QUANTITATIVE GENETICS OF MAIZE *(Zea mays L.)* DURING SEEDLING ESTABLISHMENT UNDER COOL CONDITIONS

A thesis presented in partial fulfillment of the requirements
for the degree of Master of Agricultural Science in
Plant Science at Massey University

Mohammad Chozin

1992

' Whoever recommends and helps a good cause, becomes a partner therein. And whoever recommends and helps an evil cause, shares in its burden. And Allah hath power over all things.'

(The Holy Qur'an : 4 : 85)

Abstract

Two experiments were conducted to study cool tolerance in maize *(Zea mays L.)*. The first experiment was carried out under controlled environment to evaluate several genotypes from five synthetic populations which are currently being used to develop hybrid maize for better adaptation to New Zealand climate and to study the quatitative inheritance of maize seedling growth under cool conditions. In this study, diurnal temperature of 16 °C day/6 °C night was used and characters related to seedling growth were examined.

The second experiment conducted to study the effect of temperature on maize during its early growth and to examine whether the initial seed constitution and germination characteristics could be used as selection criteria for improvement of the subsequent seedling growth. Eleven physical, chemical, and morphological characters were measured. The growth was studied in germinators under two temperature regimes of 25/20 and 16/6 °C.

The genotypic variation was highly significant for all nine characters examined in the first experiment. For the three repeatedly measured characters (i.e. chlorophyll content, shoot and root dry masses), the genotype x time interaction effect was significant. In the second experiment, the variation due to genotypic difference was highly significant only for the initial seed constitution characters and the amount of ion leakage during the early hours of germination process. It was non significant for the time to germinate, seedling growth rates, and seedling growth functions. The variation due to the difference of temperature regimes was significant for the time to germinate and seedling growth but not the growth functions.

The genotypes of synthetic line NZS3 showed the best performance for general combining ability (GCA) for almost all characters studied in the first experiment. From all genotypes evaluated, however, only few of them consistently showed good GCA over the characters.

Four of the characters studied in the first experiment had moderate to high narrow sense heritabilities, namely total leaves at 50 days after planting (82 %), chlorophyll content (46 %), anthocyanin (69%), and leaf area (62 %). In the second experiment, the estimated broad sense heritabilities observed ranged from very low to very high over all characters. The high broad sense heritabilities were recorded on most of the initial sees constitution characters, the conductivity of ion leakage, and the growth rates of root (length) and shoot (dry mass).

Both the phenotypic and genotypic correlation coefficients between pairs are in good agreement and followed the same direction. Amongst the characters examined in the first experiment only time to achieve second mature leaf, total leaf number at 50 day after planting, chlorophyll content, leaf area had considerable correlations to the dry masses. In the second experiment a good correlation with growth rate was observed for the seed weight, nitrogen and maltose contents.

Acknowledgements

I am deeply indebted to my supervisor Dr. Ian L. Gordon for his advise, encouragement, constuctive criticism, and assitance during the entire course of study in Massey University. His guidance has been invaluable.

Special thank must also go to Mr. Alan K. Hardacre for providing maize seeds and all facilities and for assistance during climate room experimentation. Thanks are also extended to the DSIR Fruit and Trees Division for use of the climate room facilities during this study and for co-operation I recieved from the staff.

Thanks are also due to all of my friends, staff of Agronomy Department and Seed Technology Centre, who assisted in many ways at various stages of this study.

I am particularly grateful to MERT of New Zealand for granting me a scholarship.

My special gratitude is to my parents, who always give me a great support. Finally, my great appreciation is to my wife LILI and my daughter ALDILA for their patient and support.

Table of Contents

		Page
1.	Intoduction	1
2.	Review of Literature	3
	2.1. Maize distribution and adaptation	3
	2.2. Maize germination and seedling growth at low temperature	4
	2.3. Breeding for cool tolerance	9
	2.3.1. Germplasm for cool tolerance	9
	2.3.2. Genetic variation	10
	2.3.3. Nature of inheritance	11
	2.3.4. Screening technique	12
	2.3.5. Plant improvement	13
	2.4. Quantitative genetic analysis	15
	2.4.1. Quantitative character and plant improvement	15
	2.4.2. Estimation of variance component	18
	2.4.3. Estimation of genetic variation	20
	2.4.4. Estimation of heritability	21
	2.4.5. Correlation among characters	23
3.	Testcross Evaluation for Cool Tolerance During Seedling Establishment	25
	3.1. Objective	25
	3.2. Materials	25
	3.3. Environment	29
	3.4. Cultural	31
	3.5. Harvest	31
	3.6. Data collection and measurement	31

3.7. Data analysis 34
3.7.1. Statistical analysis 34
3.7.2. Genetical analysis 44
3.7.2.1. Genetic variance estimation 44
3.7.2.2. Heritability estimation 45
3.7.2.3. Correlation analysis 49
3.8. Results 50
3.8.1. General values 50
3.8.2. Variance component estimates 50
3.8.2.1. Variance components of environmental effects 50
3.8.2.2. Variance of genotypic effects 56
3.8.3. Means discrimination 57
3.8.4. Genetic variance and heritability estimates 87
3.8.5. Phenotypic and genetic correlation estimates 89

4. Genotypic Variability in Initial Seed Constitution, Germinability, and Seedling Growth 92
4.1. Objectives 92
4.2. Materials 92
4.3. Experimental 93
4.3.1. Determination of initial chemical composition of the seeds 93
4.3.2. Determination of ion leakage 94
4.3.3. Evaluation of germination and seedling growth performance 94
4.4. Data analysis 95
4.4.1. Statistical analysis 95
4.4.2. Genetical analysis 101
4.4.2.1. Estimation of Heritability 101
4.4.2.2. Correlation analysis 102

4.5. Results 102
4.5.1. General values 102
4.5.2. Germinative change 104
4.5.3. Heritability estimate 114
4.5.4. Phenotypic and genotypic correlation estimates 117

5. Discussion 121
5.1. Genetic study 121
5.2. Lines evaluation 122
5.3. Physiological study 123

6. Conclusion 124

References 126

Appendices 135

List of Tables

		Page
3.1.	Exp.I. Genotypes and their generation	26
3.2.	Exp.I. Pedigree of the synthetic populations	30
3.3.	Exp.I. The degree of freedom, expectation of Mean Square, and F-ratio for nested design (Model I)	38
3.4.	Exp.I. The degree of freedom, expectation of Mean Square, and F-ratio for Randomized Complete Block design (Model II)	39
3.5.	Exp.I. The degree of freedom, expectation of Mean Square, and F-ratio for Split Plot in Time (Model III)	41
3.6.	Exp.I. The degree of freedom, expectation of Mean Square, and F-ratio for Extended Split Plot in time (Model IV)	43
3.7.	Exp.I. Genotypes and their inbreeding coefficient	46
3.8.	Exp.I. The grand Means, their range and coefficient of variations	51
3.9.	Exp.I. Variance component estimates involving genotypic effect, their standard errors and significances	52
3.10.	Exp.I. Variance component estimates involving environmental effect, their standard errors and significances	53
3.11.	Exp.I. Contrast amongst the origin group of the genotypes	58
3.12.	Exp.I. Discrimination among genotypic means for emergence time (EMERGE)	59
3.13.	Exp.I. Discrimination among genotypic means for number of day to attain second mature leaf(2MATLEAF)	62
3.14.	Exp.I. Discrimination among genotypic means for total leaves at 50 days after planting (TOTLF50D)	65
3.15.	Exp.I. Discrimination among genotypic means for chlorophyll concentration (CHPHYLL)	68

3.16.	Exp.I. Discrimination among genotypic means for anthocynin score (ANTHOCY)	71
3.17.	Exp.I. Discrimination among genotypic means for leaf area (LFAREA)	74
3.18.	Exp.I. Discrimination among genotypic means for leaf thickness (LFTHICK)	77
3.19.	Exp.I. Discrimination among genotypic means for shoot mass (SHOOT)	80
3.20.	Exp.I. Discrimination among genotypic means for root mass (ROOT)	83
3.21.	Exp.I. Additive genetic varianc and heritability estimates	88
3.22.	Exp.I.Phenotypic correlation (r_P) amongst characters pairs and their significances	90
3.23.	Exp.I.Genotypic correlation (r_G) amongst characters pairs and their significances	91
4.1.	Exp.II. The degree of freedom, Expectation of Mean square, and F-ratio for Completely Random Design (Model I)	97
4.2.	Exp.II. The degree of freedom, Expectation of Mean square, and F-ratio for Pooled-RCBD (Model I)	99
4.3.	Exp.II. The grand means, their range values and coefficient of variations	103
4.4.	Exp.II. Variance components and their standard errors and significances for the initial seed constitution characters	105
4.5.	Exp.II. Variance components and their standard errors and significances for germinability and seedling growth rate characters.	106
4.6.	Exp.II. Wilks' generalized variance components and their standard errors and significances for seedling growth functions	107
4.7.	Exp.II. Zhivotovsky's generalized variance components and their standard errors and significances for seedling growth functions	108

4.8. Exp.II. Discrimination amongst genotypic means for the initial seed constitution characters 109
4.9. Exp.II. Discrimination amongst genotypic means for germination and seedling growth characters 110
4.10. Exp.II. Discrimination between two environmental means for germination and seedling growth characters 111
4.11. Exp.II. Discrimination amongst Least Square Means of Genotypic-Environmental interaction for germination and seedling growth rate characters 112
4.12. Exp.II. Heritability estimates of the initial seed constitution characters 115
4.13. Exp.II. Heritability estimates of the germination and seedling growth rate characters 115
4.14. Exp.II. Heritability estimates of the seedling growth function characters (Estimated from Wilks' generalized variances) 116
4.15. Exp.II. Heritability estimates of the seedling growth function characters (Estimated from Zhivotovsky's generalized variances) 116
4.16. Exp.II. Phenotypic correlation (r_p) amongst characters pair and their significances 118
4.17. Exp.II. Genotypic correlation (r_G) amongst characters pair and their significances 119

1. Introduction

Maize (*Zea mays L.*) is generally recognized as a thermophylic crop. It requires a relatively high temperature to achieve an optimal growth and development. Nevertheless, for several reasons maize cultivation has been extended to areas that cannot fulfill this condition. Indeed, maize has become a crop of increasing importance in temperate regions situated at lattitudes ranging from 30-55° (Shaw, 1977) of which the northern United States, Canada, and Western Europe are outstanding examples. At these lattitudes a frost free growing period is relatively short. Of equally importance is that in spring, in which maize is commonly sown, the temperature is above freezing but still below the treshold of the plant growth and this condition is often responsible for the crop failure. Consequently, the availability of maize varieties that are capable of rapid emergence and of becoming well established in such environments would be most important.

For many years, considerable efford has been expended to understand cool tolerance and how maize lines can be developed toward more endurance in cool conditions. To date, some physiological and genetical aspects of the cool tolerance in maize have revealed. Furthermore, the source of germplasms from which the cool tolerance genes can be obtained have been reported several workers. Mock and Eberhart (1972), for instance, have demonstrated that maize germplasm of the U.S. Corn Belt Dent possessed adequate genetic variation for cool tolerance to permit its improvement through selection. Recent researches (Eagles and Hardacre, 1979; Eagles *et al.*, 1983) showed that populations containing germplasm of highland tropical origin had better seedling performances under 10 °C compared to the U.S. Corn Belt Dent.

Recurrent selection method has been extensively used in maize breeding programmes to improve many characters of economic importants. With respect to the improvement for cool tolerance, Mock and Bakri (1976) have

showed that recurrent selection could be used effectively to improve this character of maize genotypes adapted to the Central U.S. Corn Belt.

In maize hybrid breeding program, the value of a population for improvement by recurrent selection and as a source of inbred line depends on the mean performance of the population and on the genetic variability in the population for the traits of economic importance. To determine such value, progeny testing is commonly used.

The present study is conducted in two experiments. The first experiment, described in chapter 2, focused on evaluation of maize populations which are currently being use to developed maize hybrid with better adaptation to New Zealand climate and to study the quantititative inheritance of seedling growth under cool conditions. The second experiment, described in chapter 3, was aimed to study the effect of temperature on maize during its early growth and to examine if the initial seed constitution and germination characteristics could be used as selection criteria for improvement of the subsequent seedling growth.

2. Review of Literature

2.1. Maize distribution and adaptation

Maize *(Zea mays L.)* is one of the oldest crops cultivated by mankind. It probably originates from subtropicals region of Mexico (Wilkes, 1979). The first domestication was dated back to some 7000 to 10,000 years ago in south-central or southwestern Mexico. Early exploration showed that the maize-growing area extended throughout the Americas, reaching the northeastern U.S./southeastern Canada and central Chile just prior to European colonisation of The New World (Mangelsdorf, 1974). For the Old World civilisation, maize was effectively introduced for the first time in 1493 by Columbus upon his return to Spain from his first voyage. Afterward, a further extension of maize cultivation occurred which brought about its spread northward to the short growing-season areas of France, Germany, Austria, and Eastern Europe and southward to Africa and Asia (Benson and Pearce, 1987). In New Zealand, the earliest recorded introduction of maize was in 1772 during one of Marion de Fresne's voyages (Yen, 1959).

Although nowadays maize is a leading crop in many temperate regions, its adaption to such environments, which have long days and cool temperatures, seems to have been difficult. Leng *et al.* (1962) stated that maize was poorly adapted to environments of Spain, but because of repeated collections by explorers of Western Hemispehere, germplasm was continuously introduced to the European continent resulting in a range of variation available for use. Furthermore, about four hundred years of selection was required to develop varieties that were adaptable to the broad spectrum of environmental conditions, from the arid conditions surrounding the Mediterranean Sea to the short growing seasons of northern Europe.

Compared with adaptation to day length, adaptation to cool temperature is more difficult in maize, as it requires a number of features such as resistence to frost, resistence to chilling, resistence to fungi during germination, and the ability to germinate, grow, and mature at low temperatures (Miedema, 1984). Moreover, maize has a great genetic variability with respect to day-length response (Stevenson and Goodman, 1972). Day-neutral types and even a long-day line have been reported (Francis *et al.*, 1969). In contrast, the genetic variability of most maize plant responses to cool temperature is small and most of the desired characters are putatively genetically unrelated (Miedema, 1984).

2.2. Maize germination and seedling growth at low temperature

Planting maize before the soil temperature exceeds 10 °C is commonly advised against, otherwise poor stand establishment will be obtained (Bunting, 1978). Inadequate stands, in turn, will reduce the potential yield of the crop. Miedema (1984) described that exposing young maize seedlings to temperature below 16 °C are led to various types of physiological dysfunction, manifested in abnormal development of the plants, the so called 'chilling injury'. He also pointed out that temperature below about 6 °C for a period of time is low enough to to kill maize seedlings.

From the following description on the ontogeny of the maize seedling, it can be seen how important is low temperature in determining the rate of growth and development of maize during seedling establishment.

The process of germination is initiated by absorbtion of water (imbibition), proceeds through intermediate phases of metabolic re-activation and cell division, and concludes with radicle elongation (Wellington, 1966). This process can be successful only when the temperature is in an appropriate range.

Berlyn (1972) noted that water uptake occurs primarily through the pericarp despite the presence of the fractured pedicel which superficially would appear to offer less resistance to water movement. As seeds imbibe, their volume increases and this swelling is in part a reversal of the shrinkage that occurred during the final stage of grain development, when cells decreased in size and their walls became corrugated (Lott, 1974).

Blacklow (1972a) has studied the effect of temperature on imbibition of maize seeds. He described that the curves of imbibition increase in slope with temperature. However, even at low temperature, the water content of seeds increased considerably within the first hours. It seems unlikely, therefore, that temperature restricts germination by its effect on imbibition.

In early accounts of imbibition, many different subtances, including amino acids and organic acid, sugar, phenol, phosphate and potassium ions, gibrellic acid, and protein, leak out from the seeds and embryo (Simon, 1974). This is because membranes of dry seeds have lost some of their integrity during grain dehydration. During imbibition, membranes re-assemble themselves, but before their integrity is fully restored the gradient of water potential disrupts their organisation and scatters the component phospholipid and protein far from their original position (Larson, 1968; Perry and Harrison, 1970). Simon (1979) pointed out that the leakage is most rapid during the early phase of imbibition when dry seeds are first in contact with water. However, as imbibition progresses the rate of leakage declines. He also indicated that the leakage of solute from seeds reduces seedling vigour.

Leakage from imbibing seed and embryo is notably temperature sensitive and is intensified at low temperature. In peas, for instance, Perry and Harrison (1970) have demonstrated that the rate of leakage is doubled when the temperature is reduced from 25 to 5 °C. This is mainly due to a slower restitution of membrane integrity as the temperature decreases (Simon, 1979).

In quiescent seed, the rate of metabolic activitiy is very low because of the lack of water. It begins to rise rapidly when the seed becomes rehydrated. Bryant (1985) described that the first metabolic revival are increases in the activation of pre-existing mRNA, which is accompanied by an increase in the capacity of the embryo to synthesize protein, and an activation of enzymes associated with energy production. There is no attemp to catalogue here all the changes in enzyme activities and metabolic pathways that occur during the process of germination. It can be noted, however, that on a gross scale the change in the level of metabolism is reflected in large increase in the rate of gas exchange.

The next phase involves synthesis of DNA and RNA, supported by utilisation of immediate embryonic reserve materials. This is followed by rapid cell division and differentiation of the tissues within the embryo, which lead to the emergence of radicle from coleorhiza.

As the growth of the embryo continues, a significant amount of gibberellin is secreted by the embryo, possibly from the scutellar region. This diffuses to the aleurone cells and stimulates them to synthesize and release hydrolytic enzymes, particularly alpha-amylase and proteases, into endosperm. Consequently, reserve carbohydrate and protein are hydrolysed to form simple sugars and amino acids which are then translocated to the developing seedling. This phase continuous until the seedling is established as a photosynthetic organism (Ching, 1972).

The effects of temperature on the metabolic activity may be indicated by the amount of energy production and the rate of gas exchange; although on overall this is very complex (see Stamp, 1984 for detailed review). Various metabolic studies performed with plants of tropical origin indicate that mitochondrial activity is affected by low temperature (Lyons and Raison, 1970; Duke *et al.*, 1977). Stewart and Guinn (1969, 1971) demonstrated that

transferring young cotton seedlings from temperature 35 / 30 °C to 5 °C have resulted in decreased concentration of adenosin triphophate (ATP) and other nucleotides. Woodstock and Pollock (1965) have found that the rate of O_2 uptake of lima bean seed during imbibition was reduced by temperature of 15 °C and markedly reduced by 5 °C. Furthermore, Guinn (1971) showed that RNA, protein, and lipid-soluble phosphate in cotton seedling were also decreased by these temperatures.

Studies on the morphological characteristics also indicated that maize germination and seedling growth were retarded by low temperature. Working with cv. Fronica, Miedema *et al.* (1982) found a linear relationship between temperature and the rate of radicle emergence existed in the range of 8° to 32°C. The minimum temperature for this character was recorded at around 6°C and the optimum around 34° - 36°C. The time to 50% radicle emergence ranged from 10.6 days at 8°C to 17.5 hr at 36°C.

Shoot and primary root elongation of maize seedling are similar to one another in their response to temperature. Blacklow (1972 b) showed that the minimum temperature for both processes was at 9°C and optimum at 30°C. He also showed the temperature-response curves for these characters were nearly linear between 9.5 and 30°C. A similar result for shoot elongation was reported by Miedema *et al.* (1982). In this study the minimum temperature was just above 8°C and the optimum was 32°C. Between 8° and 32°C the temperature curve for this character consisted of two linear parts, from 8 to 18 °C and from 18 to 32 °C, with a slightly steeper slope at the higher range.

The effect of temperature on shoot elongation rate can also be seen from the time to emergence, which is time to the appearance of the coleoptile tip above soil level. Study on 12 maize inbred lines sown at 2.5 cm depth under three controlled root zone temperatures (10, 14, 18°C), Menkir and Later (1987) noted that at 14° and 10°C, the time required for emergence was

approximately 5 and 12 days, respectively; both of which were slower than emergence at 18 °C, which averaged 8 days from sowing. In the Midema *et al.* (1982) study, the time to emergence from a depth of 4 cm sown was recorded 23 days at 10 °C, 8 days at 15 °C, 4 days at 21 °C and 2 days at 32 °C.

The effects of temperature on the leaf appearance rate and the leaf expansion rate were described by Hardacre and Turnbull (1986). Two hybrids of U.S. Corn Belt Dent were grown in a diurnal temperature regime of 16/6 °C day/night and in constant temperatures of 16, 20, 24 and 28 °C. It was found that the visible-leaf appearance rate decreased markedly with temperature. The leaf appearance rate ranged from 0.127 leaf/day at 16/6°C to 0.577 leaf/day at 28 °C. The optimum temperature of 30 °C, and the extrapolated minimum 7 °C, for leaf appearance rate was reported by Tollenaar *et al.* (1979). The leaf expansion rate in this study was similar to the leaf appearance rate with respect to temperature. The temperature 16/6 °C resulted in the lowest value, while 28 °C had the highest expansion rate.

Reductions of seedling mass due to low temperature has also been reported by some workers. In the Menkir and Later (1987) study, for instance, both shoot and root dry weight were reduced in all inbred lines studied with lower temperatures. On average, the shoot dry weights were 6.57 g (18 °C), 1.12 g (14 °C), and 0.18 g (10 °C), while root dry weight were 3.09 g (18 °C), 0.59 g (14 °C) and 0.32 g (10 °C), respectively.

Another plant characteristic which is strongly influenced by low temperature is chlorophyll production. Alberda (1969) reported that the chlorophyll concentration was geatly reduced in the maize plant grown from emergence at temperature below 15 °C. This was worsened under conditions of high light intensity (McWilliam and Naylor, 1967).

With respect to anatomical features, Erickson (1959) found that the rates of cell division and cell elongation were equally reduced when the temperature was decreased from 30 to 15 °C; the optimum for both process was 30 °C. At 10 °C, the rate of cell elongation was much more reduced than the rate of cell division and swellings appeared in the growing region of the roots.

2.3. Breeding for cool tolerance

Breeding for cool tolerance has importance practical requirements. The genotypes representing the gen pool to be drawn need to be identified. In addition, the number of genes and the nature of the inheritance for tolerance should be explored in order to optimise progress of the breeding programme. Rapid and simple techniques for selecting desirable genotypes need to be established as well.

2.3.1. Germplasm for cool tolerance

Maize is not know as a wild plant, but the gene pools from which the cool tolerance genes can be obtained have been reported. Mock and Skrdla (1978) evaluated a representative sample of 144 maize populations introduced from different countries or ecological regions where maize is grown and found at least 25 of the populations evaluated showed sufficient tolerance to temperature of 10 °C. These include some U.S. Corn Belt Dent hybrids that are used extensively for maize production in temperate regions of the world (Hallauer and Miranda, 1981; Goodman, 1988).

Eagle and Hardacre (1979) and Eagles *et al.* (1983) identified that line and families from Highland Early Yellow Dent population (Pool 5) developed by CIMMYT for highland areas of tropic have outstanding seedling growth at 10 °C and emerged faster than Corn Belt Dent population included in the

Mock and Skrdla 's (1978) evaluation. Conico race, such as Criolo de Toluca, from the highland of Mexico or San Geronimo from highland of Peru should be excellent sources of cool tolerance genes for maize breeding programmes. Criolo de Toluca is emerged more rapidly and more reliably from cool soil than Corn Belt Dent races (Eagle and Brooking, 1981). San Geronimo has a lower temperature threshold for autotrophic based growth than Corn Belt Dent (Hardacre and Eagles, 1980). Similarly, the landrace Confite Puneno which is grown in Peru and Bolivia at altitudes between 3600 and 4000 m is a potential source of cool tolerance genes (Vallejos, 1979) because it has a resistance to chilling temperature near 0 °C (Hetherington *et al.*, 1983).

2.3.2. Genetic variation

According to Mock and McNeill (1979) or Hardacre and Eagles (1980), cool tolerance is the ability of a genotype to emerge from the soil and to grow vigorously after emergence in cool soil and air temperature. Maryam and Jones (1979) claimed that the cool tolerance was an heritable character. However, genetic studies indicated that cool tolerance is a complex quantitative character which is strongly influenced by environment (e.g. Pinell, 1949 and Grogan, 1970).

To some degree the existence of genetic variation for cool tolerance has been reported. Eagles and Hardacre (1979) found considerable genetic variation occurred for time to emergence, shoot weight, and leaf number. Stamp (1984) listed the genetic variation and the heritabilities of some seedling characteristics, including leaf area, shoot dry weight, length of primary root, number of lateral root, length of lateral root, number of mesocotyl and crown root, root surface area, and root dry weight. Further listings on the existance of genetic variation have also been made by other workers (e.g. Crosbie *et al.*, 1980 and Miedema, 1984).

Complications due to genotype-environment interaction have been reported. Percentage of emergence, seedling dry weight, juvenile plant height, juvenile leaf number, and grain yield were found to be significantly affected by genotype-environment interaction (Mock and Bakri, 1976). More complicated features were reported by McConnel and Gardner (1979). Their results showed that significant effects of genotype-environment interaction were not consistent over different generations.

2.3.3. Nature of inheritance

Few detailed studies have been made to reveal the way in which cool tolerance is inherited. Haskell and Singleton (1949) demonstrated that the genetic constitution of the embryo determined the behaviour of the germinating seed under suboptimal conditions (cool temperature). Other studies sugested that maternal or cytoplasmic seed factors are associated with low temperature tolerance (Pinnel, 1949; Haskell, 1952; Pesev, 1970; Maryam and Jones, 1983).

