A diallel study of *Secale cereale*

by

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DECLARATION

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ABSTRACT

Rye (*Secale cereale*) originated and was domesticated in the fertile cresent in the Middel East. It has been part of the human staple diet for thousands of years in those areas as well as Eastern Europe. It is known for its ability to grow and produce grain and animal feed in harsh environments. Therefore, as a result of its hardiness, rye is cultivated in many countries across the globe.

In a rapid changing environment, due to climate change and human population growth, the importance of food security cannot be over emphasised. Therefore, this study aimed to select superior parent lines for the following characteristics: days to heading, plant length, spike number, thousand kernel weight and yield to be used in the Stellenbosch University's Plant Breeding programme.

In the first part of the study seed, from eight randomly selected plants from a synthetic population, were planted in planting pots. Due to it's outbreeding nature and high degree of inbreeding depression, the first filial from each individual plant are half-siblings. DNA from three half-siblings from each parent line was extracted to determine variance at molecular level. Eight clones were made from the half-sibling showing the greatest variance for each line.

In the second part of the study these clones were planted according to a Griffing full diallel mating design in all possible combinations. The progeny of these crosses was planted in a random block design with three repititions and the results were measured and compared to determine the general as well as specific combining ability of the diverent lines.

Althouth no significant differences were observed, promising general combiners were identified for days to heading, plant length, spike number, thousand kernel weight and yield. One line may also be considered as a potential parent line for use in a synthetic population to improve qualities for animal fodder and yield. It was also found that one cross performed better than the means for four of the five traits and may therefore be considered for use in a hybrid production program.

OPSOMMING

Die oorsprong en domestikasie van Rog (*Secale cereale*) kan gevind word in die vrugbare halfmaangebied van die Midde Ooste. Dit is alreeds vir duisende jare deel van die mens se stapelvoedsel in hierdie streek, sowel as Oos-Europa en is bekend vir die vermoë om graan en dierevoer in moeilike omgewings te produseer. As gevolg van sy gehardheid, word rog in baie lande regoor die wêreld verbou.

In 'n snelveranderende omgewing, as gevolg van klimaatsverandering en menslike bevolkingsgroei, kan die belangrikheid van voedselsekuriteit nie oorbeklemtoon word nie. Hierdie studie is dus daarop gemik om beter ouerlyne vir die volgende eienskappe te selekteer: dae tot aarvorming, plant lengte, aantal are per plant, duisend korrel massa en opbrengs vir verdere gebruik in die Universiteit Stellenbosch se Planteteelt program.

In die eerste deel van die studie is saad, van agt lukraak gekose plante uit 'n sintetiese bevolking, in plant potte geplant. As gevolg van die kruistelende aard van die gewas, asook die hoë mate van inteelt depressie is die eerste filiaal van elke individuele plant dus half sibbe. DNA ekstraksies vanuit drie half sibbe van elke ouerlyn is gemaak om variansie op molekulêre vlak te bepaal. Die halfsib, van elke ouerlyn, wat die grootste variasie getoon het is agt keer gekloon.

In die tweede deel van die studie was hierdie klone geplant volgens 'n Griffing volle dialeel kruisingsplan in alle moontlike kruisings kombinasies. Die nageslag van hierdie kruisings is geplant in ewekansige blok ontwerp met drie herhalings en die resultate is gemeet en vergelyk om die algemene- sowel as spesifieke kombinerings vermoë van die onderskeie lyne te bepaal.

Alhoewel geen betekenisvolle verskille gevind is nie, is die belowendste algemene kombineerdes geïdentifiseer vir dae tot aarvorming, plant lengte, aantal are per plant, duisend korrel massa en opbrengs. Een lyn, met beter eienskappe vir dierevoer en opbrengs is ook geïdentifiseer as 'n potensiële ouerlyn vir gebruik in 'n sintetiese populasie. Daar is ook bevind dat een van die kruisings beter presteer vir vier van die vyf eienskappe en kan daarom oorweeg word vir gebruik as ‘n ouerlyn vir baster produksies.

ABBREVIATIONS

AFLP	Amplified fragment length polymorphic DNA
ALP	Amplicon length polymorphism
CMS	Cytoplasmic male sterility
FSF	Full-sub family
GCA	General combining ability
GD	Gene Diversity
H^2	Broad-sense heritability
h^2	Narrow-sense heritability
HSF	Half-sub family
ISA	Inter-simple sequence repeat amplification
MAF	Major allele frequency
MAS	Marker assisted selection
MEP	Mean excluding parents
MIP	Mean including parents
MT	Million tons
OPV	Open pollinating varieties
PIC	Polymorphic Information Content
RAPD	Random-amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RS	Recurrent selection
SCA	Specific combining ability
SCAR	Sequence characterised amplified region
SCM	*Secale sereale* microsatellite
SSR	Simple sequence repeat
STR	Simple tandem repeat
STS	Sequence-tagged sites

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Language and style used in this thesis are in accordance with the requirements of the South African Journal of Plant and Soil. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Chapter 1: Introduction

Introduction

Rye (*Secale cereale* L.) has played an important role in the diet of Europeans since the Middle Ages. This can be ascribed to the winter hardiness of the crop (Singh and Jauhar, 2006).

Rye is the result of hybridisation between *Secale vavilovir Grossh*. and perennials *Secale anatolicum Boiss*. and *Secale montanum Guss*. Before its domestication, the crop grew wild in wheat- and barley fields of Turkey, Syria, Lebanon, Iraq and Palestine. Scientific evidence suggests that the selection and domestication of the crop happened around 3 000 years ago in Iran, Turkey, and the Ukraine north of the Black sea (Singh and Jauhar, 2006).

Rye grains of wild origin were found at New Stone age sites in Poland and Austria which may be an indication of the role it played in early people's diet. Early evidence of cultivated rye, 1 000 to 500 B.C, is found in central Europe. From around 500 AD, production moved towards Sweden in the northwest (Persson and Von Bothmer, 2002). There was an increase of rye cultivation during the 16th century. In the early 20th century, it even exceeded wheat in hectares cultivated (Singh and Jauhar, 2006).

Rye has always been a particularly important crop in the Russian Federation, Poland, Germany, Belarus, China and the Ukraine and occupies an important economic position in many other countries (FAOSTAT, 2013). The hardiness of the plant will ensure that there will probably always be some interest in the utilisation of the crop (Bushuk, 2001).

In the South African context the emphasis is mainly on biomass. According to FAOSTAT (2013) an estimated 3 650 ha rye were planted in 2012. According to Dr J van Zyl (personal communication 29 July 2013) this is mainly for use as livestock pasture, as green manure in crop rotation, as a cover crop on potato fields in the Sandveld to prevent wind erosion and as a parent species for triticale.

Climate change may urge agriculturalists and plant breeders to rethink the use and importance of rye as an alternative crop in the traditional wheat producing areas of the

Western Cape. With the predicted change in weather and more unpredictable rainfall patterns, farmers in the Swartland and Rûens regions might be forced to rely more on other components like small stock to stay profitable (Benhin, 2006).

The extreme hardiness and adaptability of the rye plant enable it to grow in areas that are generally not suitable for growing other cereal grains. Most productions are done in the cool temperate zones of the world, but it is also well adapted to the semi-arid regions near deserts and at high altitudes (Bushuk, 2001).

With the general risks associated with agriculture and in particular grain production, producers can simply not keep on cultivating in the hope of making a profit. Therefore, the stimulation of renewed interest in rye production may eventually offer an ancillary crop to wheat. This will only be achieved once new rye cultivars can compete successfully in increasingly more arid parts of traditional wheat producing areas.

Internationally collaborative efforts to sequence parts of the rye genome and establishing genetic maps are well advanced (GrainGenes, 2013). In this study *Secale cereale* microsatellite markers (SCMs), described by Saal and Wrickle (1999) and Hackauf and Wehling (2002) and optimised at Stellenbosch University's Plant Breeding Laboratory (SU-PBL), were used to determine variance at molecular level (Botes and Bitalo, 2013).

Breeding cereals for yield, disease resistance, bio-mass and pre-harvest sprouting resistance is vital especially for diseases that cannot be chemically managed (Miedaner and Geiger, 1999). Improved agronomical practices and plant breeding may offer possible solutions to ensure food security. Another feature that may be considered by rye breeders in their breeding programmes is improved grain weight (Carena, 2009).

Only the flour produced from wheat and rye can be used for baking leavened bread. However, because of the versatility of rye, it is used for various other purposes including pastures, green manure, in crop rotation and feedstock for cattle and pigs. Substantial quantities of rye grain are also used for the production of alcoholic beverages like beer and whiskey (Hamaker, 2008).

Rye breeding has long been neglected in South Africa, and not much has been done regarding marker assisted selection in order to improve breeding efficiency. The focus of the cereal breeding program at the SU-PBL is more on the improvement of wheat and triticale cultivars and not on pure rye cultivars *per se* (Botes and Bitalo, 2013). The hope is that this study may trigger renewed interest in the development of rye cultivars that are more suitable for the winter rain fall area of South Africa.

In order to obtain this, the results of the study may be used to propose the best parental lines that can be used to in the current breeding programme of the University of Stellenbosch for the development of new open pollinated, spring type, rye cultivars for the Western Cape.

The primary aim of this study was to use the selected parent lines in a full 8x8 diallel cross combination mating scheme to determine the combining ability of the parents. In order to achieve this aim the following objectives were identified and pursued during this study:

i. Selection of potential parental material from an existing synthetic open pollinated rye breeding population;
ii. Determination of variance between selected plants and their clones on a molecular level;
iii. To evaluate progeny obtained from diallel crosses, according to performance, with regard to:
    - Yield
    - Agronomical characteristics

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Chapter 2: A review of rye breeding, the diallel mating scheme and diallel analysis

A review of rye breeding, the diallel mating scheme and diallel analysis

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Abstract

Breeding methods for rye are much influenced by the outbreeding nature of the crop with its high degree of self-incompatibility. A diallel study is a mating scheme used by plant breeders to investigate the genetic underpinnings of quantitative traits. Plant breeders want to determine the combining ability of various lines, clones and varieties in order to select the best combinations that can be used in a breeding programme. Common analysis methods utilise general linear models to identify heterotic groups, estimate general combining ability (GCA), specific combining ability (SCA), interactions with testing environments, or estimates of additive, dominant, and epistatic genetic effects, and genetic correlations.

2.1 Introduction

Rye traditionally grows higher than a meter. The long straw was therefore ideally suited for thatching roofs. The development of new cultivars, open pollinated and hybrid, as well as the improvement in agronomy, has also resulted in an increase in seed yield (Singh and Jauhar, 2006).

In modern cultivars, rye exhibits the agronomic traits associated with modern crops, such as wheat and maize with regard to yield. Planted hectares and production has decreased on a global scale by more than half the past four decades. The cool temperature zones of Europe continue to be the main growing regions for production (Singh and Jauhar, 2006). According to FAOSTAT (2013), approximately 16.68 million tons (MT) was produced globally in 2013 and Russia contributed (20.1%), Poland (20.1%), Germany (28%), China (3.89%), Belarus (3.88%), Denmark (3.11%), Austria (1.4%) and Canada (1.24%) of the total tonnage. South Africa only produced an estimated 1950 tons in 2013 (FAOSTAT 2013).

The USA also experienced a decrease in planted hectares. Only winter rye is produced in the USA which is mainly used for grain production. Most of the global annual harvest, 50% to 75%, is used for baking rich, dark bread that stays fresh longer. The rest is used for the production of alcoholic beverages and animal feed (Singh and Juahar, 2006).

In South Africa, rye is sown in the South Western Cape on nutrient poor, acidic sandy soils which are also used for pasture, hay and grain production. It is a small grain of either spring or winter growth habit. According to Mr Kobus van der Merwe (personal communication 10 March 2014) a small quantity of rye grain is produced in Piekenierskloof near Citrusdal, Graafwater in the Sandveld and Langebaanweg on the West Coast and sold to Citrusdal Roller mill for baking bread. This is produced on a total of 650 Ha.

2.2 Botany

Like all other cereal crops, rye belongs to the grass family, *Poaceae* (*Gramineae*), subfamily *Pooideae*, tribe *Triticeae*. The evolutionary split between wheat- and rye progenitors occurred from the *Pooideae* approximately 7 million years ago. Common names for the crop include Rye, feral rye, or cereal rye. Ryegrass (*Lolium* spp.) should not be confused with cereal rye (*Secale cereale* L.) (Singh and Juahar, 2006).

2.3 Genetics

Members of the *Triticeae* have a basic chromosome number of n=7. Polyploidy (the multiplication of the normal diploid number of chromosomes) has played a major role in the evolution of most of the genera. Although, in the case of *S. cereale*, naturally occurring polyploids are rare (Zeven, 1979), and all species are characterized by a diploid chromosome number of 2n=2x=14 plus variable numbers of B chromosomes (Bushuk, 2001; Singh and Juahar, 2006). B chromosomes are extra parts or pieces of the genome. It does not contribute to any benefits for the organisms that contain them. It is maintained in populations because it is transferred at rates higher than Mendelian frequencies (Ali M.*et al*, 2012).

Despite the low chromosome number and the fairly large size of the chromosomes, the exact karyotype of *S. cereale* was subject to much disagreement. Oinuma (1953) cleared the confusion after a study of several European and oriental cultivars. Although he was able to distinguish the seven pairs of chromosomes in each cultivar, he noted significant karyotype differences.

Similar variation has been reported between different inbred lines (Bose, 1957; Heneen, 1962). Bhattacharya and Jenkins (1960) presented a karyotype of cultivar “Dakold” in which the seven chromosomes were distinguished on the basis of length, arm ratio, and the occurrence and location of secondary constrictions. The chromomeric structure of rye chromosomes, first reported by Shmargon (1938) and later studied extensively by Lima de

Faria (1952), enabled the latter to map each of the chromosomes of rye and to identify them on this basis.

Rye is a characteristic cross pollinating plant species, and demonstrates degrees of self-incompatibility due to a gametophytic S-Z multiallelic incompatibility system (Lundquist, 1956). The S and Z are independently inherited and many different S-Z combinations are possible. Each combination brings about an incompatible response between the pistil and pollen (Lundquist, 1959). This effective out-breeding mechanism is found in all open-pollinating rye cultivars (Geiger and Schnell, 1970). The fact that it is a cross-pollinated species and that inbred lines usually lack vigour have restricted genetic analyses (Newell and Butler, 2013).

2.4 Rye breeding methods

Breeding methods for rye have inevitably been influenced by its out-breeding nature (Lundquist, 1956). Early breeding methods greatly relied on simple repeated selection. Although a high degree of inbred depression in rye is observed, inbred lines of acceptable vigour do occur and can be used in the creation of synthetic varieties, but only after progeny tests for combining ability has been done (Acquaah, 2007).

A cross-pollinating population growing in the field will have both homo- and heterozygous gene loci, and is in a continuous state of hybridisation. As a result, new recombinants are constantly formed. It is almost impossible to find two identical plants in a cross-pollinating crop, because in each generation new recombinations of genes occur (Acquaah, 2007).

Recently, the focus of rye breeding is to improve stability of grain yield, fast growth, fine stems, resistance or tolerance to diseases such as powdery mildew and stem and leaf rust, protein content and quality, cold tolerance and shorter straws. Cultivars with a high leaf index are also suitable for making silage and as green fodder for livestock (Singh and Juahar, 2006).

2.4.1 Population breeding

Open pollinated (OP) and synthetic cultivars are developed by means of population breeding. In a population breeding program, both OP and synthetic cultivars involves random mating within the breeding population. Therefore a new population is obtained by random pollination in the last generation of seed production. Inbreeding depression is evaded by the gametophytic self-incompatibility mechanism in an open pollinating rye population (Singh and Juahar, 2006).

Only self-incompatible cultivars are generally produced by population breeding. The improvement of populations is the result of a pollinated cultivar. A pollinated cultivar is the result of the improvement of a population. When performance levels of a breeding population exceeds or is similar to existing cultivars, a new cultivar may be considered (Singh and Juahar, 2006).

Several selection procedures for the improvement of rye populations have been described by Ferwerda (1956), Wolski (1975) and Geiger (1982). The objectives for the improvement of self-compatible populations are to improve the performance and potential of the population for synthetic cultivar production.

Another one of the usual objectives for the selection of self-fertile lines is the probable improvement of the population for hybrid cultivar production (Voylokov, 2007). Different selection procedures are applicable depending on available experimental facilities. These procedures can either be applied successively or consecutively in a specific selection scheme. Where a generalised population improvement scheme is followed, the procedures are divided into various selection cycles. Every cycle includes a parent line, selection- and a recombination unit and each cycle includes plants, clones, pairs of plants, or pairs of clones to be assessed, selected and recombined to form the improved population (Halauer and Miranda, 1988).

2.4.2 Hybrid breeding

Hybrid breeding mainly aims at achieving higher yields and to unlock hybrid vigour. Vigorous hybrids usually exhibit better tolerance to nutrient deficiencies and drought stress. Rye is commonly sown on marginal soils where it even out performs other cereals like wheat and triticale (Budar and Pelletier, 2001). Superior hybrid performance to OPV's are documented for a number of crops including rice (*Oryza sativa* L.) (Budar and Pelletier, 2001) and maize (*Zea mays* L.) (Duvick, 1999).

All small grain cereals except rye are self-pollinating, although self-pollinating forms have been found in a number of rye populations. These self-fertile forms are regularly used in the development of inbred lines. The result of these selfings is high levels of inbreeding depression but a high degree of vigour is shown when crossed with other appropriate inbred lines (Newell and Butler, 2013).

Hybrid vigour is exploited when different inbred lines, obtained from different gene pools, are crossed to an F_1 hybrid. This is achieved with the use of male sterile plants. Male sterile plants are unable to produce functional anthers or pollen but their ovaries function normally. When producing hybrid seed a male sterile line and a normal line is planted together. The male sterile line will act as the seed parent and the normal line as the pollen donor or pollen parent. By doing this, selfings on the seed parent is prevented and cross pollination imposed (Newell and Butler, 2013).

Geiger and Miedaner (2009), successfully applied this model for hybrid rye production since the 1970's and it is still widely in use. The system depend on the following:

- self-fertile, inbred parent lines
- cytoplasmic male sterility (CMS), and
- heterotic pools to exploit heterosis.

For rye, there are different sources of CMS of which Pampa (P) cytoplasm is found to be the most stable in various environments and therefore is commonly used (Miedaner *et al*. 2005). The source was found in Argentina by Geiger and Schnell in 1970 (Kolasiñska, 2003).

All these approaches aimed at improving the intra- and inter population's general combining ability (GCA) with regard to plants, clones, pairs of plants, or pairs of clones of the parent lines. Furthermore, for hybrid breeding, it is important to reduce the mutational load of the population with the intention to minimise inbreeding depression in established lines (Voylokov, 2007).

2.4.3 Breeding open pollinating varieties (OPV's)

Over the years, numerous variations of half-sib family (HSF) recurrent selection (RS) schemes have been used by breeders to improve rye populations with the aim to develop better OPVs. The procedure typically includes four steps over a 4 year period (Carena, 2009):

Year 1. Equally spaced (e.g. 25 x 25 cm^2) "mother plants" are cultivated. From these plants individuals are selected for disease tolerance and/or resistance, formation of productive shoots (tillering), straw stiffness, spike characteristics and general appearance.

Year 2. The HSFs offspring of the selected mother plants are evaluated. The evaluations are done in non-replicated observation plots at two to three locations. At this stage, plants are selected for lodging resistance, and quality.

Year 3. Seed from the selected HSFs are multiplied by open-pollination. This is done in plots that are isolated from each other either by distance or physical barriers like walls or nets.

Year 4. In the last year multi-environment trials of the advanced HSFs, are done for yield improvement. This is done on 5-10 m^2 plots consisting of six- to eight-rows each with one or two replications per environment. In the last phase improved grain yield, stress tolerance and lodging resistance are the more important objectives.

Over time, several rye breeders have changed from the selection of HSF to full-sib families (FSF). In year one FSF pair crosses are produced in breeding tents. Because of the self-

incompatible nature of rye, emasculation is not necessary. In year two, the best individual plants are selected from FSFs which were grown on plots. In the third year, seed is multiplied under pollen isolation to prevent genetic contaminations from another source. Year four entails the evaluation of yield trails $(FSF)^2$ at various locations (Carena, 2009).

