 726
M_6						221 974	213 904	287 1115	236 1021	166 416	261 1393	225 720	280 1082	247 1002	293 1016
M_7							289 1733	307 1405	236 983	259 1229	191 554	202 1027	298 1178	177 454	231 926
M_8								257 1266	317 1364	243 969	216 762	256 872	266 1011	236 825	236 1125
Leo parental means									269 1209	216 1138	239 1104	243 1044	273 957	255 1168	191 714

*Spring growth in upper row and forage yield in lower row.

TABLE 32. Analysis of variance for field and greenhouse agronomic characters of 84 maternal F_2 and 15 parental[1] lines of two cultivars of birdsfoot trefoil

Source of variation	d.f.	Mean squares (greenhouse)				
		Erectness	No. of stems	Vigor index	Seedling vigor	Aftermath growth
Replications	1	.89	.96	3.18**	.74	1.0**
Treatments	99	.69*	.31	.47*	1.91*	8.75**
Error	99	.51	.28	.33	1.40	5.43

Source of variation	d.f.	Mean squares[2] (field)					
		Growth habit	Pod setting	Winter-hardiness	Vigor index	Spring growth	Forage yield
Replications	2	.05	17.30**	33.06**	4.34**	651872**	18102016**
Blocks within reps.	27	.232	3.03	7.11	.56	9096	900267
Component B	27	.232	3.03	7.11	.56	9095	900256
Treatments[3]	99	.605**	5.96**	3.29**	.52**	6254*	511428**
Intrablock error	171	.147	1.76	1.36	.26	4305	174016
Randomized complete block error[4]	198	.159	1.93	2.14	.30	4959	273050
Total	299	.306	3.37	2.41	.39	9456	402472

*,**Significant at the .05 and .01 levels of probability, respectively.

[1] Parental seed was produced on mother clones from a polycross nursery.

[2] Mean squares for the last 4 characters have been adjusted when the results of the triple lattice analysis showed high efficiency with respect to randomized complete block design.

[3] By treatments is meant the 84 F_2 and 15 parental clones + 1 check entry.

[4] When the lattice analysis was equal to RCBD analysis the RCB error was used to test for significance.

characters, however, the lattice design was more efficient, and thus adjusted treatment means and variances were tested. The results indicate significant variation among the 99 entries for all the characters studied. Significant variation due to replication was also noted for all characters except growth habit.

The comparison of means of the 99 entries on an individual basis utilizing a Duncan's Multiple Range test, has not been made since firstly it does not serve the purpose behind the evaluation of the F_2's and, secondly, because such comparisons cannot be reported in a summarized form for 99 entries. It is of interest, however, to compare the average performance of the parental progeny with the Mirabel x Leo and Mirabel x Mirabel F_2 progeny (Table 33). If we use the standard error of the difference between two means as our test value, we note that, on the average, the means of the three groups are not significantly different from each other for any of the characters. Nevertheless, if we examine the actual mean performance (Tables 27 to 31), we note that the F_2 progeny were superior to their parents in seedling vigor and aftermath growth in the greenhouse, and spring growth and pod setting in the field. Their performance, however, was equal to the overall performance of all the 99 entries. These results, as they appear in Table 33, may oversimplify the picture and indicate non significant differences among treatments. This is not so, however, as indicated by the analysis of variance (Table 32). Nevertheless, we should mention that such results were

TABLE 33. Mean performance (greenhouse and field) of intra-cultivar, inter-cultivar and parental progeny corresponding to 84 maternal F_2 and 15 parental families of birdsfoot trefoil for several agronomic characters

Group	Greenhouse studies				Field studies					
	Erect-ness	Vigor index	Seedling vigor	After-math	Growth habit	Pod setting	Vigor index	Winter-hardi-ness	Spring growth	Forage yield
Mirabel x Mirabel	4.00	2.04	5.35	13.5	3.48	4.93	2.35	4.04	248	1025
Mirabel x Leo	3.96	2.49	5.03	13.5	3.35	4.90	2.70	4.13	245	1012
Leo parental	4.04	2.10	5.01	12.3	3.26	4.13	2.87	4.00	240	1047
Mirabel parental	4.24	2.10	4.8	13.3	3.56	3.04	2.59	4.16	238	1076
Overall mean	4.00	2.30	5.1	13.4	3.4	4.7	2.6	4.09	245	1023
$S_{\overline{d}} = \frac{\sqrt{2\ s^2}}{R}$	0.71	0.57	1.18	2.33	0.31	1.08	0.42	0.95	53.5	340
L.S.D. (.05)	1.4	1.2	2.3	4.6	0.6	2.2	0.8	1.8	105	667
C.V.	18%	24%	23%	17%	12%	29%	19%	29%	26%	41%

not unexpected, since the parental lines can themselves be considered as hybrids that are very likely to contain some good general or specific combinations due to pollination by pollen from clones that are themselves good general or specific combiners, as found in section 4.2.3. In spite of that, however, we can still identify some F_2 combinations that significantly surpass their parental or the overall mean performance (Tables 27 to 31). It was on the basis of existence of such superior F_2 lines that selection has been done and progeny evaluated.

If field performance of these lines can be predicted from greenhouse performance, then one can start a selection program of superior materials in the offseason and test resulting progeny under field growing conditions. The degree of association between different characters under greenhouse and field conditions is reported in Table 34a,b. Correlation between greenhouse characters and field performance has also been calculated to find out which character best predicts field performance (Table 34c). Number of stems was highly and positively correlated with seedling vigor and aftermath. Vigor index also reflects seedling vigor significantly. In the field, however, growth habit was negatively correlated with pod setting, spring growth and forage yield, indicating the superiority of prostrate types over ascending types. Considering, however, the low correlation coefficients (-.16 and -.14) and the non importance of such a correlation in the F_1 hybrids, we can only presume that such

TABLE 34. Correlations among characters: (a) in greenhouse, (b) in field, (c) correlations among some greenhouse and field performance characters, in 84 maternal F_2 and 15 parental birdsfoot trefoil lines

(a)	Erectness	Vigor index	Seedling vigor	Aftermath
No. of stems	-.12	.66**	.52**	.46**
Erectness		-.13**	-.33**	-.15*
Vigor index			.50**	.25**
Seedling vigor				.62**