Analysis on the mode of gene action has revealed that additive, dominance and epistatic effects all contibute to cool tolerance. Based on the generation means analysis, McConnel and Gardner (1979) reported that the rate of germination was significantly conditioned by all these types of gene action, while growth after emergence and grain yield were conditioned predominantly by additive and dominance effects. Recently Eagle and Hardacre (1989) examined cool tolerance characterisitics in a five-parent diallel and found significant effects for both general combining ability (GCA) and specific combining ability (SCA) on all of the characters studied, indicating that both additive and non-additive effects were important. In addition, they found also that in most characters the variation due to the GCA was much larger than variation due to SCA, again indicating the importance of additive effect.

2.3.4. Screening technique

Selection for cool tolerance may be effective if the selection techniques have been established, although good heritability is also necessary to achieve results. Identification of plant characteristics which are greatly affected by low temperature and are heritable is therefore critical. Furthermore, according to Stushnoff *et al.* (1984) a screen for cool tolerance should be highly repeatable, i.e. the genetic variation is stable. It should be simple to conduct, rapid and non-destructive and it should require only part of a single plant for analyses.

Studies of the components of cool germination, such as those done with dissected embryonic axes (Christeller, 1984) show that more stable and repeatable result can be obtained when germination is addressed at lower levels of organisation. Such analyses, however, are impractical for screening of large populations. It still remains a fact that large scale of work can only be performed with whole germinating seed in either controlled environments or under early planting in the field.

Because of the variabilty inherent in field trials, the controlled environment is beneficial and sometimes essential for selection of germination emergence, and the growth performances after emergence. This may be seedling growth or later stage of plant growth. In the controlled environment the critical temperature can be programmed within the reasonable dynamic temperature regime that approaches a simulation of the natural environment.

With regard to the selection criteria, Miedema (1984) has devised some screens on the basis of plant response to cool temperature. These include chilling injury to imbibied seed or to seedling, rate of germination or radicle emergence, vigour before and after emergence, chlorosis, and fungal attact. Regrettably not all of these selection criteria are applicable in each and every case. Vigour-based selection criteria, which are measured on dry matter, are

destructive and therefore can not be practised in early stage of selection. Linear measurements of plant or organs are not always correlated with plant dry matter (e.g. Stamp, 1984). Similarly, measurements on germination have shown no phenotypic correlation to emergence or growth of seedling (e.g. Bocsi and Kovac, 1991). Again, fungal attact has become lesser important since seed dressing with such fungicide as Captan® is common practice (McConnell and Gardner, 1979; Eagles and Hardacre, 1979; Hardacre and Eagles, 1980). Also, chlorophyll content is critical as a selection criterion. Although chlorophyll loss is a prominent symptom in low temperature, a clear relationship between chlorophyll content and plant growth has not yet been established. Hardacre and Eagles (1989) suspected that high chlrophyll content is not a prerequisite for higher growth rate. In another instance Dolstra and Miedema (1986) suggested the use of leaf elongation or leaf area as a screen but these suggestions still require further confirmation.

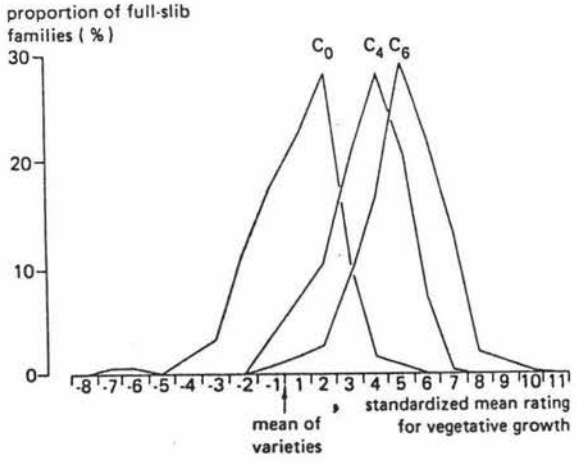
It is therefore concluded that as long as the critical information is lacking, selection for cool tolerance in maize must proceed from empirical criteria. Rapid screening techniques, or a genetic marker for cool tolerance, are required.

2.3.5. Plant improvement

Vallejos (1979) pointed out that, although some breeding has been attempted, the results have generally been contradictory and not spectacular. McConnell and Eberhart (1979) attempted to improve cool germination by combining both controlled and field environments. The seedlings germinated at 7.2 °C were transfered to the field, selected for agronomic characteristics, and recombined to form a composite population for the next cycles of selection. After four cycles of selection, cool germination at 7.2 °C was improved by 9% but only small improvement was realised in field emergence and seedling vigour.

Recurrent selection for cool tolerance was performed by Mock and Bakri (1976). Using two population from Iowa Stiff Stalk Synthetic (BSSS): BSSS2(SCT) and BSSS13(SCT), they found that percentage emergence and dry weight of BSSS13 were improved 8.4 % per cycle and 0.6 dg per cycle, respectively, but only 1.7 % per cycle improvement in percentage emergence and no improvement in dry weight on BSSS2. In addition, emergence index was not changed by selection in either population. With the same materials Hoard and Crosbie (1985) found that recurrent selection has improved slightly percentage emergence (2.1 % per cycle), seedling dry weight (0.04 g per cycle) and seedling vigour score (0.3 units per cycle). Earlier, Mock and Eberhart (1972), also with the same material but employing selection index, found that predicted gain for cool tolerance (as an agregate of the three characters evaluated by Mock and Bakri, 1976) was subtantial and that 85 to 90 % of the predicted advance was due to gain for percentage emergence.

Recurrent selection has also been used by Dolstra and Miedema (1986). They claimed that vegetative growth was improved by this selection method. Based on the standardised family means they presented the follwing figure.



In another case Dolstra *et al.* (1988) have improved chlorosis resistance with divergent mass selection. They also noted that resistance for chlorosis was followed by the chance of young plant to survive and grow at low temperature.

Looking those observation as an illustration, although the progress is relatively slow there is no reason to be pessimistic that progress can be made toward the improvement of cool tolerance in maize.

2.4. Quantitative genetic analysis

2.4.1. Quantitative character and plant improvement

The majority of characters for which crop plant are raised, e.g. growth and yield, are quantitative. Any attempt by the plant breeder to develop new cultivars must therefore concern the selection of superior genotypes from a population consisting of an array of genotypes. As a consequence, an understanding of the inheritance of quantitative characters appears to be a prerequisite for efficient breeding procedures. Fisher (1918) provided the initial framework for the study of the inheritance of quantitative characters. Since that time, his developments have been clarified, elaborated, and extended by numerous geneticists and statisticians to become a branch of science, so called 'quantitative genetic' or 'biometrical genetics'.

The basic premise of quantitative genetics is that a quantitative character is continuous phenotypically and involves many loci with small individual effects (Falconer, 1981). As a consequence, statistics appropriate for continuous variables, such as mean, variance, and covariance, become necessary to understand the inheritance of such characters.

In order to deduce the performance exhibited in a quantitative character by particular genotype, it should be realised that the masurable attribute, called 'the phenotype', is the expression of various different causal factors which can categorised ('partitioned') as genetic effect and non-genetic effects. The latter are attributable to environment and interactions between genetic and environment (Comstock and Robinson, 1948). Following Falconer (1981),

the model to describe this relationship can be presented as follows:

$$P_i = G_i + E_i + GE_i$$

where P_i is the phenotypic expression of indiviudal measured for character i, G_i is the genetic effect potentially inherited for the character, E_i is environment effect cousing variation in the character, and GEi is the interaction effect of the genetic and environment. Thus, if the deduction is made on population, the total phenotypic variation, σ^2_P, would be as follows:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{GE}$$

where σ^2_G represent the genotypic variance, or variance of genetic effects, σ^2_E is the variance of environmental effects, and σ^2_{GE} is the discrepancies of a behaviour of genotypes in environments.

The genotypic variance, σ^2_G, may be further partitioned into its components to describe the type of gene action that involve the performance of the character.

$$\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_I$$

where σ^2_A is the additive genetic variance, or simply additive variance (that due to average allele effects), σ^2_D is the dominance variance (that due to heterozygote effects), and σ^2_I is the epistatic variance or interaction between additive and dominance effects (that due to non allelic or interlocus effects).

The epistatic variance, σ^2_I, may be also further partitioned as follows:

$$\sigma^2_I = \sigma^2_{AA} + \sigma^2_{AD} + \sigma^2_{DD} + \sigma^2_{AAA} + \ldots$$

where σ^2_{AA} is the variance due to additive x additive interaction effects, σ^2_{AD} is the variance due to additive x dominance interaction effects, σ^2_{DD} is the variance due to dominance x dominance interaction effects, etc.

The availability of genetic variation and knowledge of the type of gene action are of primary improtant in the improvement of quantitative characters as selection may be in vain without the availability of genetic variation. Knowledge of the type of gene action is useful in determining the selection strategy. To estimate the contribution of each component described above there are mainly two methods of analysis which can be employed, those based on generation means analysis and those based on the variance components analysis.

The generation means analysis has been extensively studied and developed by Anderson and Kempthorne (1954), Hayman (1958, 1960), Van der Veen (1959) and Gardner and Eberhart (1966). Typically the estimation of the components is based upon the relative genetic effect deduced from the means of different generation. However, this method lack in general utility for various reason. As Sprague (1966) has pointed out, the generation means method is strickly applicable only where gene frequencies are known. While this method may provide information on the extsitance of different types of gene action, it provides no measure of their relative importance. Furthermore, the result cannot be related to any ancestral population as the estimates obtained from each pair of inbred parents may be unique in variying degrees. Also only genetic variation which generate means variability is analysed.

Analysis of variance components is much more widely used than that of generation means. This method was formerly introduced by Fisher (1925) and, since then, developed by various workers. Wright (1935), Comstock and Robinson (1948), and Mather (1949) are responsible for much of the

development of this method. Basically the estimation procedures involve a mating scheme to generate progenies. Using appropriate experimental design and statistical analysis, variance component can be calculated. To describe the type and magnitude of gene action involved, the biometrical components of variance are translated into covariances of relatives, which reflect the degree of the relationship amongst individuals in the populations. These covariance translated, in terms of gene model, into genetic (and its components), and environmental variance components.

2.4.2. Estimation of variance component

According to Steel and Torrie (1980) and others, experimental observations may be described as linear model which consist of mean, several components, and a residual. The analysis of variance technique was particularly developed to estimate the magnitude and significance of these components. In this technique the variability due to each partition is included in the model, and is calculated in term of sums of squares of deviations from the overall mean. These sums of squares are, then, converted to mean squares which have definable expectation. However, the application of analysis of variance to the experimental data is appropriate only when the data conform to the basic assumptions underlying these procedures of analysis. Failure to fulfill the assumptions will affect the significance levels and the sensitivity tests (the F-test and the t-test).

Four asumptions are considered essential for the analysis of variance (Cochran, 1947; Eisenhart, 1947). These include:

a. Independence distribution of experimental errors
b. Normal distribution of experimental errors
c. Homogeniety of experimental errors across subset of data
d. Additivity of treatment and environmental effects.

In practice, one can never certain that all the above assumptions are fulfilled with a particular set of data. The detailed discussions on the possible cosequences of failures and remedial procedures when these assumptions are not satisfied have been given by Bartlett (1947), Cochran (1947), and Eisenhart (1947). Fortunately, for most types of biological data, it weel accepted that the disturbance resulting form failure of the data to fulfill these requirements are not invalidate the procedure (Steel and Torrie, 1980). Therefore, the procedures for testing significant levels and estimating confidence limits should be considered as approximate rather than exact (Cochran, 1947).

For a proper estimate of variance components, the model must be specifically stated (Steel and Torrie, 1980). There are two philosophies that are commonly used, fixed and random effects, the choice depends on the breadth of the population of inference. For the fixed model, inference is drawn about just the population actually being studied; while for the random model, inference is drawn about a broader population. Arithmetic manipulation is required to calculate the magnitude of each variance components from the expectation of mean square. In addition, Crump (1946 and 1951) has developed a standard error that determine the precision of the variance component estimate, which can be presented as follows:

$$S.E.\hat{\sigma}_t = \sqrt{\sum \frac{2\,(MS_n)^2}{(f_n+2)}}$$

Where $\hat{\sigma}^2_t$ is the variance component estimate, MS_n is the mean squares in estimating σ^2_t, and f_n is the degree of freedom of n-th mean square.

The significance of each variance component is determined by F-test. Satterthwaite (1946) suggested to use an approximate F-test (F') when the mean squares involved in the test are linear functions. For such purpose he devised the approprite degrees of freedom as follows:

$$f' = \frac{(\sum MS_n)^2}{\sum \frac{MS_n^2}{f_n}}$$

Where f' is the degree of freedom for the linear combination of mean square, to permit approximation to the F-distribution, MS_n is the n-th mean square, and fn is the degree of freedom appropriate to n-th mean square.

2.4.3. Estimation of genetic variation

In the previous section, it can be noted that the covariance of relative serve as a pivot-point to relate the biometrical variance component estimates to the genetic information available. Therefore, theoretical consideration of the formulation of this covariance assists in attaining unbiased estimates. Cockerham (1963) has emphasized the underlying genetical assumptions including normal Mendelian diploid inheritance, no environmental correlation among relatives, the relatives are random members of some non-inbred population and linkage equilibrium.

In many situations, however, a breeding programme involves continual selfing, though it may start from a non-inbred population. Therefore, adjustment for inbreeding should be made. Excellent discussion on the covariance of relatives when inbreeding is considered have been given by Kempthorne (1954), Comstock (1963), and Falconer (1981). Following

Kempthorne (1954), the covariance of full sib can be expressed as:

$$CovFS = \left(\frac{1+F}{2}\right)\sigma_A^2 + \left(\frac{1+F}{2}\right)^2\sigma_D^2 + \left(\frac{1+F}{2}\right)^2\sigma_{AA}^2 + \left(\frac{1+F}{2}\right)^3\sigma_{AD}^2 + \left(\frac{1+F}{2}\right)^4\sigma_{DD}^2$$

while covariance of half sib can be expressed as:

$$CovHS = \left(\frac{1+F}{4}\right)\sigma_A^2 + \left(\frac{1+F}{4}\right)^2\sigma_{AA}^2$$

where F represent the inbreeding coefficient of the parents.

2.4.4. Estimation of heritability

The idea of heritability originated as an attempt to describe whether variation in characters arose from the different genetypes or from different environmental (Hanson, 1963). Mather and Jinks (1977) defined heritability as the proportion of the total phenotypic variation attributtable to heritable variation.

In practice, a distinction is made between heritability in the broad sense (h^2_B) and that in narrow sense (h^2_N or simply h^2). The broad sense heritability is then defined as the proportion of total genotype variance to the phenotypic variance (σ^2_G/σ^2_P). The estimate of broad sense heritability provide quantitative information on the relative magnitude of genetic and environment variation for a given character in a specific population but is not usually an indication of response to selection which might be made on that population (Duddley and Moll, 1969).

Gordon *et al.* (1972) proposed two forms of broad sense heritability estimates applicable to phenotypic partitioning models commonly employed in plant breeding programme. These are *'full'* heritability (when all components of phenotypic variance are included in the denominator) and *'restricted'* heritability (when only parts of the total phenotypic variance are included in the denominator), as was common practice in the literature of the 1960's.

The narrow sense heritability, on the other hand, is the proportion of additive genetic variance to the total variance (σ^2_A/σ^2_P). The estimate of narrow sense heritability is of interest to the plant breeder as the effectiveness of many selection schemes is judged with respect to the average effects of alleles (the additive of genetic variance estimates) (Falconer, 1981). Furthermore, it is useful to choose among alternative selection criteria and strategies (Dudley and Moll, 1969) and to estimate the expected improvement due to selection (Robinson, 1963).

The narrow sense heritability can be estimated by a variety of methods. Warner (1952) classified them into three categories: (i) those based on variance component from an analysis of variance, (ii) those based on parent-offspring regression, and (iii) those based on the approximation of non heritable variance from genetically uniform population. However, only the first two methods are used commonly.

The estimation of narrow sense heritability based on the variance component analysis involves only one further step from the estimation the genetic variance component themselves. The general procedure of the estimation can be followed from many standard references, e.g. Falconer (1981) and Becker (1985).

In parent-offspring regression method, narrow sense heritability is equated to slope of linear regression line of the measurement among offspring on the mean of the measurement on their parents (Jacquard, 1983). The two variations most commonly used as the estimator of heritability are $2b = h^2$, when measurement is based on offspring on one parent, and $b = h^2$, when measurement is based on offspring and the mid parent.

2.4.5. Correlation among characters

Correlations between characters are frequent features in plant. To a plant breeder, a knowledge of the correlations that exist between important characters is valuable because it may provide basis for planning more efficient selection programme. Also, correlation between important and non-important characters may reveal that some of the latter are useful as indicators of one or more of the former.

As Falconer (1981) described, the correlation that is directly observable is phenotypic correlation. This is a compound of genetic and environmental causal components. The genetic correlation arise from pleotropy and from linkage that have not reached equilibrium. Pleotropy implies that a gene affects two or more characters, so that if the gene is segregating it cause simultaneous variation in the characters its affects.

The phenotypic correlation coefficient (r_p) between character X and Y can be defined as the ratio of the phenotypic covariance between the two characters to the geometric mean of their phenotypic variances, that is :

$$r_p = \frac{Cov_{XY}}{\sqrt{\sigma^2_X} \cdot \sqrt{\sigma^2_Y}}$$

Where r_P is the phenotypic correlation coefficient, Cov_{XY} is the phenotypic covariance between characters X and Y, σ^2_X and σ^2_Y are the phenotypic variances of character X and Y, respectively.

The genotypic correlation can be separated from the phenotypic one by analysis of covariance by method analogous to those used for the partition of variance (Falconer, 1981; Baker, 1986). Similarly, the genotypic correlation coeficient can be estimated as the ratio of the resulting genotypic covariance of the two characters being considered to the geometric mean of their estimated genotypic variances.

3. Testcross Evaluation for Cool Tolerance During Seedling Establishment

3.1. Objective

The objective of this study was to evaluate five cool tolerance synthetic populations that were developed through recurrent phenotypic selections by Department of Scientific and Industrial Research (DSIR) Palmerston North. These were: NZS1, NZS2, NZS3, AS3, and BS22. The evaluations included here were:

(a). the estimation of general combining ability (GCA) of those population;

(b). the estimation of their phenotypic, genotypic (GCA) and additive genetic variances;

(c). the estimation of heritability of those GCA;

(d). the estimation of the phenotypic and genetic correlations amongst characters.

3.2. Materials

In this study 54 genotypes (test cross progenies) of the fourth selfing (S4) of NZS1 and AS3, and of S3 of NZS2, NZS3, and BS22 and 14 check hybrids were used (Table 3.1). AS3-57-2-1-1 and three of the check hybrids were entered twice, making a total of 72 entries of 68 different genotypes.

The test cross progenies were produced in Palmerston North during 1989-1990 season with A665 x CM105 as a GCA tester. A665 has the pedigree ND203 x A635[4] and CM105 has the pedigree V3 x B14[2]. Check hybrids were produced using the tester as the male parent. The seeds were produced on ears that were hand pollinated, hand picked and dried at 25-30 °C with low humidity until the seed had reached approximately 12 % moisture.

Table 3.1. Exp1. Genotypes and their generation

	Genotype	Generation
1	NZS1-48-1-1-1	S4
2	NZS1-100-1-1-1	S4
3	NZS1-100-1-2-1	S4
4	NZS1-101-1-1-2	S4
5	NZS1-101-1-2-1	S4
6	NZS1-101-4-1-1	S4
7	NZS1-101-4-2-1	S4
8	NZS1-123-1-1-1	S4
9	NZS1-141-1-1-3	S4
10	NZS1-141-1-2-1	S4
11	AS3-50-1-1-1	S4
12	AS3-51-2-1-2	S4
13	AS3-57-2-1-1	S4
14	AS3-94-1-1-1	S4
15	AS3-94-1-2-1	S4
16	AS3-94-2-1-1	S4
17	NZS3-14-1-1	S3
18	NZS3-14-2-1	S3
19	NZS3-18-2-1	S3
20	NZS3-19-1-1	S3
21	NZS3-19-2-1	S3
22	NZS3-25-2-1	S3
23	NZS3-28-1-1	S3
24	NZS3-28-2-1	S3
25	NZS3-29-1-1	S3
26	NZS3-29-2-1	S3
27	NZS3-32-1-1	S3

Table 3.1. (Continued)

	Genotype	Generation
28	NZS3-38-2-1	S3
29	NZS3-49-1-1	S3
30	NZS3-49-2-1	S3
31	NZS3-51-1-1	S3
32	NZS3-51-2-1	S3
33	NZS3-53-1-1	S3
34	NZS3-53-2-1	S3
35	NZS3-57-2-1	S3
36	NZS3-59-1-1	S3
37	NZS3-59-2-1	S3
38	NZS3-61-1-1	S3
39	BS22-3-1-1	S3
40	BS22-8-1-2	S3
41	BS22-22-2-1	S3
42	BS22-22-2-2	S3
43	BS22-34-1-1	S3
44	BS22-39-1-1	S3
45	BS22-39-1-2	S3
46	BS22-78-1-1	S3
47	BS22-84-1-1	S3
48	BS22-92-2-1	S3
49	BS22-92-2-2	S3
50	BS22-151-2-1	S3
51	NZS2-5-2-1	S3
52	NZS2-21-1-2	S3
53	NZS2-70-1-1	S3
54	NZS2-70-1-2	S3

Table 3.1. (Continued)

	Genotype	Generation
55	NZS2-92-1-1	S3
56	NZS2-124-1-2	S3
57	HUN946-1-1-1	S3
58	M378-83-2-1-1-1	S5
59	M378-80-2-1-2-2	S5
60	M396-9-1-1-1	S4
61	M396-9-2-1-1	S4
62	M396-22-2-1-1	S4
63	M396-33-1-1-1	S4
64	NZ2	Inbred
65	NZ3	Inbred
66	H99	Inbred
67	W153R	Inbred
68	A659	Inbred

S3, S4 and S5 : the 3rd, 4th, and 5th selfed generations.

The pedigrees of the five synthetic popultions are presented in Table 3.2. Strictly, NZS1 and NZS2 were composites, as defined by Hallauer and Miranda (1981), but the term synthetic will be used for all five populations.

NZS1 was a cross between Criolo de Toluca and AS3 followed by backcrossing to AS3. Criolo de Toluca is a landrace population of Conico race from the Highlands of Mexico. AS3 is an elite synthetic of eight Corn Belt Dent inbreds. The version of AS3 used was AS-3(HT)C3.

NZS2 originated from the cross between San Geronimo and BS22 and backcrossed to BS22. San Geronimo is a landrace composite from The Mantaro Valley of Peru. BS22 is a synthetic of 16 Corn Belt Dent inbreds.

NZS3 was constructed by intercrossing 4 inbred lines of Corn Belt Dent origin and 4 partially inbred lines from CIMMYT Pool 5. The CIMMYT Pool 5 contains mainly germplasm of highland Mexican origin, but it also contains some germplasm of highland Andean origin and some of temperate origin.

3.3. Environment

The experiment was carried out in a controlled environment (phytotron). The plants were grown at day/night temperatures of 16/6 °C that alternated every 12 hours and at constant humidity of 85%. The changeover between day and night took 2 hours. Throughout the experimental period temperatures and humidity were maintained within 0.3 °C and 3% , respectively, of the nominal values. The light intensity was 700 uE/m2/s from 4 x 1 kW Philips tungsen halogen lamps. The temperatures of 16/6 °C were chosen because they are close to the minimum for sustained growth of maize (Hardacre and Turnbull, 1986).

Table 3.2. Pedigrees of the synthetic populations

Synthetic	Pedigree
NZS1	Criolo de Toluca X $AS3^2$
NZS2	San Geronimo X $BS22^2$
NZS3	(A239 x A658 x A671 x H99) x (5-154 x 5-250 x 5-514 x 5-536)
AS3	A73 x A286 x A295 x A375 x Oh5 x Oh43 x Oh51A x W22
BS22	A619 x A632 x CH9 x CM37 x C123 x MS214 x W153R x SD10 x Va43 x Mo17 x B68 x B55 x SD15 x M14 x Pa884P x (CMV3 X B14) sel.

(After Eagles and Hardacre, 1985; 1989; and 1990).

3.4. Cultural

Kernels were sown individually with the embryo in lower position at a standard depth of 40 mm in 150 mm^2 pots containing steam-sterilised potting mix composed of fine gravel: peat: vermiculite (70:15:15 v/v). The pots were placed on a trolley that was subdivided into two replicates. Each genotype was randomly entered into each replicate. There were six trollies representing complete blocks (see Plate 1). Twice each week the trollies were relocated to minimise the positional effects within the phytotron (Hardacre and Turnbull, 1986). A complete North Carolina mineral solution was applied up to three times a week according to the growth stage of the plant (see appendix 1 for the recipe).

3.5. Harvest

During the experiment two harvests were carried out. The first harvest was conducted at 33 days after sowing using all the plants in the first replicate of each block. The second harvest was at 62 days after sowing using all the plants in the second replicate. Harvests were done by cutting the plants at the media-surface, and harvesting separately shoot and root parts of the plant. Root parts were washed under running water.