There is a greater selection response for the FS scheme compared to that of HS. The reason is the complete parental control as well as more genetic variance between test units [FSF vs. HSF and $(FSF)^2$ vs. $(HSF)^2$] respectively. Unfortunately, much more experimental input is required when producing the pair crosses than the matching steps in the HS scheme (Walsh 2004). With HSF the following variance components needs to be managed: between male families, between female families and within each of the HSF, whereas with FSF it is only within families and between families where variance needs to be managed.

By cloning the FS parent plants, the cycle length of the FS scheme can be reduced by half. This procedure is applied to rapidly increase the yield of breeding populations (Carena, 2009).

Modern OPVs are typically produced when two or more heterogenuous populations are combined to express overdominance or heterosis at population level. Random mating, within the breeding population, is used to improve the performance of a cultivar (Lamkey & Edwards, 1999). Experimental data point to yield increases of 10-20%, compared to parent populations, where two genetically distant rye populations were crossed (Hepting, 1978). Unfortunately, during the seed multiplication stage, Hardy-Weinberg equilibrium is quickly reached resulting in the loss of almost half of this increase due to an equivalent drop in heterozygosity (Carena, 2009).

2.4.4 Breeding synthetic varieties

In breeding cross-pollinated crops, the basis for improvement lies in the controlled utilisation of the heterosis that occurs in hybrids among certain genotypes. This controlled utilisation of heterosis has had its greatest development in maize, where the floral morphology permits the large amounts of seed required for commercial production of hybrid

varieties to be produced economically. It had been found that male sterility allows maize methods to be extended, with appropriate modifications, to a few other species, and the prospects seem good that male sterility will ultimately allow these methods to be applied to a considerable number of cross-pollinated crops (Hayes and Garber 1919).

Synthetic crops and synthetic rye in particular is the term used to describe cultivars that are produced when selected parents are allowed to cross (open pollinate) among themselves under isolated conditions. Selected parents can be any of the following; clones, inbred families or other genotypes. The genotypic potential as a component of a synthetic cultivar is determined by its general combinig ability (GCA) (Allard, 1960).

There are many crops in which the annual production of first-generation seed is impractical. When this is true, synthetic varieties seem to offer a good opportunity for controlled utilisation of an appreciable amount of heterosis (Allard, 1960).

Hayes and Garber (1919) were the first to suggest the commercial utilisation of synthetic varieties. The suggestion grew out of some results they obtained with maize. They concluded that variety improvements as a result of recombinations of various selfed strains are more beneficial in the sense that farmers can use their own seed which was saved from the previous harvest. This is not possible with seed obtained from single or double crosses.

It is therefore imperative to determine the yielding ability of all F_1 combinations before selfed lines are recombined. Recombinations of selfed lines which offer the best results in combination with all others can then be used (Hayes and Garber, 1919).

The key point of distinction between synthetic varieties and varieties developed by mass selection or line breeding lies in the way the constituent genotypes are chosen (Jenkin, 1931). A synthetic variety is synthesised from genotypes which have been tested, for combining ability. Only genotypes which combine well with each other in all combinations are put into the synthetic variety. This prior testing of hybrid performance distinguishes a synthetic from a variety developed by simple mass selection, in that the latter is made up of

genotypes that are bulked without previous testing of progeny performance or performance in hybrid combination (Jenkins and Sprague, 1943).

This prior testing of hybrid performance also distinguishes synthetic from line-bred varieties in which progenies from superior lines are composited on the basis of the performance of lines tested individually. Thus the goal of testing in the development of synthetic varieties is to identify the genotypes that will combine well when crossed among themselves (Allard, 1960).

Many different procedures can be used to determine the combining ability of different genotypes. These procedures vary from simple visual inspection for highly heritable characters to tests of yield for the ability of one parent to transmit individual traits to an offspring, to the exclusion of the other parent as the primary criterion of selection for complex ones (Jenkins, 1940).

The possible advantages of synthetic varieties in utilising hybrid vigour in cross-pollinating crops, in which floral structure causes difficulties in pollination control, are obvious. These advantages have not been overlooked, especially in Europe, where synthetic varieties are widely used to improve forage crops (Allard, 1960).

On the other hand, the success of hybrid maize varieties tended to suppress interest in other methods of breeding. As a result not much attention has been given to the development of synthetic varieties (Allard, 1960). However, Jenkins and Sprague (1943) noted that synthetic varieties are valuable reservoirs of desired germplasm, and that they might be used for that purpose.

A sharp decline of genetic variance among synthetics was found in studies on rye where the numbers of parents increased (Geiger, 1982). As a result, synthetic rye varieties have not been accepted well by the seed market (Singh and Juahar, 2006).

2.5 The use of marker-assisted selection in plant breeding

2.5.1 Introduction

Three types of genetic markers can be distinguished. These are phenotype markers as first used and described by Gregor Mendel (Agarwal *et al*., 2008), protein markers that are associated with gene products (Weising *et al*., 2005) and DNA markers that are fragments of DNA that exhibit differences in the base pair sequences (Agarwal *et al*., 2008).

Molecular techniques, using DNA markers, have huge potential for plant breeding because the time taken to develop new cultivars with desirable traits can greatly be reduced. It is difficult to analyse polygenic characters when traditional plant breeding methods are used but with molecular markers these are easily tagged (Mohan *et al*., 1997).

Molecular markers have been used to map and tag many agriculturally important genes. This forms the basis of marker-assisted selection (MAS) in crop plants. Molecular markers linked to a trait of interest, which are a prerequisite for MAS, have been developed for a number of crops using a various types of molecular markers. The advantages of molecular markers over traditional phenotypic markers are:

1. It offers more possibility for improving the efficiency of conventional plant breeding because selection is based on molecular markers associated with the trait of interest.

2. The markers are not affected by environmental conditions and can be detected in all stages of plant development (Mohan *et al*., 1997).

2.5.2 Gene mapping

The sequencing and mapping of plant genomes is helpful to understand gene function, gene regulation and gene expression. A map based on genes, such as the large genomes of flowering plants, cannot be detailed because the genes are far apart with large gaps in

between. Only a small amount of the total number of genes is in allelic forms which make it difficult to tell apart (Brown, 2007).

Techniques for assisting selection for desirable characters include molecular markers such as random-amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), sequence-tagged sites (STS) and inter-simple sequence repeat amplification (ISA), amplified fragment length polymorphic DNAs (AFLPs), amplicon length polymorphisms (ALPs) and Microsatellites and PCR-based DNA markers like sequence characterised amplified regions (SCARs), (Brown, 2007), (Mohan *et al*., 1997). It is therefore important to select and use the most efficient molecular markers for a breeding program (Gupta and Varshney, 2000).

2.5.3 Microsatellites

Microsatellites, also called simple tandem repeat (STR) or simple sequence repeats (SSR), are very short repetitive base pairs (up to 6 bp in length) of DNA such as di-, tri- or tetranucleotides. Their function, in the genome, is not clear. It is believed that they are products of genome replication and it show great variability as a result of insertions and deletions that occur, during the replication process (Akkaya *et al*., 1992). Therefore no two individuals, except clones, have the same combination of microsatellite length variants and therefore are highly polymorphic and multiallelic, codominant and chromosome specific (Röder *et al*., 1998). Microsatellites are mainly used in the construction of molecular maps as well as phylogenetic studies to determine kinship and population affinities (Brown, 2007).

Tautz *et al*. (1986) and Litt and Luty (1989) found tandem repeats of 2 to 6 nucleotides richly dispersed throughout the genomes of all studied plant species. Microsatellite characteristics such as co-dominant inheritance, high polymorphism, the convenience of PCR and good reproducibility has made it the genetic markers of choice in the study of plant genomes. A great amount of work has been done to identify and optimise microsatellites in the rye genome (Bolibok *et al*, 2006) and at least 184 *S*. *cereale* microsatellite markers (SCMs) have been developed (Saal and Wricke, 1999; Hackauf and Wehling, 2002).

The advances made with regard to MAS strategies to improve cereal crops has become a useful tool in the hands of plant breeders. By using data obtained from molecular markers, cultivars can be accurately identified. Of more importance to this study is that the degree of genetic diversity among individual plants and plant clones of the same variety can be established (Bitalo, 2012).

2.6 Quantitative inheritance in plant breeding

2.6.1 Introduction

Traits that are simply inherited are controlled by a few genes with major effects on the phenotype. These phenotypes can be classed into a number of easily distinguished or discrete classes. For example, a rye plant may be rust resistant or susceptible. These simply inherited traits are referred to as qualitative inheritance (Griffiths *et al*., 1997; Klug *et al*., 2006).

In natural populations, many of the traits are not inherited in this simple manner. The inheritance of these traits is dependent on many genes at different loci. Each gene contributes a small effect to the phenotypic expression of the character and are said to be quantitative characters or referred to as quantitative inheritance.

Quantitative characters show continuous variation and in statistical terms can be described by averages and variance. Yield is an example of such a trait. When the genotypes are classed into small groups according to yielding ability, the groups tend to fit into the pattern of a normal distribution (bell curve) (Griffiths *et al*., 1997; Klug *et al*., 2006).

Nilsson-Ehle (1908) established the concept of quantitative inheritance. From experiments on the inheritance of seed colour in wheat, the distribution was explained on the basis of two gene pairs, which segregate independently with a dominant allele which contribute to the intensity of the red colour. For example, when a plant with red seeds is crossed with a plant with white seeds, the F_1-plants had intermediate seed colour. In the F_2 generation, resulting

from mating two F_1-plants, the seed colour of the different classes varied from red to white (Griffiths *et al*., 1997; Klug *et al*., 2006).

2.6.2 Features of quantitative inheritance

The inheritance of multiple genes follows the same pattern as that of single genes, but as mentioned, there are characteristic differences in the number of genes involved and the expression of the genes. These differences are:

1. Polygenes having a small effect on the expression of a phenotype to the relative variation. Usually it is impossible to identify individual gene effects.

2. The number of genes at the different loci contributes to the expression of a certain characteristic. Therefore, there are no clear segregation ratios.

3. The individual effects of the genes are cumulative.

4. The phenotypical value of a quantitative trait includes genotype-environment interaction, which causes overlapping of genetic classes.

5. The effect of multiple genes is expressed by different kinds of gene action such as additive effects, dominance, epistasis and over dominance.

6. Transgressive segregation (fig 2.1) where some of the progeny fall outside the range of the parents. This is useful to obtain segregates which are better than the parents for one or more characteristics. When two parents with high yield are crossed, it is possible to select from the F_2-segregants plants which have more positive genes for yield than the individual parents (Griffiths *et al*., 1997; Klug *et al*., 2006).

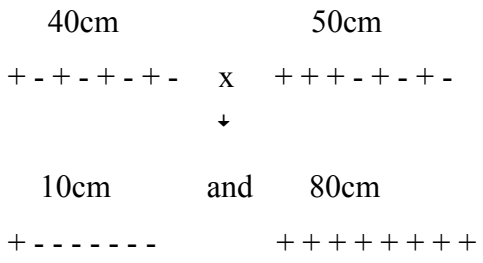

Figure 2.1: Transgressive segregation with plant length as an example and + = 10cm and - = 0cm

The numerical values of a quantitative phenotypic character like yield are continuous and are studied by using statistical procedures. Statistics commonly used are the range, mean, variance, standard deviation, standard error of the mean, covariance and correlation coefficient. The mean and variance are considered to be most important. The mean is important to compare different populations, while the variance can be used to calculate the heritability of a trait. A small variation and standard deviation imply that individual values are concentrated in a narrow distribution around the mean (Griffiths *et al*., 1997; Klug *et al*., 2006).

When the variability of two populations are compared and the population with the larger average has larger variances, the correlation coefficient can be used to compare the variability of the different populations (Griffiths *et al*., 1997; Klug *et al*., 2006).

The many genes that determine the phenotypic value of a quantitative trait may be groups of multiple alleles at a single locus, or sets of genes at different loci. Therefore the number of allelic combinations in a population is determined by different alleles, which are crossed in the population. Thus, with three alleles (A_1 A_2 A_3) six combinations (A_1 A_1, A_1 A_2, A_1 A_3, A_2 A_2, A_2 A_3, A_3 A_3) are possible and with n alleles n(n+1)/2 combinations are possible (Griffiths *et al*., 1997).

These gene combinations may affect the phenotypic expression of a quantitative trait in various ways, namely:

1. Additive genes all make a small but equal contribution to the expression of a trait. For example: aabb = 0, Aabb = 1, AAbb = 2, AABb = 3, AABB = 4.

2. Dominance effects refer to deviations from the additive value so that the heterozygote resembles to one parent more than the other. For example: aa = 0, Aa = 2, AA = 2.

3. Epistasis is the result of nonallelic gene interaction i.e. the interaction of genes at different loci. Two genes may have no effect individually, yet have an effect when combined. For example: AAbb = 0, aaBB = 0, A-B- = 4.

4. Over dominance occurs when each allele contributes a separate effect and the combined alleles contribute an effect greater than that of the either allele separately. If the effect of each allele is one then aa = 0, AA = 1, and Aa = 2 (Griffiths *et al*., 1997).

2.6.3 Heritability estimates

Heritability refers to the degree to which the variability of a quantitative trait is transferred from the parents to the progeny or the proportion of the total variability transferred to the progeny. Therefore, it is the portion of total phenotypic variation due to genetic factors (Griffiths *et al*., 1997; Klug *et al*., 2006).

Woltereck (1909), showed that the expression of traits that are influenced by the environment may also be inherited. The phenotypic expression of a trait is a product of both its genotype and the environment. Nilsson-Ehle (1909), reconciled continuous variation and Mendelian inheritance with his work on kernel colour in wheat and Fisher (1918), formulated the mathematical theory of quantitative genetics (Klug *et al*., 2006).

When estimating heritability, phenotypic variance (V_P) is partitioned into genotypic (V_G) and environmental (V_E) components.

Therefore:

$$V_P = V_G + V_E$$

(Equation 2.1)

A high heritability estimate for a multifactorial trait is an indication of the part of phenotypic variation that can be credited to genetic variation within a given population in a specific environment (Griffiths *et al*., 1997; Klug *et al*., 2006).

Plant breeders use different techniques to establish heritability. One approach is the use of inbred lines which contain genetically homogenous individuals with highly homozygous genotypes. Variation between different inbred lines grown in a constant environment can mostly be contributed to genetic factors; and where members of the same inbred lines are grown under different environments, variation will probably be due to environmental factors (Klug *et al*., 2006).

A diallel approach was followed in this study where variance for quantitative traits among the progeny from different crosses were analysed and compared among progeny and parents grown in the same environment.

Genetic variance is composed of additive genetic variance (V_A), dominance variance (V_D) and non-allelic interaction, epistasis (V_I) and is written as:

$$V_G = V_A + V_D + V_I$$

(Equation 2.2)

The additive component of genetic variance is the variance which contributes to genes with a linear effect. The similarity between parents and progeny is largely due to additive genetic effects which is also responsible for the response to selection. The dominance component represents the deviation of the heterozygote from the average of the parents and the interaction deviation is the result of epistasis (Griffiths *et al*., 1997; Klug *et al*., 2006).

2.6.4 Quantifying heritability

A distinction is made between broad-sense heritability (H^2) and narrow-sense (h^2) heritability.

The degree of heritability off a trait can be quantified once it is shown to have heritability. Phenotypic variation in a population arises from variation between genotypic variance and environmental variance. The degree of broad-sense variance of the character is defined as the proportion of the total variance that can be attributed to genetic variance:

$$H^2 = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_E}$$

(Equation 2.3)

This degree of genetic influence quantifies what proportion of the population's variation in phenotype can be assigned to variation in genotype and makes no distinction between additive-, dominance- and epistatic effects. It is inclusive of all types of genetic variation in a population (Griffiths *et al*., 1997; Klug *et al*., 2006).

The trait values range from 0.0 to 1.0. The higher the trait value, the lower the environmental impact on phenotypic variance and the higher the impact of genotypic differences among individuals in a population and *vice versa* (Klug *et al*., 2006).

Although H^2 is widely used as a means to determine the importance of genes in influencing a trait, its meaning is limited. H^2 does not take the genotype-by-environment variance into account (Griffiths *et al*., 1997; Klug *et al*., 2006).

Two conclusions can be drawn from H^2 studies:

1. When populations are measured in the environments in which they have developed and the H^2 values are higher than zero, genetic differences can be attributed to a trait and the variation between individuals was influenced by genetic differences.

2. The H^2 value only gives a limited prediction of the effect of environmental modification under specific conditions. Thus, the H^2 is an estimation of phenotypic variation, attributed to genetic function, still present when all significant environmental variation is excluded and the new constant environment is similar to the mean environment in the initial population (Griffiths *et al*., 1997; Klug *et al*., 2006).

A more precise, widely used, method for the estimation of traits that needs to be manipulated in a population is h^2, where genetic and environmental variation is further subdivided:

$$V_G = V_A + V_D + V_I$$

(Equation 2.4)

This is done to make available more information on gene action and the possibility of shaping the genetic composition of a population. It is defined as:

$$h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_E + V_A + V_D + V_I}$$

(Equation 2.5)

In practise, V_I cannot accurately be separated from V_D and is omitted, therefore:

$$h^2 = \frac{V_A}{V_E + V_A + V_D}$$

(Equation 2.6)

and h^2 is the portion of phenotypic variance due only to additive genotypic variance.

Although it is possible to subdivide V_E, such studies of variation are applicable only to a particular population in a given distribution of environments (Griffiths *et al*., 1997).

For any breeder it is important to be able to predict the expected genetic progress on selection from parent to progeny and can be defined as:

$$h^2 = \frac{selection\ response}{selection\ differential} = \frac{R}{S}$$

(Equation 2.7)

Selection differential (S) is the difference of the base population mean and the mean of the selected parents:

$$S = i\,\sigma_p$$

(Equation 2.8)

where i = selection intensity and σ_p = phenotypical standard deviation.

The value of (i) is determined by the proportion of selected plants. The value of (i) is the reciprocal of the percentage of selected plants. Therefore the more plants selected to make a contribution to the next generation, the smaller the value of (i) and *vice versa* (Griffiths *et al*., 1997). The σ_p is a function of the segregating loci in the population and environment. If the σ_p is large, it reflects a large component of genetic- and environmental variations present (Griffiths *et al*., 1997).

For this study, rye individuals were selected from a synthetic heterogeneous population. These individuals were planted, cloned, crossed in a diallel mating scheme and the off spring measured and evaluated in the same environment and then compared against their parents.

2.7 The diallel mating scheme

Schmidt (1919) was the first to use the term diallel to describe the factorial design where two females are paired with two males in all possible combinations.
Breeders use diallel schemes to study the genetic basis of quantitative traits (Hallauer and Filho, 1988). Plant breeders want to determine the combining ability of various lines, clones or varieties in order to select the best combinations that can be used in a breeding program.

According to Bos and Caligan (1995), diallel crosses are made for the following reasons:

1. To envisage the performance of a three way-cross hybrid (TC) or a double-cross hybrid (DC) of a cross-pollinating crop. This application is used by plant breeders to develop hybrid varieties.

2. To determine the GCA and / or the SCA of pure lines. This application is frequently used by breeders at research stations as a method for developing new cultivars.

3. To analyse the genetic control of quantitative variation for a trait. This application is seldom directly connected with the development of a new variety.

4. The advantage of applying the diallel cross is that it offers an overall picture of genetic control of a character in the lines used but still keeping the work down to convenient levels (Gilbert, 1958; Jinks, 1954).

A full diallel mating scheme requires that all the parents are crossed in all possible pairwise combinations to produce hybrids in all possible combinations. Variations of the full diallel may include partial diallels with parents or without parents. A full diallel requires twice as many crosses and entries in experiments, but both maternal and paternal effects are tested for (Crusio, 1987). When reciprocal effects are assumed to be minor a half diallel without reciprocals can be done.

The genotypes involved are designated as P_1, P_2, …, P_N. A diallel cross is complete when it yields N^2 progenies thus; NS_1-lines owed to self-fertilisation and N^2-N FS-families as a result of pair wise crosses. Where selfings are disregarded and no reciprocal crosses are made, a total of ½ N (N-1) FS-families are obtained. The progeny is designated as F_{ij}, and i refers to the maternal parent P_i, j refers to the paternal parent P_j, and i,j =1, … , N (Bos and Caligan, 1995).

Each progeny may be represented by either a single plant or a number of plants that were cultivated as individual randomised plants, or as J plots that each contains K plants. The interpretation of quantitative genetic observations that characterise F_{ij} may vary from the phenotypic value of only one plant, to an exact estimation of the genotypic value. Therefore, the observation is designated by the general symbol $x_{ij.}$ Table 2.1 summarises the observations derived from all off-spring resulting from a complete diallel mating scheme (Bos and Caligan 1995).