(b)	Pod setting	Winter-hardiness	Vigor rating	Spring vigor	Forage yield
Growth habit	-.42**	-.11	.00	-.16*	-.14*
Pod setting		.07	.09	.30**	.17**
Winterhardiness			-.12*	.31**	.78**
Vigor rating				.26**	.08
Spring growth					.80**

(c) Characters evaluated in greenhouse	Characters evaluated in field			
	Winter hardiness	Vigor rating	Spring growth	Forage yield
No.of stems	.09	-.07	.12	.12
Erectness	-.07	.03	.07	.00
Vigor index	.09	.02	.27**	.18*
Seedling vigor	-.03	.15	.25**	.19*
Aftermath	-.01	.13	.25**	.22*

*,**Significantly different from 0 at .05 and .01 levels of probability respectively.

small negative associations do not prevent selection of highly productive and upright growing lines. The negative correlation between growth habit and pod setting was also seen in the F_1 hybrids. In general, therefore, the correlations among characters both in the F_1 and maternal F_2 material were of the same direction and magnitude.

It is generally believed that greenhouse and controlled environment experiments do not often reflect field performance. If greenhouse and growth cabinet experiments were to be used to predict field performance, the degree of association between them should be established.

In our investigation, the 99 entries studied during the winter under greenhouse conditions were also studied in the field during the summer. Some selections of maternal F_2 crosses have been made according to greenhouse performance. For this selection to be of value, the majority of greenhouse selected crosses should prove their superiority under field conditions. Table 34c shows the correlation between greenhouse characters and field performance. Most of the correlations were low and insignificantly different from zero. Vigor index under greenhouse conditions had positive but low correlations with field spring growth and forage yield. The correlations were, however, significantly different from zero. Greenhouse seedling vigor and aftermath were positively and similarly correlated with spring growth and forage yield. The correlation coefficients individually were low and do not indicate a large reliability of using seedling vigor or aftermath growth as indicators of field performance. However, if the two characters, seedling vigor and aftermath, were used together to discriminate among the studied lines for superior performance, they would most probably act as a better combined indicator of field performance.

4.3.2 Performance of greenhouse selected genotypes in maternal F_2 and F_3 generations

Twenty-nine maternal F_2 genotypes whose combined seedling vigor and aftermath greenhouse performance exceeded the overall mean of the 99 entries by at least one s.$\bar{d}$., were selected to form a genotypically selected group. Furthermore, the best four seedlings within each of the 29 maternal F_2 genotypes were selected to form a geno-phenotypically selected group. The purpose was to compare these two methods of selection through the performance of their maternal F_3 progeny. From the two isolated nurseries set up containing these two groups, maternal F_3 seed was collected only from the geno-phenotypically selected group as the genotypic group nursery was discarded due to contamination. The resulting 29 F_3 families were evaluated together with 15 parental lines in a microplot technique under controlled environments (see section 3.3.2 and appendix for description of technique).

The comparative performance of the selected genotypes, both in maternal F_2 and F_3 generations, is presented in Table 35, along with parental means and heterosis of the F_3 genotypes over their

TABLE 35. Comparative mean performance of selected maternal F_2 crosses in greenhouse and field studies, and their respective maternal F_3 mean seedling vigor performance in growth cabinets

Selected cross	Greenhouse (F_2)		Field (F_2)		Growth cabinet (F_3)	Heterosis		Growth cabinet parental means
	Seedling vigor(g)[1]	Aftermath[2]	Spring growth[3]	Forage yield[4]	Vigor[5]	% H.P.[6]	% M.P.[6]	
M_1M_3	6.3	15.5	286	982	6.20	170**	176**	M_1=3.40
M_1L_3	5.8	14.9	221	1160	4.40	129	129	M_2=3.42
M_1L_4	5.8	14.6	277	848	5.88	173**	176**	M_3=3.64
M_2M_4	6.1	15.5	280	1053	4.78	131	135	M_4=3.66
M_2M_6	5.9	17.3	268	1245	5.08	217	137*	M_5=3.94
M_2M_8	5.7	15.5	256	854	5.27	417**	150**	M_6=3.98
M_2L_3	6.0	16.9	284	1331	5.19	152**	152**	M_7=3.88
M_2L_4	5.7	16.3	298	1076	5.39	158**	160**	M_8=3.59
M_2L_5	6.2	14.0	303	1294	4.76	139*	139*	L_1=4.06
M_3M_7	6.4	15.9	270	920	5.10	131*	134*	L_2=3.27
M_3M_8	5.6	16.0	252	1413	4.92	135*	136*	L_3=3.40
M_3L_5	6.2	16.3	301	1302	5.46	150*	155**	L_4=3.30
M_3L_6	5.8	14.9	291	1323	4.72	120	125*	L_5=3.41
M_4M_6	5.9	15.5	326	1353	5.50	138**	144**	L_6=3.94
M_4L_2	5.6	14.8	272	1192	5.96	163**	172**	L_7=3.57
M_4L_3	5.6	15.6	271	1316	4.77	130	134*	
M_4L_4	6.1	15.7	277	958	5.12	140*	147**	

(table continued)

TABLE 35 (continued)

Selected cross	Greenhouse (F_2)		Field (F_2)		Growth cabinet (F_3)	Heterosis	
	Seedling vigor(g)[1]	Aftermath[2]	Spring growth[3]	Forage yield[4]	Vigor[5]	% H.P.[6]	% M.P.[6]
M_4L_5	5.9	16.5	311	1417	4.75	130	134*
M_4L_7	5.7	15.0	182	472	4.20	115	116
M_5L_3	5.8	16.6	294	1334	4.63	117	132
M_6M_8	6.8	16.2	287	1115	4.96	125	131*
M_6L_3	5.8	14.8	261	1393	5.18	130	140**
M_6L_5	6.6	15.0	280	1082	5.72	144**	155**
M_6L_7	6.1	15.3	293	1016	5.31	133	141**
M_7M_8	6.4	15.1	307	1405	5.16	133*	138*
M_7L_5	5.8	15.8	298	1178	5.05	130	139*
M_7L_6	5.8	16.5	177	454	4.32	109	110
M_8L_1	5.7	14.8	317	1364	4.40	108	115
M_8L_5	6.5	19.8	266	1011	5.06	141*	145**
$\overline{X}$	5.98	15.7	276	1133	4.96		

