In the name of GOD the most beneficent the most merciful

QUANTITATIVE GENETICS OF PROSTRATENESS AND OTHER RELATED ATTRIBUTES IN RED CLOVER (*Trifolium pratense* L.)

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy
in Plant Breeding and Genetics
Department of Plant Science
Massey University
New Zealand

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1993

ABSTRACT

Three major experiments were conducted to investigate quantitative genetic aspects of prostrateness and related attributes in red clover (*Trifolium pratense* L.) during the years 1991-1993. These were done on several red clover genotypes with prostrate growth habit, nodal rooting ability, and early flowering characteristics, together with several other genotypes from semierect and erect types.

Three types of experiments were carried out:

1) Since genotype environment interaction is believed to be ubiquitous in affecting the performance of plants, a series of experiments were carried out in order to get general information on a range of red clover germplasm representative of the three distinct types of red clover. Twelve genotypes (four per type) were studied in a randomized complete block design with three replications at two sites for two successive years. Several techniques of univariate and multivariate analysis were applied in order to quantify and qualify the magnitude and pattern of the possible genotype-environment interaction effects. Phenotypic and genotypic correlation values were estimated for each year and type separately as well as for the whole data set in genotype-environment interaction experiment.

As a result of GE interaction analysis, a large amount of genetic variation was found in the genotypes examined. Several attributes presented significant first and second order interaction effects. Multivariate discriminant analysis based on these effects revealed discriminant scores by which the contribution and importance of each attribute in the response of genotypes examined in the environments was studied. Cluster analysis revealed that each of the three red clover types have their own particular responses to the environment effect. Phenotypic and genotypic correlation patterns were different from year to year and type to type. Prostrate growth habit reduced dry matter yield through significant negative correlation with yield components.

2) One accession from each of the two extreme types, erect and prostrate, were examined using a hierarchical mating design to investigate their genetic structure and to obtain more detailed genetic information on a narrower germplasm. Nine random plants from each type were cloned and used as male parent. Each male parent was crossed to six different random plants as female parents, three from the same population and three from opposite population. In other words four sets of crosses, two intra- and two inter-population sets, were made. To evaluate the 108 progeny families produced, male groups were divided into six sets, each containing three male groups from the same type. Each set was examined in a randomized

complete block design with three replications. Various genetic parameters including genetic variance components and heritability of several morphological attributes were estimated.

The two plant populations examined by the mating design, presented different patterns of genetic variation. Although the prostrate population did not have much genetic variation, its additive genetic variance components were of more importance than dominant components. However, in the erect population, dominance components of variance were more important than additive. In inter-population crosses, additive components were more important than dominance components. Stem length, number of internodes, number of branches, and plant diameter presented high level of heterosis. Number of stems, plant height, and stem thickness presented fairly high hybrid depression (negative heterosis).

Heritability broad sense and narrow sense were estimated in genotype-environment interaction experiment and hierarchical mating design. Heritability values in GE interaction experiment were different from the heritability broad sense values in hierarchical mating design for most of the attributes, indicating the influence of GE interaction effect. This difference was not noticeable in prostrateness. Heritability narrow sense estimated in hierarchical mating design varied from intra- to inter-population crosses.

3) Three sets of generation mean analysis were carried out to obtain the most detailed genetic information including function of genes, and number of genes controlling the attributes. To achieve these, three pairs of parent plants were used (one erect and one prostrate in each pair) to produce F_1, F_2, Bc_1, and Bc_2. Several attributes which were distinct enough in the two types so that it could be assumed that parent populations were nearly homozygous in opposite directions, were studied in these crosses. Three, six, and the best parsimonious models were presented for the studied attributes.

Prostrateness and stem thickness were partially to completely dominant over erectness and stem thinness. Small leaf size was over-dominant over large leaf size. There were strong evidences for additive x additive non-allelic interaction for stem thickness, additive x dominance interaction for leaf size, and dominance x dominance interaction for prostrateness and leaf size. Nodal rooting ability, prostrateness, and stem thickness seemed to be controlled by a low number of genes, whereas leaf size seemed to be controlled by several genes.

ACKNOWLEDGEMENT

First of all thanks be to Almighty God the Lord of the Universe Who guides us to the right path.

Then I would like to express my particular thanks and appreciation to my sponsor, Ministry of Jahad Sazandegi of Islamic Republic of Iran for enabling me to extend my studies towards PhD by providing financial support.

I would like to express my special thanks to my chief supervisor, Dr. I.L. Gordon. I can perhaps never thank him enough for his guidance, close assistance and very useful suggestions throughout this study.

My thanks are also extended to my co-supervisor Dr. W. Rumball at Grassland, Agresearch, Palmerston North, for his useful and friendly suggestions and also for providing required seed.

It was a very delightful occasion working with such a wise and excellent staff of Plant Science Department. In particular, my especial thanks are extended to Dr. K. Harrington for his guidance on weed controlling and Mrs. Karen Hill for her valuable helps in seed germinating tests.

I am very grateful and indebted to professor J. Hodgson and Professor M. Hill for their guidance, warm encouragement, and pleasant interactions.

Thanks to Professor P.G. Fenemore for pest identification and Dr. P. Long for disease verification.

I am deeply grateful to Mr. Ray Johnstone and his excellent colleagues in Plant Growth Unit (PGU). The way they handle problems is far beyond their duty.

Many thanks to field staff Mr. Terry Lynch and his colleagues for field technical assistance, F. Brown, C. McKenzie and D. Sollit for technical assistance, J. Cave and C. Gwynne for providing assistance at various ways.

Finally I would like to thank my wife, and children for their moral support, encouragement and tolerance.

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CHAPTER ONE : INTRODUCTION

1.1 Introduction

Most of the important characters with which plant breeders have to work are controlled by several genes and those characters are referred to as quantitative characters. Quantitative characters specially in cross-fertilized plants show a vast amount of variability. Genetic variation is essential for predicting genetic advance. Mean phenotype cannot be changed by selection in genetically uniform plant populations. The rapidity of a population's response to selection is indicated by the kind and magnitude of the genetic variability. In Goodrich *et al.*'s (1975) words, "consideration of the amount of genetic variance in a population is important in choosing populations for improvement". The long-range response to selection is a function of the amount of useful genetic variance in the population, and its rate of decline over time.

The importance of quantitative characterization of genetic variability in plant populations has been recognized and emphasized by numerous scientists from a long time ago (e.g. Robinson *et al.*, 1949, Welsh, 1981). The total variation in a plant population is the result of a combination of genotypic and environmental effects. The proportion of variation due to each source is of importance in plant breeding (Welsh, 1981). This includes a knowledge of the magnitude of the genotype-environment interactions, and the relationship among characters. When information on these points is available, the breeder can decide which one of the numerous breeding procedures is most likely to succeed. In other words the genetic architecture of a plant population will determine the breeding method. In essence, progress in plant breeding is conditioned by the magnitude, nature and interrelations of genetic variation in the various significant plant characters.

A breeder sometimes is faced with lack of genetic variation and large environmental effects or some combination of them on plant performance. In this case the breeder has several alternatives to consider. For example a

breeder may use an established variety as a base population for his breeding programme, or he may form a genetically diverse population using introduced materials from exotic sources, or by intercrossing divergent populations. In the first option, progress depends on the extent of the variation within the variety and the selection pressure applied. In the second case utilization will depend on the predominant kind of genetic variance (e.g. hybrid cultivars for dominance and epistasis, selection for average allele variation).

In cross-fertilized plants, such as red clover, plant populations are genetically heterogeneous internally. This not only makes selection more complex, but also as Mather & Jinks (1982) have mentioned, the range of statistics available from the population is commonly more limited.

As noted earlier, a uniform population does not respond to selection. But sometimes even a variable population does not respond well to some kinds of selection strategies. In Hartl's (1980) opinion certain populations can have ample genetic variation and yet fail to respond to selection. The part of the genetic variation amenable to selection can be clarified by partitioning the genetic variance. In other words such information is needed in designing the most effective breeding programmes.

Red clover is adapted to a wide range of soil types, pH level and environmental conditions (Smith *et al.* 1985). This plasticity and economic importance of red clover are the reasons for laying emphasis on it. Red clover is valuable because it produces large quantities of highly nutritive feed during the warm season specially with irrigation. In high rainfall areas a red clover with good persistence would be a major asset to farmers (Smith and Bishop, 1993). In spite of the importance of forage legumes for animal production, including red clover, the genetics of red clover characters have received little attention. Consequently, progress in improving its characters has been slow. Lack of persistence (Montpetit and Coulman, 1991a) which is due to susceptibility to root rot (Leath, 1985) is a major limitation of widespread acceptance of this forage legume in temperate pastures by farmers. It has been classified as one of the least persistent clovers by Lancashire (1985) and

its persistence decreases further when grazing pressure is high (Taylor and Smith, 1977). Although red clover reaches maximum forage yield in the second year of its life (Smith *et al.,* 1985), its vigour and yield decrease as a result of disease, particularly root rot, as the stand becomes older (Leath, 1985). Literature on estimates of population genetic parameters in red clover is very scarce. There are only a few articles in which population parameters of some characters such as degree of dominance, heritability, are reported (e.g. Anderson 1960; Taylor *et al.,* 1970; Cornelius *et al.*, 1977). Very few attempts have been made to estimate important genetic parameters in red clover cultivars.

A high genetic potential which exists within different types of red clover actually is intact. This potential, which is testified by a wide range of variability in most red clover characters, should be used for improving the commercial cultivars. Consequently genetic vulnerability of commercial red clover cultivars might convince us to broaden the germplasm. Bearing this fact in mind, some new materials have just been introduced to New Zealand from Galicia, Spain, which have some capacities and characteristics such as prostrate growth habit, adventitious roots and early flowering. These materials can be used as a useful germplasm for improving existing commercial cultivars. Producing adventitious roots on the axillary buds can be used for increasing the persistence of commercial cultivars of red clover. Some genetic aspects of these materials along with some semi-erect and ordinary cultivars will be considered in this work. Smith and Bishop (1993) introduced a new red clover cultivar called Astred with such characteristics, producing roots asexually as well as from seed. They described the new introduced cultivar as persistent, which was a function of its nodal rooting ability. According to their evaluation this particular characteristic allows the plant to survive and withstand continual close grazing. In their evaluation experiment, Astred produced more dry mass during the third year of its life than the other companion red clover cultivars, which was due to newly formed daughter plants grown from the nodes of original plants during and after flowering. Despite the fact that adventitious root

and its role in the persistence of the plant was known from long time ago (Taylor *et al.*, 1962; Cressman, 1967) its quantitative genetic aspects were not investigated at all. To improve the persistence of red clover stands, adventitious roots on the stem could be promoted. It was proved that this ability can remarkably improve the persistence of red clover plants (Montpetit and Coulman, 1991a; 1991b). How heritable is this character? What is the functions of gene(s) that control this character? How does environment affect it? What is its relationship with other attributes? And lots of other questions regarding this aspect or red clover are left to be answered.

In a species like red clover, which is a cross pollinated plant, a breeder is required to achieve improved population performance by accumulation of favourable genes. In other words, the cross pollinating nature of red clover requires that the breeder obtain a population structure that is stable under subsequent seed increase. In general, most cultivars or strains of red clover in use today were developed through some forms of controlled mass selection. Large populations of space-planted or sward-planted individuals were established and evaluated for the desired characteristics (Smith *et al.*, 1985). Additive genetic variance forms the basis of selection methods. However, non-additive genetic variance constitutes an important part of genetic variance in red clover (Anderson *et al.*, 1974). Under these conditions,also, hybrids or synthetic varieties are alternative approaches (Bassiri 1971). Hybrid varieties and synthetic cultivars are among the methods in which non-additive genetic variance is used. So knowledge of the kind and magnitude of genetic variation in clones or lines becomes important when the breeder considers the basic material to be used in developing a hybrid or synthetic cultivar.

1.2 **Overview of experimental programme**

From the genetic point of view, a wide genetic diversity was going to be investigated. It was planned to examine them in three experiments.
The first experiment was a genotype-environment interaction experiment,

covering diverse genotypes with minimum genetic detail.

Experiment two, hierarchical mating design, examined two populations only but gave more genetic details.

Experiment three, generation mean analysis, examined three biparental crosses from the same types used in the experiment two. It gave us the most genetic detail for these three sets of crosses.

The overall objective of this study was to understand the way in which different characters, specially prostrateness, are inherited. The aim was to estimate the relative contributions of different genetic variance components, such as additive and non-additive variances, to the mean expression of two extreme populations, prostrate and erect. Also the genotype-environment interaction study was done to find the relative influence of genotype and environment on the different characters. Finally, non-allelic interaction, and number of genes controlling some of the attributes were intended to be studied.

CHAPTER TWO : LITERATURE REVIEW

2.1 Introduction

One of the main objectives in quantitative genetics is to find genetic architectures of plant populations by which a plant breeder is able to understand plant population behaviour under the influence of breeding programmes including different selection strategies. From early years the method of partitioning apparent variability was developed to extract that variation due to genetic and others. Fisher *et. al* (1932) said the heritable variance observable among any group of organisms may be regarded as one of many which sum to form the total phenotypic variation. It was suggested that the proportion of the variance which was heritable may be estimated from the covariances of related individuals. This made it unnecessary to recognize any single gene factor, as this method gave a direct means of estimating the total genetic contributions to the heritable variance. Later, basic attempts were extended to extracting environment and genotype-environment interaction from phenotypic variance (Comstock and Moll, 1963). Biometritician and quantitative geneticists developed together, sharing many algebraic procedures. The main procedure was to equate observed variation to expected mean squares, by which the observed variation could be partitioned into the genotype, environment, genotype-environment interaction and residual effects (Comstock & Moll, 1963). Numerous scientists were involved in developing the statistical methods for the analysis of continuous variation (e.g. Mather, 1949; Jinks, 1954; Anderson and Kempthorne, 1954; Hayman and Mather, 1955). These methods were applied to crosses between two inbred lines by which the phenomena associated with mendelian genetics were better recognized and eventually the method was extended to the analysis of data from a diallel cross between a number of inbred lines (Jinks, 1954).

Nowadays further splitting of either genetic variation or means are

possible. Still, some cases, such as linkage, are a matter of controversy. There are several methods of analysis available for breeders which enable them to partition and interpret the apparent variation into genetic and environmental components and in turn partitioning of genetic components to different types of gene action and interaction such as : additive, dominance, non-allelic, linkage, and genotype-environment interaction effects. There are two main methods by which genetic variability in plant populations can be characterized; those based on generation mean analysis and those based on the variance component analysis. It should be mentioned that the theory of the diallel analysis of parental lines and F_1 performances is considered as a method by which genetic variance components can be detected. This method was extended to the F_2 and backcross generations derived from the diallel set of crosses (Jinks, 1956). His method permits the estimation of parameters for additive, dominance and environmental effects and allows the recognition of non-allelic interaction. He also elaborated a number of methods such as regression of array covariance on array variance, joint scaling, by which non-allelic interaction effects could be detected. Much of the basis for the diallel does parallel the generation mean approach, however.

2.2 **Genotype-environment interactions**

Plant phenotype is a reflection of its genotype and environment and furthermore, the effect of genotype and environment are not independent. Genotype-environment interaction simply was defined by Eisen and Saxton (1983) as: if a particular genotype is superior in one environment but fails to be superior in a second environment, then GE interaction is said to be present. As Comstock and Moll (1963) have described, the phenotypic response to a change in environment is not the same for all genotypes; the consequences of variation in genotypes depend on environment. This inter-play (inconsistency) in effect of the genetic and non-genetic factors on plant performance is what is meant by genotype-environment interaction.

By 1970s, although plant breeders had been aware of important genotypic differences in adaptability, they had been unable to exploit them fully in breeding programmes (Finlay & Wilkinson, 1963). In the case of data analysis some believe the interaction can be removed from the data structure by transforming the data to another scale in which there are no interactions (Mather and Jinks, 1977). Mather and Jinks (1982) have dealt with this subject with an example in which interactions are eliminated by a log transformation. Others presented various methods to separate GE interaction variance component from the genetic variance components.

Some people, like geneticists, want to understand the causes of the interaction, in terms of biological genetical parameters, whereas others like plant breeders and agronomists want merely to minimize the effects of GE interactions on their field trials. In Comstock and Moll's (1963) terms, measuring genetic variance components in plant populations is one of the prime concerns of breeders.

As Kroonenberg & Basford (1989) pointed out, the existence of significant genotype-environment interaction creates difficulty in genetic analysis in several ways, such as by confounding estimates of genetic parameters and statistics, and by complicating selection and testing strategies. The significance of GE interactions in this connection lies in their impact on reliability of estimates. Depending on the kind of data employed, they may introduce upward bias. GE interactions reduce the relation between phenotype and genotype with the result that valid inference becomes more complicated.

The presence of genotype-environment interaction has been recognized in many plant species by numerous workers. In other words plant breeders are fully aware that interactions between genotype and environment have an important role in plant performance in different environments but they are not in a common agreement in the way of detecting and analysing it. The presence of genotype-environment interaction is supposed to be absolutely ubiquitous so that even the absence of it in any trial is interpreted as a result of previous selection of varieties to suit the locations under test by which the

interaction effects have been removed (Bulmer, 1985). A study of GE interaction can lead to a successful evaluation of stable genotypes which could be released to farmers and or used in future breeding programmes (Gupta and Ndoye, 1991). A sufficient knowledge of GE interaction is necessary for agronomists and plant breeders, because they are always looking for a variety which has a considerable degree of general adaptability. This aim has conflict with GE interactions. In Comstock & Moll's (1963) words GE interactions are somehow involved in most problems of plant breeding.

It is well known and documented that more than two locations are needed for an accurate genotype-environment interaction test (Misevic and Dumanoivic, 1989; Hamblin *et al.*, 1980; Fakorede, 1986). But, when a large number of genotypes are to be tested, two locations could be used to reduce the number of genotypes. It is suggested that in selection programmes if a moderate selection intensity of 20% is used, most of the overall highest yielding genotypes could be recognized and selected. If a more precise estimate of yield potential is desirable, evaluation of the genotypes at four selected locations is necessary (Misevic and Dumanoivic, 1989).

GE interaction has been recently reviewed by several authors including Knight (1970); Hill (1975); Lin *et al.* (1986); Westcott (1986); Becker & Leon (1988). Some of the reviews are very recent and also the subject is still under development. There are hundreds of papers written on the application of various methods on various crops to be referred.

Hill (1975) has reviewed a large number of papers regarding the techniques detecting genotype-environment interaction. His review has covered the emergence of genotype-environment interaction problem, the analysis of variance, and linear regression. He has also pointed the possibility of extensive use of multivariate analysis techniques in this field and reviewed the existing instances. Since then techniques and their application have been developed and increased which have lead to some other reviews.

Westcott (1986) reviewed more extensively the techniques available in multivariate analysis. He has considered the linear regression approach and

related stability parameters, cluster analysis, principal components analysis, and geometrical methods.

Lin *et al.* (1986) have surveyed the two ways of classifications of data. They classified the methods in use into nine stability statistics in which on turn were classified to four groups based on the use of either the deviation from the average genotype effect or on the GE interaction term. In their idea, fore-mentioned groups are either sum of squares (ss) or regression coefficients or deviation ms from regression.

Becker and Leon (1988) have reviewed the methods of stability analysis. They also have discussed the prospect and limitations of improving yield stability with the aid of biometrics.

Different approaches are presented to detect and evaluate the existence and magnitude of genotype-environment interaction effects, and significant progress has been made in understanding and measurement of GE interaction. Therefore, the literature on GE interaction has become so large that a comprehensive literature review on them is beyond this part of my work and an exhaustive consideration of their details would require more space than can be devoted to it here. So, I will content myself with a short glance at the most popular methods of investigating GE interaction.

2.2.1 **Analysis of variance**

The analysis of variance is perhaps the most widely used computational procedure in biometrics for the analysis of quantitative inheritance. Sprague and Federer (1951) were some of the pioneer persons who showed the way of partitioning of apparent variability into its components: genotypes, environments and their interaction effects by equating the observed mean squares in the analysis of variance to their expectations in the random model (Crump, 1946; 1951; Kearsey, 1965; Searle, 1971). Comstock and Moll (1963) presented a fully detailed work on the method of analysis of variance to detect and extract GE interaction. Moreover, the components of variance separated

by this way can be used to get an estimation of broad sense and in some cases narrow sense heritability. The model for a series of experiments, replicated in several sites and years, could be expressed as follows (Miller *et al.*, 1957; 1959).

$Y_{ijkl} = \mu + \alpha_i + \eta_k + \beta_l + \delta_{(j)kl} + \alpha\eta_{ik} + \alpha\beta_{il} + \alpha\eta\beta_{ikl} + \eta\beta_{kl} + \varepsilon_{ijkl}$

in which

μ = the grand mean common to all observations.

α_i = the effect of $_i$th genotype.

η_k = the effect of $_k$th site.

β_l = the effect of $_l$th year.

$\delta_{(j)kl}$ = effect of the $_j$th block in each $_{kl}$th combination.

$\alpha\eta_{ik}$ = the interaction effect of $_i$th genotype and $_k$th site.

$\alpha\beta_{il}$ = the interaction effect of $_i$th genotype and $_l$th year.

$\alpha\eta\beta_{ikl}$ = the second order interaction between $_i$th genotype, $_k$th location and $_l$th year.

$\eta\beta_{kl}$ = the interaction effect of $_k$th site and $_l$th year.

ε_{ijkl} = residual.

The expected mean square for those experiments, while all the effects are considered to be random, is given in Table 2.1 (Wricke & Weber, 1986).

In this form of analysis of variance σ^2 is within plot variation. It is regarded as error part of the model. As Bulmer (1985) has illustrated, when several cross-fertilized cultivars are concerned, they are genetically heterogenous, but they differ in their mean genotypic values and we are considering how these mean values behave in different environments. We are thus concerned with the variability between cultivars and between environments and the interactions between the two. The genetic variability within cultivars is treated as part of the residual error.

Table 2.1: analysis of variance for a series of experiments pooled over several sites and years.

S.O.V.	D.F.	Expectation of M.S.
Site (S)	(s-1)	$\sigma^2+y\sigma^2_{R(GS)}+r\sigma^2_{GYS}+rg\sigma^2_{YS}+ry\sigma^2_{GS}+rgy\sigma^2_{S}$
Year (Y)	(y-1)	$\sigma^2+r\sigma^2_{GYS}+rs\sigma^2_{GY}+rg\sigma^2_{YS}+rgs\sigma^2_{Y}$
S x Y	(y-1)(s-1)	$\sigma^2+r\sigma^2_{GYS}+rg\sigma^2_{YS}$
$Block_{SY}$	sg(r-1)	$\sigma^2+y\sigma^2_{R(GS)}$
Genotype(G)	(g-1)	$\sigma^2+y\sigma^2_{R(GS)}+r\sigma^2_{GYS}+rs\sigma^2_{GY}+ry\sigma^2_{GS}+rys\sigma^2_{G}$
G x S	(s-1)(g-1)	$\sigma^2+y\sigma^2_{R(GS)}+r\sigma^2_{GYS}+ry\sigma^2_{GS}$
G x Y	(g-1)(y-1)	$\sigma^2+r\sigma^2_{GYS}+rs\sigma^2_{GY}$
G x S x Y	(g-1)(y-1)(s-1)	$\sigma^2+r\sigma^2_{GYS}$
Residual	sg(r-1)(y-1)	σ^2

s = number of sites

g = number of genotypes

r = number of replication per environment

y = number of years

σ^2 = residual

$\sigma^2_{R(GS)}$ = block effect nested within year and environment

σ^2_{GYS} = second order interaction

σ^2_{YS} = the component of variance related to interaction between site and year

σ^2_{GS} = the component of variance related to interaction between genotype and site

σ^2_{S} = the component of variance attributed to site

σ^2_{GY} = the component of variance attributed to interaction between genotype and year

σ^2_{G} = the component of variance attributed to genotype

σ^2_{Y} = the component of variance attributed to year

2.2.2 **Partitioning of GE interaction variance component**

Numerous statistical methods have been invented and applied in order to detect GE interaction effects (Bucio Alanis and Hill (1960), Allard and Bradshaw, 1964; Bucio Alanis, 1966; Bucio Alanis *et al.*, 1969). The nature of the GE interaction was investigated by several workers (e.g. Moll *et al.*, 1978; Eisen and Saxton, 1983; Yamada *et al.*, 1988). To investigate the nature of genotype-environment interaction, it was partitioned to its components by various methods. Baker (1969) and Byth *et al.* (1976) suggested that when the proportion of GE interaction due to linear regression on environmental indices is very small, the regression technique could be misleading. Baker (1969) modified the use of regression technique to overcome the problem. He regressed different effects on one another. The regression coefficients for the effects regression were supposed to centre zero. Any significant effects of regression coefficients would indicate violation of one of the assumptions underlying the analysis of variance, in other words non-independence of the different effects in the model.

Yamada *et al.* 1988, Eisen and Saxton, 1983, separated the interaction effects into two parts, the part associated with heterogeneous genetic variation measured in each environment and that due to differences in genetic correlations of the same trait measured in different environments. In contrast Moll *et al.* (1978) partitioned the interaction effect into that due to heterogenous environmental variances measured for each genotype and the part due to differences in environmental correlations between genotypes. As Muir *et al.* (1992) have pointed out, in either of the cases the interaction effect can be separated into that due to differences in scale and that due to imperfect correlations or change in rank. In the first case, the scales and correlations are genetic, whereas in the second case, the scales and correlations are environmental. These alternative methods for partitioning GE interaction effects were examined in order to find which one is more appropriate or informative than the other for a given situation. They found the first one, which was based on heterogeneity among environments in the

scaling of differences among genotypes, more useful and informative for random genotypes that are to be tested in either fixed or random environments. Method 2 is more useful in evaluating fixed genotypes for sensitivity to random environments.

2.2.3 **Linear regression**

One of the most frequently used methods for investigating GE interactions is the linear regression method. This method was initially proposed by Yates and Cochran (1938) and developed by Finlay and Wilkinson (1963), Eberhart and Russel (1966), Bucio Alanis (1966), Bucio Alanis and Hill (1966) and Perkins and Jinks (1968 a and b). As Hill (1975) pointed out, this method has two parts, a conventional analysis of variance along with a joint regression analysis to determine whether the GE interaction effects are a linear function of additive environmental component. In the second part the data are transformed to a scale on which the average regression slope of the genotypes under test equals unity (Yates & Cochran, 1938; Finlay & Wilkinson, 1963; Eberhart & Russel, 1966) or zero (Perkins & Jinks, 1968a) depending upon the actual analysis employed.

Finlay and Wilkinson (1963) have considered the regression coefficient and the variety mean yield over all environments as two important indices for conclusion. The slope of the regression line for each genotype was plotted against its mean yield over environments. Genotypes with a slope near one and high mean yield were regarded as well adapted to all environments. As mean yield decreased, genotypes with high or low slopes were regarded as being specifically adapted to favourable or unfavourable environments respectively. In their terms,

b=1 ⇒ average stability

b=1 + high mean yield ⇒ general adaptability

b=1 + low mean yield ⇒ poor adaptability

b>1 ⇒ sensitivity to environmental change

b<1 ⇒ resistant to environmental change

In the method of analysis of variance the yield Y_{ijk} of the $_kth$ replicate of the $_ith$ genotype in the $_jth$ environment can be regarded as:

$$Y_{ijk} = \mu + d_i + E_j + g_{ij} + e_{ijk}$$

in which

μ = general mean

d_i =genotype effect

E_j = environmental effect

g_{ij} = interaction effect

e_{ijk} = random error effect

As Yates and Cochran (1938) suggested by calculating the regression of the yields of the separate varieties on the mean yields of all varieties, the degree of association between varietal differences and general fertility can be further investigated. In the other words g_{ij} can be partitioned to

$$g_{ij} = \beta\varepsilon_j + \delta_{ij}$$

in which β_i = linear regression coefficient for the $_ith$ genotype and δ_{ij} = deviation. Combining these equations we will get

$$Y_{ijk} = \mu + d_i + (1+\beta_i)\varepsilon_j + \delta_{ij} + e_{ijk}$$

Eberhart and Russel (1966) also used a linear regression method. In their approach deviations from the regression line was regarded as another important criterion for estimating stability. Therefore, in their terms, a stable variety is one with a regression line of slope near one with a small sum of squared deviations. In fact they considered an ideal genotype as one with a regression coefficient of 1 and squared deviation of zero. They suggested that in addition to the regression coefficient, a plant breeder may be interested in the degree to which a cultivar deviates from regression on an environmental index. Since large deviations from regression indicate unpredictable behaviour, a parameter measuring this can be used to indicate stability of a cultivar.

Freeman and Perkins (1971) have claimed that almost all forementioned works of regression analysis of GE interaction are not a correct

usage of the method to consider the problem. Even if the regression method was sometimes under criticism (e.g. Easton and Clements, 1973; Mungomery *et al.,* 1974; Becker and Leon, 1988), it has been frequently used to investigate genotype-environment interaction effects on the plant performance. Sometimes it has been argued by some workers (Hill, 1975) that where the genotypes in an experiment differ in their physiological response to physical factors in the environment, the linear regression technique may over-simplify the true response pattern to an extent which could lead to erroneous conclusions.

There are quite a large number of works on modification and application of regression analysis techniques for investigating genotype-environment interaction which are not going to be referred to in detail here. The most predominant one could be listed as the works of Simmonds (1979 and 1980); Jinks and Pooni (1979); Lin *et al.* (1986); Lin and Binns (1988).

In Byth's (1977) point of view, linear regression analysis can be simply applied and effective where strong linearity of response exists. He pointed out that characterization of patterns of interaction by linear regression can be an over-simplification, relatively uninformative, potentially mis-informative, and largely irrelevant in many agriculture situations.

2.2.4 **Other methods of investigating GE interaction**

Several other authors have presented a new way of looking at GE interaction problem. Plaisted & Peterson (1959) have partitioned GE interaction effects by doing the analysis of variance for every pair of genotypes being involved in their experiment to estimate the interaction variance for every combination of two genotypes. The interaction variances obtained for each genotype were averaged to get an indicator of contribution of that genotype to the total GE interaction.

Wricke (1962, cited in Lin *et al.*, 1986) proposed ecovalences.

$$W_i^2=\Sigma_j (X_{ij}-\overline{X}_{i.}-\overline{X}_{.j}+\overline{X}_{..})^2$$

In his approach the contribution of each genotype to the interaction sum of squares was used as a measure of its instability. Because ecovalence measures the contribution of a genotype to the GE-interactions, a genotype with $W_i = 0$ is regarded as a stable genotype. In other words low values of W_i equals high ecovalence (Lin *et al.*, 1986). The sum of all W_i is the interaction sum of squares, $\Sigma_i W_i$ = ss GE (Wricke & Weber, 1986).

The detection of GE interactions by means of an analysis of variance, however does not indicate the relative interaction of each genotype with environments (Hallauer, 1988). Performance tests over a series of environments, when analyzed in the conventional manner, give information on GE interactions but give no measurement of stability of individual entries (Eberhart & Russell, 1966). Lin & Binns (1985 & 1988) proposed a procedure for considering interaction between genotypes and test locations. They defined a measure of general superiority, p_i, as the mean square for the distance between the genotype response and the maximum response at each location, averaged over all locations, the smaller the p_i value the better the genotype.

$$p_i = \Sigma^n_{j=1} (X_{ij}-M_j)^2/(2n)$$

in which

p_i = general index

X_{ij}= attribute value of the $_i th$ genotype in the $_j th$ location

M_j =maximum response among all genotypes in the $_j th$ location

n =number of locations.

2.2.5 **Multivariate methods**

Multivariate analysis is a simultaneous analysis of several correlated variables from independent individuals within a data set. Univariate analysis is not adequate, since it may overestimate the true dimensionality of divergence as it does not separate covariance among the variables from their

apparent variances. There are a wide range of multivariate methods in addition to the univariate methods, including cluster analysis, principal component analysis, and multivariate discriminant analysis. The number of multivariate methods are increasing (Kendall, 1980). Glahn (1975) has illustrated the relationship among several multivariate techniques. The multivariate analysis techniques are wider than the space that can be devoted to them here. To meet our purposes, they can be briefly summarized as follows:

2.2.5.1 **Classification methods**

Most of the classification methods assign genotypes into qualitatively homogeneous stability subsets, so that no significant GE-interaction occurs within subsets, while differences among subsets are due to GE-interaction. Many different similarity measures and clustering strategies have been proposed to achieve these subsets (Becker & Leon, 1988). In Cormack's (1971) point of view classification is a technique for allocating entities to initially undefined classes so that individuals in a class are in some sense close to one another.

Cluster analysis: cluster analysis is classified as one method of classification. Various techniques have been invented based on the various goals, by different users. The techniques are reviewed by several workers (e.g. Everritt, 1974; McQuitty, 1987; Romesburg, 1984).

Plant breeders have been using clustering methods in order to investigate genotype-environment interaction effects (e.i. Abou-El-Fittouh *et al.*, 1969; Byth *et al.*, 1976; Mungomery *et al.*, 1974; Ghaderi *et al.*, 1980; Gates and Bilbro, 1978; Lin, 1982; Lefkovitch, 1985). For instance, in cluster analysis locations or genotypes are classified according to a specific measures. There are different dissimilarity measures and clustering strategies so that choosing between them can result in different clustering groups. According to Abou-El-Fittouh *et al.* (1969) in controlling GE interactions without requiring any

knowledge of the environmental factors responsible, locations can be classified according to the similarity of their interactions with a set of entries. In that case GE interactions within locations in one class would be small. They classified the locations in their cotton trials according to a distance coefficient and a correlation coefficient as dissimilarity measures.

Byth *et al.* (1976) used a variance-standardized squared Euclidean distance as a dissimilarity measure and an incremental sum of squares clustering strategy to analyze their data on spring wheat nursery. Lin & Thompson (1975) used the deviation mean square from a joint regression, which was proposed by Finlay and Wilkinson (1963), as the dissimilarity index for clustering.

There are a large number of researches on the application of cluster analysis with different crops using different measures and dissimilarity scores so that referring to all of them is beyond the scope of this work.

2.2.5.2 **Principal component analysis**

Principal component analysis is one of the multivariate methods that has been used in order to investigate genotype-environment interaction effects. In breeding programmes the number of variables under consideration is often large and the problems of simply assessing the available information becomes difficult. This problem would be greatly reduced if the complex pattern of information held in such a data set could be reproduced in a few new variables. In Johnson and Wichern's (1992) words, its general objectives are (1) data reduction and (2) interpretation. An analysis of principal components often reveals relationships that were not previously suspected and thereby allows interpretations that would not ordinarily result. In fact much of the information in the data matrix is effectively redundant and the same information is measured several times by highly inter-correlated variables. Principal component analysis provides a method for such an optimal reduction in the dimensionality of a multivariate data set. Principal component analysis

combines the variables in such a way so that the first principal component explains the maximum of dispersion. The second principal component explains the maximum of remaining variance and so on. Although in the most cases first and second components could explain the existing variation (Cooley and Lohnes, 1971; Morrison, 1976; Chatfield and Collins, 1980; Jobson, 1991) there are reports showing that first few components failed to explain the majority of variation in the data. Cullen (1981) in her study did not find principal component analysis useful because in the analysis the first few components did not account for large amount of the total variation and she had to consider a relatively large number of components, in which interpretation of the components and genotypic scores would be extremely difficult.

Genotypes can be characterized by their principal component scores (Westcott, 1986). In Mitchell-Olds and Rutledge's (1986) point of view, principal component analysis yields a few orthogonal combinations of characters that may be easier to interpret. It has been suggested that these orthogonal factors may elucidate groups of characters controlled by the same genes (Gale & Eaves, 1972). The central idea of principal component analysis is to reduce the dimensionality of a data set in which there are a large number of interrelated variables, while retaining as much as possible variation present in the data set (Jolliffe, 1986; Bryant & Atchley, 1975).

2.2.5.3 **Multiple discriminant analysis**

Most of the multivariate techniques are looking for parsimonious but effective models to describe the groups under study without losing much information. To do this discriminant analysis estimates a set of linear coefficients vector (V) by which the original data is transformed to a new vector of coefficients so that the differences between the new vectors are maximized (Cooley & Lohnes, 1971).

Two types of discriminant analysis are documented (Cooley and

Lohnes, 1971). (a) first type is used for discriminating between the individuals belonging to two univariate groups. (b) second type, generalized discriminant analysis, is used when more than two groups are investigated. In this type a generalized discriminant function is defined for each population by which a new individual can be allocated into one of the groups. This type sometimes is called "multiple discriminant analysis". It is also used to reduce the dimension of the data in order to facilitate the study of the data. In Clifford and Stephenson's (1975) point of view this method is used to find a set of coefficients by which the observed differences amongst the groups are maximized. In this method p (number of populations) discriminant functions are found so that the first discriminant function provides the maximum separation of the group means. The second discriminant function provides the second largest separation of the group means in an orthogonal direction to the first one and so on. A few of the most significant functions (most likely only the first two) are retained based on their discriminating ability without loosing much information (Kashirsagar, 1972).

Johnson and Wichern (1992) considered the advantages of discriminant analysis particularly when someone is interested in separating several populations for visual inspection or graphical description as follows:

1- Convenient representations of the *g* populations that reduce the dimension of the data from a very large number of characteristics to a few linear combinations.

2- Plotting of the means of the first two or three linear combinations (discriminants). This helps to display the relationships and possible groupings of the populations.

3- Scatter plots of the sample values of the first two discriminants, which can indicate outliers or other abnormalities in the data.

Multivariate discriminant analysis method was used by several researchers for various purposes. For example Valero (1991), Baum and Bailey (1991) used this method for taxonomic purposes. Using this method they identified and labelled the specimens of the species under study.

2.2.6 **Genotype-environment interaction in red clover**

General adaptation is one of the most common objectives of red clover breeding programmes. Evaluation of cultivars over a wide range of environments is the best way to examine this objective. It has been suggested that genetic mixtures rather than homogenous pure lines or cultivars be used to reduce GE interaction in plant performances (Allard and Bradshaw, 1964 Eberhart and Russel, 1966). Red clover cultivars are heterogenous populations, therefore, they should show less GE interaction than homogenous pure lines or varieties. It appears that literature on red clover GE interaction is quite scarce and it has been given much less attention than in other crops. Limited research has been conducted on the genetic variability of morphological traits of red clover and the stability of these characters over locations and years.

Choo *et al.* (1984) studied 12 cultivars of two types of red clover, single cut and double cut, at five locations in Canada. They analyzed the data for dry matter yield of the two types of red clover separately. They combined the data from different environments to study GE interactions. Following Eberhart and Russell (1966), They partitioned GE interaction effects of each cultivar into two parts; (1) the variation due to the response of the cultivar to varying environmental indexes (sums of squares due to regression) and (2) the unexplainable deviations from regression on the environmental index. They found a significant genotype-environment interaction in dry matter yield for the double cut cultivars.

Montpetit and Coulman (1991a) investigated the relationship between the presence of adventitious roots growing from the crown of red clover stems and the persistence of plants. They conducted their experiments at two sites over four years. They found a relatively high correlation between adventitious roots and spring vigour. Also they found a significant positive linear relationship between foliage and adventitious roots. They concluded that profuse production of adventitious roots improves spring vigour in red clover.

2.2.7 **Heritability**

Estimation of heritability and its interpretation have presented many challenges to plant scientists. The plant kingdom presents a great diversity of natural modes of reproduction, varying from asexual to sexual means, cross fertilized to self fertilized. Correspondingly, methods for estimating heritability also varies from species to species. Confined to a cross-pollinated plant, also procedure may change, considering the diverse array of plant populations which can arise.

Plant breeders are interested in heritability because characters with higher values can be improved more rapidly with less intensive evaluation than these with lower heritability values (Wyman, 1991). The magnitude of the genotypic variance is of critical importance. This criterion determines what effect selection can have (Mayo, 1980). According to Hartl (1980), heritability says nothing about the actual mode of inheritance of a quantitative attribute, but it is useful in predicting response to selection. However, the relation between genotypic variance and the total available variance is of breeder interest. In other words, the ratio of genetic variance to phenotypic variance is called heritability. Based on the proportion of genetic variance in the nominator of the ratio, two different senses are described for heritability.

(a) The ratio of additive genetic variance component to phenotypic variance is called heritability narrow sense ($h_{ns} = V_A / V_P$).

(b) The ratio of total genetic variance to phenotypic variance is called broad sense heritability ($h_{bs} = V_G / V_P$). In quantitative genetics broad sense heritability is used as a rough estimate of the overall level of genetic variation for a character (Mitchell-Olds & Bergelson, 1990). In Falconer's (1989) point of view broad sense heritability may be easier to obtain than narrow sense heritability estimates, but they are likely to be biased upwards by non-additive genetic variance.

Heritability can also be estimated using variance components from replicated plots, grown in a number of environments. Gordon *et at.* (1972); and

Gordon (1979) have discussed the issue regarding the estimation of broad sense heritability in a series of experiments on annual and perennial plants. In their opinion heritability estimates based on replicated plots of several genotypes can be computed in two forms:

(a) Full heritability, in which full phenotypic variance is used in the model.

$$h^2_{full} = V_G/V_{P1}$$

where

V_G = total genetic variance

$V_{P1} = V_E + V_G + V_{GE} + V_R + V_e$

and

V_E = macro-environment variance

V_{GE} =genotype-environment interaction variance

V_R =block variance (meso-environment)

V_e =error variance

(b) Restricted heritability in which only the single experiment variance components is used in the model.

$$h^2_{rest} = V_G/V_{P2}$$

in which $V_{P2} = V_G + V_{GE} + V_e$

They also have derived a formula for estimating standard errors for heritability in full and restricted, both for annual and perennial plants, grown in replicated plots in several environments. This issue was also discussed by Hanson (1989).

Based on the nature of the plant populations total genetic variance consists of different portion of the components. In self pollinated plants the majority of genetic variance is additive genetic variance. Therefore, V_G is regarded as V_A and the ratio of V_G/V_P is regarded as narrow sense heritability. Whereas in cross-pollinated crops genetic variance consists of additive, dominance, and non-allelic interaction components and the ratio of heritability

would be regarded as broad sense heritability. In such crops the genetic variance has to be partitioned further into its components to get appropriate components for narrow sense heritability.

Heritability also can be estimated in several other ways. If both of offspring and parents are concerned, narrow sense heritability ($h^2 = V_A / V_P$) could be estimated by the coefficient of simple linear regression of offspring on one parent, $b_{OP} = Cov_{OP}/V_P$ (Kempthorne, 1957). The use of parent offspring regression for estimating heritability narrow sense was also discussed in detail by Falconer (1989).

If parental effects are absent, regression of offspring on the parental average would be the simplest precise estimator of narrow sense heritability (Mayo, 1980).

$$b_{oP} = \frac{0.5 V_A}{0.5 V_P}$$

In Mitchell-Olds & Rutledge's (1986) point of view, rather than using the indirect process of estimating heritability and genetic correlation from covariances of relatives and then predicting response to selection under a particular genetic model, it is much easier and more accurate to measure the potential response to natural selection by conducting artificial selection experiments in the field. Mitchell-Olds & Rutledge (1986) also have argued that estimates of heritability and genetic correlations can provide good predictions of selection response if

1- such estimates are reasonably accurate.

2- many genes contribute to genetic variances and covariances.

3- genetic variance covariance matrices remain approximately constant over evolutionary time.

4- genotype-environment interaction does not alter genetic parameters in new or unmeasured environments.

5- populations are not inbred

This method of estimating heritability is known as "realized" heritability (Falconer, 1989).

2.2.8 **Phenotypic and genotypic correlation**

In practical instances the breeder cannot assume that observations on several attributes (variables) are entirely independent of each other. In most of the cases attributes are correlated either positively or negatively. In Falconer's (1989) point of view correlated characters are of interest for three reasons. Firstly, in connection with the genetic causes of correlation through the plieotropic action of genes. Secondly, in connection with the changes brought about by selection and thirdly in connection with natural selection. Phenotypic and genotypic correlations may change drastically from one environment to another (Kahn *et al.*, 1976; Clay, 1982).

Lande and Arnold (1983) believe that correlations between characters complicate the measurement of phenotypic selection, because selection on a particular trait not only directly affects the distribution of that trait in a population but also indirectly changes the distribution of correlated characters. They also indicated that in practice selection is tremendously oversimplified via ignoring phenotypic correlations between attributes which are ubiquitous.

Genotypic and phenotypic correlations are fully described by several workers, including Baker (1986); Falconer (1989); Wricke and Weber (1986). A general formula for estimating genotypic and phenotypic correlation can be presented as follow.

$$r_g = \frac{CovG_{(ij)}}{\sqrt{V_{G_i} V_{G_j}}}$$

$$r_p = \frac{Cov_{P_{(ij)}}}{\sqrt{V_{P_i} V_{P_j}}}$$

in which

$Cov_{G(ij)}$ = genotypic covariance component

$Cov_{P(ij)}$ =phenotypic covariance component

V_{Gi} = genotypic variance component of attribute i

V_{Gj} = genotypic variance component of attribute j

V_{Pi} = phenotypic variance component of attribute i

V_{Pj} = phenotypic variance component of attribute j

2.3 **Partitioning genetic variance**

The pioneer works of Fisher (1918), Fisher *et al.* (1932), and Wright (1921) promoted the notion of looking more deeply into genetic variation. The idea was developed in different ways, and genetic variance partitioning has become a corner stone in quantitative genetics.

In the early years covariance between relatives was used to make a genetic analysis of a population. This procedure was well defined in the classical paper written by Fisher (1918). The whole concept of genotypic variance partitioning into various genetic components also relies on the relatives' covariances. Wright (1921) defined three types of hereditary or genetic variance. Additive genetic variance, variance due to dominance deviations from the additive scheme, and variance due to deviations from the additive scheme resulting from the interaction of non-allelic factors. Estimating the degree of dominance was elaborated by Fisher *et al.* (1932). Wright (1921) presented the formulae for the mean and variance of squared deviations for the cases of no dominance and of complete dominance. He outlined procedures for estimating additive genetic effects, dominance deviations, epistatic deviations, environmental effects and non-additive joint effects of heredity and environment. Many others contributed to the development of this subject. Maize and wheat breeders have a great role in utilizing and improving the genetic variation partitioning techniques. Eventually various mating designs were formed, and the practical applications of these mating designs have been increasingly improved. A mating design is a system of mating used to develop particular sets of progenies. Several methods are used to estimate the

components of genetic variation and combining ability in plant populations, including diallel crosses of parental clones or lines, partial diallel cross, biparental progenies, hierarchical (North Carolina model 1), factorial (North Carolina model 2).

2.3.1 **Covariance of relatives**

A general formula for covariance of relatives is as follows (Becker, 1984)

$$Cov_{relatives} = \alpha V_A + \delta V_D + \alpha^2 V_{AA} + \alpha\delta V_{AD} + \delta^2 V_{DD} + \alpha^3 V_{AAA} \ldots \text{etc.} \quad (I)$$

In which,

$$\alpha = (\phi + \phi')/2 \text{ and } \delta = \phi.\phi'$$

and

ϕ = probability that two relatives have an identical allele from their male parent.