Table 2.1: The observation x_{ij} is characteristic of progeny F_{ij} which are obtained from a diallel cross involving pure lines $P_1, \ldots, P_N$, $i,j = 1, \ldots, N$

		Paternal parent			
		P_1	...	P_j	... P_N
Maternal parent	P_1	x_{11}	...	x_{1j}	... x_{1N}
	.	.		.	.
	P_i	x_{i1}	...	x_{ij}	... x_{iN}
	.	.		.	.
	P_N	x_{N1}	...	x_{Nj}	... x_{NN}

A HS-family, designated by F_i and F_j respectively, is formed by the set of progenies involved in row i, e.g. $\{F_{i1}, \ldots, F_{iN}\}$, or set of progenies involved in column j,i. e.g. $\{F_{1j}, \ldots, F_{Nj}\}$. Observations from the off-spring of the same paternal or maternal parents are respectively shown in a row or column. The average through row i, say $\bar{x}_{i}$, or through column j, say $\bar{x}_{j}$, represents the mean across the entries constituting HS-family F_i or F_{j}., respectively (Bos and Caligan, 1995).

Where the total number of ½ N (N-1) progenies are too great to manage effectively, or when breeders find it impossible to produce them all due to, for example, asynchronous flowering (poor nicking), a partial diallel cross may be studied. In a partial diallel cross, progenies may be included that were obtained from crosses made according to a scheme for a balanced, incomplete block design, or of progenies obtained as a 'wild' scheme (Bos and Caligan, 1995).

2.8 Griffing diallel analysis procedures

There are various ways of analysing diallels that was developed over the years by Gardner and Eberhart (1966), Jinks (1954), Hayman (1954) and Griffing (1956) to mention a few. The diallel cross provides a way of obtaining an overall picture of the general control of a

character in a number of inbred lines while the amount of work is kept down to a level that is manageable (Jinks, 1954; Gilbert, 1958) .

Griffing (1956) developed four methods to determine GCA and SCA for the analyses of diallel-cross data. The method to be selected will depend on whether the parental inbreds or reciprocal F_1's are included or not.

Method 1: parents (p), one set of F_1's [p(p-1)/2, **and** reciprocal F_1's [p(p -1)/2]; a total of p^2 combinations.

Method 2: parents (p) and one set of F_1's [p(p -1)/2; **no** reciprocal F_1's; a total of p(p +1)/2 combinations.

Method 3: one set of F_1's [p(p -1)/2] **and** reciprocal F_1's [p(p -1)/2]; **no** parents; a total of p(p -1) combinations.

Method 4: One set of F_1's [p(p -1)/2 only; **no** parents and **no** reciprocal F_1's.

For each method, a different form of analysis is applied. Different sampling assumptions give rise to different estimation problems regarding combining ability effects. In situations where (1) parent lines are randomly sampled from a population, or (2) where lines are chosen for specific phenotypic traits, the assumptions are expressed differently. In the second case, the lines cannot be regarded as representative of the entire population thus; no valid interpretations can be made (Griffing, 1956).

For the plant breeder, it is important to know if a pure line has a good GCA with regard to a tester population and if or not two pure lines possesses good SCA. It is therefore clear that the interest, when analysing the GCA and SCA, is in the parents and not their off-spring. In this respect a diallel cross analysis is a unique type of progeny testing (Bos and Caligan, 1995).

Sprague and Tatum (1942) defined the terms GCA and SCA as follows: "'General combining ability' is used to designate the average performance of a line in hybrid combinations… The term 'specific combining ability' is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of average performance of the lines involved".

Analysing methods commonly utilise general linear models to detect heterotic groups (Griffing, 1956), estimate GCA (Gardner and Eberhart, 1966) and SCA (Gardner and Eberhart, 1966), determine interactions with testing environments and to estimates additive, dominant, and epistatic genetic effects (Sprague and Tatum, 1942; Hayman, 1954) and genetic correlations (Crusio, 1993).

Situations where parent lines were randomly selected from a population, and where deliberate parent selections were made, should be clearly distinguished. The two situations give rise to different estimation problems with regard to combining ability effects (Griffing, 1956). In the first scenario the genotypic effects are considered to be random variables where in the second case they are seen to be constants (Dey, 2002).

The progeny of the crosses can either be planted in random- or constant block designs. The randomised-block design is commonly used for this type of study. Such a design contains a varieties, each assigned at random to each of b blocks with c individuals in the ab plots (Griffing, 1956). The mathematical formula for the $ijkl$th observation is expressed as:

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

(Equation 2.9)

where u = population mean effect, v_{ij} is the effect for the ijth genotype, b_k is the kth block effect, $(bv)_{ijk}$ is the interaction between the ijth genotype and the kth block, and e_{ijkl} is the environmental effect atypical to the $ijkl$th individual (Griffing, 1956).

Double subscript notation is used for the variety effect. The genotypic means in the combining ability analyses is indicated as x_{ij} , where x_{ii} is the mean for the ith parent, an x_{ij} is the mean for the F_1 following from crossing the ith and jth parents. In the combining ability

analyses for methods in which reciprocal F_1's are included, the variety effects are expressed in terms of GCA and SCA ability effects as:

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

(Equation 2.10)

where g_i and g_j is the GCA effect of the parents, s_{ij} is the SCA effect for the cross between the *i*th and *j*th parents and r_{ij} the reciprocal effect between the *i*th and *j*th parents (Griffing, 1956).

The correct analysis of the combining ability effects and variance depends on the particular diallel method applied, the assumptions regarding the experimental material, and the conditions imposed on the combining ability effects. Four sets of assumptions are considered with regard to the variety and block effects and are summarised as follow:

1. The variety and block effects are constant. (model I)

2. The variety effects are random variables and the block effects are constants. (model II) or (mixed A)

3. The variety effects are constants and the block effects are random variables. (model III) or (mixed B)

4. The variety and block effects are both random variables (Griffing 1956). (model IV)

From assumption 1, a model (model I) is presented in which all effects, excluding the error, are regarded as constants. The last set, assumption 4, leads to a second model, (model IV) where all effects except *u* (population mean effect) are random variables. Assumptions 2 and 3 lead to mixed models which are designated as mixed A and mixed B (Eisenhart, 1947).

The objectives in model I are to compare combining abilities of the parents where the parents are used as testers and to identify higher yield combinations. Thus, the experimental material is to be regarded as the population about which inferences are to be made (Griffing

1956). The importance is in estimating combining ability effects and calculating standard errors for differences between effects. For testing procedures, the assumption is that the e_{ijkl} are normally and independently distributed with mean zero and variance $\sigma_e^{\ 2}$ (Griffing, 1956). The mathematical formula for combining ability analysis is:

$$x_{ij} = u + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{bc_{kl}} \Sigma\Sigma e_{ijkl}$$

(Equation 2.11)

where u = population mean; g_i (g_j) = GCA for the ith (jth) parents and $s_{ij} = s_{ji}$; r_{ij} = reciprocal effect involving the reciprocal crosses between the ith and jth parents and $r_{ij} = r_{ji}$; e_{ijkl} = environmental effect associated with the $ijkl$th individual observation (Griffing, 1956).

Model IV deals with random samples from a parent population in order to make assumptions about the parameters in the parent population and not individual lines. Thus, the importance is in estimating the genetic and environmental components of the population variance. The assumption is that the effects in this model are normally and independently distributed with means = zero and variances σ_θ^2 where θ = b, g, s or r. Component estimations for variance are obtained for any given diallel crossing method by equating the observed to the expected mean squares in the appropriate analysis of variance. Standard errors for variance component estimates are then calculated from the variances of the appropriate mean squares (Griffing, 1956). The mathematical formula for combining ability analysis is:

$$x_{ij} = u + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{b_k} \Sigma b_k + \frac{1}{b_k} \Sigma(bv)_{ijk} + \frac{1}{bc_{kl}} \Sigma\Sigma e_{ijkl}$$

(Equation 2.12)

where all except u are considered random variables (Griffing, 1956).

Interpretation of combining ability effects and variance depends on the diallel method used, assumptions regarding the experimental material, as well as the conditions imposed on the combining ability effects (Griffing, 1956). Thus, where model I is used; the equation for calculating combining ability depends on the applicable diallel method.

When using model IV, valid inferences will depend on the specific diallel crossing method applied as well as the nature of the population from which the lines were drawn (Griffing, 1956).

Mixed model A can be used for all four diallel crossing methods. For the methods that exclude reciprocal F_1's the mathematical formula for calculating combining ability is:

$$x_{ij} = u + g_i + g_j + s_{ij} + \frac{1}{bc_{kl}} \Sigma\Sigma e_{ijkl}$$

(Equation 2.13)

For those diallel methods including the reciprocal F_1's the formula is as follows:

$$x_{ij} = u + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{bc_{kl}} \Sigma\Sigma e_{ijkl}$$

(Equation 2.14)

In both cases, all except *u* are considered random variables (Griffing, 1956).

Mixed model B is used when the 'mixed' elements $(bv)_{ijk}$ are introduced into the calculation of combining ability. For the methods that exclude reciprocal F_1's the mathematical formula for calculating combining ability is:

$$x_{ij} = u + g_i + g_j + s_{ij} + \frac{1}{b_k} \Sigma b_k + \frac{1}{b_k} \Sigma(bv)_{ijk} + \frac{1}{bc_{kl}} \Sigma\Sigma e_{ijkl}$$

(Equation 2.15)

and for those diallel methods including the reciprocal F_1's the formula is as follows (Griffing, 1956):

$$x_{ij} = u + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{b_k} \Sigma b_k + \frac{1}{b_k} \Sigma(bv)_{ijk} + \frac{1}{bc_{kl}} \Sigma\Sigma e_{ijkl}.$$

(Equation 2.16)

In the recent past, diallel studies have routinely been performed on a number of crops including maize (Malik *et al*., 2004), wheat (Ahmad *et al*., 2006), rice (Ahangar *et al*., 2008) and rye (Goncharenko *et al*., 2013) to mention a few.

Goncharenko *et al*. (2013) analysed grain quality traits in inbred winter rye lines in a full diallel design. Five inbred lines were selected to determine their combining ability and genetic characteristics for the following traits: grain test weight, water extraction viscosity, falling number, protein content, hearth bread form ration and pan loaf volume. The parent lines, as well as their F_1 hybrids, were found to differ greatly with regard to quality traits. This enables them to identify lines with high GCA estimates for traits like high falling number and higher water extract viscosity and to calculate combining ability on the basis of the value of quality traits.

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Chapter 3: Initiation of molecular marker selection of rye

Initiation of molecular marker selection of rye

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Abstract

Variance at molecular level between selected inbred parent lines were determined by using seven (7) polymorphic simple sequence repeat (SSR) markers. Seed, phenotypically selected, from eight (8) parent lines from a synthetic open pollinated population were grown. DNA was extracted from the young leaves of each line to establish kinship and variance. A polymorphic information content (PIC) value was culculated for each marker to determine the level of diversity among the breeding material. From the data a cladogram was created to plot kinship and variance. Plants showing the greatest variance were cloned to be used in a full diallel mating scheme.

3.1 Introduction

Genetic markers can be described as representations of the genetic differences between species, individuals of the same species or between siblings (Collard *et al*. 2005). Three types of genetic markers can be distinguished:

1. Phenotype markers as first used and described by Gregor Mendel (Agarwal *et al*., 2008),

2. Protein markers that are associated with gene products (Weising *et al*., 2005) and

3. DNA markers that are fragments of DNA that exhibits differences in the base pair sequences (Agarwal *et al*., 2008).

3.1.1 The use of molecular marker-assisted selection (MAS) in plant breeding

Molecular techniques, using DNA markers, have huge potential for plant breeding because the time taken to develop new cultivars with desirable traits can greatly be reduced. It is difficult to analyse polygenic characters when traditional plant breeding methods are used. This is due to the greater influence of the environment and developmental stage of the plant on the phenotype. With molecular markers, genes affecting phenotype are easily tagged and the environmental influences as well as the developmental stage of the crop is eliminated (Mohan *et al*., 1997).

Molecular markers have been used to map and tag many agriculturally important genes. This forms the basis of marker-assisted selection (MAS) in crop plants and include molecular markers linked to a trait of interest, which are a prerequisite for MAS. Markers have been developed for a number of crops using various types of molecular markers.

A number of crops where MAS is used extensively includes maize to improve yield, wheat to improve disease resistance, barley to improve yellow mosaic and rust resistance, rye in triticale breeding programs and soybean to improve resistance to soybean cyst nematodes to

mention a few (Brumlop and Finckh, 2011). The advantages of molecular markers over traditional phenotypic markers are:

1. It offers more possibility for improving the efficiency of conventional plant breeding because selection is based on molecular markers associated with the trait of interest.

2. The markers are not affected by environmental conditions and can be detected in all stages of the plants development (Mohan *et al*., 1997).

Molecular markers are also used to determine diversity or variation and kinship between selected plants. Therefore, in this study seven molecular markers was used to select parent lines that exhibit the greatest variation for use it in a diallel mating scheme.

3.1.2 Gene mapping

Techniques for assisting selection for desirable characters include molecular markers such as random-amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), sequence-tagged sites (STS) and inter-simple sequence repeat amplification (ISA), amplified fragment length polymorphic DNAs (AFLPs), amplicon length polymorphisms (ALPs) and Microsatellites and PCR-based DNA markers like sequence characterised amplified regions (SCARs), (Brown, 2007; Mohan *et al*., 1997). It is therefore important to select and use the most efficient molecular markers for a breeding program (Gupta and Varshney, 2000).

3.1.3 Microsatellites

Microsatellites, also called simple tandem repeat (STR) or simple sequence repeats (SSR), are very short repetitive base pairs (up to 6 bp in length) of DNA such as di-, tri- or tetranucleotides. Their function, in the genome, is not clear. It is believed that they are products of genome replication (Akkaya *et al*., 1992). They show great variability as a result of indels, during replication. Therefore no two individuals, except clones, have the same combination of microsatellite length variants and therefore are highly polymorphic and

multiallelic, codominant and mostly chromosome specific (Röder *et al*., 1998). Microsatellites are mainly used in the construction of molecular maps as well as phylogenetic studies to determine kinship and population affinities (Brown 2007).

Tautz *et al*. (1986) and Litt and Luty (1989) found tandem repeats of 2 to 6 nucleotides richly dispersed throughout the genomes of all studied plant species. Microsatellite characteristics such as co-dominant inheritance, high polymorphism, the convenience of PCR and good reproducibility has made it the genetic markers of choice in the study of plant genomes. A great amount of work has been done to identify and optimise microsatellites in the rye genome (Bolibok, 2006) and at least 184 *S*. *cereale* microsatellite markers (SCMs) have been developed (Saal and Wricke, 1999; Hackauf and Wehling, 2002).

The advances made with regard to MAS strategies to improve cereal crops has become a useful tool in the hands of plant breeders. By using data obtained from molecular markers, cultivars can be accurately identified. Of more importance to this study is that the degree of genetic diversity among individual plants and plant clones of the same variety can be established (Botes, 2013). Therefore, the following two objectives were set to determine variance and kinship of parent lines:

1. Parent material was phenotypically selected from an existing synthetic open pollinated rye breeding population.
2. Variation was determined between selected plants and their clones on a molecular level.

3.2.1 Selection of parent material

In the years preceding the study a number of inbredlines were established by the SU-PBL rye breeder (personal communication WC Botes). These lines were planted at Mariendal Experimental Station (MES) (GPS co-ordinates: 33°50'59.19"S; 18°49'31.20"E) and starting material was selected from it. One hundred individual plants were phenotypically selected. The seed was harvested and cleaned and from these selected plants a further phenotypic

selection of eight parent lines was made based on variation with regard to seed size and seed mass. Seed mass is expressed as a thousand kernel weight.

Seed size and seed mass are seen as indicators of quality with regard to higher germination rates, better plant vigour, better yields and higher gradings (Ambika *et al*. 2014; Khan *et al* 2014).

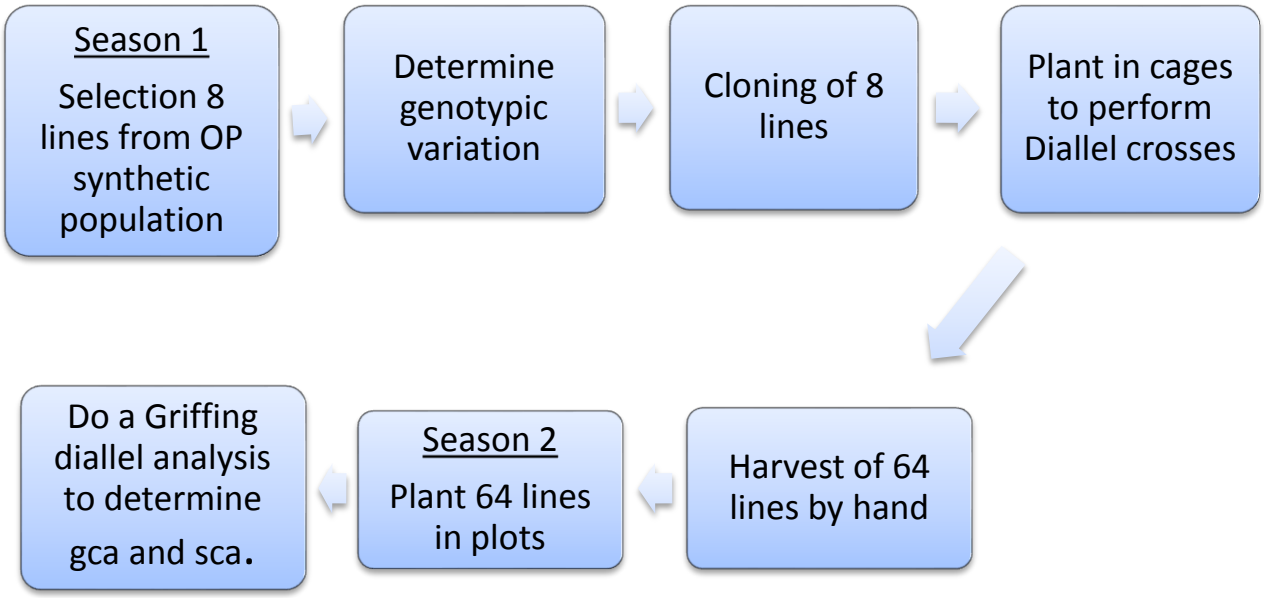

Figure 3.1: Flow diagram of study

3.2.2 Determination of variation on a molecular level

In order to determine variation between the selected plants and their clones on a molecular level, five to six seeds from each of the eight selected lines, as well as seed from "Duiker" and "Henoch", were planted in seed trays. The seedlings were transplanted into planting pots with a capacity of 1000 mℓ and kept in the greenhouse to obtain a minimum of three plants per line. "Duiker" and "Henoch" are two established cultivars and is still widely planted in the Western Cape.

Three plants per line were randomly selected and designated e.g. 1A, 1B, 1C and summerised below in table 3.1. Genomic DNA was extracted (see 3.2.3.) from young leaves

of the three plants from each of the eight selected parent lines to establish kinship and variance. The plant showing the greatest variance from each line of three was cloned by breaking of young tillers at the base of the mother plant.

Table 3.1: Table of plant material

Parent lines	"Duiker"	"Henoch"	1	2	3	4	5	6	7	8
Three plants per line	DA	HA	1A	2A	3A	4A	5A	6A	7A	8A
	DB	HB	1B	2B	3B	4B	5B	6B	7B	8B
	DC	HC	1C	2C	3C	4C	5C	6C	7C	8C

Eight clones from each selected parent line were made, planted in 1000 mℓ planting pots and maintained for the duration of the study. Sterile, coarse river sand was used as growth medium and the following nutrient mixture was used to fertigate the clones:

164 g Sol-u-fert (Kynoch Fertilizers (Edms) Bpk, Milnerton, RSA), 2 g Microplex (Ocean Agriculture (Edms) Bpk, Muldersdrift, RSA) and 77 mℓ Calsuim nitrate in 100 ℓ water.

Duett (BASF, Halfway House 1685) (Epoxiconazole – triazole and Carbendazim – benzimidazole 125g/ℓ) dosage 16mℓ/5ℓ water for the control of foliar, fungal disease and Mospilan (Nulandis, Jan Celliers Rd, Stellenbosch, 7600) (Acetamiprid – acetamidine 200g/kg) dosage 1.25 mℓ/5ℓ water for aphids control in the green house were applied.