[1] First cut = fresh weight/seedling in grams

[2] Aftermath = mean dry weight/plot in grams

[3] Spring growth = first cut after field establishment year (g)

[4] Forage yield = spring vigor x winterhardiness, where winterhardiness = number of living plants per plot

[5] Maternal F vigor = dry weight per microplot

[6] H.P., M.P. are high parent and low parent respectively

*,**Significant from H.P. or M.P. at the 5% and 1% level of probability according to L.S.D. = t(.05 or .01) $\sqrt{\frac{2\ EMS}{2}}$

respective parents. When correlations were calculated between F_3 performance and F_2 greenhouse and field performance, the coefficients were positive and relatively high. The ability of the maternal F_2 selected plants to pass their seedling vigor attributes to their progeny can be measured by the regression of the F_3 offsprings on the F_2 parents. The regression coefficients can be taken as a measure of heritability. The regression equation of mean performance of 29 F_3 offsprings on their greenhouse F_2 mother seedling vigor was found to be $Y = 3.67 + .4X$. Their correlation was .38. The regression equation of mean performance of 29 selected F_3 offsprings on their field F_2 spring vigor was found to be $Y = 4.4 + .006X$. Their correlation coefficient was .43. The positive regression coefficients found in both cases are indicators that F_3 mean performance is directly proportional to the performance of the parental F_2 and that their relation can be represented by a straight line.

The effectiveness and reliability of selection by the geno-phenotypic method is thus demonstrated. Such results agree with Twamley (1972) who used the same selection method and demonstrated its increased efficiency in identifying superior genotypes relative to the genotypic or phenotypic methods alone.

The superiority of the selected genotypes relative to their parents can be shown by the degree of heterosis maintained by the maternal F_3 over the high parent or mid-parent mean performance. Twenty-two such F_3 families showed significant heterosis, ranging between 25 and 76% over the mid-parental value. It should be noted that among the selected genotypes, the three F_2 lines stemming from the crosses among the M_2, M_6, and L_3 clones, are included. These three clones were earlier demonstrated to have high general combining ability. The evidence of the performance of the crosses M_2M_6, M_2L_3 and M_6L_3 among the selected genotypes at the F_3 level, substantiates their potential performance in producing a synthetic cultivar. The superior performance of the crosses of the selected genotypes can be attributed to the good general combiner clones or good specific hybrid combinations. This further substantiates the importance of both additive and non additive genetic variance in the expression of seedling vigor and plant performance.

4.4 Evaluation of selection methods

Three methods have been basically used for the identification of superior genotypes of birdsfoot trefoil for seedling vigor and forage productivity. The first method made use of deep seeding in a turface medium under controlled environments. Seedling vigor was measured by the mean seedling dry weight. It was demonstrated that even though such vigor was associated with the amount and rate of the seedling hypocotyl, selected vigorous seedlings failed to transmit their vigor to their progeny. However, when single F_1 crosses were made among the selected seedlings and evaluated in the field along with other F_1 diallel crosses, their performance was equal to F_1

diallel crosses and, in general, superior to check entries of unselected genotypes. Table 36 shows the mean field performance of 29 F_1 single crosses among seedlings selected by deep planting technique. In general, single F_1 crosses made among seedlings coming from small size seeds were significantly inferior in field performance to those coming from large seeds, especially in spring vigor, forage yield and total yield. Single F_1 crosses made among seedlings coming from the 2.5 cm depth were generally superior to those made among seedlings coming from the 2.0 cm depth, irrespective of seed size. Some F_1 single crosses, LeL_1 x LeL_2 (2.5), LeL_1 x MiL_2 (2.5), MiL_5 x LeL_5 (2.5), MiL_4 x MiL_5 (2.5), MiL_1 x MiL_2 (2.0), MiL_3 x MiL_4 (2.0), were consistently superior in their field performance to all other crosses in spring vigor, forage yield and total yield. The identification of these crosses was only possible after evaluation of the performance of all the single crosses under field conditions. If, however, no such evaluation is carried out and all the seedlings arising from large seeds planted at 2.5 cm are taken for production of superior synthetic cultivars, the overall performance of their progeny would be inferior to a synthetic containing only the seedlings involved in the identified superior 6 single F_1 crosses. Table 37 shows the mean performance of different groups of single F_1 crosses made among selected seedlings. The performance of the inter-cultivar F_1 crosses among large seeded selections from the 2.5 cm depth, is superior to the performance of the intra-cultivar crosses,

TABLE 36. Mean performance in the field for several agronomic characters of 29 F_1 birdsfoot trefoil crosses made among Leo and Mirabel selected plants arising from two depths of seeding

Cross	Depth (cm)	Growth habit	Pod setting	Winter-hardiness	Seedling vigor	Spring vigor	Forage yield	Total yield
LeL1 x LeL2 (1)	2.5	1.1	1.6	5.5	152*	202*	1130*	2044*
LeL1 x LeL3	2.5	1.5	4.7	5.0	115	166	801	1489
LeL2 x LeL3	2.5	2.3	1.0	5.0	139	170	849	1682
LeL3 x LeL4	2.5	1.6	3.2	4.5	140	158	712	1553
LeL5 x LeL1	2.5	1.7	5.5*	4.0	110	174	706	1366
LeL1 x MiL2	2.5	1.6	2.8	5.0	136	235*	1210*	2028*
MiL5 x LeL5	2.5	1.7	7.1*	4.5	153*	200	900	1816
MiL2 x MiL3	2.5	3.7*	1.8	5.5*	114	180	990	1674
MiL4 x MiL5	2.5	2.5	1.4	5.0	118	212	1062	1770
MiL5 x MiL6	2.5	3.6*	4.8	5.5*	120	155	851	1569
MiL6 x MiL1	2.5	3.5*	3.4	5.0	119	171	854	1565
MiS1 x LeS1	2.5	2.6	4.5	5.0	94	158	790	1354
LeL (check)		1.7	2.1	4.1	104	145	595	1219
Leo (check)		1.5	4.7	4.5	96	136	613	1191
MiL (check)		2.3	2.8	5.0	121	156	782	1508
Mirabel (check)		2.5	2.8	3.5	124	154	614	1359