3.6. Data collection and measurement

a. Time to emergence (EMERGE) was recorded for each seedling as the number of days from sowing to the emergence of the seedling to about 1 mm above the medium. This is meaningful because seeds were sown at standard depth (see section 3.4.). The data were observed from both internal replicates per block.

Plate 1. Experimental Lay-out

b. Leaf appearance was recorded on the plants from the second replicate. Both the total number of visible leaf tips and the number of mature leaves were counted. Leaves were defined as mature when the ligule had appeared. This was done at 30, 37, 40, 50, 56, and 62 days following planting. The serial data provided a means of estimating rate of leaf appearance.

c. Chlorophyll concentration (CHPHYLL) was measured on youngest mature leaf of the plants in second replicate at a point about one third of the distance from the leaf tip to the ligule. The measurements were taken using a chlorophyll sensitive photometer (Hardacre et al., 1984). During the experiment, four repeated measurements were taken on the same plant at 39, 45, 56, and 62 days after planting. The data collected from these measurements were calibrated with the chlorophyll analysis of wide range of leaf samples using Moran's procedure (1980). The callibrated data were, then, expressed as mg/g leaf sample.

d. Anthocyanin (ANTHOCY) was scored on a scale of 0 - 9, where 9 represent 50% or more of the leaves including ligule showed purple colour. This was recorded on the plants of second replicate at one day before the end of the experiment (61 days after planting).

e. Leaf area (LFAREA) was measured in square centimeter (cm^2) at the second harvest using all dissected leaves through the use of a leaf-area machine LICOR LI300.

f. Leaf thickness (LFTHICK) was measured in millimeters (mm) using a micrometer at the point where chlolophyll concentration was measured. The measurement was conducted at the second harvest.

g. Shoot and root weight (SHOOT and ROOT) were expressed in milligrams (mg) dry weight obtained from both harvests by drying shoot and root parts to constant weight under vacuum drier for seven days at 40 °C.

3.7. Data analysis

3.7.1. Statistical analysis

Two missing data out of 408 were obtained in characters EMERGE, SHOOT, and ROOT of the first replicate and prior to any statistical analysis the missing data were estimated using Yates's procedure (1933). As the number of misses was so trivial, it was assumed that any bias in using standard (balanced) analysis would be neglegible.

To provide appropriate data for analysis of variance of the character leaf appearance, a simple regression analysis was carried out separately on each experimental unit. Time of measurements were used as independent variable while leaf number was dependent variable. The function statistics (bo, b1) obtained from these analyses were used to estimate two characters, namely the number of days to attain two mature leaves (2MATLEAF), and the total of visible leaves at 50 days after planting (TOTLF50D). The character 2MATLEAF was estimated because it can be used as indication of the completion of heterotrophic growth (Cooper and MCDonald, 1970). The character TOT50D was more for agronomical interest.

Several models of analysis of variance were used because different characters had different data structures (replication, block, etc). The analyses were conducted under random effects philosophy for all components with the individual plant as the experimental unit. For those genotypes entered twice (AS3-57-2-1-1, H99, W153R, and A659), the average of the duplicates within each replicate formed the analysis unit. Otherwise, the analysis unit was the experimental unit.

The computer program SAS (Anon., 1988) was involved in the analyses of variance and the subsequent means discrimination. The expectation of mean squares were constructed following the procedures suggested by Crump (1946).

The variance components were estimated from the linear functions of the appropriate mean square expectations (see the following sections for the detailed discriptions). The standard errors of the variance components were estimated following Crump (1951) (see section 2.4.2).

F-test for the significance of variance component was constructed by choosing the appropriate mean square estimates, such that the numerator mean square expectations differed to the denominator mean square expectation by only the variance component being considered (e.g. see Crump, 1951; Steel and Torrie, 1980; Le Clerg et al., 1962).

In the case that the appropriate numerator or denominator was a linear fuction of mean squares (i.e. in the analysis model III and IV below), complex F-tests (Crump, 1946 and Satterthwaite, 1946) were applied. The degrees of freedom required for these linear functions (f') was estimated according to Satterthwaite (1946) so that a good approximation to the F-distribution could be made (see section 2.4.2).

The estimations of those variance components, and their standard errors, appropriate degrees of freedom and F-significances, were carried out using the computer program THWAITE (Gordon, unpublished).

The mean discriminations were conducted to compare the General Combining Ability (GCA) of the testcross genotypes being evaluated. The Student Newman Keul (SNK) procedure was choosen for this purpose because it provides an appropriate value for a particular comparison being

dependent upon relative ranking of the two means being compared, and gives adequate protection on type I error rate (Balaam, 1963; Gill, 1973; Chew, 1976). As well as amongst genotypic mean, the mean discrimination was made on the origin of the genotypes basis by making contrast amongst the synthetic populations and hybrid checks.

As mentioned earlier, several models were employed in the analysis of variance, as dictated by the various data structures. The description of these models are given as follows:

(1). Nested design (Model I)

The analysis of variance using this design was conducted for the character time to emergence (EMERGE) which had two replicates nested within six blocks (trolleys).
The model is:

$$X_{ijk} = \mu + \beta_i + \varrho_{j(i)} + \alpha_k + \varepsilon_{ijk}$$

Where: X_{ijk} = the ijk-th phenotypic variate
i=1...b, b=number of blocks
j=1..r, r=number of replications within block
k=1..g, g=number of genotypes

μ = the grand mean
β_i = the i-th block effect (trollies)
$\varrho_{j(i)}$ = the j-th replication effect, nested within block
α_k = the k-th genotype effect
ε_{ijk} = the residual effect.

The analysis of variance including degrees of freedom, mean square expectation, and appropriate F-ratio for this model are given in Table 3.3. The variance components of genotype (σ^2_G), replication ($\sigma^2_{R(B)}$), block (σ^2_B), and residual (σ^2) were estimated as $(MS_g - MS_e)/rb$, $(MS_r - MS_e)/g$, $(MS_b - MS_r)/rg$, and MS_e, respectively.

(2). Randomised Completely Block Design (Model II)

The analyses of variance using the RCBD model with six blocks (the trolleys) were conducted for characters 2MATLEAF, TOTLF50D, ANTHOCY, LFAREA, and LFTHICK.

The analysis was based on the following model:

$$X_{ij} = \mu + \beta_i + \alpha_j + \varepsilon_{ij}$$

Where: X_{ij} = the ijk-th phenotypic variate
i=1...b, b=number of blocks
j=1...g, g=number of genotypes

μ = the grand mean
β_i = the i-th block effect (Trollies)
α_j = the j-th genotype effect
ε_{ij} = the residual effect.

The analysis of variance including the degrees of freedom, expectations of the Mean Squares, and F-ratios for this model are given in Table 3.4. The variance component of genotype (σ^2_G), block (σ^2_B), and residual (σ^2) were calculated as $(MS_g - MS_e)/b$, $(MS_b - MS_e)/g$, and MS_e, respectively.

Table 3.3. Exp.I. The degree of freedom, Expectations of Mean Square, and F-ratio for nested design (model I)

Source	df	MSE	(MS)	F-ratio
Block	b-1	MS_b	$\sigma^2 + g\sigma^2_{R(B)} + rg\sigma^2_B$	MS_b/MS_r
Rep.(Block)	b(r-1)	MS_r	$\sigma^2 + g\sigma^2_{R(B)}$	MS_r/MS_e
Genotype	g-1	MS_g	$\sigma^2 + rb\sigma^2_G$	MS_g/MS_e
Residual	(br-1)(g-1)	MS_e	σ^2	

Table 3.4. Exp.I. The degree of freedom, Expectations of Mean Square, and F-ratio for Randomized Complete Block design (model II).

Source	df	MS	E (MS)	F-ratio
Block	b-1	MS_b	$\sigma^2 + g\sigma^2_B$	MS_b/MS_e
Genotype	g-1	MS_g	$\sigma^2 + b\sigma^2_G$	MS_g/MS_e
Residual	(b-1)(g-1)	MS_e	σ^2	

(3). Split plot in time (Model III)

This design was applied to pool the four repeated measurements on character CHPHYLL. As the basis for analysis, the following model was used.

$$X_{ijk} = \mu + \beta_i + \alpha_j + \delta_{ij} + \tau_k + \alpha\tau_{jk} + \varepsilon_{ijk}$$

Where: X_{ijk} = the ijk-th phenotypic variate

i=1...b, b=number of blocks

j=1...g, g=number of genotypes

k=1...t, t= time

μ = the grand mean

β_i = the i-th block effect (Trollies)

α_j = the j-th genotype effect

δ_{ij} = the interaction between block and genotype effects ($Error_{(a)}$)

= covariance across repeats (Gill, 1986)

τ_k = the k-th time effect

$\alpha\tau_{jk}$ = the interaction between genotype and time effects

ε_{ijk} = the residual effect ($Error_{(b)}$).

The analysis of variance including the degrees of freedom, Expectations of the Mean Squares, and F-ratios for this model are given in Table 3.5. In this table, the Expected Mean Square was constructed to provide correction for the time correlation bias, where: ρ is the correlation between two repeated measurements on the same unit and σ^2 is the base error variance when $\rho = 0$.

The components of variance were estimated using the following equations.

Block $(\sigma^2_B) = (MS_b - MS_{e(a)}/gt;$

Genotype $(\sigma^2_G) = [(MS_g+MS_{e(b)}) - (MS_{(a)}+MS_{gt})]/bt;$

Covariance $(\sigma^2_{e(a)}) = (MS_{e(a)} - MS_{e(b)})/t;$

Table 3.5. Exp.I. The degree of freedom, mean square, expected mean square, and F-ratio for Split Plot in Time (Model III)

Source	df	MS	E (MS)	F-ratio
Block (B)	b-1	MS_b	$\sigma 2\ [1+(t-1)\rho] + gt\sigma^2_B$	$(MS_b/MS_{e(a)})$
Genotype (G)	g-1	MS_g	$\sigma^2\ [1+(t-1)\rho] + b\sigma^2_{GT} + bt\sigma^2_G$	$(MS_g+MS_{e(b)})/(MS_{gt}+MS_{e(a)})$
Covariance	(b-1)(g-1)	$MS_{e(a)}$	$\sigma^2\ [1+(t-1)\rho]$	$MS_{e(a)}/MS_e$
Time (T)	t-1	MS_t	$\sigma^2\ (1-\rho) + b\sigma^2_{GT} + bg\sigma^2_T$	$(MS_t)/MS_{gt})$
G x T	(g-1)(t-1)	MS_{gt}	$\sigma^2\ (1-\rho) + b\sigma^2_{GT}$	$MS_{gt}/MS_e(b)$
Error (b)	g(b-1)(t-1)	$MS_{e(b)}$	$\sigma^2\ (1-\rho)$	

Time (σ^2_T) = (MS_t - MS_{gt}) / bg;
Genotype x Time (σ^2_{GT}) =(MS_{gt}-$MS_{e(b)}$)/b.
Base error (σ^2)= ($MS_{e(a)}$ + (t-1) $MS_{e(b)}$)t

(4). Extended Split plot in time (Model IV)

This design was used to pool the two separate harvests of characters SHOOT and ROOT. As there were two separate replications in each of these characters, each harvested at a separate time, it was assumed that the two times were independent samples, so that correction for time correlation bias (Gill, 1986) was not needed.

The model was an extension of the usual split plot in that the Block x Time interaction was partitioned out from the error(b) (Steel and Torrie, 1980). The model was:

$$X_{ijk} = \mu + \beta_i + \alpha_j + \delta_{ij} + \tau_k + \beta\tau_{ik} + \alpha\tau_{jk} + \varepsilon_{ijk}$$

Where: X_{ijk} = the ijk-th phenotypic variate
i=1...b, b=number of blocks
j=1...g, g=number of genotypes
k=1...t, t= times

μ = the grand mean
β_i = the i-th block effect (Trollies)
α_j = the j-th genotype effect
δ_{ij} = the interaction between block and genotype effects ($Error_a$)
τ_k = the k-th time effect
$\beta\tau_{ik}$ = the interaction between block and time effects
$\alpha\tau_{jk}$ = the interaction between genotype and time effects
ε_{ijk} = the residual effect ($Error_b$).

Table 3.6. Exp.I. The degree of freedom, expectation of Mean Square, and F-ratio for Extended Split Plot in Time (Model IV)

Source	df	MS	E (MS)	F-ratio
Block (B)	b-1	MS_b	$\sigma^2_b + t\sigma^2_a + g\sigma^2_{BT} + gt\sigma^2_B$	$(MS_b+MS_e(b))/(MS_{bt}+MS_{e(a)})$
Genotype (G)	g-1	MS_g	$\sigma^2_b + t\sigma^2_a + b\sigma^2_{GT} + bt\sigma^2_G$	$(MS_g+MS_e(b))/(MS_{gt}+MS_{e(a)})$
Error (a)	(b-1)(g-1)	$MS_{e(a)}$	$\sigma^2_b + t\sigma^2_a$	$MS_{e(a)}/MS_{e(b)}$
Time (T)	(t-1)	MS_t	$\sigma^2_b + g\sigma^2_{BT} + b\sigma^2_{GT} + bg\sigma 2_T$	$(MS_t+MS_{e(a)})/(MS_{bt}+MS_{gt})$
B x T	(b-1)(t-1)	MS_{bt}	$\sigma^2_b + g\sigma^2_{BT}$	$MS_{bt}/MS_e(a)$
G x T	(g-1)(t-1)	MS_{gt}	$\sigma^2_b + b\sigma^2_{GT}$	$MS_{gt}/MS_{e(a)}$
Error (b)	(b-1)(g-1)(t-1)	$MS_{e(b)}$	σ^2_b	

The analysis of variance including the degrees of freedom, expectations of the Mean Squares, and F-ratios for this model are given in Table 3.6. The complex-F and their degrees of freedom were estimated as discussed earlier (Satterthwaite, 1946).

The components of variance were estimated using the following equations.

Block $(\sigma^2_B) = [(MS_b+MS_{e(a)})-(MS_{bg}+MS_{bt})]/gt;$

Genotype $(\sigma^2_G) = [(MS_g+MS_{e(a)})-(MS_{bg}+MS_{gt})]/bt;$

Error $_{(a)}$ $(\sigma^2_{e(a)})= (MS_{e(a)}-MS_{e(b)})/t;$

Time $(\sigma^2_T) = [(MS_t + MS_{e(b)}) - (MS_{bt} + MS_{gt})] / bg;$

Block x Time $(\sigma^2_{BT})= (MS_{bt}-MS_{e(b)})/g;$

Genotype x Time $(\sigma^2_{GT}) =(MS_{gt}-MS_{e(b)})/b.$

3.7.2. Genetical analysis

3.7.2.1. Genetic variance estimation

The biometrical genotypic variance estimates were interrelated with genetical variances via covariance among relatives (Falconer, 1981). As all genotypes involved in this experiment have a common male parent, they represent half-sib families. Therefore, their genotype variance is equivalent to covariances amongst half-sib. This can be interpreted in terms of genetic variance by the following equation:

$$\sigma^2_G = COV_{HS} = [(1+F)/4]\ \sigma^2_A, \quad \text{(Kempthorne, 1957),}$$

$$\sigma^2_G = k\ \sigma^2_A$$

thus, $\sigma^2_A = 1/k\ \sigma^2_G$

where F is the inbreeding coefficient of the plants being tested. As the genotypes being tested in this experiment have different level of inbreeding, the harmonic mean of their inbreeding coefficients was used (Table 3.7).

In addition, the phenotypic variances (σ^2_P) were obtained by equating them to the total variance calculated in variance component estimations above.

3.7.2.2. Heritability estimation

The heritability (h^2) was expressed in two ways, broad and narrow senses heritabilities as described by Becker (1984) and Falconer (1981). The broad sense heritability (h^2_B) was estimated as the ratio of the genotype variance to its corresponding phenotypic variance (see above). The narrow sense heritability (h^2) was estimated as the ratio of the additive variance to its corresponding phenotypic variance. The phenotypic variance later mentioned was constructed as :

$$\sigma^2_P = k\sigma^2_A + \sigma^2_E$$

where k denotes (1+F)/4, as described by Kempthorne (1957), and σ^2E represents the sum of all variance components excluding the genotypic variance component.

As the methods of the estimation for standard error of narrow sense heritability have not been available, only those of the broad sense heritabilites were estimated. These were calculated following the procedures described by Gordon et al (1972) and Gordon (1979).

Table 3.7. Exp.I. Genotypes and their inbreeding coefficient

	Genotype	Inbreding coef.
1	NZS1-48-1-1-1	0.938
2	NZS1-100-1-1-1	0.938
3	NZS1-100-1-2-1	0.938
4	NZS1-101-1-1-2	0.938
5	NZS1-101-1-2-1	0.938
6	NZS1-101-4-1-1	0.938
7	NZS1-101-4-2-1	0.938
8	NZS1-123-1-1-1	0.938
9	NZS1-141-1-1-3	0.938
10	NZS1-141-1-2-1	0.938
11	AS3-50-1-1-1	0.938
12	AS3-51-2-1-2	0.938
13	AS3-57-2-1-1	0.938
14	AS3-94-1-1-1	0.938
15	AS3-94-1-2-1	0.938
16	AS3-94-2-1-1	0.938
17	NZS3-14-1-1	0.875
18	NZS3-14-2-1	0.875
19	NZS3-18-2-1	0.875
20	NZS3-19-1-1	0.875
21	NZS3-19-2-1	0.875
22	NZS3-25-2-1	0.875
23	NZS3-28-1-1	0.875
24	NZS3-28-2-1	0.875
25	NZS3-29-1-1	0.875
26	NZS3-29-2-1	0.875
27	NZS3-32-1-1	0.875

Table 3.7. (Continued)

	Genotype	Inbreding coef.
28	NZS3-38-2-1	0.875
29	NZS3-49-1-1	0.875
30	NZS3-49-2-1	0.875
31	NZS3-51-1-1	0.875
32	NZS3-51-2-1	0.875
33	NZS3-53-1-1	0.875
34	NZS3-53-2-1	0.875
35	NZS3-57-2-1	0.875
36	NZS3-59-1-1	0.875
37	NZS3-59-2-1	0.875
38	NZS3-61-1-1	0.875
39	BS22-3-1-1	0.875
40	BS22-8-1-2	0.875
41	BS22-22-2-1	0.875
42	BS22-22-2-2	0.875
43	BS22-34-1-1	0.875
44	BS22-39-1-1	0.875
45	BS22-39-1-2	0.875
46	BS22-78-1-1	0.875
47	BS22-84-1-1	0.875
48	BS22-92-2-1	0.875
49	BS22-92-2-2	0.875
50	BS22-151-2-1	0.875
51	NZS2-5-2-1	0.875
52	NZS2-21-1-2	0.875
53	NZS2-70-1-1	0.875
54	NZS2-70-1-2	0.875

Table 3.7. (Continued)

	Genotype	Inbreding coef.
55	NZS2-92-1-1	0.875
56	NZS2-124-1-2	0.875
57	HUN946-1-1-1	0.875
58	M378-83-2-1-1-1	0.969
59	M378-80-2-1-2-2	0.969
60	M396-9-1-1-1	0.938
61	M396-9-2-1-1	0.938
62	M396-22-2-1-1	0.938
63	M396-33-1-1-1	0.938
64	NZ2	1.000
65	NZ3	1.000
66	H99	1.000
67	W153R	1.000
68	A659	1.000

3.7.2.3. Correlation analysis

The correlation amongst characters were expressed as phenotypic and genetic correlations. In order to provide data for the correlation analysis, some characters having more than one sample or measurement (i.e. EMERGE, CHPHYLL, SHOOT, and ROOT) were re-arranged to provide a common structure equivalent to RCB design.

For character EMERGE the data were averaged over the two replicates. Similarly, for character CHPHYLL the data were averaged over four repeated measurements. For each of characters SHOOT and ROOT, an RCBD multivariate analysis of variance (MANOVA) as implemented in PROC ANOVA of SAS program was performed to obtain the standardized multiple discriminant scores. The first scores of this analysis were, then, used to combine those two measurements for each those characters.

After these several adjustments were made, all characters had the same data structure thereby, enabling all SSCP's to be obtained. The phenotypic correlation coefficients (r_p) were estimated as simple correlation coefficient (Falconer, 1981). Proc CORR as implemented in computer program SAS (Anon, 1988) was employed in the analysis.

The genetic correlations were estimated as the genotypic correlations. Again, an RCBD multivariate analysis of variance was carried out across all characters, using the same data for phenotypic correlation analysis. This provided all the partitioned SSCP matices. The genotypic cross-product were estimated thereby, in addition to the SS. The correlation coefficients (r_G) were estimated following Falconer (1981) and Baker (1986) (see section 2.4.5).

3.8. Results

3.8.1. General values

The general values of the testcross progenies under investigation are indicated by their grand means which are presented in the Table 3.8. This provides a basis for comparison and focusing of idea. This table also summarises the overall variability in two ways, the range (minimum and maximum) and coefficient of variation. According to Balaam (1963) a coefficient of variation less than 20 % is acceptable for most biological experiments. In this experiment, there are several characters that exhibit relatively high coefficients of variation. These included SHOOT1, SHOOT2, ROOT2, ANTHOCY and LAREA. Consequently the significances of their means and variance components are tested less efficiently.

3.8.2. Variance component estimates

The variance component estimates involving environmental and genotypic effects are presented in Table 3.9 and Table 3.10, respectively. The associated standard errors indicate the precision of the estimates. The levels of significance for their F-tests are also given in these Tables.

3.8.2.1. Variance components of environmental effects

As there were several statistical models involved in the analysis, the composition of the environmental variance component varies according to the model used (see Table 3.9). From this table it can be noted that most characters were influenced significantly by environment. The relativity of this effect will become evident later in discussion of heritability.

Table 3.8. Exp.I. The grand means, their range values and coefficients of variations

Character	Grand Mean	Minimum	Maximum	C.V. (%)	Unit
EMERGE	13.64	12.67	14.75	5.57	days
2MATLEAF	39.08	31.06	60.05	5.77	days
TOTLF50D	5.31	4.02	6.71	5.58	no.
CHPHYLL	1.31	0.42	2.02	13.68	mg/g
ANTHOCY	2.73	0.00	9.00	75.79	ord.score
LFAREA	89.50	34.00	176.00	21.56	cm^2
LFTHICK	17.16	6.00	21.00	7.87	m
SHOOT	0.20	0.02	0.69	31.46	g
ROOT	0.38	0.11	1.05	24.34	g

Table 3.9. Exp.I Variance component estimates involving genotypic effect, their standard errors (in brackets) and significances

Character	σ^2_G	$\sigma^2_{(TxG)}$
EMERGE	0.1504	-
	(0.0339)	
	**	
MATLEAF	1.3585	-
	(0.381)	
	**	
TOTLF50D	0.0574	-
	(0.0123)	
	**	
CHPHYLL	0.0130	0.0013
	(0.003)	(0.0007)
	**	**
ANTHOCY	2.2049	-
	(0.500)	
	**	
LFAREA	159.9623	-
	(38.106)	
	**	
LFTHICK	0.4545	-
	(0.131)	
	**	
SHOOT	0.0008	0.0005
	(0.0004)	(0.0003)
	**	**
ROOT	0.003	0.0008
	(0.001)	(0.0005)
	**	**

- not applicable, ns non significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level.

Table 3.10. Exp.I. Variance component estimates involving environmental effect, their standard errors (in brackets) and significances

Character	σ^2_B	$\sigma^2_{R(B)}$	σ^2_δ	σ^2_T	$\sigma^2_{(TxB)}$	σ^2
EMERGE	0.0090	0.0430	-	-	-	0.5975
	(0.0023)	(0.0258)				(0.0003)
	ns	**				
MATLEAF	0.0902	-	-	-	-	5.0914
	(0.088)					(0.3922)
	ns					
TOTLF50D	0.0014	-	-	-	-	0.0876
	(0.001)					(0.0067)
	ns					
CHPHYLL	0.0036	-	0.0009	0.0089	-	0.0321
	(0.002)		(0.0001)	(0.0057)		(0.0014)
	**		**	**		
ANTHOCY	0.2358	-	-	-	-	4.2783
	(0.159)					(0.3296)
	**					

Table 3.10. (Continued)

Character	σ^2_B	$\sigma^2_{R(B)}$	σ^2_δ	σ^2_T	$\sigma^2_{(TxB)}$	σ^2
LFAREA	13.2634	-	-	-	-	372.5420
	(0.027)					(28.6996)
	**					
LFTHICK	0.3176	-	-	-	-	1.8232
	(0.184)					(0.1404)
	**					
SHOOT	-0.0001	-	0.0001	0.0222	0.0005	0.0046
	(0.0001)		(0.0002)	(0.0182)	(0.0003)	(0.0003)
	ns		ns	**	**	
ROOT	-0.0002	-	0.0004	0.0385	0.0008	0.0085
	(0.0002)		(0.0003)	(0.0320)	(0.0005)	(0.0007)
	ns		ns	**	**	

- not applicable, ns non-significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level

The replication within block component ($\sigma^2_{R(B)}$) was the most significant environmental component that contributed the variation on character EMERGE, while the block components (σ^2_B) gave only an unimportant and non-significant contribution to this character. Presence of significant on $\sigma^2_{R(B)}$ component for character EMERGE indicated that internal replication was more important compared to blocking (trolleys). The absence of blocking effect probably resulted from the regular relocation of the trolleys during the experiment.

For characters CHPHYLL, ANTHOCY, LFAREA, and LFTHICK, however, the block effect showed highly significant. These results were unexpected. In climate room (phytotron) experiment block effect would normally be very small because of the eveness of the conditions within the room. Furthermore, regular relocation of the blocks (trolleys) would ensure of this eveness. Therefore, the significant of the block effects on these characters are difficult to explain.