ϕ' = probability that two relatives have an identical allele from their female parent.

Therefore, in the case of two half-sibs:

$$\alpha = (0.5 + 0)/2 = 0.25 \quad \text{and} \quad \delta = (0.5)(0) = 0$$

In the case of two full-sibs:

$$\alpha = (0.5 + 0.5)/2 = 0.5 \quad \text{and} \quad \delta = (0.5)(0.5) = 0.25$$

When F=0, such as in a random mating population the components of variance can be translated to genetic components using the coefficient in the Table 2.3 and the equation (I).

For instance, $Cov_{fs} = 1/2\ V_A + 1/4\ V_D + 1/4\ V_{AA} + 1/8\ V_{AD} + 1/16\ V_{DD}$

Typically plants are crossed in the mentioned well defined designs allowing components of variance to be obtained from parents, full-sib, and half-sib families. But unfortunately components of variance are confounded with environmental effects and their interactions with genetic effects. This

would lead to some sort of uncertainty in purity of genetic variance components in plant populations. One way to solve the problem of induced genotype-environment interaction is to grow plants under a variety of environmental conditions in order to subtract the mentioned effects from the total phenotypic variation. Here the most common mating designs will be discussed briefly.

2.3.2 **Diallel**

The term diallel cross is used to describe a mating design in which a set of *p* fixed lines are chosen as male and female parents. Crosses are made between these parents in all possible combinations. There are maximum of p^2 possible crosses, which can be divided into three groups: (1) the *p* selfings of parental lines themselves; (2) $p(p-1)/2$ F_1s; (3) $p(p-1)/2$ reciprocal F_1s.

From early 1940's techniques involving diallel crosses have been invented and used to investigate quantitative inheritance problems. This design probably has been used more frequently than any other design to estimate general and specific combining ability. Genetic interpretation and analysis are presented in numerous papers. Griffing (1956 a & b), Hayman and Jinks (Hayman, 1954b; 1957; 1958; Jinks, 1954) and Kempthorne (1956) presented different approaches in diallel crossing method. Since then a number of workers have used these approaches to investigate quantitative genetic parameters in plant populations. Illustrations of and improvements to the theory have also been made. Mather and Jinks (1982) developed an analysis of diallel cross data based on the variance and covariance estimates of a sample of parents and their F_1s following work done by Hayman (1954b). The most important feature of their method is regressing of W_r on V_r (covariance and variances of parental arrays respectively) by which an average degree of dominance and genetical diversity among parents can be estimated. The graph based on the ratio W_r/V_r is linear and its slope does not depart significantly from one if additive dominance model describe the data,

otherwise, in the presence of non-allelic interactions (epistasis), the linear relationship does not exist.

Depending on the material under investigation and postulated underlying mechanism and methods of estimation there are two view points for diallel analysis. As mentioned by Hayman (1954b) the interest may lie in a particular set of parental lines (fixed effect model) or it may lie in a base population from which these lines were unbiasedly sampled. Kempthorne and Hayman and Jinks have based their methods on different models. Hayman (1954b; 1957; 1958), Jinks (1954; 1956), and Gardner and Eberhart (1966) attempted to investigate the genetics of the difference between a set of inbred lines whereas Kempthorne (1956) developed a model which was adapted to random mating populations. Griffing (1956b) amalgamated both of those approaches. He was one of the first plant breeders who introduced and utilized general and specific combining ability in terms of population genetics. The analysis of Griffing (1956a) is less demanding in terms of its genetical assumptions. Kempthorne's (1956) model allows a complete orthogonal partitioning of the total epistatic variance.

As was pointed out by Sprague (1966) and Christie and Shattuck (1992), in general any model developed for the estimation of genetic variances involves a series of biological assumptions. These vary somewhat with the model but the more common restrictions are:

1- normal diploid behaviour at meiosis.
2- no reciprocal (maternal or cytoplasmic) effects.
3- no multiple alleles.
4- linkage equilibrium.
5- no selection (random sample from a population).
6- no epistasis (no non-allelic interaction).

Hayman and Jinks developed some ideas on this theory in the 1960s in a series of papers (mainly in Jinks and Hayman, 1953; Jinks, 1954; Hayman, 1954a), in which they were mainly concerned about a particular set

of inbred lines. Although their method and its assumptions have been criticized by several authors (Gilbert, 1958; Baker, 1978) and some of the assumptions have been regarded as unrealistic (Baker, 1978), it has been widely used to investigate quantitative genetic aspects of metric characters. One of the features of the Hayman and Jinks method is the presentation of epistasis based on graphical analysis, in which the values related to V_r (the variance of all of the offspring of the ,th parent) are plotted against W_r (the covariance between those offspring and their nonrecurrent parents). This graph made it possible to study the relative dominance and epistasis properties of the parents used.

Kempthorne (1956) developed a model for analysis of the diallel crosses. His genetic interpretation was in terms of genetic variances and covariances in a random mating population. Therefore, he has mentioned the fact that his analysis is useful only when it is going to be used to make inferences about a base random mating population.

Griffing (1956b) has introduced four models depending upon whether or not the inbred lines and/or the reciprocal F_1's are included:

1- all p_2 combinations are included
2- inbreds and one set of F_1's are included
3- F_1's and reciprocal F_1's are included
4- only one set of F_1's are included.

Usually the experimental data are used to estimate genetic statistics of the population from which the parents were sampled, this being random effect model. A fixed effect model exists also, in which parent lines are considered to be a fixed set of lines and the results are not going to be extended to a further reference population.

Based on the paper written by Fisher (1918) and Kempthorne (1956), Griffing (1956a) developed his analysis. He has derived his model which represents the genotypic value in terms of additive and non-additive genetic effects. His final model symbolically was written as:

$$\sigma^2_G = \sigma^2_A + \sigma^2_{NA} = \sigma^2_A + \sigma^2_D + \sigma^2_I$$

Griffing (1956a) also defined general combining ability as a function of additive genetic effects and specific combining ability as a function of dominance genetic effects. Griffing (1956b) has further elaborated his idea. He presented the model for the $_{ijkl}th$ observation in a randomized-blocks design as follows:

$$x_{ijkl} = u + v_{ij} + b_k + (bv)_{ijk} + e_{ijkl}$$

in which u is the population mean effect v_{ij} is the effect for the $_{ij}th$ genotype, b_k is the $_kth$ block effect, $(bv)_{ijk}$ is the interaction between the $_{ij}th$ genotype and the $_kth$ block, and e_{ijkl} is the environmental effect peculiar to the $_{ijkl}th$ individual. The variety effects for those diallel crossing methods in which reciprocal F_1's are not included, are considered in terms of general and specific combining ability effects,

$$v_{ij} = g_i + g_j + s_{ij}$$

and the same effect for those diallel crossing methods in which reciprocal F1's are included were:

$$v_{ij} = g_i + g_j + s_{ij} + r_{ij}$$

The major disadvantage of diallel methods of analysis is the limitation of usage of parents in crossing scheme. This would subject the estimates of variance components to large sampling errors. In the other words estimates of variance components could not be significant estimates of population parameters unless the number of parents exceeds ten. Otherwise, a fixed model is recommended (Hayman, 1963).

Diallel cross between inbred plants can be used for measuring general combining ability in the development of open pollinated or synthetic cultivars

(Baker, 1978). It also can make useful information for measuring hybrid performances or in assessing prediction of the potential of hybrid breeding programme. In fixed models of diallel crosses, genetic interpretation of the results should be attempted only when the parents of the diallel cross are homozygous. In cross pollinated crops such as maize, this needs a laborious and time-consuming process. Self-incompatibility in plants such as red clover makes things more complicated. Because of these limitations most of the applications of diallel cross in cross pollinated plants is confined to estimating general and specific combining ability means and effects.

2.3.3 **Factorial mating design (North Carolina model II)**

This mating design, invented by Comstock and Robinson (1952) is also used to estimate genetic variance components within random-mating populations. This mating design also was initially planned to be used in F_2 populations derived by crossing 2 inbred lines. Using this scheme, with multi-flowered species, m males are crossed in all combinations with n females to produce mn full-sib families. In this method it is possible to distinguish both maternal and paternal arrays, therefore, comparing variances of maternal and paternal arrays, possible maternal effects can be measured (Kearsey, 1965, 1970). In this design covariance between half-sibs is estimated from two variance components. One from the sample of males and one from the sample of females, therefore, this design allows greater precision in the estimation of covariance between half-sibs than the hierarchical mating design (Wricke & Weber, 1986) which will be discussed later in this chapter, but there is a limitation of sampling enough representative individuals, from the base population.

Statistical model for this mating design is as follows:

$$Y_{hijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + R_h + e_{hijk}$$

in which

Y_{hijk} =phenotypic value of the $_{hijk}$th plant

μ = population mean

α_i = the effect of $_i$th male

β_j = the effect of $_j$th female

$(\alpha\beta)_{ij}$ = the interaction effects of $_i$th male and $_j$th female.

R_h = the effect of the $_h$th replication.

e_{hijk} = non-genetic effects.

Where progenies are compared in several replications in a simple RCB design, analysis of variance can be summarized in the following Table 2.2:

Table 2.2 : Analysis of variance appropriate for design II.

S.O.V.	D.F.	Expectations of M.S.
Reps	r-1	$\sigma_w^2 + k\sigma_e^2 + kmn\sigma_r^2$
Males	m-1	$\sigma_w^2 + k\sigma_e^2 + rk\sigma_{mf}^2 + rkn\sigma_m^2$
Females	n-1	$\sigma_w^2 + k\sigma_e^2 + rk\sigma_{mf}^2 + rkm\sigma_f^2$
Male X Female	(m-1)(n-1)	$\sigma_w^2 + k\sigma_e^2 + rk\sigma_{mf}^2$
Between plots	(mn-1)(r-1)	$\sigma_w^2 + k\sigma_e^2$

In which:

r = number of replication

m = number of male groups

n = number of females per male

k = plants per plot

σ^2_w = within plot variance

σ^2_e = among plot variance

σ^2_f = female parents components of variance

σ^2_m = male parents component of variance

σ^2_{mf} = interaction between male and female component of variance

σ^2_r = component of variance related to replication

The coefficients of genetic components of variance for F=0 can be summarized as follows:

Table 2.3 : Coefficients of genetic components of variance

Component	Covariance	V_A	V_D	V_{AA}	V_{AD}	V_{DD}	V_m	V_e
Male	$Cov_{HS(m)}$	1/4	0	1/16	0	0	0	0
Female	$Cov_{HS(f)}$	1/4	0	1/16	0	0	1	0
Interaction	Cov_{fs} - ($Cov_{HS(m)}$+ $Cov_{HS(f)}$)	0	1/4	1/8	1/8	1/16	0	0
Within plot		1/2	3/4	3/4	7/8	15/16	0	1

2.3.4 **Hierarchical mating design (North Carolina model I)**

This is one of the commonly used mating design that originally was invented by maize breeders, Comstock and Robinson (1948), and modified and developed by other animal and plant breeders. This scheme was initially designed at North Carolina Experiment station to be applied to F_2 populations of crosses between inbred lines, the design eventually has been applied to the estimation of variances and covariances in random mating populations (Comstock & Robinson, 1952). This design might be considered as a multipurpose mating design. It can be used both to estimate genetic variance components and to generate families for use in either full-sib or half-sib recurrent selection schemes (Stuber, 1980). Those who have made the most contribution in developing this mating design are: Robinson *et al.* (1949); Kempthorne (1957); Compton *et al.* (1965); Goodrich *et al.* (1975); Obilana *et al.* (1979); Becker (1984).

In this design each one of random male parents (m) is crossed with several random female parents (n) and each mating produces several

progenies. In Kempthorne's (1957) words, the genetical structure of the entries in the experiment is as follows:

1 The individuals in the same or different replicates resulting from a particular cross are full-sibs.

2 The individuals in the same or different replicates resulting from a common male parent but different female parents are half-sibs. Therefore, this design offers estimates based on both full-sib and half-sib family structure. As Becker (1984) has pointed out, the reference population is non-inbred random mating population (inbreeding coefficient F=0). Inbred lines can be formed from the population with no selection among or within the lines. The lines are crossed and the estimates of parameters refer to the base non-inbred population.

One possible experimental design for evaluating progeny families in this design can be outlined as below. The offspring of the mn crosses are grown in r replications with k plants per plot. The expectation of mean squares is presented in Table 2.4 (Wricke & Weber, 1986). The procedure for estimating variance components for this design was reported by Comstock and Robinson (1948) and simply involves equating observed mean squares to their expectations and solving the equations. The standard error of an estimated component can be computed as follows: (Satterthwaite, 1946; Crump, 1951; Compton *et al.*, 1965).

$$SE=\frac{1}{c}\sqrt{2\sum_i M_i^2/(F_i+2)}$$

in which M_i = the $_i$th mean square in the function by which the component is estimated.

F_i = the degree of freedom for the $_i$th mean square

c = the divisor of the mean square function.

There are some disputations on the form of the denominator of this equation. Some authors do not support the use of $(F_i + 2)$ in place of F_i as a

correction factor (Satterthwaite, 1946). But most of the authors would rather use (F_i + 2) (Compton *et al.*, 1965; Becker, 1984) particularly when some negative estimates have been estimated which makes estimates more sensitive to the test.

Table 2.4: Analysis of variance of hierarchical mating design

S. O. V.	D.F.	M.S.	Expectation of M.S.
Block	r-1	M_3	$\sigma_e^2 + mn\sigma_r^2$
Males	m-1	M_1	$\sigma_e^2 + r\sigma_f^2 + nr\sigma_m^2$
Females in males	m(n-1)	M_2	$\sigma_e^2 + r\sigma_f^2$
Error	(mn-1)(r-1)	M_4	σ_e^2
Total	mnrk-1		

σ^2_e is the sum of the intra "plot" environmental variance and the genetic variance among individuals of the same progeny.

σ^2_f is the variance of female effects.

σ^2_m is the variance of male effects.

In their notation

$$\sigma^2_m = \text{cov (half-sibs)} = \sigma^2_g/4 \Rightarrow \sigma^2_g = 4\,\sigma^2_m$$

and

$$\sigma^2_f = \text{cov(full-sibs)- cov(half-sibs)} = 1/4\,\sigma^2_g + 1/4\,\sigma^2_d$$

provided that q, the frequency of favourable allele, has the same value for all gene pairs the weighted degree of dominance for all loci equals:

$$a=\sqrt{2\sigma^2_d/\sigma^2_g}$$

In Comstock and Robinson's (1948) point of view "a" cannot exceed unity unless one or more of the d's, departure of the heterozygote from mid-point,

are larger than one. Therefore, if the estimate of "a" is significantly greater than one, it can be concluded that there is over dominance of genes at one or more locus.

The linear model of the hierarchical mating design is as follows (Wricke & Weber, 1986).

$$Y_{ijk} = \mu + m_i + F_{ij} + r_k + e_{ijk}$$

in which

Y_{ijk} = phenotypic value of the ijkth plant

μ = population mean

m_i = effect of male i, $m_i \sim N(0,\sigma^2 m)$

F_{ij} = effect of the female ij crossed with male i $F_{ij} \sim N(0,\sigma^2 f)$

r_k = block effect

e_{ijk} = non-genetic effect, $e_{ijk} \sim N(0, \sigma^2)$

Covariance between Y_{ijk} and $Y_{ijk'}$ (Full-sibs) equals to:

$$\text{Cov(full-sibs)} = \text{Cov}(Y_{ijk}, Y_{ijk'})$$

$$= E((m_i + F_{ij} + r_k + e_{ijk})(m_i + F_{ij} + r_{k'} + e_{ijk'}))$$

$$= E(m_i^2) + E(F_{ij}^2) = \sigma^2 m + \sigma^2 F$$

Since no covariance between different effects exists.

In a similar way Cov (half-sibs) equals to:

$$\text{Cov (Half-sibs)} = \text{Cov}(Y_{ijk}, Y_{ij'k'})$$

$$= E((m_i + F_{ij} + r_k + e_{ijk})(m_i + F_{ij'} + r_{k'} + e_{ij'k'}))$$

$$= E(m_i^2) = \sigma^2 m$$

In this mating design when the inbreeding coefficient is zero (F=0), such as in random mating populations, the genetic explanations of male and female components of variance can be summarized in Table 2.5 (Becker, 1984).

Table 2.5 : Genetic expectations of various components of variance.

Component	Covariance	V_A	V_D	V_{AA}	V_{AD}	V_{DD}	V_{AAA}
Male	Half-sibs	1/4	0	1/16	0	0	1/64
Female	Full-sibs - Half-sib	1/4	1/4	3/16	1/8	1/16	7/64
Within-plot	Total - Full-sibs	1/2	3/4	3/4	7/8	15/16	7/8
Male + Female	Full-sibs	1/2	1/4	1/4	1/8	1/16	1/8

Robinson *et al.* (1949; 1955) and Robinson & Comstock (1955) used this mating design to consider genetic variances within maize F_2 and open pollinated populations. They produced a large number of progenies, therefore, they modified the field experimental design to test progenies. In their work male groups were divided into s sets for field testing. The expectations of mean squares are presented in Table 2.6.

Table 2.6. Analysis of variance appropriate with design 1 when the male groups are divided to s sets.

S.O.V.	D.F.	MS	EMS
Sets	s-1		
Replications in sets	s(r-1)		
Males in sets	s(m-1)	M_{11}	$\sigma^2 + r\sigma^2_f + rn\sigma^2_m$
Females in males in sets	sm(n-1)	M_{12}	$\sigma^2 + r\sigma^2_f$
Remainder among plots	s(mn-1)(r-1)	M_{13}	σ^2

Numerous authors have used this mating design to investigate genetic variance components within and between plant populations, through which they have developed the efficiency of the design.

The previous references all investigated intra-population genetic variation. Robinson *et al.* (1958) arranged their crosses in such a way as to

consider inter-population genetic variation as well as intra-population genetic variation in maze. They crossed random plants as males from one variety (population) to four random plants each as female from another variety (population). Under the assumptions of no reciprocal effects (maternal or cytoplasmic effects) and that the varieties are in Hardy-Weinberg equilibrium as well as in linkage equilibrium they derived the following parameters.

(a) Heterosis measured as the variety cross mean minus the average of the two varieties.

(b) intra-population male component of variance (σ^2_{m11} *and* σ^2_{m22}).

(c) intra-population component of variance for females mated to a common male (σ^2_{f11} *and* σ^2_{f22}).

(d) Inter-population male component of variance (σ^2_{m12} *and* σ^2_{m21}).

(e) Inter-population female component of variance (σ^2_{f12} *and* σ^2_{f21}).

Robinson *et al.*(1958) also showed that if

$$a>0 \ , \ 1.0 < p+q < 1+1/a$$

or if

$$a<0 \ , \ 1+1/a < (p+q) < 1$$

where

a describes dominance, a<0 is negative dominance, a=0 no dominance and so on. p and q are relative frequencies of the favourable allele in the two populations, then the expected value of the average of the intra-varietal male components is larger in magnitude than that of the inter-crosses. Therefore, the ratio of $(\sigma^2_{m11}+\sigma^2_{m22})/(\sigma^2_{m12}+\sigma^2_{m21})$ is then expected to be larger than 1.

Conversely, if

$$a>0 \ , \ 1 < p+q < 1+2/a$$

and

$$a<0 \ , \ 1+2/a > p+q >1$$

The expected value of the ratio of

$$(\sigma^2_{f11}+\sigma^2_{f22})/(\sigma^2_{f12}+\sigma^2_{f21})$$

would be larger than one.

Goodrich *et al.* (1975) examined genetic variance among full-sib and half-sib families within two varieties of corn and in crosses between them. Following closely the method presented by Robinson *et al.* (1958). They added an extra step by having selfed the male parents. They designed their studies so that they could derive expressions for the variance among s_1 lines (σ^2_{s1}, σ^2_{s2}) and the genetic covariances among s_1 lines and the intra- and inter-population male components of variance (cov_{s1m11}, cov_{s2m22}, cov_{s1m12}, cov_{s2m21}). They also used the ratios $\sigma^2 s_1/\sigma^2 s_2$, $\sigma s_1 m_{11}/\sigma s_1 m_{12}$, and $\sigma s_2 m_{22}/\sigma s_2 m_{21}$, as a base for comparison to estimate average gene frequencies from the observed ratios.

Obilana *et al.* (1979) used this mating design in a slightly different way to examine genetic variation in an inter-population crosses, and also to compare this mating design with factorial mating design. They suggested that linkage bias may be the only reason for the occurrence of negative estimates in model I and II. They also conducted one cycle of selection on the population under study and the observed response was in a good agreement with expected response.

Gouesnard and Gallais (1992), examined the genetic variance components in maize, assuming negative estimations of variance components can arise in hierarchical mating design. They believe that in addition to inaccurate estimates, experimental problems, sampling error, or failure of the genetical or statistical assumptions might be other reasons for negative estimates of variance components. Sowing date of female and male parent comparing to each other could lead to over or under estimating of genetic variance components. Also assortative mating can cause a serious bias in estimates of genetic variance components. Therefore, they studied the effects of assortative mating on estimates of genetic variance components. They pointed out that under positive assortative mating conditions, additive variance components would be overestimated by $4(2\sigma_{MF}+\sigma_{FF})$ and dominance variance

would be underestimated by $8(\sigma_{FF'} + \sigma_{MF})$.

In which

σ_{MF} = the covariance of the male parent M_i and female parent F_{ij}.

$\sigma_{FF'}$ = the covariance of the females F_{ij} and $F_{ij'}$ mated to the same male.

Gouesnard and Gallais (1992) also confirmed that if the females crossed to the same male are related, the estimate of the male mean square increases and the female within male mean square estimate decreases. Subsequently the additive variance will be overestimated by $1 + (r + 2ar') / a^2$ and the non-additive variance will be underestimated by $1-2(r+ar')/(1-a^2)$, in which "r" is the correlation between two females, "r'" is the correlation between the mated male and a female, and "a" is the degree of dominance.

2.3.5 **Genetic variance components in red clover**

There are some published reports available in red clover on the estimation of the nature and magnitude of genetic variance components within populations (intra-population). Diallel crossing schemes have been more extensively used in red clover than has any other mating design.

Anderson, (1960) used a diallel cross mating design to examine genetic architecture of some characters in a late flowering red clover. They found significant differences in general combining ability and specific combining ability between seven non-inbred parents for flowering date and several other characters including growth habit. In their study the estimation of general combining ability variance for flowering date and growth habit was greater than specific combining ability variance.

Brandon & Leffel (1968) used a diallel cross scheme to consider pseudo-self-compatibility (PSC) in red clover. In their study sterility-allele genotypes occurred in abnormal ratios, and differed significantly for mean PSC, in two of the three F_1 progenies involving the 6 I_o clones as parents.

Taylor *et al.* (1970) employed a diallel cross mating design to compare genetic variance components in two generations from several red clover

parent plants. Effects of inbreeding and heterosis in red clover were investigated in two separate experiments. They concluded that selfing had significant effects on yield and hybrid vigour.

Anderson *et al.* (1974) evaluated 10 I_0 red clover parental clones to investigate genetic variance components in several characters including days to first flowering and yield. They concluded that general combining ability was the only significant source of genetic variance components for all of the measured characters. They suggested non-additive genetic variance component may not be important enough to warrant production of hybrid red clover cultivars.

Cornelius *et al.* (1977) found significant non-additive genetic variance for survival and vigour in progenies resulting from a diallel cross involving 10 I_1 (plants were selfed for one generation) red clover parent plants. They came to the conclusion that hybrid progenies can significantly increase red clover yield.

All forementioned works were done on the estimation of the nature and magnitude of genetic variance components within populations (intra-population). But there is no report available on genetic variability between population crosses (inter-population). These sorts of information are useful when a breeder is concerned about formulating a breeding programme based on several populations.

2.4 **Generation mean and variance analyses**

In generation mean analysis usually two parents with contrasting phenotypes for the attributes under study are selected and their F_1 and subsequent generations as well as backcrosses are produced. These are then grown in an appropriate field design and then the analysis of variance can be done as well as analysis of generation mean.

Using different approaches, various genetic parameters could be estimated, assuming no epistasis, mean value, additive, and dominance

effects could be estimated and tested for the perfect fit of the model. The mean value has different definitions in different approaches which will be discussed later in this chapter. In the case of the existence of epistasis (non-allelic interaction), a six parameter model was developed by several scientists (e.g. Hayman 1958; Mather and Jinks 1982), in which the first order non-allelic interaction effects between genetic effects were incorporated into the model. Also a twelve parameter model was developed (Gamble, 1962) in which the environment and its interaction with genetic effects were incorporated. These will be discussed briefly later in this chapter.

Based on the work done by Fisher *et al.* (1932), generation mean analysis was developed by Mather (1949) and was elaborated by several scientists (Anderson & Kempthorne, 1954; Jinks, 1956; Hayman, 1954; 1957; 1958; Gardner & Eberhart, 1966). This method is based on the performance of two homozygous lines that may be different by any number of unlinked genes and sets of their descendants. Fisher (1918) introduced a gene model which included dominance at a single locus. He coined the term dual epistacy to indicate the interaction of all possible pairs of loci affecting a given character. He extended this model later on, with his coworkers (1932) to investigate the function of any number of genes on a given attribute assuming no epistacy. Mather (1949) and Griffing (1950) introduced tests of generation mean for epistasis or non-allelic interactions.

Anderson and Kempthorne (1954) adapted a model based on the factorial model used in the field experimental design. They developed their model in such a way that they could explain the existence of epistasis or non-allelic interaction. They assumed that linkage and lethal genes were absent and that viability was constant for all genotypes.

Hayman (1958) further developed the possibility of a useful measures of epistasis and he investigated the problem of separating additive and dominance effects from epistatic effects.

If two homozygous lines differ by any number of unlinked genes according to Hayman (1958) the expectations of their means and some of their

descendant family and generation means may be expressed as

P_1	=	m	+d	-1/2h	+i	-j	+1/4l
P_2	=	m	-d	-1/2h	+i	+j	+1/4l
F_1	=	m		+1/2h			+1/4l
F_2	=	m					
B_1	=	m	+1/2d		+1/4i		
B_2	=	m	-1/2d		+1/4i		
F_3	=	m		-1/4h			+1/16l
B_1S_1	=	m	+1/2d	-1/4h	+1/4i	-1/4j	+1/16l
B_2S_1	=	m	-1/2d	-1/4h	+1/4i	+1/4j	+1/16l
F_4	=	m		-3/8h			+9/64l

$$the\ Y_i = m + \alpha d + \beta h + \alpha^2 i + 2\alpha\beta j + \beta^2 l$$

In which

P_1 and P_2 = means of two parent lines.

F_1 = mean of F_1 progenies.

F_2, F_3 and F_4 = means of generations descending from this cross by selfing.

B_1 and B_2 = means of the first backcrosses to the parents.

B_1S_1 and B_2S_1 = means of the progeny of selfing these first backcross families.

Y_i= $_i$th generation mean,

m= mean of F_2 generation,

d= Pooled additive effects,

h= Pooled dominance effects,

i= Pooled interactions between additive effects,

j= pooled interactions between additive and dominance effects,

l= Pooled interactions between dominance effects,

α and β = coefficients of corresponding parameters.

Mather and Jinks (1982) have further developed the theory of generation mean analysis. In their terminology,

m= mid point between two homozygote genotypes (P_1 and P_2),

d, h, i, j, and I have the same meaning as those for Hayman's method.

As it can be seen one of the major differences between Hayman's method and Mather & Jinks' method can be summarized in the definition of '*m*'. In the Hayman's method *m* represents the F_2 mean, whereas in Mather and Jinks' method '*m*' represents the mid-point between the two homozygous parents. The expected deviation of the mean of any population can be specified in terms of *d* and *h*. In the F_2 generation the homozygotes cancel out each other and the heterozygote will contribute to the generation mean. Hence summing over all segregating genes the F_2 generation mean [F_2] will equal

$$m + 1/2\,[h]$$

Thus the F_2 will deviate from the mid-point value by an amount which is equal to the half the deviation of the F_1 from the mid-parent. This result can be generalized to any generation derived by selfing the successive generations derived from an F_1. For example in respect of each gene segregating in the cross in F_n generation $(1/2)^{n-1}$ individuals will be heterozygous. Therefore, the generation mean will be

$$F_n = m + (1/2)^{n-1}[h]$$

Using the same premise the expected generation means for a backcrossing series from an F_1 can be obtained. So that

$$B_1 = m + 1/2\,[d] + 1/2\,[h]$$

$$B_2 = m - 1/2\,[d] + 1/2\,[h]$$

Other generation mean expectations like sib-mating series and further generations of backcrossing are fully described in Mather and Jinks (1982).

Mather and Jinks (1982) first introduced a three parameter model by which m, d, and h could be estimated. If there are interactions between non-allelic genes, this simple additive-dominance model is insufficient. Adequacy of the additive-dominance model can be tested in a number of ways including "scaling" test and "joint scaling" test.

In the scaling test the quantities A, B, and C and their variances are calculated using following equations

$$A = 2B_1 - P_1 - F_1$$

$$B = 2B_2 - P_2 - F_1$$

$$C = 4F_2 - 2F_1 - P_1 - P_2$$

Based on the variance of the functions we have :

$$\sigma^2(x-y) = \sigma^2x + \sigma^2y - 2cov\ xy$$

Apparently Mather and Jinks (1982) have assumed that the covariance between various generations are zero then they have calculated the variance of the mentioned functions as follows:

$$V_A = 4V_{B1} + V_{P1} + V_{F1}$$

$$V_B = 4V_{B2} + V_{P2} + V_{F1}$$

$$V_C = 16V_{F2} + 4V_{F2} + V_{P1} + V_{P2}$$

These equations are widely used along with joint scaling test in order to test the adequacy of three parameter model. The number of applications are too large to be mentioned here. If the model is adequate these quantities should equal zero within the limits of sampling error.

A joint scaling test was introduced by Cavalli (1952) and was elaborated by Mather and Jinks (1982). It provides a suitable way for estimating the parameters, *m, [d]* and *[h]* from the means of the available types of generations as well as comparing observed generation means with their expected values.

In practice because different generations have different expectations, family size is deliberately varied with the kind of family. Genetically uniform populations usually have smaller family size than segregating generations. Therefore, means have different variations. The remedy for this is to use the inverse of the variance of each family mean as a weight for that family.

A twelve parameter model was developed by Gamble (1962) in which he has incorporated environmental effects and their interaction with genetic effects. In his notation the mean of each generation would be as follows:

$$Y_{ik} = m+\alpha a+\beta d+\alpha^2 aa+2\alpha\beta ad+\beta^2 dd+\gamma e+\alpha\gamma ae+\beta\gamma de+\alpha^2\gamma aae+2\alpha\beta\gamma ade+\beta^2\gamma dde$$

in which

Y_{ik} = $_i$th generation mean in the $_k$th environment

m = F_2 generation mean which is called background mean

α, β, and γ= Coefficients of corresponding parameters

a = Pooled additive effects (average allele effect)

d = Pooled dominance effects

e = Environmental effects

aa = Interaction between additive effects

ad = Interaction between additive and dominance effects

dd = Interaction between dominance effects

ae, de, aae, ade, and dde are the interactions between genetic and environmental effects.

In all of the three models, three, six, and twelve parameter, the equations could be expressed in matrix notation as follows:

$$\mathbf{G= J^{-1} M}$$

in which

G = the vector containing estimated parameters.

$\mathbf{J^{-1}}$ = information matrix (variance covariance matrix).

M = the vector of weighted observed means.

The dimension of the vectors and matrix depends on the model used.

Generation mean analysis has been widely used to estimate the genetic parameters in some crops like wheat. As was pointed out by Sprague (1966) it does not require extremely sophisticated field designs. In spite of its simplicity, however, the method lacks general utility due to some limitations imposed by the method. As it is applicable only where gene frequencies are known, as in F_2 or other derived populations resulting from a cross between two homozygous lines. Furthermore, although this method can provide evidence of the existence of different types of gene action it provides no

measure of their relative importance. The results can not be related to any ancestral population as the estimates obtained from each pair of inbred parents may be unique in varying degrees. For these reasons using methods based on partitioning genetic variance components are much more widely used than that of generation mean analysis.

If a particular population has been isolated under a natural or artificial selection in favour of particular attributes for a long time, the alleles controlling the attributes may be concentrated in the population, therefore, parent plants from such a population could be regarded as a representative plant and the results could be extended to the reference population.

2.5 **Estimating the number of genes**

Since genetic architecture of quantitative variation affects the response to selection (Mitchell-Olds& Bergelson, 1990), the number of loci influencing quantitative attributes in plant populations and magnitude of their effects have been the topic of a considerable discussion for many years (Lande, 1981; Lande and Arnold 1983; Mather and Jinks, 1982; Cockerham, 1986; Mayo, 1980). Based on the measurements on the different generations resulted from a cross between two parents, several statistical techniques have been suggested for estimating the number of genes that control the quantitative characters between two inbred lines (e.g. Wehrhahn and Allard, 1965; Tan and Chang, 1972; Jinks and Towey, 1976; Towey and Jinks, 1977; Choo and Reinberg, 1982; Mather and Jinks, 1982) and between diverse random mating populations (e.g. Wright, 1968; Lande, 1981; Cockerham, 1986). Mulitze and Baker (1985a and 1985b) have evaluated the existing biometrical methods for estimating the number of genes. They have investigated the effects of sample size of estimating the number of genes using various approaches. Eventually, although, they have mentioned that both ways of estimating the number of genes, assay procedure (used by Jinks and Towey, 1976 and Towey and Jinks, 1977) and inbred backcrosses procedure (used by Wehrhahn and

Allard, 1965) are subjected to upward or downward biases. They have concluded that the estimation of the number of genes are theoretically less reliable when estimated by the genotype assay procedure than by the inbred-backcross procedure.

Regarding two diverse plant populations, Lande (1981) suggested a basic theoretical formula for estimating the minimum number of genes affecting a metrical trait as follows:

$$n_E = \frac{(\mu_{P2} - \mu_{P1})^2}{8\sigma^2_s}$$

Its variance also could be estimated by the following equation:

$$Var[n_E] = n_E^2 \left(\frac{4\left[\frac{\sigma^2_{P1}}{N_{P1}} + \frac{\sigma^2_{P2}}{N_{P2}}\right]}{[\mu_{P2} - \mu_{P1}]^2} + \frac{Var[\sigma^2_s]}{\sigma^4_s} \right)$$

in which n_E is minimum number of genes and μ_{P2} and μ_{P2} are the mean values for parents. σ^2_{P1}, σ^2_{P2} are the variances of parental populations. σ^2_s is the extra genetic variance in F_2 population beyond that in F_1. Lande (1981) presented several alternatives for estimating σ^2_s. Cockerham (1986) combined them into one estimate by least squares ending with the following equation.

$$\sigma^2_s = 0.2(4\sigma^2_{F2} + \sigma^2_{B1} + \sigma^2_{B2}) - 0.4(\sigma^2_{P1} + \sigma^2_{P2} + \sigma^2_{F1})$$

and

$$Var(\sigma^2_s) = 0.08\left[\frac{16\sigma^4_{F2}}{N_{F2}} + \frac{\sigma^4_{B1}}{N_{B1}} + \frac{\sigma^4_{B2}}{N_{B2}}\right] + 0.32\left[\frac{\sigma^4_{P1}}{N_{P1}} + \frac{\sigma^4_{P2}}{N_{P2}} + \frac{\sigma^4_{F1}}{N_{F1}}\right]$$

As was pointed out by Lande (1981) the effective number (n_E) may be greatly underestimated by decreasing the mean difference between the parental populations, also linkage of loci influencing the character increases σ^2_s that in turn underestimates the actual number of genes. Furthermore, non-

allelic interactions are likely also to produce a downward bias in estimates of n_E. Although Lande (1981) generalized the procedure proposed by Wright (1968) to be applied on two heterogenous parental populations but the assumptions that he supposed could be easily violated. Cockerham (1986) elaborated the procedure in order to eliminate the probable biases as much as possible.

CHAPTER THREE: MATERIALS AND METHODS

Three major experiments were carried out in Massey University's glasshouses and fields during the years 1991 - 1993 to investigate the quantitative genetics of prostrateness and other related characters in red clover. A wide range of genetic materials were studied in these experiments. Depending on the model of each experiment itself and the amount of genetic material covered, certain genetic parameters can be estimated as in any particular model certain violations from the assumptions may be ignored which will be discussed later in chapters four, five, and six.

3.1 Genotypes

Several red clover accessions with specific characteristics, such as prostrate growth habit, early flowering and adventitious roots on the stems, were introduced from Spain to New Zealand by the late Dr. Margot Forde from Agresearch, Palmerston North, New Zealand. Four accessions of this type of red clover, along with four accessions of semierect and four cultivars of erect red clover were employed in this work. Accession numbers and the structure of these accessions are listed in Appendix 1, Table 1.

This germplasm was examined at three levels of sophistication; (1) all twelve genotypes were examined at two sites for two years in a genotype-environment interaction experiment; (2) two accessions from two diverse types, erect and prostrate, were randomly taken as two populations. These two populations were investigated in a hierarchical mating design to estimate broadly their quantitative genetics; and (3) six generations of three such crosses were examined, using Mather and Jinks (1982) generation mean analysis method, to estimate fine detail of the quantitative genetics for prostrateness and other related characters.

3.2 **Objectives**

The overall objectives of these experiments were:

1- Investigation of the quantitative genetic aspects of prostrate growth habit and other related characters in red clover (*Trifolium pratense* L.);

2- Investigation of the influence of genotype-environment interaction in red clover for the prostrate growth habit and other related attributes.

3- Defining prostrateness gene action.

4- Estimating the number of genes (factors) controlling the attributes.

5- Investigation of the phenotypic and genotypic correlations between the attributes.

6- Investigation of genetic architecture of two diverse red clover plant populations.

3.3 **Genotype-environment interactions**

The main concern in these experiments was to estimate genotypic variances, phenotypic and genotypic correlations, and heritability from the wider gene pool, to estimate genotype-environment interaction and to investigate the possible pattern of the response of genotypes studied to genotype-environment interaction. These experiments were started in mid-July 1991. Four accessions from each type were used, i.e. four prostrates, four semi-erects and four erects (Appendix 1, Table 1).

3.3.1 **Test locations and land preparation**

Experiments were carried out for two years, 1991 and 1992, in two sites at Massey University research fields. The two sites were at Frewans and Mogini paddocks. These differed in altitude, soil type and soil fertility. Based on New Zealand Land Resource Inventory survey done by National Water and Soil Conservation Organization (Aokautere Science Centre, Palmerston North), Frewans block is classified as 111w1 with a deep fertile soil on the Manawatu Plains. Its grazing capacity for top farmers is 23 stock units per hectare and

the soil type is Manawatu fine sandy loam. Mogini Block is classified as 11s2 with a yellow-grey earth soils developed on loess. and its grazing capacity for top farmers is 15 stock units per hectare. There is limitation due to seasonal soil moisture deficiencies and a sub-surface pan which impedes drainage. Climatic type in both sites, as defined by Fletcher (1987), is D_1. This climate has an annual rainfall of 900 to 1300 mm, with prevaling west to northwest wind. Rainfall is reliable and evenly distributed throughout the year. Both sites generally have warm summers and mild winters.

To prepare the land, the vegetation on the ground was sprayed in March with 3.3 kg/ha of glyphosate (Roundup) plus 0.4 kg/ha of dicamba (Banvel 200). The high rate of glyphosate killed all perennial weeds present including docks, the dominant perennial weed in the plots. The dicamba was added to ensure that the white clover (*Trifolium repens*) was killed. After several weeks, the land was cultivated. For the remaining months prior to the sowing date, weeds which established were killed by applying 0.5 kg/ha paraquat (Preeglone). Trifluralin (Treflan) at 1.0 Kg/ha was applied on the land and incorporated into the soil using a powered implement, one day prior to transplanting.

3.3.2 **Evaluation**

3.3.2.1 **Seedling raising and Experimental design**

Seeds were germinated on wet filter paper, in a germinator, under 20°C and dark conditions. 0.5% KNO_3 solution was used as imbibant to help germination. The germinated seeds were sown into planting medium in individual peat-pots (Jiffy pot) in a medium made by sand and peat in the proportion of 3:1 by volume and 50 gram Osmocote (a commercial preparation of mixed fertilizers including NPK in the form of granules) per bucket of media. Medium was steam sterilized and the pots were watered with distilled water for two weeks to prevent fungi contamination. 0.5 g/L benomyl solution (Benlate) was mist sprayed every fortnight on seedlings to prevent damping

off. Methiocarb granules was used to prevent slug damage. Taufluvalinate (Mavrik Aquaflow) at 48 mg/L was sprayed once a week to control aphids. The environmental conditions in glasshouse were: 20°C day and night, with natural day length. After eight weeks the intact pots were transplanted into the field. A RCB design with three replications was conducted in each location. Each plot consisted of one row with five single plants. Within and between row spacing was sixty centimetres. Weeds were controlled by hand.

3.3.2.2 **Studied attributes**

The following characters were recorded on all individual plants over the two years.

Prostrateness: Prostrateness was scored from 1-5 in the following manner.

Score 1 for those plants which were completely erect and the angle between the main stem and horizontal line was in 75°-90° interval.

Score 2 for those plants that the angle between the line connects the stem tip and crown and soil surface was 50°-75° interval.

Score 3 for those plants that the fore-mentioned angle was in 25°-50° interval.

Score 4 for those plants that the fore-mentioned angle was less than 25° but the end part of stems were upward growing.

Score 5 for those plants which were absolutely prostrate.

Rooting ability on stems: This character was scored from 1-5 on following basis.

Score 1 for those plants on which no root were formed on the nodes.

Score 2 for those plants on which adventitious roots were formed on the first and second nodes.

Score 3 for those plants on which adventitious roots were formed on the first to third nodes.

Score 4 for those plants on which adventitious roots were formed on the first to fourth nodes.

Score 5 for those plants on which adventitious roots were formed on the first to fourth + nodes.

Days to first flowering: Number of days after sowing date on which each plant had one floret fully open was recorded for all individual plants. This character plays an important role in the life of the plant. According to Choo (1984) the flowering response can be used as a selection criterion for persistent plants.

Median flowering date: Number of days after sowing date on which the plant had fifty percent of its flowers open, as estimated visually.

Leaf size: Leaves were scored according to their area from one to five at median flowering date. The smallest ones were scored 1 and the biggest one 5. Half scores were used as required.

Stem length: Stem length was measured at median flowering stage. Stem length was measured in centimetres, from plant crown to stem tip, on three main stems. These were averaged for analysis where the plant was the observational unit.

Stem thickness: Stem thickness was measured in millimetres on three random main stems per plant at the third internode at median flowering stage. The average of the three measurements became the value for analysis.

Number of main stems per plant: Number of stems per individual plants was recorded at median flowering stage.

Number of internodes: Number of internodes was counted on three random main stems per individual plant at median flowering stage.

Number of branches: Number of branches was counted on the same stems used for obtaining internode number at the same time.

Dry matter yield: Plants were cut at median flowering stage, and weighed. A sample of 200 gram from each plant was dried in an oven at 75°C immediately after field sampling. From the total and sample fresh weights, and the sample dry weight, the whole-plant dry matter weights were estimated in grams.

3.3.2.3 **Statistical analysis**

3.3.2.3.1 **Univariate analysis**

The data recorded on the attributes prostrateness, nodal rooting ability, and leaf size were based on a score from one to five. Therefore, the distance between 1 and 2 may not be the same as between 3 and 4 and so on. To fix this problem the scores were transformed to rankit to overcome this problem. Rankit transformation returns the *p*th quantile from the standard normal distribution. Where *p* is a numeric probability, with $0 \leq p \leq 1$. To get the transformed data the original data was multiplied by two then divided by 10, then it was subjected to rankit transformation. The result was summed with five to get a positive value after transformation: $X_t = \text{probit}((X_o * 2)/10) + 5$, in which X_t is the transformed data, and X_o is data in original scale.

Analysis were done using General Linear Model (GLM) procedure in SAS package (SAS institute Inc., 1990). One of the assumptions underlying the pooled analysis of variance is homogeneity of experimental errors, although Cochran (1947) pointed out that, even with heterogeneity, the pooled estimate is still the only practical single estimate obtainable. In order to test the homogeneity of error variances, Bartlett's test (Steel & Torrie, 1980) was conducted. The results showed error variance of some characters in different experiments to be heterogenous which were not serious. The results of the test of homogeneity are presented in the Table 2 Appendix 1. Pooled analysis of variance was done on the data. For the pooled analysis of variance the random effects model was applied. The intention in applying this model was to consider both environments and genotypes as random samples from their respective population of inference.

The model is as follows:

$$Y_{ijklm} = \mu + \eta_l + \rho_{k(l)} + \alpha_i + (\alpha\eta)_{il} + \delta_{ik(l)} + \beta_j + (\beta\eta)_{jl} + (\alpha\beta)_{ij} + (\alpha\beta\eta)_{ijl} + \varepsilon_{ijkl} + \omega_{ijklm}$$

in which

Y_{ijklm} = the phenotypic value of the $_n$th plant of the $_i$th genotype in the $_k$th block grown in the $_l$th location in the $_j$th year.

μ = the grand mean.

η_l = additive effect of the $_l$th location.

$\rho_{k(l)}$ = the effect of $_k$th replication at the $_l$th location.

α_i =the effect of the $_i$th genotype.

$(\alpha\eta)_{il}$ = the interaction between $_i$th genotype and $_l$th location.

$\delta_{ik(l)}$ = the main plot "error"

β_j = the effects of $_j$th year.

$(\beta\eta)_{jl}$ = the interaction between $_l$th location and $_j$th year

$(\alpha\beta)_{ij}$ = the interaction between $_l$th genotype and $_l$th location.

$(\alpha\beta\eta)_{ijl}$ = the interaction between $_i$th genotype, $_l$th location and $_j$th year.

ε_{ijkl} = the random error associated with the individual plots.

ω_{ijklm} = residual

i = index of genotypes = 1...g, where g = 12

j = index of years = 1...y, where y = 2

k = index of replications = 1...r, where r = 3

l = index of locations = 1...s, where s = 2

m = index of plants per plot = 1...k, where k = 5 (plants per plot)

The expectations of mean squares and the degree of freedom for the analysis of variance of a series of experiments pooled over sites nested within year given all the effects are random are presented in Table 3.1.

Table 3.1: The expectations of mean squares and the degree of freedom for the analysis of variance of a series of experiments pooled over sites nested within year given all the effects are random.