(table continued)

TABLE 36 (continued)

Cross	Depth (cm)	Growth habit	Pod setting	Winter-hardiness	Seedling vigor	Spring vigor	Forage yield	Total yield
LeL1 x LeL3	2	2.7	2.8	5.5	101	154	821	1425
LeL2 x LeL3	2	2.3	1.6	4.5	134	128	574	1378
MiL1 x LeL2	2	1.1	2.8	3.5	127	153	537	1299
LeL1 x MiL2	2	3.3	3.6	5.0	104	157	783	1408
MiL1 x MiL2	2	2.8	1.2	5.5*	102	195	1068	1681
MiL2 x MiL3	2	6.9*	1.3	5.5*	123	164	912	1651
MiL3 x MiL4	2	3.0	1.3	6.0*	105	168	1010	1639
MiL4 x MiL5	2	1.8	4.7	5.0	119	171	895	1611
MiL5 x MiL6	2	2.7	1.5	4.0	120	194	776	1494
MiL6 x MiL1	2	2.1	2.6	5.0	100	186	916	1516
LeS3 x MiS1	2	2.6	4.4	5.0	90	159	793	1332
MiS5 x LeS3	2	3.2	4.9	4.5	93	152	685	1242
MiS1 x MiS2	2	2.5	4.7	3.5	110	131	516	1175
MiS2 x MiS3	2	1.1	5.3	5.0*	121	144	726	1453
MiS3 x MiS4	2	3.3	4.2	6.0*	127	193	1157*	1918*
MiS5 x MiS6	2	2.2	1.9	4.0	83	70	285	781
MiS6 x MiS1	2	3.8*	1.1*	5.0*	110	146	732	1390
MiS (check)		2.5	2.8	3.5	106	136	465	1101
LeS (check)		2.0	2.7	3.5	115	139	363	874
L.S.D. (.05)		1.01	2.7	1.9	51.6	64.8	478.9	582.3

*Significantly different from their respective check entries at the .05 level of probability as determined by an L.S.D. test.

(1) Le = Leo; Mi = Mirabel; L = large; S = small.

TABLE 37. Mean performance in the field of groups of F_1 single crosses made among growth cabinet seedling selections chosen by the depth of seeding technique

Group [1]	Depth[2] (cm)	Growth habit	Pod setting	Winter-hardiness	Seedling vigor	Spring growth	Forage yield	Total yield
LeL x LeL	2.5	1.6	3.8	4.8	131	174	840	1627
MiL x LeL	2.5	1.6	4.9	4.9	144	218*	1055	1922*
MiL x MiL	2.5	3.3	2.9	5.1	118	179	919	1627
LeL x LeL	2.0	2.5	2.2	5.0	117	141	697	1399
MiL x LeL	2.0	2.2	3.2	4.3	115	155	659	1353
MiL x MiL	2.0	3.2	2.1	5.2	112	179	927	1599
MiS x LeS	2.0	2.9	4.7	4.8	91	155	739	1294
LeS x MiS	2.5	2.6	4.5	5.0	94	158	790	1354
MiS x MiS	2.0	2.6	3.4	4.7	110	137	683	1341
(Mi + Le)/2		2.0	3.8	4.0	110	145	613	1275
(MiS + LeS)/2		2.3	2.8	3.5	111	137	414	987
(MiL + LeL)/2		2.0	2.5	4.5	112	151	678	1352
L.S.D. .05		1.01	2.7	1.9	51.6	64.8	478.9	582.3

*Significantly larger than the (Mi + Le)/2 at the .05 level of probability.

[1] Groups signify inter- and intra-cultivar F_1 single crosses among seedlings chosen from large or small seeds, as well as non selected check entries. Le = Leo; Mi = Mirabel; L = large; S = small.

[2] Depths from which parent seedlings were chosen by the depth of seeding technique.

although not significantly so, but is significantly superior to the mean performance of the non-selected Mirabel and Leo checks ((Mi + Le)/2). The mean performance of both intra-cultivar groups was similar.

The second selection method depended on a more complex evaluation of selected clones for their combining abilities when diallel crosses were made among all the clones involved. The diallel analysis permits the identification of clones that have superior general combining abilities. These clones can be combined into a synthetic cultivar. We have shown earlier that through the combining ability analysis three good general combiners were identified: M_2, M_6 and L_3. The performance of the F_1 single crosses arising from these clones was compared with other F_1 single crosses and the overall mean of the 105 F_1 diallel crosses, and established the superiority of such a potential synthetic. Nevertheless, some specific hybrids had also indicated superior performance. Their superiority was due to non additive gene action, and their maternal F_2 performance was, in general, not associated with their F_1 performance, an indication that non additive gene effects were not completely passed from parents to offsprings.

The third selection method (geno-phenotypic method) is not based on the knowledge of general or specific combining ability of selected clones, and can be applied to any series of crosses made among selected clones. The effectiveness of this method depends on identifying single F_1 crosses that were able to transmit their performance to F_2 progeny regardless of the type of genetic variance involved. The selection of the F_1 crosses is really done on the basis of maternal F_2 and F_3 progeny tests.

It should be realized that all the three methods started out from selected genotypes. The first method was based on the superiority of seedling vigor and forage productivity associated with large seeds of birdsfoot trefoil. The two other methods were based on the performance of clones selected in the field for fall vigor.

The critical evaluation of the three methods can only be done at the F_1 level since only data related to F_1 single crosses is available for the genotypes identified by all the three methods. Comparisons of the performance of maternal F_2 selected crosses can be done for the last two methods, but not the first. We shall focus, in this evaluation, only on the superior genotypes identified by each of the three methods.