Table 3.2: Fertiliser composition used to grow and maintain parent lines

Kynoch Sol-u-fert: 164g/100ℓ H_2O		Microplex: 2g/100ℓ H_2O		Calsium nitrate: 77mℓ/100mℓ H_2O	
N	$57g.kg^{-1}$	Fe	1.68 p.p.m	Ca	$180g.ℓ^{-1}$
K	$27g.kg^{-1}$	Mn	0.04 p.p.m	N	$125g.ℓ^{-1}$
P	$25g.kg^{-1}$	Zn	0.2 p.p.m		
Mg	$30g.kg^{-1}$	Cu	0.03 p.p.m		
S	$40g.kg^{-1}$	B	0.5 p.p.m		
		Mo	0.05 p.p.m		

3.2.3 DNA extraction and PCR protocols

A modified DNA protocol as described by Doyle and Doyle, 1990 was used to extract and determine genotypic variance:

Approximately 0.1g young leaf tissue was cut in small pieces and placed in 2.2 ml microcentrifuge tubes with three steel bearings in each tube. A 800 µl of 2% (w/v) CTAB [5M NaCl, 0.5M EDTA (pH 8), 1M Tris-Cl (pH 8) and 1.6 µl 0.2 % (v/v) ß-Mercaptoethanol (ß-ME) were added and preheated to 60°C and put in a Qiagen Tissuelizer (company) for three cycles of 90 seconds at 30 Hz each. The mixtures were incubated at 60°C for 60 minutes.

A 800 µl Chloroform: Isoamyl (C:I) mixture made up to 24:1 ratio was added and centrifuged at 12 000 rpm for 10 minutes. The supernatant was then transferred to clean 1.5 ml tubes. 0.5 volume Phenol and 0.5 volume C:I were added and centrifuge at 12 000 rpm for 5 minutes. The supernatant was again transferred to clean 1.5 ml tubes, 1 volume C:I added and centrifuged at 12 000 rpm for 5 minutes. The supernatant was tranfered for a third time and 1 volume Isopropanol added and left to incubate overnight at -20°C.

After incubation the samples were centrifuged at 12 000 rpm for 10 minutes at 4°C. The supernatant was carefully discarded, 0.5 ml of 70% (v/v) cold Ethanol added and centrifuge for 10 minutes at 12 000 rpm at 4°C. The supernatant was again discarded and the pellets left to air-dry. The dry pellets were then dissolved in 50 µl TE [1M Tris-Cl, 0.5M EDTA (pH 8)] containing 40 µg/ml RNase A and incubated for 30 minutes at 37°C. About one tenth volume (5µl) soduim acetate (pH 5) and 2.5 volume (110µl) 100% cold Ethanol were centrifuged at 12 000 rpm for 10 minutes at 4°C.

The supernatant was discarded, 1 ml of 70% Ethanol added and centrifuged for 5 minutes at 12 000 rpm. This was repeated once, where after the supernatant was discarded and the pellets left to air-dry. The air-dry pellets were then dissolved in 20-50 µl dH_2O (SABEX) water.

Quantification of the extracted DNA was done according to the NanoDrop® ND-1000 Spectrophotometer Thermo SCIENTIFIC (Wilmington, Delaware USA) user's guide. Samples were diluted to 100 ng/ µl and stored at 4°C. The stock samples were stored at -20°C.

The PCRs were done in a Thermo Cycler 2720 (Applied Biosystems: Bio-Rad Laboratories Ltd. 34 Bolton Ave, Rosebank Johannesburg South Africa) under conditions described by Röder *et al*, (1998). Seven selected SCMs (Table 3.3) designed for the rye genome and described by Saal and Wrickle (1999) and Hackauf and Wehling (2002) and optimised by Botes and Bitalo (2013), were used. These SCMs were selected based on PIC and gene diversity values. Calculations, to indicate the ability of each marker to detect polymorphisms, were done using PowerMarker v3.25 (Liu and Muse, 2005). The final reaction volume was 20 µl and it contained about 300 ng template DNA, 0.5 µM of each primer, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 1.5 mM $MgCl_2$, one unit of BIOTAQ^{TM} (rTaq) DNA polymerase (Bioline) and 1 x PCR NH_4 buffer (Bioline [16mM $(NH_4)2SO_4$, 67 mM Tris-HCl (pH 8.8) at 25°C, 0.01% (v/v) Tween-20]. Conditions for thermal cycling were set at 1 cycle for 3 minutes at 94°C, 45 cycles for 1 minute each at 94°C, 1 cycle annealing temperature for 1 minute at 72°C and one cycle for 10 minutes at 72°C.

Table 3.3: SSR marker sequences (R genome), annealing temperature and repeat length

Marker Name	Forward / Reveres sequence (5'-3')	T_A (°C)	Repeat
SCM2-6RL	GATGACTATGACTACCAGGATGAA	55	$(GT)_{10}$
SCM9-1RS	TGACAACCCCCTTTCCCTCGT	60	$(GT)_8$
SCM40-7R	CCCTTCAGCGGTCATTGTTG	60	$(GT)_{18}$
SCM152(F1)-4R	TAAAACGACGGCCAGTGACGA	68	$(AG)_7$
SCM152(F2)-4R	ACGGCCAGTGGAGCAGCAGCAG	68	$(AG)_7$
SCM159(F1)-4R	AAGAGCCAGTTTGGACTTGGAG	68	$(GAAA)_5$
SCM159(F2)- 4R	CGGCCAGTGGTTCCTTGGAT	68	$(GAAA)_5$

3.2.4 DNA analysis

The products of the PCR process were visualized employing a 6% (w/v) denatured polyacrylamide gel (acrylamide:bisacrylamide 19:1). A 40% acrylamide stock solution was first made up by adding 76g Acrylamide and 4g Bis-acrylamide in a 250ml Schott flask and filled to 200ml with dH_2O. The flask was totally covered with aluminium foil and stored at 4°C for up to two weeks.

The 6% sequencing gel mix (6M urea, 1 x TBE buffer [Tris-HCl, EDTA, Boric acid]) was prepared by adding 75ml 40% stock solution, 180g urea and 100ml 5 x TBE in a 500ml Schott flask and made up to 500ml with dH_2O. The flask was covered with aluminium and stored at 4°C for up to 1 week. The gel was prepared by adding 800µl 10% ammonium persulphate (APS) and 160µl TEMED to 160 ml of 6% gel mix in a glass beaker. The 10% APS solution was prepared by dissolving 0.1g APS in 1ml dH_2O in a 2.2ml micro tube. The gel mix was then casted with great care to prevent any air bubbles from forming between the glass plates and left for 1 hour to set. A pre-run of the gel was done at 70W for 30 minutes.

The amplification samples were prepared for loading by adding equal volumes of loading buffer (98% formamide, 10mM EDTA pH8, 0.05% w/v bromo phenol blue, 0.05% w/v xylene cyanol FF) to each of the PCR samples. The samples were denaturated at 95°C for 5 minutes and immediately quenched on ice. A 12-15µl of each sample and 1µl 50-100bp

ladder (Promega, Madison, Wisconsin, USA) were loaded and separated by electrophoresis at a constant power of 70W for 4 hours in a 1 x TBE buffer.

Band fragments were visualised by silver staining according to the following protocol described by Tixier *et al*. (1997); The gels were bathed in freshly prepared fixing solution (10% (v/v) ethanol, 0.5% (v/v) acetic acid) for 20 minutes on a belly dancer and then rinsed twice for 5 minutes per rinse in 2 l dH_2O. It was then stained in staining solution (1% (w/v) $AgNO_3$) for 20 minutes on the belly dancer and rinsed for 10 seconds in dH_2O. It was again placed back on the belly dancer and in developing solution (1.5% (w/v) NaOH , 0.16% (v/v) formaldehyde) until the bands appear. Gels were rinsed in dH_2O.

The gels were covered with plastic film, placed on a light table and photographed using a Kodak (Easy Share LS753) camera.

3.2.5 Data analysis

The visualised bands were scored as alleles, and their sizes determined by making use of a 50-100bp ladder (Promega, Madison, Wisconsin, USA). The frequency based distances between parent lines were determined by the scored band sizes using the CS Chord (Cavalli-Sforza and Edwards, 1967) method. Major Allele Frequency (MAF), Gene Diversity (GD) and Polymorphic Information Content (PIC) was calculated and analised by using Power Marker V3.25 (Liu and Muse, 2005) (Table 3.4 and Figure 3.2).

The ability of each of the markers used in this study to detect polymorphisms was determined by calculating each one's discriminatory power. The capacity of the markers, GD and the alternative PIC, were determined by using the software PowerMarker v3.25 (Weir 1996).

MAF is defined as the frequency at which the most common allele occurs in a given population, and expressed as a fraction of all the alleles in a gene pool at a specific locus (Berg and Hamrick 1997).

GD is applied as a way to measure the possible heterozygosity of gene copies in a sample collected at a particular locus and is used to characterise molecular diversity patterns (Berg and Hamrick 1997).

PIC values are used to quantify the informativeness of a marker or to detect polymorphism in a population. This depends on the number of alleles observed and is calculated by multiplying the frequency of mating types by the fractions that are expected of informative offspring:

$$\text{PIC} = 1 - \sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2P_i^2 P_j^2 \qquad \text{(Equation 3.1)}$$

Where P_i and P_j represent the population frequency of the *i*th and *j*th allele. The greater the number of alleles involved, the higher the PIC values will be (Sándor *et al*. 2012).

3.3 Results and discussion

For the initial selection of the eight parent lines, the lines with the biggest and heaviest seeds were selected. Seed were visually evaluated for size and weight to determine a thousand kernel mass. This was done, based on the assumption that bigger and heavier seed germinate better and the plants growing from such seed are more vigorous (Ambika *et al*. 2014; Khan *et al* 2014).

The second selection was made after DNA was extracted from all potential parents. The quantification of the extracted genomic DNA was done according to the NanoDrop® ND-1000 Spectrophotometer protocol as prescribed by the Thermo SCIENTIFIC user's manual guide. Samples were diluted to 50 ng/µl and stored at 4 ºC. All stock samples were stored at -20 ºC for further use. The 260/280 and 260/230 ratios are an indication of sample purity. Nucleic acids peak absorbance of UV light is at 260 nm and that of proteins and phenolic acids are 280 nm. A ~1.8 ratio can be regarded as a pure DNA sample. A second measure of purity is the 260/230 ratio since a number of organic compounds have strong absorbance at 230 nm. A ~2.0 ratio can be regarded as pure (Table 3.4). This is in line with the results obtained.

Variance was visualised based on electrophoresis results obtained from polyacrylamide gels (see 3.3.1 Parent line selection). A cladogram was created in Power Marker v.3.25 to indicate genetic variation at molecular level (Figure 3.2).

Table 3.4: **The results from extracted DNA**

Sample ID	ng/ul	260/280 nm	260/230 nm	DNA ul/100ul dH_2O
1A	731.74	1.95	2.22	6.83
1B	477.46	1.88	2.23	10.47
1C	523.03	1.83	2.31	9.52
2A	368.89	1.89	2.39	13.55
2B	1395.24	1.95	2.22	3.58
2C	276.5	1.98	2.54	18.08
3A	541.74	1.86	2.28	9.23
3B	246.64	1.91	2.29	20.27
3C	292.06	1.9	2.31	17.12
4A	734.5	1.96	2.27	6.81
4B	541.65	1.85	2.26	9.23
4C	819.89	1.93	2.19	6.10
5A	340.58	1.85	2.14	14.68
5B	258.29	1.9	2.44	19.36
5C	260.73	1.89	2.22	19.18
6A	306.48	1.88	2.29	16.31
6B	574.64	1.85	2.28	8.70
6C	357.62	1.86	2.25	13.98
7A	767.67	1.94	2.3	6.51
7B	570.79	1.84	2.32	8.76
7C	548.46	1.85	2.31	9.12
8A	725.85	1.94	2.15	6.89
8B	285.64	1.86	2.33	17.50
8C	303.15	1.88	2.36	16.49
DA	360.62	1.88	2.24	13.87

Sample ID	ng/ul	260/280 nm	260/230 nm	DNA ul/100 ul dH_2O
DB	570.26	1.88	2.31	8.77
DC	504.09	1.86	2.24	9.92
HA	456.64	1.87	2.2	10.95
HB	769.04	1.92	2.22	6.50
HC	670.79	1.93	2.22	7.45

3.3.1 Parent line selection

A total of seven microsatellite primers were used to establish kinship between the siblings in each line and the results were summarised in Table 3.5. In total, 66 alleles were detected with and average number of 9.43 per locus. The maximum number were detected at SCM 9-1RS, SCM 159 (F1) and SCM 2-6RL. The calculated major allele frequency (MAF) ranged from 0.13 to 0.83 with an average of 0.45. It was detected that the higher the MAF values, the lower the PIC. For instance SCM 9 has a MAF value of 0.13 but a PIC of 0.92 and SCM 159 (F2) a MAF of 0.83 and the lowest PIC of 0.29.

PIC values higher than 0.7 is seen to be highly informative and a value of 0.44 moderately informative (Hildebrand, *et al*. 1992). The average PIC value was 0.66 which imply a moderate to high diversity level among the parent lines. SCM 152(F2) with PIC of 0.43 and SCM 159(F_2) with PIC of 0.29 respectively has a low value but were included because of the number of alleles involved. A neibour-joining (NJ) cladogram (Figure 3.2) was then generated to visualise kinship and to identify the final parents which were cloned and used in the diallel crossing scheme.

Table 3.5: **Summary of statistics generated in Power Marker v.3.25**

Marker	MAF	Sample Size	No. of obs.	Allele No	Gene Diversity	PIC
SCM 2-6RL	0.37	30	30	11	0.83	0.79
SCM 152(F_1)	0.40	30	30	7	0.71	0.67
SCM 152(F_2)	0.73	30	30	6	0.45	0.43
SCM 159(F_1)	0.23	30	30	13	0.87	0.86
SCM 159(F_2)	0.83	30	30	6	0.30	0.29
SCM 40-7RA	0.47	30	30	6	0.68	0.63
SCM 9-1RS	0.13	30	30	17	0.93	0.92
Mean	0.45	30	30	9.43	0.68	0.66

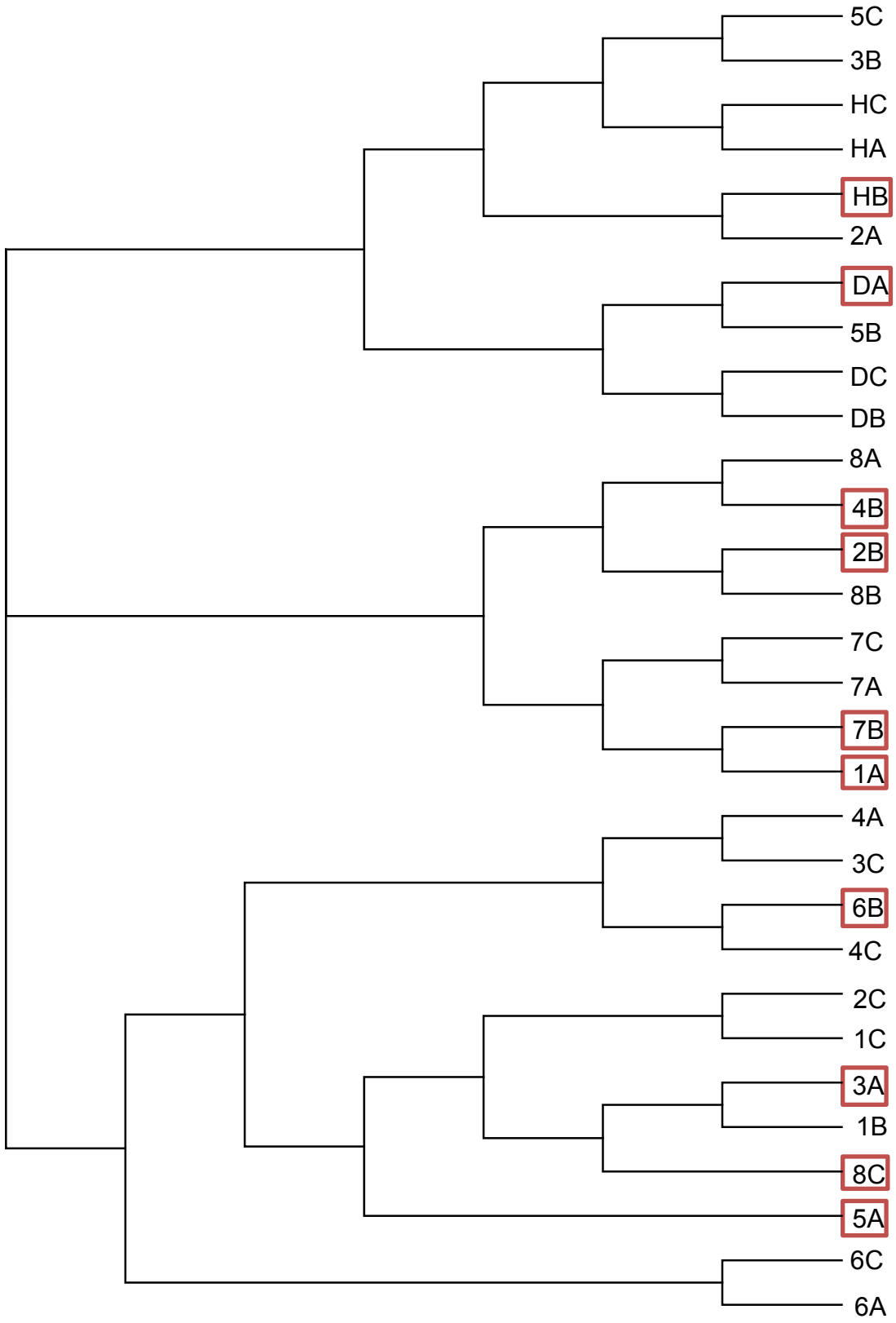

Figure 3.2: Cladogram indicating genetic variation at molecular level.

3.4 Conclusion

The individual parents, that are genetically the furthest removed from its other two half-siblings (HS), were selected to be used as parent lines e.g. 1A has the least in common with 1B and 1C and is, according to the results, much closer related to line 7B (Figure 3.2).

The following parent lines found to have the most variance are indicated on the cladogram (Figure 3.2) in red boxes. Therefore the second objective, ensuring maximum variance between selected plants from each set of HS, was achieved.

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Chapter 4: Diallel analysis of rye

Diallel analysis of rye

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Abstract

Selected parent lines were crossed according to a full diallel mating design. Twelve to fifteen seeds, from each cross, were planted in the three randomised blocks of 76 rows each with an average plant density of 28.8 plants / m^2. Results with regard to days to heading, plant length, spike number, thousand kernel weight and yield were evaluated by making use of a full Griffing diallel analysis to determine GCA and SCA. Results from this study indicate the best general combiners to be line 5 for days to heading, lines 5 and 6 for length, line 1 for spike number and line 6 for thousand kernel weight and yield. Although insignificant p-values were obtained ($p > 0.05$), line 6 may be considered as a potential parent line in a synthetic population to improve both morphological qualities for animal fodder, and yield qualities for rye bread production. No significant reciprocal effects were observed for any of the traits. The specific cross 8 x 4 performed significantly better than the means for four of the five traits namely: length, spikes, thousand kernel weight and yield and may be considered for use in a hybrid production program.

4.1 Introduction

Griffing diallel analysis procedures were thoroughly discussed in chapter two. The Griffing's diallel method 1: parent (p), one set of F_1's [p(p-1)/2, and reciprocal F_1's [p(p-1)/2]; a total of p^2 combinations was the design used in this study to analyse GCA and SCA effects.

The following two objectives were set, to determine variance and kinship of parent lines:

i. Perform a diallel mating scheme to estimate general- and specific combining ability between selected lines.
ii. Selection of lines, according to performance, with regard to:
    - Yield
    - Agronomical characteristics
    - Seed quality

4.1 Materials and methods

To achieve the first objective one plant for each parent line, showing the greatest variance at molecular level, was selected and used in a full diallel cross design (Figure 4.1). These plants were cloned and planted in breeding cages on 15/09/2010. The flowering date was 15-30/10/2010 and the seed harvested on 18/12/2010. Sixty four (8x8) diallel reciprocal crosses were done at Welgevallen, experimental farm- of the University of Stellenbosch (GPS co-ordinates: 33°56'35.57"S; 18°51'57.94"E).