S.O.V.	D.F.	df values	MS	Expectation of M.S.	F test
Site (S)	(s-1)	1	11	$\sigma^2_w+n\sigma^2_\varepsilon+ny\sigma^2_{RG(S)}+nyg\sigma^2_{R(S)}+nr\sigma^2_{GSY}+nrg\sigma^2_{SY}+nry\sigma^2_{SG}+nryg\sigma^2_S$	(11+7+3)/(10+2+5)
Block(SY)	s(r-1)	4	10	$\sigma^2_w+n\sigma^2_\varepsilon+ny\sigma^2_{RG(S)}+nyg\sigma^2_{R(S)}$	10 / 7
Genotype(G)	(g-1)	11	9	$\sigma^2_w+n\sigma^2_\varepsilon+ny\sigma^2_{RG(S)}+nr\sigma^2_{GSY}+nry\sigma^2_{SG}+nrs\sigma^2_{GY}+nrsy\sigma^2_G$	(9+3) / (8+4)
G x S	(g-1)(s-1)	11	8	$\sigma^2_w+n\sigma^2_\varepsilon+ny\sigma^2_{RG(S)}+nr\sigma^2_{GSY}+nry\sigma^2_{SG}$	(8+2) / (7+3)
$Error_a$	s(r-1)(g-1)	44	7	$\sigma^2_w+n\sigma^2_\varepsilon+ny\sigma^2_{RG(S)}$	7 / 2
Year (Y)	(y-1)	1	6	$\sigma^2_w+n\sigma^2_\varepsilon+nr\sigma^2_{GSY}+nrg\sigma^2_{SY}+nrs\sigma^2_{GY}+nrsg\sigma^2_Y$	(6+3) / (5+4)
S x Y	(y-1)(s-1)	1	5	$\sigma^2_w+n\sigma^2_\varepsilon+nr\sigma^2_{GSY}+nrg\sigma^2_{SY}$	5 / 3
G x Y	(g-1)(y-1)	11	4	$\sigma^2_w+n\sigma^2_\varepsilon+nr\sigma^2_{GSY}+nrs\sigma^2_{GY}$	4 / 3
G x S x Y	(g-1)(s-1)(y-1)	11	3	$\sigma^2_w+n\sigma^2_\varepsilon+nr\sigma^2_{GSY}$	3 / 2
$Error_b$	sm(y-1)(r-1)	48	2	$\sigma^2_w+n\sigma^2_\varepsilon$	2 / 1
Within	sgyr(n-1)	576	1	σ^2_w	

in which

σ^2_w = within plot variance component

σ^2_ε = error b

$\sigma^2_{RG(S)}$ = error a

$\sigma^2_{R(S)}$ = the component of variance related to block nested within site.

σ^2_{GSY} =second order interaction component of variance

σ^2_{SY} =the component of variance attributed to interaction between site and year

σ^2_{SG} =the component of variance attributed to interaction between site and genotype

σ^2_{GY} =the component of variance attributed to interaction between year and genotype

σ^2_S =the component of variance attributed to environment

σ^2_G =the component of variance attributed to genotype

σ^2_Y =the component of variance attributed to year

Complex F-tests were required in this model (last column of Table 3.1). The appropriate degrees of freedom for F-test for any effect can be estimated using the Satterthwaite equation (1946). The numerator or denominator of the complex F-test equals:

$$= \frac{(\sum_n MS_n)^2}{\sum_n (MS_n^2 / f_n)}$$

in which

n = number of MS's in the numerator or denominator of the F-test. f_n is the degree of freedom associated with MS_n, the $_n$th mean square in the linear function.

It is believed that since in perennial crops experimental layouts remain constant over the years, environments sampled from any consecutive years may be correlated. Therefore, the correlation between time was estimated by

following equation (Gill 1986) to see how much the two successive years are correlated.

$$r_t = \frac{\text{error(a) - error(b)}}{\text{error(a) + (y-1)*(error(b))}}$$

While the r_t estimates suggested that correlations are negligible, standard analysis of variance was first carried out for each character at each location and year.

3.3.2.3.2 **Genetical analysis**

Variance components including first and second order interaction effects were estimated for each character by equating observed mean squares to their expectations. These were used in estimating phenotypic and genotypic correlation and heritability values. Standard error of estimated components of variance was estimated by a programme called THWAITE (unpublished, written by Gordon, Massey University).

3.3.2.3.3 **Phenotypic and genotypic correlation**

Overall phenotypic correlation was estimated for the whole data set in this experiment. It is well documented that phenotypic and genotypic correlations may change from year to year, population to population (Falconer, 1989). Therefore, phenotypic and genotypic correlation were estimated for each year and type separately as well as whole the data set. Procedure CORR in SAS package (SAS institute Inc., 1990) was used in order to estimate phenotypic correlations and their significance. Phenotypic correlations could be estimated by the following equation.

$$r_p = \frac{Cov_{P_{ij}}}{\sqrt{V_{P_i} X V_{P_j}}}$$

in which

Cov_{Pij} = phenotypic covariance component

V_{Pi} = phenotypic variance component of attribute i

V_{Pj} = phenotypic variance component of attribute j

To estimate genotypic correlation the following equation was used (Baker, 1986; Kempthorne, 1957).

$$r_g = \frac{CovG_{ij}}{\sqrt{V_{Gi} X V_{Gj}}}$$

in which

$CovG_{ij}$ is genotypic covariance between the two characters.

V_{Gi} = genotypic variance of the character i

V_{Gj} = genotypic variance of the character j

Using the same data for phenotypic correlation analysis a pooled randomized complete block design multivariate analysis of variance was carried out on all characters in order to get $CovG_{ij}$, σ^2G_i, and σ^2G_j. Proc GLM and the statement MANOVA from SAS package (SAS institute Inc., 1990) were used. The MANOVA statement supplied the genetic variance/covariance matrix, which was used to estimate genotypic correlations.

3.3.2.3.4 **Heritability and genetic advance**

Full and restricted heritability (Gordon *et al.*, 1972; Gordon, 1979) were estimated for all studied attributes in this experiment using following equations.

$$h^2_{full} = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_s + \sigma^2_{SG} + \sigma^2_y + \sigma^2_{ys} + \sigma^2_{YG} + \sigma^2_{YGS} + \sigma^2_{R(S)} + \sigma^2_W + \sigma^2_{RG(S)} + \sigma^2_\epsilon + \sigma^2}$$

$$h^2_{res} = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_{GS} + \sigma^2_{GY} + \sigma^2_{YGS} + \sigma^2_W + \sigma^2_{R(S)}}$$

in which the components are the same as what were described in Table 3.1.

The standard errors of the heritability estimates were estimated using the procedure presented by Gordon (1979) called variance of ratio approximation.

Using phenotypic variance of the genotypes under study, genetic advance (GA) was predicted by the following equation (Falconer, 1989) assuming 10% of the population would be selected (i=1.4).

$$GA = i\,\sigma P\,h^2$$

in which i is selection intensity and its value depends on the proportion of the population included in the selected population. It is assumed that the selection is based on both sexes and individual selection is applied.

3.3.2.4 **Multivariate analysis**

Several multivariate analysis methods were applied on this data set in order to investigate the behaviour of these genotypes in environments examined.

3.3.2.4.1 **Multivariate discriminant analysis**

Multivariate discriminant analysis was used to find possible pattern of genotype-environment interaction effects amongst the examined genotypes. First a full model of the GE interaction experiments was applied using procedure GLM in SAS package (SAS Institute Inc., 1990). Apart from the results of univariate analysis, a multivariate partitioning of GE interaction was

carried out using the MANOVA statement in conjunction with the CANONICAL option from procedure GLM in SAS. This was done using GxSxY effects as the effect being tested and YxB(GxS) as error term (Table 3.1). To find the possible pattern between examined genotypes in responding to environment by estimating discriminant functions.

Since stem thickness, internodes, and dry matter yield were the only attributes which showed a significant GxSxY effect, a multivariate partitioning of variance for these attributes was conducted in conjunction with pooled analysis of variance to investigate the possible pattern of environmental response amongst the genotypes in the data set regarding attributes with significant second order interaction effect.

Prostrateness, first flowering, stem thickness, number of stems, and number of branches showed a moderate to high significant first order interaction of GxY. Therefore, another multivariate discriminant analysis of variance was carried out on the mentioned attributes following a pooled analysis of variance, in order to investigate the possible pattern of environment response amongst the genotypes regarding GxY interaction effect. All these analysis were carried out using procedure GLM, Statement MANOVA, and option CANONICAL in SAS package (SAS Institute, Inc., 1990).

The same procedures were used to estimate discriminant functions and to investigate the role of different attributes in forming different types. In this case types were regarded as genotype treatments.

In all of the cases mentioned above at least 70% of the discriminating power was used to specify the number of functions from structure matrix which can retain the majority of the variation in the data. Then the specified discriminant functions were named based on their association with attributes studied. These functions also were used to specify the importance of the measured attributes in forming the function. The same number of standardized coefficients as discriminant functions were used in conjunction with discriminant functions in order to interpret the role and importance of variables.

An unpublished lexicon from Gordon (Massey University) was used to name the variables based on their contribution in discriminant function and standardized coefficients.

3.3.2.4.2 **Cluster analysis**

Doing multivariate discriminant analysis of variance based on GxSxY effect, for all attributes, attributes with significant GxSxY and GxY effects, two discriminant functions were qualified as the best discriminator of the variables under study. Although those parsimonious scores would reduce the dimension of the data set and make the existing pattern of the different effect on the data much easier to understand and interpret but still there are a wide range of statistics to consider. Therefore, discriminant scores on which the extent of contribution of each particular attribute to those scores could be explained were used along with some other estimates such as environmental mean values of variables, grand mean and their standard errors, to generate another score for cluster analysis by a programme called SCOREST written by Gordon (unpublished, Massey University). SCOREST calculated scores which were used in the cluster analysis to recognize the groups of genotype regarding to their interaction with the imposed environments. Procedure CLUSTER in SAS package (SAS institute Inc., 1990) and Ward method of clustering was used in which minimal increase of within cluster sum of squares is used as the criterion of merging.

Based on the classification of the clustering method done by Williams (1971), the Ward method is an agglomerative, exclusive classification method. This method sometimes is known as incremental sums of squares. In a method of clustering such as Ward (Agglomerative hierarchical clustering) the cut off point is a matter of controversy. It may be arbitrary, but more objective methods are derivable. A method suggested by Gordon (unpublished) and used by Mozafari (1992) defines the cut off point at that clustering stage where the amongst-cluster mean square is most significant. The original attributes

are reanalysed using MANOVA and cluster membership defined by successive stages of clustering. This was done with procedure GLM in SAS package (SAS Institute Inc., 1990), using the same model and data as for the original MANOVA, but defining groups as just discussed, rather than by original genotypes. The successive F tests for clusters were examined, and the clustering stage where this was most significant was used to define the truncation point.

Plate 1: General performance of the two types of red clover, prostrate and erect

3.4 Hierarchical mating design

3.4.1 Parent plants

Two extreme types of red clover, prostrate (F2427, Table 1, Appendix 1) and erect (F2256), were used in this mating design experiment as two separate populations. A general performance of the two types is presented in plate 1. Proper randomization of crosses and planting sites precluded most sources of bias. Therefore, parental plants were randomly taken to prevent bias in estimations. Nine randomly taken plants from prostrate accession (F2427) and 9 randomly taken plants from erect accession (F2256) were used as male parent plants. 54 individual plants were taken randomly from erect population and 54 from prostrate population as female parents. On the whole, 136 individual plants were sampled and used as a representative sample from two populations.

3.4.2 Cloning

Red clover is a cross pollinated crop. As a result considerable variation exist between individual plants. Consequently, studies on red clover are complicated, since observations on plants grown from seed must be made on single plants rather than a replicated groups of plants. Mating designs are more readily interpreted if the parents are fixed genotypes. One way to fix the parental genotype is by inbreeding, of which selfing is the most rapid and useful method. However, in the case of red clover selfed lines are very hard and time consuming to produce because of self-incompatibility. The majority of the investigations in breeding of red clover have been conducted through sib-mating schemes. Compared to selfing, sib-mating is a slower way of advancing towards homozygosity.

Cloning is another approach to produce a fixed genotype, each of which could be used as fixed parental lines in genetic studies. To provide fixed

genotype parental plants, the randomly taken male parent plants were cloned using crown and stem cutting procedures.

Individual plants were grown under natural day length in glasshouse conditions during early winter. When the plants had produced long stems and suitable number of shoots, individual plants were cloned by crown or stem cutting procedures. In the case of prostrate parent plants, individual plants produced long stems in natural day length. All stem cuttings were taken in such a way that each included two nodes. One leaf was left attached to the upper node of cuttings. All expanded leaves were removed in order to reduce transpiration. The lower end one cm of cuttings was dipped in a rooting hormone (Seradix 2, a commercial preparation containing 3 g/kg beta-indolylbutyric acid in the form of dust), and the excess shaken off. The cuttings were planted in moist sand, one node being immersed and the other just above the sand.

In the case of erect parent plants, these didn't produce long stems in natural day length. Instead they produced a large number of short shoots. Therefore, crown cutting was applied in such a way so that each single plant was divided into several parts at the crown region. Cuttings initially were planted in moist sand. Environmental conditions were set up to produce optimum rooting. The containers were kept in a high humidity cabinet with a warm bed (25° C). Overhead misting was applied automatically for five seconds every five minutes. As soon as they set enough roots they were transplanted into pot with a mixture of sand and peat and fertilizer media. Aspects of genetic variability in rooting on these materials have been discussed in Mirzaie-Nodoushan & Gordon (1993).

3.4.3 **Crossing**

3.4.3.1 **Field crossing nursery**

The parent plants were transplanted into the crossing nursery late in October according to a crossing plan following the procedure described by Robinson *et al.* (1958). Crosses were set up in such a way that two sets of intra- and two sets of inter-population crosses were produced. To generate these progeny families, 9 randomly taken clones from erect populations and 9 randomly taken clones from the prostrate population were used as male parents. Each male parent plant was crossed to six female parent plants, three randomly taken from the same population and three randomly taken from the other population. A male group thus consisted of three half-sib intra-population families and three half sib inter-population families. Crosses were done in the field, using cages enclosing bumble bees (*Bombus ruderatus*) as pollinators around December 1991. Flowers were not emasculated because previous studies have indicated gametophytic self-incompatibility in red clover (Smith *et al.*, 1985). However to check the amount of selfing, one inflorescence of each plant was bagged with a glassine paper bag. At full maturity the number of self-set seeds was counted. After crossing, hybrid seeds and reciprocals were thrashed by hand and were kept in cold room at 4° C.

3.4.3.2 **Hand crossing**

As insurance against seed inadequacies, two hand crosses for each male group were made in glasshouse, one for intra- and one for inter-population crosses. Also two complete male group crosses, one in each type, were done by hand to replace any crosses in which male parents failed to flower on time to ensure achieving complete sets of crosses.

3.4.4 Evaluation

3.4.4.1 Field design, and experimental establishment

Because a high number of families was examined (in this case 108), the males were grouped into 6 sets. Three male groups from the same population were assigned at random to each set. Each progeny family was replicated three times within the set. Each set was treated as a separate experiment with sums of squares and degrees of freedom pooled for a combined analysis of variance.

Plate 2: General view of plots after transplanting the jiffy pots in the field.

Progeny seeds were germinated in a germinator on wet filter paper late in July, 1992 at 20°C and after germination they were planted in the Jiffy pots. Insect and disease control in glasshouse were the same as was described for GE interaction experiments' seedlings. Finally, after eight weeks they were transplanted to the field late in September 1992. A plot consisted of one three metre row with plants spaced 60 cm apart within and between rows. A general view of the field experiment after transplanting the jiffy pots is presented in plate 2.

3.4.4.2 **Studied attributes**

Data were recorded for the same characters as mentioned for the GE interaction experiment. In addition seedling vigour, leafiness, plant height and plant diameter were assessed. Because of practical limitations, dry matter yield was not recorded on this experiment.

Leafiness: Leafiness was scored on all individual plants at median flowering stage. The most leafy plants were scored five and the least scored one. Half scores also were used as required. Two extreme situations of this attribute are presented in plate 3.

Plant height: Plant height was recorded in decimeters before harvesting the plants.

Plant diameter: Plant average diameter was recorded in decimeters before harvesting the plants.

Seedling vigour: Seedling vigour was scored from 1 to 5 according to the size of individual plants. The smallest plants were scored 1 and the biggest ones were scored 5.

The measured attributes, the abbreviations used to refer to them and their units of measurements are presented in Table 3.2

Plate 3: Variation in leafiness in inter-population crosses.

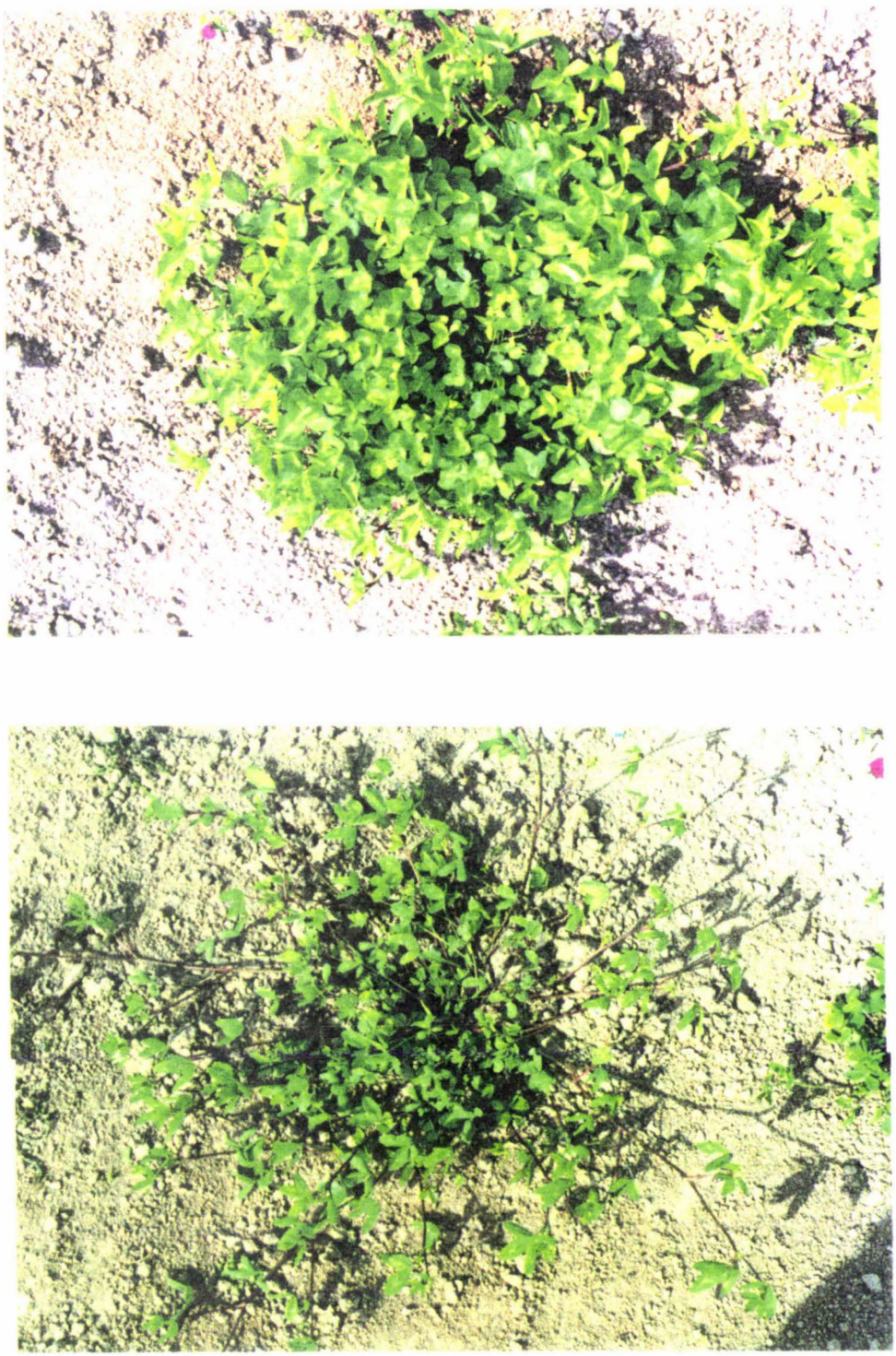


Table 3.2: Measured attributes, their abbreviations and unit of measurement.

Characters	Abbreviation	Unit of measurement
Prostrateness	PRO	Score 1-5
Nodal rooting	NRT	Score 1-5
First flowering	FFW	Days after sowing date
Median flowering	MFW	Days after sowing date
Leaf size	LSZ	Score 1-5
Leafiness	LNS	Score 1-5
Stem thickness	STT	Millimetre
No. of stems	NST	Number of stems per plant
Stem length	STL	Centimetre
No. of internodes	NIN	Average No. of internodes/3 stems
No. of branches	NBR	Average No. of branches/3 stems
Plant height	PHT	Decimeter
Plant diameter	PDI	Decimeter
Seedling vigour	SVG	Score 1-5

3.4.4.3 **Data analysis**

3.4.4.3.1 **Statistical model**

In order to compare the overall mean values for different sets of crosses, a combined analysis of variance was carried out in which four sets

of crosses were regarded as four treatments, using ANOVA procedure in SAS package (SAS institute Inc., 1990).

According to Becker (1984) the statistical model for this design can be written as follows:

$$Y_{ijklm} = \mu + \eta_l + \rho_{k(l)} + \alpha_{j(l)} + \beta_{i(jl)} + \varepsilon_{ijkl} + \omega_{ijklm}$$

where

Y_{ijklm} =the phenotypic value of the $_i$th female parent nested within $_j$th male parent of the $_k$th block of the $_l$th set.

μ = grand mean common to all observations

η_l = the effects of the $_l$th set

$\rho_{k(l)}$ = the effects of the $_k$th replication in the $_l$th set

$\alpha_{j(l)}$ = the effect of $_j$th male nested within $_l$th set

$\beta_{i(jl)}$ = the effect of $_i$th female nested within $_j$th male and $_l$th set

ε_{ijkl} = the random error associated with the individual plots

ω_{ijklm} = residual

Rankit transformation was applied on the characters measured on the 1-5 scoring scale. It was assumed that there were no maternal effects and populations were in allelic and linkage equilibrium. Analysis of variance was based on individual plant values. The data for each set of progenies, either inter- or intra-population crosses were analyzed as individual sets. Finally a pooled analysis of variance was carried similar to that of Robinson *et al.* (1958) Table 3.3.

Table 3.3 : Expectations of mean square for a random effect, balance, hierarchical mating design.

S.O.V.	D.F.	MS	Expectation of M.S.	F
Sets	s-1	M_6	$\sigma^2_w + k\sigma^2_e + mn\sigma^2_{r(s)} + rk\sigma^2_{f(ms)} + rnk\sigma^2_{m(s)} + mn\sigma^2_s$	$(M_6+M_3)/(M_5+M_1)$
Reps (sets)	s(r-1)	M_5	$\sigma^2_w + k\sigma^2_e + mn\sigma^2_{r(s)}$	M_5/M_3
Males (sets)	s(m-1) (D_1)	M_1	$\sigma^2_w + k\sigma^2_e + rk\sigma^2_{f(ms)} + rnk\sigma^2_{m(s)}$	M_1/M_2
Females (males sets)	sm(n-1) (D_2)	M_2	$\sigma^2_w + k\sigma^2_e + rk\sigma^2_{f(ms)}$	M_2/M_3
Remainder (plot error)	s(mn-1)(r-1) (D_3)	M_3	$\sigma^2_w + k\sigma^2_e$	M_3/M_4
Plants in plots		M_4	σ^2_w	

k = plants per plot

m = male groups per set

n = female per male

r = replications per sets

s = sets per experiments

σ^2_w = intra-plot variance (sum of genetic and environmental variances among individuals of the same progeny).

σ^2_e = inter-plot variance (plot error).

$\sigma^2_{f(ms)}$ = variance attributed to female parents mated to the same male.

$\sigma^2_{m(s)}$ = variance attributed to male parents nested within the same set.

σ^2_s = variance attributed to set.

Following Compton *et al.* (1965) intra- and inter-population male and female components of variance were estimated. Therefore, σ^2_m (variance component due to male parents), σ^2_f (variance component due to female parents) were obtained by equating observed mean squares to their expectations. The mentioned components were translated to genetical terms as follows (Gouesnard & Gallais, 1992):

for intra-population crosses

$$\sigma^2_A = 4\,\sigma^2 m$$

$$\sigma^2_D = 4\sigma^2 f - \sigma^2_g$$

and for inter population crosses

$$\sigma^2 A = 2(\sigma^2 m_{ep} + \sigma^2 m_{pe})$$

$$\sigma^2 D = 2(\sigma^2 f_{ep} + \sigma^2 f_{pe} - \sigma^2 m_{ep} - \sigma^2 m_{pe}).$$

in which $\sigma^2 A$ is the additive genetic variance component, $\sigma^2 D$ is the dominance variance component, $\sigma^2 f$ and $\sigma^2 m$ as defined previously. Suffixes refer to population, the suffix ep, for example, denotes population 1 (erect) as male parents and population 2 (prostrate) as female parents.

The ratios of the intra-population male and female component to inter-population male and female components were estimated.

$$(\sigma^2 m_{ee} + \sigma^2 m_{pp}) / (\sigma^2 m_{ep} + \sigma^2 m_{pe})$$
$$(\sigma^2 f_{ee} + \sigma^2 f_{pp}) / (\sigma^2 f_{ep} + \sigma^2 f_{pe})$$

Referring to tables containing these ratios of dominance to additive genetic variance components (Singh *et al.*, 1984; Compton *et al.*, 1965) inferences can be made about average gene frequencies in the population.

3.4.4.3.2 **Estimating standard errors**

The standard error of an estimated component of variance was estimated using the equation presented by Compton *et al.* (1965) which is similar to that of Crump (1951) and Satterthwaite (1946).

$$S.E. = \frac{1}{C}\sqrt{2\Sigma_i \left(\frac{M_i^2}{F_i+2}\right)}$$

in which M_i = the $_i$th mean square in the function by which the component is estimated. F_i = the degrees of freedom for $_i$th mean square and C is the divisor of the component mean square function. For example from Table 3.3, the standard error of $\sigma^2 m_{ee}$ in this case was estimated as follows:

$$= \frac{1}{A}\sqrt{2\left(\frac{M_1^2}{D_1+2} + \frac{M_2^2}{D_2+2}\right)}$$

and standard error of $\sigma^2 f_{ee}$ equals:

$$= \frac{1}{B}\sqrt{2\left(\frac{M_2^2}{D_2+2} + \frac{M_3^2}{D_3+2}\right)}$$

The same procedure applied for estimating standard errors for $\sigma^2 m_{pp}$, $\sigma^2 f_{pp}$, $\sigma^2 m_{ep}$, $\sigma^2 f_{ep}$, $\sigma^2 m_{pe}$, *and* $\sigma^2 f_{pe}$.

Standard error for additive variance component for intra-population crosses equals to:

$$=\frac{1}{A}\sqrt{32\left(\frac{M_1^2}{D_1+2}+\frac{M_2^2}{D_2+2}\right)}$$

Standard error for dominance variance component for intra crosses equals to:

$$=\sqrt{16*\left[\left(\frac{2}{B^2}*\left(\frac{M_2^2}{D_4+2}+\frac{M_3^2}{D_3+2}\right)\right)+\frac{2}{A^2}*\left(\frac{M_1^2}{D_1+2}+\frac{M_2^2}{D_2+2}\right)\right]}$$

Standard error for additive variance component for inter population crosses equals to:

$$=\sqrt{\frac{8}{A^2}*\left[\frac{(M_1)^2_{ep}}{D_1+2}+\frac{(M_2)^2_{ep}}{D_2+2}+\frac{(M_1)^2_{pe}}{D_1+2}+\frac{(M_2)^2_{pe}}{D_2+2}\right]}$$

Standard error for dominance variance component for inter population crosses equals to:

$$=\sqrt{\frac{8}{B^2}\left[\frac{(M_2)^2_{ep}}{D_2+2}+\frac{(M_3)^2_{ep}}{D_3+2}+\frac{(M_2)^2_{pe}}{D_2+2}+\frac{(M_3)^2_{pe}}{D_3+2}\right]+\frac{8}{A^2}\left[\frac{(M_1)^2_{ep}}{D_1+2}+\frac{(M_2)^2_{ep}}{D_2+2}+\frac{(M_1)^2_{pe}}{D_1+2}+\frac{(M_2)^2_{pe}}{D_2+2}\right.}$$

where A and B are the coefficients of male and female expectation of variances respectively. Referring to the Table 3.3, A = *rnk* and B = *rk*. Suffixes ep and pe refer to the variance for inter-crosses sets 1 and 2.

3.4.4.3.3 **Heritability and genetic advance**

Heritability (narrow sense) was estimated for all characters in the usual way, since $\sigma^2_m = 1/4\ \sigma^2_A$

$$h^2_{ns}=\frac{4(\sigma^2_m)}{\sigma^2_m+\sigma^2_f+\sigma^2_e+\sigma^2_w}$$

Also, broad sense heritability was estimated for all traits using the following equation.

$$h^2_{bs}=\frac{\sigma^2_A+\sigma^2_D}{\sigma^2_m+\sigma^2_f+\sigma^2_e+\sigma^2_w}$$

These are analogous to restricted heritability based on a series of experiments done on several years and sites described by Gordon *et al.* (1972) and Gordon (1979). Heritability narrow sense and broad sense were also estimated using the following equations which is analogous to full heritability suggested by the same authors.

$$h^2_{ns}=\frac{4(\sigma^2_m)}{\sigma^2_m+\sigma^2_f+\sigma^2_s+\sigma^2_{r(s)}+\sigma^2_e+\sigma^2_w}$$

$$h^2_{bs}=\frac{\sigma^2_A+\sigma^2_D}{\sigma^2_m+\sigma^2_f+\sigma^2_s+\sigma^2_{r(s)}+\sigma^2_e+\sigma^2_w}$$

According to Falconer (1989) this one is more likely to be upward biased by non-allelic genetic variance components.

A major role of quantitative genetics is to provide a genetic basis for the development of effective and efficient selection schemes for particular objectives. Assuming the phenotypic values of the measured attributes were normally distributed and selection would be by truncation and individual selection would be applied on the both sexes, expected genetic advance was estimated for all characters using following equation (Falconer, 1989).

$$GA = K\,\sigma^2_P\,h^2$$

in which

GA = expected response to selection

K = a constant depends on the percentage of population which is going to be selected as a base population for next generation.

σ^2_p = phenotypic variance

h^2 = narrow sense heritability of attribute

3.4.4.3.4 **Reciprocal crossing effects**

In an ordinary crossing scheme in the hierarchical mating design the estimates are inflated by reciprocal crossing effects as well as non-allelic interaction effects. Using this scheme of crossing the investigator is able to investigate the possible existence of reciprocal crossing effects in which maternal effects are included. In the genetic point of view, the two inter-population sets of crosses were analogous. The only difference between them was the position of male and female parents had changed. In greater detail, in the first inter-population crosses male parents were erect and female parents were prostrate, whereas, in second inter-population crosses, male parents were prostrate and female parents were erect. Therefore, both populations had the same background and they were expected to perform in a similar way. Any significant difference in mean values of the attributes could be regarded as a sign of reciprocal crossing effects.

3.4.4.3.5 **Heterosis and hybrid depression**

Heterosis in quantitative genetic terminology, is usually measured as the superiority of a hybrid over the average of its parents. The two inter-population crosses sets could be regarded as F_1 populations and as a result their mean value could be regarded as F_1 mean of crosses between two parental populations, erect and prostrate. Therefore, the percentage of the difference between the mean values for the inter- and intra-population crosses could be regarded as a form of heterosis.

3.5 **Generation mean analysis**

3.5.1 **Crossing nursery**

Three pairs of parents were used (one erect and one prostrate in each pair) in this experiment. Two pairs originated from the same population as was

used in hierarchical mating design and the other pair from genotypes F2265 and F2420. Parent plants were induced to flower by artificial long day length and heat during October and December 1991 in Massey University's glasshouses. Crosses were made by hand crossing between contrasting parent plants. F_1 seeds were kept in 4°C for 10 days while they were scattered on wet Whatman filter paper in petri dishes as a prechilling treatment. F_1 plants were backcrossed to their parent plants (P_1 & P_2) during April 1992 to produce backcross generations, B_1 & B_2. Due to self incompatibility F_2 generation seeds were produced through full-sib mating.

3.5.2 **Progeny tests**

Parents, F_1, F_2, B_1 and B_2's seeds were pre-germinated and then transplanted into jiffy pots in glasshouse. Seedling management in the glasshouse was the same as described for genotype-environment interaction experiments' seedlings. Intact jiffy pots were transplanted into the field, Frewans block, early October 1992. Randomized complete block design with three replications was used as experimental layout for all three sets of crosses. Field rows consisted of 8 plants in a single four metre row with 50 and 60 cm space between individual plants within and between rows respectively. The number of rows per plot differed according to segregating and non-segregating generations. Therefore, in each replication one plot consisted of one row for each parents, two rows for F_1s, three rows for B_2 five rows for B_1 and F_2. In the case of B_2 generation in all three sets, there were not enough germinated seeds. Therefore, the number of rows per replication allotted to these generations were reduced to available resources. All actual plot sizes were recorded. All measurements were made on individual plants. The same characters were measured as in the hierarchical mating design. Since generation mean analysis is applicable on homozygous lines, the statistical and genetical analyses were done only on the characters which

were quite diverse and in opposite directions in both populations so that it could be assumed that the two populations are nearly homozygous, or at least that the alleles controlling opposite extremes of attributes are accumulated in the two populations.

3.5.3 **Statistical analysis**

An ordinary randomized complete block analysis of variance was conducted for each character separately using procedure GLM of the SAS statistical package (SAS Institute Inc., 1990) to determine whether differences exist among generations or not. The number of plants per generation for each replication varied based on the segregating or non-segregating generation, therefore, within plot variance was used as a weight using weight command in SAS Package (SAS institute Inc., 1990). Analysis of variance was carried out for each set of crosses separately. The model for analysis of variance for one set can be expressed as below:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

in which, Y_{ij} is the observed value for $_i$th generation in the $_j$th block, μ, α_i, β_j and ε_{ij} are the general mean, $_i$th generation, $_j$th block and error term associated with $_j$th observation effects, respectively.

3.5.4 **Genetical analysis**

3.5.4.1 **Function of gene**

When the analysis of variance of field experimental design indicated significant differences between generation means, generation mean analysis was carried out for that variable. The erect parent was designated P_1, and prostrate parent plant P_2, regardless of the mean values of parents in different characters. Usually the parent with the greater phenotypic value was designated P_1 to get positive values for [d]. But here in this experiment some characters such as stem thickness, erect the parent plant had greater value

than the prostrate parent plant. In contrast the prostrate parent plant had a greater value in prostrateness than the erect parent plant.

Weighted generation mean analysis were done by a QBASIC programme called HOMINO written by the author, in which the generation variances were weighted and analyzed following the procedure presented by Mather and Jinks (1982) and modified by Gordon (unpublished lecture notes). The programme is presented is Appendix 5. The genetic analysis was done in three separate stages with three different models, (1) three parameters model, or additive-dominance model; (2) six parameter model; (3) the best parsimonious fit model. The reciprocals of the internal variances of the generations were used as weights for means. The procedure followed can be expressed in the matrix notation as follows:

$$\mathbf{J = E' W E}$$

in which

J = the information matrix,

E = the coefficient matrix,

E′ = the transpose of coefficient matrix,

W = the diagonal matrix containing the inverse of variances of the generations.

$$\mathbf{M = E' W O}$$

In which

M = the vector containing weighted observations,

O = the vector containing observed generation means.

Then: $$\mathbf{G = J^{-1} M}$$

where

G = the vector containing estimated parameters,

$\mathbf{J^{-1}}$ = the inverse of **J**, information matrix (or variance covariance matrix).

3.5.4.2 **Testing the model**

There are two ways to test the adequacy of the three parameters model. (a) simple scaling test (Mather and Jinks, 1982) in which observed generation means and their variances are used to test the goodness of fit, (b) joint scaling test, presented by Cavalli (1952) and elaborated by Mather and Jinks (1982). These two ways are discussed in the previous chapter. First, simple scaling tests were applied and A,B and C types of scales were calculated to test the presence of epistasis. According to Mather and Jinks (1982), the expectations of A,B and C in a simple additive dominance model are zero but their amounts are biased by the non-allelic interactions. The outcome of A and B tests are affected by [j] type of interaction while the amount of C is affected mostly by [l] type of interaction.

Using estimated parameters, m, [d], and [h], expected means of generations were estimated, followed by comparing expected and observed means of different generations to test the goodness of fit of the model. If chi-square was significant the data were transformed to log and then square root and transformed data were analyzed again. In any case, simple additive dominance model was adequate or not, a six parameter model was estimated to get mean, additive, dominance, the pooled additive x additive interactions, the pooled dominance x dominance interactions and the pooled dominance x dominance effects (m, [d], [h], [i], [j] and [l]).

In some cases simple additive dominance model was not adequate for the variables according to χ^2 test. In other words at least one of the interaction terms were significant. In those cases estimated values of gene effects were tested by t-test. In this manner significance of all the parameters were estimated. To calculate t values, standard errors of estimated

parameters and associated degrees of freedom, which were equal to the sum of the number of individual plants in the generations involved in the estimation of the parameter minus the number of generations were used. For example the calculated t value for the parameter *m* the following procedure was used:

$$V_m = 1/4\ V_{P1} + 1/4V_{P2} + 16V_{F2} + 4\ V_{B1} + 4\ V_{B2}$$

$$\text{and S.E.}_m = \sqrt{V_m}$$

$$t = m/\text{S.E.}_m$$

$$df_m = (N_1 + N_2 + .. + N_5 - 5)$$

in which N_1 to N_5 are the number of plants in P_1, P_2, F_2, B_1, and B_2 generations. The parameter with non-significant t value was deleted in order to test the adequacy of the model. The significance of the parameters could be clarified by standard errors of the estimated parameters which are the diagonal elements of $\mathbf{J}^{-1}$ matrix. Both ways are done by the HOMINO programme. Obviously in those cases that χ^2 in joint scaling test was significant at least one of the interactions was significant.

Using six family means and fewer than six parameters, the adequacy of the new model could be tested. After deleting non-significant parameters, the usual procedure was carried out again and the test of goodness of fit of the model was done by χ^2. In some other cases none of the t values were non-significant to enable me to delete at least one of the parameters to test the adequacy of the model. As Mather and Jinks (1982) have pointed out if all six parameters are significant there is no way to test the model with six family means and normally we would have to raise further generations to provide such a test.

3.6 **Estimating minimum number of genes**

The minimum number of genes was estimated by the procedure presented by Lande (1981) and Cockerham (1986). This procedure was reviewed in detail in chapter two.

3.7 **Estimating heterosis**

Heterosis was calculated on the basis of estimated value of gene effects proposed by Moreno-Gonzales and Dudley (1981) as follows:

$$\text{Heterosis} = [d] + [dd] - [aa]$$

Also heterosis was estimated using F_1 and mid parent generation means.

$$\text{Heterosis} = F_1 - mP$$

A t value to evaluate the significance of this statistic was calculated by the following formula.

$$t = \frac{M_{F1} - (M_{P1} + M_{P2})/2}{\sqrt{\frac{V_{P1}}{4} + \frac{V_{P2}}{4} + V_{F1}}}$$

The degrees of freedom were calculated for the above t value as follows:

$$df = n_1 + n_2 + n_3 - 3$$

in which n_1, n_2, and n_3 are the number of individual plants in P_1, P_2, and F_1 generations respectively.

CHAPTER FOUR: GENOTYPE-ENVIRONMENT INTERACTIONS RESULTS AND ASSOCIATED DISCUSSION

Twelve accessions (genotypes) were examined for two years in two sites in order to estimate general trend in prostrateness and related attributes. These genotypes adequately sampled finite populations of prostrate, semi-erect and erect types available in the local collections. Since the experiments over the two years were repeated with the same blocking, the correlations between years performances were estimated. The results of this analysis are also presented in Table 4.1. The majority of the results suggested the absence of a correlation between time, therefore, it was decided to analyze the data recorded on the two successive years as a split plot in time. General estimates of genotypic variances, genotype-environment interaction effects, heritability values, phenotypic and genotypic correlations were obtained on the basis of examined genotypes. Subsequently, more detailed genetic analysis will follow on subset of this gene-pool. The genotype-environment interaction results here were examined firstly on a single variate basis, and, secondly, on a multivariate basis. The first provides a traditional but piecemeal view; the second one unbiased, optimum combined view.

4.1 Univariate analysis

4.1.1 Analysis of variance

The results of the pooled analysis of variance for these twelve genotypes of two sites and for two years (mean square estimates, their F values and degrees of freedom) are presented in Appendix 3. The F values are partly repeated in Tables 4.3 and 4.5. These biometrical mean squares were translated to the estimates of variance components. Variance components were estimated from these mean squares using the random-effect

expectation (see methods). These estimates and their standard errors are presented in Table 4.2.

Table 4.1 : The abbreviations used to refer to the attributes, the units of measurement, the grand mean, their coefficient of variance, and the values for correlation between time (r_t) on which GE interaction experiments were conducted.

Traits	Abbreviation	Unit of measurement	Unit of analysis	Grand mean	C.V.	r_t
Prostrateness	PRO	Score 1-5	Rankit	5.19	4.52	0.453
Nodal rooting	NRT	Score 1-5	Rankit	4.29	7.25	0.018
First flowering	FFW	Days	Days	143.00	7.31	0.289
Median flowering	MFW	Days	Days	160.60	5.37	0.130
Leaf size	LSZ	Score 1-5	Rankit	5.02	5.07	-0.069
Stem thickness	STT	mm	mm	2.98	11.88	0.100
No. of stems	NST	Number/plant	ratio	59.21	38.86	0.053
Stem length	STL	cm	cm	48.89	12.27	0.084
No. of internodes	NIN	Average No./3 stems	ratio	8.39	14.50	0.194
No. of branches	NBR	Average No./3 stems	ratio	4.94	25.14	0.068
Dry matter yield	DMY	Gram/plant	gram	196.40	37.65	0.129

4.1.1.1 **Environmental variances**

Variation in plant performance is a mixture of environmental and genetical variances. Environmental variation can be subdivided to macro- and micro-environmental variance, such as sites, years, blocks, and within plot variation. In the case of first flowering, stem length, and dry matter yield site effects were significant, indicating variation between sites examined. There were some negative estimates for site variation, indicating trivial values for this source. In these cases negative values were so small relative to their standard errors, that could be safely neglected and regarded as zero. Except for prostrateness, leaf size, and stem thickness, year effect was significant for the rest of attributes. Interaction between year and site was significant for

prostrateness, median flowering, leaf size, and number of stems. Block effect was not significant for most of the attributes, except for leaf size and stem thickness. This indicates that the blocks within site were fairly homogeneous. Error a which is interaction between block and genotype nested within site was only significant for prostrateness and first flowering at one and five percent respectively. In contrast error b was significant for most of the attributes except for first flowering (Appendix 3).

4.1.1.2 **Genotypic variances**

In this experiment, genotype, site X genotype, year X genotype and year X site X genotype were considered as the components of variance in which genetic variation is involved.

In general the analysis showed that genotypes were highly significantly different in most of the studied attributes except for stem number, and branch per stem which were not significant. There appears to be substantial genetic variability among different types of red clover for most of the traits and improving the traits by breeding programmes seems to be possible because of the vast amount of variability.

Genotypes did not interact with these sites significantly for any trait, indicating the relative ranking of the genotypes over sites has been constant. The non-significance of the site X genotype interaction for the studied traits and the comparable rankings of genotypes in the sites also indicated that selection for some of the studied attributes such as stem thickness could be done in a single site, particularly if such a broad based breeding material was going to be evaluated.

Year X genotype interaction effect was moderately to highly significant for prostrateness, first flowering, stem thickness, number of stems, and branches (Table 4.2). These attributes were further studied using multivariate discriminant analysis and eventually cluster analysis to find the possible pattern of response to environment between the genotypes studied.

The second order interaction (genotype X site X year) was significant just for stem thickness, internodes, branches, and dry matter. The general result showed reasonable stability of these genotypes over these environments, which reflects, probably, the narrow sample of environments included in the study. Also it may be due to stability of most the studied attributes. Under these conditions, extensive analysis of GE interaction pattern is not important. A GxSxY multivariate analysis for stem thickness, internodes, branches, and dry matter would be reasonable, and a GxY multivariate for prostrateness, first flowering, stem thickness, number of stems, and rooting is also indicated.

Table 4.2: Variance components (and their standard errors) for pooled analysis of variance of twelve genotypes at two sites over two years.

Traits	Site (S)	Block	Genotype (G)	G x S	Error a	Year (Y)
PRO	-0.0075 ns[1] (0.0056)	0.0030 ns (0.0036)	0.8582 ** (0.3455)	0.0081 ns (0.0084)	0.0240 ** (0.0085)	0.0039 ns (0.0116)
NRT	-0.0006 ns (0.0012)	0.0018 ns (0.0028)	0.2029 ** (0.0854)	0.0039 ns (0.0071)	0.0013 ns (0.0101)	0.0198 ** (0.0174)
FFW	18.28 ** (15.48)	1.2091 ns (1.9033)	195.93 ** (81.491)	-1.7517 ns (3.1453)	10.7300 * (5.654)	689.73 ** (564.47)
MFW	7.944 ns (10.824)	-0.4971 ns (0.7891)	152.76 ** (62.55)	3.7556 ns (6.788)	4.6886 ns (5.320)	546.250 ** (449.466)
LSZ	-0.010 ns (0.0133)	0.0055 * (0.0044)	0.1203 ** (0.0493)	-0.0022 ns (0.0079)	-0.0037 ns (0.0079)	-0.0129 ns (0.0123)
STL	132.64 * (112.90)	-0.3615 ns (1.7024)	132.66 ** (63.0424)	9.8006 ns (13.544)	5.6993 ns (9.927)	261.522 ** (219.003)
STT	0.0274 ns (0.0355)	0.0037 * (0.0034)	1.0547 ** (0.4570)	0.0121 ns (0.0273)	0.0044 ns (0.0064)	-0.0159 ns (0.0141)
NST	47.837 ns (59.662)	5.0153 ns (9.4794)	-188.86 ns (117.824)	42.0005 ns (28.893)	13.1540 ns (35.99)	1499.81 ** (1275.97)
NIN	0.0141 ns (0.0826)	0.0191 ns (0.0327)	2.9765 ** (1.5094)	0.6574 ns (0.5374)	0.1390 ns (0.1066)	0.2175 * (0.2052)
NBR	0.295 ns (0.2753)	0.0002 ns (0.0238)	0.9418 ns (0.7054)	0.0145 ns (0.1759)	0.0588 ns (0.1252)	10.6060 ** (8.7649)
DMY	8320.03 * (7226.2)	64.488 ns (92.222)	8245.52 ** (3913.5)	1129.87 ns (1506.50)	250.588 ns (284.619)	3434.27 ** (3130.8)

Table 4.2, continued : Variance components (and their standard errors) for pooled analysis of variance of twelve genotypes at two sites over two years.