Mean field performance of the top genotypes identified by the three selection methods at the F_1 and F_2 levels is presented in Table 38. Means of all selected genotypes by any one method for seven field agronomic characters are also shown in Table 38. A comparison of the performance of the selected genotypes on an individual basis reveals a certain degree of variation within groups or among groups. Individual comparisons, however, are not indicative of the average merits of any one selection method. The comparison of

TABLE 38. Comparative F_1 and F_2 mean field performance of birdsfoot trefoil crosses of genotypes chosen by three selection methods

Cross		Growth habit	Pod setting	Winter-hardiness	Seedling vigor	Spring growth	Forage yield	Total yield
LeL1 x LeL2 (2.5)	(1)	1.1	1.6	5.5	152	202	1130	2044
LeL1 x MiL2 (2.5)		1.6	2.8	5.0	136	235	1210	2028
MiL5 x LeL5 (2.5)		1.7	7.1	4.5	153	200	900	1816
MiL4 x MiL5 (2.5		2.5	1.4	5.0	118	212	1062	1770
M1L1 x M1L2 (2.0)		2.8	1.2	5.5	102	195	1068	1681
M1L3 x M1L4 (2.0)		3.0	1.3	6.0	105	168	1010	1638
Method 1 mean		2.1	2.6	5.3	128	202	1063	1830
M2M6	(2)	5.6	1.0	5.0	124	193	1057	1798
		3.8	5.0	4.1	- (3)	268	1245	-
M2L3		3.7	1.0	5.5	117	179	970	1672
		3.1	5.0	3.8	-	233	820	-
M6L3		4.1	1.8	5.5	141	230	1268	2116
		2.7	5.6	4.4	-	261	1393	-
Method 2 mean		4.5	1.3	5.3	127	201	1098	1862
		3.2	5.2	4.1	-	254	1153	-

(table continued)

TABLE 38 (continued)

Cross	Growth habit	Pod setting	Winter-hardiness	Seedling vigor	Spring growth	Forage yield	Total yield
M2L3	3.7	1.0	5.5	117	179	970	1672
	3.1	5.0	3.8	-	233	820	-
M3M8	4.3	1.0	5.5	58	122	671	1016
	3.9	4.1	5.6	-	252	1413	-
M3L5	3.5	1.7	5.5	141	150	828	1671
	2.9	5.5	4.0	-	301	1302	-
M3L6	3.6	1.8	5.5	130	170	922	1702
	2.9	7.0	4.6	-	291	1323	-
M4M6	5.0	1.1	3.5	125	173	617	1365
	2.8	5.2	4.0	-	326	1353	-
M4L3	3.7	1.4	4.5	118	133	604	1313
	2.9	6.3	4.9	-	271	1316	-
M4L5	3.1	2.7	3.5	100	151	523	1125
	3.2	6.9	4.7	-	311	1417	-
M6L3	4.1	1.8	5.5	141	230	1268	2116
	2.7	5.6	4.4	-	261	1393	-
M7M8	3.2	4.6	3.5	93	127	447	1007
	3.1	5.0	4.6	-	307	1405	-
M8L1	3.0	1.7	5.5	126	158	857	1611
	3.4	4.6	3.8	-	266	1011	-
Method 3 mean	3.7	1.9	4.8	115	179	771	1460
	3.1	5.5	4.4	-	282	1275	-

(1) Depth of seeding from which selection was made, and figures show only the F_1 mean performance.

(2) F_1 and F_2 mean performance in upper and lower rows respectively.

(3) Seedling vigor and forage yield of F_2 crosses were not measured.

the means of the three groups permits us to make some conclusions around the effectiveness of each individual method in identifying superior genotypes. On the basis of F_1 mean performance, both methods 2 and 3 identified genotypes significantly superior in upright growth habit to method 1, while they themselves were not significantly different at both F_1 and F_2 levels. In pod setting method 1 was superior to both methods 2 and 3. For F_1 winterhardiness and seedling vigor, methods 1 and 2 were equally effective and both superior to method 3, while for F_2, methods 2 and 3 were equally reliable in identifying genotypes with superior winterhardiness. For F_1 spring growth the first two methods were of equal effectiveness and were both superior to method 3, while for F_2, methods 2 and 3 were equally effective. If the combined effect of winterhardiness and spring growth, i.e., forage yields, are compared at the F_1 level, methods 1 and 2 are obviously superior to method 3. The same is true for the total yield when assessed at the F_1 level. At the F_2 level, however, methods 2 and 3 were equally effective in identifying genotypes of superior forage yields.

On the average, therefore, if the selection of superior genetic material can only be done on the basis of F_1 mean performance, methods 1 and 2 can be equally effective and more so than method 3. But if the superiority of the identified crosses in the F_1 generation is to be utilized in upgrading cultivars, this superiority must be passed to maternal F_2 and later generations. In other words, this superiority must be due to a genetic variance that can be transmitted from parent to progeny. In this respect, the merits of the first method cannot be established with confidence since we do not have any idea about the stability in further generations of the progeny of the genotypes selected by this method. We must appreciate the fact that the vigorous seedlings (parents of the identified superior F_1 single crosses) selected by a deep seeding technique, originated from open pollinated seed from the two cultivars. This seed could conceivably have equally come from plants (clones) possessing high general or specific combining ability. This combining ability could either cause increased or decreased vigor in the F_2 or F_3 maternal progeny. The best way of finding out the combining ability and performance of the six crosses identified by the first method is to evaluate their polycross maternal F_2 and F_3 progeny produced in isolated nurseries. However, if selection method 1 were used with large seed taken from a restricted nursery of clones previously evaluated and selected, it would have considerable merit in identifying superior genotypes within these clones.

In evaluating method 2 the choice of the three clones (parents of the 3 F_1 crosses) was done on the basis of high general combining ability measured by a diallel analysis of 105 diallel crosses among 15 clones preselected for fall vigor. The basis of selection, therefore, was mainly the additive genetic effects possessed by these clones for most of the characters studied. The variances associated with these clones were predominantly additive in nature. What all this means is that the performance of these clones or crosses among them is most likely to be passed to advanced generations. Moreover, totally on the basis of maternal contribution of these crosses, they were found to maintain this superiority in the F_2 and F_3 generations. Evidence for this is quite clear from the mere fact of identifying the three single crosses, M_2M_6, M_2L_3, and M_6L_3, by the geno-phenotypic selection method 3, as well as the heterosis of their F_3 progeny demonstrated over the parents (Table 35).