For character SHOOT and ROOT, block variance component estimate exhibited a negative value and was non-significant. This is typical of sampling from a population of effects with mean close to zero. This is also one of the problems often encountered when using the analysis of variance technique in estimating a variance component. Searle (1971) showed several possibilities in handling a negative variance components. These include :

1. It can be taken as evidence that the true value of the component is zero.
2. Accepting a negative estimate as evidence that the true value of the corresponding component is zero suggests changing the estimate which is negative to be zero.
3. Ignoring that component in the model, but retaining the factor so far as the lines in the analysis of variance tables are concerned.
4. Interpreting a negative estimate as indication of a wrong model and change model that have finite rather than infinite populations.

5. Use estimation procedure other than the analysis of variance methods, such as maximum likelihood (ML) and maximum norm quadratic unbiased estimator (MINQUE).
6. Collect more data and repeat the analysis, either on the new or on the new and old pooled together.

The time variance component (σ^2_T) was found to be significant on characters CHPHYLL, SHOOT and ROOT which were subjected to repeated measurements. A significance of time variance component on character SHOOT and ROOT was expected because as the plant grows the dry mass will also increase. Whereas that on character CHPHYLL indicated that chlorophyll concentration was changed during the course of the experiment. This may reflect changes in light penetration in the growing conditions. In addition, for characters SHOOT and ROOT, the estimates of block-time interaction variance component (σ^2_{BT}) indicated significant but their values were relatively small. This suggested that the effect of location were changing as the root and shoot biomass changed.

3.8.2.2. Variance of genotypic effects

As different character had different data structure and analysis, the composition of this effect was vary accordingly. For characters EMERGE, 2MATLEAF, TOTLF50D, ANTHOCY, LFAREA, and LFTHICK this category contains genotypic components (σ^2_G) only, while for charaters CHPHYLL, SHOOT, and ROOT it also includes the first order interaction with time (σ^2_{GT}) (see Table 3.10).

Highly significant variations due to the mean genotypic effect (σ^2_G) were found in all characters. As the genotypic variance in this experiment arised from test-crosses, this component respresent the general combining ability (GCA) variance among genotypes with regard to these characters. This

evidence was very strong as the standard errors of variances estimates were relatively low, except for SHOOT, for which there was about 50% of the variance estimate.

Presence of such genotypic variations are highly expected, as the genotype evaluated in this study were derived from very diverse origin (see section 3.2). It should be noted, however, that the magnitude of the variations were not solely due to the test-line genotypic differences, but also due to gametes sample of the tester. In this evaluation the tester used to generate the testcross progenies was a weak hybrid, i.e. it was derived from a cross between non-homozygous parents. Consequently, the individual plants of the tester would be expected to have constrained genotypic variations.

For character CHPHYLL, SHOOT and ROOT, the variance component of genotype-time was significant. This indicate that one or more genotypes had different trends for these characters over time. Furthermore, the presence of pronounced levels of genotypic variance for all characters studied suggest that notable heritabilities may be found. This will be discussed subsequently.

3.8.3. Means discrimination

The means of the five synthetic populations for nine characters studied are presented in Table 3.11. It can be noted that the NZS3 group was superior for most of the characters studied compared to the other groups. An interesting feature is that both NZS1 and NZS2 groups showed similar performances to their corresponding synthetic parents (AS3 and BS22, respectively), except on the leaf pigmentation (CHPHYLL and ANTHOCY) and leaf thickness (LFTHICK). Both NZS1 and NZS2 were lower in CHPHYLL but higher in ANTHOCY compared to their synthetic parents. For LFTHICK, only NZS1 was less than its synthetic parent (AS3), while NZS2 was similar to BS22 with respect to this character.

Table 3.11. Exp.I. Contrasts amongst the origin-group of the genotypes

Origin	EMERGE (days)	2MATLEAF (days)	TOTLF50D	CHPHYLL (mg/g)	ANTHOCY (score)
NZS1	13.80 bc	39.0 b	5.31 b	1.289 bc	4.2 a
AS3	14.06 d	39.8 b	5.22 b	1.338 ab	2.5 bc
NZS3	13.30 a	38.5 a	5.42 a	1.381 a	2.9 b
BS22	13.94 cd	39.5 b	5.20 b	1.230 c	2.1 c
NZS2	13.64 b	38.8 ab	5.21 b	1.145 d	2.7 bc
Checks	13.67 b	39.4 b	5.29 b	1.354 ab	1.9 c

Table 3.11. (Continued)

Origin	LFAREA (cm)	LFTHICK (m)	SHOOT (g)	ROOT (g)
NZS1	91.12 b	16.88 b	0.218 a	0.404 ab
AS3	90.53 b	16.92 b	0.215 a	0.412 ab
NZS3	98.61 a	17.44 a	0.222 a	0.415 a
BS22	79.21 c	16.93 b	0.153 c	0.304 c
NZS2	78.56 c	16.19 c	0.159 c	0.296 c
Checks	86.71 b	17.71 a	0.187 b	0.388 b

Table 3.12. Exp. I. Means discrimination among genotype for emergence time (EMERGE)

	Genotype	Mean (days)	Comparison
1	NZS1-48-1-1-1	13.58	abcdefg
2	NZS1-100-1-1-1	13.42	cdefg
3	NZS1-100-1-2-1	13.67	abcdefg
4	NZS1-101-1-1-2	13.75	abcdefg
5	NZS1-101-1-2-1	13.58	abcdefg
6	NZS1-101-4-1-1	14.17	abcdef
7	NZS1-101-4-2-1	14.08	abcdef
8	NZS1-123-1-1-1	14.75	a
9	NZS1-141-1-1-3	13.58	abcdefg
10	NZS1-141-1-2-1	13.42	cdefg
11	AS3-50-1-1-1	14.67	ab
12	AS3-51-2-1-2	14.00	abcdef
13	AS3-57-2-1-1	14.13	abcdef
14	AS3-94-1-1-1	13.58	abcdefg
15	AS3-94-1-2-1	13.50	bcdefg
16	AS3-94-2-1-1	14.50	abc
17	NZS3-14-1-1	13.25	defg
18	NZS3-14-2-1	13.17	defg
19	NZS3-18-2-1	13.42	cdefg
20	NZS3-19-1-1	13.08	efg
21	NZS3-19-2-1	13.58	abcdefg
22	NZS3-25-2-1	13.50	bcdefg
23	NZS3-28-1-1	13.08	defg
24	NZS3-28-2-1	13.08	defg
25	NZS3-29-1-1	13.08	defg
26	NZS3-29-2-1	13.17	defg

Table 3.12. (Continued)

	Genotype	Mean (day)	Comparison
27	NZS3-32-1-1	13.08	defg
28	NZS3-38-2-1	13.83	abcdefg
29	NZS3-49-1-1	13.08	defg
30	NZS3-49-2-1	12.92	g
31	NZS3-51-1-1	13.92	abcdef
32	NZS3-51-2-1	13.42	cdefg
33	NZS3-53-1-1	13.50	bcdefg
34	NZS3-53-2-1	13.75	abcdefg
35	NZS3-57-2-1	12.92	fg
36	NZS3-59-1-1	13.17	defg
37	NZS3-59-2-1	12.67	g
38	NZS3-61-1-1	13.92	abcdef
39	BS22-3-1-1	13.50	bcdefg
40	BS22-8-1-2	14.17	abcdef
41	BS22-22-2-1	13.58	abcdefg
42	BS22-22-2-2	13.92	abcdef
43	BS22-34-1-1	14.25	abcde
44	BS22-39-1-1	14.00	abcdef
45	BS22-39-1-2	14.00	abcdef
46	BS22-78-1-1	13.67	abcdefg
47	BS22-84-1-1	13.17	defg
48	BS22-92-2-1	14.17	abcdef
49	BS22-92-2-2	14.50	abc
50	BS22-151-2-1	14.33	abcd
51	NZS2-5-2-1	14.17	abcdef
52	NZS2-21-1-2	13.67	abcdefg
53	NZS2-70-1-1	13.50	bcdefg

Table 3.12. (Continued)

	Genotype	Mean (day)	Comparison
54	NZS2-70-1-2	13.50	cdefg
55	NZS2-92-1-1	13.00	fg
56	NZS2-124-1-2	14.00	abcdef
57	HUN946-1-1-1	14.25	abcde
58	M378-83-2-1-1-1	13.58	bcdefg
59	M378-80-2-1-2-2	13.75	abcdefg
60	M396-9-1-1-1	13.75	abcdefg
61	M396-9-2-1-1	13.75	abcdefg
62	M396-22-2-1-1	13.42	cdefg
63	M396-33-1-1-1	13.50	bcdefg
64	NZ2	13.83	abcdefg
65	NZ3	13.33	cdefg
66	H99	13.83	abcdefg
67	W153R	13.42	cdefg
68	A659	13.63	abcdefg

Means with the same letter are not significantly different according to SNK test at 5% level.

Table 3.13. Exp.I. Discrimination among genotypic means for number of days to attain second mature leaf (2MATLEAF)

	Genotype	Mean (days)	Comparison
1	NZS1-48-1-1-1	38.5	bcde
2	NZS1-100-1-1-1	36.6	bcde
3	NZS1-100-1-2-1	34.6	e
4	NZS1-101-1-1-2	39.9	abcde
5	NZS1-101-1-2-1	40.3	abcd
6	NZS1-101-4-1-1	42.0	ab
7	NZS1-101-4-2-1	41.1	abc
8	NZS1-123-1-1-1	39.2	bcde
9	NZS1-141-1-1-3	38.4	bcde
10	NZS1-141-1-2-1	39.9	abcde
11	AS3-50-1-1-1	39.6	abcde
12	AS3-51-2-1-2	40.4	abcd
13	AS3-57-2-1-1	41.0	abc
14	AS3-94-1-1-1	39.1	bcde
15	AS3-94-1-2-1	39.4	abcde
16	AS3-94-2-1-1	39.4	abcde
17	NZS3-14-1-1	38.9	bcde
18	NZS3-14-2-1	39.0	bcde
19	NZS3-18-2-1	36.8	bcde
20	NZS3-19-1-1	39.3	bcde
21	NZS3-19-2-1	39.9	abcde
22	NZS3-25-2-1	36.3	cde
23	NZS3-28-1-1	38.7	bcde
24	NZS3-28-2-1	39.9	abcde
25	NZS3-29-1-1	38.0	bcde
26	NZS3-29-2-1	35.5	de

Table 3.13. (Continued)

	Genotype	Mean (days)	Comparison
27	NZS3-32-1-1	39.4	abcde
28	NZS3-38-2-1	37.7	bcde
29	NZS3-49-1-1	37.2	bcde
30	NZS3-49-2-1	42.0	ab
31	NZS3-51-1-1	39.6	abcde
32	NZS3-51-2-1	38.7	bcde
33	NZS3-53-1-1	39.7	abcde
34	NZS3-53-2-1	38.3	bcde
35	NZS3-57-2-1	38.8	bcde
36	NZS3-59-1-1	37.5	bcde
37	NZS3-59-2-1	37.3	bcde
38	NZS3-61-1-1	39.9	abcde
39	BS22-3-1-1	39.9	abcde
40	BS22-8-1-2	40.1	abcd
41	BS22-22-2-1	39.0	bcde
42	BS22-22-2-2	39.0	bcde
43	BS22-34-1-1	39.6	abcde
44	BS22-39-1-1	38.9	bcde
45	BS22-39-1-2	39.2	bcde
46	BS22-78-1-1	38.8	bcde
47	BS22-84-1-1	38.9	bcde
48	BS22-92-2-1	40.2	abcd
49	BS22-92-2-2	40.1	abcd
50	BS22-151-2-1	40.3	abcd
51	NZS2-5-2-1	37.3	bcde
52	NZS2-21-1-2	40.1	abcd
53	NZS2-70-1-1	40.4	abcd

Table 3.13. (Continued)

	Genotype	Mean (days)	Comparison
54	NZS2-70-1-2	40.0	abcd
55	NZS2-92-1-1	38.1	bcde
56	NZS2-124-1-2	37.1	bcde
57	HUN946-1-1-1	44.2	a
58	M378-83-2-1-1-1	38.9	bcde
59	M378-80-2-1-2-2	39.0	bcde
60	M396-9-1-1-1	40.4	abcd
61	M396-9-2-1-1	39.8	abcde
62	M396-22-2-1-1	39.5	abcde
63	M396-33-1-1-1	38.5	bcde
64	NZ2	38.1	bcde
65	NZ3	38.4	bcde
66	H99	38.9	bcde
67	W153R	39.0	bcde
68	A659	38.4	bcde

Means with the same letter are not significantly different accodring to SNK test at 5% level.

Table 3.14. Exp.I. Discrimination among genotypic means for total leaves at 50 days (TOTLF50D)

	Genotype	Mean (no.)	Comparison
1	NZS1-48-1-1-1	5.64	abcdef
2	NZS1-100-1-1-1	5.44	abcdefgh
3	NZS1-100-1-2-1	5.87	a
4	NZS1-101-1-1-2	5.06	efghij
5	NZS1-101-1-2-1	5.26	bcdefghij
6	NZS1-101-4-1-1	4.73	j
7	NZS1-101-4-2-1	4.95	ghij
8	NZS1-123-1-1-1	5.42	abcdefgh
9	NZS1-141-1-1-3	5.54	abcdefgh
10	NZS1-141-1-2-1	5.15	cdefghij
11	AS3-50-1-1-1	5.12	cdefghij
12	AS3-51-2-1-2	4.91	hij
13	AS3-57-2-1-1	4.94	ghij
14	AS3-94-1-1-1	5.28	abcdefghij
15	AS3-94-1-2-1	5.57	abcdefg
16	AS3-94-2-1-1	5.49	abcdefgh
17	NZS3-14-1-1	5.40	abcdefghi
18	NZS3-14-2-1	5.61	abcdefg
19	NZS3-18-2-1	5.48	abcdefgh
20	NZS3-19-1-1	5.32	abcdefghij
21	NZS3-19-2-1	5.28	abcdefghij
22	NZS3-25-2-1	5.92	a
23	NZS3-28-1-1	5.15	cdefghij
24	NZS3-28-2-1	5.14	cdefghij
25	NZS3-29-1-1	5.74	abcd
26	NZS3-29-2-1	5.84	ab

Table 3.14. (Continued)

	Genotype	Mean (no.)	Comparison
27	NZS3-32-1-1	5.42	abcdefgh
28	NZS3-38-2-1	5.35	abcdefghij
29	NZS3-49-1-1	5.69	abcde
30	NZS3-49-2-1	5.40	abcdefghi
31	NZS3-51-1-1	4.97	fghij
32	NZS3-51-2-1	5.46	abcdefgh
33	NZS3-53-1-1	5.19	bcdefghij
34	NZS3-53-2-1	5.31	abcdefghij
35	NZS3-57-2-1	5.42	abcdefgh
36	NZS3-59-1-1	5.76	abc
37	NZS3-59-2-1	5.42	abcdefgh
38	NZS3-61-1-1	4.93	ghij
39	BS22-3-1-1	5.19	bcdefghij
40	BS22-8-1-2	5.32	abcdefghij
41	BS22-22-2-1	5.48	abcdefgh
42	BS22-22-2-2	5.54	abcdefgh
43	BS22-34-1-1	5.12	cdefghij
44	BS22-39-1-1	5.02	efghij
45	BS22-39-1-2	5.08	defghij
46	BS22-78-1-1	5.28	abcdefghij
47	BS22-84-1-1	5.54	abcdefgh
48	BS22-92-2-1	5.07	defghij
49	BS22-92-2-2	4.85	ij
50	BS22-151-2-1	4.92	ghij
51	NZS2-5-2-1	5.34	abcdefghij
52	NZS2-21-1-2	5.04	efghij
53	NZS2-70-1-1	4.99	fghij

Table 3.14. (Continued)

	Genotype	Mean (no.)	Comparison
54	NZS2-70-1-2	5.01	fghij
55	NZS2-92-1-1	5.38	abcdefghij
56	NZS2-124-1-2	5.52	abcdefgh
57	HUN946-1-1-1	4.72	j
58	M378-83-2-1-1-1	5.19	cdefghij
59	M378-80-2-1-2-2	5.47	abcdefgh
60	M396-9-1-1-1	5.11	cdefghij
61	M396-9-2-1-1	5.13	cdefghij
62	M396-22-2-1-1	5.39	abcdefghij
63	M396-33-1-1-1	5.31	abcdefghij
64	NZ2	5.54	abcdefgh
65	NZ3	5.51	abcdefgh
66	H99	5.34	abcdefghij
67	W153R	5.42	abcdefgh
68	A659	5.39	abcdefghij

Means with the same letter are not significantly different according to SNK test at 5% level.

Table 3.15. Exp.I. Discrimination among genotypic means for chlorophyl concentration (CHPHYLL)

	Genotype	Mean (mg/g)	Comparison
1	NZS1-48-1-1-1	1.39	abcde
2	NZS1-100-1-1-1	1.36	abcdef
3	NZS1-100-1-2-1	1.35	abcdef
4	NZS1-101-1-1-2	1.33	abcdef
5	NZS1-101-1-2-1	1.38	abcde
6	NZS1-101-4-1-1	1.35	abcdef
7	NZS1-101-4-2-1	1.23	bcdefghi
8	NZS1-123-1-1-1	1.19	cdefghi
9	NZS1-141-1-1-3	1.29	abcdefg
10	NZS1-141-1-2-1	1.03	fghi
11	AS3-50-1-1-1	1.29	abcdefg
12	AS3-51-2-1-2	1.38	abcde
13	AS3-57-2-1-1	1.35	abcdef
14	AS3-94-1-1-1	1.35	abcdef
15	AS3-94-1-2-1	1.31	abcdefg
16	AS3-94-2-1-1	1.35	abcdef
17	NZS3-14-1-1	1.45	abcde
18	NZS3-14-2-1	1.39	abcde
19	NZS3-18-2-1	1.62	a
20	NZS3-19-1-1	1.21	cdefghi
21	NZS3-19-2-1	1.38	abcde
22	NZS3-25-2-1	1.26	bcdefghi
23	NZS3-28-1-1	1.40	abcde
24	NZS3-28-2-1	1.29	abcdefg
25	NZS3-29-1-1	1.38	abcde
26	NZS3-29-2-1	1.48	abcd

Table 3.15. (Continued)

	Genotype	Mean (mg/g)	Comparison
27	NZS3-32-1-1	1.24	bcdefghi
28	NZS3-38-2-1	1.49	abcd
29	NZS3-49-1-1	1.45	abcde
30	NZS3-49-2-1	1.46	abcde
31	NZS3-51-1-1	1.22	cdefghi
32	NZS3-51-2-1	1.32	abcdef
33	NZS3-53-1-1	1.44	abcde
34	NZS3-53-2-1	1.58	ab
35	NZS3-57-2-1	1.38	abcde
36	NZS3-59-1-1	1.31	abcdefg
37	NZS3-59-2-1	1.38	abcde
38	NZS3-61-1-1	1.26	bcdefgh
39	BS22-3-1-1	1.19	cdefghi
40	BS22-8-1-2	1.16	defghi
41	BS22-22-2-1	1.30	abcdefg
42	BS22-22-2-2	1.31	abcdef
43	BS22-34-1-1	0.96	hi
44	BS22-39-1-1	1.23	bcdefghi
45	BS22-39-1-2	1.18	cdefghi
46	BS22-78-1-1	1.34	abcdef
47	BS22-84-1-1	1.28	abcdefg
48	BS22-92-2-1	1.26	bcdefgh
49	BS22-92-2-2	1.42	abcde
50	BS22-151-2-1	1.12	efghi
51	NZS2-5-2-1	1.32	abcdef
52	NZS2-21-1-2	1.20	cdefghi
53	NZS2-70-1-1	0.95	i

Table 3.15. (Continued)

	Genotype	Mean (mg/g)	Comparison
54	NZS2-70-1-2	0.98	ghi
55	NZS2-92-1-1	1.15	defghi
56	NZS2-124-1-2	1.26	bcdefgh
57	HUN946-1-1-1	1.17	cdefghi
58	M378-83-2-1-1-1	1.34	abcdef
59	M378-80-2-1-2-2	1.53	abc
60	M396-9-1-1-1	1.33	abcdef
61	M396-9-2-1-1	1.28	abcdefg
62	M396-22-2-1-1	1.31	abcdef
63	M396-33-1-1-1	1.40	abcde
64	NZ2	1.33	abcdef
65	NZ3	1.46	abcde
66	H99	1.42	abcde
67	W153R	1.29	abcdefg
68	A659	1.43	abcde

Means with the same letter are not significantly different accodring to SNK test at 5% level.

Table 3.16. Exp.I. Discrimination among genotypic means for anthocyanin score (ANTHOCY))

	Genotype	Mean (score)	Comparison
1	NZS1-48-1-1-1	1.2	de
2	NZS1-100-1-1-1	6.8	ab
3	NZS1-100-1-2-1	7.8	a
4	NZS1-101-1-1-2	6.8	ab
5	NZS1-101-1-2-1	3.5	bcde
6	NZS1-101-4-1-1	5.0	abcde
7	NZS1-101-4-2-1	3.2	bcde
8	NZS1-123-1-1-1	0.3	e
9	NZS1-141-1-1-3	2.2	cde
10	NZS1-141-1-2-1	4.7	abcde
11	AS3-50-1-1-1	3.0	bcde
12	AS3-51-2-1-2	1.0	de
13	AS3-57-2-1-1	1.7	cde
14	AS3-94-1-1-1	3.2	bcde
15	AS3-94-1-2-1	5.0	abcde
16	AS3-94-2-1-1	1.3	de
17	NZS3-14-1-1	2.2	cde
18	NZS3-14-2-1	1.8	cde
19	NZS3-18-2-1	4.7	abcde
20	NZS3-19-1-1	2.0	cde
21	NZS3-19-2-1	1.2	de
22	NZS3-25-2-1	3.7	abcde
23	NZS3-28-1-1	1.7	cde
24	NZS3-28-2-1	3.5	bcde
25	NZS3-29-1-1	3.3	bcde
26	NZS3-29-2-1	5.0	abcde

Table 3.16. (Continued)

	Genotype	Mean (score)	Comparison
27	NZS3-32-1-1	2.0	cde
28	NZS3-38-2-1	1.3	de
29	NZS3-49-1-1	4.0	abcde
30	NZS3-49-2-1	4.2	abcde
31	NZS3-51-1-1	1.8	cde
32	NZS3-51-2-1	1.0	de
33	NZS3-53-1-1	4.2	abcde
34	NZS3-53-2-1	2.3	bcde
35	NZS3-57-2-1	6.3	abc
36	NZS3-59-1-1	3.2	bcde
37	NZS3-59-2-1	3.7	abcde
38	NZS3-61-1-1	1.2	de
39	BS22-3-1-1	2.0	cde
40	BS22-8-1-2	1.5	de
41	BS22-22-2-1	2.8	bcde
42	BS22-22-2-2	4.2	abcde
43	BS22-34-1-1	2.0	cde
44	BS22-39-1-1	1.5	de
45	BS22-39-1-2	0.8	de
46	BS22-78-1-1	3.0	bcde
47	BS22-84-1-1	4.2	abcde
48	BS22-92-2-1	0.3	e
49	BS22-92-2-2	1.8	cde
50	BS22-151-2-1	1.3	de
51	NZS2-5-2-1	2.5	bcde
52	NZS2-21-1-2	2.2	cde
53	NZS2-70-1-1	0.7	de

Table 3.16. (Continued)

	Genotype	Mean (score)	Comparison
54	NZS2-70-1-2	2.2	cde
55	NZS2-92-1-1	3.0	bcde
56	NZS2-124-1-2	5.5	abcd
57	HUN946-1-1-1	0.2	e
58	M378-83-2-1-1-1	3.7	abcde
59	M378-80-2-1-2-2	0.3	e
60	M396-9-1-1-1	2.3	bcde
61	M396-9-2-1-1	3.3	bcde
62	M396-22-2-1-1	1.5	de
63	M396-33-1-1-1	4.5	abcde
64	NZ2	0.3	e
65	NZ3	1.7	cde
66	H99	1.6	cde
67	W153R	1.4	de
68	A659	2.4	bcde

Means with the same letter are not significantly different according to SNK test at 5% level.