Traits	S x Y	G x Y	G x S x Y	Error b	Within plot
PRO	0.01248 ** (0.0107)	0.02497 ** (0.0114)	-0.0019 ns (0.0037)	0.0175 ** (0.0059)	0.0549
NRT	-0.0011 ns (0.0007)	0.01534 ns (0.0102)	-0.0049 ns (0.0085)	0.0488 ** (0.0138)	0.0967
FFW	-0.3849 ns (0.2933)	17.8361 ** (8.5226)	-1.7139 ns (3.3434)	3.708 ns (5.4733)	109.29
MFW	7.14590 * (7.082)	-1.356 ns (4.6186)	7.3220 ns (7.3625)	16.037 ** (6.4003)	74.257
LSZ	0.02730 ** (0.0244)	-0.0019 ns (0.0080)	0.0112 ns (0.0127)	0.044 ** (0.0117)	0.0648
STL	6.54217 ns (7.7867)	23.100 ns (17.26)	13.663 ns (14.247)	35.258 ** (12.618)	129.44
STT	0.01690 ns (0.0198)	0.1571 ** (0.080)	0.0718 ** (0.0335)	0.0130 * (0.0080)	0.1252
NST	31.9587 ** (28.627)	540.31 ** (219.27)	-42.15 ns (21.518)	123.653 ** (47.49)	529.45
NIN	-0.0848 ns (0.0370)	0.3146 ns (0.4053)	0.9174 ** (0.4381)	0.2693 ** (0.1176)	1.481
NBR	0.02531 ns (0.0638)	1.0939 ** (0.5568)	0.3193 * (0.2375)	0.4773 ** (0.1620)	1.546
DMY	453.570 ns (665.33)	-26.60 ns (1074.8)	3558.20 ** (1622.9)	549.394 * (345.589)	5470.65

1 : ns = non-significant * = F test was significant at the 5% level of probability
** = F test was significant at the 1% level of probability.

4.1.2 **Genotypic performance**

4.1.2.1 **Grand mean values**

The central tendencies (grand means) of the studied attributes, their coefficients of variation, abbreviations used to refer to the attributes, and the units of measurement are presented in Table 4.1. Coefficients of variation provide us with an estimate of the relative precision of the attributes. Genotypic overall means are also presented in Table 4.1 together with their Duncan significance. These means provide a criterion for comparing the general performance of the genotypes across all environments.

4.1.2.2 **Environments and their interaction means**

Site and year means for all measured attributes are presented in Table 4.5. Although as a result of differences in soil type and other minor differences such as altitude and available moisture in the two sites, the mean values for attributes leaf size, stem length, stem thickness, number of stems, branches, and dry matter yield were bigger at Frewans than them at Mogini (Table 4.5), but just the site effect was significant only for first flowering, stem length, and dry matter yield. Also year effect was highly significant for most of the characters except for prostrateness, leaf size and stem thickness. The mean values for the attributes first flowering, median flowering, stem length, number of stems, branches, and dry matter had changed remarkably, indicating considerable change in the plant characteristics, so that in the second year plants tend to be earlier flowering with more and shorter stems, fewer branches, and lower dry matter yield. This is in agreement with Bowley *et al.* (1988).

Site x year interaction means are presented in Table 4.3. There was not a consistent trend between sites and years. A significant F value for prostrateness, median flowering, leaf size, and number of stems indicated inconsistency of the mean from year to year and site to site. Second order interaction means (GxSxY) were not significant for the majority of attributes,

except for stem thickness, internodes, branches, and dry matter (Appendix 2, Table 3). The trait of most interest, nodal rooting, was not significantly affected by environment.

The pattern of response of the genotypes to various environments is an important aspect of a genotype, as well as its general performance. There were large differences between genotypes trends across environments. The ranges of the traits also varied from environment to environment. In traits such as prostrateness, leaf size, first flowering, and stem thickness the ranking inconsistency was more within type than between types. But in traits such as branches, and number of internodes the ranking inconsistency belonged to both within and between types. The values for genotype X site and genotype X year and genotype X site X year interaction mean, and their trends as indicated by Duncan multiple range test, significant at 5% level, are presented in Tables 1, 2, and 3, Appendix 2. Following the analysis of variance the environmental response patterns are considered again, using multiple discriminant analysis and cluster analysis.

Table 4.3 : Site by year overall means (environment effect) for the measured characters.

Trait	Frewans		Mogini		F value
	1992	1993	1992	1993	
PRO	5.29	5.01	5.29	5.18	20.1 **
NRT	4.15	4.37	4.22	4.4	0.25 ns
FFW	158.1	121.3	166.2	126.8	0.36 ns
MFW	175.9	139.8	179.1	147.7	5.77 *
LSZ	5.03	5.14	5.07	4.85	11.6 **
STL	70.2	44.2	50.67	30.41	3.26 ns
STT	3.29	2.99	2.79	2.85	3.38 ns
NST	34.38	95.9	28.28	77.66	11.4 **
NIN	8.94	8.17	8.58	7.86	0.09 ns
NBR	7.73	2.87	6.80	2.36	1.51 ns
DMY	319.5	205.3	159.6	100.4	2.32 ns

Table 4.4 : Genotypic means across all environments.

Genotype	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR	DMY
F2210[1]	4.02 e	3.98 c	150.56 cd	167.95 bc	5.45 ab	58.86 bc	4.44 a	49.54	6.32 fg	3.38	223.21 c
F2256	4.06 e	4.00 c	157.05 ab	174.68 a	5.33 bc	53.25 c	4.37 a	57.09	7.04 ef	4.21	325.69 ab
F2367	4.03 e	4.01 c	159.93 a	176.18 a	5.38 abc	65.17 ab	4.28 a	57.07	7.38 de	3.59	292.22 b
F2378	4.17 e	4.00 c	152.18 cd	168.52 bc	5.56 a	57.68 bc	4.01 b	62.70	6.99 ef	4.35	291.16 b
F2505	5.13 d	4.07 c	155.51 abc	171.77 ab	5.21 c	66.68 a	3.13 c	57.12	9.24 c	5.74	360.95 a
F2497	5.26 d	3.92 c	124.90 g	144.26 e	5.01 d	46.09 d	2.71 d	72.79	7.15 e	4.25	127.37 e
F2496	5.26 d	4.02 c	111.09 h	135.05 f	4.77 e	37.91 ef	2.75 d	35.00	5.93 g	3.26	61.00 f
F2414	5.25 d	4.01 c	134.15 f	148.53 e	4.95 de	57.96 bc	3.00 c	74.16	8.10 d	4.91	176.76 d
F2427	6.38 a	5.18 a	140.69 e	158.25 d	4.56 f	37.95 ef	1.70 gf	68.50	10.7 b	7.03	123.94 e
F2424	6.50 a	5.11 a	148.76 d	163.20 cd	4.54 f	30.02 g	1.62 g	56.17	12.2 a	6.48	119.29 e
F2420	6.15 b	4.61 b	141.67 e	159.72 d	4.76 e	33.67 fg	2.09 e	62.57	9.42 c	5.71	139.64 ed
F2419	5.88 c	4.44 b	138.57 ef	158.00 d	4.79 e	44.14 de	1.83 f	58.23	9.81 c	6.11	125.53 e
F sig.	**	**	**	**	**	**	**	ns	**	ns	**

Mean values with the same letter were not significantly different at 5% level of probability, using Duncan multiple range.

1: The first four accessions are erect, the next four ones semierect, and the last four ones are prostrate (Refer to Appendix 1 for details).

Table 4.5 : Site and year overall means and their F values for measured characters.

Trait	Frewans	Mogini	F	1992	1993	F
PRO	5.16	5.22	0.27 ns (4.12,2.40)[1]	5.27	5.11	1.43 ns (1.05,1.85)
NRT	4.27	4.31	0.81 ns (12.5,10.7)	4.19	4.39	9.91 ** (1.07,12.0)
FFW	139.6	146.46	12.22 ** (1.10,8.37)	161.2	124.2	364.5 ** (1.00,11.90)
MFW	157.8	163.4	2.35 ns (1.22,1.86)	176.9	143.7	111.1 ** (1.00,1.31)
LSZ	5.08	4.96	0.45 ns (1.71,1.52)	5.05	4.99	0.20 ns (2.69,1.15)
STL	56.94	40.54	17.54 * (1.04,2.83)	60.2	37.36	33.3 ** (1.01,2.81)
STT	3.13	2.82	2.48 ns (1.21,2.36)	3.04	2.92	0.45 ns (1.89,4.85)
NST	65.26	52.97	2.70 ns (1.15,2.53)	31.82	87.0	24.96 ** (1.00,8.15)
NIN	8.56	8.22	1.11 ns (2.79,14.7)	8.72	8.05	3.83 * (1.41,11.9)
NBR	5.3	4.58	4.81 ns (1.23,4.03)	7.22	2.63	70.20 ** (1.00,8.99)
DMY	260.9	130.0	12.53 * (1.05,3.16)	238.9	153.4	7.24 ** (1.09,1.87)

1: Nominator and denominator degree of freedom for complex F test.

4.1.3 **Heritability and genetic advance**

Heritability estimates and their corresponding standard errors are presented in Table 4.9 in full and restricted form. Because red clover is a cross pollinated plant and genetic variance components estimated in this experiment were combination of additive and dominance genetic variance, these values would be equivalent to broad sense heritability. As was expected, full heritability estimates were usually smaller in magnitude than restricted

heritability (Table 4.9). That is because in restricted heritability the purely environmental effects (site, year, site*year, Block(site*year)) in denominator are ignored. The attributes first flowering, branches, stem length, median flowering, and dry matter presented lowest full heritability (6-27%) and it was medium for rooting, leaf size, and number of internodes (43-65%). The traits prostrateness and stem thickness, had high full heritability (72-87%). Due to negative genetic estimate, number of stems had a negative heritability value which comparing with its corresponding standard error it would be zero.

Restricted heritability estimates were comparable with full heritability in some attributes (prostrateness, rooting, first flowering, median flowering stem thickness, leaf size, and internodes). In the case of branches, stem length, and dry matter the estimate values for restricted heritability were considerably bigger than the corresponding values for full heritability, indicating more influence from the macro environmental effects on the mentioned attributes.

4.1.4 **Phenotypic and genotypic correlation**

The association between two characters that can be directly observed is the correlation of phenotypic values or the phenotypic correlation (Falconer, 1989). The estimated phenotypic correlations between all possible pairs of the traits evaluated in this experiment are listed in Tables 4.6 and 4.7. On the whole, phenotypic correlation values were highly significant. Prostrateness was negatively correlated with first flowering, median flowering, leaf size, stem length, stem thickness, and dry matter but positively correlated with branches and internodes, indicating that prostrateness leads to a low value for first flowering, smaller and less leaf, thinner and lower stems and finally lower dry matter yield. Dry matter was highly correlated with first flowering, median flowering, leaf size, stem length, stem thickness, and branches in positive direction and with prostrateness, rooting and number of

stems in negative direction. A similar result can be seen for other attributes (Table 4.6).

In perennial plants such as red clover phenotypic correlations may change from first year to second year of the plant life. For instance, prostrateness was highly correlated with first flowering negatively in first year (-0.420**) whereas, those traits were not so strongly correlated in second year (-0.113*). This indicates that either prostrate plants tend to flower later in the second year compared with erect and semierect ones or vice versa. Another more straightforward example is the correlation between prostrateness and number of stems which was -0.60** for first year and 0.34** for second year. This indicated prostrate plants had changed their performance in second year. In other words, prostrate plants produced for fewer stems than erect plants in first year but many more stems than erect plants in second year.

Genotypic correlation values are presented in Table 4.8. Because of the dual nature of the phenotypic correlation (involvement of environment and genetical causes) magnitude and even the sign of the genetic correlation can not be determined from the phenotypic correlation alone (Falconer, 1989). Most of the genetic correlation values (Table 4.8) in this experiment were comparable with the overall phenotypic correlation values (Table 4.6). This indicates that most of the studied characters are controlled by genetic causes rather than environmental factors. A very large value for genotypic correlations between prostrateness and other traits such as rooting, leaf size, stem thickness, internodes, branches suggests that these characters are either controlled by common genetic factors or by linked genes. The same conclusion can come from other large values such as correlation between first flowering and median flowering. In such a case there is no need to record both attributes on the plants under study. Obviously the large positive values indicate the genetic factor or linked genes increase both traits and the large negative values indicate the genetic factor or linked genes increase one attribute and decrease the other one. These are discussed further in chapter seven. Generally it can be seen that the genotypic correlation estimates were

higher in absolute magnitude than the corresponding phenotypic correlation. This situation arises either due to random sampling error (Cheverude, 1988) or a low correlation between the environments in relation to the expression of characters with similar governing genes (Searle, 1961). In Baker's (1986) opinion, since r_{Gij} is a function of r_{Pij} and heritability values of two traits, i.e.

$$r_G = \frac{r_p}{\sqrt{h_x^2 h_y^2}} = \frac{r_p}{h_x h_y}$$

h_x and h_y can never exceed one. Therefore, the genotypic correlation will be larger in absolute magnitude than the phenotypic correlation particularly if at least one of the heritability values are low.

Since genotypic and phenotypic correlations varied a lot from one plant type to another one, they were estimated separately for each type (Tables 1 to 3, Appendix 4).

Table 4.6 : Phenotypic correlation between traits for twelve genotypes at two sites over two years.

Trait	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR
NRT	0.603 **									
FFW	-0.122 **	-0.086 *								
MFW	-0.132 **	-0.128 **	0.909 **							
LSZ	-0.659 **	-0.450 **	0.185 **	0.199 **						
STL	-0.378 **	-0.385 **	0.475 **	0.444 **	0.380 **					
STT	-0.839 **	-0.530 **	0.238 **	0.259 **	0.635 **	0.405 **				
NST	-0.021 ns	0.207 **	-0.479 **	-0.518 **	-0.033 ns	-0.228 **	-0.006 ns			
NIN	0.592 **	0.494 **	0.193 **	0.150 **	-0.353 **	-0.051 ns	-0.495 **	0.046 ns		
NBR	0.374 **	0.102 **	0.534 **	0.548 **	-0.101 **	0.390 **	-0.298 **	-0.502 **	0.550 **	
DMY	-0.407 **	-0.257 **	0.412 **	0.443 **	0.441 **	0.670 **	0.521 **	-0.020 ns	0.008 ns	0.240 **

ns = non-significant, * =significant at the 5% level of probability, ** = significant at the 1% level of probability.

Table 4.7 : Phenotypic correlation between traits for twelve genotypes at two sites for first and second year. The correlations for first year are above the diagonal and those for second year are below the diagonal.

Trait	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR	DMY
PRO		0.538**	-0.420**	-0.306**	-0.682**	-0.495**	-0.875**	-0.602**	0.495**	0.574**	-0.475**
NRT	0.737**		0.026 ns	0.035 ns	-0.426**	-0.299**	-0.558**	-0.296**	0.459**	0.428**	-0.187**
FFW	-0.113*	0.080 ns		0.717**	0.275**	0.346**	0.250**	0.287**	0.269**	0.058 ns	0.402**
MFW	-0.264**	-0.037 ns	0.884**		0.162**	0.236**	0.202**	0.253**	0.319**	0.106*	0.431**
LSZ	-0.676**	-0.468**	0.155**	0.272**		0.419**	0.616**	0.346**	-0.306**	-0.306**	0.417**
STL	-0.484**	-0.460**	0.022 ns	0.036 ns	0.443**		0.434**	0.293**	-0.058 ns	0.041 ns	0.629**
STT	-0.815**	-0.513**	0.361**	0.499**	0.708**	0.449**		0.633**	-0.473**	-0.554**	0.549**
NST	0.340**	0.297**	-0.093 ns	-0.179**	-0.091 ns	0.137*	-0.273**		-0.303**	-0.439**	0.485**
NIN	0.704**	0.606**	0.023 ns	-0.152**	-0.423**	-0.277**	-0.567**	0.413**		0.829**	0.161**
NBR	0.410**	0.283**	-0.108*	-0.182**	-0.145**	0.103 ns	-0.369**	0.502**	0.481**		0.050 ns
DMY	-0.411**	-0.280**	0.252**	0.322**	0.506**	0.673**	0.485**	0.154**	-0.318**	0.152**	

ns = non-significant, * = significant at the 5% level of probability, ** = significant at the 1% level of probability.

Table 4.8 : Genotypic correlation between traits for 12 genotypes at two sites over two years.

Trait	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR
NRT	0.822									
FFW	-0.393	0.024								
MFW	-0.440	-0.013	0.990							
LSZ	-0.952	-0.769	0.533	0.568						
STL	-0.796	-0.709	0.515	0.518	0.847					
STT	-0.987	-0.771	0.465	0.504	0.945	0.793				
NST	0.253	0.200	0.113	0.031	-0.121	0.060	-0.243			
NIN	0.818	0.889	0.180	0.117	-0.694	-0.510	-0.774	0.348		
NBR	0.817	0.830	0.117	0.058	-0.666	-0.456	-0.784	0.481	0.945	
DMY	-0.690	-.451	0.800	0.818	0.799	0.824	0.730	0.043	-.255	-0.220

Table 4.9 : Full and restricted heritability and their standard error.

Trait	h^2_{full}	S.E. of h^2_{full}	$h^2_{res.}$	S.E. of h^2_{res}
PRO	0.847 **	0.049	0.878 **	0.046
NRT	0.531 **	0.112	0.541 **	0.110
FFW	0.528 **	0.120	0.587 **	0.103
MFW	0.564 **	0.121	0.593 **	0.101
LSZ	0.483 **	0.108	0.522 **	0.108
STL	0.259 *	0.092	0.379 **	0.117
STT	0.640 **	0.100	0.729 **	0.099
NST	-.115 ns	0.057	-.186 ns	0.101
NIN	0.424 **	0.136	0.441 **	0.138
NBR	0.160 ns	0.056	0.211 (*)	0.137
DMY	0.300 *	0.118	0.435 **	0.127

ns = non-significant (*) = F test significant at 10% level of probability * = F test was significant at 5% level of probability ** = F test was significant at 1% level of probability.

4.2 **Multivariate analysis**

4.2.1 **Multivariate discriminant analysis**

4.2.1.1 **Based on GE interaction effect (all traits)**

Despite the paucity of evidence for GxSxY interaction (ANOVA F test) it was relevant to check on possible patterns of environmental response amongst the genotypes. Some weak evidence for GxY and GxSxY did exist for a small number of characters. The pooled analysis provided a MANOVA partition for GxSxY, across all attributes simultaneously, and this has been

used to coordinate any incipient GE interaction. Multiple discriminant functions were estimated from this MANOVA partitioning. Cumulative proportion of discriminating power and the significance of discriminating ability of the first two functions are presented in Table 4.10.

Table 4.10 : Multiple discriminant of GxSxY patterns (discrimination based on all attributes)

Discriminant	Cumulative	F	Numerator d.f.	Denominator d.f.	Probability
1	0.4100	2.43	121	305.9	0.0001
2	0.6874	1.90	100	283.9	0.0001

Based on a cumulative discrimination cut-off point of 0.70, the first two discriminants could describe the majority of existing GxSxY variation. These two multiple discriminant functions (Table 4.11) suggested that dry matter yield and to lesser extent stem thickness and first flowering are the attributes best correlated with the first discriminant function (Table 4.11). In fact these attributes appear to be good diagnostic ones that allows a good discrimination between the genotypes studied. These statistics are the correlations between the discriminant function and the original variables. These enable a name to be given to the discriminant and to indicate its meaning. The second discriminant function correlated 0.7600 with median flowering, -0.6909 with stem thickness, 0.5550 with prostrateness and -0.4002 with internodes. From these values the first function appeared to be measuring dry matter and stem thickness (+) versus first flowering and internodes, and the second function is measuring median flowering, prostrateness, and branches (+) versus stem thickness and rooting (-). Based on the magnitude of the correlation of various attributes with the two functions, first function can be named vigourous and second function can be named stoloniforous.

Table 4.11 : First and second canonical structure values describing the most part of variation exist in data set (discrimination based on all attributes).

Traits	Between canonical structure		Standardized discriminant coefficients	
	DIS1	DIS2	DIS1	DIS2
PRO	-0.3767	0.5550	-0.0108	0.7067
NRT	-0.0090	-0.6264	0.2303	-0.4718
FFW	-0.6722	0.1359	-0.3044	-0.1920
MFW	-0.1371	0.7600	-0.4178	1.1506
LSZ	-0.5089	0.0486	-0.2873	0.1401
STL	0.1612	0.3307	0.0191	-0.0222
STT	0.6977	-0.6909	1.3144	-2.3037
NST	-0.0011	0.2112	-0.3235	0.1367
NIN	-0.5485	-0.4002	-1.1033	-0.8953
NBR	0.3014	0.3816	0.8846	0.8972
DMY	0.9208	0.1342	1.2701	0.5094

The values of the standardized canonical coefficients for the first two discriminants are also presented in Table 4.11. Based on a lexicon presented by Gordon (unpublished, Massey University) by which variables can be named by jointly consideration of discriminant function and standardized coefficient, dry matter yield, stem thickness, and branches are consensual variables which decrease the score and they are so counteracted by other variables which consensually increase the score. First flowering, leaf size, and internodes are consensual variables which are negatively correlated to the score and they are negatively correlated with other attributes so that the score consensually decreases through those correlations. Rooting is a suppressed attribute which is independent from the score but it is counteracted by other attributes which increase the score. Prostrateness is a pseudo variable which is negatively correlated to the score but there is no contribution through other attributes. Median flowering and number of stems are also suppressed attributes which are independent from the score but they are so contracted by other attributes by which score is decreased.

Based on the second function and standardized coefficient prostrateness, median flowering and branches are consensual determinant variables which increase the function both through positive correlation with the function and other variables. Nodal rooting, stem thickness and internodes are consensual variables which decrease the function both through negative correlation with the function and other variables.

These scores also seem to be obscure to describe the existing pattern of GE interaction effect in the data set, therefore, they were used in cluster analysis which is discussed later in this chapter in order to clarify the pattern.

4.2.1.2 **Based on GE interaction effect (traits with significant GxSxY effect)**

Since a limited number of attributes showed significant second order interaction effect (GxSxY), a pooled analysis of variance was carried out for these attributes, stem thickness, internodes, branches, and dry matter, followed by a MANOVA partitioning of GxSxY across the mentioned attributes. Cumulative discriminating ability, their F values and probability of discriminating ability of the two discriminants indicated to retain the majority of GxSxY variation, existing in the data (Table 4.12). This is in agreement with multivariate discriminant analysis based on all attributes.

Table 4.12 : Multiple discriminant of GxSxY patterns in attributes with significant GxSxY effect in pooled analysis.

Discriminant	Cumulative	F	Numerator d.f.	Denominator d.f.	Probability
1	0.6081	4.97	44	170.3	0.0001
2	0.8325	3.27	30	132.8	0.0001

The structures of these discriminants are presented in Table 4.13. As it can be seen (Table 4.13) all the four studied attributes in this analysis were highly, positively or negatively, correlated with the first discriminant function. Since first discriminant function accounted for the most of the variation existing in the data set, regarding second order interaction (Table 4.12), one may conclude that based on the first discriminant function, the major variable responsible for the pattern existing in the environmental response in the genotypes examined was dry matter. In contrast, dry matter did not have a major role in determining the second discriminant function. Stem thickness and internodes were highly correlated to this function, respectively. A cluster analysis based on these scores is presented later in this chapter.

Table 4.13: First and second canonical structure values describing the most part of variation exist in data set (discrimination based on attributes with significant GxSxY effect in ANOVA).

Traits	Between canonical structure		Standardized discriminant coefficients	
	DIS1	DIS2	DIS1	DIS2
STT	0.4915	0.8511	0.8025	2.4692
NIN	-0.6645	0.4319	-1.4584	0.7093
NBR	0.3860	-0.0359	1.3498	-0.1816
DMY	0.9076	0.2221	1.0978	-0.2449

From the statistics related to the first function this function appeared to be measuring dry matter and stem thickness versus internodes. This function also could be named vigorous. Second function seemed to be measuring stem thickness and internodes and could be named stoloniferous. Regarding the sign and magnitude of the first discriminant functions and standardized coefficients, stem thickness and dry matter are consensual determinant variables which algebraically increase the score, whereas, internodes is a consensual variable which reduces the score.

4.2.1.3 **Based on first order interaction (Traits with significant GxY effect)**

Nodal rooting, first flowering, stem thickness, number of stems, and branches, presented significant GxY effect. Therefore, a pooled analysis of variance in conjunction with multivariate discriminant analysis of variance by which GxY effect was multivariately partitioned across the mentioned attributes was carried out. Cumulative discriminating power, their F values, probability of discriminating ability of the discriminant functions are presented in table 4.14. In this case also the first two discriminant functions can retain the majority of the variation existing in the data (about 85%, Table 4.14).

Table 4.14 : Multiple discriminant of GxY patterns in attributes with significant GxY effect in pooled analysis.

Discriminant	Cumulative	F	Numerator d.f.	Denominator d.f.	Probability
1	0.6380	8.296	55	202.6	0.0001
2	0.8490	5.225	40	168.7	0.0001

All the studied attributes in this analysis presented a very high correlation with first discriminant function, whereas, stem thickness and number of stems were the only attributes with a high correlation value with the second discriminant function (Table 4.15). Based on the sign of the discriminant function values and standardized coefficients, number of branches is a consensual variable which decreases the score. In contrast stem thickness and number of stems are consensual variables which increase the score. First flowering can be regarded as a pseudo variable which does not contribute to the score but its positive correlation with other variables increases the score. Prostrateness is also a pseudo variable which is negatively correlated with the score. Therefore, the first function measures stem thickness, number of stems, and first flowering (+) versus branches and prostrateness. This function could be named as "late flowering vigorous" score. The second function measures number of stems, first flowering, and

branches (+) versus prostrateness and stem thickness (-) and could be named as "erect stemy" score.

Table 4.15: The structures of discriminant functions and standardized coefficients of the analysis based on the attributes with significant GxY effect.

Traits	Between canonical structure		Standardized discriminant coefficients	
	DIS1	DIS2	DIS1	DIS2
PRO	-0.4954	-0.4448	-0.1973	-1.0420
FFW	0.5102	0.3947	0.1772	0.8954
STT	0.9256	-0.3712	1.9202	-1.6635
NST	0.7202	0.5754	0.5708	1.0002
NBR	-0.9456	0.1024	-0.5495	-0.0144

4.2.2 **Cluster analysis**

In spite of doing multivariate discriminant analysis there was still too many statistics to consider and it may not be so clear to understand the real pattern of GE interaction effect on the genotypes. Therefore, cluster analysis was done using the Ward's method of clustering. The aim of clustering was to specify the most similar genotypes based on their genotype-environment interaction pattern and to segregate the genotypes with dissimilar response to genotype-environment interaction. However, as clustering proceeds and sub-clusters merge, the internal homogeneity of clusters decreases. In other words, within cluster sums of squares increase and between cluster sums of squares decrease. Therefore, a method suggested by Gordon (pers. com.), based on the ratio of amongst cluster sums of squares to within cluster sums of squares, was used to determine the cut off point on the dendrogram and specify the number of clusters. The cut off points have been marked with an interrupted line on dendrograms.

With all attributes regarding GxSxY effect the genotypes were divided to three groups (Figure 4.1). MANOVA degrees of freedom, F ratios and the corresponding probabilities are presented in Table 4.16.

Table 4.16 : The probability of F test for different clustering stages (clustering based on GxSxY effect and all attributes).

Clustering stage	Numerator DF	Denominator DF	F ratio	Probability
9	24	3.50	416.36	0.0005
10	16	4.00	19.369	0.008
11	8	3.00	9.2542	0.05

One of the objectives of clustering is to fined whether the boundaries between clusters reflect any discontinuity. Based on the results of this analysis, clustering corresponded with the growth habit. In other words all erect genotypes were appointed to a common cluster, and the same was for prostrate and semi-erect genotypes. This indicates that each growth habit has its own particular response to environment. Means for the three cluster levels, each environment, and attribute are presented in Table 4.19.

Clustering was also done based on multivariate partitioning of GxSxY effect for the attributes which showed significant GxSxY effect in ANOVA. In this analysis also the studied genotypes were divided into the same three clusters and clusters corresponded with the growth habit of the studied genotypes (Figure 4.2). Degrees of freedom, F ratio and the corresponding probabilities are presented in Table 4.17.

Table 4.17 : The probability of F test for different clustering stages (clustering based on attributes with significant GxSxY effect).

Clustering stage	Numerator DF	Denominator DF	F ratio	Probability
9	24	3.50	4.2630	0.09
10	16	4.00	12.640	0.015
11	8	3.00	28.374	0.01

The same procedure of cluster analysis was done on the basis of attributes which showed significant GxY effect in ANOVA. The clustering process was quite similar to the previous ones, that is, genotypes with the same growth habit merged together to form larger clusters. The cut-off point divided the studied genotypes into four clusters (Figure 4.3). In other words, prostrate genotypes formed two clusters. Degrees of freedom, F ratios and the corresponding probabilities are presented in Table 4.18. Mean values of all attributes in the four clusters are presented in Table 4.20. The clustering of genotypes based on all the three cases confirmed that growth habit responds quite differently to the environmental effects.

Table 4.18 : The probability of F test for different clustering stages (clustering based on attributes with significant GxY effect).

Clustering stage	Numerator DF	Denominator DF	F ratio	Probability
9	24	3.50	18.68	0.008
10	16	4.00	34.26	0.004
11	8	3.00	70.95	0.003

Table 4.19 : Cluster mean values for all environments (clustering based on GxSxY effect).

Trait	Cluster	Frewans 1991	Mogini 1991	Frewans 1992	Mogini 1992
PRO	one	4.04 b	4.01 b	4.06 b	4.16 a
NRT		3.93 a	3.96 a	4.04 a	4.04 a
FFW		172.22 b	179.71 a	132.63 c	134.32 c
MFW		186.66 a	188.24 a	153.81 c	158.07 b
LSZ		5.36 b	5.39 b	5.73 a	5.20 c
STL		84.45 a	65.70 b	46.13 c	37.76 d
STT		4.90 a	3.90 d	4.21 b	4.05 c
NST		48.96 c	39.92 d	80.18 a	56.78 b
NIN		8.42 a	7.21 b	6.79 c	5.18 d
NBR		6.43 a	4.85 b	2.13 c	2.01 c
DMY		483.03 a	224.64 b	236.35 b	182.76 c
PRO	Two	5.43 a	5.23 b	5.00 c	5.21 b
NRT		3.91 c	3.94 bc	4.06 ab	4.09 a
FFW		145.81 b	151.21 a	110.44 d	118.41 c
MFW		165.93 a	166.87 a	128.70 c	138.43 b
LSZ		5.00 a	5.18 a	4.94 b	4.79 c
STL		71.25 a	47.92 c	57.12 b	30.29 d
STT		3.42 a	3.08 b	2.61 d	2.46 c
NST		29.91 c	29.07 c	96.18 a	81.33 b
NIN		7.66 a	7.05 b	7.68 a	7.99 a
NBR		6.95 a	5.66 b	3.09 c	2.37 d
DMY		276.55 a	127.67 c	236.84 b	76.69 d
PRO	Three	6.38 a	6.38 a	5.95 c	6.21 b
NRT		4.60 b	4.71 b	5.01 a	5.06 a
FFW		155.81 b	161.96 a	120.35 d	129.92 c
MFW		174.85 b	178.48 a	136.53 d	147.63 c
LSZ		4.70 a	4.67 a	4.71 a	4.52 b
STL		54.36 a	38.24 b	29.06 c	23.07 d
STT		1.53 b	1.51 b	2.12 a	2.06 a
NST		24.68 c	19.16 c	111.43 a	94.48 b
NIN		10.68 ab	10.97 a	9.99 c	10.50 b
NBR		9.75 a	9.30 a	3.36 b	2.69 c
DMY		194.81 a	125.67 b	140.56 b	42.71 c

t test results goes across the rows. Means with the same letter were not significantly different.

Table 4.20: Cluster mean values for both years (clustering based on attributes with significant GxY effect).

Traits	Cluster	Year 1991a	Year 1992
PRO		4.03 b	4.11 a
NRT		3.94 a	4.04 a
FFW		175.90 a	133.46 b
MFW		187.43 a	155.88 b
LSZ	One	5.38 a	5.47 a
STL		75.24 a	42.06 b
STT		4.41 a	4.13 b
NST		44.52 b	68.79 a
NIN		7.82 a	6.01 b
NBR		5.65 a	2.07 b
DMY		356.06 a	210.26 b
PRO		5.33 a	5.10 b
NRT		3.92 b	4.07 a
FFW		148.44 a	114.32 b
MFW		166.38 a	133.44 b
LSZ	Two	5.09 a	4.87 b
STL		59.90 a	44.06 b
STT		3.25 a	2.53 b
NST		29.50 b	88.82 a
NIN		7.36 b	7.83 a
NBR		6.32 a	2.74 b
DMY		204.08 a	158.17 b
PRO		6.48 a	6.39 b
NRT		4.97 b	5.32 a
FFW		160.96 a	126.76 b
MFW		178.18 a	141.57 b
LSZ		4.62 a	4.47 b
STL	Three	43.18 a	24.33 b
STT		1.37 b	1.97 a
NST		19.75 b	109.05 a
NIN		11.60 a	11.21 b
NBR		10.12 a	3.12 b
DMY		162.21 a	77.76 b
PRO		6.27 a	5.75 b
NRT		4.31 b	4.74 a
FFW		156.70 a	123.00 b
MFW		175.05 a	142.12 b
LSZ	Four	4.76 a	4.78 a
STL		49.46 a	28.07 b
STT		1.68 b	2.22 a
NST		24.22 b	97.24 a
NIN		9.97 a	9.25 b
NBR		8.86 a	2.95 b
DMY		156.87 a	108.29 b

t test results goes across the rows. Means with the same letters were not significantly different.

Figure 4.1: Dendrogram for cluster analysis based on the first and second discriminant scores for the GxSxY partition (all attributes).

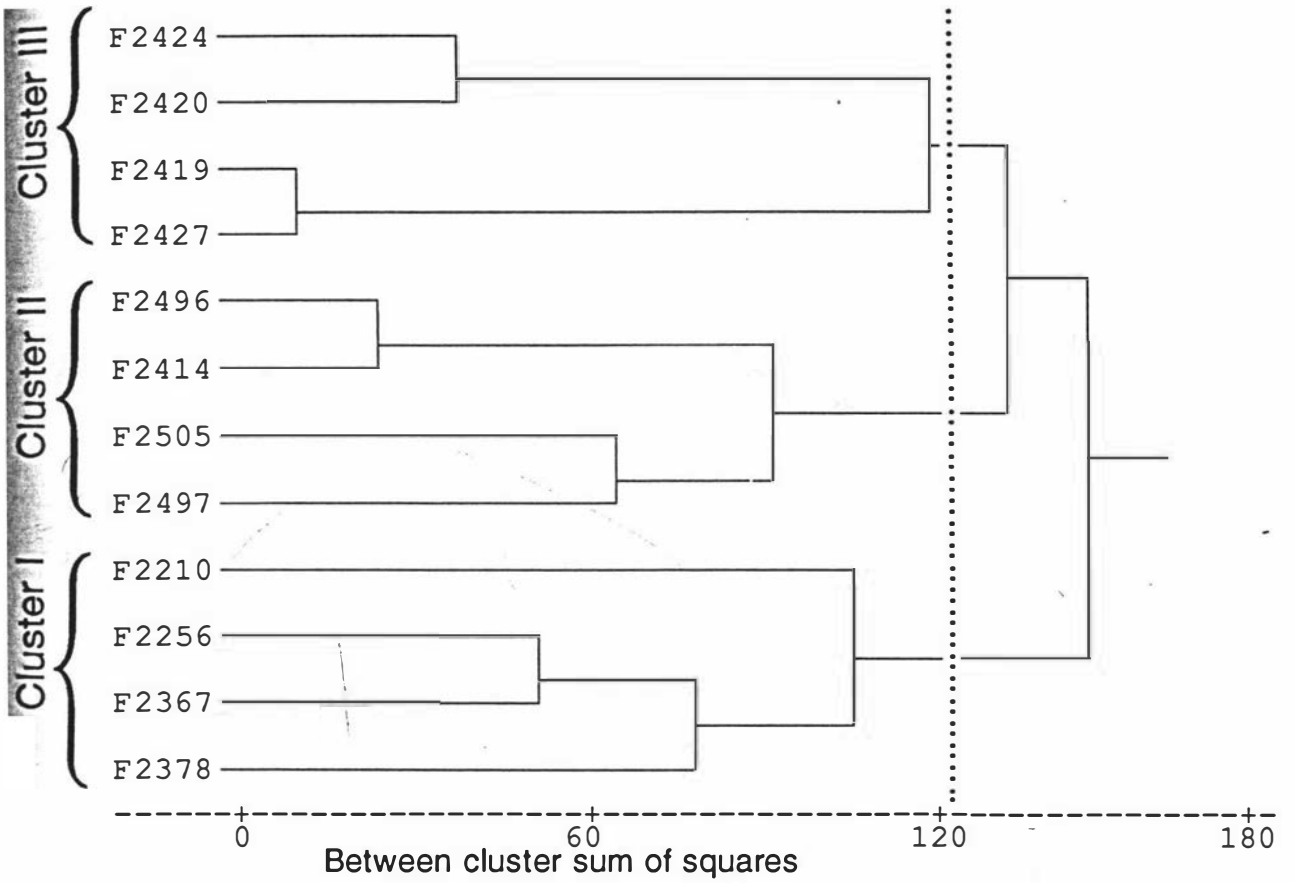


Figure 4.2 : Dendrogram for cluster analysis based on the first and second discriminant scores for the GxSxY partitioning (attributes with significant GxSxY effect in ANOVA).

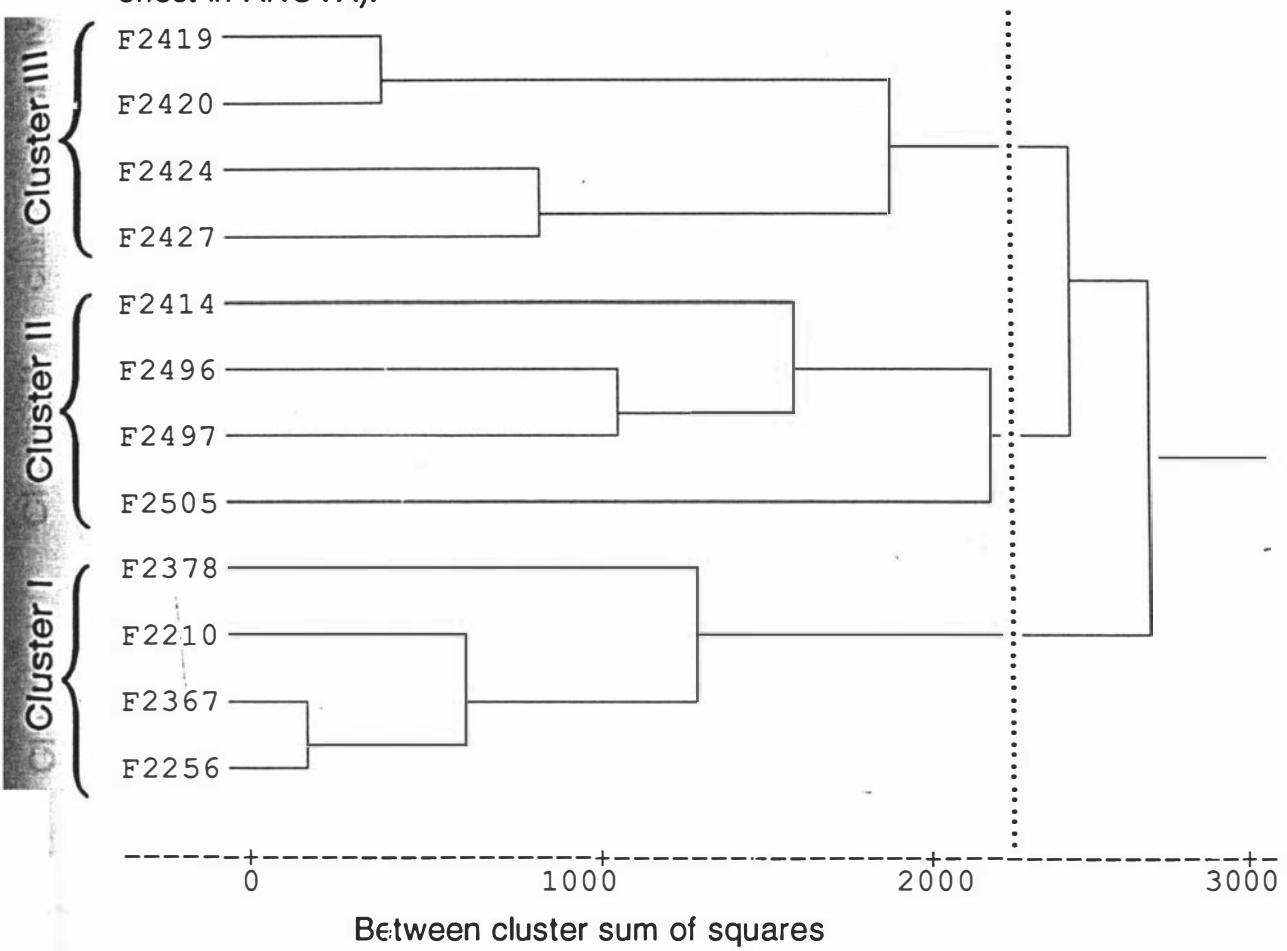
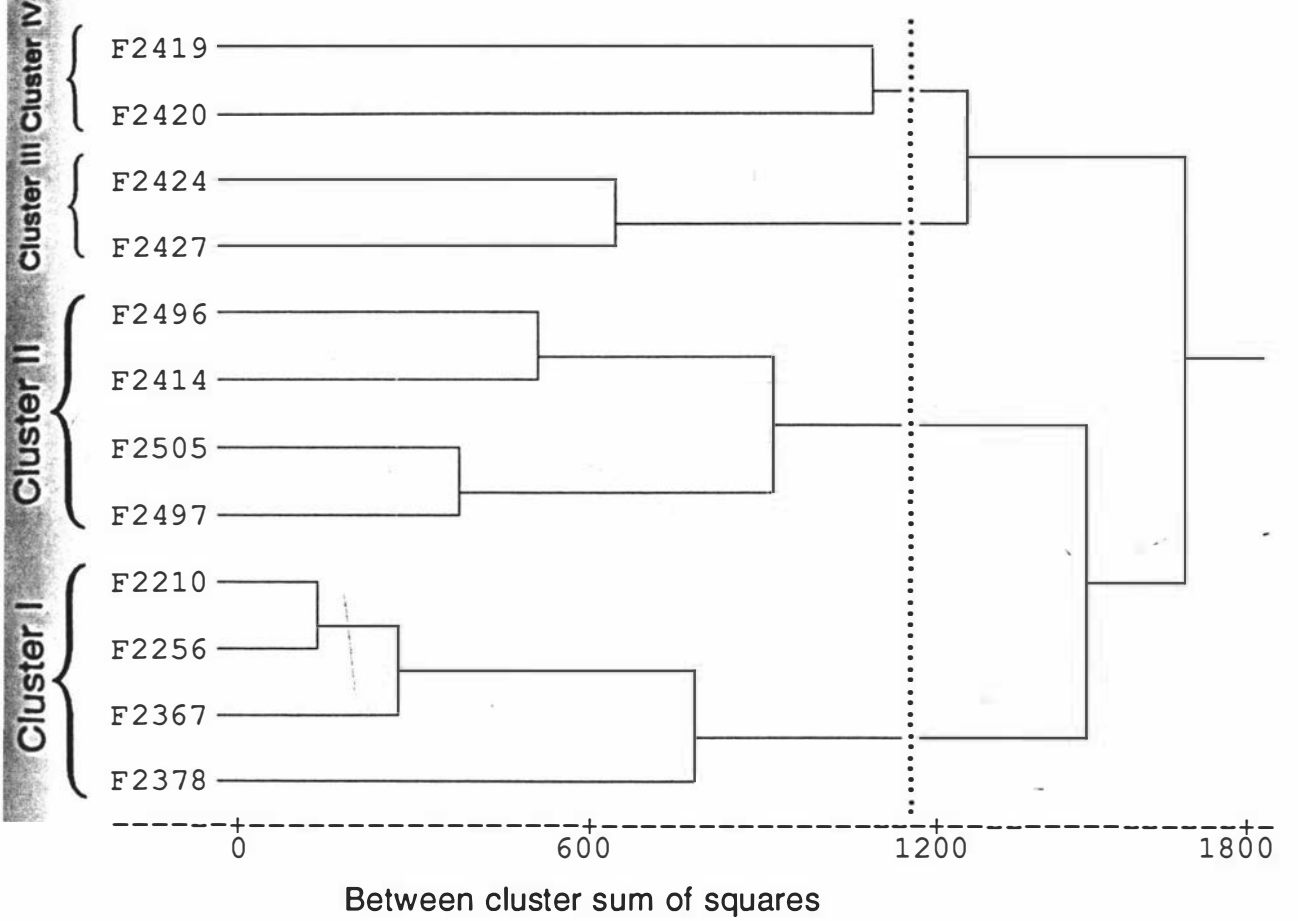


Figure 4.3 : Dendrogram for cluster analysis based on the first and second discriminant scores for the GxY partitioning (attributes with significant GxY effect in ANOVA).



4.2.3 Type discrimination

To investigate the role of different attributes in forming types a multivariate discriminant analysis was done on the data regarding types as treatments following a pooled analysis of variance. With three types, two canonical correlations were estimated using GLM procedure and MANOVA statement in SAS (SAS Institute, Inc., 1990). Cumulative discriminating power and the probability of significance of discriminating ability of successive functions are presented in Table 4.21. The cumulative discrimination indicated that the first function can describe the majority of the variation in the data, regarding type discriminating.

Table 4.21 : Multiple discriminant analysis results based on the growth habit.

Discriminant	Cumulative	F	Numerator d.f	Denominator d.f.	Probability
1	0.897	168.06	22	4	0.0001
2	1.000	93.90	10	3	0.0016

It can be concluded that by this way of analysis to discriminate between types, the first function is enough to retain sufficient amount of the original variation. Canonical structure correlations and standardized canonical coefficients are presented in Table 4.22.

As was mentioned earlier, the values of this function represent the correlation between the function and the original variables. Prostrateness, nodal rooting, number of stems, branches, and internodes were highly and positively correlated with the first canonical structure function, and the rest of the attributes were highly negatively correlated with the function.

Table 4.22 : First and second canonical structure values and standardized canonical structures.

Traits	Between canonical structure	Standardized discriminant coefficients
PRO	0.9992	5.5899
NRT	0.8334	0.0169
FFW	-0.6018	-6.7172
MFW	-0.6192	6.6991
LSZ	-0.9999	0.3862
STL	-0.9571	0.7301
STT	-1.0000	0.3598
NST	0.9985	3.0504
NIN	0.9135	1.7383
NBR	0.9463	-3.2552
DMY	-0.9950	-6.1336

Regarding the sign and magnitude of canonical and standardized scores, prostrateness, number of stems, and internodes are determinant attributes which consensually increase the score. First flowering and dry matter yield are other determinant attributes which consensually decrease the scores. Number of branches is a determinant reversed attribute. Stem thickness and leaf size are pseudo attributes which are negatively correlated to the score but they do not contribute to the score through other attributes. Nodal rooting is also a pseudo attribute which does not contribute to the score, but it is positively correlated with score. Median flowering and stem length are determinant reversed attributes which increase the score but it is counteracted with other attributes.