An inconsistency of performance between the F_1 and F_2 generations within the crosses identified by method 3 was observed, especially for the characters spring growth and forage yield. The F_1 performance of the crosses identified by method 3 was significantly lower than the performance of the crosses identified by either method 1 or 2. Their F_2 performance in these two characters was, however, superior to method 2 mean performance. This throws some doubt on the type and origin of gene action causing such variation in performance. The F_3 mean performance of these crosses was also inconsistant with either F_1 or F_2 performance, and on the average, some of these crosses identified by method 3 were not significantly superior to their parental means (Table 35). The variability in performance from F_1 through F_2 and F_3 can be due to a number of factors. Firstly, the hand-crossed F_1 generation represented systematic interpollination. The field nurseries used for the production of F_2 and F_3 seed were dependent on pollinators that are believed to behave in a non-random manner. A second possible cause of this generation to generation fluctuation may be the small sample size used in the evaluation. By chance an atypical seed sample can be drawn that does not accurately reflect gene frequencies. A third possibility is associated with the progress of the population towards the Hardy-Weinberg equilibrium. The possibility of *Lotus corniculatus* being an autotetraploid and not reaching equilibrium in the F_2 cannot be ignored.

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1 Introduction

It is generally agreed among forage breeders that the most important problem limiting the acceptance of an expansion of birdsfoot trefoil culture is the difficulty of stand establishment believed to be mainly due to inferior seedling vigor. Although it does not compare with alfalfa in forage productivity, its ability to persist and produce under conditions unfavorable to alfalfa growth makes it rather unique.

In their effort to increase seedling vigor and forage productivity in the crop, birdsfoot trefoil breeders attempted to increase seed size and introduce more genetic variability. Increasing seed size by recurrent selection has been found to increase seedling vigor, but not all large seeded lines displayed comparable seedling vigor response. Crosses among cultivars of widely separated gene pools have been noted to exhibit higher vigor characteristics.

In the present investigation attempts were made to select vigorous lines within large-seeded families and evaluate the efficiency of this selection. Furthermore, projections were made on the amount of variation attributed to genotype as opposed to environment. The breeding behaviour of seedling vigor, forage productivity and other agronomic characters was studied in diallel crosses among 15 clones selected for fall vigor from two diverse birdsfoot trefoil cultivars, Leo and Mirabel. The possibility of selection of superior genotypes from the F_1 or maternal F_2 generations was evaluated. Three possible selection methods were discussed. The investigations were carried out at three levels of environment and by two different procedures. Studies on the effect of seed size and depth of seeding on seedling vigor and other characters were carried out mainly under controlled environment growth cabinets. The diallel crosses and advanced generations were investigated mainly under field conditions. Several seedling and plant characters were measured, and different experimental designs, depending on the nature of the material, were utilized. The statistical analysis and evaluation of breeding behaviour indicated the following results.

5.2 Effect of size, cultivar, and depth on seedling vigor characters

While the two cultivars were significantly different in stem thickness and plant height, depth of seeding caused variation only in root weight. The interaction of depth and cultivar showed differences in aftermath growth, root weights, and total yield. The most striking differences for almost all seedling characters were found among different seed sizes. Large seeds seem to have imparted an increase in all characters except seedling erectness and number of stems. The per cent and rapidity of emergence of different size seeds indicated a large degree of variation. When the data for seedling vigor of seedlings coming from large seeds planted at different depths were plotted (Figures 1, 2, 3), the frequency of vigorous seedlings identified from large seeds placed at 2.5 cm was higher than others. The depth of seeding seemed to have some selective pressure against seeds with inferior vigor. If seedling vigor was measured by the ability of the seed to produce a long hypocotyl within a short period of time, irrespective of the physiological and biochemical factors inducing this, then the depth of seeding can be utilized to select for inherently vigorous seedlings. When such a technique was used and the progeny of the selected seedlings were evaluated, they seemed to sustain their increased vigor over non selected seedlings in the same size category. The increase was, however, not statistically significant, and this was taken as an indication that the type of seedling vigor selected for by deep seeding was not completely controlled genetically. Explanations were presented to cover these findings.

5.3 Diallel analysis, combining abilities, heritabilities and genetic advance, correlations among characters, and predictions of superior genotypes

A diallel analysis was conducted on the 105 inter-cultivar diallel F_1 crosses according to Griffing's (1956b) method 4 model 1. The same analysis was conducted on the Mirabel intra-cultivar F_1 crosses and the Leo intra-cultivar F_1 crosses.

The analysis of variance indicated significant differences among the 105 F_1 crosses in all characters studied. The intra-Mirabel F_1 crosses were significantly different for all characters except winterhardiness and spring growth, while the intra-Leo F_1 crosses were significantly different only in pod setting and spring growth. Diallel analysis was not conducted on characters for which the F_1 crosses were not significantly different.

Variance due to general combining ability, GCA, was significant for all characters studied in both the inter-cultivar and the two intra-cultivar diallels, whereas specific combining ability, SCA, variances were not significant for any of the characters. The relative importance of general and specific combining abilities was evaluated by three methods: firstly, by comparing the magnitudes of their variances; secondly, by the ratio of SCA sum of squares to the GCA sum of squares; and thirdly, by the relative ratio of GCA sum of squares to the total genotypic sum of squares. It was shown that direct comparisons of variance magnitudes almost always result in the under-estimation of the importance of non additive gene action. The second method often identified larger variation due to SCA compared with GCA, and is thought to be very useful in reflecting the importance of both types of gene action. The coefficient of determination (third method) reflected the magnitude of additive genetic variance compared with the total genetic variance and enhanced the power of discriminating between the two types of gene action. In a further attempt to study the variance of various characters in birdsfoot trefoil, the phenotypic variance was subdivided into its components, the genotypic and the environmental variances. The genotypic variance is composed of both additive and non additive variance. Comparisons among the components of phenotypic variance indicated that for growth habit and pod setting, additive variance was more important than any other component, and contributed around 60% to phenotypic variance. It was concluded that, for these two characters, selection can be started in early generations. The large environmental variance associated with winterhardiness makes it mandatory to conduct selection in advanced generations and over environments and years. The contribution of different variance components was almost equal for seedling vigor, spring growth, and forage yield.

Broad sense (BS) heritability estimates were always larger than narrow sense (NS) estimates for all characters. Narrow sense estimates were high (.55 to .69) for the two characters, growth habit and pod setting; around .25 to .33 for winterhardiness; .27 to .48 for seedling vigor, spring growth, forage yield, and total yield.