The specific cross design used for this study attempted to ensure cross pollination in a controlled environment. Due to the high degree of self-incompatibility of rye, both plants in a cage acted as both pollen parent as well as seed parent for each other e.g. cage 2 where 1A acted as pollen donor for 2B and 1A is the seed parent for 2B and *vice versa*.

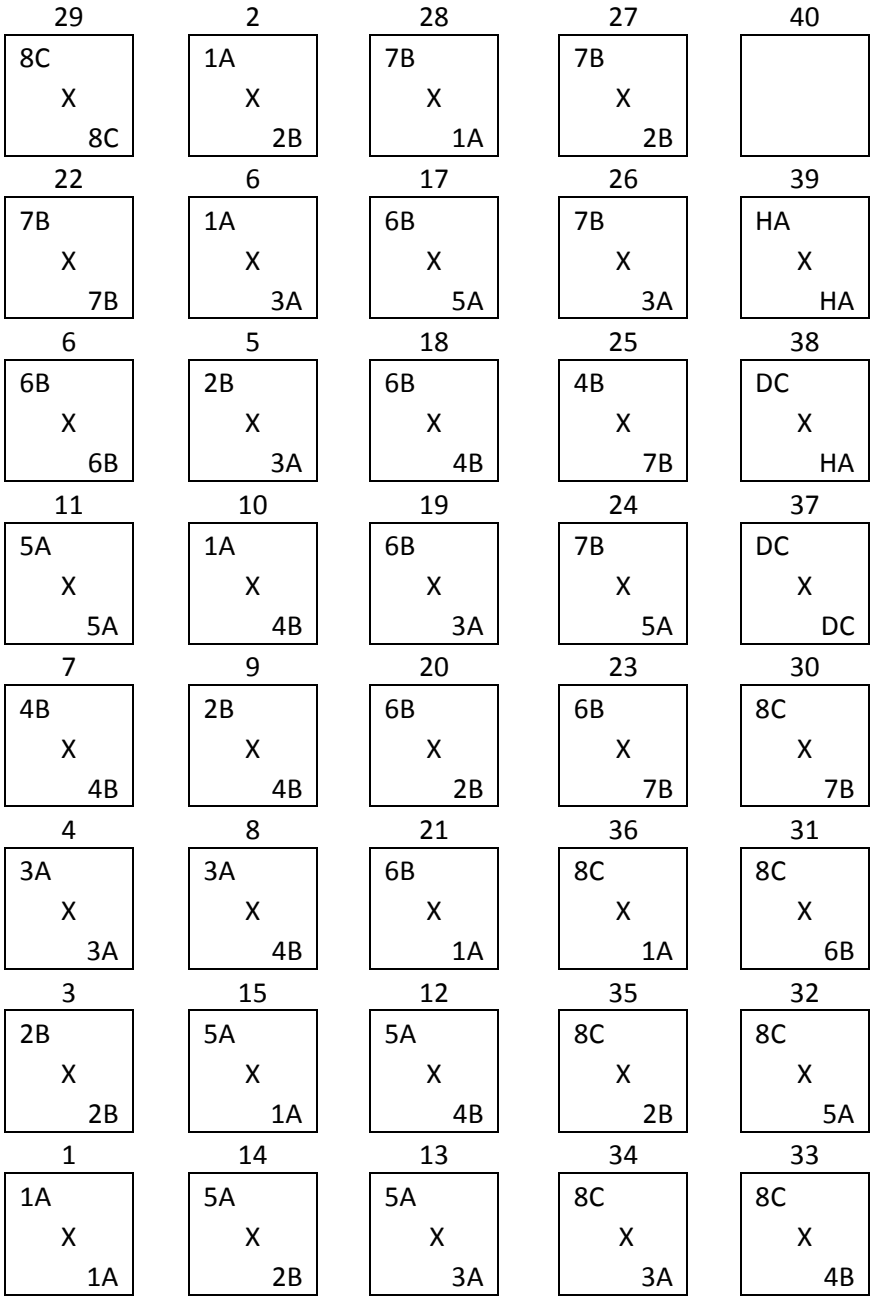

Figure 4.1: Plant plan of diallel cross design

Each cage consisted of an 8 mm iron rod frame with the following dimensions: width 0.74 m, breadth 0.74 m and height 1.62 m (Figure 4.2). The frames were covered by white shade netting (Alnet, Epping) with a 19% shade factor (Figure 4.3). Each cage was protected against wind damage by four anchoring ropes. The plant pairs were planted hexagonal in the cages to allow maximum air flow and pollination. Micro sprinklers were positioned to

irrigate the plants from above (Figures 4.2 and 4.3). The sprinklers were placed at the top and inside the cages to keep the shade netting wet. This was done to form wet walls which acted as a barrier to prevent pollen from escaping or entering. Each sprinkler emitted 4.2 ℓ per hour (Figure 4.4). The cages were spaced 8 m apart (Figure 4.5).

Although wind damage occurred on 11/12/2010, one week before harvest, it had no impact on the outcome of the trial. The highest temperature, 37.39°C, was recorded on 7/12/2010 (Addendum D).

This design intended to maximize air and wind flow for improved pollination. At the end of the season, seed from each plant was manually harvested, cleaned, counted and weighted.

4.2.1 Randomised block design and determination of GCA and SCA

Twelve to fifteen seeds, from each cross, were planted in three randomised blocks of 80 plots/rows each with an average plant density of 28.8 plants / m^2 (Table 4.1). This included the Duiker and Henoch cultivars used as controls (Table 4.1). The seed was sown on 20/05/2011 at Welgevallen and harvested on 12/12/2011. No abnormal weather was experienced during the 2011 season; the highest temperatures were recorded on 09/10/2011 (37.51°C) and just before harvest on 04/12/2011 (37.78°C) (Addendum D). No wind damage was observed. A Wintersteiger Nursery Elite Plot Combine was used for harvesting and the seed was cleaned with a mini air screen.

Kynoch's Turbo 31 (6:9:15) was used as fertiliser. Mospilan SP (acetamiprid), 50g/ha was used in the cages and on the field for aphid control.

To achieve the second objective, an analysis of variance (ANOVA) was done for the following traits:

A. Morphological

1. Days to heading
2. Plant height

B. Yield components

3. Number of spikes per plant
4. 1 000 kernel weight
5. Yield per plant.

The data analysis to determine GCA and SCA was done using Agrobase software and user's manual.

Table 4.1: Field plan of F_1 progeny

First repeat		Second repeat (Random)		Third repeat (Random)	
Row number	**F1**	**Row number**	**F1**	**Row number**	**F1**
1	SP 1A x PP 1A	**1**	SP 6B x PP 6B	**1**	SP 1A x PP 5C
2	SP 1A x PP 1A	**2**	SP 3A x PP 2B	**2**	SP 5A x PP 7B
3	SP 1A x PP 2B	**3**	SP 1A x PP 5C	**3**	SP 6B x PP 2B
4	SP 2B x PP 1A	**4**	SP 5A x PP 4B	**4**	SP 5A x PP 5A
5	SP 2B x PP 2B	**5**	SP 3A x PP 1A	**5**	SP 5C x PP 1A
6	SP 2B x PP 2B	**6**	SP 2B x PP 5C	**6**	SP 3A x PP 2B
7	SP 3A x PP 3A	**7**	SP 8C x PP 1C	**7**	SP 2B x PP 4B
8	SP 3A x PP 3A	**8**	SP DC x PP DC	**8**	SP 2B x PP 7B
9	SP 2B x PP 3A	**9**	SP 3A x PP 3A	**9**	SP 8C x PP 5C
10	SP 3A x PP 2B	**10**	SP 6B x PP 4B	**10**	SP 1A x PP 6B
11	SP 1A x PP 3A	**11**	SP 5C x PP 3A	**11**	SP 2B x PP 2B
12	SP 3A x PP 1A	**12**	SP 4C x PP 8C	**12**	SP 5C x PP 3A
13	SP 4B x PP 4B	**13**	SP HA x PP DC	**13**	SP 8C x PP 1C
14	SP 4B x PP 4B	**14**	SP 2B x PP 6B	**14**	SP 49 x PP 7B
15	SP 3A x PP 4B	**15**	SP 2B x PP 4B	**15**	SP 5C x PP 8C
16	SP 4B x PP 3A	**16**	SP 6B x PP 6B	**16**	SP 4C x PP 8C
17	SP 2B x PP 4B	**17**	SP 7A x PP 6B	**17**	SP 7B x PP 1A
18	SP 4B x PP 2B	**18**	SP 1A x PP 4B	**18**	SP 8A x PP 6A
19	SP 1A x PP 4B	**19**	SP 1C x PP 8C	**19**	SP 7B x PP 5A
20	SP 4B x PP 1A	**20**	SP 3A x PP 3A	**20**	SP DC x PP HA
21	SP 5A x PP 5A	**21**	SP 5A x PP 5A	**21**	SP 7B x PP 2B
22	SP 5A x PP 5A	**22**	SP 5A x PP 6B	**22**	SP 8C x PP 4C
23	SP 5A x PP 4B	**23**	SP 1A x PP 2B	**23**	SP 6B x PP 5A
24	SP 4B x PP 5A	**24**	SP 6B x PP 1A	**24**	SP 7B x PP 3A
25	SP 5C x PP 3A	**25**	SP HA x PP HA	**25**	SP 5A x PP 6B
26	SP 3A x PP 5C	**26**	SP 3A x PP 4B	**26**	SP 6B x PP 4B
27	SP 5C x PP 2B	**27**	SP 4B x PP 7B	**27**	SP 1A x PP 3A
28	SP 2B x PP 5C	**28**	SP 7B x PP 2B	**28**	SP 1 x PP 2
29	SP 5C x PP 1A	**29**	SP 7B x PP 7B	**29**	SP 6B x PP 7A

First repeat		Second repeat (Random)		Third repeat (Random)	
Row number	**F1**	**Row number**	**F1**	**Row number**	**F1**
30	SP 1A x PP 5C	**30**	SP 8C x PP 8C	**30**	SP 4B x PP 4B
31	SP 6B x PP 6B	**31**	SP 4B x PP 4B	**31**	SP 7B x PP 7B
32	SP 6B x PP 6B	**32**	SP 7B x PP 7B	**32**	SP 7A x PP 6B
33	SP 6B x PP 5A	**33**	SP 2 x PP 1	**33**	SP 7B x PP 4B
34	SP 5A x PP 6B	**34**	SP 5C x PP 2B	**34**	SP 4B x PP 1A
35	SP 6B x PP 4B	**35**	SP 1A x PP 6B	**35**	SP 2B x PP 1A
36	SP 4B x PP 6B	**36**	SP 2B x PP 1A	**36**	SP 8C x PP 8C
37	SP 6B x PP 3A	**37**	SP 8C x PP7A	**37**	SP 1A x PP 1A
38	SP 3A x PP 6B	**38**	SP 8C x PP 3B	**38**	SP 8C x PP 2C
39	SP 6B x PP 2B	**39**	SP 3A x PP 5C	**39**	SP 1A x PP 4B
40	SP 2B x PP 6B	**40**	SP 7B x PP 5A	**40**	SP 6A x PP 8A
41	SP 6B x PP 1A	**41**	SP 2B x PP 3A	**41**	SP 6B x PP 3A
42	SP 1A x PP 6B	**42**	SP 7B x PP 3A	**42**	SP 3A x PP 5C
43	SP 7B x PP 7B	**43**	SP 1A x PP 1A	**43**	SP 8C x PP 8C
44	SP 7B x PP 7B	**44**	SP 7B x PP 4B	**44**	SP 1A x PP 1A
45	SP 6B x PP 7A	**45**	SP 6A x PP 8A	**45**	SP 4B x PP 3A
46	SP 7A x PP 6B	**46**	SP 6B x PP 3A	**46**	SP 3A x PP 3A
47	SP 7B x PP 5A	**47**	SP 4B x PP 2B	**47**	SP 4B x PP 2B
48	SP 5A x PP 7B	**48**	SP 1 x PP 2	**48**	SP 3A x PP 4B
49	SP 4B x PP 7B	**49**	SP 9C x PP 2C	**49**	SP 2B x PP 6B
50	SP 7B x PP 4B	**50**	SP 4B x PP 3A	**50**	SP 4B x PP 4B
51	SP 7B x PP 3A	**51**	SP 4B x PP 4B	**51**	SP 2 x PP 1
52	SP 3A x PP 7B	**52**	SP 2C x PP 8C	**52**	SP 3A x PP 7B
53	SP 7B x PP 2B	**53**	SP 8C x PP 5C	**53**	SP 1A x PP 7B
54	SP 2B x PP 7B	**54**	SP 5C x PP 8C	**54**	SP 4B x PP 6B
55	SP 7B x PP 1A	**55**	SP 5A x PP 5A	**55**	SP 2B x PP 5C
56	SP 1A x PP 7B	**56**	SP 6B x PP 7A	**56**	SP HA x PP DC
57	SP 8C x PP 8C	**57**	SP 1A x PP 1A	**57**	SP 3A x PP 1A
58	SP 8C x PP 8C	**58**	SP 8C x PP 4C	**58**	SP 1C x PP 8C
59	SP 8C x PP 7A	**59**	SP 5A x PP 7B	**59**	SP 3A x PP 6B
60	SP 7A x PP 8C	**60**	SP HA x PP HA	**60**	SP 5A x PP 5A
61	SP 8A x PP 6A	**61**	SP 6B x PP 5A	**61**	SP 4B x PP 5A
62	SP 6A x PP 8A	**62**	SP DC x PP HA	**62**	SP DC x PP DC
63	SP 8C x PP 5C	**63**	SP 8C x PP 8C	**63**	SP 6B x PP 6B
64	SP 5C x PP 8C	**64**	SP 2B x PP 7B	**64**	SP 5A x PP 4B
65	SP 8C x PP 4C	**65**	SP 4B x PP 1A	**65**	SP 2B x PP 2B
66	SP 4C x PP 8C	**66**	SP 1A x PP 3A	**66**	SP 8C x PP 3B
67	SP 8C x PP 3B	**67**	SP 4B x PP 5A	**67**	SP 3B x PP 8C
68	SP 3B x PP 8C	**68**	SP 3A x PP 7B	**68**	SP 1A x PP 2B
69	SP 8C x PP 2C	**69**	SP 7B x PP 1A	**69**	SP 6B x PP 1A

First repeat		Second repeat (Random)		Third repeat (Random)	
Row number	**F1**	**Row number**	**F1**	**Row number**	**F1**
70	SP 2C x PP 8C	**70**	SP 2B x PP 2B	**70**	SP 7A x PP 8C
71	SP 8C x PP 1C	**71**	SP 6B x PP 2B	**71**	SP DC x PP DC
72	SP 1C x PP 8C	**72**	SP 5C x PP 1A	**72**	SP 3A x PP 3A
73	SP DC x PP DC	**73**	SP 4B x PP 6B	**73**	SP HA x PP HA
74	SP DC x PP DC	**74**	SP 8A x PP 6A	**74**	SP 3C x PP 8C
75	SP DC x PP HA	**75**	SP 3A x PP 6B	**75**	SP HA x PP HA
76	SP HA x PP DC	**76**	SP 7A x PP 8C	**76**	SP 8C x PP 7A
77	SP HA x PP HA	**77**	SP 3B x PP 8C	**77**	SP 2B x PP 3A
78	SP HA x PP HA	**78**	SP 1A x PP 7B	**78**	SP 6B x PP 6B
79	SP 1 x PP 2	**79**	SP DC x PP DC	**79**	SP 5C x PP 2B
80	SP 2 x PP 1	**80**	SP 2B x PP 2B	**80**	SP 7B x PP 7B

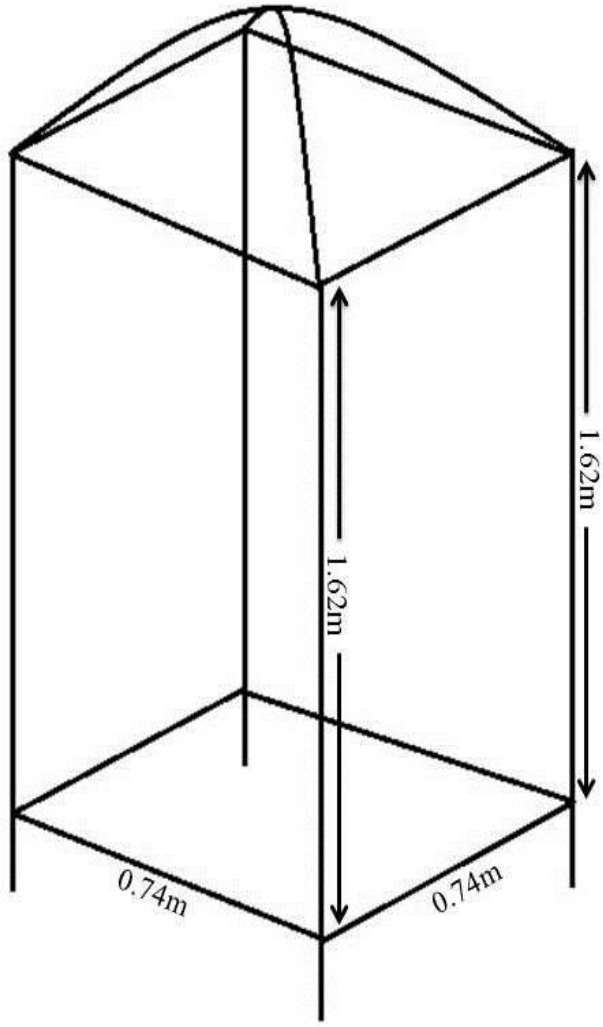

Figure 4.2: Breeding cage frame design.

Figure 4.3: Photo of breeding cage.

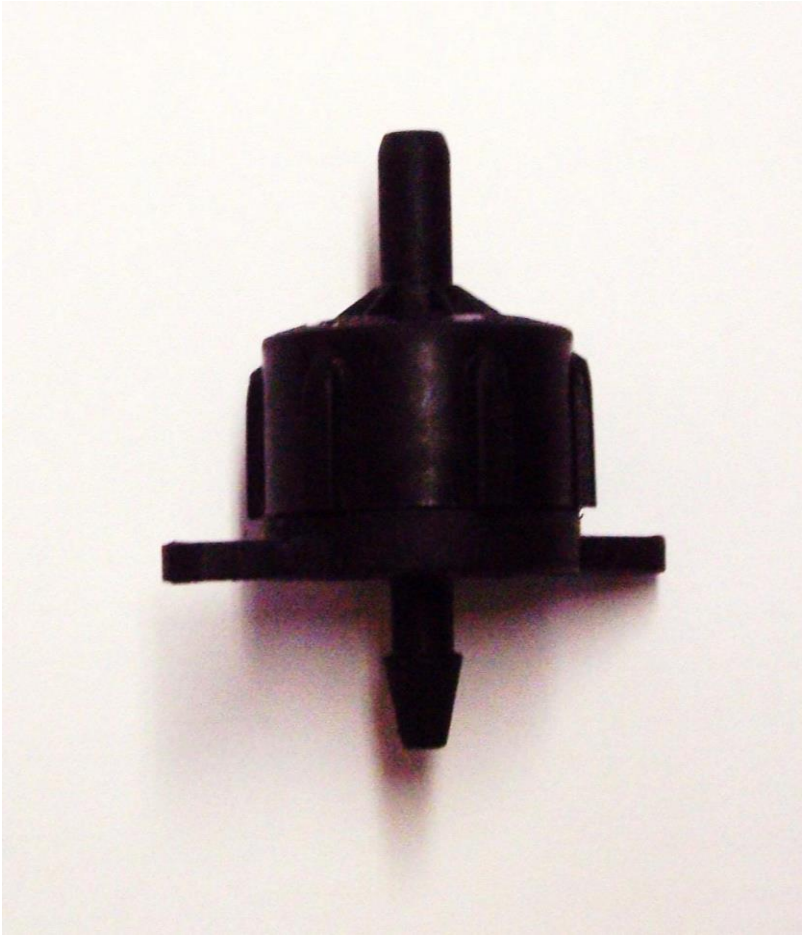

Figure 4.4: Photo of sprinkler used in breeding cage

Figure 4.5: Photo of breeding cages

4.3 Results and discussion

An analysis of variance (ANOVA) among the 64 genotypes are summarised in Table 4.2. No significant variation was revealed for DTH, Spikes, and Yield ($p > 0.05$). A p-value of $p = 0.05$ for plant length is not significant but may indicate a tendency for a slight improvement in the F_1. Significant variation was revealed for TKW (Table 4.2).