CHAPTER FIVE : HIERARCHICAL MATING DESIGN RESULTS AND ASSOCIATED DISCUSSION

Following a genotypic overview with the wider germplasm in the genotype-environment interaction experiment, a more detailed genetic analysis follows, but within a narrower gene pool. The data for each type of cross was analyzed separately. The estimated parameters also were calculated for each set of crosses independently. Due to the nature of the design some parameters such as σ^2_A, σ^2_D, h^2_{ns}, h^2_{bs}, and R_1, (σ^2D/σ^2A), for inter-population crosses had to be calculated jointly for both inter-population crosses.

5.1 Intra- and inter-crosses mean values

Mean values, their coefficient of variation, and the results of combined analysis of variance are presented in Table 5.1. Mean values were significantly different between the two intra-population sets of crosses for all traits. Since individuals from both intra-population crosses were grown together in the same environment any significant differences in phenotypic means indicated genetic divergence between the two populations. Also mean values were significantly different in the two inter-population crosses for prostrateness, first flowering, median flowering, leafiness, number of stems, stem length, and height.

5.2 Heterosis and hybrid depression

Heterosis is the enhancement of trait expression with increased heterozygosity. It results from crossing unrelated strains. The percentage difference between overall mean values for the two inter- and intra-population sets of crosses was considered as an estimate of heterosis (if positive) or hybrid depression (if negative). Heterosis and hybrid depression values are presented in the Table 5.1. Some attributes such as stem length, internodes,

branches, and diameter showed a relatively large heterosis, whereas some attributes such as number of stems, and height showed a large hybrid depression.

5.3 **Biometrical components of variance**

Biometrical components of variance and their standard errors for the measured traits in four sets of intra and inter-population crosses are presented in Tables 5.2 to 5.5. Generally male and female components of variance (σ^2m & σ^2f) showed a different pattern of significance in inter- and intra-population crosses. Female components of variance were highly significant in all population combinations, but male components were rarely significant at 5% level of probability (Tables 5.2 to 5.5). In some cases, such as nodal rooting, median flowering, first flowering, and branches the values for male and female components of variance were somewhat smaller when estimated from erect intra-population crosses than from the prostrate intra-population crosses. When both male and female components of variance were smaller it suggests generally less genetic variance in the population. This suggests that the erect population might be more highly selected than the prostrate population based on the mentioned attributes. The erect population is from research material, whereas the prostrate population is a simple accession. Components of variance related to set and replication (σ^2s & σ^2r) were usually non-significant, and between plot component (σ^2b) was usually highly significant. Negative estimates were detected in some cases, particularly in erect population, but the estimates were far less than their corresponding standard errors. Therefore, they could be safely regarded as zero value.

Table 5.1: Mean values, their percentage of coefficient of variation (in brackets), heterosis, hybrid depression for inter and intra-population sets of crosses (F_1).

Traits	Intra-population crosses			Inter-population crosses			Heterosis[4]
	Erect	Pros.	O.M[1]	♀Ex♂P[1]	♀Px♂E	O.M	
PRO[3]	4.60 b (3.38)	6.41 a (2.61)	5.50	5.50 c (2.35)	5.62 b (2.41)	5.56	1.00
NRT	4.07 c (3.46)	4.51 a (5.08)	4.29	4.37 b (5.04)	4.34 b (4.56)	4.36	1.52
FFW	160.5 a (1.94)	136.2 d (3.24)	148.4	146.9 c (2.15)	151.0 b (2.42)	148.9	0.40
MFW	173.4 a (1.80)	149.3 d (1.98)	161.4	161.1 c (1.57)	164.7 b (1.62)	162.9	0.96
LSZ	5.72 a (4.16)	4.29 c (3.42)	5.01	5.03 b (2.66)	5.05 b (2.42)	5.04	0.70
LNS	5.52 a (5.26)	5.14 b (4.27)	5.33	5.60 a (5.25)	5.08 b (4.18)	5.34	0.19
STT	4.83 a (3.74)	2.71 c (6.82)	3.77	3.62 b (4.96)	3.6 b (4.28)	3.61	-4.24
NST	17.50 a (16.55)	6.52 d (18.76)	12.01	9.91 c (16.86)	10.75b (19.08)	10.33	-13.99
STL	38.48 a (9.76)	21.50 d (14.67)	29.99	35.85 b (9.20)	34.58 c (11.10)	35.22	17.42
NIN	7.93 b (4.39)	7.82 b (7.50)	7.88	8.80 a (4.22)	8.76 a (7.21)	8.78	11.49
NBR	6.76 c (5.16)	7.00 b (7.82)	6.88	8.39 a (4.50)	8.46 a (5.33)	8.42	22.46
PHT	3.63 a (8.68)	0.59 d (12.88)	2.11	2.12 b (12.64)	1.87 c (14.0)	2.00	-5.45
PDI	6.30 b (7.40)	3.56 c (14.9)	4.93	6.44 a (9.64)	6.20 b (7.35)	6.32	28.19
SVG	4.98 b (5.75)	4.91 c (5.42)	4.94	5.20 a (5.12)	4.79 d (5.83)	5.00	1.01

1: overall mean for the two inter or intra-populations of crosses
2: E: Erect P : Prostrate
3: scores were transformed to rankit with the following SAS equation: X_t = probit (X_O*2/10 - 0.05)+5, in which X_t and X_O are the value in transformed and original scale respectively.
LSD result goes across the rows, and the means with the same letter were not significantly different.
4: refer to section 3.4.4.3.5.

Table 5.2 : Biometrical components of variance and their standard errors in erect intra-population crosses.

Traits	$\sigma^2 m_{(ee)}$	$\sigma^2 f_{(ee)}$	$\sigma^2 s$	$\sigma^2 r$	$\sigma^2 b$	$\sigma^2 w$
PRO	0.020 (*) (0.014)	0.020 ** (0.008)	0.001 ns (0.009)	0.001 ns (0.002)	0.008 ** (0.003)	0.024
NRT	0.004 ns (0.004)	0.005 * (0.003)	0.004 ns (0.004)	-0.001 ns (0.001)	0.006 ** (0.002)	0.020
FFW	0.621 ns (1.208)	3.516 * (1.535)	0.570 ns (1.199)	0.784 ns (0.847)	1.660 * (0.734)	9.659
MFW	-0.987 ns (1.864)	9.718 ** (3.687)	-0.133 ns (0.845)	0.372 ns (0.689)	3.238 ** (1.046)	9.689
LSZ	0.022 ns (0.024)	0.059 ** (0.022)	0.014 ns (0.024)	0.008 ns (0.008)	0.018 ** (0.006)	0.057
LNS	0.110 * (0.068)	0.055 * (0.024)	-0.026 ns (0.027)	0.006 ns (0.009)	0.037 ** (0.011)	0.085
STT	-0.049 ns (0.028)	0.222 ** (0.074)	0.103 ns (0.079)	-0.001 ns (0.001)	0.022 ** (0.006)	0.033
NST	11.126 ns (9.935)	22.530 ** (7.770)	5.420 ns (9.177)	0.981 ns (1.115)	3.489 ** (1.042)	8.389
STL	-0.404 ns (2.786)	14.020 ** (4.795)	8.459 ns (7.228)	0.516 ns (0.599)	0.044 ns (0.616)	14.113
NIN	0.450 (*) (0.333)	0.582 ** (0.196)	-0.141 ns (0.123)	0.003 ns (0.010)	0.072 ** (0.019)	0.121
NBR	0.212 ns (0.252)	0.735 ** (0.254)	-0.003 ns (0.144)	0.036 ns (0.040)	0.154 ** (0.036)	0.122
PHT	-0.012 ns (0.029)	0.147 ** (0.054)	-0.002 ns (0.012)	0.001 ns (0.006)	0.046 ** (0.013)	0.099
PDI	0.030 ns (0.122)	0.519 ** (0.178)	0.031 ns (0.081)	-0.001 ns (0.008)	0.066 ** (0.022)	0.217
SVG	0.035 * (0.028)	0.045 ** (0.019)	0.017 ns (0.027)	0.001 ns (0.004)	0.022 ** (0.008)	0.082

** : significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level
ns : non-significant

Table 5.3 : Biometrical components of variance and their standard errors in prostrate intra-population crosses.

Traits	$\sigma^2 m_{(ee)}$	$\sigma^2 f_{(ee)}$	$\sigma^2 s$	$\sigma^2 r$	$\sigma^2 b$	$\sigma^2 w$
PRO	0.022 ns (0.019)	0.038 * (0.014)	-0.006 ns (0.008)	0.001 ns (0.002)	0.009 ** (0.003)	0.028
NRT	0.058 ns (0.055)	0.136 ** (0.046)	-0.003 ns (0.030)	0.004 ns (0.005)	0.018 ** (0.006)	0.052
FFW	8.561 ns (7.210)	12.527 ** (5.292)	-3.129 ns (2.767)	1.194 ns (1.759)	7.273 ** (2.258)	19.547
MFW	9.622 ns (7.838)	15.027 ** (5.442)	-1.666 ns (3.562)	0.276 ns (0.657)	3.860 ** (1.131)	8.751
LSZ	0.034 * (0.020)	0.014 * (0.006)	-0.010 ns (0.007)	-0.001 ns (0.001)	0.014 ** (0.004)	0.021
LNS	0.168 * (0.095)	0.052 ** (0.020)	-0.039 ns (0.036)	-0.001 ns (0.002)	0.021 ** (0.006)	0.048
STT	0.041 ns (0.035)	0.075 ** (0.026)	-0.009 ns (0.015)	0.003 ns (0.003)	0.008 ** (0.003)	0.034
NST	0.172 ns (0.289)	0.805 * (0.349)	0.622 ns (0.574)	0.009 ns (0.073)	0.501 ** (0.162)	1.497
STL	4.496 ns (4.301)	9.801 ** (3.622)	-1.450 ns (1.685)	0.148 ns (0.473)	2.455 ** (0.903)	9.943
NIN	0.283 ns (0.289)	0.709 ** (0.260)	-0.127 ns (0.101)	0.002 ns (0.026)	0.224 ** (0.058)	0.344
NBR	0.616 (*) (0.474)	0.824 ** (0.300)	-0.302 ns (0.155)	-0.001 ns (0.026)	0.260 ** (0.064)	0.300
PHT	0.002 ns (0.006)	0.022 ** (0.008)	-0.001 ns (0.003)	-0.001 ns (0.001)	0.008 ** (0.002)	0.006
PDI	0.363 (*) (0.225)	0.195 ** (0.078)	-0.130 ns (0.076)	-0.004 ns (0.011)	0.103 ** (0.032)	0.278
SVG	0.016 (*) (0.011)	0.007 ns (0.006)	-0.004 ns (0.005)	0.001 ns (0.004)	0.020 ** (0.007)	0.071

** : significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level
ns : non-significant

Table 5.4 : Biometrical components of variance and their standard errors in first inter-population crosses where erect population was as male parent population.

Traits	$\sigma^2 m_{(ee)}$	$\sigma^2 f_{(ee)}$	$\sigma^2 s$	$\sigma^2 r$	$\sigma^2 b$	$\sigma^2 w$
PRO	0.013 ns (0.014)	0.037 ** (0.014)	0.002 ns (0.007)	-0.001 ns (0.001)	0.013 ** (0.003)	0.018
NRT	0.016 ns (0.013)	0.022 * (0.010)	0.008 * (0.004)	-0.001 ns (0.002)	0.020 ** (0.006)	0.039
FFW	-0.249 ns (3.407)	14.621 ** (5.669)	-1.918 ns (0.987)	0.091 ns (0.844)	56.477 ** (1.843)	13.388
MFW	1.734 ns (3.215)	11.133 ** (3.936)	-1.595 ns (0.994)	-0.331 ns (0.110)	2.260 ** (0.747)	7.157
LSZ	0.007 ns (0.009)	0.025 ** (0.009)	-0.001 ns (0.004)	-0.001 ns (0.001)	0.009 ** (0.002)	0.015
LNS	0.014 ns (0.040)	0.164 ** (0.054)	-0.042 ns (0.048)	0.001 ns (0.003)	0.012 ** (0.004)	0.045
STT	-0.022 ns (0.036)	0.116 ** (0.043)	0.035 ns (0.041)	-0.002 ns (0.004)	0.050 ** (0.011)	0.024
NST	7.417 ns (8.002)	22.805 * (7.555)	11.709 ns (12.152)	0.095 ns (0.324)	2.1799 ** (0.608)	4.205
STL	6.415 (*) (4.282)	3.770 * (2.004)	2.874 ns (4.219)	-0.211 ns (0.442)	3.896 ** (1.388)	14.729
NIN	0.122 ns (0.244)	0.878 ** (0.305)	-0.041 ns (0.152)	-0.002 ns (0.020)	0.168 ** (0.050)	0.398
NBR	0.311 ns (0.343)	0.921 ** (0.324)	0.055 ns (0.223)	0.009 ns (0.031)	0.251 ** (0.058)	0.204
PHT	-0.097 (*) (0.063)	0.066 ** (0.027)	-0.076 ns (0.085)	-0.003 ns (0.002)	0.037 ** (0.010)	0.068
PDI	0.186 (*) (0.149)	0.256 ** (0.099)	0.041 ns (0.109)	-0.001 ns (0.013)	0.114 ** (0.032)	0.217
SVG	0.054 * (0.030)	-0.001 ns (0.007)	-0.005 ns (0.015)	-0.001 ns (0.004)	0.038 ** (0.011)	0.078

** : significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level
ns : non-significant

Table 5.5 : Biometrical components of variance and their standard errors in second inter-population crosses where prostrate population was as male parent population.

Traits	$\sigma^2 m_{(ee)}$	$\sigma^2 f_{(ee)}$	$\sigma^2 s$	$\sigma^2 r$	$\sigma^2 b$	$\sigma^2 w$
PRO	0.030 (*) (0.019)	0.021 ** (0.008)	-0.001 ns (0.010)	-0.001 ns (0.001)	0.004 * (0.002)	0.017
NRT	0.057 * (0.034)	0.024 ** (0.010)	-0.008 ns (0.016)	0.004 ns (0.005)	0.011 ** (0.004)	0.049
FFW	6.144 (*) (4.819)	7.786 ** (3.124)	0.029 ns (2.906)	0.865 ns (1.052)	3.425 ** (1.096)	9.999
MFW	6.456 (*) (4.516)	5.677 ** (2.324)	-0.844 ns (2.131)	0.192 ns (0.493)	3.079 ** (0.877)	6.391
LSZ	0.046 (*) (0.029)	0.026 ** (0.010)	-0.018 ns (0.009)	0.001 ns (0.002)	0.011 ** (0.003)	0.018
LNS	0.134 (*) (0.083)	0.071 ** (0.029)	00.032 ns (0.068)	0.004 ns (0.007)	0.037 ** (0.011)	0.086
STT	0.056 ns (0.059)	0.139 ** (0.054)	-0.025 ns (0.020)	-0.007 ns (0.003)	0.076 ** (0.017)	0.032
NST	6.380 * (3.858)	2.946 ** (1.210)	-1.616 ns (1.418)	-0.100 ns (0.124)	1.737 ** (0.461)	2.791
STL	4.781 ns (4.158)	7.967 ** (3.145)	0.347 ns (2.495)	-0.132 ns (0.340)	2.993 ** (1.048)	10.878
NIN	0.034 ns (0.136)	0.586 ** (0.247)	0.049 ns (0.085)	-0.037 ns (0.017)	0.468 ** (0.099)	0.138
NBR	-0.199 ns (0.220)	0.457 * (0.212)	-0.093 ns (0.074)	-0.026 ns (0.028)	0.526 ** (0.111)	0.143
PHT	0.087 (*) (0.059)	0.063 * (0.029)	0.024 ns (0.047)	-0.004 ns (0.003)	0.059 ** (0.015)	0.072
PDI	0.339 (*) (0.215)	0.184 * (0.084)	0.134 ns (0.205)	-0.009 ns (0.012)	0.134 ** (0.043)	0.386
SVG	0.014 ns (0.014)	0.025 * (0.012)	-0.006 ns (0.005)	0.001 ns (0.004)	0.023 ** (0.008)	0.071

** : significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level
ns : non-significant

5.4 **Genetic components of variance**

Additive and dominance components of variance were estimated for both sets of intra-population crosses (erect and prostrate populations) and the pooled inter-populations crosses. These components of variance and their corresponding standard errors are presented in tables 5.6 to 5.8, generally, values for genetic variance components indicated that the additive genetic variance components were the most important component of variance in prostrate population, but dominance was the most important component of genetic variance in the erect and inter-population crosses. Only number of stems and seedling vigour had a bigger additive component of variance in erect population than that in prostrate population. It was pointed out by Robinson *et al.* (1955) that if there is a considerable dominance variance component but only a negligible amount of additive genetic variance in a population, it would be strong evidence for the presence of over-dominance. It is also a possibility, as pointed out by Mitchell-Olds & Bergelson (1990), that the female component of genetic variance may be inflated by maternal effect. This would result in dominance genetic variance being biased upward. This is particularly so for seedling vigour which was measured at the early stages of life of the plants.

5.5 **Dominance ratio**

The degree of dominance, called the R_1 ratio, (see materials and methods) was estimated for all the measured attributes, and is presented also in Tables 5.6 to 5.8, This ratio could be regarded as a degree of dominance for those traits controlled by one gene. Consequently the values bigger than one would lead to the conclusion of over dominance; but in quantitative characters which are controlled by several genes, this value should be regarded simply as a ratio (Obilana *et al.*, 1979).

Table 5.6 : Estimates of genetic variance components and their standard errors in erect population.

Traits	σ^2A		σ^2D		Ratio
	Estimate	S.E.	Estimate	S.E.	σ^2D/σ^2A
Prostrateness	0.082 (*)	0.058	-0.002 ns	0.066	0.0
Nodal rooting	0.016 ns	0.014	0.004 ns	0.018	0.2
First flowering	2.486 ns	4.830	11.58 (*)	7.810	4.7
Median flowering	-3.947 ns	7.45	42.82 **	16.51	ne
Leaf size	0.087 ns	0.094	0.147 ns	0.129	1.7
Leafiness	0.441 (*)	0.272	-0.22 ns	0.288	0.0
Stem thickness	-0.197 ns	0.112	1.084 **	0.317	ne
No. of stems	44.50 ns	39.74	45.62 ns	50.44	1.0
Stem length	-1.615 ns	11.13	57.687 **	22.17	ne
No. of internodes	1.801 (*)	1.331	0.527 ns	1.545	0.3
No. of branches	0.849 ns	1.009	2.092 (*)	1.431	2.5
Plant height	-0.047 ns	0.114	0.635 **	0.245	ne
Plant diameter	0.119 ns	0.490	1.958 *	0.864	16.4
Seedling vigour	0.139 ns	0.113	0.041 ns	0.136	0.3

** : significantly different from zero at 1% probability level

* : significantly different from zero at 5% probability level

(*): significantly different from zero at 10% probability level

ns : non-significant

ne : non-estimable

Table 5.7 : Estimates of genetic variance components and their standard errors in prostrate population.

Traits	σ^2A		σ^2D		Ratio
	Estimate	S.E.	Estimate	S.E.	σ^2D/σ^2A
Prostrateness	0.089 ns	0.076	0.063 ns	0.094	0.71
Nodal rooting	0.231 ns	0.221	0.311 ns	0.289	1.35
First flowering	34.24 ns	28.90	15.87 ns	35.81	0.46
Median flowering	38.489 ns	31.42	21.61 ns	38.21	0.56
Leaf size	0.1346 *	0.081	-0.08 ns	0.085	0
Leafiness	0.673 *	0.380	-0.464 ns	0.389	0
Stem thickness	0.163 ns	0.139	0.137 ns	0.173	0.84
No. of stems	0.689 ns	1.155	2.529 ns	1.811	3.67
Stem length	17.98 ns	17.16	21.22 ns	22.45	1.18
No. of internodes	1.133 ns	1.159	1.701 ns	1.556	1.50
No. of branches	2.462 (*)	1.898	0.833 ns	2.245	0.34
Plant height	0.009 ns	0.024	0.080 ns	0.040	8.50
Plant diameter	1.460 (*)	0.896	-0.706 ns	0.949	0
Seedling vigour	0.064 (*)	0.046	-0.036 ns	0.053	0

** : significantly different from zero at 1% probability level

* : significantly different from zero at 5% probability level

(*): significantly different from zero at 10% probability level

ns : non-significant

Table 5.8 :Estimates of genetic variance components and their standard errors in inter-population crosses.

Traits	σ^2A		σ^2D		Ratio σ^2D/σ^2A
	Estimate	S.E.	Estimate	S.E.	
Prostrateness	0.086 *	0.048	0.031 ns	0.057	0.36
Nodal rooting	0.146 *	0.073	-0.056 ns	0.078	0
First flowering	11.79 ns	11.72	33.03 *	17.38	2.8
Median flowering	16.38 *	11.01	17.24 ns	14.26	1.05
Leaf size	0.106 *	0.060	-0.005 ns	0.066	0
Leafiness	0.297 (*)	0.184	0.173 ns	0.221	0.58
Stem thickness	0.156 ns	0.138	0.355 *	0.195	2.28
No. of stems	27.59 (*)	17.41	23.92 ns	22.87	0.87
Stem length	22.39 (*)	11.80	1.084 ns	13.95	0.05
No. of internodes	0.176 ns	0.548	2.752 **	0.952	0.10
No. of branches	1.019 ns	0.802	1.738 (*)	1.110	1.70
Plant height	0.368 *	0.171	-0.109 ns	0.188	0
Plant diameter	1.056 *	0.521	-0.177 ns	0.581	0
Seedling vigour	0.137 *	0.067	-0.089 ns	0.073	0

** : significantly different from zero at 1% probability level

* : significantly different from zero at 5% probability level

(*): significantly different from zero at 10% probability level

ns : non-significant

5.6 **Heritability and genetic advance**

Heritability narrow sense and broad sense, restricted and full, were estimated and are presented in the tables 5.9 and 5.10. Full and restricted heritability values are discussed by Gordon *et al.* (1972) and Gordon (1979).

When breeding a new cultivar based on an unknown population is being considered, the breeder is usually eager to predict the magnitude of change produced by selection after one cycle of selection. This is achieved by estimating a parameter called genetic advance or what is called response to selection by Falconer (1989). Genetic advance per cycle of selection was estimated for both inter and intra-population crosses assuming 10% of parental population would be selected. These are also presented in table 5.9. There were some negative estimates for heritability which lead to negative estimates for genetic advance. In some cases, due to negative estimates for dominance variance, heritability was inflated, leading to inflation of genetic advance also.

5.7 **The ratio of intra- to inter-population male and female components of variance**

These estimates, called R_2 and R_3 ratios (see chapter three), also could be used in order to make inferences about allele frequencies in the base populations (Singh *et al.*, 1984). The estimated values are presented in table 5.9. Based on the estimates, the sum of the frequency of alleles controlling the attribute under question in the two parental populations was generally larger than one.

Table 5.9 : Full heritability narrow sense and broad sense estimates and expected genetic advance per cycle of selection in intra and inter-population crosses.

Traits	Erect			Prostrate			Inter-population		
	h^2_{ns}	h^2_{bs}	GA[1]	h^2_{ns}	h^2_{bs}	GA	h^2_{ns}	h^2_{bs}	GA
PRO	0.73	0.71	0.50	0.47	0.80	0.42	0.60	0.82	0.47
NRT	0.36	0.43	0.16	0.38	0.88	0.61	0.98	0.60	0.78
FFW	0.10	0.55	1.02	0.44	0.65	8.04	0.19	0.73	3.10
MFW	*	0.75	*	0.53	0.83	9.30	0.38	0.78	5.14
LSZ	0.28	0.76	0.32	1.49	0.61	0.92	0.83	0.79	0.61
LNS	1.29	0.64	1.55	2.42	0.75	2.63	0.53	0.84	0.82
STT	*	0.94	*	0.48	0.88	0.57	0.26	0.85	0.42
NST	0.44	0.88	9.08	0.13	0.62	0.62	0.48	0.90	7.53
STL	*	0.80	*	0.35	0.76	5.16	0.56	0.59	7.32
NIN	0.71	0.92	2.34	0.33	0.83	1.27	0.05	0.83	0.19
NBR	0.26	0.91	0.98	0.64	0.85	2.58	0.31	0.83	1.15
PHT	*	0.80	*	0.09	0.87	0.06	0.97	0.69	1.23
PDI	0.05	0.88	0.16	1.29	0.67	2.83	0.81	0.67	1.90
SVG	0.49	0.63	0.54	0.54	0.24	0.38	0.89	0.31	0.72

* : negative estimates for either heritability or genetic advance.

1 : GA= genetic advance or response to selection based on the individual selection on both sexes (selection pressure = 5% of parental population).

Table 5.10 : Restricted heritability narrow sense and broad sense estimates and expected genetic advance per cycle of selection in intra and inter-population crosses.

Traits	Erect			Prostrate			Inter-population		
	h^2_{ns}	h^2_{bs}	GA[1]	h^2_{ns}	h^2_{bs}	GA	h^2_{ns}	h^2_{bs}	GA
PRO	1.09	0.86	0.61	0.97	0.96	0.60	0.61	0.83	0.47
NRT	0.43	0.75	0.17	0.88	0.93	0.93	1.01	0.63	0.79
FFW	0.15	0.82	1.25	0.75	0.88	10.4	0.19	0.74	3.10
MFW	*	0.84	*	1.07	0.93	13.2	0.39	0.81	5.22
LSZ	0.49	0.77	0.43	1.86	0.95	1.03	0.89	0.85	0.63
LNS	1.65	0.94	1.76	2.60	1.07	2.78	0.50	0.78	0.79
STT	*	0.62	*	1.07	0.99	0.86	0.26	0.85	0.41
NST	0.86	0.81	12.7	0.19	0.69	0.75	0.45	0.83	7.21
STL	*	0.75	*	0.71	0.95	7.35	0.54	0.57	7.19
NIN	1.65	1.06	3.56	0.79	0.93	1.95	0.05	0.83	0.19
NBR	0.67	0.85	1.56	1.45	1.02	3.89	0.31	0.84	1.16
PHT	*	0.84	*	0.26	0.82	0.10	0.87	0.61	1.16
PDI	0.14	0.89	0.26	1.80	1.03	3.34	0.76	0.63	1.84
SVG	0.69	0.80	0.64	0.57	0.84	0.40	0.93	0.32	0.73

* : negative estimates for either heritability or genetic advance.

1 : GA= genetic advance or response to selection based on the individual selection on both sexes (selection pressure = 5% of parental population).

Table 5.11 : Ratios of intra to inter-population male and female components of variances.

Traits	$\frac{(\sigma^2_{m(ee)} + \sigma^2_{m(pp)})}{(\sigma^2_{m(ep)} + \sigma^2_{m(pe)})}$	$\frac{(\sigma^2_{f(ee)} + \sigma^2_{f(pp)})}{(\sigma^2_{f(ep)} + \sigma^2_{f(pe)})}$
prostrateness	0.987	0.989
Nodal rooting ability	0.849	3.119
First flowering	1.558	0.716
Median flowering	1.054	1.472
Leaf size	1.045	1.429
Leafiness	1.875	0.457
Stem thickness	-0.108	1.16
No. of stem	0.819	0.906
Stem length	0.365	2.029
No. of internodes	8.335	0.882
No. of branches	1.624	1.131
Plant height	-0.051	1.308
Plant diameter	0.748	1.612
Seedling vigour	0.743	2.164

CHAPTER SIX : GENERATION MEAN ANALYSIS RESULTS AND ASSOCIATED DISCUSSION

The hierarchical mating design experiments provided estimates of genetic variance components from two diverse populations and their inter-population crosses. The next objective was to obtain more detailed genetic information on prostrateness and other related attributes; but this requires a reduction of base gene-pool because of the workload of the experiment. The generation mean analysis, employing six generations (P_1, P_2, F_1, F_2, B_1, and B_2) from three single crosses, provides this extra insight.

In this experiment only those attributes which were quite diverse in the two types, erect and prostrate, were analyzed to correspond the assumptions of this design.

As some of the generations were segregating, whilst others were not, heterogeneity of the within plot variances were expected. For this reason, a weighted analysis was done, utilising the inverse of the plant-to-plant within-plot variance as a weight. As a result of analysis of variance generations were significantly different for the studied attributes. Therefore, generation means and their within plot variances were used to carry out the genetical analysis. The estimates of observed generation means and their within plot variances for the three sets of crosses are presented in Tables 6.2 to 6.4. Within plot variances were usually greater for the segregating generations (F_2, B_1, B_2) than for the parents and F_1 (Tables 6.2 to 6.4). The heterosis estimates were calculated based on the differences between mid-parent and F_1 and are presented in tables 6.2 to 6.4.

Table 6.1 : Degrees of freedom and mean squares from the weighted analyses of variance of parental, F_1, F_2, B_1, and B_2 for 4 characters in three sets of crosses (erect x prostrate).

Set	Source	df	Prostrateness	Stem thickness	Leaf size	Nodal rooting
set 1	Replication	2	0.31^{ns}	0.739^{ns}	0.158^{ns}	0.929^{ns}
	Generation	5	12.41^{**}	13.85^{**}	7.39^{**}	6.733^{**}
	Error	10	0.12	0.37	0.05	0.242
set 2	Replication	2	0.001^{ns}	0.692^{ns}	0.09^{ns}	0.79^{ns}
	Generation	5	8.1^{**}	29.66^{**}	6.77^{**}	10.53^{**}
	Error	10	0.295	0.27	0.21	0.68
set 3	Replication	2	0.01^{ns}	0.68^{ns}	0.02^{ns}	0.39^{ns}
	Generation	5	9.92^{**}	17.12^{**}	6.11^{**}	11.3^{**}
	Error	10	0.25	0.39	0.18	0.41

*: Significantly greater than the error mean square at p≤.05
**: Significantly greater than the error mean square at p≤ .01
ns: non-significant at p≤ .05

Table 6.2 : Observed generation means, their within plot variances and F_1 mid-parent deviations (F_1-MP) for four attributes in cross one.

Generation or parameter	Prostrateness	Nodal rooting	Stem thickness	Leaf size
P_1 (erect)	4.56 (0.0250)	3.87 (0.1385)	4.87 (0.2647)	5.84 (0.1474)
P_2 (prostrate)	6.26 (0.0922)	4.97 (0.2734)	3.07 (0.1533)	4.39 (0.0671)
F_1	5.27 (0.1074)	4.09 (0.2141)	3.79 (0.3097)	4.92 (0.0613)
F_2	5.19 (0.1842)	4.18 (0.2966)	3.42 (0.4411)	4.71 (0.1254)
B_1	4.70 (0.1316)	3.89 (0.1196)	4.00 (0.4180)	5.08 (0.1350)
B_2	5.52 (0.1747)	4.59 (0.4824)	3.12 (0.2474)	4.42 (0.0853)
F_1 - MP	-0.142ns	-0.329ns	-0.174ns	-0.199ns
heterosis[1]	-2.6	-7.4	-4.4	-3.9

1: Heterosis based on the percentage of the difference of F_1's mean and mid-parent value. The figure in brackets are within plot variances.

Table 6.3 : Observed generation means, their within plot variances and F_1 mid-parent deviations (F_1-MP) for four attributes in cross two.

Generation or parameter	Prostrateness	Nodal rooting	Stem thickness	Leaf size
P_1 (erect)	4.48 (0.0927)	3.76 (0.0183)	4.96 (0.1855)	5.73 (0.2646)
P_2 (prostrate)	6.25 (0.0154)	5.78 (0.3626)	2.26 (0.0704)	4.20 (0.0841)
F_1	5.57 (0.0554)	4.51 (0.2338)	2.79 (0.2825)	4.92 (0.0623)
F_2	5.58 (0.2295)	4.50 (0.2797)	2.48 (0.2141)	4.65 (0.0708)
B_1	5.43 (0.0596)	4.34 (0.2649)	3.69 (0.2749)	5.03 (0.1450)
B_2	5.94 (0.1416)	4.58 (0.2694)	2.42 (0.2569)	4.53 (0.1370)
F_1 - MP	0.21ns	-0.26ns	-0.82ns	-0.05ns
heterosis[1]	3.9	-5.4	-22.7	-1.0

1: Heterosis based on the percentage of the difference of F_1's mean and mid-parent value.

Table 6.4 : Observed generation means, their within plot variances and F_1 mid-parent deviations (F_1-MP) for four attributes in cross three.

Generation or parameter	Prostrateness	Nodal rooting	Stem thickness	Leaf size
P_1 (erect)	4.54 (0.0488)	3.82 (0.0680)	4.55 (0.2526)	5.52 (0.1198)
P_2 (prostrate)	6.19 (0.0456)	5.30 (0.3073)	2.85 (0.1093)	4.38 (0.0455)
F_1	5.26 (0.2162)	4.41 (0.2454)	3.55 (0.1956)	4.89 (0.0872)
F_2	5.23 (0.1344)	4.18 (0.2518)	3.21 (0.3322)	4.64 (0.1206)
B_1	4.85 (0.0863)	3.88 (0.1289)	3.84 (0.3762)	4.87 (0.0737)
B_2	5.47 (0.1499)	4.66 (0.5118)	2.82 (0.2418)	4.35 (0.0934)
F_1 - MP	-0.11ns	-0.15	-0.15ns	-0.06
heterosis[1]	2.1	-3.3	-4.1	-1.2

1: Heterosis based on the percentage of the difference of F_1's mean and mid-parent value.

6.1 Gene function

Three and six parameter models will be presented. In those cases in which chi-square was significant for three parameter model, a parsimonious model (what was called the best fit model by Mather and Jinks, 1982) is suggested. The procedure to find the parsimonious model was described in chapter three. The results of simple scaling test showed the presence of non-allelic interactions in most cases. In other words simple additive model failed to describe the existing variation in the data. The joint scaling test verified the adequacy of the three parameter models, m, (d) and (h). This test revealed a lack of fit in most cases and the results were in a good agreement with the simple scaling tests. Based on the results of the various tests for goodness of fit and presence of epistasis, the six parameter model result was presented and the removal of non-significant components such as (i), (j) and (l) caused a considerable reduction in standard errors of the remnant components and chi-square test results. The results are presented in Tables 6.5 to 6.8 and they are described here for each attribute separately.

6.1.1 Prostrateness

The results of analysis of variance showed significant differences between generations (Table 6.1). Generation means and within plot variances are presented in Tables 6.2 to 6.4. Segregating generations had bigger within plot variances than non-segregating ones.

In the first cross, simple and joint scaling test indicated the presence of epistasis (Table 6.5). In six parameter model, additive dominance interaction was not significant. Additive x additive interaction was significant at ten percent level. Removal of these two components, (i) and (j) led to a parsimonious four parameter model in which additive and dominance, (d) and (h), components of the means played an equal role. The negative value for (d) was due to a bigger value of the mean for parent two in this particular

character. A negative value for (h) showed a partial to complete dominance of prostrateness to erectness.

In cross two a simple additive dominance model failed to explain gene functions for the trait under discussion. Joint scaling test strongly revealed the existence of epistasis in function of the gene(s) controlling the trait. Simple scaling test also showed the presence of epistasis, although, not as strong as joint scaling test did. In the six parameter model, only the additive x additive interaction component was not significant. Therefore, it was deleted from the model. The chi-square value for five parameter model involving m, (d), (h), (j), and (l) was highly non-significant suggesting the major part of the variation observed in this character and set was due to these components. In the other words, there was nothing else beyond these five parameters to explain prostrateness. A negative value for (l) indicated a duplicate nature of interaction. Dominance effect showed a greater role than additive effect.

In cross three also both simple and joint scaling tests showed the presence of non-allelic gene interaction. In the six parameter model additive x additive parameter was not highly significant (significant at 10% level, Table 6.5). Deleting this part of the model greatly increased the precision of estimates (by the decreasing of standard errors) and consequently a remarkable drop in residual deviance suggested the five parameter model could be strong enough to explain the most part of genetic variation in this particular trait. A negative value for (*h*) indicated that the function of prostrateness gene is partially dominant to erectness.

Table 6.5: Gene effects estimated for **prostrateness** using three and six parameter models on means and their variances of parents, F_1, F_2, B_1 and B_2 in a cross between erect and prostrate plants.

Model	Effect	cross one		cross two		cross three	
		estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Three parameter	m	5.378**	±0.020	5.591**	±0.025	5.335**	±0.030
	d	-0.869**	±0.020	-0.651**	±0.025	0.782**	±0.027
	h	-0.302**	±0.048	0.103**	±0.049	-0.207**	±0.063
	Scaling test						
	A	-0.432**	±0.097	0.807**	±0.090	-0.088ns	±0.101
	B	-0.489**	±0.121	0.072ns	±0.157	-0.502**	±0.126
	C	-0.618**	±0.202	0.467ns	±0.382	-0.305(*)	±0.212
	Joint scaling						
	χ^2	30.58		80		16.3	
	p	<.005		<.005		<.005	
Six parameter model	m	5.716**	±0.212	4.949**	±0.403	5.647**	±0.186
	d	-0.848**	±0.021	-0.883**	±0.046	-0.825**	±0.032
	h	-1.671**	±0.518	1.916*	±0.884	-1.266**	±0.456
	i	-0.304(*)	±0.211	0.412ns	±0.401	-0.285(*)	±0.183
	j	-0.057ns	±0.136	0.736**	±0.174	0.414**	±0.128
	l	1.225**	±0.328	-1.291**	±0.496	0.875**	±0.306
The best fit model	m	5.413**	±0.021	5.362**	±0.036	5.362**	±0.032
	d	-0.847**	±0.020	-0.883**	±0.036	-0.825**	±0.032
	h	-0.948**	±0.130	1.028**	±0.185	-0.592**	±0.149
	i	0	0	0	0	0	0
	j	0	0	0.788**	±0.166	0.365**	±0.124
	l	0.805**	±0.151	-0.815**	±0.177	0.487**	±0.178
	χ^2	2.09		1.06		2.44	
	p	.5-.25		.5-.25		.25-.1	

**: significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level
ns: non-significant

6.1.2 **Nodal rooting ability**

According to the results of analysis of variance, generations were significantly different in all three sets (Table 6.1). The estimation of the components of generation means based on three parameter, six parameter and the parsimonious model are presented in Table 6.6 along with the results of simple and joint scaling tests. The corresponding generation means and within plot variances are presented in Table 6.2 to 6.4. Within plot variances and the means of generations were quite different in all three sets. Within plot variances for segregating generations were bigger than non-segregating generations.

In cross one the results of simple scaling and joint scaling tests were in common agreement that there was nothing else except three parameters, m, (d), and (h) to explain the nodal rooting ability in this set. The negative value for (d) was because of greater value for P_2 in this character and negative value for dominance effect indicated that rooting ability is partially dominant to rooting disability. All non-allelic interaction effects were non-significant. Non-significance of (*h*) in the six parameter model was due to inadequacy of this model. As it can be seen from the Table 6.6 in six parameter model standard errors have increased i.e. precision has decreased. The best parsimonious model for this case was the three parameter model.

In cross two both simple and joint scaling tests revealed the existence of non-allelic interaction effects. Dominance and additive x additive interaction effect were not significant in the full model (Table 6.6). Removal of the (i) effect from the six parameter model considerably reduced the standard errors of the remnant parameters and the chi-square. Therefore, the five parameters, m, (d), (h), (j), and (l) were considered the best fit to the observed means of generations in this set. In this case also nodal rooting ability was partially dominant.

In cross three again both simple and joint scaling tests suggested the existence of epistasis or non-allelic interactions. But except m and (d) the rest of the parameters were non-significant. In the six parameter model the trend was quite similar to that of first set. The removal of (j) and (l) gave a good fit to the means of families (Table 6.6).

Table 6.6 : Gene effects estimated for **nodal rooting ability** using three and six parameter models on means and their variances of parents, F_1, F_2, Bc_1 and Bc_2 in a cross between erect and prostrate plants.

Model	Effect	cross one		cross two		cross three	
		estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Three parameter	m	4.389**	±0.059	4.620**	±0.054	4.432**	±0.054
	d	-0.608**	±0.054	-0.829**	±0.054	-0.717**	±0.051
	h	-0.351**	±0.102	-0.059ns	±0.087	-0.275**	±0.091
	Scaling test						
	A	-0.191(*)	±0.134	0.409**	±0.127	-0.466**	±0.115
	B	0.119ns	±0.224	-1.141**	±0.252	-0.402*	±0.224
	C	-0.300ns	±0.296	-0.561ns	±0.451	-1.220**	±0.281
	Joint scaling						
	χ^2	3.16		37.6		24.58	
	p	.5-.25		<.005		<.005	
Six parameter model	m	4.195**	±0.294	4.944**	±0.472	4.209**	±0.282
	d	-0.549**	±0.072	-1.009**	±0.063	-0.744**	±0.065
	h	0.056ns	±0.747	-1.338ns	±1.087	-0.314ns	±0.721
	i	0.228ns	±0.284	-0.172ns	±0.468	0.352ns	±0.274
	j	-0.310(*)	±0.239	1.550**	±0.263	-0.065ns	±0.228
	l	-0.156ns	±0.483	0.905(*)	±0.644	0.517ns	±0.469
The best fit model	m	-	-	4.772**	±0.063	3.915**	±0.122
	d	-	-	-1.009**	±0.063	-0.773**	±0.052
	h	-	-	-0.952**	±0.286	0.451**	±0.179
	i	-	-	0	0	0.645**	±0.137
	j	-	-	1.524**	±0.253	0	0
	l	-	-	0.691**	±0.278	0	0
	χ^2			.14		2.51	
	p			.75-.5		.5-.25	

**: significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level
ns: non-significant

The similarity of the results of sets one and three again indicated that in extremely diverse populations of cross pollinated plants one single plant can be a relatively good representative of the population to be used in determining the function of gene(s) controlling the character.

6.1.3 **Leaf size**

The results of analysis of variance showed a highly significant difference for this trait in all three sets (Table 6.1). Within plot variances and generation means were reasonably different. The within plot variance for P_1 in cross one was bigger than for the segregating generations. But its mean was in proper order, which may be in part due to more sensitivity of bigger leaf size to the environment and in part due to possible heterozygosity of this trait in parent populations.

In cross one, both simple and joint scaling tests showed the interferences of non-allelic interactions (Table 6.7). According to these tests there are something else beyond simple additive dominance model controlling the expression of leaf size. The full model parameters are presented in Table 6.7. Additive x additive interaction parameter was not significant. Therefore, the removal of (i) enabled a test of the five parameter model for goodness of fit. The deletion of (l) reduced χ^2 dramatically to 0.13. Also the absolute value of the standard errors were remarkably reduced, resulting in an increment of precision in the five estimated parameters. The high value for (h) indicated the importance of dominance gene action in leaf size and the negative sign indicated that the small leaf size was dominant to the big leaf size. The absolute value of (h) was larger than that for (d), which may suggest that the function of genes control small leaf size is over-dominance.

In cross two also, the results of simple and joint scaling tests were in common agreement, indicating the existence of epistasis. The results of the six parameter model confirmed the existence of non-allelic interaction. Dominance x dominance interaction value was much smaller than its

corresponding standard error. Removal of this parameter gave a good fit of remaining five parameters. The parsimonious model was not in a good agreement with the results of the other two sets of crosses.

In cross three again both simple and joint scaling tests showed the presence of non-allelic interaction. Further analysis of the data related to this cross included the interaction components. The six parameter model is set out in Table 6.7. (i) and (j) parameters were not significant. The elimination of non-significant parameters resulted in a good fit of the four parameters model. Apart from the difference of significance in interaction components, the general trend was quite similar in cross one and three, i.e. absolute value of dominance effect was much bigger than additive effect, indicating the importance of dominance gene action in leaf size.

6.1.4 **Stem thickness**

The results of analysis of variance on the field design revealed that generations were significantly different on this trait (Table 6.1). The generation mean analysis results of the character are presented in Table 6.8 along with the simple and joint scaling test results.

In cross one simple and joint scaling tests suggested the existence of non-allelic gene interactions. In the six parameter model, m, (d), (i) were significant. The removal of additive x dominance interaction and dominance x dominance, (j) and (l), gave us the parsimonious model and the parameters m, (d), (h), and (i) could be the best explanatory statistic of stem thickness gene functions in this cross. the additive and additive x additive interaction effects part of the model seems to be the most important part of the means of the character in question (Table 6.8). Since the (*h*) value is very similar to (*d*), the function of the genes control thickness in stem seems to be partially dominance.

In cross two also simple and joint scaling tests on three parameter model indicated the existence of epistasis. Therefore, simple additive dominance cannot explain the gene functions in this character. The (j) part of

six parameter model was not significant. Therefore, it was deleted to get the parsimonious model. The remaining five parameters were assessed. Results on the relative magnitude of components with their corresponding standard errors revealed that the precision of estimates increased in the remaining parameters, i.e. the values of standard errors were reduced.

In cross three simple additive dominance model failed to be the best explanatory of the gene functions and both simple and joint scaling tests indicated the existence of at least one kind of non-allelic gene interaction. The removal of the least important component of non-allelic interaction, (l), produced a good fitted model with the rest of the parameters i.e. m, (d), (h), (i), (j). In this cross also the results indicated that the function of genes that control of thickness of stem is partially dominant.

Table 6.7 : Gene effects estimated for **leaf size** using three and six parameter models on means and their variances of parents, F_1, F_2, Bc_1 and Bc_2 in a cross between erect and prostrate plants.

Model	Effect	cross one		cross two		cross three	
		estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Three parameter	m	4.823**	±0.038	4.730**	±0.042	4.739**	±0.033
	d	0.583**	±0.036	0.573**	±0.043	0.469**	±0.030
	h	-0.064ns	±0.063	0.126*	±0.065	-0.073**	±0.060
	Scaling test						
	A	-0.763**	±0.123	-0.598**	±0.142	-.661**	±0.099
	B	-0.453**	±0.102	-0.066ns	±0.164	-0.563**	±0.098
	C	-1.278**	±0.191	-1.175**	±0.254	-1.132**	±0.185
	Joint scaling						
	χ^2	62.7		27.3		76.76	
	p	<.005		<.005		<.005	
Six parameter model	m	5.052**	±0.181	4.455**	±0.271	5.042**	±0.171
	d	0.725**	±0.053	0.768**	±0.065	0.569**	±0.043
	h	-1.292**	±0.448	0.312ns	±0.672	-1.467**	±0.411
	i	0.061ns	±0.173	0.512*	±0.263	-0.092ns	±0.166
	j	-0.311*	±0.149	-0.532**	±0.210	-0.099ns	±0.125
	l	1.155**	±0.283	0.152ns	±0.417	1.316**	±0.260
The best fit model	m	5.113**	±0.053	4.363**	±0.100	4.939**	±0.041
	d	0.725**	±0.053	0.763**	±0.064	0.543**	±0.031
	h	-1.438**	±0.185	0.553**	±0.122	-1.236**	±0.154
	i	0	0	0.596**	±0.125	0	0
	j	-0.310*	±0.149	-0.552**	±0.202	0	0
	l	1.239**	±0.157	0	0	1.188**	±0.145
	χ^2	.13		.13		.14	
	p	.75-.5		.75-.5		.95-.9	

ns : non-significant
**: significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level

Table 6.8 : Gene effects estimated for **stem thickness** using three and six parameter models on means and their variances of parents, F_1, F_2, Bc_1 and Bc_2 in a cross between erect and prostrate plants.