Table 3.17. Exp.I. Discrimination among genotypic means for leaf area (LFAREA)

	Genotype	Mean (cm^2)	Comparison
1	NZS1-48-1-1-1	91.83	bcde
2	NZS1-100-1-1-1	99.83	abcde
3	NZS1-100-1-2-1	116.67	ab
4	NZS1-101-1-1-2	108.50	abc
5	NZS1-101-1-2-1	99.17	abcde
6	NZS1-101-4-1-1	79.83	bcde
7	NZS1-101-4-2-1	77.50	bcde
8	NZS1-123-1-1-1	81.67	bcde
9	NZS1-141-1-1-3	79.83	bcde
10	NZS1-141-1-2-1	76.33	bcde
11	AS3-50-1-1-1	89.50	bcde
12	AS3-51-2-1-2	76.33	bcde
13	AS3-57-2-1-1	81.67	bcde
14	AS3-94-1-1-1	93.33	bcde
15	AS3-94-1-2-1	98.17	abcde
16	AS3-94-2-1-1	104.17	abcd
17	NZS3-14-1-1	111.83	ab
18	NZS3-14-2-1	90.67	bcde
19	NZS3-18-2-1	105.50	abcd
20	NZS3-19-1-1	86.67	bcde
21	NZS3-19-2-1	81.17	bcde
22	NZS3-25-2-1	135.67	a
23	NZS3-28-1-1	86.67	bcde
24	NZS3-28-2-1	93.83	bcde
25	NZS3-29-1-1	115.33	ab
26	NZS3-29-2-1	113.50	ab

Table 3.17. (Continued)

	Genotype	Mean (cm^2)	Comparison
27	NZS3-32-1-1	98.17	abcde
28	NZS3-38-2-1	95.17	bcde
29	NZS3-49-1-1	114.67	ab
30	NZS3-49-2-1	102.00	abcde
31	NZS3-51-1-1	75.33	bcde
32	NZS3-51-2-1	99.50	abcde
33	NZS3-53-1-1	81.00	bcde
34	NZS3-53-2-1	89.33	bcde
35	NZS3-57-2-1	91.00	bcde
36	NZS3-59-1-1	92.17	bcde
37	NZS3-59-2-1	115.00	ab
38	NZS3-61-1-1	95.33	bcde
39	BS22-3-1-1	72.33	bcde
40	BS22-8-1-2	78.83	bcde
41	BS22-22-2-1	91.50	bcde
42	BS22-22-2-2	87.67	bcde
43	BS22-34-1-1	76.67	bcde
44	BS22-39-1-1	90.00	bcde
45	BS22-39-1-2	65.00	cde
46	BS22-78-1-1	101.83	abcde
47	BS22-84-1-1	85.17	bcde
48	BS22-92-2-1	72.33	bcde
49	BS22-92-2-2	57.83	e
50	BS22-151-2-1	71.33	bcde
51	NZS2-5-2-1	90.17	bcde
52	NZS2-21-1-2	64.67	cde
53	NZS2-70-1-1	65.67	cde

Table 3.17. (Continued)

	Genotype	Mean (cm^2)	Comparison
54	NZS2-70-1-2	61.67	de
55	NZS2-92-1-1	87.83	bcde
56	NZS2-124-1-2	101.33	abcde
57	HUN946-1-1-1	79.17	bcde
58	M378-83-2-1-1-1	78.67	bcde
59	M378-80-2-1-2-2	83.33	bcde
60	M396-9-1-1-1	83.50	bcde
61	M396-9-2-1-1	84.67	bcde
62	M396-22-2-1-1	87.83	bcde
63	M396-33-1-1-1	76.17	bcde
64	NZ2	83.50	bcde
65	NZ3	108.83	abc
66	H99	87.67	bcde
67	W153R	82.67	bcde
68	A659	104.50	abcd

Means with the same letter are not significantly different according to SNK test at 5% level.

Table 3.18. Exp.I. Discrimination among genotypic means for leaf thickness (LFTHICK)

	Genotype	Mean (m)	Comparison
1	NZS1-48-1-1-1	15.67	ab
2	NZS1-100-1-1-1	17.00	ab
3	NZS1-100-1-2-1	16.83	ab
4	NZS1-101-1-1-2	17.67	ab
5	NZS1-101-1-2-1	16.50	ab
6	NZS1-101-4-1-1	16.83	ab
7	NZS1-101-4-2-1	18.00	ab
8	NZS1-123-1-1-1	15.50	b
9	NZS1-141-1-1-3	18.17	ab
10	NZS1-141-1-2-1	16.67	ab
11	AS3-50-1-1-1	16.67	ab
12	AS3-51-2-1-2	17.83	ab
13	AS3-57-2-1-1	17.00	ab
14	AS3-94-1-1-1	17.50	ab
15	AS3-94-1-2-1	15.67	ab
16	AS3-94-2-1-1	16.83	ab
17	NZS3-14-1-1	17.50	ab
18	NZS3-14-2-1	17.33	ab
19	NZS3-18-2-1	18.67	ab
20	NZS3-19-1-1	15.67	ab
21	NZS3-19-2-1	18.00	ab
22	NZS3-25-2-1	17.50	ab
23	NZS3-28-1-1	16.00	ab
24	NZS3-28-2-1	17.33	ab
25	NZS3-29-1-1	17.83	ab
26	NZS3-29-2-1	18.33	ab

Table 3.18. (Continued)

	Genotype	Mean (m)	Comparison
27	NZS3-32-1-1	17.33	ab
28	NZS3-38-2-1	17.67	ab
29	NZS3-49-1-1	17.67	ab
30	NZS3-49-2-1	17.83	ab
31	NZS3-51-1-1	16.83	ab
32	NZS3-51-2-1	16.83	ab
33	NZS3-53-1-1	18.50	ab
34	NZS3-53-2-1	17.83	ab
35	NZS3-57-2-1	16.17	ab
36	NZS3-59-1-1	18.17	ab
37	NZS3-59-2-1	17.00	ab
38	NZS3-61-1-1	17.67	ab
39	BS22-3-1-1	16.33	ab
40	BS22-8-1-2	16.50	ab
41	BS22-22-2-1	17.33	ab
42	BS22-22-2-2	17.83	ab
43	BS22-34-1-1	15.50	b
44	BS22-39-1-1	15.83	ab
45	BS22-39-1-2	17.00	ab
46	BS22-78-1-1	16.83	ab
47	BS22-84-1-1	16.33	ab
48	BS22-92-2-1	18.17	ab
49	BS22-92-2-2	17.83	ab
50	BS22-151-2-1	17.67	ab
51	NZS2-5-2-1	16.83	ab
52	NZS2-21-1-2	15.50	b
53	NZS2-70-1-1	15.50	b

Table 3.18. (Continued)

	Genotype	Mean (m)	Comparison
54	NZS2-70-1-2	15.67	ab
55	NZS2-92-1-1	16.67	ab
56	NZS2-124-1-2	17.00	ab
57	HUN946-1-1-1	17.17	ab
58	M378-83-2-1-1-1	18.67	ab
59	M378-80-2-1-2-2	18.83	a
60	M396-9-1-1-1	17.33	ab
61	M396-9-2-1-1	17.67	ab
62	M396-22-2-1-1	18.33	ab
63	M396-33-1-1-1	17.67	ab
64	NZ2	18.17	ab
65	NZ3	17.17	ab
66	H99	17.50	ab
67	W153R	16.58	ab
68	A659	17.50	ab

Means with the same letter are not significantly different according to SNK test at 5% level.

Table 3.19. Exp.I. Discrimination among genotypic means for shoot mass (SHOOT)

	Genotype	Mean (g)	Comparison
1	NZS1-48-1-1-1	0.217	abcdefghi
2	NZS1-100-1-1-1	0.251	abcdefg
3	NZS1-100-1-2-1	0.273	abcd
4	NZS1-101-1-1-2	0.282	abc
5	NZS1-101-1-2-1	0.250	abcdefgh
6	NZS1-101-4-1-1	0.207	abcdefghi
7	NZS1-101-4-2-1	0.182	cdefghijk
8	NZS1-123-1-1-1	0.167	efghijkl
9	NZS1-141-1-1-3	0.188	bcdefghij
10	NZS1-141-1-2-1	0.159	ghijkl
11	AS3-50-1-1-1	0.208	abcdefghi
12	AS3-51-2-1-2	0.189	bcdefghij
13	AS3-57-2-1-1	0.193	bcdefghij
14	AS3-94-1-1-1	0.220	abcdefghi
15	AS3-94-1-2-1	0.227	abcdefghi
16	AS3-94-2-1-1	0.252	abcdefg
17	NZS3-14-1-1	0.273	abcd
18	NZS3-14-2-1	0.221	abcdefghi
19	NZS3-18-2-1	0.284	ab
20	NZS3-19-1-1	0.180	defghijkl
21	NZS3-19-2-1	0.180	defghijkl
22	NZS3-25-2-1	0.297	a
23	NZS3-28-1-1	0.201	bcdefghij
24	NZS3-28-2-1	0.190	bcdefghij
25	NZS3-29-1-1	0.247	abcdefgh
26	NZS3-29-2-1	0.267	abcde

Table 3.19. (Continued)

	Genotype	Mean (g)	Comparison
27	NZS3-32-1-1	0.202	bcdefghij
28	NZS3-38-2-1	0.215	abcdefghi
29	NZS3-49-1-1	0.263	abcdef
30	NZS3-49-2-1	0.254	abcdefg
31	NZS3-51-1-1	0.164	efghijkl
32	NZS3-51-2-1	0.219	abcdefghi
33	NZS3-53-1-1	0.186	bcdefghij
34	NZS3-53-2-1	0.199	bcdefghij
35	NZS3-57-2-1	0.207	abcdefghi
36	NZS3-59-1-1	0.194	bcdefghij
37	NZS3-59-2-1	0.233	abcdefghi
38	NZS3-61-1-1	0.204	abcdefghi
39	BS22-3-1-1	0.147	hijkl
40	BS22-8-1-2	0.151	ghijkl
41	BS22-22-2-1	0.173	defghijkl
42	BS22-22-2-2	0.183	bcdefghij
43	BS22-34-1-1	0.132	jkl
44	BS22-39-1-1	0.162	fghijkl
45	BS22-39-1-2	0.124	kl
46	BS22-78-1-1	0.204	abcdefghi
47	BS22-84-1-1	0.178	defghijkl
48	BS22-92-2-1	0.141	ijkl
49	BS22-92-2-2	0.126	kl
50	BS22-151-2-1	0.120	l
51	NZS2-5-2-1	0.192	bcdefghij
52	NZS2-21-1-2	0.129	kl
53	NZS2-70-1-1	0.121	l

Table 3.19. (Continued)

	Genotype	Mean (g)	Comparison
54	NZS2-70-1-2	0.139	ijkl
55	NZS2-92-1-1	0.180	defghijkl
56	NZS2-124-1-2	0.199	bcdefghij
57	HUN946-1-1-1	0.162	fghijkl
58	M378-83-2-1-1-1	0.187	bcdefghij
59	M378-80-2-1-2-2	0.192	bcdefghij
60	M396-9-1-1-1	0.175	defghijkl
61	M396-9-2-1-1	0.185	bcdefghij
62	M396-22-2-1-1	0.194	bcdefghij
63	M396-33-1-1-1	0.178	defghijkl
64	NZ2	0.174	defghijkl
65	NZ3	0.211	abcdefghi
66	H99	0.184	bcdefghij
67	W153R	0.166	efghijkl
68	A659	0.238	abcdefghi

Means with the same letter are not significantly different according to SNK test at 5% level.

Table 3.20. Exp.I. Discrimination among genotypic means for root dry weight (ROOT)

	Genotype	Mean (g)	Comparison
1	NZS1-48-1-1-1	0.509	abc
2	NZS1-100-1-1-1	0.443	abcdefg
3	NZS1-100-1-2-1	0.541	a
4	NZS1-101-1-1-2	0.409	abcdefghi
5	NZS1-101-1-2-1	0.419	abcdefghi
6	NZS1-101-4-1-1	0.367	cdefghijk
7	NZS1-101-4-2-1	0.342	defghijkl
8	NZS1-123-1-1-1	0.354	defghijkl
9	NZS1-141-1-1-3	0.382	bcdefghij
10	NZS1-141-1-2-1	0.277	hijklm
11	AS3-50-1-1-1	0.404	abcdefghi
12	AS3-51-2-1-2	0.348	defghijkl
13	AS3-57-2-1-1	0.330	fghijklm
14	AS3-94-1-1-1	0.452	abcdefg
15	AS3-94-1-2-1	0.447	abcdefg
16	AS3-94-2-1-1	0.490	abcd
17	NZS3-14-1-1	0.431	abcdefgh
18	NZS3-14-2-1	0.445	abcdefg
19	NZS3-18-2-1	0.526	ab
20	NZS3-19-1-1	0.315	ghijklm
21	NZS3-19-2-1	0.345	defghijkl
22	NZS3-25-2-1	0.480	abcdef
23	NZS3-28-1-1	0.443	abcdefg
24	NZS3-28-2-1	0.362	cdefghijk
25	NZS3-29-1-1	0.421	abcdefghi
26	NZS3-29-2-1	0.473	abcdefg

Table 3.20. (Continued)

	Genotype	Mean (g)	Comparison
27	NZS3-32-1-1	0.398	abcdefghi
28	NZS3-38-2-1	0.448	abcdefg
29	NZS3-49-1-1	0.489	abcde
30	NZS3-49-2-1	0.417	abcdefghi
31	NZS3-51-1-1	0.343	defghijkl
32	NZS3-51-2-1	0.467	abcdefg
33	NZS3-53-1-1	0.341	defghijkl
34	NZS3-53-2-1	0.391	bcdefghij
35	NZS3-57-2-1	0.377	bcdefghij
36	NZS3-59-1-1	0.367	cdefghijk
37	NZS3-59-2-1	0.447	abcdefg
38	NZS3-61-1-1	0.405	abcdefghi
39	BS22-3-1-1	0.283	hijklm
40	BS22-8-1-2	0.319	ghijklm
41	BS22-22-2-1	0.325	fghijklm
42	BS22-22-2-2	0.355	cdefghijk
43	BS22-34-1-1	0.228	klm
44	BS22-39-1-1	0.344	defghijkl
45	BS22-39-1-2	0.264	ijklm
46	BS22-78-1-1	0.328	fghijklm
47	BS22-84-1-1	0.346	defghijkl
48	BS22-92-2-1	0.324	fghijklm
49	BS22-92-2-2	0.285	hijklm
50	BS22-151-2-1	0.249	jklm
51	NZS2-5-2-1	0.396	abcdefghi
52	NZS2-21-1-2	0.274	ijklm
53	NZS2-70-1-1	0.201	m

Table 3.20. (Continued)

	Genotype	Mean (g)	Comparison
54	NZS2-70-1-2	0.218	lm
55	NZS2-92-1-1	0.356	cdefghijk
56	NZS2-124-1-2	0.332	efghijklm
57	HUN946-1-1-1	0.333	defghijkl
58	M378-83-2-1-1-1	0.373	cdefghijk
59	M378-80-2-1-2-2	0.406	abcdefghi
60	M396-9-1-1-1	0.386	bcdefghij
61	M396-9-2-1-1	0.398	abcdefghi
62	M396-22-2-1-1	0.336	defghijkl
63	M396-33-1-1-1	0.398	abcdefghi
64	NZ2	0.396	abcdefghi
65	NZ3	0.446	abcdefg
66	H99	0.409	abcdefghi
67	W153R	0.356	cdefghijk
68	A659	0.418	abcdefghi

Means with the same letter are not significantly different according to SNK test at 5% level.

Closer inspection on the genotypic means analysis, which are presented in Table 3.12 to Table 3.20, it can be noticed that significant differences among genotypes were found for all characters studied. These indicate that there were different trend in the general combining ability (GCA) among the genotypes studied.

In these tables the differences amongst genotypes were not clearly defined, but showed overlapping graduation in each character. Furthermore, for character CHPHYLL the discrimination might be too conservative as there were time correlation between each measurement with $\rho=0.32$ and the computer program available did not permit to use the base error variance (Gill, 1986) in the mean dicrimination. Useful information, however, can be made by looking on the magnitude of the estimates of each character.

The best GCA for emergence time (EMERGE) was showed by NZS3-59-2-1 as it grew faster than the other genotypes (Table 3.12). The superiority of this genotype, however, could not be held any longer as the seedling grew. This can be indicated that the shortest time to attain two mature leaves stage (2MATLEAF) was gained by NZS1-100-1-2-1 which brought it to be the best GCA for this character (Table 3.13).

Although NZS1-100-1-2-1 was not the best CGA for TOTLF50D (Table 3.14), it at least occupied the second best GCA for that character and non-significantly different to NZS3-25-2-1 which was the best GCA for the same character. Similar feature was occured for character LFAREA (Table 3.17), where NZS3-25-2-1 and NZS3-100-1-2-1 performed the best and second best GCA with no significant different between them. For character ANTHOCY and ROOT, again, NZS100-1-2-1 was the best in GCA (Tabel 3.16 and Table 3.20).

With regard to character CHPHYLL and SHOOT (Table 3.15 and Table 3.19), the best GCA was performed NZS3-18-2-1, whereas the worst GCA was recorded, respectively, in NZS2-70-1-1 and BS22-151-2-1. The thickest leaves was showed by hybrid check M378-80-2-1-2-2 but it was not significantly distinctive from most of the genotypes being evaluated.

3.8.4. Genetic variance and heritability estimates

As the genotypic variances, which were the variance of general combining abilities (GCA), genetically only accounted for covariance among halfsib, neither variances of dominant nor of its epistases could be estimated in the analysis, by definition (see Section 3.7.2.1). Consequently, the genetic variances ($\sigma 2_A$), which determine the variances due to average allele effects, were estimated higher than their corresponding genotypic variances. This applied for all characters studied in this experiment (see Table 3.10 and Table 3.21 for comparison)

Relative contribution of either genotypic variance ($\sigma 2G$) or genetic variance ($\sigma 2A$) to the phenotypic variance can viewed in the forms of broad sense heritability (h^2_B) and narrow sense heritability (h^2) respectively. The comparison between those two estimates can be made in Table 3.21. The broad sense heritability estimates varied among character from low to moderate while the additive heritability estimates varied from low to high. Characters that indicated relatively high h^2 were TOTLF50D (82%), ANTHOCY (69%) and LFAREA (61%).

Table 3.21. Exp.I. Additive genetic variance and heritability estimates

Character	σ^2_A	h^2_B	Se	h^2
EMERGE	0.3160	0.1924	0.2436	0.4042
MATLEAF2	2.8543	0.2077	0.0813	0.4364
TOTLF50D	0.1207	0.3924	0.5422	0.8246
CHPHYLL	0.0273	0.2171	0.0810	0.4562
ANTHOCY	4.6329	0.3282	0.0821	0.6895
LFAREA	336.1016	0.2931	0.0089	0.6158
LFTHICK	0.9549	0.1751	0.1256	0.3679
SHOOT	0.0017	0.0290	0.7024	0.0610
ROOT	0.0063	0.0556	0.6176	0.1168

3.8.5. Phenotypic and genetic correlation estimates

The estimated phenotypic and genotypic correlations between all possible pairs of the characters studied are presented in Table 3.22 and Table 3.23. respectively. Most of the estimated correlation coefficients (either phenotypic or genetic) were significant. However, a significant phenotypic correlation was not always accompanied by a significance in the corresponding genotypic correlation and vice-versa. This can be noted on several estimates which exhibited significant at phenotypic level, but they came to be non-significant at genotypic level. For example the correlation between CHPHYLL and ANTHOCY was phenotypically significant but it was genotypically non-significant.

Looking to the magnitude of the estimates, both phenotypic and genotypic correlations were ranged from very low to very high. Characters showed very high correlation, both phenotypically and genotypically, were between LFAREA and SHOOT, LFAREA and ROOT, and between SHOOT and ROOT. The correlation between LFTHICK and CHPHYLL or between 2MATLEAF and TOTLF50D was recorded on medium at the phenotypic level but it became very high at genotypic level. Moreover, most of the characters were negatively associated with EMERGE, which in turn also negatively associated with 2MATLEAF.

It can be noted in these tables that that most of the genotypic correlations were estimated higher than the corresponding phenotypic correlations. This is a common feature that genotypic correlation tends to be higher than the phenotypic counterpart (e.g. Robinson et al., 1951 and Johnson et al., 1955). Such feature arise due to the genes governing two characters are similar but the environments pertaining the expression of these characters have a low correlation (Searle, 1961) and/or due to random sampling error present in estimates of true population values (Cheverud, 1988).

Table 3.22. Exp.I. Phenotypic correlation (r_p) amongst character pairs

	EMERGE	2MATLEAF	TOTLF50D	CHPHYLL	ANTHOCY	LFAREA	LFTHICK	SHOOT
2MATLEAF	0.21							
	**							
TOTLF50D	-0.29	-0.58						
	**	**						
CHPHYLL	-0.09	-0.26	0.36					
	ns	**	**					
ANTHOCY	-0.11	-0.33	0.29	0.24				
	*	**	**	**				
LFAREA	-0.22	-0.49	0.66	0.46	0.34			
	**	**	**	**	**			
LFTHICK	-0.02	-0.08	0.12	0.42	0.06	0.19		
	ns	ns	*	**	ns	**		
SHOOT	-0.19	-0.47	0.62	0.56	0.41	0.89	0.23	
	**	**	**	**	**	**	**	
ROOT	-0.15	-0.43	0.58	0.63	0.31	0.72	0.22	0.81
	**	**	**	**	**	**	**	**

ns non significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level

Table 3.23. Exp.I. Genotypic correlation (r_G) amongst character pairs

	EMERGE	2MATLEAF	TOTLF50D	CHPHYLL	ANTHOCY	LFAREA	LFTHICK	SHOOT
2MATLEAF	0.45							
	**							
TOTLF50D	-0.57	-0.91						
	**	**						
CHPHYLL	-0.21	-0.41	0.38					
	*	**	**					
ANTHOCY	-0.39	-0.45	0.32	0.18				
	**	**	**	ns				
LFAREA	-0.38	-0.65	0.70	0.49	0.50			
	**	**	**	**	**			
LFTHICK	-0.11	-0.18	0.17	0.71	0.05	0.20		
	ns	ns	ns	**	ns	*		
SHOOT	-0.32	-0.51	0.56	0.64	0.59	0.92	0.34	
	**	**	**	**	**	**	**	
ROOT	-0.23	-0.63	0.63	0.76	0.34	0.82	0.34	0.88
	*	**	**	**	**	**	**	**

ns non significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level

4. Genotypic Variability in Initial Seed Constitution, Germinability, and Seedling Growth

4.1. Objectives

The objectives of this study were, firstly, to describe the effects of temperature on germinability and seedling growth of five genotypes expected to have different levels of cool tolerance and, secondly, to find suitable selection criteria for cool tolerant. To accomplish these objectives the following approaches were used:

(a). Description the germinative change of genotypes under two temperature regimes by estimating the variance components and means of several characters related to germination and seedling growth.
(b). Estimation of heritability of characters related to the initial seed constitution and those related to germination and seedling growth.
(c). Estimation of both the phenotypic and genotypic correlations amongst all these characters.

4.2. Materials

Five maize hybrids expected to have different degrees of tolerance to cool environment were used in this experiment. These were: (1) 3Mo71, (2) H99x(A665x CM105), (3) A665xW153R, (4) NZ1AxA665, and (5) NZ1Ax5-113.

3Mo71 is a lowland tropic origin and expected to be a cool-sensitive hybrid. H99, A665, CM105 and W153R are all Corn Belt Dent origins and expected to contain some cool tolerance characteristics. NZ1A and 5-113 are pure highland tropical origin and expected to be the most tolerant to

cool temperature (Hardacre and Eagles, 1989). Seeds of hybrids were grown at Palmerston North during 1985/1986 season. The seeds were produced on ears that were hand pollinated, hand picked and dried at 25-30 °C with low humidity until the seed had reached approximately 12 % moisture. Kernels were screened for damage or infection prior to evaluation.

4.3. Experimental

Kernels were weighed to obtain the weight of 100 seeds (W100SEED). Attributes relating to the initial seed constitution and to germinability and seedling growth were, then, investigated. The former included determinations of initial chemical compositions of the seed. The latter included deterimination ion leakage of the seed during early hours of germination process, time to germinate, and time-course evaluations of the seedling growth under day/night temperatures of 16/6 °C and 25/20 °C. There were three replications for each measurement.

4.3.1. Determination of initial chemical composition of the seeds

Twenty seeds were tempered with water spray to bring the moisture to about 20% before grinding. The meal was used to determine the nitrogen, sugars contents, and alpha-amylase activity.

Nitrogen was determined by macro-Kjeldahl method using a Kjeltec Auto 1030 Analyzer (appendix 2). The nitrogen content was expressed in percent of 0.5 g meal sample.

Sugars content were measured as milligram reducing sugar maltose (MALTOSE) and non-reducing sugar sucrose (SUCROSE) in 10 gram meal sample. These were determined according to AACC method (appendix 3).

Alpha-amylase activity (AMYLASE) was determined following Barnes and Blakeney's method (1974; appendix 4). This was expressed as Enzyme units per litre (U/ℓ).

4.3.2. Determination of ion leakage

Ten seeds were weighed and placed in 100 mℓ glass beaker containing 30 mℓ destiled water. The beaker was incubated in darkness at either 16/6 °C or 25/20 °C. After 24 hours of incubation, the conductivity of leachate (CONDUCT) was measured using Radiometer CDM 83 conductivity meter and recorded in Siement/g seed.

4.3.3. Evaluation of germination and seedling growth performance

Seeds were dusted with fungicide Captan and germinated in rolled paper towels. Each roll contained 20 seeds and was arranged randomly onto three shelves, which represent blocks, in a germinator. Two germinators were used in this experiment. One was run under temperatures day/night of 16/6 °C and the other was run under 25/20 °C

Attributes associated with germinability and seedling growth were recorded for these two environments. The germinability was recorded as the time to germinate (GERMTIME) which was counted from sowing to the emergence of radicle to about 1 mm from the caryopsis.

The seedling growth was expressed both as linear extentions and as dry masses. These included root length (ROOTLEN), seedling height (HEIGHT), root mass (ROOT), and shoot mass (SHOOT). The root length and height were measured in millimeter (mm) from the base at caryopsis to the tip of primary root and to the highest point of the leaves, respectively. The root and shoot masses was recorded in gram (g) dry weight obtained by drying the plant materials for four days under oven at 65 °C.