No significant reciprocal differences were found for any of the traits. Therefore in the light of the data observed, the five best genotypes for each of the five traits tested for were selected and the results are summarised in Table 4.3.

The mean values of dominant gene action were found to be significantly higher than additive gene action for all five traits and therefore governed by non-additive or dominant gene action and no single parent line was found suitable to be used as a general parent (Table 4.2). Therefore, each of the five traits were analysed *per se* and the results are summarised in Tables 4.4 to 4.8. The means including parents (MIP) are indicated in yellow and the means excluding parents (MEP) are indicated in blue.

Various degrees of inbreeding depression were observed. Inbred depression can be described as the reduced ability of related parents to produce offspring with characteristics superior to theirs (Charlesworth and Willis 2009). Parent lines 4, 5 and 8 were significantly affected by inbreeding depression for all five traits while lines 1, 2, 3, 6 and 7 were not affected. In many instances these seemingly unaffected lines even produced better results than the hybrids (Tables 4.4 to 4.8).

The results also show that the mean squeres for GCA were significant for DTH, Height, Spike and TWK. No significance was revealed for Yield. For SCA all characteristics showed significance differences for DTH, Height, Spike, TWK and Yield (Machikowa *et al*. 2011).

Table 4.2: Genotypic differences, GCA, SCA and reciprocal mean squares for days to heading, plant height, number of spikes, thousand kernel weight and yield obtained from an 8x8 diallel analysis as applied in this study using Griffing's method -1.

		Mean Squares				
Source	.df	DTH	Height	Spikes	TKW	Yield
Replication	2	19.29	24447.4	67.72	1010.9	911.2
Genotype	63	737.58	250376.2	10.62	1644.8	128.6
GCA	7	464.65	197251.2	1.84	1069.5	72.2
SCA	28	326.19	93569.1	5.26	689.7	64.5
Reciprocal	28	110.83	44900.2	2.25	276.5	14.0
Residual	126	548.48	177718.8	8.12	714.0	20.9
CV		19.06	27.3	43.85	49.9	12.1
P		0.081	0.053	0.103	0	0.0832
Means		122.90	1545.4	6.50	53.5	37.8
Additive		17.6	13035.6	0.42	48.4	1.04
Dominance		80.5	19272.7	1.43	253.6	17.7

4.3.1 Days to heading

Selection for DTH was based on the shortest possible number of days from plant to head and those crosses were then selected.

From the analysis, the overall mean for DTH was 123 days and for the hybrids 125 days (Table 4.4). Significant inbred depression was observed for parents 4, 5 and 8. Cross 6 x 5 is shown to be the most promising with 80 DTH and the difference between the means of the five most promising crosses and the overall hybrid means is 27 days. Parent 5 is the most promising general combiner (Table 4.9). Significant differences were observed for reciprocal crosses 5 x 6 (43 days), 7 x 1 (47 days) and 8 x 1 (40 days) but not for 1 x 5 and 4 x 7.

4.3.2 Plant height

For plant heigth, depending on the season, the criterion for mechanical harvesting was 1500 mm while for silage production the tallest plants producing the highest bio mass were selected.

The ideal heigth of rye, to enable mechanical harvesting with a combine, is in the order of 1.5 m to 1.7 m. Therefore, the five progeny with a length closest to 1.5 m were selected (Table 4.5).

The means for the five most promising crosses was 1523 mm with cross 8 x 4 offering the best results with a heigth of 1500 mm. The MIP mean was 1545 mm and that of the hybrids 1564 mm. Although the mean for parent line 8 is the least at 1304 mm line 5, with a mean of 1484 mm, is closer to 1500 mm and therefore more suitable for its GCA (Table 4.9). Crosses 8 x 4, 7 x 3 and 6 x 3 offered the best results in terms off SCA with a means off 1511 mm.

For the production of silage, F_1's with maximum heigth were selected to ensure greater bio mass. The five tallest crosses were 5 x 6 (1883 mm), 6 x 8 (1850 mm), 2 x 1 (1850 mm), 1

x 2 (1800 mm) and 3 x 6 (1800 mm) with a means of 1837 mm. That is 273 mm taller than the hybrid means (Table 4.3). The most promising general combiner was line 6 (Table 4.9).

4.3.3 Spike number

Crosses for spike number were selected based on results obtained from the five best crosses (Table 4.2). Single crosses 7 x 4, 6 x 8 and 8 x 4 had the best SCA effect (Table 4.3). The means for the five best specific combiners was 10.3 spikes per plant which is a significant improvement of 3.6 spikes per plant (Addendum C). The overall mean spike number for the diallel was 6.5 spikes per plant. The most promising general combiner was line 1 with 7 spikes per plant (Table 4.9).

4.3.4 Thousand kernel weight

Crosses for thousand kernel weight were selected based on results obtained from the five best crosses (Table 4.2). Significant differences between the means for the five best TKW crosses (Table 4.3) and the MEP were observed (Table 4.7). Line 6 was identified as the best general combiner while cross 7 x 4 performed significantly better than the means of 53g/ 1000 as a specific combiner (Tables 4.3 and 4.7).

4.3.5 Yield

Crosses for yield were selected based on results obtained from the five best crosses (Table 4.2). The most promising general combiner was line 6 with a mean of 38.8 g per plant which is 4.1 g per plant better than the mean (Tables 4.8 and 4.9). The five most promising crosses were 5 x 3, 8 x 5, 7 x 1, 6 x 3 and 3 x 5 (Table 4.3). The means for these crosses were 42.3 g which was significantly better than the overall means of 34.7 g (Table 4.8).

Table 4.3: Summary of the F_1 results for the five best crosses for each of the five quantitative traits evaluated in a randomised block design with 3 replications.

DTH Crosses	Days	HEIGTH Crosses	mm	SPIKE Crosses	Number per plant	TKW Crosses	g/1000	Yield Crosses	g/plant
6 x 5	80.3	8 x 4 (5 x 6)	1500.0 (1883)	7 x 4	11.2	7 x 4	100.0	5 x 3	42.8
1 x 7	82.3	7 x 3 (6 x 8)	1516.7 (1850)	6 x 8	10.7	1 x 2	97.6	8 x 5	42.7
1 x 8	86.0	6 x 3 (2 x 1)	1516.7 (1850)	8 x 4	10.6	3 x 6	96.4	6 x 3	41.9
5 x 1	121.0	7 x 2 (1 x 2)	1533.3 (1800)	1 x 5	9.87	8 x 4	95.5	7 x 1	41.6
7 x 4	121.3	3 x 1 (3 x 5)	1550.0 (1800)	1 x 2	8.97	1 x 5	93.3	3 x 5	41.6
Means	98.2		1523.3 (1837)		10.3		96.6		42.1

Table 4.4: Mean DTH of F_1 parental and reciprocal populations above, and below the diagonal.

	1	2	3	4	5	6	7	8	MIP	MEP
1	126.0	126.3	128.3	134.3	123.3	128.3	82.3	86.0	116.9	115.6
2	123.0	136.7	132.0	134.0	125.3	129.3	125.0	123.0	128.5	127.4
3	124.7	127.7	130.3	133.0	126.0	125.3	130.7	127.0	128.1	127.8
4	126.7	131.7	131.7	91.3	126.7	129.0	121.7	126.0	123.1	127.6
5	121.0	131.7	124.3	128.7	42.3	123.3	122.3	129.3	115.4	125.8
6	128.3	131.7	129.7	124.0	80.3	124.7	126.0	125.3	121.3	120.8
7	129.3	132.7	131.0	121.3	131.3	125.3	120.3	133.7	128.1	129.2
8	126.3	132.3	126.3	123.7	124.0	125.3	126.7	89.0	121.7	126.4
MIP	125.7	131.3	129.2	123.8	109.9	126.3	119.4	117.4	122.9	
MEP	125.6	130.6	129.0	128.4	119.6	126.6	119.2	121.5		125.1

Mean including parents (MIP) Mean excluding parents (MEP)

Table 4.5: Mean Heigth (mm) of F_1 parental and reciprocal populations above, and below the diagonal.

	1	2	3	4	5	6	7	8	MIP	MEP
1	1716.7	1800.0	1700.0	1616.7	1666.7	1716.7	1183.3	533.3	1491.7	1459.5
2	1850.0	1483.3	1633.3	1583.3	1800.0	1683.3	1636.7	833.3	1562.9	1574.3
3	1550.0	1733.3	1750.0	1783.3	1750.0	1800.0	1716.7	1483.3	1695.8	1688.1
4	1433.3	1733.3	1716.7	1350.0	1616.7	1550.0	1733.3	1353.3	1560.8	1591.0
5	1683.3	1466.7	1750.0	1666.7	633.3	1883.3	1700.0	1550.0	1541.7	1671.4
6	1733.3	1566.7	1516.7	1683.3	1083.3	1683.3	1683.3	1850.0	1600.0	1588.1
7	1666.7	1533.3	1516.7	1766.7	1116.7	1683.3	1716.7	1333.3	1541.7	1516.7
8	1233.3	1066.7	1050.0	1500.0	1750.0	1700.0	1666.7	983.3	1368.8	1423.8
MIP	1608.3	1547.9	1579.2	1618.8	1427.1	1712.5	1629.6	1240.0	1545.4	
MEP	1592.9	1557.1	1554.8	1657.1	1540.5	1716.7	1617.1	1276.7		1564.1

Table 4.6: Mean Spike number (no/plant) of F_1 parental and reciprocal populations above, and below the diagonal.

	1	2	3	4	5	6	7	8	MIP	MEP
1	5.73	8.23	6.00	7.10	9.87	7.03	6.03	5.40	6.92	7.10
2	8.13	4.63	4.73	7.10	7.40	6.17	7.53	7.73	6.68	6.97
3	6.07	7.77	7.03	7.30	7.40	7.30	5.53	5.70	6.76	6.72
4	8.70	8.03	7.17	1.50	5.37	5.70	6.50	7.10	6.26	6.94
5	8.10	5.77	7.17	5.57	1.90	7.67	4.53	8.30	6.13	6.73
6	4.77	5.77	5.87	5.43	2.67	4.33	7.03	10.37	5.78	5.99
7	6.07	6.33	5.27	11.20	3.27	7.43	8.53	4.50	6.58	6.30
8	8.97	5.50	6.83	10.60	8.00	6.13	4.87	4.10	6.88	7.27
MIP	7.07	6.50	6.26	6.98	5.73	6.47	6.32	6.65	6.50	
MEP	7.26	6.77	6.15	7.76	6.28	6.78	6.00	7.01		6.75

Table 4.7: Mean TKW (g/1000) of F_1 parental and reciprocal populations above, and below the diagonal.

	1	2	3	4	5	6	7	8	MIP	MEP
1	45.1	97.6	49.9	46.5	93.3	66.8	37.7	4.2	55.1	56.6
2	68.4	35.1	42.8	58.2	71.6	80.0	57.9	16.8	53.9	56.5
3	63.8	77.6	35.7	56.2	51.3	96.4	38.3	22.5	55.2	58.0
4	56.2	60.1	42.8	5.9	48.5	68.6	65.9	39.2	48.4	54.5
5	85.8	61.6	83.0	44.0	20.3	73.2	40.6	45.8	56.8	62.0
6	75.0	63.1	58.3	55.6	27.9	50.5	75.0	61.5	58.4	59.5
7	46.5	40.6	30.4	100.0	20.0	80.5	58.0	24.4	50.1	48.9
8	39.5	6.6	48.3	95.5	59.1	76.0	35.9	16.3	47.2	51.6
MIP	60.1	55.3	48.9	57.7	49.0	74.0	51.2	28.8	53.1	
MEP	62.2	58.2	50.8	65.1	53.1	77.4	50.2	30.6		55.9

Table 4.8: Mean Yield (g) of F_1 parental and reciprocal populations above, and below the diagonal.

	1	2	3	4	5	6	7	8	MIP	MEP
1	38.0	38.7	38.7	36.1	40.9	38.0	24.3	8.6	32.9	33.6
2	37.1	30.5	38.3	32.3	38.5	40.3	40.9	20.5	34.8	34.9
3	38.5	40.9	37.7	37.3	41.6	38.8	34.7	25.7	36.9	37.1
4	37.7	36.5	40.1	15.7	33.2	39.5	35.7	32.9	33.9	33.9
5	40.9	40.8	42.8	34.0	8.4	37.5	38.1	37.7	35.0	35.1
6	38.0	34.0	41.9	32.1	20.1	36.8	38.3	40.5	35.2	35.3
7	41.6	40.0	34.8	32.5	33.3	38.8	41.1	35.8	37.2	36.7
8	23.3	21.1	25.5	37.9	42.7	40.4	38.0	24.5	31.3	33.6
MIP	36.9	35.3	37.5	32.2	32.3	38.8	36.4	27.9	34.7	
MEP	37.2	35.3	37.7	32.2	32.5	38.8	37.9	31.7		36.0

Table 4.9: Summary of F_1 and reciprocal means to determine GCA. Most promising parents are printed in bold.

Parent lines		1	2	3	4	5	6	7	8
DTH	MIP	121.3	129.9	128.7	123.5	**112.7**	123.8	123.8	119.6
	MEP	120.6	129.0	128.4	128.0	122.7	123.7	124.2	124.0
Heigth	MIP	1550	1555	1638	1590	**1484**	**1656**	1586	1304
	MEP	1526	1566	1621	1624	1606	1652	1567	1350
Spikes	MIP	**7.00**	6.59	6.51	6.62	5.93	6.13	6.45	6.77
	MEP	7.18	6.87	6.44	7.35	6.51	6.39	6.15	7.14
TKW	MIP	60.1	54.6	52.1	53.1	52.9	**66.2**	50.7	38.0
	MEP	62.2	57.4	54.4	59.8	57.6	68.5	49.6	41.1
Yield	MIP	36.9	35.3	37.5	32.2	32.3	**38.8**	36.4	27.9
	MEP	34.5	35.7	37.1	35.6	37.3	37.0	36.2	30.8

4.4 Conclusion

Characteristics such as yield, days to heading, plant height, spikes per plant and 1 000 kernal weight are all important traits in ray. Therefore objectives were set, to select panent lines with superior GCA and SCA.

No significant reciprocal differences were found for any of the traits. Therefore in the light of the data observed, the five best genotypes for each of the five traits tested for were selected.

Dominant gene action were found to be significantly higher than additive gene action for all five traits and therefore governed by non-additive or dominant gene action. No single parent line was found suitable to be used as a general parent. Therefore, each of the five traits were analysed *per se*.

Various degrees of inbreeding depression, which can be described as the reduced ability of related parents to produce offspring with characteristics superior to theirs, were observed (Charlesworth and Willis 2009). Parent lines 4, 5 and 8 were significantly affected by inbred depression for all five traits while lines 1, 2, 3, 6 and 7 were not affected. In many instances these seemingly unaffected lines even produced better results than the hybrids.

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Chapter 5: Conclusion

The pupose of the study was to identify and select promising parent lines, from an existing synthetic rye population, for use in the breeding program of the Genetics and Plant Breeding Department of the University of Stellenbosch. To achieve this goal, the study was done in two parts.

In the first part, seed from each of eight plants which were selected phenotypically, were planted. Variation was determined between selected plants and their clones on a molecular level. In the second part of the study, the siblings with the greatest variation per line were crossed in a full diallel mating scheme, including parents, with the aim of determining the general- and specific combining abilities of selected lines and crosses (Griffing 1956). The offspring of these crosses were evaluated for yield and agronomical characteristics (Miller et al., 1980; Kadkol et al.,1984 and Sherrif et al., 1985).

In assessing these lines, the most important factors that are considered are firstly the characteristics of the line and secondly its behaviour in a specific cross. Superior lines with high GCA can be used effectively in the development of synthetic cultivars while parents of high yielding specific crosses can be utilised in selecting parental material for hybrid production. Furthermore, the indication that mainly dominant gene action is involved in the traits investigated in this study, should therefore be considered when lines are selected for use in a breeding program (Falconer 1967).

Therefore, the following recommendations are made: The best general combiners were line 5 for DTH, lines 5 and 6 for heigth, line 1 for Spike number and line 6 for TKW and Yield. Although insignificant p values were obtained ($p > 0.05$), line 6 may be considered as a potential parent line in a synthetic population to improve both morphological qualities for animal fodder, and yield qualities for rye bread production (Machikowa *et al*., 2011).

No significant reciprocal effects were observed for any of the traits indicating that the maternal effect was minimal and gene action is mainly governed by nucleus DNA. The specific cross 8 x 4 performed significantly better than the means for three of the five traits namely: Heigth, Spikes and TKW and may therefore be considered for use in a hybrid production program.

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ADENDUM A: Summary of PIC values

1						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM 2-6RL	0.428571429	7	7	4	0.693877551	0.641399417
SCM9	0.285714286	7	7	6	0.816326531	0.791336943
SCM40	0.571428571	7	7	3	0.571428571	0.501457726
SCM69	1	7	7	1	0	0
SCM152(F1)	0.428571429	7	7	5	0.734693878	0.699708455
SCM 152(F2)	0.285714286	7	7	6	0.816326531	0.791336943
SCM159(F1)	0.857142857	7	7	2	0.244897959	0.214910454
SCM159(F2)	0.428571429	7	7	3	0.653061224	0.579758434
Mean	0.535714286	7	7	3.75	0.566326531	0.527488546
2						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.285714286	7	7	6	0.816326531	0.791336943
SCM9	0.571428571	7	7	3	0.571428571	0.501457726
SCM40	0.857142857	7	7	2	0.244897959	0.214910454
SCM69	0.428571429	7	7	5	0.734693878	0.699708455
SCM152(F1)	0.571428571	7	7	4	0.612244898	0.569762599
SCM152(F2)	0.142857143	7	7	7	0.857142857	0.839650146
SCM159(1)	0.142857143	7	7	7	0.857142857	0.839650146
SCM159(F2)	1	7	7	1	0	0
Mean	0.5	7	7	4.375	0.586734694	0.557059559

3						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.571428571	7	7	4	0.612244898	0.569762599
SCM9	0.571428571	7	7	4	0.612244898	0.569762599
SCM40	0.428571429	7	7	4	0.693877551	0.641399417
SCM69	1	7	7	1	0	0
SCM152(F1)	0.714285714	7	7	3	0.448979592	0.406497293
SCM152(F2)	0.142857143	7	7	7	0.857142857	0.839650146
SCM159(F1)	0.571428571	7	7	2	0.489795918	0.369845898
SCM159(F2)	0.714285714	7	7	2	0.408163265	0.32486464
Mean	0.589285714	7	7	3.375	0.515306122	0.465222824
4						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.833333333	6	6	2	0.277777778	0.239197531
SCM9	0.333333333	6	6	4	0.722222222	0.671296296
SCM40	0.833333333	6	6	2	0.277777778	0.239197531
SCM69	0.333333333	6	6	5	0.777777778	0.74382716
SCM152(F1)	0.666666667	6	6	3	0.5	0.449074074
SCM152(F2)	0.166666667	6	6	6	0.833333333	0.810185185
SCM159(F1)	0.833333333	6	6	2	0.277777778	0.239197531
SCM159(F2)	0.833333333	6	6	2	0.277777778	0.239197531
Mean	0.604166667	6	6	3.25	0.493055556	0.453896605

5						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.25	4	4	4	0.75	0.703125
SCM9	0.5	4	4	3	0.625	0.5546875
SCM40	0.5	4	4	3	0.625	0.5546875
SCM69	0.25	4	4	4	0.75	0.703125
SCM152(F1)	0.75	4	4	2	0.375	0.3046875
SCM152(F2)	0.75	4	4	2	0.375	0.3046875
SCM159(F1)	0.75	4	4	2	0.375	0.3046875
SCM159(F2)	1	4	4	1	0	0
Mean	0.59375	4	4	2.625	0.484375	0.428710938
6						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.285714286	7	7	6	0.816326531	0.791336943
SCM9	0.571428571	7	7	4	0.612244898	0.569762599
SCM40	0.285714286	7	7	6	0.816326531	0.791336943
SCM69	0.571428571	7	7	4	0.612244898	0.569762599
SCM152(F1)	0.428571429	7	7	5	0.734693878	0.699708455
SCM152(F2)	0.142857143	7	7	7	0.857142857	0.839650146
SCM159(F1)	0.714285714	7	7	3	0.448979592	0.406497293
SCM159(F2)	0.714285714	7	7	3	0.448979592	0.406497293
Mean	0.464285714	7	7	4.75	0.668367347	0.634319034

7						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.428571429	7	7	3	0.612244898	0.529779259
SCM9	0.428571429	7	7	5	0.734693878	0.699708455
SCM40	0.571428571	7	7	3	0.571428571	0.501457726
SCM69	0.285714286	7	7	5	0.775510204	0.739691795
SCM152(F1)	1	7	7	1	0	0
SCM152(2)	0.428571429	7	7	5	0.734693878	0.699708455
SCM159(1)	0.285714286	7	7	5	0.775510204	0.739691795
SCM159(F2)	0.428571429	7	7	3	0.653061224	0.579758434
Mean	0.482142857	7	7	3.75	0.607142857	0.56122449
8						
Marker	**Major.Allele. Frquency**	**SampleSize**	**No. of obs.**	**AlleleNo**	**GeneDiversity**	**PIC**
SCM2-6RL	0.857142857	7	7	2	0.244897959	0.214910454
SCM9	0.714285714	7	7	3	0.448979592	0.406497293
SCM40	0.428571429	7	7	3	0.612244898	0.529779259
SCM69	0.428571429	7	7	5	0.734693878	0.699708455
SCM152(F1)	0.428571429	7	7	5	0.734693878	0.699708455
SCM152(F2)	0.285714286	7	7	6	0.816326531	0.791336943
SCM159(F1)	0.714285714	7	7	3	0.448979592	0.406497293
SCM159(F2)	0.428571429	7	7	3	0.653061224	0.579758434
Mean	0.535714286	7	7	3.75	0.586734694	0.541024573

ADENDUM B: Genotypic means of days to heading, number of spikes per plant, thousand kernel weight, yield and length.