Model	Effect	cross one		cross two		cross three	
		estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Three parameter	m	3.760**	±0.061	3.506**	±.047	3.517**	±0.052
	d	0.849**	±0.057	1.296**	±0.046	0.859**	±0.050
	h	-0.276**	±0.113	-0.930**	±0.094	-0.208*	±0.091
	Scaling test						
	A	-0.659**	±0.198	-0.379**	±0.161	-0.415**	±0.171
	B	-0.616**	±0.177	-0.203ns	±0.224	-0.764**	±0.154
	C	-1.820**	±0.347	-2.899**	±0.406	-1.662**	±0.294
	Joint scaling						
	χ^2	31.13		51.1		43	
	p	<.005		<.005		<.005	
Six parameter model	m	3.422**	±0.323	1.295**	±0.426	3.216**	±0.291
	d	0.900**	±0.074	1.353**	±0.055	0.849**	±0.063
	h	-0.361ns	±0.787	3.231**	±1.002	-0.363ns	±0.711
	i	0.544*	±0.315	2.316**	±0.422	0.483*	±0.284
	j	-0.043ns	±0.234	-0.175ns	±0.252	0.349*	±0.210
	l	0.731(*)	±0.501	-1.734**	±0.609	0.697(*)	±0.446
The best fit model	m	3.006**	±0.153	1.372**	±0.411	2.807**	±0.127
	d	0.885**	±0.057	1.337**	±0.050	0.838**	±0.063
	h	0.740**	±0.219	3.003**	±0.946	0.712**	±0.175
	i	0.927**	±0.172	2.231**	±0.404	0.864**	±0.145
	j	0	0	0	0	0.366*	0.210
	l	0	0	-1.582**	±0.568	0	0
	χ^2	2.13		.48		2.43	
	p	.5-.25		.5-.25		.25-.1	

ns : non-significant
** : significantly different from zero at 1% probability level
* : significantly different from zero at 5% probability level
(*): significantly different from zero at 10% probability level

6.2 **Minimum number of genes**

Wright (1968) presented a formula for estimating the number of genes which was elaborated by Lande (1981) and eventually by Cockerham (1986) so that it could be applied to crosses between genetically heterogenous populations. Using two divergent populations and the mentioned procedure, the number of genes was estimated for the studied attributes which are presented in Table 6.9. Except for the estimates of number of genes for prostrateness in cross three the results of crosses one and three were very similar in magnitude, indicating that in such populations individual plants could be used in order to estimate genetic parameters.

Table 6.9 : The estimated number of genes control the attributes (and their standard errors).

Attributes	Cross one	Cross two	Cross three
Prostrateness	2.36 **	2.46 **	11.16 ns
	(0.39)	(0.84)	(9.48)
Nodal rooting	1.41 *	6.02 ns	3.40 (*)
	(0.83)	(5.62)	(2.37)
Stem thickness	2.08 **	14.71 ns	2.16 **
	(0.82)	(14.29)	(0.77)
Leaf size	7.72 ns	-5.74 ns	5.59 ns
	(6.10)	(4.51)	(4.33)

CHAPTER SEVEN: GENERAL DISCUSSION

7.1 Introduction

To understand the way in which attributes are inherited and the extent to which they are transmitted to the next generation, the phenotypic variance is partitioned into its environmental and genetical components. Gene function and number of genes involved in the attributes have to be estimated. The three separate experiments reported in this thesis estimated some of these components in order to investigate inheritance of prostrateness and other related attributes, using the three types of red clover. Various univariate and multivariate techniques have been used. In this chapter the intention was to focus on the general results. In this chapter, the results are presented in conjunction with one another both for breeding programmes and for understanding the genetics of these attributes. The possible use of these genotypes in improving the existing commercial cultivars and breeding a new cultivar will be discussed.

7.2 Genotype-environment interaction

In a wide range of genetic resources, such as was examined in the first experiment, the pooled analysis of variance, provided an indication of the presence and magnitude of genetic and environmental variation, and indicated the necessity of further study, which also follows.

Genotype-environment interaction experiments were used as a typical design for estimating variance components, including interaction between genotypes and environments. These experiments were conducted at two sites for two years. It was pointed out by several authors (Finlay and Wilkinson, 1963; Hamblin *et al.*, 1980; Fakorede, 1986; Misevic and Dumanoivic, 1989) that testing the hybrids or cultivars at two selected

locations is not good enough for an unbiased prediction of their yield performance over a wider region. However, it was suggested that testing at two environments could be appropriate enough for a preliminary screening of a large number of hybrids or cultivars. We were aware that when the reference population of environments is made more homogenous by constriction, genotypic variance may be increased by incorporation of variance that may represent GE interaction variance in a wider population of environments (Schutz and Bernard, 1967). However, testing the performance of cultivars or accessions in two locations can present a rough estimate of GE interactions, in addition to being a preliminary test. These will provide some indication of possible bias in genetic variance components estimated from different mating designs (Comstock and Moll, 1963).

It was pointed out by Taliaferro *et al.* (1973) that environments sampled from any consecutive years will tend to be correlated. With perennial crops like red clover experimental layouts remain constant over the years and plot yields from year to year may be correlated and this correlation in its turn may cause genotype-environment interaction to be underestimated. The correlation between time periods in which the two GE interaction experiments (presented in chapter four) suggested that it could be safely ignored. However, being aware of all these premises, these experiments were pooled over locations to get an estimate of GE interaction in materials.

There are a series of assumptions underlying the analysis of variance discussed in chapter two. Some of the mentioned assumptions may fail to hold in practice. Therefore, transformation of the data may help to meet the assumptions (Steel and Torrie, 1980; Eisenhart, 1947). Although in biological cases the failure of the data to some extent could be ignored, but it is a common practice to modify the data in order to meet the assumptions. As it is suggested (Steel and Torrie, 1980; Cochran, 1947), non-additivity is one of the major failures of the data set by which error variance is confounded but the level of F value for all treatment means may be affected only slightly.

There were some heterogeneity of variance in some attributes based on the results of Bartlett's test. Since it happened only in a low number of attributes the disturbances were ignored and the error variances were assumed to be homogenous, although this may lead to a slight bias in estimates of significance levels (Cochran,1947).

7.2.1 **Genetic variation**

In the pooled analysis of variance over both sites and years based on the data related to the first experiment the genotype effect was highly significant for most of the attributes (Table 4.2) indicating the presence of genetic variation in the attributes studied. Since red clover is a cross pollinated plant and examined genotypes have been cross-pollinated for a long time, genetic variation is compounded of additive and non-additive components and their possible interaction effects. The data from second experiment were used to part the genotypic variance into its components, additive and non-additive which will be discussed later in this chapter.

7.2.2 **Environments and GE interaction**

In this particular experiment Frewans and Mogini were considered as two typical diverse environments in Manawatu region with enough diversity in soil type. The experiment block in Frewans was located down in a valley type location, whereas, the experiment in Mogini was located up on a hill type location. Therefore, although rainfall was not much different at the two sites, the amount of moisture available for the plants were quite different as well as soil differences which was mentioned in chapter three.

The results of pooled analysis of variance showed that site effect was significant just for three characters. Although the results may suggest that the tested sites have not been diverse enough, differences between mean

values (Table 4.5) for the two sites would indicate the existence of difference between sites, but apparently not enough to change the means significantly.

The non-significant values of site by genotype interaction and interaction effects for some attributes indicated that the changes in mean phenotypes over years and sites were constant and if the examined genotypes are going to be used as a gene pool for a breeding programmes, most of the initial work can be done in one site. In contrast, significant genotype x year values indicated that genotypes did not respond to the different years in the same way and evaluation of breeding material have to be done in more than one year. In the cases like yield, the measurement is an absolute measure, whereas in cases such as prostrateness and nodal rooting ability measurements were based on scoring from 1-5. As Helgadottir and Kristjansdottir (1991) have pointed out, scores are relative values, which depend on the observer, it is, therefore, possible to get discrepancies in scoring between experimental locations or successive years. In these experiments scoring was done by the same person all through the experimental period, mostly based on a definite rule (see chapter three). Therefore, this controversy is not the case here, and the significant interaction between genotype and year and the detected differences between years could not be as a result of wrong scoring.

The second order interaction effects were highly significant for stem thickness, number of internodes, number of branches, and dry matter yield indicating that some of the first order interaction effects involving two variables (SxY, GxY, GxS) were not consistent. The existence of this kind of interaction would introduce considerable difficulty in identifying the generally superior genotypes, unless the difference between the genotypes under study is so large that could cover all the interaction effects. The results indicated that if evaluation of red clover is intended, evaluation has to be done on the basis of a data set collected from more than one year. Conflict inevitably exists between breeding for general adaptation (minimizing interaction effects) and

specific adaptation (emphasizing favourable interaction effects). Therefore, if specific adaptation is intended such an emphasis is not required on testing at numeric sites but still testing over several successive years is necessary.

Prostrateness and nodal rooting ability which were the attributes of most interest seemed to be constant over environments. Prostrateness presented significant GxY and SxY interaction effect, but the other first and second order interaction effects were not significant for either of these attributes. This would indicate that either genotypes were examined in a narrow sample of environments or these attributes were not so affected by environment.

7.2.3 **Phenotypic and genotypic correlation**

In quantitative attributes, the genetic and phenotypic correlation coefficient between two attributes play an important role in the discussion of correlated characters response to selection. Pleiotropy is regarded as chiefly responsible for genetic correlation, though linkage is a cause of transient correlation (Falconer, 1989).

In overall genetic and phenotypic correlations, genetic correlation values had the same trend as phenotypic, but in all the cases absolute values in genetic correlation were larger than that in phenotypic correlation.

Since phenotypic correlation is a compound of genetic and environmental effects (Falconer, 1989) genetic correlation will be considered as a base for discussion on the subject. Prostrateness was highly correlated with most of the other traits, either positively or negatively. Very high negative correlations between prostrateness and stem thickness, (-0.987), leaf size (-0.952), and stem length (-0.796) indicated that these attributes may be controlled by common genetic factors which increases prostrateness and decreases the other ones. In these cases, the response to selection would be slowed down if both attributes are selected for increase. This is a typical case

when selection indices are useful. In contrast, high positive values for correlations between prostrate and other traits such as nodal rooting, number of internodes and number of branches indicated that these attributes may be either controlled by common genetics/physiology or the genes are closely linked. In these cases also the response to selection might be slowed if one attribute state is selected for increase and the other state is selected for decrease. Such a high positive correlation was found between nodal rooting and number of internodes, nodal rooting and number of branches, first flowering and median flowering, first flowering and dry matter yield, leaf size and stem length, leaf size and stem thickness and finally between number of internodes and number of branches. In terms of yield production it seems prostrateness is associated with low dry matter yield (-0.407). Negative estimates for the genetic and phenotypic correlation between prostrateness and flowering characters indicated that prostrateness is also associated with early flowering date. Since low genetic correlations between characters do not necessarily imply a lack of pleiotropic gene action, changes in gene frequency may be sufficient to alter genetic correlations (Mitchell-Olds and Rutledge, 1986).

As was discussed in chapter three, in biennual plants such as red clover plant performance may change in the second year also the pattern of genetic and phenotypic correlation may change. For this reason genotypic and phenotypic correlation values were estimated for from the data for the first and second year separately (Table 4.7). The general trend for phenotypic correlations was almost the same in both years. But in some cases correlation values for one attribute either changed by level of significance or even sometimes changed the direction of correlation. For instance number of stems and prostrateness had a very strong negative correlation in first year (-0.602 Table 4.7) but a highly significant positive correlation in the second year (+0.34). A similar trend can be seen for the correlation among number of stems and first flowering, number of stems and median flowering, and finally

number of internodes and median flowering with one difference, that they had positive correlation in first year but negative less significant correlation for second year. In fact in the case of number of stems the pattern and direction of correlation with almost all of the other attributes had changed in the second year. These results may suggest that phenotypic or genotypic correlation values for one year may be misleading and to get a more reliable results inferences have to be made on the results based on the data collected over more than one year, particularly when it comes to biannual plants when the difference may increase. Therefore, the estimates based on the data collected for two years (Table 4.6 and 4.8) would be more reliable.

Since phenotypic and genotypic correlations may be different from one population to another population (Falconer, 1989) or one type to another type the phenotypic and genotypic correlation values were estimated from the data for each of the three types of plants under study (Table 1 to 3 Appendix 4). As was expected, genotypic and phenotypic correlation values were remarkably different from one population to the other. For instance, prostrateness had a moderate to high positive genotypic correlation with number of internodes, dry matter yield, nodal rooting, number of stems and number of branches in erect type genotypes, whereas, it had moderate to highly negative genotypic correlation with the same attributes in semi-erect type genotypes. On the whole, the pattern of genotypic and phenotypic correlation in the different types had changed dramatically. This may indicate that the estimation of these values has to be restricted to each type to be valid.

Finally, to get an accurate figure of genetic and phenotypic correlations an accurate assessment of a large number of individuals with a broad genotypic base is required. This is a constraint in most quantitative genetic methods in plant breeding. In this study the intention was to examine as many individual plants as practically possible.

A noticeable positive correlation between adventitious roots and spring vigour was found in Montpetit and Coulman's (1991a) study, both in research plots and production fields. They came to this conclusion that "the presence of adventitious roots is the cause rather than the consequence of superior spring vigour." In this study nodal rooting had positive significant correlation with prostrateness, number of stems, number of internodes, and number of branches and negative significant correlation with other attributes. Since adventitious roots effects would be more pronounced in later years of the life of red clover plants, to get a better impression of the role of adventitious roots in red clover the research plots have to be extended to the third and fourth year of the life of the plant. In that case correlations between adventitious roots and plant persistence would be more clear. Also red clover may behave differently when it is mixed with other pasture species under grazing. Therefore, an estimate of the effects of adventitious roots on red clover would be closer to reality if a series of experiments could be conducted under different managements and mixed with other pasture species in several successive years and sites.

7.2.4 **Multivariate analysis**

Multivariate analysis can be defined as the simultaneous analysis of data set which is formed from several correlated variables, originated from independent individuals. The use of univariate analysis in a big data set with various variables is often inadequate. In univariate analysis in genotype-environment interaction experiment the effects of different environments were investigated on the basis of one character at a time. Univariate analysis was cumbersome and repetitive and gave us no easy understanding of overall variation. Multivariate analysis methods used in this study were primarily to summarize and simplify multivariate data in order to view the data in such a way as to enable interpretation of the genotype-environment interaction

effects, secondly to determine the most discriminant characters between the populations.

7.2.4.1 **Multiple discriminant analysis**

The results of this analysis on the genotype-environment interaction data set, were presented in chapter four. It is concluded that discriminant analysis of various morphological attributes can be used in order to obtain a parsimonious expression and ranking of variable effects in the structure of canonical discriminant functions which avoid the confusion of numerous univariate analysis of variance.

Despite the fact that some attributes did not show significant GxSxY effect in ANOVA, they were subjected to multivariate discriminant analysis of variance to reveal the possible pattern of the response of studied genotypes to GE interaction effects. Also the same analysis was done for just the attributes with significant GxSxY and GxY effects in ANOVA. The results and associated discussion are presented in chapter four. But in integrated form, in the both analysis based on GxSxY effect, dry matter yield and stem thickness and internodes were amongst the attributes with the most influence in forming first discriminant function and standardized coefficients. Any name given to the first functions in the two analysis would have to certainly consider these attributes as an important part of the function. One of the most interesting result of this analysis was : in both analysis (multivariate discriminant analysis based on all attributes and attributes with significant GxSxY in ANOVA) dry matter yield and stem thickness were a determinant consensual attribute which increase score, whereas, number of internodes was a determinant consensual attribute which reduces the score and it is so counteracted by other attributes which results in decreasing the score as well.

The results based on GxY effect were somehow different. This analysis was done on the attributes with significant GxY effect in ANOVA.

Regarding first discriminant function in this analysis also stem thickness was one of the determinant attributes which increases the score and other attributes consensually. In contrast, prostrateness and branches were determinant attributes which decrease the score and other attributes consensually.

In growth habit examination where types were classifying variables results were tremendously different from previous ones. The first canonical discriminant function explained the majority of the variation existing in the data set. All of the studied attributes had a very high positive or negative correlation with the first function. In other words if the intention is to classify a particular plant to one of the three groups all of the attributes studied have almost equal discriminating ability by which the original type of the plant could be recognized.

7.2.4.2 **Cluster analysis**

Various reviews on cluster analysis (e g. Mungomery *et al.* 1974, Eisemann *et al.*, 1977) have discussed the advantages of using cluster analysis to interpret genotype-environment interaction results. They have pointed out that Ward's method of clustering is the best method. This method is based on the minimum increment of pooled sums of squares within clusters at each stage of clustering.

The genotype-environment interaction pooled analysis of variance indicated that first and second order interaction effects (GxY and GxSxY) were highly significant for some of the attributes.

The cluster analysis was used to clarify the results of the discriminant analysis, which were the pure GE analysis (see previous section). However, in each case, more than one discriminant was required to account for at least 70% of the discriminatory power, so that patterns of response

amongst the genotypes were not obvious. The cluster analysis revealed the patterns inherent in these discriminant scores.

In all of the three cases of multivariate discriminant analysis based on GxSxY and GxY effects, cluster analysis was done based on the first two discriminant functions produced by multivariate discriminant analysis separately. The results for the analysis based on GxSxY effects, all attributes and attribute with significant GxSxY effect in ANOVA were quite the same in general. In other words, based on the both functions, cluster analysis classified the genotypes examined into three separate groups which were exactly corresponding to their type of growth habit. This would indicate that clustering the genotypes based on the coefficients produced by multivariate discriminant analysis did successfully summarize the response patterns to the environments involved. One of the most important results of this analysis was: revealing the fact that each type has its own particular response to various environments which is distinct from other types examined.

The results of clustering based on attributes with significant GxY effect in ANOVA in principal was similar to those of clustering based on GxSxY effect with one difference; that was the groups were divided to four clusters, but in sub-clusters genotypes with the same growth habit merged to form new clusters. In table 4.20 the attributes with significant GxY effect in ANOVA reveal that the examined prostrate genotypes in clusters three and four presented different patterns of change over the two years. For instance, number of stems in cluster three had changed from 19.75 in year 1991 to 109.05 in year 1992, whereas, the same attribute in cluster four had changed from 24.22 in year 1991 to 97.24 in year 1992. These kinds of effects could be seen more clearly between other clusters.

One particular aspect of clustering is selecting parental plants in a breeding programme. In most of the breeding programmes a great deal of genetic variation is required. Therefore, assuming phenotypic similarity is a reflection of genotypic similarity, selecting parental plants from one particular

cluster is not supposed to produce enough genetic variation in F_2 and later generations. Consequently a great phenotypic variation amongst clusters is assumed to reflect greater genotypic variation. Therefore, selecting the parental plants from extreme clusters would create a much wider genetic variation.

7.3 **Hierarchical mating design**

The estimation of the components of genetic variance in plant populations is useful primarily for predicting response to selection. In other words the response of a quantitative character to selection depends on the strength of selection and on the additive genetic variance component. The components of variance are called genetic architecture or the genetic parameter (Obliana *et al.*, 1979; Khehra *et al.*, 1985). The genetic architecture of plant populations can be investigated through various mating designs. Two extreme plant populations, one from erect type and the other one from prostrate type were studied in hierarchical mating design.

7.3.1 **Why hierarchical mating design?**

Before choosing a specific mating design, the researcher ought to carefully evaluate the objectives of the project and determine which design will effectively satisfy those objectives with available resources. The relative usefulness and efficiency of mating designs has been a matter of controversy for a long time (Kearsey, 1965; Pederson, 1972). For an open pollinated crop such as red clover, the diallel cross mating design to estimate genetic variance components leads to truncation sampling, due to using a small number of parents from a population, specially when two populations are concerned. As Steel and Torrie (1980) have said, the intention of sampling is to use sample information to make an inference about a population. For this reason it is

particularly important to define the population under discussion and to obtain a representative sample from the population defined.

Within limits imposed by the nature of the materials choices existed between alternative mating designs that will vary in their sampling of the inference population. Hierarchical mating designs was employed because a wider gene-pool could be examined with the crosses employed than with other designs.

7.3.2 **Additive component of genetic variance**

In erect population additive component of variance was either non-significant or significant at 10% level for prostrateness, leafiness, and number of internodes. Negative estimates of σ^2_A was obtained for attributes median flowering, stem thickness, stem length, and height. The possible reasons for these negative values were mentioned in chapter four. Regarding the small values, comparing with their corresponding standard errors, sampling error variance could be the most probable reason, although, the intention was to remove the errors by the relatively big sample size of crosses, and the field design used. These negative values and other small positive values indicated that there is not enough proper genetic variation available to respond to selection in the mentioned attributes in erect population. Hayman (1958) has recommended that in case of failure of statistical tests in such crosses particular lines may be removed until the remnant satisfy the test. This procedure has been disputed by Kempthorne (1956) because if the original set of parents is regarded as a random sample from a large base population the reduced set cannot be regarded as a random sample from the base population. Gilbert (1958) has argued that if a breeder is concerned only with some particular parents this objection looses its force and removing particular parental line(s) is justified. In this study, Kempthorne argument is more relevant, because some particular parents were not concerned. The intention

was to study genetic architecture of two plant populations from two diverse types and their inter-population crosses. Therefore, removal of particular half-sib or full-sib family might have discredited the assumption of the parents being random sample from the base populations. Therefore, negative estimates were regarded as a result of sampling error, particularly when they were so smaller than their corresponding standard errors.

In the prostrate population, in general, additive variance component was more important than dominance variance component (Table 5.7) for leaf size, leafiness, number of branches, diameter, and seedling vigour. Therefore, in contrast to erect population for the mentioned attributes, the population mean can be altered by selection strategies. It should be mentioned that if this experiment had been conducted over a range of diverse environments (different sites and years), then additive genetic variance might have been found to be less than what is now. In the case of attributes such as first flowering and median flowering, although the estimates of additive genetic variance component were larger than the corresponding standard errors, they were associated with relatively large standard errors, thus making them non-significant.

In the inter-population crosses, reference population for which the genetic parameters were estimated was inter-population crosses were formed by crossing random individuals in pairs from two diverse populations. In this population in general additive components were of more importance than dominance components of variance. With only one exception, number of internodes, the estimates for σ^2_A were larger than their corresponding standard errors, indicating that the mean values for this population can be altered by selection programme.

It was pointed out that because this experiment was done in one year and one site, therefore, the estimates of genetic variance components are subjected to upward bias from genotype-environment interaction effects. In fact almost any of the violations from the basic assumptions would lead to upward

bias for genetic variance component and hence the other estimates such as the ratio of dominance, which will be discussed later in this chapter, and genetic advance would be subjected to corresponding bias.

It is well known that substantial changes happen in quantitative genetic parameters following artificial selection among plant populations (Falconer, 1989; Mather and Jinks; 1982). Therefore, estimates obtained from these particular populations should not be extrapolated to the species as a whole.

In essence there was little or no additive genetic variance for studied attributes in erect population and most of the attributes in prostrate population. Consequently short term response to selection may be limited by low level of heritable genetic variation.

7.3.3 **Non-additive component of genetic variance**

Using hierarchical mating design in order to partition genetic variance into its components, dominance component is confounded with possible non-allelic interaction effect, therefore, it is better to call this component the non-additive component.

In the erect population the non-additive genetic component of variance was of more importance than the additive component for most of the measured attributes (Table 5.6). For first flowering, median flowering, stem thickness, stem length, number of branches, height, and diameter non-additive component was highly to lowly significant indicating that the development of hybrids or synthetic cultivars would be rewarding if the breeding programme is to be based on this population. Becker (1984) believes a small number of male parent plants would lead to a large standard error and a wide confidence interval which in turn makes the corresponding variance component non-significant. With this study, in spite of the practical limitations in the case of

crossing nursery, the intention was to produce as many male groups as possible, in order to increase precision of the estimates.

Breese and Hayward (1972) have pointed out that genetic analysis of plant populations of cross pollinated pasture species have indicated that genetic variance for characters relevant to vegetative persistence are mainly additive with little evidence for dominance or epistatic effects. They also suggested that attributes related to reproductive growth are expected to have less additive genetic variance component than dominance and epistasis. Therefore, plant populations increased in gene pools are expected to present more dominance and epistasis, because seed increasing is usually done in first year of life of the plant, therefore, successive generations of vegetative persistence have not eroded their dominance variation. This might be the possible reason for σ^2D being the most important component of variance in erect population.

In the prostrate population the non-additive component was of less importance than additive component of variance. In fact, comparing with their corresponding standard errors, none of the attributes studied presented significant dominant variance. This might indicate that, regarding these attributes, breeding programmes based on non-additive genetic variance would not be rewardable in this population. Prostrateness and nodal rooting, which were of our particular interest, although presented larger mean value than the erect population, did not seem to have any additive or dominance variance in this population.

In inter-population crosses branches, first flowering, stem thickness, and internodes showed low to highly significant non-additive component of variance respectively. These attributes are of particular importance, therefore, if intention is to alter these attributes based on this population, breeding programmes based on non-additive component of variance seems to be rewardable. Although the magnitude of genetic variance components in some cases are comparable in all inter- and intra-population

crosses, whether the populations should be subjected to the selection programmes or not depends on the purpose of the selection programme and the mean values of the populations which are presented in Table 5.1. For instance, obviously if someone intends to reduce first flowering date, he or she would use the prostrate population among the populations studied.

7.3.4 **Degree of dominance**

The degree of dominance did not have a particular trend in intra- and inter-population crosses. In fact it varied from character to character. Nodal rooting ability, internodes, and seedling vigour in erect population and first flowering and branches in prostrate population presented a small degree of dominance which indicate relative importance of additive genetic variance, regarding these attributes in corresponding plant populations. Both in erect and prostrate populations, there are some large values for the ratio (Tables 5.6 & 5.7) which could be explained by the various reasons. There was a strong belief in the 1960's considering the values larger than one for this ratio as a sign of the existence of over-dominance (Brieger, 1950; Crow, 1948), whereas, in quantitative characters which are controlled by several factors or genes, the values larger than one for this ratio can not necessarily indicate the existence of over-dominance. Any violation in the basic assumptions of the mating design may lead to overestimating of the components of variance by which consequently the degree of dominance also would be subjected to the same source of bias. Genotype-environment interaction is believed to be one of the major source of bias in the estimation of genetic variance components (Comstock and Moll, 1963; Mather and Jinks, 1982). If the magnitude of the bias is proportional to that of the variance estimated, there is no net effect on the estimate of the ratio (Robinson *et al.*, 1955). Otherwise bias would be greater in female component (σ^2_f). Since $\sigma^2_D = \sigma^2_f - \sigma^2_m$, the effect of this would have been to cause upward bias in the estimates of σ^2_D/σ^2_A. The other

possibility of this ratio being large is: the expected value of σ^2_D/σ^2_A increases with the increase in the frequency of the favourable allele in the population (Singh *et al.*, 1985). On the other hand the existence of a considerable dominance variance but a negligible amount of additive genetic variance component which would lead to a large ratio strongly supports the existence of over-dominance (Robinson *et al.*, 1955). Also at a certain level of dominance the value would increase at higher levels of frequency of favourable allele (Singh *et al.*, 1984). In essence, over-dominance, negligible additive genetic variance, high frequency of favourable allele, and eventually any violation from the basic assumptions of the mating design may result in a high value for the ratio of σ^2_D/σ^2_A.

Linkage disequilibrium could be another source of the upward bias in estimates of level of dominance (Mitchell-Olds & Bergelson, 1990), although linkage disequilibrium is not a major problem in cross pollinated plants which have been cross-pollinated for several successive generations (Sprague, 1966). The estimates of σ^2_D/σ^2_A in F_2 generation of hybrid population, are certainly subject to the upward bias due to linkage. Robinson *et al.* (1958) also suggested that sometimes over-dominance or a large value for the degree of variance would indicate a repulsion type of linkage of the genes controlling the attribute. Therefore, sometimes the degree of dominance is not a total effect of one particular gene but it can be considered as the effect of the gene at a particular generation. Of course this is less applicable in red clover because of several successive generations of cross-pollinating but it can be seriously argued for the plant populations which have been produced by a cross between two homozygous lines.

Zero and non-estimable values were due to the negative values for one of the components of variance, nominator or denominator of the ratio, which were regarded as zero. Maintaining the varieties in successive generations should be considered carefully, because it is quite reasonable to suggest a variety is a partially inbred population due to a low number of plants

in cycles of seed increasing which results to significant amount of inbreeding leading to the change in the ratio value.

7.3.5 **Frequency of favourable alleles**

Plant breeders in their endeavours to develop better cultivars, attempt to manipulate gene frequencies in plant populations. A plant population with a relatively low frequency of favourable alleles is expected to respond to selection over a longer period of time than populations with high gene frequencies (Singh *et al.*, 1984). The degree of dominance was used to estimate allele frequency using the theoretical tables presented by several authors (Singh *et al.*, 1984; Robinson *et al.*, 1955). These estimates would indicate probability of developing a highly productive cultivar from the base populations.

Based on the mentioned theoretical values calculated for a single locus and various levels of dominance, in the erect population the frequency of favourable alleles which increase the attribute under question at a certain level of dominance (say 1) for first flowering date, stem thickness, stem length, number of branches, plant height, and plant diameter would be larger than 0.8. In contrast the frequency of favourable alleles for nodal rooting ability, number of internodes, seedling vigour, and obviously the attributes with zero value would be less than 0.4 and for the rest of the attributes the frequency of desirable allele (which increase the attribute) would be in the range of 0.4 -0.8.

In the prostrate population, number of stem, and plant height had an allele frequency value larger than 0.8 and less than 0.4 for number of branches, leaf size, leafiness, plant diameter, and seedling vigour. The ratio values for prostrateness and nodal rooting in erect population which were of our particular interest were negligible, 0 and 0.2 respectively, indicating the low frequency of the favourable allele as well as low degree of dominance. The same values in prostrate population were considerably larger, 0.711 and

1.346, which suggest a high frequency of the favourable allele and high degree of dominance.

7.3.6 **Heritability**

Knowledge of the heritability of the characters is important to breeders since it indicates the possibility and extent to which improvement is possible through selection. A considerable research effort has gone into estimating heritability of many quantitative attributes in many commercial plants.

Two types of heritability, restricted and full, were estimated in genotype-environment interaction experiment. These were fully discussed by Gordon *et al*., 1972; and Gordon, 1979. In essence restricted heritability ignores all the macro- and meso-environmental components of variance, whereas, full heritability does not. Therefore, full heritability could be extended safely to other regions and cultivars. Full heritability will be considered here in this discussion. Since red clover is a cross pollinated plant the estimated genetic component of variance from genotype-environment interaction experiment would be regarded as a combination of additive and non-additive genetic variance, hence the estimated heritability in this particular experiment would be considered as broad sense heritability. In hierarchical mating design experiment, heritability narrow sense and broad sense were estimated in all intra- and inter-population crosses.

Heritability values estimated from the data related to genotype-environment interaction and hierarchical mating design experiment, were considerably different. If there is any attempt to generalize the heritability results to other red clover plant populations, the results of genotype-environment interaction experiment is the more appropriate one because in that experiment a wider diversity genotypes have been examined.

Estimates of broad sense heritability for prostrateness and stem thickness were very high in both GE interaction and hierarchical mating design experiments, but in the other characters the estimated broad sense heritability value in GE interaction experiment were lower than that in hierarchical mating design one. In the later case it was expected to be so, since the several other effects including first and second order interaction were subtracted from the genetic variance in GE interaction experiment. In former case, therefore, comparable heritability values in these two experiments such as the values for prostrateness and stem thickness may suggest that the attributes were not affected by interaction effects.

Narrow sense heritability was also estimated for intra- and inter-population crosses in hierarchical mating design experiment. As was mentioned earlier, there was not a considerable amount of additive genetic variance in the erect population, leading to very small or even negative estimates for heritability narrow sense for most of the attributes. In contrast in attributes such as prostrateness and number of internodes most of the observed genetic variance was additive which in turn led to larger values for heritability narrow sense. Because of the negative value for dominance component of variance which in turn reduced the denominator of the ratio of heritability caused a value larger than one for heritability narrow sense in leafiness. For the same reason heritability narrow sense in prostrateness is higher than heritability broad sense. In essence a negative estimate for additive component of variance leads to a negative estimate for heritability narrow sense. In the other hand negative estimate for heritability broad sense leads to a larger value for heritability narrow sense than heritability broad sense and even values larger than one. The heritability narrow sense larger than one for the leaf size, leafiness, and diameter was due to this problem. Except for prostrateness, number of stems, and number of internodes heritability narrow sense in the prostrate population was higher than that in the

erect population, indicating the potentiality of this population for being used as a base population in possible selection programmes.

A similar low narrow sense heritability estimate was found for nodal rooting ability in both erect and prostrate populations, 0.36 and 0.38 respectively. This low heritability would indicate that selecting within existing commercial red clover cultivars to root on their stems in order to increase their persistence would not be so fast and rewardable. This result is in agreement with Montpetit and Coulman (1991b). The narrow sense heritability of first flowering in the populations examined ranged from 10 to 44%. This is in a good agreement with Bowly *et al.* (1987).

Mean values for most of the characters were different in these two populations, therefore, if the intention is to choose one of these populations as base population for plant improvement, the mean values have to come into account as well as heritability. In essence since in prostrate population the genetic architecture was in contrast with erect population, σ^2_A was more important than σ^2_D in most of the attributes, different patterns of heritability narrow sense were observed in the two populations.

For attributes such as dry matter yield it is very difficult to obtain unbiased estimates of heritability that can be generalized (Yadava & Yadav, 1977). These kind of attributes which are highly affected by environment, adequate sample of environments and successive years have to come into consideration. In Mayo's (1980) point of view a very low heritability for an important attribute such as clover foliage yield may not indicate no potential for selection, it rather implies that simple truncation selection would be relatively ineffective.

7.3.7 **Predicted genetic advance**

Presence of a large additive genetic variance for some of the attributes studied led to high expected genetic advance in intra- and inter-

population crosses in hierarchical mating design experiment (Tables 5.9 and 5.10). It is obvious that the selection of one of these populations as a base population depends on the purpose of the breeding programme. For instance to develop a very late flowering variety, one would certainly choose an erect population regardless of genetic advance value being larger for prostrate population. This is because the mean value for erect population in this particular trait is already 24 days larger than that for prostrate population. To illustrate this properly another instance is presented. Assuming the objective of a breeding programme is to increase stem thickness; one may select an erect population instead of a prostrate one or even their inter-cross, because even in inter-population crosses, although in later generations we may get a wider variation but because the aim is thicker stem the other tail of variation will not be of much use. In breeding programmes other related attributes such as persistence, disease resistance, high productivity also should be taken into account as well as genetic architecture of plant population. How much the response would be after one cycle of selection depends on the expected genetic advance. Cockerham and Matzinger (1985) have pointed out that in breeding populations epistasis may cause the initial response to selection to be substantially different from the permanent response. Therefore, it would be necessary to estimate h^2 and genetic advance for each cycle of selection.

In essence, heritability and genetic advance values have very wide implications in plant breeding particularly in the evaluation of examined genotypes in order to develop new varieties of red clover. For instance, the choice of plant population as gene pool is not only based on regional success of material but also it depends on the extent to which the favourable attribute can be transmitted to future generations. Hence the choice of parental populations for crosses can be rationalized by estimated heritability and expected genetic advance. On the whole if a breeding programme is based on some sort of selection strategy, reference plant populations have to be characterized with high heritable characters and less genotype-environment

interaction to ensure a large genetic gain and good stability of performance. In the other words when proper experimental design and large samples provide accurate estimates of genetic parameters the response to selection may be reliably predicted for a particular population in a particular set of environments.

Prostrateness presented similar genetic advance values in the two intra- and inter-population crosses. Therefore, selecting one of the studied populations as a base population for a selection programme depends on the purpose of the programme and other aspects of the population. In other words, selecting parental population for selection programmes needs other considerations as well as genetic variation. The expected response to one cycle of selection for lengthening or shortening the flowering date ranged from 1.02 days in erect population to 8.04 days in prostrate population under natural conditions.

7.3.8 **Heterosis and hybrid depression**

Heterosis results from crossing unrelated lines with high dominance ratio and it enhances the trait expression. In crops such as maize, studies have involved a variety of crosses of parents of different geographical origin and show that the association of increased heterosis with increased diversity extends over a considerable range of maize types (Mole and Stuber, 1974).

Although traits such as diameter, number of branches, stem length and number of internodes showed relatively large heterosis, there were some attributes such as number of stems and height which presented relatively high hybrid depression (negative heterosis). However, there are some evidences in other crops such as maize, that when the range of diversity was expanded crosses of the most distantly related populations showed less heterosis than crosses of populations assumed to be less distantly related (Moll *et al.,* 1965).

This would suggest that maximum heterosis occurs at an optimal or intermediate level of genetic diversity. The hybrid depression expressed by some of the attributes may be due to this fact that the two populations had been isolated for a long time and they were more distant than what required.

There are three possible genetic causes of heterosis: (1) partial to full dominance; (2) over dominance; (3) several types of epistasis. From these results, all three cases of genetic variance were detected in the studied germplasm and attributes. For instance plant diameter, number of branches, and stem length showed a high degree of dominance in erect population, which could be due to partial and full dominance or over dominance. Also all the studied attributes in generation mean analysis, including prostrateness and nodal rooting ability, presented some indications of non-allelic interaction (epistasis).

7.4 Generation mean analysis

7.4.1 Introduction

The type of breeding programme and genetic analysis that can be used to investigate genetic parameters is dependent on the breeding system of species concerned. This controls the ease with which different generations such as F_1, F_2, and backcrosses can be produced. Red clover is a self-incompatible species, therefore, producing selfed seeds is not possible and pure line can be produced only through full-sib mating which takes a longer time than doing through selfing. Consequently the starting points for genetic analysis in red clover are some heterozygote varieties or individual plants and this complicates the theoretical basis for the analysis of both qualitative and quantitative variation. Therefore, using generation mean analysis in a plant population like red clover might be in vain. Nevertheless, in some cases when a character shows two extremes, two clear and discrete phenotypic classes in two populations, it can be assumed that most likely the character is oligogenic and the populations are either homozygous in loci that control the

character or at least alleles of opposite directions are accumulated in those two different populations. Therefore, using single plants from each population as parent plants, the generation mean analysis can be done to get an estimate of genetic parameters particularly non-allelic interaction effects in that character.

It was pointed out by Snape (1987) that the first step in the genetic analysis of a character is to establish whether the variation should be considered qualitative or quantitative. To find the answer it should be clarified whether there are clear, discrete phenotypic classes in segregating generations of crosses between two contrasting genotypes or not. There are two extreme discrete phenotypic classes in erect and prostrate plant populations. First type is characterized by up standing and thick stems on which few adventitious roots grow. The prostrate type is characterized by creeping, thin stems with more adventitious roots on the nodes. Therefore, it was thought these characters might be controlled by one or low number of genes (oligogenic characters) or at least it could be assumed that alleles of opposite directions are accumulated in two different types. The second type may be useful to change architecture of present erect red clover commercial cultivars to increase their persistence potential. Understanding of the genetic architecture, qualitative and quantitative aspects of characters involved is a primary step for such breeding efforts. It was decided to carry out several generation mean analysis sets assuming the parent plants are homozygous in those loci controlling the attributes which were diverse in the two types. With this assumption three sets of generation mean analysis were done to partition genetic variance components further to the allelic and non-allelic interaction effects. As was mentioned before, generation mean analysis was done only on the attributes that were diverse enough in the parental populations so that they could be nearly homozygous in those attributes.

7.4.2 **Gene effects**

Almost in all of the studied attributes and sets of crosses in generation mean analysis non-allelic interaction was observed. It should be mentioned that this experiment partitions the effects into allelic and non-allelic interaction effects, but these components also may be inflated by the existence of genotype-environment interaction effects. It is believed that the analysis of generation means for a single environment will only give information about the gene effects in that particular environment. One can only assume that there is no genotype-environment interaction to be able to apply the results in other environments.

To my knowledge, there is nothing known about quantitative genetic aspects of prostrateness in red clover, although this type of red clover was known from long time ago. The analysis of generation means proved to be a useful procedure for investigating the gene function involved in the inheritance of the mentioned attributes in all three sets of crosses.

In prostrateness (*h*) and (*l*) had different sign in all three sets of crosses. This would indicate duplicate epistasis (Mather and Jinks, 1982) which is not suitable in selection programmes. Based on the crosses one and three, prostrateness is controlled by a few genes which are partial to completely dominant to erectness. It was discussed in chapter six that large reduction in magnitude of standard errors in the best parsimonious model indicated the significant usefulness of this model and higher precision of the model. So the large reduction in the magnitude of standard errors in the best parsimonious model was optimistic.

In the case of nodal rooting ability, since the results in the sets one and two were not similar, this attribute seemed not to be homozygous in the parental populations. However, in this particular character non-allelic interaction effects were not as important as they were in prostrateness. This implies that the estimates for this attribute in other experiments such as hierarchical mating design experiment's results are not much biased by the existence of non-allelic interaction effect. In other words estimates such as

heritability were not inflated by large contributions of epistasis (non-allelic interaction). Negative estimates for (*d*) in all three sets of crosses indicated that the parent with larger value for this particular attribute was allotted as P_2 and negative estimate for (*h*) indicated that the function of gene is partially dominance toward erectness. Regarding the magnitude of the parameters estimated, additive effects appeared to be the most important one suggesting the usefulness of selection from the crosses, though in set two different sign for (*h*) and (*l*) indicated a duplicate type of epistasis for this attribute which suggests that difficulty would be encountered in selecting for nodal rooting ability.

Stem thickness (*d*) and (*h*) presented a similar value in both sets one and three. This would indicate that stem thickness is partial to complete dominance to stem thinness. Although in set three (*j*) was significant at five percent, the other parameters in the parsimonious models were very similar. This would confirm that in such a situation, extreme situations in two diverse populations, generation mean analysis could be applied in order to investigate function of the genes involved.

Leaf size was another characteristic with an opposite situation in the two populations. Erect population was characterized with large leaves, whereas, prostrate population was characterized with very small leaves (Plate 1, chapter three). Based on the results of sets one and two, the genes controlling small leaf size are completely to over dominant to the genes controlling large leaf size. A remarkable reduction in standard errors and chi-square value indicated the efficiency of the best fitted model.

On the whole the analysis of generation means proved to be an efficient procedure for investigating function of genes involved in the inheritance of the examined attributes. It has to be emphasized that in cross pollinated plants parental populations had to be isolated for enough time, and natural or artificial selection have to be applied in opposite directions on them so that the populations can be regarded as either homozygous lines for the character under question or at least the majority of opposite alleles concentrate in the opposite directions. The crosses investigated each involved

two parents with quite contrasting characteristics, particularly in terms of prostrateness, nodal rooting ability, stem thickness, and leaf size. This study also provided the opportunity to estimate the amount of possible heterosis which will be discussed later.

7.5 **Number of genes**

Estimating number of genes contributing to the variance of quantitative characters in plant populations is fundamental for the study of mechanisms of heredity. If one line is fixed with alleles decreasing the character of interest and the other line is fixed with alleles increasing it, the number of genes, or the minimum effective factors, can be estimated. In cross pollinated plant populations the requirement of inbred lines which is sometimes violated in practice, may lead to unwanted complications of inbreeding depression on the mean of the populations. However, this fixation of lines occur most likely when two populations are divergent, and this divergence may be caused by sustained artificial or natural selection on the trait of interest or on some characters highly correlated with it (Lande, 1981).

The data analyzed in generation mean analysis are typical of crosses between populations that differ greatly in some quantitative characters as a result of natural or artificial selection. Generation mean analysis on several diverse attributes in the two types provided us with a possibility of estimating the number of genes or factors controlling the attributes. Because of lack of enough germinated seeds, the number of individual plants in F_2 and B_2 in set two was not the same as sets one and three. Therefore, the results of this set were more readily subjected to bias and this may be the reason for non-significance of the results.

The effective or minimum number of genes estimated for prostrateness in set one and two indicated that this character is oligogenic (controlled by a few of genes). The corresponding standard errors for

estimated number of genes for sets one and two were very small. This would indicate that when the assumptions for this procedure are approximately satisfied, the estimates of the number of genes will be reasonably accurate.

The minimum number of genes estimated for nodal rooting ability also indicated that this character is oligogenic. Since the parents in set one and three had originated from the same population, it was expected to get a similar result for these two crosses.

The values estimated for stem thickness was surprisingly very similar in sets one and three. The high level of significance also would indicate the precision of the estimate. These results strongly suggested that this attribute is oligogenic.

The minimum number of genes estimated for leaf size were very high in set one and three, and due to correspondingly large standard errors they were not significant. Also the results indicated that on the chosen scale of measurement multiple genetic factors are involved in the inheritance of the character.

7.6 Conclusions

1- Genotype main effect was highly significant for most of the attributes. The pooled analysis of variance suggested that first and second order interaction effects were present in the performance of examined genotypes of red clover.

2- In the analysis based on all attributes regarding second order interaction effect (GxSxY) the first two discriminant scores accounted for 68.74% of the total GxSxY variation. In the cases of analysis based on attributes with significant GxY and GxSxY the first two discriminant scores could explain more than 70% of the total GxY and GxSxY variation existing in the data set.

3- In cluster analysis in the cases based on GxSxY effect genotypes were grouped corresponding to their types, this suggested that each type of red clover responds to genotype-environment interaction effects in a particular way. In cluster analysis based on GxY effect, clustering proceeding was the same as the ones mentioned before but eventually genotypes were divided to four clusters. In fact prostrate genotypes formed two clusters, indicating different response to successive years between the genotypes within prostrate type.

4- Genotypic and phenotypic correlation patterns changed from type to type and year to year. Genotypic and phenotypic correlations based on all three types at two sites over two years suggested that prostrateness has a negative correlation with dry matter yield, first flowering date, leaf size, stem length, and stem thickness. This would indicate that prostrateness growth habit would reduce dry matter yield through reducing yield components.

5- A very high negative genotypic and phenotypic correlation between prostrateness and stem thickness in all of the cases indicated that these two attributes are either controlled by the same genes or they are closely linked. In fact one of the reasons for being prostrate might be thin, weak stems.

6- As a result of hierarchical mating design, the two intra-population crosses, erect and prostrate, presented opposite genetic architecture. Dominance components of genetic variance were more important than additive in erect population, whereas in the prostrate population additive components of genetic variance were more important. Inter-population crosses presented larger additive and dominance components of variance than both of the intra-population crosses.