There were five measurements on these seedling growth characters for each environment with four samples in each measurement. For 16/6 °C environment the measurements were taken at eight days interval, while for 25/20 °C environment were taken at three days intervals.

4.4. Data analysis

There were two stages of analyses: routine statistical analysis and a subsequent genetical analysis.

4.4.1. Statistical analysis

The analysis of variance was conducted under random effects philosophy for all components with samples as the experimental unit. For characters W100SEED, NITROGEN, MALTOSE, SUCROSE, AMYLASE, and CONDUCT the analysis units were formed from the experimental units, while characters GERMTIME, ROOTLEN, ROOT, HEIGHT, and SHOOT were the average of the internal replications.

As the seedling growth characteristics (ROOTLEN, ROOT, HEIGHT, and SHOOT) were time-course measurements, the analysis of variance these attributes were conducted on the basis of their 'growth rates' and 'growth functions' over the five time measurements. To obtain these, simple regressions were carried out separately on each analysis unit. The slopes of regression lines (β_1's) were used as estimates of the growth rates, while the intercepts (β_0's) together with their coresponding slope (β_1's) were used to estimate the growth function of each analysis unit.

Both linear and logarithmic regressions were explored to find the suitable model. The linear model was chosen for all four growth attributes because it had higher coefficients of determination and better fit amongst the observations for most of the analysis units of those characters compared to

the logarithmic model. A complete list of the individual coefficient of determination (R^2) and the regression functions (β_0 and β_1) estimates are given in Appendix 5 to Appendix 12. Furthermore as there were considerable heterogeniety among the estimated error variance of the analysis units in the regression analysis, the square root of these error variance was used as a weighting factor in the analysis of variance.

Two models of analysis of variance were performed to conform with the two different data structures. The variance components and standard errors of the variance components were estimated according to Crump (1951). These models were as follows:

(1). Completely Random Design / CRD

The analysis of variance using CRD was conducted for characters W100SEED, NITROGEN, MALTOSE, SUCROSE, and AMYLASE. This was based on the following linear model.

$$X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where: X_{ij} = the ijk-th phenotypic variate
i=1...g, g=number of genotype
j=1...r, r=number of replication
μ = the grand mean
α_i = the j-th genotype effect
ε_{ij} = the residual effect.

The analysis of variance including the degrees of freedom, expectations of the Mean Squares, and F-ratios for this model are given in table 4.1. The genotype variance component (σ^2_G) was calculated as (MS_g - MS_e)/r and the error variance component was σ^2.

Table 4.1. Exp.II. Degree of freedom, Expectation of Mean Square, and F-ratio for Completely Random Design (Model I)

Source	df	MS	E (MS)	F-ratio
Genotype	g-1	MS_g	$\sigma^2 + r\sigma^2_G$	MS_g/MS_e
Residual	g(r-1)	MS_e	σ^2	

(2). Pooled Randomized Complete Block design/Pool-RCBD

This was performed to pool the measurements over two environments of characters CONDUCT, GERMTIME, ROOTLEN, ROOT, HEIGHT and SHOOT.

The following model was used as the basis of the analysis.

$$X_{ijk} = \mu + \eta_i + \rho_{j(i)} + \alpha_k + \eta\alpha_{ik} + \varepsilon_{ijk}$$

Where: X_{ijk} = the ijk-th phenotypic variate

i=1...t, t=number of environment

j=1...r, g=number of replication

k=1...r, r=number of genotype

μ = the grand mean

η_i = the i-th environment effect

$\rho_{j(i)}$= the j-th replication, nested within environment

α_k = the k-th genotype effect

$\eta\alpha_{ik}$ = the interaction between environment and genotype

ε_{ijk} = the residual effect

The analysis of variance including the degrees of freedom, expectations of the Mean Squares, and F-ratio for this model is given in table 4.2. The components of variance were estimated using following equations :

Environment $(\sigma^2_E) = [(MS_t + MS_e) - (MS_R + MS_{GE})] / rg;$

Rep.(Env.) $(\sigma^2_{R(E)} = (MS_r - MS_e)/g$

Genotype $(\sigma^2_G) = (MS_g - MS_{gt})/rt;$

Genot. x Env. $(\sigma^2_{GT}) = (MS_{gt} - MS_e)/r.$

Error $= \sigma^2$

Table 4.2. Exp.II. The degree of freedom, Mean Square, Expectation of Mean Square, and F-ratio for Pooled-RCBD (model II).

Source	df	MS	E (MS)	F-ratio
Envir. (E)	t-1	MS_t	$\sigma^2 + g\sigma^2_{R(E)} + r\sigma^2_{GE} + rg\sigma^2_E$	$(MS_t+MS_e)/(MS_r+MS_{ge})$ [a]
Rep.(Envir.)	t(r-1)	MS_r	$\sigma^2 + g\sigma^2_{R(E)}$	MS_r/MS_e
Genot.(G)	g-1	MS_g	$\sigma^2 + r\sigma^2_{GE} + re\sigma^2_G$	MS_g/MS_{ge}
G x E	(g-1)(e-1)	MS_{ge}	$\sigma^2 + r\sigma^2_{GE}$	MS_{ge}/MS_e
Residual	t(r-1)(g-1)	MS_e	σ^2	

[a] this complex F-test was calculated according Satterthwaite (1946).

The least significant different (LSD) test was used for the subsquent mean discrimination. LSD was chosen because the number of means involved in the discrimination were relatively small, so it would not be expected to suffer from Type I error problems (Balaam, 1963; Gill, 1973; Chew, 1976).

The pool-RCBD model was also used to perform multivariate analysis of variance (MANOVA). This was conducted to incorporate, into a single analysis, the two regression statistics (β_0,β_1) of the growth attributes (ROOTLEN, ROOT, HEIGHT and SHOOT). It is appropriate that these be analysed a set, thereby analysing differences in the entire function at once. As β_0 and β_1 are correlated (Draper and Smith, 1981), MANOVA is necessary for this purpose.

The variance components of these growth functions were presented in terms of the generalized variance components as described both by Wilks (1932) and Zhivotovsky (1988). To obtain the MSCP matrices the same procedure in obtaining mean squares of the associated univariate character was used. The Wilks' Generalized Mean Square of each component was expressed as the determinant of the corresponding MSCP matrix, while the Zhivotovsky's Generalized Mean Square was the n^{th} root of the Wilks', where n is the order of the MSCP matrix. The generalized variance, denoted as $\omega 2$ (for Wilks') and as $\psi 2$ (for Zhivotovsky's), was estimated by applying the same expectations to generalized mean squares as to the univariate mean squares. This conforms with the usual approach underlying all MANOVA's (Anderson, 1958 ; Cooley and Lohnes, 1971)

Two computer programs were employed to analyse the experimental data. SAS program (Anon, 1988) was used for the regression analysis, the analysis of variance, and the mean dicriminations. THWAITE (Gordon, unpublished) was used for the variance component estimations, their appropriate F-tests and Standard errors.

4.4.2. Genetical analysis

4.4.2.1. Estimation of Heritability

The heritability was expressed as broad sense heritability (h^2). For the characters involved in analysis model I, the heritability was estimated as the ratio of the genotypic variance to the total (phenotypic) variance. For the characters in the analysis model II, the heritability was estimated in two forms, full and restricted heritability (Gordon et al., 1972; Gordon 1979)).

$$h^2 \text{ (full)} = \frac{\sigma^2_G}{\sigma^2_G+\sigma^2_{GE}+\sigma^2_{R(E)}+\sigma^2_E}$$

$$h^2 \text{ (restricted)} = \frac{\sigma^2_G}{\sigma^2_G+\sigma^2_{GE}+\sigma^2}$$

These two forms of broad sense heritability were also estimated from the generalized variance components. These were to perform the heritabilities of the growth fuctions (the multivariate characters). The symbols $\theta 2$ and $\eta 2$ were used to denote the heritability derived from Wilks' and Zhivotovsky's generalized variance components, respectively.

The standard error of the heritability estimate was determined using variance of a ratio approximation as described by Gordon et al. (1972) and Gordon (1979).

4.4.2.2. Correlation analysis

The correlation analysis was performed to estimate the phenotypic and genotypic correlations between the characters of the initial seed constituent, germinability, and growth rates. Prior to this analysis the characters involved in the analysis model II were averaged over two environments to provide a common data structure equivalent to analysis model I. Although this removes the information about the temperature regime, its permits these characters to be correlated.

The phenotypic correlation coefficients (r_P) were estimated as simple unpartitioned correlation coefficients (Falconer, 1981). Proc CORR in SAS (Anon, 1988) was used for the analysis. The genotypic correlation coefficients (r_G) were estimated following Falconer (1981) and Baker (1986). To obtain the necessary partitioned SSCP matrices, a CRD MANOVA was carried out using the same data as used in the corresponding phenotypic correlation analysis. The genotypic correlations were estimated in the same manner as the phenotypic ones, but using the partitioned genotypic SSCP. These correlation represent the correlation amongst attributes for their genotypic effects.

4.5. Results

4.5.1. General values

The general values as indicated by grand means, minimum and maximum values, and coefficients of variation are presented in table 4.3. In this experiment high coefficient of variations were found in some characters. These included SUCROSE, ROOTLEN (βo), ROOT (βo and β1), SHOOT (β_o and β_1). As mentioned earlier (section 3.8.1) that high coefficient of variation would reduce the efficiency in testing the significances of the means and variance components.

Table 4.3. Exp.II. The grand means, their range values and coefficients of variations

Character	Grand Mean	Minimum	Maximum	C.V. (%)	Unit
W100SEED	33.633	27.500	42.780	2.39	g
NITROGEN	1.919	1.448	2.310	1.82	%
MALTOSE	49.000	26.200	68.000	12.57	mg/10g
SUCROSE	7.567	4.000	14.000	34.63	mg/10g
AMYLASE	68.067	32.000	128.000	11.40	U/ℓ
CONDUCT	33.935	21.445	51.585	9.42	uS/g
GERMTIME	3.848	3.575	4.395	14.68	day
ROOTLENGTH (β_0)	-12.956	-46.425	44.843	-23.14	function
ROOTLENGTH (β_1)	13.143	5.830	25.904	3.11	function
HEIGHT (β_0)	-54.071	-88.905	-25.501	3.11	function
HEIGHT (β_1)	12.785	4.803	22.742	1.52	function
ROOT (β_0)	-0.012	-0.027	-0.001	-342.87	function
ROOT (β_1)	0.005	0.002	0.008	106.60	function
SHOOT (β_0)	-0.014	-0.028	-0.005	-197.19	function
SHOOT (β_1)	0.004	0.001	0.008	76.85	function

4.5.2. Germinative change

The results of the analyses of variance and means discriminations are summarised in Table 4.4 to Table 4.11. Partition of total variance into genotypic and non-genotypic variance components together with their standard errors and significances enable to identify the magnitude and significance of variation in a given character resulted by the different causal components. The mean discriminations enable to make comparison / classification of each of these causal components. Although Table 4.4 and Table 4.8 do not indicate the germinative change, they are presented in this section to show give the idea of the genotypic difference in the initial seed constitution.

Table 4.5 showed that environmental variance was highly significant for CONDUCT, GERMTIME, and all characters of seedling growth rates. This result agrees with common conlusion that temperature change the amount of ion (electrolyte) leakage (e.g. Tatum, 1954), time to germinate (e.g. Blacklow, 1972b) and the growth rates of the seedling (e.g. Hardacre and Turnbull, 1986).

Closer inspection on the environmental means (Table 4.10), it can be noted that the conductivity of ion leakage (CONDUCT) was recorded higher in 16/6 °C compared to that in 25/20 °C. In contrast, the time to germinate (GERMTIME) and all characters of seedling growth rates were slower under 16/6 °C temperature. This result suggested that temperature of 16/6 °C was low enough to enhance ion leakage and to supress the germination and the subsequent growth.

It was somewhat surprising that even though the environmental (temperature) variance was noticable in magnitude and highly significant for the seedling growth rates, it was hardly detectable and non significant for the seedling growth functions (Table 4.6 and 4.7). These discrepancies were

Table 4.4. Exp.II. Variance components and their standard errors (in bracket) and significances for the initial seed constitution characters

Character	σ^2_G	σ^2
W100SEED	24.0085 (13.9868) **	0.6504 (0.2655)
NITROGEN	0.0790 (0.0458) **	0.0012 (0.0005)
MALTOSE	121.5749 (77.6640) **	37.9487 (15.4925)
SUCROSE	1.4333 (1.0567) ns	6.8667 (2.8033)
AMYLASE	860.4333 (508.436) **	60.2667 (24.6038)

ns non-significant at 0.05 level
* significant at 0.05 level
** significant at 0.01 level

Table 4.5. Exp.II. Variance components and their standard error (in brackets) and significances for germinability and seedling growth rate characters

Character	$\sigma 2_E$	$\sigma^2_{R(E)}$	σ^2_G	σ^2_{GE}	σ^2
CONDUCT	64.2998 (53.9133) **	0.5321 (1.6371) ns	133.2999 (79.2155) **	4.2931 (4.5901) ns	10.2288 (3.4096)
GERMTIME	5.5652 (4.5920) **	-0.0377 (0.0152) ns	0.1782 (0.2095) ns	0.2873 (0.1750) **	0.3167 (0.0186)
ROOTLENGTH	1.4538 (1.1981) **	0.0062 (0.0255) ns	0.0132 (0.0292) ns	0.0011 (0.0378) ns	0.1676 (0.0559)
ROOT	0.0006 (0.0005) **	0.00001 (0.00004) ns	0.00001 (0.00003) ns	0.00001 (0.00006) ns	0.00002 (0.00018)
HEIGHT	4.9689 (4.0714) **	0.0005 (0.0053) ns	0.0273 (0.0480) ns	0.0746 (0.0505) **	0.0377 (0.1510)
SHOOT	0.0009 (0.0007) **	0.00001 (0.00004) ns	0.0001 (0.00005) ns	0.00001 (0.00006) ns	0.00001 (0.00018)

ns non-significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level

Table 4.6. Exp.II. Wilks' Generalized Variance components and their standard error (in bracket) and significances for seedling growth fuction characters

Character	ω^2_E	$\omega^2_{R(E)}$	ω^2_G	ω^2_{GE}	ω^2
ROOTLENGTH	-0.0339 (0.0266) ns	0.0012 0.0548 ns	0.3147 0.2350 ns	0.1539 0.0968 **	0.4065 0.1355
ROOT	0.0000 (0.0000) ns	3.6E-10 (4.0E-5) ns	1.8E-8 (3.3E-5) ns	1.1E-8 (6.7E-5) ns	1.8E-8 (1.8E-4)
HEIGHT	-0.0103 (0.0051) ns	0.0105 (0.0099) ns	0.8230 (0.4851) **	0.0237 (0.0199) *	0.0311 (0.0137)
SHOOT	0.0000 (0.0000) ns	-5.4E-10 (4.1E-5) ns	7.14E-8 (3.4E-5) ns	1.9E-8 (6.6E-5) ns	4.5E-9 (2.0E-5)

ns non-significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level

Table 4.7. Exp.II. Zhivotovsky's Generalized variance components and their standard error (in bracket) and significances for seedling growth function characters

Character	ψ^2_E	$\psi^2_{R(E)}$	ψ^2_G	ψ^2_{GE}	ψ^2
ROOTLENGTH	-0.0466 (0.0395) ns	0.0010 (0.0855) ns	0.1395 (0.1637) ns	0.2377 (0.1537) ns	0.6376 (0.2125)
ROOT	0.0000 (0.0000) ns	1.3E-6 (4.0E-5) ns	2.9E-5 (4.0E-5) ns	3.0E-5 (4.0E-6) ns	1.3E-4 (1.8E-4) ns
HEIGHT	-0.0268 (0.0171) ns	0.0226 (0.0354) ns	0.6418 (0.4364) *	0.0478 (0.0646) ns	0.1763 (0.0588) ns
SHOOT	0.0000 (0.0000) ns	-5.0E-6 (7.8E-6) ns	7.5E-5 (7.2E-5) ns	6.1E-5 (4.9E-5) *	6.7E-5 (3.1E-5)

ns non-significant at 0.05 level, * significant at 0.05 level, ** significant at 0.01 level

Table 4.8. Exp.II. Discrimination amongst genotypic means for the intial seed constitution characteristics

Genotype	W100SEED	NITROGEN	MALTOSE	SUCROSE	AMYLASE
3Mo71	41.69 a	1.49 d	35.90 d	7.67 a	55.00 c
H99x(A665xCM105)	34.15 b	1.89 c	40.83 cd	7.67 a	34.67 d
A665xW153R	31.94 c	1.95 b	65.77 a	9.00 a	59.67 c
NZ1AxA665	28.58 d	2.28 a	53.17 b	6.83 a	77.33 b
NZ1Ax5-113	31.81 c	1.97 b	49.33 bc	6.67 a	113.67 a

Means with the same letter are non-significantly different according LSD test at 5% level.

Table 4.9. Exp.II. Discrimination amongst genotypic means for the germination and seedling grwoth rate characters

Genotype	CONCUCT	GERMTIME	ROOTLENGHT	ROOT	HEIGHT	SHOOT
3Mo71	24.587 bc	4.39 a	12.2940 a	0.0055 a	14.4907 a	0.0057 a
H99x(A665xCM105)	46.665 a	3.62 a	12.2467 a	0.0049 a	12.9617 a	0.0040 a
A665xW153R	21.767 c	3.59 a	11.5206 a	0.0043 a	10.3837 a	0.0033 a
NZ1AxA665	45.828 a	3.67 a	13.8254 a	0.0047 a	11.3989 a	0.0037 a
NZ1Ax5-113	30.830 b	3.96 a	15.7273 a	0.0041 a	15.3818 a	0.0046 a

Means with the same letter are non-significantly different according to LSD test at 5% level.

Table 4.10. Exp.II. Discrimination between two environmental means for germination and growth rate characters

Environment	CONCUCT	GERMTIME	ROOTLENGHT	ROOT	HEIGHT	SHOOT
16/6 °C	39.681 a	4.82 a	7.6916 a	0.0025 a	5.7138 a	0.0021 a
25/20 °C	28.190 b	2.87 b	19.3284 b	0.0064 b	19.9100 b	0.0061 b

Table 4.11. Exp. II. Least Square mean of Genotypic-Environment for germination and growth rate characters

Genotype	CONCUCT	GERMTIME	ROOTLENGHT	ROOT	HEIGHT	SHOOT
		---------- 16/6 °C ----------				
3Mo71	28.71 c	5.72 a	7.0931 a	0.0028 a	6.1967 c	0.0028 a
H99x(A665xCM105)	54.24 a	4.47 cd	6.9617 a	0.0029 a	5.5317 c	0.0020 a
A665xW153R	25.87 c	4.33 d	8.0907 a	0.0023 a	5.0159 c	0.0016 a
NZ1AxA665	53.98 a	4.62 c	8.5912 a	0.0023 a	5.8112 c	0.0020 a
NZ1Ax5-113	35.60 b	4.96 b	7.4532 a	0.0022 a	6.6954 c	0.0025 a
		---------- 25/20 °C ----------				
3Mo71	20.46 f	3.05 e	18.3946 b	0.0075 b	21.3006 a	0.0079 b
H99x(A665xCM105)	39.09 d	2.78 f	16.6820 b	0.0063 b	21.9297 a	0.0059 b
A665xW153R	17.66 f	2.85 ef	16.8881 b	0.0063 b	17.7976 b	0.0055 b
NZ1AxA665	37.68 d	2.72 f	20.8617 b	0.0064 b	17.5732 b	0.0051 b
NZ1Ax5-113	26.06 e	2.97 ef	22.1697 b	0.0057 b	20.7899 a	0.0062 b

Means with the same letter are not significantly different at 5% level.

appeared possibly because they were different attributes. The growth functions measured not only on the relative changes over time (the slope of regression line) as the growth rates did, but they also accounted for the initial states before the application of temperature treatments (the intercept). A non significant on the growth fuction characters, therefore, could be due to the inflation by their intercepts. Environmental variances were non-significant for the intercepts (see Appendix 13).

Genotypic variance was significant for CONDUCT and growth function HEIGHT only. The genotypes used in this study had a relatively narrow genetic base, as some of them were genetically related (see section 4.2). Hence absence of average genetic differences for germination and most of the growth characteristic were reasonable.

The significant of genotypic different for CONDUCT suggested that the rapidity of membrane restitution is a characteristic of genotype. A similar result was reported recently by Zemetra and Cuany (1991), where they found significant genetic variation with respect to the amount of the ion leakage among eleven inbred lines.

Significant genotypic-environment (GE) interaction was found on GERMTIME, indicating that the genotypes ranking based on time to germinate changed as temperature changed. This means that the germination of some genotypes were delayed longer than were others as temperature decreased. However, as the genotypic variance, this GE interaction was non significant for most of the growth characteristics. A non significant on GE interaction indicates that the genotypes used in this study had similar growth performances over the two environments. The lack GE interaction may also mean that the sampling of genotypes and/or environment used was insufficient.

Noticable features on Table 4.6 and Table 4.7 are some growth functions were significant when they were estimated as Wilks' generalized variance but were not significant when estimated as Zhivotovsky's generalized variance, vice-versa. These inconsistencies were possibly due to different method in estimatimation, which were resulted from two different philosophies. The Wilks' generalized variance was derived on the basis of the product of determinant of a dispersion matrix, as described by Wilks (1932). However, Zhivotovsky (1988) claimed that the theoritical basis underlying this estimation is lacking as the use of product as a measure of multivariate variability is questionable. Instead, he suggest to use the geometric mean of the determinant of dispersion matrix which mimics correlation coefficient estimation, which includes a geometric mean of two variance in its denominator.

4.5.3. Heritability estimate

All the broad sense heritability estimates, including the seed characteristics, are presented in Table 4.12 to 4.15. Most of the seed characteristics showed high heritability, except SUCROSE. These indicated that the measurable variations on those characters were mostly due to genetic factors. However, their relevance as selection criteria for cool tolerant breeding must be confirmed with their relation to the subsequent growth. This will be discussed later in correlation analysis.

For the germination and growth characteristics, two forms of broad sense heritability were estimated (Table 4.13 to Table 4.15). The different magnitude between these two form indicates the relative important of 'macro' and 'meso' environments in determining the measurable (phenotypic) variation. Of these two forms, the restricted heritability is the more commonly used in conjuction with this type of experiment (Allard, 1960).

Table 4.12. Exp.II. Heritability estimates of the initial seed constitution characters

Character	h^2	Se
W100SEED	0.9736	0.4523
NITROGEN	0.9850	0.4221
MALTOSE	0.7621	0.0525
SUCROSE	0.1727	0.0209
AMYLASE	0.9345	0.1150

Table 4.13. Exp.II. Heritability estimates of the germination and growth rate characters

Character	h^2 (full)	Se	h^2(restd)	Se
CONDUCT	0.6585	0.2927	0.9650	0.0171
GERMINTN	0.0297	0.0756	0.4165	0.1894
ROOTLENGTH	0.0089	0.0115	0.6439	0.9040
ROOT	0.0159	0.0865	0.3333	0.9876
HEIGHT	0.0054	0.0436	0.2666	0.9428
SHOOT	0.0980	0.1701	0.8333	0.7918

Table 4.14. Exp.II. Heritability estimates of the growth function characters (Estimated from Wilks' generalized variances)

Character	θ^2 (full)	Se	θ^2(restd)	Se
ROOTLENGTH	0.3736	0.4208	0.3596	0.4176
ROOT	0.3796	0.1246	0.3825	0.1389
HEIGHT	0.9374	0.1731	0.9376	0.1784
SHOOT	0.7556	0.7049	0.7514	0.7848

Table 4.15. Exp.II. Heritability estimates of the growth function characters (Estimated from Zhivotovsky's generalized variances)

Character	η^2 (full)	Se	η^2 (restd)	Se
ROOTLENGTH	0.1439	0.3818	0.1375	0.306
ROOT	0.1524	0.2794	0.1534	0.2804
HEIGHT	0.7448	0.3309	0.7412	0.3398
SHOOT	0.3787	0.3260	0.3694	0.3382

The restricted heritability estimates were ranged from low to very high. The highest estimate was recorded on CONDUCT, indicating that this character warrants for improvement. Again, it usefullness as selection criterion for cool tolerance breeding, however, has to be confirmed with it relation to the subsequent growth.

Noticable feature on Table 4.14 and Table 4.15 is that the Zhivotovsky's heritabilities were estimated lower that Wilks' as were the estimated generalized variance. However, both method of estimation were consistent over all characters. This means that either Wilks' or Zhivotovsky's method can be used to estimate heritability. The choice, however, depends on which philosophy is followed.

4.5.4. Phenotypic and genotypic correlation estimates

The phenotypic and genotypic correlations between pairs of characters are presented in Table 4.16 and Table 4.17. General comparison between phenotypic and genotypic correlations are that they were in the same direction. However, it was occurred in the first experiment (see section 3.8.5) that the genotypic correlations were estimated higher than the phenotypic counterparts. In addition, twenty one of fifty five pairs of characters showed significant phenotypic correlations but only six of them were genotypically significant.

W100SEED, NITROGEN, and MALTOSE were the most prevalent among seed characteristics, by means, that they showed the most considerable both phenotypic and genotypic correlation with the germination time and the growth rate characters. This suggest that these characters could be useful as selection criteria for cool tolerant breeding.