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
1x1	126	1716.7	5.73	71.37	38
1x2	126.3	1800	8.23	97.57	38.67
1x3	128.3	1700	6	49.9	38.67
1x4	134.3	1616.7	7.1	46.5	36.13
1x5	123.3	1666.7	9.9	93.3	40.93
1x6	128.3	1716.7	7.03	66.83	38
1x7	82.3	1183.3	6.03	37.73	27.33
1x8	86	533.3	5.4	4.23	14.67
Mean	**116.9**	**1491.7**	6.93	**58.43**	**34.05**
Hybrid mean excluding parents	115.6	1459.5	**7.10**	56.6	33.5

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
2x1	123	1850	8.13	68.4	37.07
2x2	136.7	1483.3	4.63	35.1	30.53
2x3	132	1633.3	4.73	42.83	38.27
2x4	134	1583.3	7.1	58.2	32.27
2x5	125.3	1800	7.4	71.63	38.53
2x6	129.3	1683.3	6.17	79.97	40.27
2x7	125	1636.7	7.53	57.9	40.93
2x8	123	833.3	7.73	16.8	23.5
Mean	**128.5**	1562.9	6.68	53.85	35.17
Hybrid mean excluding parents	127.4	**1574.3**	**6.97**	**56.5**	**35.8**

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
3x1	124.7	1550	6.07	63.83	38.53
3x2	127.7	1733.3	7.77	77.57	40.93
3x3	130.3	1750	7.03	35.7	37.73
3x4	133.3	1783.3	7.3	56.17	37.33
3x5	126	1750	7.4	51.27	41.6
3x6	125.3	1800	7.3	96.37	38.8
3x7	130.7	1716.7	5.53	38.27	34.67
3x8	127	1483.3	5.7	22.53	28.67
Mean	**128.1**	**1695.8**	**6.76**	55.21	**37.28**
Hybrid mean excluding parents	127.8	1688.1	6.72	**58.0**	37.2

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
4x1	126.7	1433.3	8.7	56.23	37.73
4x2	131.7	1733.3	8.03	60.07	36.53
4x3	131.7	1716.7	7.17	42.8	40.13
4x4	91.3	1350	1.5	5.87	18.67
4x5	126.7	1616.7	5.37	48.53	33.2
4x6	129	1550	5.7	68.63	39.47
4x7	121.7	1733.3	6.5	65.87	35.73
4x8	126	1353.3	7.1	39.23	32.93
Mean	123.1	1560.8	6.26	48.4	34.3
Hybrid mean excluding parents	**127.6**	**1590.9**	**6.94**	**54.5**	**36.5**

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
5x1	121	1683.3	8.1	85.83	40.93
5x2	131.7	1466.7	5.77	61.57	40.8
5x3	124.3	1750	7.17	83.03	42.8
5x4	128.7	1666.7	5.57	44.04	34
5x5	42.3	633.3	1.9	20.33	14.4
5x6	123.3	1883.3	7.67	73.17	37.47
5x7	122.3	1700	4.53	40.63	38.13
5x8	129.3	1550	8.3	45.83	37.73
Mean	115.4	1541.7	6.13	56.8	35.78
Hybrid mean excluding parents	**125.8**	**1671.4**	**6.73**	**62.0**	**38.8**

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
6x1	128.3	1733.3	4.77	75.03	38
6x2	131.7	1566.7	5.77	63.07	34
6x3	129.7	1516.7	5.87	58.33	41.87
6x4	124	1683.3	5.43	55.63	32.13
6x5	80.3	1083.3	2.67	27.93	23.07
6x6	124.7	1683.3	4.33	50.53	36.8
6x7	126	1683.3	7.03	75	38.27
6x8	125.3	1850	10.37	61.47	40.53
Mean	**121.3**	**1600**	5.78	58.37	**35.58**
Hybrid mean excluding parents	120.8	1588.1	**6.0**	**59.5**	35.41

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
7x1	129.3	1666.7	6.07	46.47	41.6
7x2	132.7	1533.3	6.33	40.63	40
7x3	131	1516.7	5.27	30.43	34.8
7x4	121.3	1766.7	11.2	100	32.53
7x5	131.3	1116.7	3.27	20.03	33.3
7x6	125.3	1683.3	7.43	80.5	38.8
7x7	120.3	1716.7	8.53	58	41.07
7x8	133.7	1333.3	4.5	24.37	35.77
Mean	128.1	**1541.7**	**6.58**	**50.05**	**37.23**
Hybrid mean excluding parents	**129.2**	1516.7	6.30	48.9	36.7

CROSSES	DTH (Days)	LENGTH (mm)	SPIKES (no./plant)	TKW (g/1000)	YIELD (g)
8x1	126.3	1233.3	8.97	39.53	26.27
8x2	132.3	1066.7	5.5	6.63	24.07
8x3	126.3	1050	6.83	48.3	28.53
8x4	123.7	1500	10.6	95.5	37.87
8x5	124	1750	8	59.07	42.67
8x6	125.3	1700	6.13	76.03	40.4
8x7	126.7	1666.7	4.87	35.9	38
8x8	89	983.3	4.1	16.27	24.53
Mean	121.7	1368.7	6.88	47.15	31.71
Hybrid mean excluding parents	**126.4**	**1423.8**	**7.27**	**51.6**	**32.9**
Grand mean	122.9	1545.4	6.50	53.53	35.27
Parental mean	107.6	1414.6	4.7	36.64	30.21
Hybrid mean	125.1	1564.1	6.75	55.95	38
S.E.D.	±19.12	±344.21	±2.33	±21.82	±3.73
LSD	37.8	681.2	4.6	43.18	7.39
P	0.081	0.053	0.103	0	0.083

ADENDUM C: Analysis of variance to determine combining ability (Methods 1 to 4).

PXP DIALLEL TABLE OF **YIELD** MEANS

	1	2	3	4	5	6	7	8
1	38.0	38.7	38. 7	36.1	40.9	38.0	24.3	8.7
2	37.1	30.5	38.3	32.3	38.5	40.3	40.9	20.5
3	38.5	40.9	37.7	37.3	41.6	38.8	34.7	25.7
4	37.7	36.5	40.1	15. 7	33.2	39.5	35.7	32.9
5	40.9	40.8	42.8	34.0	8.4	37.5	38.2	37.7
6	38.0	34.0	41.9	32.1	20.1	36.8	38.3	40.5
7	41.6	40.0	34.8	32.5	33.3	38.8	41.1	35.8
8	23.3	21. 7	25.5	37.9	42.7	40.4	38.0	21.5

ANALYSIS OF VARIANCE

Method 1 - Parents, F1s, and Reciprocals - Random effects

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	191	31616.3			
Replications	2	2129.3	1064.7	7.52	0.0008
Genotypes	63	11655.3	185.0	1.31	0.10
Residual	126	17831.7	141.5		

Grand mean = 34.6651 CV = 34.318% Heritability = 0.093

ANALYSIS OF VARIANCE

ANOVA For Combining Abilities - method 1

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	63	3885.101			
GCA	7	730.188	104.313	1.12	0.3778
SCA	28	2629.505	93.911	1.99	0.0054
Reciprocal	28	525.408	18.765	0.40	0.9970
Residual	126	17831.69	47.174		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	0.701351	3.810418
S.C.A.	26.23838	14.480184
Reciprocal	-14.204594	3.888249
Residual	47.17377	5.943337

Additive 1.402703

Dominance 26.238379

PXP DIALLEL TABLE OF **DTH** MEANS

	1	2	3	4	5	6	7	8
1	126.000	126.333	128.333	134.333	123.333	128.333	82.333	86.000
2	123.000	136.667	132.000	134.000	125.333	129.333	125.000	123.000
3	124.667	127.667	130.333	133.000	126.000	125.333	130.667	127.000
4	126.667	131.667	131.667	91.333	126.667	129.000	121.667	126.000
5	121.000	131.667	124.333	128.667	42.333	123.333	122.333	129.333
6	128.333	131.667	129.667	124.000	80.333	124.667	126.000	125.333
7	129.333	132.667	131.000	121.333	131.333	125.333	120.333	133.667
8	126.333	132.333	126.333	123.667	124.000	125.333	126.667	89.000

ANALYSIS OF VARIANCE

Method 1 - Parents, F1s, and Reciprocals - Random effects

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	191	115614.25			
Replications	2	38.573	19.286	0.04	0.9655
Genotypes	63	46467.578	737.581	1.34	0.0810
Residual	126	69108.094	548.477		

Grand mean = 122.8802 CV = 19.059% Heritability = 0.103

ANALYSIS OF VARIANCE

ANOVA For Combining Abilities - method 1

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	63	15489.193			
GCA	7	3252.566	464.652	1.44	0.2300
SCA	28	9133.349	326.191	1.78	0.0165
Reciprocal	28	3103.278	110.831	0.61	0.9376
Residual	126	69108.094	182.826		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	8.811027	16.420035
S.C.A.	80.485833	50.621668
Reciprocal	-35.997148	18.761384
Residual	182.825645	23.033866

Additive 17.622054

Dominance 80.485833

PXP DIALLEL TABLE OF **LENGTH** MEANS

	1	2	3	4	5	6	7	8
1	1716.67	1800.00	1700.00	1616.67	1666.67	1716.67	1183.33	533.33
2	1850.00	1483.33	1633.33	1583.33	1800.00	1683.33	1636.67	833.33
3	1550.00	1733.33	1750.00	1783.33	1750.00	1800.00	1716.67	1483.33
4	1433.33	1733.33	1716.67	1350.00	1616.67	1550.00	1733.33	1353.33
5	1683.33	1466.67	1750.00	1666.67	633.33	1883.33	1700.00	1550.00
6	1733.33	1566.67	1516.67	1683.33	1083.33	1683.33	1683.33	1850.00
7	1666.67	1533.33	1516.67	1766.67	1116.67	1683.33	1716.67	1333.33
8	1233.33	1066.67	1050.00	1500.00	1750.00	1700.00	1666.67	983.33

ANALYSIS OF VARIANCE

Method 1 - Parents, F1s, and Reciprocals - Random effects

Variable: LENGTH

Source	df	SS	MS	F-value	Pr> F
Total	191	38215166.7			
Replications	2	48894.792	24447.4	0.14	0.8716
Genotypes	63	15773700	250376.2	1.41	0.0529
Residual	126	22392571.9	177718.8		

Grand mean = 1545.4167 CV = 27.279% Heritability = 0.120

ANALYSIS OF VARIANCE

ANOVA For Combining Abilities - method 1

Variable: LENGTH

Source	df	SS	MS	F-value	Pr> F
Total	63	5257900			
GCA	7	1380758.33	197251.2	2.12	0.0734
SCA	28	2619936.11	93569.15	1.58	0.0468
Reciprocal	28	1257205.56	44900.2	0.76	0.8006
Residual	126	22392571.9	59239.61		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	6517.76977	6766.247169
S.C.A.	19272.7235	14651.169313
Reciprocal	-7169.704861	7065.862586
Residual	59239.6081	7463.489090

Additive 13035.539532

Dominance 19272.723475

PXP DIALLEL TABLE OF **SPIKE** MEANS

	1	2	3	4	5	6	7	8
1	5.7333	8.2333	6.0000	7.1000	9.8667	7.0333	6.0333	5.4000
2	8.1333	4.6333	4.7333	7.1000	7.4000	6.1667	7.5333	7.7333
3	6.0667	7.7667	7.0333	7.3000	7.4000	7.3000	5.5333	5.7000
4	8.7000	8.0333	7.1667	1.5000	5.3667	5.7000	6.5000	7.1000
5	8.1000	5.7667	7.1667	5.5667	1.9000	7.6667	4.5333	8.3000
6	4.7667	5.7667	5.8667	5.4333	2.6667	4.3333	7.0333	10.3667
7	6.0667	6.3333	5.2667	11.2000	3.2667	7.4333	8.5333	4.5000
8	8.9667	5.5000	6.8333	10.6000	8.0000	6.1333	4.8667	4.1000

ANALYSIS OF VARIANCE

Method 1 - Parents, F1s, and Reciprocals - Random effects

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	191	1827.009			
Replications	2	135.444	67.722	8.34	0.0004
Genotypes	63	668.715	10.615	1.31	0.1027
Residual	126	1022.849	8.118		

Grand mean = 6.4974 CV = 43.851% Heritability = 0.093

ANALYSIS OF VARIANCE

ANOVA For Combining Abilities - method 1

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	63	222.905			
GCA	7	12.896	1.842	0.35	0.9213
SCA	28	147.146	5.255	1.94	0.0071
Reciprocal	28	62.863	2.245	0.83	0.7102
Residual	126	1022.849	2.706		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	-0.210510	0.105952
S.C.A.	1.431157	0.811393
Reciprocal	-0.230416	0.345060
Residual	2.705951	0.340918

Additive -0.421020

Dominance 1.431157

PXP DIALLEL TABLE OF **TKW** MEANS

	1	2	3	4	5	6	7	8
1	71.3667	97.5667	49.9000	46.5000	93.3000	66.8333	37.7333	4.2333
2	68.4000	35.1000	42.8333	58.2000	71.6333	79.9667	57.9000	16.8
3	63.8333	77.5667	35.7000	56.1667	51.2667	96.3667	38.2667	22.5333
4	56.2333	60.0667	42.8000	5.8667	48.5333	68.6333	65.8667	39.2333
5	85.8333	61.5667	83.0333	44.0333	20.3333	73.1667	40.6333	45.8333
6	75.0333	63.0667	58.3333	55.6333	27.9333	50.5333	75.0000	61.4667
7	46.4667	40.6333	30.4333	100.0000	20.0333	80.5000	58.0000	24.3667
8	39.5333	6.6333	48.3000	95.5000	59.0667	76.0333	35.9000	16.2667

ANALYSIS OF

VARIANCE

Method 1 - Parents, F1s, and Reciprocals - Random effects

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	191	195609.98			
Replications	2	2021.873	1010.936	1.42	0.2466
Genotypes	63	103619.25	1644.75	2.30	0.0000
Residual	126	89968.861	714.039		

Grand mean = 53.5359 CV = 49.913% Heritability = 0.303

ANALYSIS OF

VARIANCE

ANOVA For Combining Abilities - method 1

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	63	34539.75			
GCA	7	7486.93	1069.561	1.57	0.1853
SCA	28	19310.756	689.67	2.90	0.0000
Reciprocal	28	7742.063	276.502	1.16	0.2823
Residual	126	89968.861	238.013		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	24.23846	37.481217
S.C.A.	253.5618	104.839480
Reciprocal	19.2447	39.875345
Residual	238.0129	29.986802

Additive 48.476927

Dominance 253.561824

P X P DIALLEL TABLE OF **YIELD** MEANS

	2	3	4	5	6	7	8
1	37.7	38.5	37.7	40.9	38.0	41.6	23.3
2		40.9	36.5	40.8	34.0	40.0	21.1
3			40.1	42.8	41.9	34.8	25.5
4				34.0	32.1	32.5	37.9
5					20.1	33.3	42.7
6						38.8	40.4
7							38.0

A N A L Y S I S O F V A R I A N C E

Method 2 - Parents and F1s - Random Effects

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	107	19234.3			
Replications	2	983.7	491.8	3.16	0.05
Genotypes	35	7343.2	209.8	1.35	0.14
Residual	70	10907.5	155.8		

Grand mean = 34.3083 CV = 36.384% Heritability = 0.104

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 2

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	35	2447.7			
GCA	7	619.5	88.5	1.36	0.26
SCA	28	1828.2	65.3	1.26	0.22
Residual	70	10907.5	52.0		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	2.32	5.04
S.C.A.	13.4	19.5
Residual	51.94	8.78

Additive 4.64

Dominance 13.4

P X P DIALLEL TABLE OF **DTH** MEANS

	2	3	4	5	6	7	8
1	123.0	124.7	126.7	121.0	128.3	129.3	126.3
2		127.7	131.7	131.7	131.7	132.7	132.3
3			131.7	124.3	129.7	131.0	126.3
4				128.7	124.0	121.3	123.7
5					80.3	131.3	124.0
6						125.3	125.3
7							126.7

A N A L Y S I S O F V A R I A N C E

Method 2 - Parents and F1s - Random Effects

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	107	81194.52			
Replications	2	1.35	0.68	0.00	1.00
Genotypes	35	34621.2	989.2	1.49	0.08
Residual	70	46572.0	665.3		

Grand mean = 121.7037 CV = 21.194% Heritability = 0.140

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 2

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	35	11540.4			
GCA	7	4505.5	643.6	2.56	0.04
SCA	28	7034.9	251.2	1.13	0.33
Residual	70	46572.0	221.8		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	39.2	35.1
S.C.A.	29.5	77.0
Residual	221.8	37.5

Additive 78.5

Dominance 29.5

P X P DIALLEL TABLE OF **LENGTH** MEANS

	2	3	4	5	6	7	8
1	1850.0	1550.0	1433.3	1683.3	1733.3	1666.7	1233.3
2		1733.3	1733.3	1466.7	1566.7	1533.3	1066.7
3			1716.7	1750.0	1516.7	1516.7	1050.0
4				1666.7	1683.3	1766.7	1500.0
5					1083.3	1116.6	1750.0
6						1683.3	1700.0
7							1666.7

A N A L Y S I S O F V A R I A N C E

Method 2 - Parents and F1s - Random Effects

Variable: LENTGH

Source	df	SS	MS	F-value	Pr> F
Total	107	24145185.2			
Replications	2	94629.6	47314.8	0.21	0.81
Genotypes	35	8295185.2	237005.3	1.05	0.42
Residual	70	15755370.4	225076.7		

Grand mean = 1520.3704 CV = 31.204% Heritability = 0.017

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 2

Variable: LENTGH

Source	df	SS	MS	F-value	Pr> F
Total	35	2765061.3			
GCA	7	913888.9	130555.6	1.97	0.09
SCA	28	1851172.8	66113.3	0.88	0.64
Residual	70	15755370.4	75025.6		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	6444.2	7198.7
S.C.A.	-8912.3	21749.4
Residual	75025.6	12681.6

Additive 12888.4

Dominance -8912.3

P X P DIALLEL TABLE OF **SPIKE** MEANS

	2	3	4	5	6	7	8
1	8.13	6.17	8.70	8.10	4.87	6.17	9.07
2		7.87	8.03	5.87	5.87	6.33	5.50
3			7.17	7.20	5.97	5.37	6.83
4				5.67	5.43	11.2	10.6
5					2.77	3.37	8.00
6						7.43	6.13
7							4.97

A N A L Y S I S O F V A R I A N C E

Method 2 - Parents and F1s - Random Effects

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	107	975.2			
Replications	2	50.42	25.2	4.06	0.02
Genotypes	35	490.3	14.01	2.26	0.002
Residual	70	434.5	6.21		