7- Plant diameter, branches, and stem length presented high level of heterosis which is optimistic in producing synthetic and hybrid varieties based on these populations. In contrast number of stems, plant height, and stem thickness presented an indication of hybrid depression (negative heterosis) which may be due to an excessive genetic diversity between the two examined types.

8- General performance and mean values of F_1 plants in generation mean analysis and hierarchical mating design experiment were quite similar to that of semi-erect populations in the genotype-environment interaction study. This raises the question as to whether semi-erect genotypes originated by such inter-type crosses.

9- Prostrateness, rooting ability, and stem thickness are oligogenic attributes and leaf size is a polygenic attribute.

10- Gene functions controlling prostrateness and stem thickness which were partially to completely dominance respectively. There was over-dominant of alleles controlling large leaf size.

11- Prostrateness, nodal rooting ability, stem thickness, and leaf size presented non-allelic interaction effects which would interfere in the results of possible selection programme based on the examined populations.

7.7 **Suggested further studies**

1- To extend the results of genotype-environment interaction to other regions, experiments need to be done on more diverse sites as representative sample of environments.

2- To get a stronger estimation of genetic variance components, progeny families produced need to be tested in a wide sample of sites and successive years in order to subtract the interaction effects from the genetic variance component.

3- Performance of spaced individual plants may be poorly correlated with the performance under sward conditions. To get a practical result a further study of the attributes studied under sward conditions is suggested.

4- The inter-population crosses produced in hierarchical mating design could possibly be used as base populations for breeding programmes. It should be possible to produce a highly productive cultivar with nodal rooting ability from these populations.

5- It would be a sound idea to develop selection indices based on the estimated parameters in these study regarding breeding purposes in red clover.

6- As was suggested by other authors (see chapter two) nodal rooting ability in red clover would affect plant performance from second year of the plant life and thereafter. To get definite effects of nodal rooting ability, it is suggested to carry out the same studies over more than two years to get a net effect of nodal rooting ability.

REFERENCES

Abou-El-Fittouh, H. A.; Rawlings. J.O., & Miller, P. A. ; 1969. Classification of environments to control genotype by environment interactions with an application to cotton. *Crop Sci.*; 9: 135-140.

Allard, R. W., & Bradshaw, A. D. ; 1964. Implication of genotype environment interactions in applied plant breeding. *Crop Sci.*; 4: 503-508.

Anderberg, M. R.; 1973. Cluster analysis for applications. New York, Academic press

Anderson, L. B. ; 1960. Evaluation of general and specific combining ability in a late flowering variety of red clover (*Trifolium pratense* L.). *N.Z.J. of Agric. Res.*; 3: 680-692.

Anderson, M. K.; Taylor, N. L., & Hill, j. R. R. ; 1974. Combining ability in I0 single crosses of red clover. *Crop Sci.*; 14: 417-419.

Anderson, V. L., & Kempthorne, O. ; 1954. A model for the study of quantitative inheritance. *Genetics*; 39: 883-898.

Baker, R. J. ; 1969. Genotype environment interactions in yield of wheat. *Can. J. Plant Sci.*; 49: 743-751.

Baker, R. J. ; 1978. Issues in diallel analysis. *Crop Sci.*; 18: 533-536.

Baker, R. J.; 1986. Selection indices in plant breeding. Florida, USA, CRC Press, Inc.

Basford, K. E., & Delacy, I. H. ; 1979. The use of matrix specification in defining gene action in genotypic value models and generation mean analysis. *Theor. Appl. Genet.*; 55: 225-229.

Bassiri, A.; 1971. Variation in Quantitative characters of red clover, *Trifolium pratense* L., after two generations of selfing. PhD Thesis. University of Wisconsin , USA,

Baum, B. R., & Bailey, G. ; 1991. A numerical taxonomic investigation of the Hordeum brevisubulatum aggregate. *Can. J. Of Botany*; 69: 2001-2019.

Becker, H. C., & Leon, J. ; 1988. Stability analysis in plant breeding. *Plant Breeding*; 101: 1-23.

Becker, W. A.; 1984. Manual of quantitative genetics. Academic enterprises

Bowley, S. R.; Dougherty, C. T.; Taylor, N. L., & Cornelius, P. L. ; 1988. Comparison of yield components of red clover and alfalfa. *Can. J. Of Plant Sci.*; 68: 103-114.

Bowley, S. R.; Taylor, N. L., & Dougherty, C. T. ; 1987. Photoperiodic response and heritability of the pre flowering interval of two red clover (*Trifolium pratense* L.) populations. *Ann. Appl. Biol.*; 111: 455-461.

Bowley, S. R.; Taylor, N. L., & Dougherty, C. T. ; 1983. Physiology and morphology of red clover. *Advances In Agro.*; 37: 317-347.

Brandon, R. A., & Leffel, R. C. ; 1968. Pseudo-self-compatibility of a diallel cross and sterility-allele genotypic ratios in red clover (*Trifolium pratense* L.). *Crop Sci.*; 8: 185-186.

Breese, E. L., & Hayward, M. D. ; 1972. The genetic basis of present breeding methods in forage crops. *Euphytica*; 21: 324-336.

Brieger, F. G. ; 1950. The genetic basis of heterosis in maize. *Genetics*; 35: 420-445.

Bryant, E. H., & Atchley, W. R.; 1975. Multivariate statistical methods, within-groups covariation. U.S.A., Dowsen, Hutchinson & Ross, Inc.

Bucio Alanis, L. ; 1966. Environmental and genotype-environmental components of variability. I. inbred lines. *Heredity*; 21: 387-397

Bucio Alanis, L., & Hill, J. ; 1966. Environmental and genotype environmental components of variability. II. Heterozygotes. *Heredity*; 21: 399-405.

Bucio Alanis, L.; Perkins, J. M., & Jinks, J. L. ; 1969. Environmental and genotype-environmental components of variability. V. Segregating generations. *Heredity*; 24: 115-127.

Bulmer, M. G.; 1985. The mathematical theory of quantitative genetics. Oxford, Clarendon Press Oxford

Byth, D. E.; 1977. A conceptual basis of genotype environment interactions for plant improvement. in: Anonymous, Plant breeding papers :1 3rd international congress of the society for the advancement of breeding researches in Asia and Oceanic 1 pp:3(d)-1 to 3(d)-11 Australia,

Byth, D. E.; Eisemann, R. L., & Delacy, I. H. ; 1976. Two way pattern analysis of a large data set to evaluate genotypic adaptation. *Heredity*; 37: 215-230.

Cavalli; 1952. An analysis of linkage in quantitative inheritance. in: E. C. R. Reeve, & C. H. Waddington, Quantitative inheritance pp:pp 135-144 London, H.M.S.O.

Chatfield, C., & Collins, A. J.; 1980. Introduction to multivariate analysis. London, Chapman and Hall

Cheverud, J. M. ; 1988. A comparison of genetic and phenotypic correlations. *Evolutions*; 42: 958-68.

Choo, T. M. ; 1984. Association between growth habit and persistence in red clover. *Euphytica*; 33: 177-185.

Choo, T. M.; Langille, J. E.; Rayment, A. F.; Bubar, J. S.; Walton, R. B., & Coulson, N. N. ; 1984. Cultivar environment interactions in red clover. *Can. J. Plant Sci.*; 64: 139-144.

Choo, T. M., & Reinberg, S. E. ; 1982. Estimation of the number of genes in doubled haploid populations of barley (*Hordeum Vulgare*). *Can. J. Genet. Cytol.*; 24: 337-341

Christie, B.R., & Shattuck, V.I.; 1992. The diallel cross : Design, analysis, and use for plant breeders. *Plant breeding reviews*; 9: 9-36

Clay, K. ; 1982. Environmental and genetic determinants of cleistogamy in a natural population of the grass *Danthonia spicata*. *Evolution*; 36: 4734-741.

Clifford, H. T., & Stephenson, W.; 1975. An introduction to numerical classification. New York, Academic Press

Cochran, W. G. ; 1947. Some consequences when the assumptions for the analysis of variance are not satisfied. *Biometrics*; 3: 22-39.

Cockerham, C. C. ; 1961. Implication of genetic variances in a hybrid breeding programm. *Crop Sci.*; 1: 47-52.

Cockerham, C. C. ; 1986. Modifications in estimating the number of genes for a quantitative character. *Genetics*; 114: 659-664.

Cockerham, C. C., & Matzinger, D. F. ; 1985. Selection response based on selfed progenies. *Crop Sci.*; 3: 483-488.

Compton, W. A.; Gardner, C. O., & Lonnquist, J. H. ; 1965. Genetic variability in two open pollinated variety of corn (*Zea mayze* L.) and their F1 progenies. *Crop Sci.*; 5: 506-508.

Comstock, R. E., & Moll, R. H.; 1963. Genotype-environment interaction. in: Hanson, W.D., Statistical Genetics And Plant Breeding; pp: 164-196; Washington, NAS-NRC

Comstock, R. E., & Robinson, H. F. ; 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics*; 4: 254-256.

Comstock, R. E., & Robinson, H. F.; 1952. Estimation of average dominance of genes. in: Heterosis Iowa, Iowa State College Press

Cooley, W. W., & Lohnes, P. R.; 1971. Multivariate data analysis. New York, John Wiley & Sons, Inc.

Cormack, R. M. ; 1971. A review of classification. *J. Statis. Soc.*; 134: 321-359.

Cornelius, P. L.; Taylor, N. L., & Anderson, M. K. ; 1977. Combining ability in I1 single crosses of red clover. *Crop Sci.*; 17: 9709-713.

Cressman, R. M. ; 1967. Internal breakdown and persistence of red clover. *Crop Sci.*; 7: 357-361.

Crow, J. F. ; 1948. Alternative hypothesis of hybrid vigour. *Genetics*; 33: 477-487.

Crump, S. L. ; 1946. The estimation of variance components in analysis of variance. *Biometrics Buletin*; 2: 7-11.

Crump, S. L. ; 1951. The present status of variance component analysis. *Biometrics*; 7: 1-16.

Cruz, R. M. ; 1992. More about the multiplicative model for the analysis of genotype-environment interaction. *Heredity*; 68: 135-140.

Cullen, C. ; 1981. Pattern analysis of genotype-environment interaction and comparison with alternative analysis. *M.Agr.Sc. thesis, Massey University, New Zealand*;

Easton, A. S., & Clements, R. J. ; 1973. The interaction of wheat genotypes with a specific factor of the environment. *J. Agric. Sci. Camb.*; 80: 43-52.

Eberhart, S. A., & Russell, W. A. ; 1966. Stability parameters for comparing varieties. *Crop Sci.*; 6: 36-40.

Eisemann, R. L.; Byth, D. E.; DeLacy, I. H., & Taylor, P. J.; 1977. A new approach to the analysis of genotypic adaptation and genotype environment interactions. in: Anonymous, Plant breeding papers 1 pp:3(d)-41 to 3(d)-47 3rd SABRAO Proceedings

Eisen, E. J., & Saxton, A. M. ; 1983. Genotype by environment interactions and genetic correlations involving two environmental factors. *Theor. Appl. Genet.*; 67: 75-86.

Eisenhart, C. ; 1947. The assumptions underlying the analysis of variance. *Biometrics*; 3: 1-21.

Everritt, B.; 1974. Cluster analysis. London, Heinemann Educational Books Ltd

Fakorede, M. A. B. ; 1986. Selection of sites for preliminary maize yield trials in the rainforest zone of south-western Nigeria. *Euphytica*; 35: 441-447.

Falconer, D. S.; 1989. Introduction to quantitative genetics. 3rd edition; New York, John Wiley & Sons, Inc.

Ferreira, P. E. ; 1988. A new look at Jinks-Hayman's method for the estimation of genetical components in diallel crosses. *Heredity*; 60: 347-353.

Finlay, K. W., & Wilkinson, G. N. ; 1963. The analysis of adaptation in a plant breeding programme. *Aust. J. Agric. Res.*; 14: 742-754.

Fisher, R. A. ; 1918. The correlation between relatives on the supposition of mendelian inheritance. *Trans. royal soc.*; 52: 399-433.

Fisher, R. A.; Immer, F. R., & Tedin, O. ; 1932. The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. *Genetics*; 17: 107-124.

Fletcher, J.R., 1987. Land use capability classification of the Taranaki-Manawatu region (with associated map N149 and extended legends), National water and soil conservation, New Zealand.

Freeman, G. H., & Perkins, J. M. ; 1971. Environmental and genotype-environmental components of variability. VIII. relations between genotypes grown in different environments and measures of these environments. *Heredity*; 27: 15-23.

Gale, J. S., & Eaves, L. J. ; 1972. Variation in wild populations of Papaver dubium, V. The application of factor analysis to the study of variation. *Heredity*; 29: 135-149.

Gamble, E. E. ; 1962. Gene effects in corn (*Zea mayze* L.). I. Separation and relative importance of the gene effects for yield. *Can. J. Plant Sci.*; 42: 339-348.

Gardner, C. O., & Eberhart, S. A. ; 1966. Analysis and interpretation of the variety cross diallel and related populations. *Biometrics*; 22: 439-452.

Gates, C. E., & Bilbro, J. D. ; 1978. Illustration of a cluster analysis method for mean separation. *Agron. J.*; 70: 462-465.

Ghaderi, A.; Everson, E. H., & Cress, C. E. ; 1980. Classification of environments and genotypes in wheat. *Crop Sci.*; 20: 707-710.

Gilbert, N. E. G. ; 1958. Diallel cross in plant breeding. *Heredity*; 12: 477-492.

Gill, J. L. ; 1986. Repeated measurement: Sensitive tests for experiments with few animals. *Journal of Animal Science*; 63: 943-954.

Glahn, H. R.; 1975. Canonical correlation and its relationship to discriminant analysis and multiple regression. in: E. H. Bryant, & W. R. Atchley, Multivariate statistical methods, within-groups covariation U.S.A, Dowden, Hutchinson & Ross, Inc.

Goodrich, C. L.; Stuber, R. E., & Compton, W. A. ; 1975. Average gene frequency estimate in two open pollinated cultivar of corn. *Crop Sci.*; 15: 746-749.

Gordon, I. L. ; 1979. Standard errors of heritabilities based on perennial observations, with application to yorkshire grass. *Euphytica*; 28: 81-88

Gordon, I. L.; Byth, D. E., & Balam, L. N. ; 1972. Variance of heritability ratios estimated from phenotypic variance components. *Biometrics*; 28: 401-415.

Gouesnard, B., & Gallais, A. ; 1992. Genetic variance component estimation in a nested mating design with positive assortative mating, and application to maize. *Crop Sci.*; 32: 1127-1131.

Gray, E. ; 1982. Genotype-environment interactions and stability analysis for forage yield of orchardgrass clones. *Crop Sci.*; 22: 19-23.

Griffing, B. ; 1950. Analysis of quantitative gene action by constant parent regressions and related techniques. *Genetics*; 35: 303-321.

Griffing, B. ; 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.*; 9: 463-493.

Griffing, B. ; 1956a. A generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity*; 10: 31-50.

Gupta, S. C., & Ndoye, A. T. ; 1991. Yield stability analysis of promising pearl millet genotypes in senegal. *Maydica*; 36: 83-86.

Hallauer, A. R.; 1988. Genotype-environment interactions. in: B. S. Weir; E. J. Eisen; M. M. Goodman, & G. Namkoong, Proceeding of the second international conference on quantitative genetics pp:488-491 Massachusettes, Sineuer Associates, Inc. Publisher

Hamblin, J.; Fisher, H. M., & Ridings, H. I. ; 1980. The choice of locality for plant breeding when selecting for high yield and general adaptation. *Euphytica*; 20: 161-168.

Hanson, W. D. ; 1989. Standard errors for heritability and expected selection response. *Crop Sci.*; 29: 1561.

Hartl, D. L.; 1980. Principles of population genetics. Massachusetts, Sinauer Associates Inc.

Hayman, B. I. ; 1954a. The analysis of variance of diallel crosses. *Biometrics*; 10: 235-244.

Hayman, B. I. ; 1954b. The theory and analysis of diallel crosses. *Genetics*; 39: 789-809.

Hayman, B. I. ; 1957. Interaction, heterosis and diallel crosses. *Genetics*; 42: 336-355.

Hayman, B. I. ; 1958. The separation of epistatic from additive and dominance variation in generation means. *Heredity*; 12: 371-390.

Hayman, B. I. ; 1960. The theory and analysis of diallel crosses. III. *Genetics*; 45: 155-172.

Hayman, B. I.; 1963. Notes on diallel cross theory. In: Hanson, W.D., Statistical Genetics And Plant Breeding; pp:571-578; Washington, NAS-NRC

Hayman, B. I., & Mather, K. ; 1955. The description of genic interactions in continuous variation. *Biometrics*; 11: 69-82.

Helgadottir, A., & Kristjansdottir, T. A. ; 1991. Simple approach to the analysis of GxE interactions in a multilocational spaced plant trial with timothy. *Euphytica*; 54: 65-73.

Hill, B. J. ; 1975. Genotype-environment interactions- a challenge for plant breeding. *J. Agric. Sci. Camb.*; 85: 477-493.

Jacquard, A. ; 1983. Heritability: one word, three concepts. *Biometrics*; 39: 465-477.

Jinks, J. L. ; 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. *Genetics*; 39: 767-788.

Jinks, J. L. ; 1956. The F2 and backcross generations from a set of diallel crosses. *Heredity*; 10: 1-30.

Jinks, J.L., & Hayman, B.I.; 1953. The analysis of diallel crosses. *Maize Genetics Coop.* Newsletter, 27: 48-54

Jinks, J. L., & Perkins, J. M. ; 1970. Environmental and genotype-environmental components of variability. VII. simultaneous prediction across environments and generations. *Heredity*; 25: 475-480.

Jinks, J. L., & Pooni, H. S. ; 1979. Non-linear genotype-environment interactions arising from response thresholds.I. Parents, F1's and selections. *Heredity*; 43: 57-70.

Jinks, J. L., & Towey, P. ; 1976. Estimating the number of genes in a polygenic system by genotype assay. *Heredity*; 37: 69-81.

Jobson, J. D.; 1991. Applied multivariate data analysis; Volume 1: Regression and experimental design. New York, Springer-Verlag

Johnson, R. A., & Wichern, D. W.; 1992. Applied multivariate statistical analysis ; third edition. U.S.A., Prentice-Hall Inc.

Jolliffe, I. T.; 1986. Principal component analysis. New York, Springer-Verlag

Kahn, M. A.; Antonovics, J., & Bradshaw, A. D. ; 1976. Adaptation to heterogeneous environments. III The inheritance of response to spacing in flax and lineseed (*Linum usitatissimum*). *Aust. J. Agric. Res.*; 27: 519-529.

Kang, M. S., & Pham, H. N. ; 1991. Simultaneous selection for high yielding and stable crop genotypes. *Agron. J.*; 83: 161-165.

Kashirsagar, A. M.; 1972. Multivariate analysis. New York, Marcel Dekker Inc.

Kearsey, M. J. ; 1965. Biometrical analysis of a random mating population: a comparison of five experimental designs. *Heredity*; 20: 205-235.

Kearsey, M. J. ; 1970. Experimental size for detecting dominance variation. *Heredity*; 25: 529-542.

Kearsey, M. J., & Jinks, J. L. ; 1968. A general method of detecting additive, dominance and epistatic variation for metrical traits I. Theory. *Heredity*; 23: 403-409.

Kempthorne, O. ; 1954. The theoretical values of correlations between relatives in random mating populations. *Genetics*; 40: 153-167.

Kempthorne, O. ; 1956. The theory of the diallel cross. *Genetics*; 41: 451-459.

Kempthorne, O.; 1957. An introduction to genetic statistics. Ames, Iowa State Univ. Press

Kempthorne, O.; 1988. An overview of the field of quantitative genetics. in: B. S. Weir; E. J. Eisen; M. M. Goodman, & G. Namkoong, Proceedings of the second international conference on quantitative genetics (1) pp:47-56 Massachusetts, Sinaure Associates, Inc.Publishers

Kendall, Sir M.G.; 1980. Multivariate analysis. 2nd edition; London, Charles Griffin & Co. Ltd.

Ketata, H.; Edwards, L. H., & Smith, E. L. ; 1976. Inheritance of eight agronomic characters in a winter wheat Cross. *Crop Sci.*; 16: 19-22.

Khehra, A. S.; Singh, H., & Dhillon, B. S. ; 1985. Genetic architecture of a heterotic cross between two populations of maize. *Theor. Appl. Genet.*; 69: 256-261.

Knight, R. ; 1970. The measurement and interpretation of genotype environment interactions. *Euphytica*; 19: 225-235.

Kroonenberg, P. M., & Basford, K. E. ; 1989. An investigation of multi-attribute genotype response across environments using three-mode principal component analysis. *Euphytica*; 44: 109-123.

Lancashire, J. A.; 1985. Distribution and use of forage legumes in New Zealand. In: R. e. al. (Ed.) Barnes, Forage legumes for energy-efficient animal production pp:20-34 Washington, D.C., USDA

Lande, R. ; 1981. The minimum number of genes contributing to quantitative variation between and within populations. *Genetics*; 99: 541-553.

Lande, R., & Arnold, S. J. ; 1983. The measurement of selection on correlated characters. *Evolution*; 37: 61210-1226.

Leath, K. T.; 1985. General diseases. in: N. L. Taylor, Clover science and technology 25 pp:205-233 Wisconsin, USA, ASA, CSSA, SSSA

LeClerg, E. L.; Leonard, W. H., & Clark, A. G.; 1962 . Field plot technique. 2nd Edition; Minnesota, Burgess

Lefkovitch, L. P. ; 1980. Conditional clustering. *Biometrics*; 36: 43-58.

Lefkovitch, L. P. ; 1982. Conditional clusters, musters, and probability. *Mathematical Biosciences*; 60: 207-234.

Lefkovitch, L. P. ; 1985. Multi-criteria clustering in genotype-environment interaction problems. *Theor. Appl. Genet.*; 70: 585-589.

Lin, C. S. ; 1982. Grouping genotypes by a cluster method directly related to genotype- environment interaction mean square. *Theor. Appl. Genet.*; 62: 277-280.

Lin, C. S., & Binns, M. R. ; 1985. Procedural approach for assessing cultivar-location data: Pairwise genotype-environment interactions of test cultivars with checks. *Can. J. Plant Sci.*; 65: 1065-1071.

Lin, C. S.; Binns, M. R., & Lefkovitch, L. P. ; 1986. Stability analysis: where do we stand? *Crop Sci.*; 26: 894-900.

Lin, C. S., & Binns, M. R. ; 1988. A superiority measure of cultivar performance for cultivar x location data. *Can. J. Plant Sci.*; 68: 193-198.

Lin, C. S., & Butler, G. ; 1990. Cluster analysis for analysing two-way classification data. *Agron. J.*; 82: 344-348.

Lin, C. S., & Thompson, B. ; 1975. An empirical method of grouping genotypes based on a linear function of the genotype-environment interaction. *Heredity*; 34: 255-263.

Mather, K.; 1949. Biometrical genetics. First edition. London, Methuen and Co. Ltd.

Mather, K., & Jinks, J. L.; 1982. Biometrical Genetics. London, Chapman and Hall

Mather, K., & Jinks, J. L.; 1977. Introduction to biometrical genetics. London, Chapman and Hall

Mayo, O.; 1980. The theory of plant breeding. Oxford, Clarendon Press

McQuitty, L. L.; 1987. Pattern-analytic clustering (theory, method, research and configural findings). U.S.A., University Press Of America, Inc.

Miller, P. A.; Williams, J. C.; Robinson, H. F., & Comstock, R. E. ; 1957. Estimates of genotypic and environmental variances and covariances in upland cotton and their implications in selection. *Agron. J.*; 126-131.

Miller, P. A.; Williams, J. C., & Robinson, H. F. ; 1959. Variety-environment interactions in cotton variety tests and their implications on testing methods. *Agron. J.*; 51: 132-134.

Mirzaie-Nodoushan, H., & Gordon, I. L. ; 1993. Rooting ability in red clover accessions. *Proc. XVII International Grassland Congress, New Zealand and Queensland*; In press:

Misevic, D., & Dumanoivic, J. ; 1989. Examination of methods for choosing locations for preliminary maize yield testing. *Euphytica*; 44: 173-180.

Mitchell-Olds, T., & Bergelson, J. ; 1990. Statistical genetics of an annual plant, *Impatiens capensis.* I. Genetic basis of quantitative variation. *Genetics*; 124: 407-415.

Mitchell-Olds, T., & Rutledge, J. J. ; 1986. Quantitative genetics in natural plant populations: A review of the theory. *The American Naturalist*; 127: 3379-402.

Moll, R. H. ; 1965. *Genetics*; 52: 139-144.

Moll, R. H.; Cockerham, C. C.; Stuber, C. W., & Williams, W. P. ; 1978. Selection responses, genetic-environmental interactions,and heterosis with recurrent selection for yield in maize. *Crop Sci.*; 18: 641-645.

Moll, R. H., & Stuber, C. W. ; 1974. Quantitative genetics-empirical results relevant to plant breeding. *Advances In Agron.*; 26: 277-313.

Montpetit, J. M., & Coulman, B. E. ; 1991a. Relationship between spring vigour and the presence of adventitious in established stands of red clover, *Trifolium pratense* L. *Can. J. Plant Sci.*; 71: 749-754.

Montpetit, J. M., & Coulman, B. E. ; 1991b. Responses to divergent selection for adventitious root growth in red clover (*Trifolium pratense* L.). *Euphytica*; 58: 2119-127.

Moreno-Gonzales J., & Dudley, J. W. ; 1981. Epistasis in related and unrelated maize hybrids determined by three methods. *Crop Science*; 21: 644-651.

Morrison; 1976. Multivariate statistical methods. New York, Mc Grow Hill

Mozafari, J. ; 1992. Genotypic and environmental effects on growth habit in wheat (*Triticum aestivume* L.). *Msag thesis Massey University, New Zealand.*;

Muir, W.; Nyquist, W. E., & Xu, S. ; 1992. Alternative partitioning of the genotype-environment interaction. *Theor. Appl. Genet.*; 84: 193-200.

Mulitze, D. K., & Baker, R. J. ; 1985. Evaluation of biometrical methods for estimating the number of genes. I.Effect of sample size. *Theor. Appl. Genet.*; 69: 553-558.

Mulitze, D. K., & Baker, R. J. ; 1985. Evaluation of biometrical methods for estimating the number of genes. II. Effect of type I and type II statistical errors. *Theor. Appl. Genet.*; 69: 559-566.

Mungomery, V. E.; Shorter, R., & Byth, D. E. ; 1974. Genotype-environment interactions and environmental adaptation. I pattern analysis-application to soya bean populations. *Aust. J. Agric. Res.*; 25: 59-72.

Obilana, A. T.; Hallauer, A. R., & Smith, O. S. ; 1979. Estimated genetic variability in a maize interpopulation. *J. Heredity*; 70: 127-132.

Parrott, A., & Smith, R. ; 1986. Description and inheritance of new genes in red clover. *J. Heredity*; 77: 355-358.

Pederson, D. G. ; 1972. A comparison of four experimental designs for the estimation of heritability. *Theor. Appl. Genet.*; 42: 371-377.

Perkins, J. M. ; 1970. Environmental and genotype-environmental components of variability. VI. Diallel sets of crosses. *Heredity*; 25: 29-40.

Perkins, J. M., & Jinks, J. L. ; 1968a. Environmental and genotype environmental components of variability. III multiple lines and crosses. *Heredity*; 23: 339-356.

Perkins, J. M., & Jinks, J. L. ; 1968b. Environmental and genotype-environmental components of variability. IV. Non-linear interactions for multiple inbred lines. *Heredity*; 23: 525-535.

Plaisted, R. L., & Peterson, L. C. ; 1959. A technique for evaluating the ability of selections to yield consistently in different locations or seasons. *Ame. Potato Journal*; 36: 381-385.

Robertson, A. ; 1959. The sampling variance of the genetic correlation coefficient. *Biometrics*; 15: 469-485.

Robinson, H. F., & Comstock, R. E. ; 1955. Analysis of genetic variability in corn with reference to probable effects of selection. *Cold SPring Har. Sym. On Qua. Bio.*; 20: 127-136.

Robinson, H. F.; Comstock, R. E., & Harvey, P. H. ; 1949. Estimation of heritability and the degree of dominance in corn. *Agron. J.*; 41: 353-359.

Robinson, H. F.; Comstock, R. E., & Harvey, P. H. ; 1955. Genetic variances in open pollinated varieties of corn. *Genetics*; 40: 45-60.

Robinson, H. F.; Khalil, A.; Comstock, R. E., & Cockerham.C.C. ; 1958. Joint interpretation of heterosis and genetic variances in two open pollinated varieties of corn and their crosses. *Genetics*; 43: 868-877.

Romesburg, H. C.; 1984. Cluster analysis for researchers. California, Lifetime Learning Publications

SAS Institute Inc. ; 1990. SAS/STAT User's guide, version 6. Fourth edition, Vols. 1 and 2.

Satterthwaite, F. E. ; 1946. An approximate distribution of estimates of variance components. *Biometrics*; 2: 110-114.

Schutz, W.M.; Bernard, R.L.; 1967. Genotype environment interactions in the regional testing of soybean strains. *Crop Sci.*; 7: 125-130.

Searle, S. R. ; 1961. Phenotypic, genetic and environmental correlations. *Biometrics*; 17: 474-480.

Searle, S. R. ; 1971. Topics in variance component estimation. *Biometrics*; 27: 1-76.

Shorter, R.; Byth, D. E., & Mungomery, V. E. ; 1974. Genotype-environment interactions and environmental adaptation. II Assessment of environmental contributions. *Aust. J. Agric. Res.*; 223-235.

Shorter, R.; Lawn, R. J., & Hammer, G. L. ; 1991. improving genotypic adaptation in crops - A role for breeders, physiologists and modellers. *Experimental Agriculture*; 27: 2155-175.

Shukla, G. K. ; 1972. Some statistical aspects of partitioning genotype environmental components of variability. *Heredity*; 29: 237-245.

Simmonds, N. W. ; 1980. Comparisons of the yields of four potato varieties in trials and in agriculture. *Experimental Agriculture*; 16: 393-398.

Simmonds, N. W.; 1979. Principles of crop improvement. London, Longman

Singh, H.; Khehra, A. S., & Dhillon, B. S. ; 1984. Estimation of genes frequencies in two heterotic maize populations. *Theor. Appl. Genet.*; 69: 179-186.

Singh, H.; Khehra, A. S., & Dhillon, B. S. ; 1985. Genetic architecture of two heterotic populations of maize. *Maydica*; 30: 31-36

Singh, M., & Singh, R. K. ; 1984. A comparison of different methods of half-diallel analysis. *Theor. Appl. Genet.*; 67: 323-326.

Singh, O., & Paroda, R. S. ; 1984. A comparison of different diallel analysis. *Theor. Appl. Genet.*; 67: 541-545.

Smith, R. S., & Bishop, D. J. ; 1993. Astred a stoloniferous red clover. *XVII International Grasslands Congress, New Zealand and Queensland*;(In press);

Smith, R. R.; Taylor, N. L., & Bowley, S. R.; 1985. Red clover. In: N. L. Taylor, Clover science and technology 25 pp:458-470 Wisconsin, USA, ASA, CSSA, SSSA

Snape, J. W.; 1987. Conventional methods of genetic analysis in wheat. in: F. G. H. Lupton, Wheat Breeding; Its Scientific Basis pp:109-128 New York, Chapman And Hall

Sprague, G. F.; 1966. Quantitative genetic in plant improvement. in: Plant Breeding. First edition; pp:315-354 Iowa, The Iowa State University Press

Sprague, G. F., & Federer, W. T. ; 1951. A comparison of variance components in corn yield trials: II. Error, year x variety, location x variety, and variety components. *Agron. J.*; 43: 535-541.

Steel, R. G. D., & Torrie, J. H.; 1980. Principles and procedures of statistics. New York, McGraw-Hill

Stuber, C. W.; 1980. Mating designs, field nursery layouts, and breeding records. in: W. R. Fehr, & H. H. Hadley, Hybridization of crop plants pp:pp 83-104 Wisconsin, USA, ASA,CSSA

Taliaferro, C.M., Denman, C.E., Morrison, R.D., ZHolbert, D.; 1973. Cultivar environment interaction study of alfalfa yields in Oklahoma. *Crop Sci.*; 13: 619-622.

Tan, W. Y., & Chang, W. C. ; 1972. Convolution approach to the genetic analysis of quantitative characters of self-fertilized populations. *Biometrics*; 28: 1073-1090.

Taylor, N. L.; Johnston, K.; Anderson, M. K., & Williams, J. C. ; 1970. Inbreeding and heterosis in red clover. *Crop Sci.*; 10: 522-525.

Taylor, N. L., & Smith, R. ; 1977. Red clover breeding and genetics. *Advances In Agro.*; 31: 125-154.

Taylor, N. L.; Stroube, W. H.; Kendall, W. A., & Fergus, E. N. ; 1962. Variation and relation of clonal persistence and seed production in red clover. *Crop Sci.*; 2: 303-305.

Towey, P., & Jinks, J. L. ; 1977. Alternative ways of estimating the number of genes in a polygenic system by genotype assay. *Heredity*; 39: 399-410.

Valero, M. ; 1991. Discriminant alleles and discriminant analysis efficient characters to separate closely related species: The example of *Lathyrus latifolius* L. and *Lathyrus sylvestris* L. (Leguminosae). *Botanical Journal of the Linnean Society*; 107: 139-161.

Warner, J. N. ; 1952. A method for estimating heritability. *Agron. J.*; 44: 427-430.

Wehrhahn, C., & Allard, R. W. ; 1965. The detection and measurement of the effects of individual genes involved in the inheritance of a quantitative character in wheat. *Genetics*; 51: 109-119.

Welsh, J. R.; 1981. Fundamentals of plant genetics and breeding. New York, John Wiley & sons

Westcott, B. ; 1986. Some methods of analysing genotype-environment interaction. *Heredity*; 56: 243-253.

Williams, W. T. ; 1971. Principles of clustering. *Annual Review of Ecology and Systematics*; 2: 303-326.

Wricke, G. 1962. Ubereine Methode zur erfassung der okologischen Streubreite in feldversuchen z. *Pflanzenzuecht.* 47:92-96.

Wricke, G., & Weber, W. E.; 1986. Quantitative genetics and selection in plant breeding. New York, Walter de Gruyter

Wright, S. ; 1921. Systems of mating. *Genetics*; 6: 111-178.

Wright, S.; 1968. Genetic and biometric foundations. (Evolution and the genetics of populations. U.S.A., University Of Chicago Press

Wyman, E. N. ; 1991. Estimation of heritability and prediction of selection response in plant populations. *Critical reviews in plant sciences*; 10: 235-322.

Yadava, T. P., & Yadav, C. k.; 1977. Effects of simulated environments on the estimates of heritability and its implications in the improvement of indian mustard (*Brassica juncea* (L.) Czern and Coss). in: Anonymous, Plant breeding papers: 1 (3rd international congress of the society for the advancement of breeding researches in Asia and Oceania 1 pp:3(d)-26 to 3(d)-31

Yamada, Y.; Itoh, Y., & Sugimoto, I. ; 1988. Parametric relationships between genotype environment interaction and genetic correlation when two environments are involved. *Theor. Appl. Genet.*; 76: 850-854.

Yates, F., & Cochran, W. G. ; 1938. The analysis of groups of experiments. *J. Agric. Sci.*; 28: 556-580.

APPENDICES

Appendix 1, Table 1: accession number and their structure used in experiments.

F number	name	growth habit	structure
F2210	Swiss	erect	synthetic cultivar
F2256	Hannua	erect	" "
F2367	Turkish	erect	introduced population
F2378	Colenso	erect	synthetic cultivar
F2414	-	semierect	original collection
F2496	-	semierect	" "
F2497	-	semierect	" "
F2505	-	semierect	" "
F2419	-	prostrate	" "
F2420	-	prostrate	" "
F2424	-	prostrate	" "
F2427	-	prostrate	" "

Appendix 1, Table 2: The results of the test of homogeneity of variance in the genotype-environment interaction experiments.

Traits	Un-corrected Chi-square	Corrected Chi-square
Prostrateness	8.776 ns	8.768 ns
Nodal rooting	35.230 ns	35.149 ns
First flowering	11.385 ns	11.359 ns
50 % flowering	33.325 ns	33.249 ns
Leaf size	13.750 ns	13.718 ns
Stem thickness	39.767 *	39.675 *
No. of stems	65.668 **	65.517 **
stem length	39.436 *	39.345 *
No. of internodes	12.617 ns	12.588 ns
No. of branches	93.590 *	93.376 *
Dry matter yield	10.923 ns	10.898 ns

Appendix 2, Table 1 : Genotype X site interaction mean values.

Genotypes	Site	PRO	NRT	FFW	MFW	LSZ
F2210		4.01 d	3.96 de	152.1 ab	168.8 ab	5.53 ab
F2256		4.03 d	3.99 de	155.1 a	172.6 a	5.45 b
F2367		4.06 d	4.05 de	155.6 a	170.0 a	5.48 b
F2378		4.13 d	3.96 e	146.9 b	169.5 a	5.76 a
F2505		5.09 c	4.14 d	151.2 b	169.6 a	5.30 b
F2497		5.21 c	3.81 e	121.8 f	142.1 g	4.98 c
F2496	Site 1	5.33 c	4.02 de	107.4 g	130.5 h	4.75 de
F2414		5.25 c	3.98 de	133.3 de	148.6 fg	4.87 c
F2427		6.53 a	5.28 a	136.0 de	152.0 de	4.58 e
F2424		6.46 a	4.94 b	144.8 bc	162.1 bc	4.62 de
F2420		5.88 b	4.50 c	138.7 cd	158.8 cd	4.80 de
F2419		5.81 b	4.51 c	132.8 e	149.9 ef	4.85 d
F2210		4.02 d	4.00 d	149.0 cd	166.9 cd	5.34 ab
F2256		4.11 d	4.00 d	158.4 ab	176.5 ab	5.21abc
F2367		4.00 d	3.98 d	163.8 a	181.8 a	5.30 ab
F2378		4.25 d	4.05 cd	156.1 ab	165.3 d	5.36 a
F2505		5.16 c	4.00 d	159.3 ab	173.8 bc	5.10 bc
F2497	Site 2	5.31 c	4.03 d	127.1 f	145.8 e	5.01 cd
F2496		5.17 c	4.02 d	111.8 g	138.3 e	4.78 de
F2414		5.25 c	4.06 cd	135.5 e	148.5 e	5.02 bc
F2427		6.36 a	5.21 a	142.6 d	160.7 d	4.51 fg
F2424		6.54 a	5.30 a	153.3 bc	164.8 d	4.47 g
F2420		6.43 a	4.74 b	144.6 d	160.6 d	4.72 ef
F2419		5.87 b	4.37 c	143.0 d	165.9 d	4.74 ef

Mean values with the same letter are not significantly different at 5% level of probability.

Appendix 2, Table 1, continued : Genotype X site interaction mean values.

Genotypes	Site	STL	STT	NST	NIN	NBR	DMY
F2210		64.57 bc	4.61 ab	59.17 bcd	6.59 e	3.46 f	289.9 c
F2256		62.53 bc	4.54 ab	58.87 bcd	7.70 d	4.94 e	402.0 b
F2367		72.32 b	4.70 a	70.12 acb	8.19 cd	3.76 f	353.5 b
F2378		62.83 bc	4.41 b	70.40 abc	8.01 d	4.98 de	392.9 b
F2505		83.30 a	3.39 c	53.64 cd	7.90 d	6.21 bc	535.3 a
F2497		58.71 cd	2.88 d	73.02 ab	8.46 cd	5.04 de	190.1 de
F2496	Site 1	46.60 ef	2.89 d	43.43 d	6.26 e	3.59 f	87.4 f
F2414		68.37 bc	2.92 d	80.77 a	8.16 cd	5.33 cde	226.6 d
F2427		43.63 ef	1.70 fg	80.13 a	11.24 b	7.60 a	153.9 e
F2424		35.93 g	1.58 g	53.90 cd	12.55 a	7.30 ab	159.2 e
F2420		38.33 fg	2.20 e	75.47 ab	8.49 cd	5.29 cde	205.4 de
F2419		48.97 de	1.85 f	62.73 abc	9.07 c	6.06 cd	152.3 e
F2210		52.43 ab	4.25 a	38.23 de	6.05 d	3.32 b	146.2 bc
F2256		43.07 bcd	4.20 a	55.61 abcd	6.32 d	3.42 b	245.9 a
F2367		59.10 a	3.89 b	44.77 cde	6.58 d	3.39 b	235.0 a
F2378		52.81 ab	3.52 c	54.33 abcd	5.76 d	3.68 b	177.0 b
F2505		48.57 bc	2.89 d	61.28 abc	10.5 b	5.17 a	191.5 ab
F2497	Site 2	33.61 ef	2.54 d	72.66 a	5.98 d	3.45 b	63.05 de
F2496		28.21 ef	2.54 e	26.52 e	5.60 d	2.76 b	31.58 e
F2414		47.56 bcd	3.00 c	67.56 ab	8.05 c	4.49 a	126.9 c
F2427		30.75 ef	1.62 e	65.67 ab	10.54 b	6.18 a	86.02 d
F2424		24.15 f	1.65 e	57.08 abcd	11.80 a	5.77 a	78.26 de
F2420		28.78 ef	1.99 e	48.81 bcd	10.47 b	6.18 a	69.85 de
F2419		38.46 cde	1.81 e	54.38 abcd	10.47 b	6.18 a	95.22 cd

Mean values with the same letter are not significantly different at 5% level of probability.

Appendix 2, Table 2 : Genotype X year interaction mean values.

Genotypes	Year	PRO	NRT	FFW	MFW	LSZ
F2210		3.99 f	3.92 de	169.7 b	183.0 bc	5.39 a
F2256		4.00 f	3.94 de	178.5 a	189.5 ab	5.25 ab
F2367		4.02 f	3.99 de	178.1 a	191.3 a	5.43 a
F2378		4.10 f	3.94 de	176.9 a	184.0 bc	5.48 a
F2505		5.20 e	4.06 cd	167.5 b	184.2 bc	5.28 ab
F2497	Year 1	5.31 de	3.75 e	142.5 e	160.4 e	5.07 bc
F2496		5.43 d	3.96 de	129.8 f	152.3 f	4.95 c
F2414		5.39 de	3.95 de	151.7 d	166.2 de	5.04 bc
F2427		6.48 ab	5.29 a	159.0 cd	175.7 d	4.59 d
F2424		6.62 a	4.81 b	164.0 bc	181.0 c	4.61 d
F2420		6.38 b	4.30 c	158.4 cd	175.6 d	4.74 d
F2419		6.17 c	4.32 c	155.4 d	174.8 d	4.79 d
F2210		4.05 f	4.04 d	131.4 bc	152.6 bc	5.49 ab
F2256		4.14 ef	4.05 d	135.0 b	159.5 a	5.41 ab
F2367		4.04 f	4.03 d	141.4 a	160.6 a	5.35 bc
F2378		4.27 e	4.07 d	126.0 cd	150.9 cd	5.65 a
F2505		5.06 d	4.07 d	143.0 a	159.1 ab	5.12 dc
F2497	Year 2	5.21 d	4.09 d	106.4 g	127.5 g	4.92 de
F2496		5.07 d	4.08 d	89.42 h	116.5 h	4.58 fg
F2414		5.11 d	4.07 d	116.6 f	130.9 g	4.85 ef
F2427		6.41 a	5.21 ab	119.5 ef	137.0 fg	4.49 g
F2424		6.38 a	5.43 a	134.1 b	145.9 de	4.47 g
F2420		5.93 b	4.93 bc	124.9 de	143.8 e	4.78 ef
F2419		5.51 c	4.56 c	120.5 def	141.0 f	4.80 ef

Mean values with the same letter are not significantly different at 5% level of probability.

Appendix 2, Table 2, continued : Genotype X year interaction mean values.

Genotypes	Year	STL	STT	NST	NIN	NBR	DMY
F2210		71.09 b	4.48 a	34.10 bcd	7.26 cd	5.19 de	261.6 c
F2256		69.97 b	4.57 a	52.80 a	8.13 c	5.68 d	408.2 a
F2367		83.29 a	4.42 a	47.51 ab	7.92 c	5.67 d	389.0 ab
F2378		77.38 ab	4.12 b	41.51 abc	7.91 c	6.09 d	344.6 b
F2505		76.32 ab	3.26 c	23.60 de	10.19 b	8.39 c	404.3 a
F2497	Year 1	53.25 cd	3.13 c	28.23 cde	6.42 d	5.64 d	140.0 d
F2496		44.73 def	3.32 c	31.40 bcde	5.39 e	4.56 e	71.83 c
F2414		65.89 bc	3.28 c	41.43 abc	7.67 c	6.59 d	207.5 cd
F2427		48.20 cde	1.32 e	22.60 de	11.45 a	10.50 a	168.9 d
F2424		37.52 f	1.29 e	15.94 e	12.23 a	10.10 ab	154.3 d
F2420		41.77 ef	1.98 d	23.07 de	9.65 b	8.51 c	131.9 d
F2419		56.62 c	1.42 e	25.01 cde	10.36 b	9.30 bc	178.9 d
F2210		45.91 bcd	4.38 a	63.30 f	5.39 g	1.60 cd	174.5 cd
F2256		35.63 e	4.18 a	61.67 f	5.88 fg	2.68 ab	239.6 b
F2367		48.13 abc	4.17 a	67.38 ef	6.84 e	1.48 d	199.4 bc
F2378		38.26 de	3.81 b	83.22 de	5.86 fg	2.57 abc	225.4 b
F2505		55.55 a	3.01 c	91.32 cd	8.22 d	3.00 a	322.5 a
F2497	Year 2	39.07 cde	2.29 e	117.5 ab	8.01 d	2.85 ab	113.1 ef
F2496		30.07 ef	2.11 ef	38.55 g	6.47 ef	1.79 bcd	47.14 g
F2414		50.03 ab	2.71 d	106.9 abc	8.54 cd	3.23 a	146.1 de
F2427		26.18 f	2.00 fg	123.2 a	10.33 b	3.29 a	70.95 fg
F2424		22.57 f	1.94 g	95.03 cd	12.11 a	2.96 a	83.20 fg
F2420		25.35 f	2.20 ef	101.2 bcd	9.31 c	2.96 a	143.4 de
F2419		30.81 ef	2.24 e	92.10 cd	9.18 c	2.93 a	68.62 fg

Mean values with the same letter are not significantly different at 5% level of probability.

Appendix 2, Table 3 : Genotype X environment interaction mean values.