The genetic coefficient of variability was highest for growth habit and pod setting and lowest for winterhardiness. This indicates the advantages of introducing new germplasm of superior winterhardiness into the gene pools of the two studied cultivars.

Phenotypic correlations among characters indicated that by selecting for more erect type growth habit in the material studied, some loss in pod setting is most likely to occur. With the problem of shattering in birdsfoot trefoil, this can likely indicate less seed production and higher seed prices for erect birdsfoot trefoil types. A non significant low correlation between winterhardiness and seedling vigor indicates the possibility of improving these two characters separately. Thus, the generally accepted indication that more vigorous types would be lacking in winter survival is rather disputable.

Correlations among other characters were mostly positive and ranged between .20 and .88. This is a good indication that mutual improvement of these characters is likely to occur due to selection for any one of them.

The estimation of GCA effects and variances associated with each individual clone used in the diallel crosses, is a powerful tool that permits the identification of superior genotypes that can be used in the production of synthetic cultivars. Utilizing such estimates, three clones, M_2, M_6, and L_3, were identified. Their performance in hybrid combination in the F_1 and F_2 generations indicated that their superior performance can be attributed mostly to additive gene effects and slightly to specific effects.

From a separate analysis of the two intra-cultivar diallels, it was found that the same three clones can be identified. Accordingly, it was concluded that, since the number of crosses resulting from partial diallels is much less than complete or half diallels, the partial diallel mating designs are more profitable in identifying a larger number of clones for the production of synthetic cultivars in birdsfoot trefoil.

5.4 Maternal F2 performance and evaluation of selection methods

Significant differences in greenhouse performance were observed among the 84 maternal F_2 and the 15 parental types for seedling erectness, vigor index, seedling vigor, and aftermath growth. In the field the same genotypes were significantly different for all the characters studied: growth habit, pod setting, vigor score, winter-hardiness, spring growth, and forage yield. The comparison of the parental progeny with the Mirabel x Leo and Mirabel x Mirabel F_2 genotypes indicated no significant differences among their mean greenhouse or field performance for any of the characters studied. The equivalent performance of the parental progeny can be explained on the basis of existing good general combinations and specific combinations among the 15 clones. Since the seed of the parental progeny originated from a polycross nursery of the 15 original clones, it can be considered as maternal F_1 seed carrying a lot of good combinations. Nevertheless, many maternal F_2 crosses showed superior performance to the overall mean of the F_2 population. All the maternal F_2 crosses surpassing the overall average by at least one standard error were identified, and the best four plants within the best lines were selected to form a geno-phenotypic group. Polycross seed from this group (maternal F_3) was tested for seedling vigor performance. The regression of F_3 seedling vigor on both greenhouse and field F_2 seedling vigor and spring growth for the 29 selected genotypes was high and positive. This leads us to conclude that the geno-phenotypic selection method was quite efficient in identifying superior genotypes among single crosses. There was also a positive regression coefficient for the regression of field spring growth and forage yield on greenhouse seedling vigor and aftermath growth. Hence, the greenhouse performance can be utilized to predict field performance without much loss in precision. This can help speed up a recurrent selection program. Significant heterosis for most of the maternal F_3 crosses over the parental seedling vigor performance was observed.

Selection of genotypes superior to average mean performance of the materials investigated was achieved by three methods: method 1 used the advantages of deep seeding and intercrossing of vigorous seedlings for evaluation in F_1 hybrids. Twenty-one F_1 hybrids originating from seedling selections coming from large seed planted at 2.5 and 2.0 cm depths were evaluated. The results indicated the superiority of inter-cultivar F_1 crosses among seedlings selected from 2.5 cm depth compared with those selected from the 2.0 cm depths. The top six F_1 hybrids were compared with the top three hybrids identified from the diallel analysis and the top 10 hybrids identified by the geno-phenotypic method.

Although on the average the first two methods were found to be more efficient than the third in identifying superior genetic material, it was established that the three clones M_2, M_6, L_3 identified by the second method (diallel studies) were most reliable for increasing seedling vigor and forage productivity in a potential synthetic cultivar. Their superiority was due mostly to the size of additive genetic variance they exhibited in the F_1. This type of genetic variance was mostly responsible for their superior performance in F_2 and F_3.

Generally, then, it is felt that although seedling vigor in birdsfoot trefoil can be increased by recurrent selection for larger seed size, the identification of the heritable portion responsible for variation in seed size and the subsequent selection within the large seeded material would most likely enhance the improvement of seedling vigor in this species. The introduction of exotic germplasm into the two cultivars studied and the use of partial diallel mating designs for the identification of potential clones with high general combining ability that can be combined in synthetic cultivars, would also improve birdsfoot trefoil.

While simple breeding methods like mass selection can be effective for the improvement of growth habit and pod setting, more complex breeding methods are needed for the improvement of winter-hardiness, seedling vigor, and total forage productivity. It is felt, in general, that most advance can be achieved by introducing more variability in the germplasm pool, selecting and screening large seeded types, and subsequent combination of the best general combining lines as evaluated by partial diallel mating designs.

CHAPTER VI

CONTRIBUTIONS TO KNOWLEDGE

1. The effect of seed size, cultivar, and depth of seeding on seedling vigor of birdsfoot trefoil were previously established. Relations between seed size-seedling vigor and physiological and biochemical activities in the seed have also been studied.

In this investigation, the use of a deep seeding technique for the selection of inherently vigorous seedlings within large seeded classes, and the evaluation of the effectiveness of this technique have not been done previously. Through the use of the technique, information was made available on the importance of genotype and environment in the determination of seed size-seedling vigor relationship in two birdsfoot trefoil cultivars.

2. Although two earlier reports have dealt with the magnitude and importance of general and specific combining ability in some cultivars of birdsfoot trefoil, this is the first comprehensive study of the different components of genotypic and phenotypic variances, as

well as the heritabilities, genetic advance, and coefficient of genetic variability for agronomic characters in the two gene pools of Mirabel and Leo birdsfoot trefoil cultivars.

3. The comparisons of general and specific combining abilities, other variances, and heterosis as they occur within and between two diverse sources of germplasm is an original contribution.

4. The use of three methods for the estimation of the comparative importance of general and specific combining abilities in birdsfoot trefoil is also original.