Table 4.16. Exp.II. Phenotypic correlation (r_p) amongst character pairs and their significances

	W100SEED	NITROGEN	MALTOSE	SUCROSE	AMYLASE	CONDUCT	GERMTIME	ROOTLEN	ROOT	HEIGHT
NITROGEN	-0.96 **									
MALTOSE	-0.67 **	0.52 *								
SUCROSE	0.12 ns	-0.16 ns	0.07 ns							
AMYLASE	-0.39 ns	0.36 ns	0.25 ns	-0.21 ns						
CONDUCT	0.44 ns	0.54 *	-0.20 ns	-0.26 ns	-0.15 ns					
GERMTIME	0.80 **	-0.75 **	-0.61 *	-0.06 ns	0.17 ns	0.44 ns				
ROOTLEN	-0.40 ns	0.39 ns	0.09 ns	0.38 ns	0.59 *	-0.07 ns	-0.50 ns			
ROOT	0.74 **	0.57 *	-0.59 *	0.22 ns	0.44 ns	-0.16 ns	-0.48 ns	0.36 ns		
HEIGHT	0.56 *	0.56 *	-0.76 **	0.04 ns	0.02 ns	-0.07 ns	-0.57 *	0.03 ns	0.26 ns	
SHOOT	0.89 **	0.83 **	-0.70 **	0.04 ns	0.01 ns	-0.41 ns	-0.93 **	0.10 ns	0.64 *	0.64 *

ns non-significant at 0.05 level
* significant at 0.05 level
** significant at 0.01 level

Table 4.17. Exp.II. Genotypic correlation (r_G) amongst character pairs and their significances

	W100SEED	NITROGEN	MALTOSE	SUCROSE	AMYLASE	CONDUCT	GERMTIME	ROOTLEN	ROOT	HEIGHT
NITROGEN	-0.98 **									
MALTOSE	-0.70 ns	0.61 ns								
SUCROSE	0.21 ns	-0.30 ns	0.43 ns							
AMYLASE	-0.41 ns	0.37 ns	0.26 ns	-0.59 ns						
CONDUCT	0.44 ns	0.55 ns	-0.25 ns	-0.53 ns	-0.17 ns					
GERMTIME	0.81 ns	-0.76 ns	-0.67 ns	-0.25 ns	0.15 ns	0.46 ns				
ROOTLEN	-0.65 ns	0.62 ns	0.28 ns	0.71 ns	0.94 *	-0.15 ns	-0.07 ns			
ROOT	0.96 **	0.87 ns	-0.74 ns	0.25 ns	0.40 ns	-0.19 ns	-0.68 ns	0.82 ns		
HEIGHT	0.60 ns	0.60 ns	-0.86 ns	0.42 ns	0.01 ns	-0.08 ns	-0.59 ns	0.08 ns	0.40 ns	
SHOOT	0.94 *	0.90 *	-0.81 ns	0.19 ns	0.01 ns	-0.42 ns	-0.95 *	0.24 ns	0.58 ns	0.76 ns

ns non-significant at 0.05 level
* significant at 0.05 level
** significant at 0.01 level

The correlations between CONDUCT and the growth rate characters were relatively low, both phenotypically and genotypically. This means that the usefullness of this character as a selection criteria for cool tolerant breeding is lacking regardless it's heritability was high (see Table 4.13).

Medium to very high correlations were recorded on GERMTIME in its association with three of the growth rate characters. This result gives an indication that germination and seedling growth are not completely independent processes (cf. Bocsi and Kovac, 1991). Furthermore, although this character may not be easily improved as its heritability was low, it can be used as selection criterion for growth rate improvement.

5. Discussion

5.1. Genetic study

Although the physiological basis and genetical control of cool tolerance in maize have not been fully understood, a gradual improvement for these characters has been made breeding only plants in better early growth lines. Data reported by Mock and Bakri (1976), for example, showing the improvement on overall performances of the plants can be made from selection for early growth characteristics. These data also suggest that there is no detrimental effects of selection for early growth performances on the mature plant performances as well as the yield.

In this study, the results of the first experiment, presented in chapter 3, show that most of the total variation for all characters measured was associated with highly significant genetic variation. Of the advantages is that the extent of the narrow sense heritabilities for some of non destructive measurements were large enough (the h2 of total leaves number at 50 days after planting was 82 %, anthocyanin was 69%, leaf area was 62%, and chlorophyll content was 46%). This suggests that selection for these characters would be effective. Furthermore, these easily measurable characters have a relatively high genetic correlation to the dry masses, which are commonly used as measures for seedling vigour since they are the end result of several processes. The magnitude of these estimates indicate that indirect selection can be applied to improve these dry mass characters, which may be difficult to improve because of low heritabilities. However, care should be taken as these results are applicable only to the population inference referred to in this study. It was, however, a broad population since 68 genotypes were studied.

In the second experiment, described in chapter 4, the most prominent genetic variation was found for the characters of initial seed constitution.

However, as mentioned earlier, only three of these characters, namely seed weight, percent of nitrogen, and maltose contents of the seed, may characterise genotypes with superior growth potential under cool conditions. Nevertheless, more detailed work with a large number of genotypes appears desirable to verify these observation.

5.2. Lines evaluation

One of the main purpose of this study was to evaluate performances of several genotypes that currently being used to develop maize hybrids with better adaptation for New Zealand conditions. Of the five synthetic populations evaluated, NZS3 was the most promising synthetic for the devolopment of maize with reliable seedling growth under cool temperatures. The superiority of NZS3 over the rest of the synthetic populations used in this evaluation indicates that the higher level highland tropical germplasm is more desirable source of genes for improving seedling establisment of maize in temperate regions with cool temperature like New Zealand. This can be seen from the proportion of this germplasm in the five synthetics. NZS3 contains approximately 45% of highland tropical germplasm, NZS1 and NZS2 contain 25 %, while AS3 and BS22 are mainly Corn Belt Dent germplasm. This supports earlier conclusiosn by Eagles and Hardacre (1989) who worked for the agronomic performances of mature plants and the yield.

From mean analysis of 68 genotypes of these synthetics and hybrid checks, it was shown that only few of them consistenly had good general combining ability (GCA) over nine characters measured. However, as only some of these nine characters had high heritability and had genetic correlation to dry mass, only the genotypes which showed good GCA for these characters are reasonable to be selected. A selection index would be useful for this purpose. As the genotypes are early generations, mild selection pressure are suggested.

5.3. Physiological study

Temperature response for ion leakage, germination, and seedling growth were similar to those reported in literature. The amount of ion leakage, measured as the conductivity of a soaking solution (CONDUCT), increased as the temperature decreased. This confirmed with Tatum's (1954) result that ion leakage was negatively correlated with temperature. The increase in ion leakage during imbibition theoritically would be due a slower restitution of membrane integrity as the temperature decrease (Simon, 1979); and/or due to a membrane-phase change causing mitochondrial dysfunction as described by Lyons and Raison (1970). According to these theories, maize genotypes with smaller amount of ion leakage when germinated under low temperature would be the genotypes with the greater cool tolerance. However, it was found that there was no close correlation, neither phenotypically nor genetically, between the amount of ion leakage and the seedling growth rates. This discrepancy may be due to the limited number of observations involved this experiment, or to the fact that the hyphothesis is wrong.

Time to germinate and seedling growth rates are manifestation of the stability of biochemical or physiological processes at ealy stage the plant life cycle, the postponement of germination and the reduction of seedling growth rates due to declining temperature indicate that these physiological processes are temperature independent. Furthermore, as the growth of maize seedling depends on the utilisation of seed reserves until two leaves have fully emerged (Cooper and MacDonald, 1970), seed constitution must play an important role in determining the growth rate of the seedling. The existence of close correlations (both phenotypic and genotypic) between seed constitution (i.e. seed weight, nitrogen and maltose contents) and seedling growth rates is an evidence that support to this view.

6. Conclusion

1. In the first experiment, a highly significant genotypic variation was observed for all nine characters studied. The genotype x time interaction effects were significant for chlorophyl content, shoot and root dry masses which were repeatedly measured, indicating that low temperatures influenced genotypes differentially as the plants grow. Consequently, the genetic gain resulted from the selection of these characters may differ for different growth stages.

2. In the second experiment, the variation due to genotypic difference was highly significant only for the initial seed constitution characters and the amount of ion leakage during the early hours of germination process. It was non-significant for the time to germinate, seedling growth rates, and seedling growth functions. These non-significance were probably due to restricted gametes sampling in the materials used, which consisted of a narrow genetic base of genetically related lines. The variation due to the difference of temperature regimes was significant for the time to germinate and seedling growth rate but not the growth function, indicating that both radicle and plumule primordia were not affected by temperatures.

3. On average, genotypes of synthetic line NZS3 showed the best performance for general combining ability for almost all characters studied in the first experiment. Similar results were also reported by other workers who studied on the mature plant and yield. These indicate that highland tropical germplasm are useful source of cool tolerant genes for maize breeding.

4. Four of the characters studied in the first experiment had moderate to high narrow sense heritabilities, namely total leaves at 50 days after planting (82 %), chlorophyll content (46 %), anthocyanin (69%), and leaf area (62 %). These indicate that considerable progress can be made by selecting for these characters. In the second experiment, the estimated broad sense heritabilities observed ranged from very low to very high over all characters. The high broad sense heritabilities were recorded on most of the initial sees constitution characters, the conductivity of ion leakage, and the growth rates of root (length) and shoot (dry mass).

5. Both the phenotypic and genotypic correlation coefficients between pairs are in good agreement and followed the same direction. This indicates that selection on specific characters will result in a similar change in direction of the genotype. Amongst the characters examined in the first experiment only time to achieve second mature leaf, total leaf number at 50 day after planting, chlorophyll content, leaf area had considerable correlations to the dry masses. In the second experiment a good correlation with growth rate was observed for the seed weight, nitrogen and maltose contents.

6. Based on the results of the first experiment, recommendations are made on the future maize breeding works for cool tolerance. These include the use of easily measured characters as selection criteria and the use of selection index to improve the seedling dry mass. Additionally, more detailed work with a large number of genotypes appears desirable to verify the results of the second experiment.

References

Alberda, T. (1969). The effect of low temperature on dry matter production, chlorophyll concentration and photosynthesis of maize plants of different ages. Acta Bot.Neerl. 18: 39-49.

Allard, R.W. (1960). Principles of plant breeding. John Wiley & Sons, New York.

Anderson, T.W. (1958). An introduction to multivariate statistical analysis. John Wiley & Sons, Inc., New York.

Anderson, V.L. and O. Kempthorne (1954). A model for the study of quantitative inheritance. Genetics 39: 883-898.

Anonymous (1988). SAS/STAT user's guide. SAS Institue Inc.

Baker, R.J. (1986). Selection indices in plant breeding. CRC Press Inc. Florida.

Balaam, L.N. (1963). Multiple comparisons - A sampling experiment. Aust.J.Stat. 5: 62-84.

Balaam, L.N. (1972). Fundamentals of biometry. George Allen & Unwin Ltd., London.

Barnes, W.C. and A.B. Blakeney (1974). Determination of cereal alpha-amylase using a commercially available dye-labelled subtrate. Die Stärke 26: 193-197.

Bartlett, M.S. (1947). The use of transformations. Biometrics 3: 39-53.

Becker, W.A. (1985). Manual of quantitative genetics. Academic Enterprises, Washington.

Berlyn, G.P. (1972). Seed germination and morphogenesis. In: T.T. Kozlowski (Ed.). 'Physiological ecology Vol. I'. Academic Press Inc., New York : 223-312.

Blacklow, W.M. (1972a). Mathematical description of the influence of temperature and seed quality on imbibition by seed corn *(Zea mays L.)*. Crop Sci. 12 : 643-646

Blacklow, W.M. (1972b). Influence of temperature on germina tion and elongation of the radicle and shoot of corn *(Zea mays L.)*. Crop Sci. 12 : 647-650.

Benson, G.O. and R.B. Pearce (1987). Corn perspective and culture. In: S.A. Watson and P.E. Ramstad (Eds.). 'Corn chemistry and technology '. Amer.Assoc.Cereal Chem.Inc.: 1-30.

Bocsi, J. and G. Kovac (1991). Inheritance of the rate of germination and emergence at low temperatures in maize *(Zea mays L.)*. Plant Breed.Abstr. 61: 3369.

Bryant, J.A. (1985). Seed physiology. Edward Arnold (Publ.) Ltd.

Bunting, E.S. (1978). Agronomic and physiological factors affecting forage maize production. In: E.S. Bunting, B.F. Pain, R.H. Phipps, J.M. Wilkinson, and R.E. Gunn (Eds.).'Forage maize'. Agricultural Research Council, London.

Cheverud, J.M. (1988). A comparison of genetic and phenotypic correlations. Evolution 45: 958-968.

Chew, V. (1976). Comparing treatment means: A compendium. HortScience 11: 348-356

Ching, T.M. (1972). Metabolism of germinating seeds. In: T.T. Kozlowski (Ed.). 'Physiological ecology Vol. II'. Academic Press Inc., New York : 223-312.

Christeller, J.T. (1984). Seedling growth of *Zea mays* at 13 °C: Comparison of a corn belt dent hubrid and hybrid selected for rapid plumule emergence at cool temperatures. J.Exp.Bot. 35: 955-964.

Cochran, W.G. (1947). Some consequences when the assumptions for the analysis of variance are not satisfied. Biometrics 3: 22-38.

Cockerham, C.C.(1963). Estimation of genetic variance. In: W.D. Hanson and H.F. Robinson (Eds.). 'Statistical genetics and plant breeding'. NAS-NRC publ. 982: 53-94.

Comstock, R.E. and H.F. Robinson (1948). The components of genetic variance in population of biparental progenies and their use in estimating the average degree of dominance. Biometrics 4: 254-266.

Cooley, W.W. and P.R. Lohnes (1971). Multivariate data analysis. Wiley, New York.

Cooper, C.S. and P.W. MacDonald (1970). Energetic seedling growth in corn *(Zea mays L.)*. Crop Sci. 10 : 136-139.

Crosbie, T.M., J.J. Mock, and O.S. Smith (1980). Comparison of gains predicted by several selection methods for cold tolerance traits of two maize populations. Crop Sci. 20: 649-655.

Crump, S.L. (1946). The estimation of variance components in of variance. Biometrics 2: 7-11.

Crump, S.L. (1951). The present status of variance component analysis. Biometrics 7: 1-16.

Dolstra, O. and P. Miedema. (1986). Breeding for improved vegetative growth at low temperature in maize *(Zea mays L.)*. In: O. Dolstra and P. Miedema (Eds.). 'Breeding for silage maize'. Pudoc, Wageningen : 61-70.

Dolstra, O., M.A. Jongmans, and Jong, K.D. (1988). Improvement and significance of resistance to low-temperature damage in maize *(Zea mays L.)*. I. Chlorosis resistance. Euphytica Suppl.: 117-123.

Draper, N.R. and H. Smith (1981). Applied regression analysis. John Wiley, New York.

Duddley, J.W. and R.H. Moll (1969). Interpretation and use of estimates of heritability and genetic variance in plant breeding. Crop Sci. 9: 257-261.

Duke, S.H., L.E. Schrader, and M.G. Miller (1977). Low temperature effects on soybean (*Glycine max L. Mer.* cv. Wells). Mitochondrial respiration and several dehydrogenases during imbibition and germination. Plant Physiol. 60: 716-722.

Eagles, H.A. and A.K. Hardacre (1979). Genetic variation in maize for early seedling growth in a low temperature environment. N.Z. J.Agric.Res. 22: 553-559.

Eagles, H.A. and A.K. Hardacre (1985). Prospect of breeding maize cultivars specifically for New Zealand conditions. In: T.A. Williams and G.S. Wratt (eds.). 'Maize: management to market. Agron.Soc. N.Z. Spec.Publ. 4: 73-77.

Eagles, H.A. and A.K. Hardacre (1989). Synthetic populations of maize contining highland Mexican or highland Peruvian Germplasm. Crop Sci. 29:660-665.

Eagles, H.A. and A.K. Hardacre (1990). Genetic changes from introgression of highland Mexican germplasm into a Corn Belt Dent population of maize. Theor.Appl.Genet. 79: 543-549.

Eisenhart, C. (1947). The assumptions underlying the analysis of variance. Biometrics 3: 1-21.

Erickson, R.O. (1959). Integration of plant growth processes. Am.Nat. 93: 225-235.

Falconer, D.S. (1981). Introduction to quantitative genetics. 2nd ed. Longman, New York.

Fisher, R.A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. Trans.Roy.Soc. Edinburg 52: 399-433.

Fisher, R.A. (1925). Statistical methods for research workers. Oliver & Boyd.

Francis, C.A., C.O. Grogan, and D.W. Spreling (1969). Identification of photoperiod insensitive strains of maize Crop Sci. 9: 675-677.

Gardner, C.O. and S.A. Eberhart (1966). Analysis and interpretation of the variety cross diallel and related populations. Biometrics 22: 439-452.

Gill, J.L. (1973). Current status of multiple comparisons of means in designed experiments. J. Dairy Sci. 56: 973-977.

Gill, J.L. (1986). Repeated measurement: Sensitive tests for experiments with few animals. J.Anim.Sci. 63: 943-954.

Goodman, M.M. (1988). The hidtory and evolution of maize. CRC Crit.Rev. Plant Sci. 7: 197-220.

Gordon, I.L. (1979) Standart errors of heritabilities based on perenial observations, with application to yorkshire grass. Euphytica 28: 81-88.

Gordon, I.L., D.E. Byth and L.N. Balaam (1972). Variance of heritability ratios estimated from phenotypic variance components. Biometrics 28: 401-415.

Grogan, C.O. (1970). Genetic variabiltiy in aize for germintaion and seedling growth at low temperatures. Proc.Annual Corn Sorghum Res.Conf. 25: 90-98.

Guinn, G. (1971). Changes in sugar, starch, RNA, Protein and lipid-soluble phosphate in leaves of cotton plants at low temperatures. Crop Sci. 11: 262-265.

Hallauer, A.R. and J.B. Miranda (1981). Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames, Iowa.

Hanson, W.D. (1963). Heritability. In: W.D. Hanson and H.F. Robinson (Eds.). 'Statistical genetics and plant breeding'. NAS-NRC publ. 982: 53-94.

Hardacre, A.K. and H.A. Eagles (1980). Comparisons among populations of maize for growth at 13 °C. Crop Sci. 20: 780-784.

Hardacre, A.K. and H.L. Turnbull (1986). The growth and development of maize *(Zea mays L.)* at five temperature. Ann.Bot. 58: 779-787.

Hardacre, A.K. and H.A. Eagles (1989). The temperature response of young hybrid maize plants adapted to different climates. N.Z. J. Crop Hort.Sci. 17: 9-17.

Hardacre, A.K., D.H. Greer (1989). Difference in growth in response to temperature of maize hybrids varying in low temperature tolerance. Aust.J.Plant Physiol. 16: 181-187.

Hardacre, A.K., H.F. Nicholson, and M.L.P. Boyce (1984). A portable photometer for the measurement of chlorophyll in intact leaves. N.Z. J.Exp.Agric. 12: 357-362.

Haskell, G. (1952). genetics of cold tolerance in maize and sweet corn seed. Herdity 6:377-385.

Haskell G. and W.R. Singleton (1949). Use of controlled temperature in evaluating the cold hardiness of inbred and hybrid maize. Agron.J. 41: 34-40.

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 separation of epistatic from additive and dominance variation in generation means II. Genetica 31: 133-146.

Hetherington, S.E., R.M. Smillie, A.K. Hardacre, and H.A. Eagles (1983). Using chlorophyll flourescence in vivo to measure chilling tolerances of different populations of maize. Aus.J.Plant Physiol. 10: 247-256.

Hoard, K.G. and T.M. Crosbie (1985). S1-line recurrent selection for cold tolerance in two maize populations. Crop Sci. 25: 1041-1045.

Jacquard, A. (1983). Heritaility: one word, three concepts. Biometrics 39: 465-477.

Johnson, H.W., H.F. Robinson, and R.E. Comstock (1955). Genotypic and phenotypic correlations in soybeans and their implications in selection. Agron.J. 47: 477-483.

Kempthorne, O. (1957). An introduction to genetic statistics. John Wiley & Sons Inc., New York.

Larson, L.A. (1968).The effect of soaking pea seeds with or without seed coats has on seedling growth. Plant Physiol. 43: 255-259.

Le Clerg, E.L., W.H. Leonard, A.G. Clark (1962). Field plot technique. Burgess, Minneapolis, Minn.

Leng, E., R.A. Tavcar, and V. Trifunovic (1962). Maize of southeastern Europe and its potential value in breeding programs elsewhere. Euphytica 11: 263-272.

Lott, J.N. (1974). Cell walls in *Cucurbita maxima* cotyledons in relation to imbibition. Can.J.Bot. 52: 1465-1468.

Lyons, J.M. (1973). Chilling injury in plants. Annu.Rev.Plant Physiol. 24: 445-456.

Lyons, J.M. and J.K. Raison (1970). Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant Physiol. 45: 386-389.

McConnell, R.L. and C.O. Gardner (1979). Inheritance of several cold tolerance traits in corn. Crop Sci. 19: 847-852.

McWilliam, J.R. and A.W. Naylor (1967). Temperature abd plant adaptation. I. Interaction of temperature and light in the synthesis of chlorophyll in corn. Plant Physiol. 42: 1711-1715.

Mangelsdorf, P.C. (1974). Corn: its origin, evolution and improvement. Harvard Univ. Press, Cambidge, Mass.

Maryam, B. and D.A. Jones (1983). The genetics of maize *(Zea mays L.)* growing at low temperatures. I. Germination of inbred lines and their F1s. Euphytica 32: 535-542.

Mather, K. (1949). Biometrical genetics. Methuen, London.

Mather, K. and J.L. Jinks (1977). Introduction to biometrical genetics. Chapman and Hall, London.

Menkir, A. and E.N. Later (1987). Emergence and seedling growth of inbred lines of corn at suboptimal root-zone temperatures. Can.J.Plant Sci. 67: 409-415.

Miedema, P. (1982). The effect of low temperature on *Zea mays*. Adv.Agron. 35: 93-128.

Miedema, P., P. Post, and P.J. Groot (1982). The effect of low temperature on seedling growth of maize genotypes. PUDOC, Wageningen.

Mock, J.J. and S.A. Eberhart (1972). Cold tolerance in adapted maize populations. Crop Sci. 12 : 466-469.

Mock, J.J. and A.A. Bakri (1976). Recurrent selection for cold tolerance in maize. Crop Sci. 16: 230-233.

Mock, J.J. and W.H. Skrdla (1978). Evaluation of maize plant introductions for cold tolerance. Euphytica 27: 27-32.

Mock J.J. and M.J. McNeill (1979). Cold tolerance of maize inbred lines adapted to various latitudes in North America. Crop Sci. 19: 239-242.

Moran, R. (1982). Formulae for determintaion of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol. 69: 1376-1381.

Perry, D.A. and J.G. Harrison (1970). The deleterious effect of water and low temperature on germination of pea seed. J.Exp.Bot. 21:504-512.

Pesev, N.V. (1970). Genetic factors affecting maize tolerance to low temperatures at emergence and germination. Theor.Appl.Genet. 40: 351-356.

Pinell, E.L. (1949). Genetic and environmental factors affecting corn seed germination at low temperatures. Agron.J. 41: 562-568.

Robinson, H.F., R.E. Comstock, and P.H. Harvey (1951). Genotypic and phenotypic in corn and their implications in selection. Agron.J. 43: 282-287.

Robinson, P. (1963). Heritability: Second look. In: W.D. Hanson and H.F. Robinson (Eds.). 'Statistical genetics and plant breeding'. NAS-NRC publ. 982: 53-94.

Satterthwaite, F.E. (1946). An approximate distribution of estimates of variance components. Biometrics Bull. 2: 110-114.

Searle, S.R. (1961). Phenotypic, genetic and environmental correlations. Biometrics 17 : 475-480.

Searle, S.R. (1971). Topics in variance component estimation. Biometrics 27: 1-76.

Shaw, R.H. (1977). Climatic requirement. In: G.F. Sprague (Ed.). 'Corn and corn improvement'. Amer.Soc.Agron.Inc., Wisconsin : 591-623.

Simon, E.W. (1974). Phospholipids and plant membrane permiability. New Phytol. 73: 377-420.

Simon, E.W. (1979). Seed germination at low temperature. In: J.M. Lyons, D. Graham, and J.K. Raison (Eds.). 'Low temperature stress in crop plants'. Academic Press Inc., New York : 37-46.

Sprague, G.F. (1966). Quantitative genetics in plant improvement. In: K.J. Frey (Ed.). 'Plant breeding'. The Iowa State Univ. Press, Ames : 315-353.

Stamp, P. (1984). Chilling tolerance of young plants demonstrated on the example of maize *(Zea mays L.)*. Verlag Paul Parey, Berlin and Hamburg.

Steel, R.G.D. and J.H. Torrie (1980). Principles and procedures of statistics. McGraw-Hill, Auckland.

Stevenson, J.C. and M.M. Goodman (1972). Ecology of exotic races of maize. I. Leaf number and tillering of 16 races under four temperatures and two photoperiod. Crop Sci. 12: 864-868.

Stewart, J.McD., and G. Guinn (1969). Chilling injury and changes in adenosine triphosphate of cotton seedlings. Plant Physiol. 44: 605-608.

Stewart, J.McD., and G. Guinn (1971). Chilling injury and nucleotide changes in young cotton plants. Plant Physiol. 48: 166-170.