Genotype	Envir.	PRO	NRT	FFW	MFW	LSZ
F2210		4.01 e	3.88 de	170.2 ab	185.7 ab	5.21 abc
F2256		3.99 e	3.92 de	174.8 a	186.9 a	5.32 ab
F2367		4.08 e	4.02 cd	174.2 a	186.8 a	5.48 a
F2378		4.09 e	3.91 de	169.9 ab	187.2 a	5.47 a
F2505	site 1	5.26 d	4.19 bcd	163.6 bc	182.4 bc	5.32 ab
F2497		5.41 cd	3.58 e	139.7 e	164.0 e	4.89 cd
F2496	Year 1	5.64 c	3.96 de	128.8 e	149.9 f	4.86 d
F2414		5.43 cd	3.93 de	151.6 d	168.5 e	4.94 bc
F2427		6.64 a	5.12 a	156.9 cd	173.3 cde	4.64 d
F2424		6.60 a	4.59 b	160.2 c	181.1 cd	4.70 d
F2420		6.11 b	4.28 bcd	156.1 d	173.1 de	4.70 d
F2419		6.17 b	4.42 bc	150.1 d	172.0 de	4.80 d
F2210		4.01 d	4.04 c	134.0 ab	151.8 abc	5.86 ab
F2256		4.08 d	4.06 c	135.3 ab	158.3 a	5.58 b
F2367		4.04 d	4.07 c	137.0 ab	153.2 ab	5.48 bc
F2378	Site 1	4.16 d	4.01 c	123.9 cd	151.8 ab	6.05 a
F2505		4.93 c	4.09 c	138.8 a	156.8 a	5.28 cd
F2497	Year 2	5.01 c	4.04 c	103.9 f	120.2 ef	5.07 de
F2496		5.03 c	4.08 c	86.0 g	111.1 f	4.65 fg
F2414		5.08 c	4.04 c	115.0 e	128.7 de	4.80 efg
F2427		6.42 a	5.45 a	115.1 e	130.8 d	4.51 g
F2424		6.32 a	5.29 a	129.5 bc	143.1 c	4.53 g
F2420		5.64 b	4.73 b	121.0 cde	144.5 bc	4.91 def
F2419		5.46 b	4.59 b	115.5 ed	127.8 e	4.91 def
F2210		3.96 d	3.96 c	169.3 bc	180.3 c	5.57 a
F2256		4.01 d	3.96 c	182.1 a	192.2 ab	5.18 bc
F2367		3.96 d	3.96 c	181.9 a	195.7 a	5.37 ab
F2378		4.11 d	3.96 c	184.0 a	180.8 c	5.49 a
F2505	Site 2	5.15 c	3.93 c	171.3 b	186.1 bc	5.23 ab
F2497		5.21 c	3.92 c	145.3 e	156.8 de	5.26 ab
F2496	Year 1	5.23 c	3.96 c	130.9 f	154.7 e	5.04 bcd
F2414		5.36 c	3.98 c	151.8 d	163.8 d	5.17 bc
F2427		6.32 b	5.46 a	161.2 c	178.2 c	4.55 e
F2424		6.64 a	5.04 b	167.9 bc	180.9 c	4.52 e
F2420		6.64 a	4.33 c	160.7 c	178.1 c	4.79 cde
F2419		6.18 b	4.21 c	160.7 c	177.5 c	4.78 de
F2210		4.08 e	4.04 e	128.8 de	153.4 bc	5.11 ab
F2256		4.20 de	4.04 e	134.7 cd	160.8 ab	5.23 a
F2367		4.04 e	3.99 e	145.7 ab	167.9 a	5.22 a
F2378		4.39 d	4.13 de	128.1 de	149.9 cd	5.24 a
F2505	Site 2	5.18 c	4.06 e	147.3 a	161.5 ab	4.96 abc
F2497		5.40 bc	4.14 de	108.9 g	134.8 ef	4.76 bcde
F2496	Year 2	5.11 c	4.07 de	92.83 h	121.9 g	4.52 de
F2414		5.15 c	4.10 de	118.2 f	133.1 f	4.91 abce
F2427		6.40 a	4.96 bc	124.0 ef	143.3 de	4.47 e
F2424		6.43 a	5.56 a	138.7 bc	148.7 cd	4.41 e
F2420		6.21 a	5.14 ab	128.5 de	143.1 de	4.64 cde
F2419		5.56 b	4.52 cd	125.4 ef	154.3 bc	4.70 bcde

Mean values with the same letter are not significantly different at 5% level of probability.

Appendix 2, Table 3, continued: Genotype-environment interaction mean values.

Genotype	Envir.	STL	STT	NST	NIN	NBR	DMY
F2210		83.67 ab	5.00 a	42.13 abc	7.69 ef	5.49 de	388.5 b
F2256		84.67 a	4.90 a	53.13 a	8.69 cde	6.91 cd	527.5 a
F2367		88.58 a	4.97 a	53.08 ab	8.42 cde	5.99 cde	504.6 a
F2378		82.27 ab	4.77 a	47.47 ab	8.93 cd	7.35 c	513.7 a
F2505	site 1	91.97 a	3.52 b	17.60 cd	10.23 b	9.20 b	545.0 a
F2497		68.17 c	3.17 b	27.72 bcd	7.04 f	6.73 cd	210.3 cd
F2496	Year 1	53.33 def	3.53 b	35.27abcd	5.61 g	5.02 e	102.2 e
F2414		71.87 bc	3.47 b	37.40abcd	7.90 de	6.98 c	262.7 c
F2427		60.07 cde	1.27 d	21.93 cd	12.42 a	11.75 a	201.5 cd
F2424		45.40 f	1.37 d	15.60 d	12.38 a	10.96 a	199.9 cd
F2420		47.53 ef	2.13 c	30.27abcd	8.32 de	7.37 c	162.9 de
F2419		64.47 cd	1.37 d	30.93abcd	9.60 bc	8.94 b	214.9 cd
F2210		45.47 cde	4.22 ab	76.20 cde	5.49 g	1.43 d	191.3 cd
F2256		40.40 def	4.18 ab	64.60 de	6.71 fg	2.96 abc	276.5 b
F2367		56.07 bc	4.42 a	87.17 cd	7.96 de	1.53 cd	202.4 cd
F2378	Site 1	43.40 cde	4.05 b	93.33 bc	7.09 ef	2.60 abcd	272.2 b
F2505		74.63 a	3.25 c	89.68 cd	5.58 g	3.22 ab	525.6 a
F2497	Year 2	49.25 cd	2.60 d	118.3 ab	9.87 bc	3.34 ab	170.0 de
F2496		39.87 defg	2.24 f	51.60 e	6.90 ef	2.17 bcd	72.60 f
F2414		64.87 ab	2.38 de	124.1 a	8.41 d	3.68 a	190.5 cd
F2427		27.20 gh	2.14 f	138.3 a	10.07 b	3.45 ab	106.2 ef
F2424		26.47 h	1.79 g	92.20 c	12.71 a	3.63 a	118.5 ef
F2420		29.13 fgh	2.26 f	120.7 a	8.65 cd	3.21 ab	247.9 bc
F2419		33.47 efgh	2.33 de	94.53 bc	8.55 d	3.17 ab	89.73 f
F2210		58.51 c	3.97 ab	26.07 bc	6.83 cd	4.88 c	135 bcde
F2256		55.27 c	4.23 a	52.47 a	7.57 c	4.45 c	289.0 a
F2367		78.00 a	3.87 b	41.93 ab	7.43 c	5.35 c	273.5 a
F2378		72.50 ab	3.47 c	35.55 abc	6.89 cd	4.82 c	175.5 b
F2505	Site 2	60.67 bc	3.00 d	29.60 abc	10.16 b	7.57 b	263.6 a
F2497		38.33 de	3.10 d	28.73 abc	5.81 de	4.54 c	69.80 de
F2496	Year 1	36.13 de	3.10 d	27.53 bc	5.17 e	4.10 c	41.47 e
F2414		59.92 c	3.10 d	45.46 ab	7.43 c	6.38 bc	152.2 bc
F2427		36.33 de	1.38 f	23.27 bc	10.48 b	9.24 a	136.3 bcd
F2424		29.63 e	1.21 f	16.28 c	12.08 a	9.24 a	108.6 cde
F2420		36.00 de	1.82 e	15.88 c	10.98 ab	9.65 a	100.8 de
F2419		48.77 cd	1.47 f	19.08 bc	11.12 ab	9.66 a	142.9 bc
F2210		46.35 a	4.54 a	50.40 ef	5.28 ef	1.76 ab	157.7 abc
F2256		30.87 bcde	4.17 b	58.75 de	5.06 ef	2.39 ab	202.7 a
F2367		40.20 a	3.91 b	47.60 ef	5.72 ef	1.43 b	196.4 a
F2378		33.12 abcd	3.57 c	73.10 cde	4.63 f	2.55 ab	178.5 ab
F2505	Site 2	36.47 ab	2.77 d	92.97 abc	10.86 ab	2.77 ab	119.4 bcd
F2497		28.88 bcde	1.97 e	116.6 a	6.14 e	2.36 ab	56.30 def
F2496	Year 2	20.28 de	1.99 e	25.50 f	6.03 e	1.42 b	21.69 f
F2414		35.20 abc	3.05 d	89.67 bc	8.67 d	2.79 ab	101.7 cde
F2427		25.17 cde	1.86 e	108.1 ab	10.60 abc	3.13 a	35.70 ef
F2424		18.67 e	2.10 e	97.87 abc	11.51 a	2.29 ab	47.93 ef
F2420		21.57 de	2.15 e	81.73 cd	9.96 bc	2.71 ab	38.90 ef
F2419		28.15 bcde	2.15 e	89.67 bc	9.82 cd	2.69 ab	47.50 ef

Mean values with the same letter are not significantly different at 5% level of probability.

Appendix 3 : Experimental mean squares, (their corresponding F values) from pooled analysis of variance of twelve genotypes at two sites over two years.

Traits	Site (S)	Block	Genotype (G)	G x S	Error a	Year (Y)
	1[1]	4	11	11	44	1
Prostrateness	0.4594 ns (0.27)	0.7142 ns (1.93)	50.8113 ** (36.28)	0.5771 ns (1.48)	0.3707 ** (2.66)	4.3002 ns (1.43)
Nodal rooting	0.2086 ns (0.81)	0.5534 ns (1.62)	12.5323 ** (11.82)	0.3846 ns (1.19)	0.3416 ns (1.04)	7.2952 ** (9.91)
First flowering	6535.3 ** (12.2)	369.82 ns (1.6)	11970.7 ** (15.71)	155.47 ns (0.85)	230.78 * (1.81)	236711 ** (364.5)
50% flowering	4319.4 ns (2.35)	140.11 ns (0.71)	9186.2 ** (15.02)	410.9 ns (1.24)	197.28 ns (1.3)	188478 ** (111)
Leaf size	2.2016 ns (0.46)	0.8802 * (3.61)	7.2265 ** (10.59)	0.3389 ns (0.91)	0.2437 ns (0.87)	0.6531 ns (0.20)
Stem thickness	14.376 ns (2.48)	0.6622 * (2.86)	66.9568 ** (9.32)	1.6076 ns (1.24)	0.2312 ns (1.22)	3.1724 ns (0.45)
No. of stems	24429 ns (2.7)	1832.8 ns (1.46)	6427.05 ns (0.39)	1871.4 ns (1.68)	1256.1 ns (1.11)	534991 ** (24.4)
stem length	47676 * (17.5)	313.86 ns (0.88)	9150.18 ** (4.84)	835.04 ns (1.33)	355.44 ns (1.18)	91823.1 ** (33.3)
No. of internodes	28.854 ns (1.11)	6.3304 ns (1.53)	217.055 ** (3.81)	36.313 ns (1.95)	4.1301 ns (1.48)	84.838 ** (3.83)
No. of branches	115.40 ns (4.82)	4.4561 ns (1)	95.041 ns (2.11)	9.4128 ns (1.03)	4.428 ns (1.15)	3675.53 ** (70.2)
Dry matter yield	304977 * (12.5)	17969.5 ns (1.7)	562515 ** (4.26)	94202 ns (1.47)	10.553 ns (1.3)	1305201 ** (7.24)

1 : degree of freedom

Appendix 3; continued : Experimental mean squares, (their corresponding F values) from pooled analysis of variance of twelve genotypes at two sites over two years.

Traits	S * Y	G x Y	G x S x Y	Error b	Within plot
	1	11	11[1]	48	576
Prostrateness	2.2522 ** (20.05)	0.8265 ** (7.36)	0.1123 ns (0.81)	0.1395 ** (2.54)	0.0549
Nodal rooting	0.0645 ns (0.25)	0.6973 ns (2.7)	0.2585 ns (0.78)	0.3294 ** (3.41)	0.0967
First flowering	36.7974 ns (0.36)	612.88 ** (5.96)	102.76 ns (0.81)	127.27 ns (1.16)	109.29
50% flowering	1481.55 * (5.77)	217.94 ns (0.85)	256.74 ns (1.69)	152.04 ** (2.05)	74.257
Leaf size	5.1182 ** (11.6)	0.3851 ns (0.88)	0.4398 ns (1.57)	0.2796 ** (4.31)	0.0648
Stem thickness	4.1127 ns (3.38)	5.7075 ** (4.69)	1.2157 ** (6.43)	0.1891 * (1.51)	0.1252
No. of stems	6004.18 ** (11.4)	15979 ** (30.35)	526.45 ns (0.47)	1129.2 ** (2.13)	529.45
stem length	1617.16 ns (3.26)	1156.5 ns (2.33)	495.83 ns (1.65)	300.44 ** (2.32)	129.44
No. of internodes	1.3696 ns (0.09)	24.904 ns (1.57)	15.907 ** (5.7)	2.7874 ** (1.88)	1.4813
No. of branches	12.7635 ns (1.51)	39.712 ** (4.71)	8.426 ** (2.18)	3.8607 ** (2.5)	1.5457
Dry matter yield	136759 ns (2.32)	51564 ns (0.87)	59017 ** (7.25)	8135.2 * (1.49)	5470.7

1 : degree of freedom

Appendix 4, Table 1: Phenotypic and genotypic correlation between traits for three genotypes in erect type. The phenotypic correlations are above the diagonal and genotypic correlations are below the diagonal.

Trait	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR	DMY
PRO		0.172**	-0.207**	-0.136*	-0.045 ns	-0.181**	-0.103 ns	0.217**	-0.132*	-0.123 ns	-0.068 ns
NRT	0.199		-0.281**	-0.280**	0.092 ns	-0.225**	-0.156*	0.253**	-0.177**	-0.179**	-0.106 ns
FFW	-0.396	0.774		0.823**	-0.172**	0.563**	0.118 ns	-0.445**	0.451**	0.622**	0.330**
MFW	-0.442	0.692	0.989		-0.181**	0.418**	0.201**	-0.372**	0.466**	0.580**	0.367**
LSZ	-0.709	-0.152	-0.728	-0.816		0.007 ns	0.009 ns	0.238**	0.058 ns	0.026 ns	0.110 ns
STL	-0.346	0.469	0.392	0.276	0.076		0.087 ns	-0.260**	0.411**	0.668**	0.472**
STT	-0.947	-0.378	0.283	0.372	-0.778	0.026		0.112 ns	0.525**	0.214**	0.551**
NST	0.667	0.840	0.418	0.356	0.139	0.025	-0.723		-0.006 ns	-0.346**	0.165*
NIN	0.156	0.985	0.834	0.775	-0.288	0.352	-0.293	0.838		0.618**	0.589**
NBR	0.836	0.241	-0.107	-0.085	0.224	-0.661	-0.667	0.713	0.296		0.533**
DMY	0.261	0.734	0.698	0.715	-0.450	-0.217	-0.210	0.800	0.831	0.634	

ns = non-significant * = significant at the 5% level of probability ** = significant at the 1% level of probability.

Appendix 4, Table 2 : Phenotypic and genotypic correlation between traits for three genotypes in semi-erect type. The phenotypic correlations are above the diagonal and genotypic correlations are below the diagonal.

Trait	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR	DMY
PRO		-0.157 *	0.207 **	0.261 **	-0.119 ns	0.092 ns	0.268 **	-0.263 **	-0.137 *	0.295 **	-0.112 ns
NRT	-0.598		-0.139 *	-0.150 *	0.012 ns	-0.057 ns	-0.157 *	0.215 **	0.115 ns	-0.226 **	0.051 ns
FFW	-0.829	0.528		0.929 **	0.437 **	0.500 **	0.584 **	-0.358 **	0.275 **	0.675 **	0.437 **
MFW	-0.906	0.591	0.987		0.421 **	0.521 **	0.606 **	-0.440 **	0.216 **	0.724 **	0.462 **
LSZ	-0.869	0.308	0.943	0.946		0.394 **	0.422 **	-0.101 ns	0.156 *	0.403 **	0.403 **
STL	-0.746	0.507	0.991	0.958	0.905		0.483 **	-0.067 ns	0.344 **	0.695 **	0.790 **
STT	-0.680	0.732	0.921	0.896	0.739	0.942		-0.361 **	0.022 ns	0.550 **	0.430 **
NST	-0.039	-0.506	0.424	0.309	0.522	0.476	0.216		0.229 **	-0.396 **	0.034 ns
NIN	-0.777	0.442	0.994	0.965	0.945	0.994	0.901	0.521		0.360 **	0.311 **
NBR	-0.725	0.411	0.984	0.943	0.925	0.994	0.902	0.563	0.997		0.488 **
DMY	-0.929	0.608	0.976	0.998	0.943	0.939	0.880	0.267	0.949	0.922	

ns = non-significant * = significant at the 5% level of probability ** = significant at the 1% level of probability.

Appendix 4, Table 3 : Phenotypic and genotypic correlation between traits for three genotypes in prostrate type. The phenotypic correlations are above the diagonal and genotypic correlations are below the diagonal.

Trait	PRO	NRT	FFW	MFW	LSZ	STL	STT	NST	NIN	NBR	DMY
PRO		0.318**	0.436**	0.372**	-0.406**	-0.005 ns	-0.454**	-0.255**	0.457**	0.347**	-0.054 ns
NRT	0.938		-0.099 ns	-0.194**	-0.404**	-0.328**	-0.088 ns	0.286**	0.220**	-0.178**	-0.163*
FFW	0.680	0.426		0.928**	-0.101 ns	0.424**	-0.535**	-0.767**	0.319**	0.756**	0.296**
MFW	0.476	0.179	0.966		-0.035 ns	0.490**	-0.524**	-0.794**	0.234**	0.773**	0.371**
LSZ	-0.937	-0.985	-0.516	-0.279		0.290**	0.162*	-0.081 ns	-0.144*	0.129*	0.306**
STL	-0.786	-0.527	-0.867	-0.804	0.533		-0.260**	-0.471**	0.124 ns	0.676**	0.656**
STT	-0.602	-0.719	-0.403	-0.219	0.811	0.134		0.499**	-0.403**	-0.575**	-0.187**
NST	0.104	0.358	-0.652	-0.804	-0.220	0.310	0.034		-0.148*	-0.732**	-0.228**
NIN	0.815	0.772	0.776	0.616	-0.866	-0.572	-0.882	-0.278		0.466**	0.115 ns
NBR	0.665	0.857	0.169	-0.070	-0.884	-0.080	-0.914	0.366	0.741		0.479**
DMY	-0.429	-0.566	-0.315	-0.163	0.675	-0.027	0.979	0.104	-0.805	-0.851	

ns = non-significant 0.938 * = significant at the 5% level of probability ** = significant at the 1% level of probability.

Appendix 5: HOMINO programme for analysing generation means, estimating number of genes and heritability.

PURPOSE AND DESCRIPTION:

The programme, written in QBASIC language by the author, reads the data recorded on individual plants and estimates the three parameters model, *m, d,* and *h*, based on a method presented by Mather and Jinks (1982) and modified by Gordon (unpublished lecture notes). Then it tests the model by both simple and joint scaling test. It also transforms the original data to log and square root and estimates the same parameters based on the transformed data. In all three cases, original scale, log, and square root, it calculates the observed and expected generation means, within family variance, standard errors to test the significance of the parameters in the model, and simple scales. It also estimates heritability and its standard error based on the procedure presented by Ketata (1976), the difference between mid-parent and F_1 (F_1 - Mp) as an indication of heterosis and an appropriate t value. The expected genetic advance based on selecting of 5% of the most desirable individual plants between F_2 family. Then it estimates the number of genes control the attribute under question and the appropriate standard error based on a procedure presented by Lande (1981) and Cockerham (1986). Then it estimates six parameter model and the corresponding standard errors based on the original scale. Then it prompts the user to indicate if six parameter model is enough or not. It gives various options to choose from. It estimates some other parsimonious models or what is called the best fitted model (Mather and Jinks 1982), according to the option selected by the user which is based on the standard error of the estimated parameters. The programme can read in more than one attributes at a time and print the results in an output file.

INSTRUCTIONS:

The programme first prompts for the path and the name of the file that contains the original data. The data file can be format free but the first column have to be the generations indices in the order of 1 for P_1, 2 for P_2, 3 for F_1, 4 for F_2, 5 for Bc_1, and 6 for Bc_2. The rest of the columns have to be the characters recorded. The data file also have to be sorted in ascending order based on the generation indices without any

identification. Therefore, a data file containing 5 attributes recorded on six generations have to be set up as follows:

1	5	120	10	30	55
1	6	115	10	29	58
1	5.5	121	9	31	61
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
4	3	101	6	21	39
4	6	125	10	31	67
4	9	113	12	36	38
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
6	4	99	8	20	50
6	3	100	9	23	49
6	3.5	96	7.5	28	54

Then the programme prompts for the path and name of the output file, the number of rows (entries) in the input file, since the user may have several sets of crosses to analyze, the programme prompts for the number of sets. To facilitate proper input, the user will then be prompted to indicate how many characters are included in the data set (in the case of former example, 5). As soon as these information are received by the programme it would print the appropriate results into the out put file. It also prints the six parameter model, and related parameters including standard errors of the parameters, on the screen in order to facilitate the selecting the options for the best fitted model. The same process would be carried out for all the attributes recorded in the data file.

PROGRAMME LISTING

```
    G$ "============================================================"
    CLS : LOCATE 1, 8: PRINT G$
    LOCATE 2, 24: PRINT "          HOMINO          ": PRINT
    LOCATE 4, 21: PRINT "Programme for generation mean analysis"
    LOCATE 5, 13: PRINT "The programme has used Hayman Generation Mean Analysis"
    LOCATE 6, 17: PRINT "Following the procedure described by Mather and Jinks, 1982"
    LOCATE 7, 22: PRINT "Written by HOSSEIN MIRZAIE-NODOUSHAN"
    LOCATE 8, 8: PRINT G$
    LOCATE 15, 20: PRINT "Press any key to continue"
    WHILE INKEY$ = "": WEND: CLS
    LOCATE 3, 5: INPUT "Enter the path for input data file:    "; INP$
LOCATE 5, 10: INPUT "Enter the path for output file:    "; OUT$
    LOCATE 7, 15: INPUT "How many entries?  "; ENT
    LOCATE 9, 20: INPUT "which set?  "; DIS$
    LOCATE 11, 25: INPUT "How many characters"; var: CLS
    LOCATE 12, 18: PRINT " It may take a while. Please wait"
    cha = var + 1
    DIM DOU(ENT, cha), MAI(ENT, cha), EE(6, 6), pp(6), EA(6, 6)
    DIM AA(6, 6), BB(6, 6), W(6, 6), QQ(6, 6), XY(6, 6), W%(6, 3)
    OPEN INP$ FOR INPUT AS #1
    OPEN OUT$ FOR OUTPUT AS #2
    PRINT #2, "          ANALYSIS OF GENERATION MEAN DATA BY HOMINO PROGRAMME":
PRINT #2,
    PRINT #2, "              WRITTEN BY HOSSEIN MIRZAIE NODOUSHAN"
    PRINT #2,
    FOR I = 1 TO ENT
    FOR J = 1 TO cha
    INPUT #1, MAI(I, J)
    NEXT: NEXT
    FOR I = 1 TO 6
    READ PAR$(I): READ GEN$(I): NEXT
    DATA "M ","P1","A ","P2","D ","F1","AA","F2","AD","B1","DD","B2"
    FOR I = 1 TO 6
    FOR J = 1 TO 6
    READ EE(I, J)
    NEXT
```

```
NEXT
DATA 1,1,0,1,0,0,1,-1,0,1,0,0,1,0,1,0,0,1,1,0, .5,0,0,.25,1,.5,.5,.25,.25,.25,1,-.5,.5,.25,-.25,.25
FOR I = 1 TO ENT
FOR J = 1 TO cha
DOU(I, J) = MAI(I, J)
NEXT: NEXT
FOR tv = 2 TO cha
PRINT #2,
PRINT #2, "TIME:"; TIME$; "  DATE:"; DATE$; "          THE HOMINO PROGRAMME": PRINT #2,
FOR I = 1 TO 6
FOR J = 1 TO 6
EA(I, J) = EE(I, J)
NEXT: NEXT
CH = tv - 1
IP = 3
FOR T = 1 TO 3
GOSUB 1400
pp(1) = B(1) + B(2)
pp(2) = B(1) - B(2)
pp(3) = B(1) + B(3)
pp(4) = B(1) + .5 * B(3)
pp(5) = B(1) + .5 * B(2) + .5 * B(3)
pp(6) = B(1) - .5 * B(2) + .5 * B(3)
REM
CHI = 0
FOR I = 1 TO 6
CHI = CHI + ((R(I) - pp(I)) ^ 2) * (E(I))
NEXT
PRINT #2,
PRINT #2, "                  "; DIS$; "      CHARACTER NUMBER "; CH: PRINT #2,
PRINT #2, G$
PRINT #2, "GEN.  No.O.P. Variance      1/Vxbar          Ob.mean       Ex.mean"
PRINT #2, G$
FOR I = 1 TO 6
PRINT #2, GEN$(I); NU(I), VV(I), E(I), R(I), pp(I)
NEXT
PRINT #2, G$: PRINT #2, "          THREE PARAMETERS MODEL"
PRINT #2, "              Parameter    Signi.          S.E. "
```

```
PRINT #2, G$
FOR I = 1 TO IP
BK(I) = B(I)
NEXT
GOSUB 1540
FOR I = 1 TO IP
TTES(I) = ABS(BK(I)) / SD(I)
NEXT
FOR I = 1 TO IP
IF TTES(I) < 1.29 THEN TTTE$(I) = "ns "
IF TTES(I) > 1.29 THEN TTTE$(I) = "(*)"
IF TTES(I) > 1.64 THEN TTTE$(I) = "** "
IF TTES(I) > 2.33 THEN TTTE$(I) = "*** "
NEXT
AZ(1) = 2 * R(5) - R(1) - R(3)
VAZ = 4 * VM(5) + VM(1) + VM(3)
SVAZ(1) = SQR(VAZ)
TVAZ = ABS(AZ(1)) / SVAZ(1)
IF TVAZ < 1.29 THEN TVAZ$(1) = "ns "
IF TVAZ > 1.29 THEN TVAZ$(1) = "(*)"
IF TVAZ > 1.64 THEN TVAZ$(1) = "** "
IF TVAZ > 2.33 THEN TVAZ$(1) = "*** "
AZ(2) = 2 * R(6) - R(2) - R(3)
VBZ = 4 * VM(6) + VM(2) + VM(3)
SVAZ(2) = SQR(VBZ)
TVBZ = ABS(AZ(2)) / SVAZ(2)
IF TVBZ < 1.29 THEN TVAZ$(2) = "ns "
IF TVBZ > 1.29 THEN TVAZ$(2) = "(*)"
IF TVBZ > 1.64 THEN TVAZ$(2) = "** "
IF TVBZ > 2.33 THEN TVAZ$(2) = "*** "
AZ(3) = 4 * R(4) - R(1) - R(2) - 2 * R(3)
VCZ = 16 * VM(4) + VM(1) + VM(2) + 4 * VM(3)
SVAZ(3) = SQR(VCZ)
TVCZ = ABS(AZ(3)) / SVAZ(3)
IF TVCZ < 1.29 THEN TVAZ$(3) = "ns "
IF TVCZ > 1.29 THEN TVAZ$(3) = "(*)"
IF TVCZ > 1.64 THEN TVAZ$(3) = "** "
IF TVCZ > 2.33 THEN TVAZ$(3) = "*** "
```

```
FOR I = 1 TO IP
PRINT #2, PAR$(I); " =", BK(I), TTTE$(I), SD(I)
NEXT
PRINT #2, G$
PRINT #2,
PRINT #2, "Simple scaling tests results"
PRINT #2, G$
PRINT #2, " Scale                Signi.                S.E."
PRINT #2, G$
FOR I = 1 TO IP
PRINT #2, AZ(I), , TVAZ$(I), , SVAZ(I)
NEXT
PRINT #2, G$
PRINT #2,
PRINT #2, "NUMBER OF GENES AND ITS STANDARD ERROR BASED ON EQUATIONS"
PRINT #2, "      PRESENTED BY LANDE, (1981) AND COCKERHAM, (1986)."
SIGNG = ABS(NGEN)/SENG
IF SIGNG < 1.29 THEN SIGG$ = "ns "
IF SIGNG > 1.29 THEN SIGG$ = "(*)"
IF SIGNG > 1.64 THEN SIGG$ = "** "
IF SIGNG > 2.33 THEN SIGG$ = "*** "
PRINT #2, G$
PRINT #2, "  NO. OF GENES     SIGNIFICANCE     STANDARD ERROR"
PRINT #2, G$
PRINT #2, NGEN, SIGG$ , SENG
PRINT #2, G$
PRINT #2,
PRINT #2,
PRINT #2, " The joint scaling test results, chi-square=  "; CHI
PRINT #2,
IF T > 1 GOTO 100
Hns = (2 * VV(4) - (VV(5) + VV(6))) / VV(4)
VHns1 = ((VV(5) + VV(6)) ^ 2 / (NU(4) - 1))
VHns2 = ((VV(5) ^ 2) / (NU(5) - 1)) + ((VV(6) ^ 2) / (NU(6) - 1))
VHns = (2 * (VHns1 + VHns2)) / (VV(4) ^ 2)
SEHns = SQR(VHns)
GS = 2.06 * SQR(VV(4)) * Hns
VIG = R(3) - ((R(1) + R(2)) / 2)
```

```
    TVIG = VIG / (SQR(VV(1) + VV(2) + VV(3)))
    DFT = NU(1) + NU(2) + NU(3) - 3
    PRINT #2, : PRINT #2,
    PRINT #2, "Heritability narrow sense ="; Hns
    PRINT #2, "Standard error of heritability ns="; SEHns
    PRINT #2, "F1's mean - mp ="; VIG
    PRINT #2, "t value for F1-MP="; TVIG
    PRINT #2, "df for the above t value ="; DFT
    PRINT #2, "Genetic advance for k=2.06 (sellecting"
    PRINT #2, "the most 5% desirable of the F2 plants)="; GS
    PRINT #2, : PRINT #2,
    IF T > 1 THEN GOTO 100
    PRINT "FOR CHARACTER No."; CH; "CHI-SQUARE FOR THREE PARAMETER MODEL = "; CHI
    FOR I = 1 TO ENT
    DOU(I, tv) = LOG(MAI(I, tv))
    DOU(I, 1) = MAI(I, 1)
    NEXT
    PRINT #2, "DATA WERE TRANSFORMED TO LOG, THEN": PRINT #2,
    GOTO 200
100 IF T > 2 GOTO 200
    FOR I = 1 TO ENT
    DOU(I, tv) = SQR(MAI(I, tv))
    DOU(I, 1) = MAI(I, 1)
    NEXT
    PRINT #2, "DATA WERE TRANSFORMED TO SQUARE ROOT, THEN": PRINT #2,
    GOTO 200
    PRINT "Still calculated chi-square is bigger than tabulated"
    PRINT "chi-square. Therefore, either additive dominance model"
    PRINT "is not adequate for these data or the data have to be"
    PRINT "transformed to other scales except log and square root"
200 NEXT T
    IP = 6
    FOR I = 1 TO ENT
    DOU(I, tv) = MAI(I, tv)
    DOU(I, 1) = MAI(I, 1)
    NEXT
    GOSUB 1400
    FOR I = 1 TO IP
```

```
BK(I) = B(I)
SDK(I) = SD(I)
NEXT
GOSUB 1540
FOR I = 1 TO 6
IF SDK(I) = 0 THEN GOTO 300
TTES(I) = ABS(BK(I)) / SDK(I)
00 NEXT
FOR I = 1 TO 6
IF TTES(I) < 1.29 THEN TTTE$(I) = "ns "
IF TTES(I) > 1.29 THEN TTTE$(I) = "(*)"
IF TTES(I) > 1.64 THEN TTTE$(I) = "* "
IF TTES(I) > 2.33 THEN TTTE$(I) = "** "
NEXT
PRINT #2,
PRINT #2, G$: PRINT #2, "            FULL PARAMETER MODEL"
PRINT #2, G$
PRINT #2, "          parameter  Signi.  t value     df          S.E."
PRINT #2, G$
PRINT G$
PRINT " parameter Sig. t value  df  S.E."
PRINT G$
FOR I = 1 TO 6
T(I) = F(I) / SD(I)
PRINT PAR$(I); B(I); TTTE$(I); T(I); DF(I); SD(I)
PRINT #2, PAR$(I), B(I), TTTE$(I), T(I), DF(I), SD(I)
NEXT
PRINT #2,
PRINT #2, G$
PRINT #2, "CHI-SQUARE IS NOT CALCULABLE IN THIS CASE": PRINT #2,
PRINT #2, G$: PRINT #2, : PRINT
400 PRINT "Which parameter(s) would you like to delete?": PRINT
PRINT "Enter"
PRINT "1 for AD and DD     2 for AA and DD     3 for AA and AD"
PRINT "4 for DD          5 for AD          6 for AA"
PRINT "7 for D, DD, and AD  8 for D and AA     9 for nothing"
INPUT GA
IF GA = 1 GOTO 500
```

```
    IF GA = 2 GOTO 600
    IF GA = 3 GOTO 700
    IF GA = 4 GOTO 800
    IF GA = 5 GOTO 900
    IF GA = 6 GOTO 1100
    IF GA = 7 GOTO 1120
    IF GA = 8 GOTO 1000
    IF GA = 9 GOTO 1380
BEEP: GOTO 400
500  IP = 4
    FOR J = 1 TO 4
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    NEXT: NEXT
    GOTO 1140
600  IP = 4
    FOR J = 1 TO 4
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    IF J = 4 THEN EA(I, J) = EE(I, J + 1)
    NEXT: NEXT
    GOTO 1140
700  IP = 4
    FOR J = 1 TO 4
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    IF J = 4 THEN EA(I, J) = EE(I, J + 2)
    NEXT: NEXT
    GOTO 1140
800  IP = 5
    FOR J = 1 TO 5
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    NEXT: NEXT
    GOTO 1140
900  IP = 5
    FOR J = 1 TO 5
    FOR I = 1 TO 6
```

```
    EA(I, J) = EE(I, J)
    IF J = 5 THEN EA(I, J) = EE(I, J + 1)
    NEXT: NEXT
    GOTO 1140
1000 IP = 2
    FOR J = 1 TO 2
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    NEXT: NEXT
    GOTO 1140
1100 IP = 5
    FOR J = 1 TO 5
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    IF J = 4 THEN EA(I, J) = EE(I, J + 1)
    IF J = 5 THEN EA(I, J) = EE(I, J + 1)
    NEXT: NEXT
    GOTO 1140
1120 IP = 3
    FOR J = 1 TO 3
    FOR I = 1 TO 6
    EA(I, J) = EE(I, J)
    IF J = 3 THEN EA(I, J) = EE(I, J + 1)
    NEXT: NEXT
1140 GOSUB 1400
    FOR I = 1 TO 6
    BK(I) = 0
    SDK(I) = 0
    NEXT
    IF GA = 1 GOTO 1160
    IF GA = 2 GOTO 1180
    IF GA = 3 GOTO 1200
    IF GA = 4 GOTO 1220
    IF GA = 5 GOTO 1240
    IF GA = 6 GOTO 1260
    IF GA = 7 GOTO 1280
    IF GA = 8 GOTO 1300
1160 FOR I = 1 TO 4
```

```
    BK(I) = B(I)
    SDK(I) = SD(I)
    NEXT
    GOTO 1320
1180 FOR I = 1 TO 3
    BK(I) = B(I)
    IF I = 3 THEN BK(5) = B(4)
    SDK(I) = SD(I)
    IF I = 3 THEN SDK(5) = SD(4)
    NEXT
    GOTO 1320
1200 FOR I = 1 TO 3
    BK(I) = B(I)
    IF I = 3 THEN BK(6) = B(4)
    SDK(I) = SD(I)
    IF I = 3 THEN SDK(6) = SD(4)
    NEXT
    GOTO 1320
1220 FOR I = 1 TO 5
    BK(I) = B(I)
    SDK(I) = SD(I)
    NEXT
    GOTO 1320
1240 FOR I = 1 TO 4
    BK(I) = B(I)
    IF I = 4 THEN BK(6) = B(5)
    SDK(I) = SD(I)
    IF I = 4 THEN SDK(6) = SD(5)
    NEXT
    GOTO 1320
1260 FOR I = 1 TO 3
    BK(I) = B(I)
    IF I = 3 THEN BK(5) = B(4)
    IF I = 3 THEN BK(6) = B(5)
    SDK(I) = SD(I)
    IF I = 3 THEN SDK(5) = SD(4)
    IF I = 3 THEN SDK(6) = SD(5)
    NEXT
```

```
    GOTO 1320
1280 FOR I = 1 TO 2
    BK(I) = B(I)
    IF I = 2 THEN BK(4) = B(3)
    SDK(I) = SD(I)
    IF I = 2 THEN SDK(4) = SD(3)
    NEXT
    GOTO 1320
1300 FOR I = 1 TO 2
    BK(I) = B(I)
    SDK(I) = SD(I)
    NEXT
1320 pp(1) = BK(1) + BK(2) + BK(4)
    pp(2) = BK(1) - BK(2) + BK(4)
    pp(3) = BK(1) + BK(3) + BK(6)
    pp(4) = BK(1) + .5 * BK(3) + .25 * BK(6)
    pp(5) = BK(1) + .5 * BK(2) + .5 * BK(3) + .25 * BK(4) + .25 * BK(5) + .25 * BK(6)
    pp(6) = BK(1) - .5 * BK(2) + .5 * BK(3) + .25 * BK(4) - .25 * BK(5) + .25 * BK(6)
    GOSUB 1540
    FOR I = 1 TO 6
    IF SDK(I) = 0 THEN GOTO 1314
    TTES(I) = ABS(BK(I)) / SDK(I)
    TTTE$(I) = "O"
1314 NEXT
    FOR I = 1 TO 6
    IF TTES(I) < 1.29 THEN TTTE$(I) = "ns "
    IF TTES(I) > 1.29 THEN TTTE$(I) = "(*)"
    IF TTES(I) > 1.64 THEN TTTE$(I) = "*  "
    IF TTES(I) > 2.33 THEN TTTE$(I) = "** "
    NEXT
    CHI = 0
    FOR I = 1 TO 6
    CHI = CHI + ((R(I) - pp(I)) ^ 2) * (E(I))
    NEXT
    PRINT G$: PRINT #2,
    PRINT #2, G$
    PRINT #2, "          THE BEST FIT MODEL"
    PRINT #2, G$
```

```
    PRINT #2, "          parameter  Signi.  t value     df          S.E."
    PRINT #2, G$
    PRINT #2,
    FOR I = 1 TO 6
    IF SDK(I) = 0 THEN GOTO 1316
    T(I) = F(I) / SDK(I)
1316 KK = SDK(I)
    IF BK(I) = 0 THEN KK = O
    IF BK(I) = 0 THEN T(I) = 0
    IF BK(I) = 0 THEN DF(I) = 0
    IF BK(I) = 0 THEN TTTE$(I) = "O"
    PRINT #2, PAR$(I); BK(I), TTTE$(I), T(I), DF(I), KK
    PRINT PAR$(I); "="; BK(I); TTTE$(I); T(I); DF(I); KK
    NEXT
    PRINT #2, G$: PRINT #2,
    HET = BK(3) + BK(6) - BK(4)
    PRINT #2, "(HETEROSIS = D+DD+AA)="; HET: PRINT #2,
    PRINT #2, G$: PRINT #2, " chi-square=  "; CHI: PRINT
    PRINT "CHI-SQUARE="; CHI: PRINT #2, : PRINT
1340 INPUT "is the result OK?  (Y or N)", Z$
    IF Z$ = "N" THEN GOTO 400
    IF Z$ = "Y" THEN GOTO 1380
    BEEP: GOTO 1340
1380 NEXT tv
    IF tv = cha THEN GOTO 1560
    REM SUBRUTIN TO MULTIPLY AND CONVERT MATRICES
    CLOSE #1, #2
    END
1400 CC = 1: DDD = 0: CCC = 0: EEE = 0
    FOR I = 1 TO ENT
    M = DOU(I, 1)
    IF M = CC GOTO 1420
    CC = CC + 1
    DDD = 0: CCC = 0: EEE = 0
1420 DDD = DDD + DOU(I, tv)
    CCC = CCC + DOU(I, tv) ^ 2
    EEE = EEE + 1
    R(M) = DDD / EEE
```

```
    NU(M) = EEE
    VU(M) = CCC - DDD ^ 2 / EEE
    NEXT
    FOR I = 1 TO 6
    VV(I) = VU(I) / (NU(I) - 1)
    VM(I) = VV(I) / NU(I)
    E(I) = 1 / VM(I)
    FOR J = 1 TO 6
    W(I, J) = 0
    IF I = J THEN W(I, J) = E(I)
    NEXT: NEXT
    VARS = .2 * (4 * VV(4) + VV(5) + VV(6)) - .4 * (VV(1) + VV(2) + VV(3))
    NGEN = ((R(2) - R(1)) ^ 2) / (8 * VARS)
    VVARS1 = .08 * ((16 * ((VV(4)) ^ 2)) / NU(4) + ((VV(5)) ^ 2) / NU(5) + ((VV(6)) ^ 2) / NU(6))
    VVARS2 = .32 * (((VV(1)) ^ 2) / NU(1) + ((VV(2)) ^ 2) / NU(2) + ((VV(3)) ^ 2) / NU(3))
    VVARS = VVARS1 + VVARS2
    VNGEN = ((NGEN) ^ 2) * ((4 * (VV(1) / NU(1) + VV(2) / NU(2))) / ((R(2) - R(1)) ^ 2) + VVARS /
((VARS) ^ 2))
    SENG = SQR(VNGEN)
    FOR I = 1 TO IP
    FOR J = 1 TO 6
    V = 0
    FOR K = 1 TO 6
    V = EA(K, I) * W(K, J) + V
    NEXT
    XY(I, J) = V
    NEXT
    NEXT
    FOR I = 1 TO IP
    FOR J = 1 TO IP
    VV = 0
    FOR K = 1 TO 6
    VV = XY(I, K) * EA(K, J) + VV
    NEXT
    QQ(I, J) = VV
    NEXT
    NEXT
    FOR I = 1 TO IP
```

```
    VVV = 0
    FOR J = 1 TO 6
    VVV = VVV + XY(I, J) * R(J)
    NEXT
    RH(I) = VVV
    NEXT
    FOR I = 1 TO IP
    FOR J = 1 TO IP
    AA(I, J) = QQ(I, J)
    BB(I, J) = QQ(I, J)
    NEXT J
    B(I) = RH(I)
    W%(I, 3) = 0
    NEXT I
    FOR I = 1 TO IP
    DT = 1
    BG = O
    FOR J = 1 TO IP
    IF W%(J, 3) = 1 THEN 1460
    FOR KV = 1 TO IP
    IF W%(KV, 3) > 1 THEN PRINT "Matrix cannot be inverted": STOP
    IF W%(KV, 3) = 1 THEN 1440
    IR = J
    IC = KV
    BG = ABS(BB(J, KV))
1440 NEXT KV
1460 NEXT J
    W%(IC, 3) = W%(IC, 3) + 1
    W%(I, 1) = IR
    W%(I, 2) = IC
    IF IR = IC THEN 1480
    DT = -DT
    FOR LV = 1 TO IP
    H = BB(IR, LV)
    BB(IR, LV) = BB(IC, LV)
    BB(IC, LV) = H
    NEXT LV
    H = B(IR)
```

```
    B(IR) = B(IC)
    B(IC) = H
1480 PV = BB(IC, IC)
    DT = DT * PV
    BB(IC, IC) = 1
    FOR LV = 1 TO IP
    BB(IC, LV) = BB(IC, LV) / PV
    NEXT LV
    B(IC) = B(IC) / PV
    FOR MV = 1 TO IP
    IF MV = IC THEN 1500
    H = BB(MV, IC)
    BB(MV, IC) = 0
    FOR LV = 1 TO IP
    BB(MV, LV) = BB(MV, LV) - BB(IC, LV) * H
    NEXT LV
    B(MV) = B(MV) - B(IC) * H
1500 NEXT MV
    NEXT I
    FOR I = 1 TO IP
    LV = IP - I + 1
    IF W%(LV, 1) = W%(LV, 2) THEN 1520
    IR = W%(LV, 1)
    IC = W%(LV, 2)
    FOR KV = 1 TO IP
    H = BB(KV, IR)
    BB(KV, IR) = BB(KV, IC)
    BB(KV, IC) = H
    NEXT KV
1520 NEXT I
    FOR KV = 1 TO IP
    IF W%(KV, 3) <> 1 THEN PRINT " Equations cannot be solved": STOP
    NEXT KV
    FOR I = 1 TO IP
    FOR J = 1 TO IP
    IF I = J THEN SD(I) = SQR(BB(I, J))
    NEXT: NEXT
    RETURN
```

```
1540 SS(1) = BK(1) + BK(2) + BK(4)
     SS(2) = BK(1) - BK(2) + BK(4)
     SS(3) = BK(1) + BK(3) + BK(6)
     SS(4) = BK(1) + .5 * BK(3) + .25 * BK(6)
     SS(5) = BK(1) + .5 * BK(2) + .5 * BK(3) + .25 * BK(4) + .25 * BK(5) + .25 * BK(6)
     SS(6) = BK(1) - .5 * BK(2) + .5 * BK(3) + .25 * BK(4) - .25 * BK(5) + .25 * BK(6)
     F(1) = .5 * SS(1) + .5 * SS(2) + 4 * SS(4) - 2 * SS(5) - 2 * SS(6)
     F(2) = .5 * SS(1) - .5 * SS(2)
     F(3) = 6 * SS(5) + 6 * SS(6) - 8 * SS(4) - SS(3) - 1.5 * SS(1) - 1.5 * SS(2)
     F(4) = 2 * SS(5) + 2 * SS(6) - 4 * SS(4)
     F(5) = 2 * SS(5) - SS(1) - 2 * SS(6) + SS(2)
     F(6) = SS(1) + SS(2) + 2 * SS(3) + 4 * SS(4) - 4 * SS(5) - 4 * SS(6)
     DF(1) = NU(1) + NU(2) + NU(4) + NU(5) + NU(6) - 5
     DF(2) = NU(1) + NU(2) - 2
     DF(3) = NU(1) + NU(2) + NU(3) + NU(4) + NU(5) + NU(6) - 6
     DF(4) = NU(4) + NU(5) + NU(6) - 3
     DF(5) = NU(1) + NU(2) + NU(5) + NU(6) - 4
     DF(6) = NU(1) + NU(2) + NU(3) + NU(4) + NU(5) + NU(6) - 6
     RETURN
1560 END
RUN
```