5. The evaluation of an advanced generation (F_3) by a micro-plot technique under controlled environments is new. The establishment of the validity of the technique through the regression of offsprings on parents is of great potential value in speeding up a breeding program.

6. Valuable information was obtained on the usefulness of half and partial diallel analyses to identify superior genotypes.

7. The use of three selection methods and the comparison of their relative effectiveness in identifying superior genotypes is also an original contribution.

In general, the author believes that the information obtained is a valuable contribution to our theoretical knowledge concerning breeding methods for forage crop improvement and indicates practical guidelines for a birdsfoot trefoil breeding program.

CHAPTER VII

SUGGESTIONS FOR FURTHER RESEARCH

It is a generally accepted belief that if most research projects are conducted with clear and well defined objectives, the results obtained will answer to most of the objectives. Nevertheless, it is also understood that some more questions may arise as a result of the investigation, and depending on the methodology used, some suggestions for modifying objectives and methodology are most likely to be made. According to the present investigation, the following suggestions for further research can be made.

1. With the objectives of further establishing the reliability of the selection by deep seeding technique, and the measurement of the relative importance of environment and genotype in the control of seed size-seedling vigor complex, investigations can be made as follows: Firstly, the reliability of the deep seeding technique can be established by using the technique to select within large seed produced on clonal selections of two cultivars, whereby existing

difference between the two genotypes can be measured. If progeny of the selected seedlings within each clone are evaluated, the correlation between their performances must be similar to the correlation between the performances of the original clones. Furthermore, the regressions of progeny on parental clone performance should be positive.

It is suggested, however, the utilization of different inactive germination media of finer texture to remove any probable physical impediments to the emerging seedling. It is suggested, also, that further studies be made to establish the magnitude of environmental effects in the control of seed size within clones of birdsfoot trefoil.

2. The diallel analysis has established the large importance of general combining ability in the control of all characters studied. Nevertheless, comparisons of non additive and additive components of genetic variance have shown the relative importance of non additive gene action in the control of most characters other than growth habit and pod setting. The Griffing's analysis cannot explain the nature of this non additive variation. We suggest that such variation in a tetraploid can be further clarified and attributed to digenic, trigenic, and quadrigenic effects or their interactions. If a computer program can be designed to handle the analysis of an autotetraploid diallel, its use in birdsfoot trefoil can very likely come out with further clarification of the importance of non additive gene action. This can help make our breeding programs based on a more sound genetic basis.

3. Considering the amount of time, effort, and money involved in the study of half diallels, and the reliability of partial diallel designs in providing similar information, the use of partial diallels on large numbers of clones representing all the variability in any gene pool of birdsfoot trefoil, is highly recommended in order to select clones for the production of synthetic cultivars.

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APPENDICES

APPENDIX 1

DESCRIPTION AND EVALUATION OF MICROPLOT TECHNIQUE

The use of a microplot technique for the evaluation of F_3 and parental lines might have introduced a confounding factor between genotypic differences and the environmental effect of the microplot (Section 3.3.2).

To test for the existence of such confounding, two phenotypically and genotypically different birdsfoot trefoil cultivars were evaluated for seedling vigor utilizing the same microplot technique and under the same set of environmental conditions. The two cultivars, Viking and Sask Comp, were seeded alternatively in microplots and were labelled V_1, S_1, V_2, S_2, V_3, S_3, etc., where V_1 = Viking plot number 1. A randomized complete block design with four replications was utilized for the test. Thirty-six days after planting, the seedlings from each microplot were cut and weighed. The data and analyses of variance are shown in Appendix Tables 1 and 2.

APPENDIX TABLE 1. Mean dry shoot weights (g) for 10 Viking and Sask Comp microplots in 4 replications

	Rep.I	Rep. II	Rep.III	Rep. IV	Average
V	6.56	6.82	6.70	6.92	6.75
S	5.23	5.14	4.98	5.32	5.17
V	6.78	6.65	6.93	7.02	6.84
S	5.15	5.07	5.18	5.36	5.20
V	6.63	6.78	6.85	6.89	6.79
S	5.03	5.25	5.06	5.18	5.13
V	6.91	6.98	6.61	6.75	6.81
S	4.98	5.09	5.13	5.23	5.11
V	6.67	6.80	6.91	7.00	6.85
S	5.31	5.02	5.17	5.09	5.15
L.S.D. .05					.24

APPENDIX TABLE 2. Analysis of variance of both cultivars combined and separated

Source of variation	d.f.	SS	MS	F
Combined				
Replications	3	.14	.046	3.01*
Entries	9	27.61	3.06	197.9***
Error	27	.419	.0155	
Total	39	28.17		
Viking alone				
Replications	3	.1067	.0355	1.87
Entries	4	.026	.0065	.34 NS
Error	12	.228	.0190	
Total	19	.361		
Sask Comp alone				
Replications	3	.0549	.0183	1.34
Entries	4	.0148	.0037	.27 NS
Error	12	.1653	.0137	
Total	19	.2351		

The analysis of variance of the whole experiment indicated significant differences among the treatments. Mean seedling vigor of the Viking cultivar was superior to that of the Sask Comp (an already known fact). The analysis of variance for differences among microplots belonging to the same cultivar did not indicate any significant differences. If confounding of genotypic differences with the environment of the microplot was existent, the following could have been indicated: (1) Viking plots were not always superior to Sask Comp plots; (2) adjacent microplots of the two cultivars were not significantly different; and (3) plots belonging to the same cultivar were significantly different from each other.

Such results were not observed and thus no confounding was existent.

APPENDIX 2

SEEDLING VIGOR EVALUATION

The method of measurement of seedling vigor is a critical consideration in studies of the sort that has been described in this thesis. In this work it was decided to allow seedlings to develop to a size large enough to overcome the manual errors in the quantitative estimate of weight due to differences in cutting height and other manual errors.

For stand establishment in the fall it is believed that a critical stage of development for winter survival is the five-leaf stage. The exact stage of growth for spring-seeded material to establish successfully under a range of environmental conditions is not so well understood. A suggested method of evaluation that could be used in further studies is the time required for a seedling to reach a critical developmental condition such as the five-leaf stage. The shortest time requirement would be a useful selection criterion.