Stushnoff, C., B. Fowler, and A. Brule-Babel (1984). Breeding and selection for resistance to lo temperature. In: ' Crop breeding'. P.B. Vose and S.G. Blixt (Eds.). Pergamaon Press, Oxford : 115-136.

Tatum, L.A. (1954). Seed permiability and 'Cold Test' reaction in *Zea mays*. Agron.J. 46: 8-10.

Tollenaar, M., T.B. Daynard, and R.B. Hunter (1979). Effect of temperature on development of maize. Crop Sci. 19: 363-366.

Vallejos, C.E. (1979). Genetic deversity of plants for response to low temperatures and its potential use in crop plants. In: J.M. Lyons, D. Graham, and J.K. Raison (Eds.). 'Low temperature stress in crop plants'. Academic Press Inc., New York : 473-490.

Van der Veen, J.H. (1959). Test of non-allelic interaction and linkage for quantitative character in generations derived from diploid pure lines. Genetica 30: 201-232.

Warner, J.N. (1952). A method for estimating heritability. Agron.J. 44: 427-430.

Wellington, P.S. (1966). Germination and seedling emergence. In: F.L. Milthorpe and J.P. Irving (Eds.). 'Growth of cereals and grasses'. Methuen, London : 3-19.

Wilks, S.S. (1932). Certain generalization in the analysis of variance. Biometrics 24: 471-494.

Wilkes, H.G. (1979). Mexico and Central America as a centre for the origin of agriculture and the evolution of maize. Crop Improv. 6: 1-18.

Wright, S. (1935). The analysis of variance and the correlations between relatives with respect to deviations from an optimum. J.Genetics 30: 243-256.

Woodstock, L.W. and B.M. Pollock (1965). Physiological predetermination: imbibition, respiration, and growth of lima bean seed. Science 150: 1031-1032.

Yates, F. (1933). The analysis of replicated experiments when the field results are incomplete. Emp.J.Exp.Agric. 1: 129-142.

Yen, D.E. (1959). The use of maize be New Zealand Maories. J.Econ.Bot. 13: 319-327.

Zhivotovsky, L.A. (1988). Some methods of analysis of correlated characters. In: B.S. Weir, M.M. Goodman, E.J. Eisen, and G. Namkoong (Eds.). 'Proceedings of the second international conference on quantitative genetics'. Sinauer Assoc.Inc., Massachusetts : 423-432.

Appendix 1

North Carolina Solution

Element	Source	Form	Concentration (ppm)
N	NH_4NO_3	NH_4^+	28.02
		NO_3^-	28.02
	$CaNO_3$	NO_3^-	49.02
	KNO_3	NO_3^-	17.71
			122.77
K	KH_2PO_4	K^+	7.18
	K_2HPO_4	K^+	4.94
	KNO_3	K^+	49.42
			61.54
Ca	$CaNO_3$	Ca^+	70.10
P	KH_2PO_4	PO_4^{3-}	5.69
	K_2HPO_4	PO_4^{3-}	1.96
			7.65
S	$MgSO_4$	SO_4^{2-}	8.02
	Na_2SO_4	SO_4^{2-}	16.02
	$ZnSO_4$	SO_4^{2-}	0.006
	$CuSO_4$	SO_4^{2-}	0.003
			24.049
Mg	$MgSO_4$	Mg^{2+}	6.08

Appendix 1

(Continued)

Element	Source	Form	Concentration (ppm)
Na	Na_2SO_4	Na^+	22.99
Zn	$ZnSO_4$	Zn^{2+}	0.0114
Mn	$MnCl_2$	Mn^{2+}	0.145
Cu	$CuSO_4$	Cu^{2+}	0.0051
B	H_3BO_3	B	0.123
Mo	H_2MoO_4	Mo	0.0021
Fe		Fe	5.96
Cl	$MnCl_2$	Cl^-	0.186

Appendix 2

Determination of Kjeldahl Nitrogen Content with a Kjeltec Auto System

Equipments :

1. Kjeltec Auto 1030 Analyser and accessories
2. Tecator Digestion System and accessories
3. Analytical balance
4. Fume Hood and sink

Reagents :

1. Sulphuric acid, concentrated analystical grade N-free
2. Kjeltabs (Se)
3. Alkali: 2 kg Sodium hydroxide analytical grade 35-40 % are disolved in 5 ℓ distilled water
4. Titrant : Hydrochloric acid 0.1 M
5. Receiver solution 1% (v/v): 100 g Boric acid are dissolved in 10 ℓ distilled water. 100 mℓ Bromocresol green (100 mg in 100 mℓ methanol), 70 mℓ Methyl red solution (70 mg in 70 mℓ methanol), and 0.5 mℓ Sodium hydroxide 1 M are added to the solution.

Procedure :

1. Meal (0.5 g) is weighed onto a weighing boat and quantitatively transfered to a digestion tube.
2. Three small tablets of Kjeltab are added to the digestion tube containing a sample to be analysed.

3. 10 mℓ concentrated Sulphuric acid from a dispenser are added and mixed by swirling the tube.
4. The digestion tube is digested in the preheated digestor to 420 °C for 45 minutes.
5. The sample solution is cooled to hand temperature and diluted with 30 mℓ distilled water.
6. The sample is then automatically distilled with receiver solution and tritrated with hydrochloric acid in Kjeltec Auto 1030 analyser.

Calculation :

$$N\ \% = \frac{14.01 \times M \times f \times (\text{mℓ titrant} - \text{mℓ blank})}{\text{g sample}}$$

where 14.01 is the atomic weight of nitrogen, M is the molarity of titrant HCL (mole/ℓ), and f is the standard Kjeldahl factor = 1.00 for % N.

Appendix 3

Reducing and Nonreducing Sugar Analysis (AACC Method 80-60)

Equipment:

1. Analytical balance
2. Glasswares
3. Filter paper
4. Waterbath
5. Thermometer

Reagent:

1. Ethyl alcohol, 95 % (v/v)
2. Acid buffer solution : 3 mℓ glacial acetic acid, 4.1 g anhydrous sodium acetate, and 4.5 sulphuric acid are dissolved and dilluted to 1 ℓ with water.
3. Sodium tungstate, 12 %: 12 g sodium tungstate are dissolved and dilluted to 100 mℓ.
4. Alkali ferricyanide solution, 0.5 N: 33 g pure dry potassium ferrycyanide and 44 g anhydrous sodium carbonate are dissolved ann dilluted to 1 ℓ.
5. Acetic acid-salt solution: 70 g potassium chloride and 40 g zincsulphate are dissolved in 750 mℓ water, added with 200 mℓ glacial acetic acid and dilluted to 1 ℓ with water.

6. Soluble starch-potassium iodide solution: 2 g soluble starch are suspended in small quantity of water and poured slowly into boilling water with constant stirring. The suspension is cooled, added 50 g potassium iodide, dilluted to 100 mℓ, and added with 1 drop of standardized sodium hydroxide solution.
7. Thiosulphate solution, 0.1 N

Procedure:

Preparation of extract:

1. Maize meal (5.675 g) is introduced into a 100 mℓ E-flask. The flask is tipped so that all meal is at one side and wetted with 5 mℓ alcohol. The flask is then tipped again so that the wet meal is at uuper side and added 50 mℓ acid buffer solution, keeping solution from coming in contact with the meal until it has all been added to the flask. The flask is then shaked to bring the meal into suspension. Sodium tingstate (2 mℓ) is added immediately and mixed thoroughly.
2. The suspension is filtered with a Whatman paper No. 4 and discarded the first 8-10 drops of filtrate.

Reducing sugars:

1. The extract (5 mℓ) is pipetted into a test tube and added with 10 mℓ alkali ferricyanide solution .
2. The test tube is immersed in vigorously boiling water bath for 20 minutes, cooled under running water, poured into a 100 mℓ E-flask, and rinsed out with 25 mℓ acetic acid-salt solution, adding rinsings to solution in E-flask.

3. The solution is added with 1 mℓ of soluble starch-KI solution, mixed thoroughly, and titrated with 0.1 N thiosulphate to complete the disappearance of blue colour.

Nonreducing sugars:

1. The extract (5 mℓ) is pipetted into a test tube, immersed in vigorously boiling water bath for 15 minutes, and cooled under running water.
2. The solution is added with 10 mℓ alkali ferricyanide solution and carried out a reduction and subsequent titration as described above for reducing sugars.

Calculation:

1. The reduced ferricyanide was calculated by subtracting mℓ thiosulophate required from thiosulphate equivalent of ferricynide reagent (blank determination).
2. The reducing sugars as mg maltose/ 10 g meal and the nonreducing sugars as mg sucrose/10 g meal are computed by reference to the following table.

FERRICYANIDE-MALTOSE-SUCROSE CONVERSION TABLE

(Final Approval, 4-13-61)

Issued December 1962

0.1 *N* Ferricyanide reduced	Maltose per 10 g flour	Sucrose per 10 g flour	0.1 *N* Ferricyanide reduced	Maltose per 10 g flour	Sucrose per 10 g flour
ml	mg	mg	ml	mg	mg
0.10	5	5	4.50	237	214
0.20	10	10	4.60	244	218
0.30	15	15	4.70	251	223
0.40	20	19	4.80	257	228
0.50	25	24	4.90	264	233
0.60	31	29	5.00	270	238
0.70	36	34	5.10	276	242
0.80	41	38	5.20	282	247
0.90	46	43	5.30	288	251
1.00	51	48	5.40	295	256
1.10	56	52	5.50	302	261
1.20	60	57	5.60	308	266
1.30	65	62	5.70	315	270
1.40	71	67	5.80	322	275
1.50	76	71	5.90	328	280
1.60	80	76	6.00	334	285
1.70	85	81	6.10	341	290
1.80	90	86	6.20	347	294
1.90	96	91	6.30	353	299
2.00	101	95	6.40	360	304
2.10	106	100	6.50	367	309
2.20	111	104	6.60	373	313
2.30	116	109	6.70	379	318
2.40	121	114	6.80	385	323
2.50	126	119	6.90	392	328
2.60	130	123	7.00	398	333
2.70	135	128	7.10	406	337
2.80	140	133	7.20	412	342
2.90	145	138	7.30	418	347
3.00	151	143	7.40	425	352
3.10	156	148	7.50	431	357
3.20	161	152	7.60	438	362
3.30	166	157	7.70	445	367
3.40	171	161	7.80	451	372
3.50	176	166	7.90	458	377
3.60	182	171	8.00	465	382
3.70	188	176	8.10	472	387
3.80	195	181	8.20	478	392
3.90	201	185	8.30	485	397
4.00	207	190	8.40	492	402
4.10	213	195	8.50	499	407
4.20	218	200	8.60	505	..
4.30	225	204	8.70	512	..
4.40	231	209	8.80	519	

Appendix 4

Alpha-Amylase Analaysis

(Barnes and Blakeney Method)

Equipments

1. Mechanical shaker
2. Centrifuge
3. Water bath
4. Spectrophotometer
5. Glassware

Reagents

1. Phadebas tablet
2. Sodium chloride
3. Calcium chloride
4. Sodium hydroxide 0.5 M

Procedure

1. Ground maize (5 g) is added to 20 mℓ destilled water containing sodium chloride 5 g/ℓ and calcium chloride 0.2 g/ℓ.
2. The suspension is gently shaken on a mechanical shaker for five minutes and centrifuged at 2000 rpm.

3. Five mℓ of the clear supernatant are added to a test tube and placed in a water bath at 50 °C to equilibrate. A timer is started on the addition of a Phadebas tablet, which is completely dispersed with gentle hand shaking.
4. The digest is incubated for 15 minutes with a hand shake each 5 minutes.
5. On the completion of of incubation 1 mℓ of 0.5 M sodium hydroxide is added, the volume made up to 10 mℓ, filtered, and absorbance read at 620 nm with a cuvette for a 1 cm light path. If absorbance exeed the scale of the spectrophotometer, the filtrate is diluted accordingly.
6. The enzyme activity is measured by converting the absorbance reading to the unit enzyme per liter (U/ℓ) as showed on the chart accompanying the Phadebas tablets.

Appendix 5

Coefficient of determination (R^2) for ROOTLENGTH

Envir.	Genot.[a]	Block	R^2 (linear)	R^2 (Log)
1	1	1	0.8504	0.6742
1	1	2	0.6906	0.6423
1	1	3	0.8578	0.8242
1	2	1	0.8983	0.8053
1	2	2	0.4655	0.0706
1	2	3	0.5153	0.1176
1	3	1	0.8169	0.7715
1	3	2	0.9202	0.7915
1	3	3	0.8572	0.7470
1	4	1	0.4032	0.0881
1	4	2	0.9434	0.7997
1	4	3	0.8948	0.7669
1	5	1	0.5294	0.1881
1	5	2	0.8433	0.7339
1	5	3	0.7989	0.7722
2	1	1	0.4191	0.0393
2	1	2	0.9223	0.7464
2	1	3	0.3934	0.0957
2	2	1	0.8269	0.6250
2	2	2	0.4064	0.0220
2	2	3	0.7455	0.6084
2	3	1	0.8155	0.6403
2	3	2	0.2469	0.0001
2	3	3	0.3771	0.0192
2	4	1	0.8945	0.7568
2	4	2	0.4261	0.0506
2	4	3	0.3657	0.0284
2	5	1	0.8548	0.7052
2	5	2	0.8217	0.6771
2	5	3	0.8779	0.6847

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113.

Appendix 6

Coefficient of determination (R^2) for HEIGHT

Envir.	Genot.[a]	Block	R^2 (linear)	R^2 (Log)
1	1	1	0.8927	0.7780
1	1	2	0.8814	0.8548
1	1	3	0.9135	0.8506
1	2	1	0.9255	0.8689
1	2	2	0.8866	0.8184
1	2	3	0.9554	0.8387
1	3	1	0.9422	0.8533
1	3	2	0.9386	0.8811
1	3	3	0.9535	0.8760
1	4	1	0.7932	0.7138
1	4	2	0.9456	0.8876
1	4	3	0.9623	0.8326
1	5	1	0.7821	0.7914
1	5	2	0.9058	0.8504
1	5	3	0.9414	0.8957
2	1	1	0.9191	0.8700
2	1	2	0.9686	0.8481
2	1	3	0.9438	0.8719
2	2	1	0.9725	0.7590
2	2	2	0.9079	0.7019
2	2	3	0.9397	0.7591
2	3	1	0.9584	0.7576
2	3	2	0.8650	0.7352
2	3	3	0.9351	0.8118
2	4	1	0.9094	0.7513
2	4	2	0.9430	0.7274
2	4	3	0.9221	0.7200
2	5	1	0.9588	0.7565
2	5	2	0.9519	0.7956
2	5	3	0.9794	0.8354

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113

Appendix 7

Coefficient of determination (R^2) for ROOT

Envir.	Genot.[a]	Block	R^2 (linear)	R^2 (Log)
1	1	1	0.8481	0.6997
1	1	2	0.7389	0.7698
1	1	3	0.8368	0.8304
1	2	1	0.7382	0.6141
1	2	2	0.8531	0.6627
1	2	3	0.9316	0.7488
1	3	1	0.8592	0.7753
1	3	2	0.9300	0.7766
1	3	3	0.7402	0.6691
1	4	1	0.7065	0.6309
1	4	2	0.5248	0.5048
1	4	3	0.7891	0.6746
1	5	1	0.8015	0.6902
1	5	2	0.7346	0.6680
1	5	3	0.7823	0.6814
2	1	1	0.9069	0.8264
2	1	2	0.9021	0.8274
2	1	3	0.8141	0.7860
2	2	1	0.9386	0.8285
2	2	2	0.8962	0.7718
2	2	3	0.8770	0.8094
2	3	1	0.8046	0.6978
2	3	2	0.9009	0.7946
2	3	3	0.8822	0.7668
2	4	1	0.7236	0.6772
2	4	2	0.9193	0.8557
2	4	3	0.6772	0.6786
2	5	1	0.7865	0.7245
2	5	2	0.8282	0.7079
2	5	3	0.8207	0.6934

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113.

Appendix 8

Coefficient of determination (R^2) for SHOOT

Envir.	Genot.[a]	Block	R^2 (linear)	R^2 (Log)
1	1	1	0.9007	0.8066
1	1	2	0.8573	0.8310
1	1	3	0.7725	0.8612
1	2	1	0.8626	0.8268
1	2	2	0.8960	0.8418
1	2	3	0.9583	0.8650
1	3	1	0.8860	0.8125
1	3	2	0.9295	0.7627
1	3	3	0.8484	0.8001
1	4	1	0.7808	0.7420
1	4	2	0.9010	0.8573
1	4	3	0.8246	0.8013
1	5	1	0.7558	0.7196
1	5	2	0.8463	0.7996
1	5	3	0.9359	0.8342
2	1	1	0.8465	0.8382
2	1	2	0.9233	0.8430
2	1	3	0.9259	0.8581
2	2	1	0.9541	0.8189
2	2	2	0.8966	0.7581
2	2	3	0.9440	0.7813
2	3	1	0.8530	0.7166
2	3	2	0.8600	0.7284
2	3	3	0.9188	0.7835
2	4	1	0.8595	0.7043
2	4	2	0.9045	0.8099
2	4	3	0.8184	0.7152
2	5	1	0.9272	0.7771
2	5	2	0.8927	0.7496
2	5	3	0.9242	0.7652

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113.

Appendix 9

Regression functions estmates and their square root of error Mean Squares for ROOTLENGTH

Envir.	Genot.[a]	Block	b_0	b_1	Root MSE
1	1	1	-22.4000	7.3250	36.6442
1	1	2	-45.6000	6.5000	51.8871
1	1	3	-46.4250	7.1406	34.6749
1	2	1	-23.1000	7.6875	30.8462
1	2	2	13.6618	5.8305	72.5185
1	2	3	-8.3500	6.2750	72.5749
1	3	1	-36.7529	8.2485	45.3296
1	3	2	-46.2000	8.8187	30.9775
1	3	3	-23.1750	7.2156	35.1212
1	4	1	19.5147	6.1210	86.4375
1	4	2	-46.2500	9.3937	27.4310
1	4	3	-36.5500	8.7375	35.7368
1	5	1	- 6.1103	6.4866	70.9839
1	5	2	-21.0000	8.5312	43.8541
1	5	3	-25.6750	7.0094	41.9409
2	1	1	20.0478	14.0538	67.1285
2	1	2	-25.3896	21.3141	25.8885
2	1	3	12.0520	13.8204	71.8125
2	2	1	8.4714	17.8891	33.6129
2	2	2	26.1046	15.7871	77.4006
2	2	3	24.7893	16.4740	40.2766
2	3	1	- 0.1282	20.8509	41.5037
2	3	2	44.8439	13.7927	97.7379
2	3	3	44.8414	14.0334	74.0716
2	4	1	-17.1432	25.9040	36.5419
2	4	2	18.8223	17.5247	85.1044
2	4	3	44.7577	15.4145	83.3825
2	5	1	-21.9780	24.4699	41.4129
2	5	2	13.2094	18.4664	35.9911
2	5	3	-14.8794	23.6662	36.9383

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113.

Appendix 10

Regression functions estmates and their square root of error Mean Squares for HEIGHT.

Envir.	Genot.[a]	Block	b_0	b_1	Root MSE
1	1	1	-58.3162	6.6682	26.8304
1	1	2	-58.9000	5.8250	25.4770
1	1	3	-64.1000	6.1187	22.4495
1	2	1	-46.1176	5.6574	18.6359
1	2	2	-46.1429	6.0714	24.2284
1	2	3	-38.9372	5.0634	12.8589
1	3	1	-37.3750	4.8031	14.1864
1	3	2	-40.1397	4.9947	14.8237
1	3	3	-44.9000	5.2250	13.7667
1	4	1	-25.5020	5.0585	28.9903
1	4	2	-54.2000	6.2687	17.9333
1	4	3	-46.3750	5.8281	13.7505
1	5	1	-37.7021	6.0187	37.3186
1	5	2	-56.6250	7.8656	28.2890
1	5	3	-54.2000	6.2375	18.5571
2	1	1	-81.6580	21.1095	25.4012
2	1	2	-82.1954	21.0647	15.8701
2	1	3	-88.9054	21.5160	21.3012
2	2	1	-74.6650	22.4962	15.8293
2	2	2	-52.4270	20.4090	26.3644
2	2	3	-77.5241	22.7418	24.1025
2	3	1	-52.7868	19.1453	16.6971
2	3	2	-39.5343	17.0103	27.5844
2	3	3	-42.8155	17.0739	18.2451
2	4	1	-49.6523	19.3426	25.5452
2	4	2	-41.2272	17.0670	17.0245
2	4	3	-38.5841	16.6100	19.5905
2	5	1	-55.9759	19.7582	17.1347
2	5	2	-62.0013	20.6904	19.4598
2	5	3	-72.6548	21.7233	13.1805

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113.

Appendix 11

Regression functions estmates and their square root of error Mean Squares for ROOT

Envir.	Genot.[a]	Rep.	b_0	b_1	Root MSE
1	1	1	-0.0193	0.0030	0.0147
1	1	2	-0.0275	0.0030	0.0210
1	1	3	-0.0226	0.0025	0.0133
1	2	1	-0.0121	0.0028	0.0194
1	2	2	-0.0094	0.0029	0.0133
1	2	3	-0.0155	0.0028	0.0090
1	3	1	-0.0133	0.0024	0.0117
1	3	2	-0.0146	0.0025	0.0081
1	3	3	-0.0026	0.0018	0.0124
1	4	1	-0.0010	0.0022	0.0156
1	4	2	-0.0027	0.0023	0.0253
1	4	3	-0.0049	0.0024	0.0144
1	5	1	-0.0085	0.0022	0.0129
1	5	2	-0.0043	0.0025	0.0171
1	5	3	-0.0041	0.0019	0.0121
2	1	1	-0.0273	0.0084	0.0109
2	1	2	-0.0226	0.0073	0.0100
2	1	3	-0.0215	0.0068	0.0131
2	2	1	-0.0166	0.0067	0.0072
2	2	2	-0.0148	0.0069	0.0090
2	2	3	-0.0086	0.0053	0.0083
2	3	1	-0.0069	0.0063	0.0130
2	3	2	-0.0131	0.0067	0.0090
2	3	3	-0.0085	0.0059	0.0088
2	4	1	-0.0062	0.0057	0.0147
2	4	2	-0.0227	0.0077	0.0093
2	4	3	-0.0044	0.0053	0.0149
2	5	1	-0.0108	0.0056	0.0123
2	5	2	-0.0081	0.0056	0.0108
2	5	3	-0.0081	0.0060	0.0118

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113.

Appendix 12

Regression functions estmates and their square root of error Mean Squares for SHOOT

Envir.	Genot.[a]	Block	b_0	b_1	Root MSE
1	1	1	-0.0284	0.0031	0.0121
1	1	2	-0.0245	0.0027	0.0132
1	1	3	-0.0252	0.0026	0.0168
1	2	1	-0.0144	0.0020	0.0093
1	2	2	-0.0154	0.0021	0.0080
1	2	3	-0.0129	0.0019	0.0045
1	3	1	-0.0072	0.0016	0.0068
1	3	2	-0.0077	0.0015	0.0049
1	3	3	-0.0084	0.0017	0.0085
1	4	1	-0.0090	0.0018	0.0108
1	4	2	-0.0166	0.0023	0.0087
1	4	3	-0.0118	0.0020	0.0105
1	5	1	-0.0122	0.0024	0.0161
1	5	2	-0.0155	0.0027	0.0129
1	5	3	-0.0158	0.0024	0.0075
2	1	1	-0.0222	0.0076	0.0132
2	1	2	-0.0275	0.0081	0.0097
2	1	3	-0.0270	0.0079	0.0090
2	2	1	-0.0179	0.0059	0.0054
2	2	2	-0.0159	0.0064	0.0084
2	2	3	-0.0138	0.0054	0.0055
2	3	1	-0.0098	0.0058	0.0100
2	3	2	-0.0070	0.0053	0.0087
2	3	3	-0.0066	0.0053	0.0064
2	4	1	-0.0064	0.0051	0.0087
2	4	2	-0.0118	0.0057	0.0075
2	4	3	-0.0050	0.0045	0.0086
2	5	1	-0.0156	0.0065	0.0076
2	5	2	-0.0106	0.0058	0.0084
2	5	3	-0.0145	0.0062	0.0074

[a] (1) 3Mo71, (2) H99x(A665xCM105), (3) A665xW153R, (4) NZ1AxA665, (5) NZ1Ax5-113

Appendix 13

Exp.II. Variance components and their standard errors (in brackets) and significant for the intercept (β_o).

Character	σ^2_E	$\sigma^2_{R(E)}$	σ^2_G	σ^2_{GE}	σ^2
ROOTLENGTH	10.9258 (9.5519) ns	0.2117 (1.3064) ns	0.1581 (1.4545) ns	0.1035 (2.2033) ns	8.9932 (2.9977)
ROOT	7.9E-05 (2.2E-04) ns	0.0001 (0.0003) ns	0.0028 (0.0018) *	-1.7E-05 (4.1E-04) ns	0.0019 (0.0006)
HEIGHT	3.3401 (3.4474) ns	0.3523 (0.5636) ns	5.3831 (4.2876) ns	2.6895 (2.1221) ns	2.8363 (0.9454)
SHOOT	-2.7E-05 5.5E-05 ns	-6.2e-05 7.8E-05 ns	0.0048 0.0029 *	1.3E-04 2.4E-04 ns	7.4E-04 3.0E-04

ns non-significant at 0.05, * significant at 0.05 level, ** significant at 0.01 level