Grand mean = 6.2556 CV = 39.826% Heritability = 0.295

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 2

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	35	163.5			
GCA	7	33.2	4.70	1.02	0.44
SCA	28	130.2	4.75	2.25	0.003
Residual	70	434.5	2.17		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	0.009	0.28
S.C.A.	2.58	1.29
Residual	2.07	0.35

Additive 0.019

Dominance 2.58

P X P DIALLEL TABLE OF **TKW** MEANS

	2	3	4	5	6	7
1	68.4	63.8	56.2	85.8	75.0	46.5
2		77.7	60.1	61.6	63.1	40.6
3			42.8	83.0	58.3	30.4
4				44.0	55.6	100.0
5					27.9	20.0
6						80.5

A N A L Y S I S O F V A R I A N C E

Method 2 - Parents and F1s - Random Effects

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	107	116810.7			
Replications	2	1647.0	823.5	1.04	0.36
Genotypes	35	59714.6	1706.1	2.15	0.003
Residual	70	55449.0	792.1		

Grand mean = 52.6546 CV = 53.452% Heritability = 0.278

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	35	19904.9			
GCA	7	2479.4	354.2	0.57	0.77
SCA	28	17425.5	622.3	2.36	0.002
Residual	70	55449.0	264.0		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	-26.8	25.2
S.C.A.	358.3	172.2
Residual	264.0	44.6

Additive -53.6

Dominance 358.3

P X P DIALLEL TABLE OF **YIELD** MEANS

	1	2	3	4	5	6	7	8
1	0.00	38.7	38.7	36.1	40.9	38.0	24.3	8.77
2	37.1	0.00	38.3	32.3	38.5	40.3	40.9	20.5
3	38.5	40.9	0.00	37.3	41.6	38.0	34.7	25.7
4	37.7	36.5	40.1	0.00	33.2	39.5	35.7	32.9
5	40.9	40.8	42.8	34.0	0.00	37.5	38.1	37.7
6	38.0	34.0	41.9	32.1	20.1	0.00	38.3	40.5
7	41.6	40.0	34.8	32.5	33.3	38.8	0.00	35.8
8	23.3	21.1	25.5	37.9	42.7	40.4	38.0	0.00

A N A L Y S I S O F V A R I A N C E

Method 3 - F1s and Reciprocals - Random Effects

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	167	22933.6			
Replications	2	2274.8	1137.4	9.59	0.0001
Genotypes	55	7614.8	138.5	1.17	0.24
Residual	110	13044.0	118.62		

Grand mean = 35.5149 CV = 30.662% Heritability = 0.053

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 3

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	55	2538.38			
GCA	7	526.0	75.1	1.01	0.45
SCA	20	1486.9	74.3	1.88	0.02
Reciprocal	28	525.4	18.8	0.47	0.99
Residual	110	13044.0	39.5		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	0.07	3.88
S.C.A.	17.4	12.1
Reciprocal	-10.4	3.66
Residual	39.5	5.33

Additive 0.13

Dominance 17.4

P X P DIALLEL TABLE OF **DTH** MEANS

	1	2	3	4	5	6	7	8
1	0.00	126.3	128.3	134.3	123.3	128.3	82.3	86.0
2	123.0	0.00	132.0	134.0	125.3	129.3	125.0	123.0
3	124.7	127.7	0.00	133.0	126.0	125.3	130.7	127.0
4	126.7	131.7	131.7	0.00	126.7	129.0	121.7	126.0
5	121.0	131.7	124.3	128.7	0.00	123.3	122.3	129.3
6	128.3	131.7	129.7	124.0	80.3	0.00	126.0	125.3
7	129.3	132.7	131.0	121.3	131.3	125.3	0.00	133.7
8	126.3	132.3	126.3	123.7	124.0	125.3	126.7	0.00

A N A L Y S I S O F V A R I A N C E

Method 3 - F1s and Reciprocals - Random Effects

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	167	52608.3			
Replications	2	261.2	130.6	0.43	0.65
Genotypes	55	18977.6	345.0	1.14	0.28
Residual	110	33369.4	303.4		

Grand mean = 125.0655 CV = 13.926% Heritability = 0.044

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 3

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	55	6325.9			
GCA	7	1057.6	151.1	1.40	0.26
SCA	20	2165.0	108.2	1.07	0.39
Reciprocal	28	3103.3	110.8	1.10	0.36
Residual	110	33369.4	101.1		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	3.57	7.31
S.C.A.	3.56	18.42
Reciprocal	4.86	16.3
Residual	101.1	13.6

Additive 7.14

Dominance 3.56

P X P DIALLEL TABLE OF **LENGTH** MEANS

	1	2	3	4	5	6	7	8
1	0.00	1800.0	1700.0	1616.7	1666.7	1716.7	1183.3	533.3
2	1850.0	0.00	1633.3	1583.3	1800.0	1683.3	1636.7	833.3
3	1550.0	1733.3	0.00	1783.3	1750.0	1800.0	1716.7	1483.3
4	1433.3	1733.3	1716.7	0.00	1616.7	1550.0	1733.3	1353.3
5	1683.3	1466.7	1750.0	1666.7	0.00	1883.3	1700.0	1550.0
6	1733.3	1566.7	1516.7	1683.3	1083.3	0.00	1683.3	1850.0
7	1666.7	1533.3	1516.7	1766.7	1116.7	1683.3	0.00	1333.3
8	1233.3	1066.7	1050.0	1500.0	1750.0	1700.0	1666.7	0.00

A N A L Y S I S O F V A R I A N C E

Method 3 - F1s and Reciprocals - Random Effects

Variable: LENTGH

Source	df	SS	MS	F-value	Pr> F
Total	167	29823266.1			
Replications	2	27046.4	13523.2	0.08	0.92
Genotypes	55	11786799.4	214305.4	1.31	0.12
Residual	110	18009420.2	163722.0		

Grand mean = 1564.1071 CV = 25.869% Heritability = 0.093

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 3

Variable: LENTGH

Source	df	SS	MS	F-value	Pr> F
Total	55	3928933.1			
GCA	7	1038962.5	148423.2	1.82	0.14
SCA	20	1632765.1	81638.3	1.50	0.10
Reciprocal	28	1257205.6	44900.2	0.82	0.72
Residual	110	18009420.2	54574.0		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	5565.4	6952.5
S.C.A.	13532.1	13422.3
Reciprocal	-4836.9	7038.3
Residual	54574.0	7358.8

Additive 11130.8

Dominance 13532.1

P X P DIALLEL TABLE OF **SPIKE** MEANS

	1	2	3	4	5	6	7	8
1	0.00	8.23	6.00	7.10	9.97	7.03	6.03	5.40
2	8.13	0.00	4.73	7.10	7.40	6.27	7.53	7.73
3	6.07	7.77	0.00	7.30	7.40	7.30	5.53	5.70
4	8.70	8.03	7.27	0.00	5.47	5.70	6.50	7.10
5	8.10	5.77	7.27	5.67	0.00	7.77	4.53	8.30
6	4.77	5.77	5.97	5.43	2.77	0.00	7.03	10.47
7	6.17	6.33	5.37	11.0	3.37	7.43	0.00	4.50
8	9.00	5.50	6.83	10.6	8.00	6.13	4.97	0.00

A N A L Y S I S O F V A R I A N C E

Method 3 - F1s and Reciprocals - Random Effects

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	167	1535.1			
Replications	2	144.0	72.0	8.53	0.0004
Genotypes	55	462.8	8.45	1.00	0.50
Residual	110	928.3	8.44		

Grand mean = 6.7512 CV = 43.029% Heritability = 0.000

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 3

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	55	154.3			
GCA	7	22.3	3.180	0.92	0.512
SCA	20	69.1	3.46	1.23	0.25
Reciprocal	28	62.9	2.25	0.80	0.75
Residual	110	928.3	2.81		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	-0.023	0.17
S.C.A.	0.32	0.58
Reciprocal	-0.28	0.35
Residual	2.81	0.38

Additive -0.046

Dominance 0.32

P X P DIALLEL TABLE OF **TKW** MEANS

	1	2	3	4	5	6	7	8
1	0.00	97.6	49.9	46.5	93.3	66.8	37.7	4.23
2	68.4	0.00	42.8	58.2	71.6	80.0	57.9	16.8
3	63.8	77.6	0.00	56.2	51.3	96.4	38.3	22.5
4	56.2	60.1	42.8	0.00	48.5	68.6	65.9	39.2
5	85.8	61.5	83.0	44.0	0.00	73.2	40.6	45.8
6	75.0	63.1	58.3	55.6	27.9	0.00	75.0	61.5
7	46.5	40.6	30.4	100.0	20.0	80.5	0.00	24.4
8	39.5	6.63	48.3	95.5	59.1	76.0	35.9	0.00

A N A L Y S I S O F V A R I A N C E

Method 3 - F1s and Reciprocals - Random Effects

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	167	170161.8			
Replications	2	2120.6	1060.3	1.41	0.25
Genotypes	55	85335.2	1551.5	2.06	0.0007
Residual	110	82706.0	751.9		

Grand mean = 55.9488 CV = 49.010% Heritability = 0.262

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 3

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	55	28445.1			
GCA	7	7361.7	1051.7	1.58	0.20
SCA	20	13341.3	667.1	2.66	0.0006
Reciprocal	28	7742.1	276.5	1.10	0.35
Residual	110	82706.0	250.6		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	32.1	50.0
S.C.A.	208.2	106.8
Reciprocal	12.9	40.6
Residual	250.6	33.8

Additive 64.1

Dominance 208.2

P X P DIALLEL TABLE OF **YIELD**

MEANS

	2	3	4	5	6	7	8
1	37.1	38.5	37.7	40.9	38.0	41.6	23.1
2		40.9	36.5	40.8	34.0	40.0	21.1
3			40.1	42.8	41.8	34.8	25.5
4				34.0	32.1	32.5	37.9
5					20.1	33.3	42.7
6						38.8	40.4
7							38.0

A N A L Y S I S O F V A R I A N C E

Method 4 - F1s Only - Random Effects

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	83	10557.4			
Replications	2	1109.8	554.9	4.88	0.01
Genotypes	27	3308.4	122.5	1.08	0.40
Residual	54	6139.2	113.7		

Grand mean = 35.9060 CV = 29.696% Heritability = 0.025

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 4

Variable: YIELD

Source	df	SS	MS	F-value	Pr> F
Total	27	1102.8			
GCA	7	137.5	19.6	0.41	0.89
SCA	20	965.3	48.7	1.27	0.24
Residual	54	6139.2	37.9		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	-4.77	3.09
S.C.A.	10.4	16.9
Residual	37.90	7.31

Additive	-9.541095
Dominance	10.369326

P X P DIALLEL TABLE OF **DTH** MEANS

	2	3	4	5	6	7	8
1	123.0	124.7	126.7	121.0	128.3	129.3	126.3
2		127.7	131.7	131.7	131.7	132.7	132.3
3			131.7	124.3	129.7	131.0	126.3
4				128.7	124.0	121.3	123.7
5					80.3	131.3	124.0
6						125.3	125.3
7							126.7

A N A L Y S I S O F V A R I A N C E

Method 4 - F1s Only - Random Effects

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	83	18454.2			
Replications	2	298.2	149.1	0.75	0.48
Genotypes	27	7396.9	274	1.38	0.16
Residual	54	10759.2	199.2		

Grand mean = 125.7381 CV = 11.226% Heritability = 0.111

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 4

Variable: DTH

Source	df	SS	MS	F-value	Pr> F
Total	27	2465.6			
GCA	7	718.7	102.7	1.18	0.36
SCA	20	1747	87.4	1.32	0.21
Residual	54	10759.2	66.4		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	2.55	10.2
S.C.A.	20.9	30.44
Residual	66.4	12.8

Additive 5.11

Dominance 20.9

P X P DIALLEL TABLE OF **LENGTH** MEANS

	2	3	4	5	6	7	8
1	1850.0	1550.0	1433.3	1683.3	1733.3	1666.7	1233.3
2		1733.3	1733.3	1466.7	1566.7	1533.3	1066.7
3			1716.7	1750.0	1516.7	1516.7	1050.0
4				1666.7	1683.3	1766.7	1500.0
5					1083.3	1116.7	1750.0
6						1683.3	1700.0
7							1666.7

A N A L Y S I S O F V A R I A N C E

Method 4 - F1s Only - Random Effects

Variable: LENTGH

Source	df	SS	MS	F-value	Pr> F
Total	83	15877470.2			
Replications	2	71666.7	35833.3	0.17	0.84
Genotypes	27	4432470.2	164165.6	0.78	0.76
Residual	54	11373333.3	210617.3		

Grand mean = 1550.5952 CV = 29.597% Heritability = 0.000

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 4

Variable: LENTGH

Source	df	SS	MS	F-value	Pr> F
Total	27	1477490.1			
GCA	7	239606.5	34229.5	0.55	0.78
SCA	20	1237883.6	61894.2	0.88	0.61
Residual	54	11373333.3	70205.8		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	-4610.8	4465.5
S.C.A.	-8311.6	23783.2
Residual	70205.8	13511.1

Additive -9221.6

Dominance -8311.6

P X P DIALLEL TABLE OF **SPIKE** MEANS

	2	3	4	5	6	7	8
1	8.13	6.10	8.70	8.10	4.87	6.17	8.97
2		7.87	8.03	5.77	5.87	6.33	5.50
3			7.27	7.27	5.97	5.37	6.83
4				5.67	5.43	11.20	10.6
5					2.77	3.37	8.00
6						7.43	6.13
7							4.87

A N A L Y S I S O F V A R I A N C E

Method 4 - F1s Only - Random Effects

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	83	697.2			
Replications	2	60.0	30.02	4.78	0.012
Genotypes	27	298.3	11.05	1.76	0.04
Residual	54	338.8	6.3		

Grand mean = 6.6940 CV = 37.419% Heritability = 0.202

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 4

Variable: SPIKES

Source	df	SS	MS	F-value	Pr> F
Total	27	99.4			
GCA	7	42.1	6.02	2.10	0.09
SCA	20	57.3	2.87	1.37	0.18
Residual	54	338.8	2.09		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	0.52	0.56
S.C.A.	0.78	0.99
Residual	2.09	0.40

Additive 1.05

Dominance 0.78

P X P DIALLEL TABLE OF **TKW**

MEANS

	2	3	4	5	6	7
1	68.4	63.8	56.2	85.8	75.0	46.4
2		77.6	60.1	61.6	63.1	40.6
3			42.8	83.0	58.3	30.4
4				44.0	55.6	100.0
5					27.9	20.0
6						80.5

A N A L Y S I S O F V A R I A N C E

Method 4 - F1s Only - Random Effects

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	83	91279.1			
Replications	2	2110.6	1055.3	1.19	0.31
Genotypes	27	41347.2	1531.4	1.73	0.04
Residual	54	47821.4	885.6		

Grand mean = 57.2286 CV = 52.000% Heritability = 0.196

A N A L Y S I S O F V A R I A N C E

ANOVA For Combining Abilities - Method 4

Variable: TKW

Source	df	SS	MS	F-value	Pr> F
Total	27	13782.4			
GCA	7	1669.2	238.5	0.39	0.89
SCA	20	12113.1	605.7	2.05	0.02
Residual	54	47821.4	295.2		

Component	Variance	Approx. Std. Dev. of the Var.
G.C.A.	-61.2	38.3
S.C.A.	310.5	199.8
Residual	295.2	56.8

Additive -122.4

Dominance 310.5

ADENDUM D: Weather data for 2010/ 2011 season (Institute for Soil, Climate and water –ARC Infruitech)

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2010	9	1	18.77	8.52	6.1
30026	2010	9	2	22.02	10.13	0
30026	2010	9	3	26.86	5.35	0
30026	2010	9	4	22.43	8.47	0
30026	2010	9	5	21.49	6.38	4.83
30026	2010	9	6	19.33	9.44	0
30026	2010	9	7	25.65	2.8	0
30026	2010	9	8	26.96	7.99	0
30026	2010	9	9	23.14	9.74	1.52
30026	2010	9	10	21.76	7.26	0
30026	2010	9	11	31.27	4.49	0
30026	2010	9	12	24.82	5.37	0
30026	2010	9	13	32.36	5.18	0
30026	2010	9	14	25.04	7.31	5.33
30026	2010	9	15	16.25	7.32	5.33
30026	2010	9	16	18.56	7.12	0
30026	2010	9	17	26.17	8.16	0
30026	2010	9	18	26.38	6.06	0
30026	2010	9	19	20.44	12.55	0
30026	2010	9	20	22.08	12.58	0.51
30026	2010	9	21	19.48	11.34	1.52
30026	2010	9	22	22.83	8.78	0
30026	2010	9	23	21.11	6.7	2.54
30026	2010	9	24	21.22	7.98	0.25
30026	2010	9	25	23.95	4.85	0
30026	2010	9	26	18.37	7.59	4.57
30026	2010	9	27	20.86	8.15	0
30026	2010	9	28	25.2	3.32	0
30026	2010	9	29	27.39	5.07	0
30026	2010	9	30	25.67	7.95	0
30026	2010	9	31	--	--	--
30026	2010	9	**Average**	23.26	7.46	1.08
30026	2010	9	**Total**	697.85	223.93	32.51
30026	2010	9	**Highest**	32.36	12.58	6.1
30026	2010	9	**Lowest**	16.25	2.8	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2010	10	1	22.87	7.97	0
30026	2010	10	2	28.44	10.71	0
30026	2010	10	3	27.91	9.48	0
30026	2010	10	4	27.04	17.17	0
30026	2010	10	5	31.7	12.78	0
30026	2010	10	6	32.07	11.83	0
30026	2010	10	7	22.41	14.56	5.59
30026	2010	10	8	22.31	9.32	0
30026	2010	10	9	29.75	6.38	0
30026	2010	10	10	18.45	10.5	32.77
30026	2010	10	11	16.44	8.64	3.3
30026	2010	10	12	20.1	5.11	0
30026	2010	10	13	21.37	12.54	0
30026	2010	10	14	21.03	10.94	0
30026	2010	10	15	22.51	8.66	0
30026	2010	10	16	26.82	5.05	0
30026	2010	10	17	34.39	8.24	0
30026	2010	10	18	25.63	10.21	0.76
30026	2010	10	19	24.49	9.31	0.51
30026	2010	10	20	24.68	7.2	5.33
30026	2010	10	21	18.55	11.25	11.18
30026	2010	10	22	20.37	8.25	0
30026	2010	10	23	23.18	6.47	0
30026	2010	10	24	23.11	8.44	0
30026	2010	10	25	23.86	9.24	0
30026	2010	10	26	29.42	11	0
30026	2010	10	27	24.71	15.4	0
30026	2010	10	28	26.34	10.54	0
30026	2010	10	29	24	9.02	0
30026	2010	10	30	25.6	9.85	0
30026	2010	10	31	30.19	7.62	0
30026	2010	10	**Average**	24.83	9.8	1.92
30026	2010	10	**Total**	769.75	303.68	59.44
30026	2010	10	**Highest**	34.39	17.17	32.77
30026	2010	10	**Lowest**	16.44	5.05	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2010	11	1	33.05	9.35	0
30026	2010	11	2	30.29	13.88	0
30026	2010	11	3	23.9	14.1	9.65
30026	2010	11	4	21.29	8.12	4.06
30026	2010	11	5	22.41	7.62	0
30026	2010	11	6	23.16	9.41	0
30026	2010	11	7	28.64	7.34	0
30026	2010	11	8	20.81	9.2	6.6
30026	2010	11	9	22.8	10.82	2.79
30026	2010	11	10	27.68	7.21	0
30026	2010	11	11	23.1	11.19	4.06
30026	2010	11	12	22.3	9.99	0.25
30026	2010	11	13	24.15	9.59	0
30026	2010	11	14	33.56	6.37	0
30026	2010	11	15	36.32	9.39	0
30026	2010	11	16	29.31	15.68	0
30026	2010	11	17	30.79	17.77	0
30026	2010	11	18	34.15	11.66	0
30026	2010	11	19	28.05	12.89	0
30026	2010	11	20	25.99	14.96	9.65
30026	2010	11	21	22.17	12.39	0.25
30026	2010	11	22	22.13	10.11	0
30026	2010	11	23	23.92	7.04	0
30026	2010	11	24	30.06	14.96	0
30026	2010	11	25	31.66	18.48	0
30026	2010	11	26	35.47	17.38	0
30026	2010	11	27	24.07	12.94	0
30026	2010	11	28	22.73	9.04	0
30026	2010	11	29	28.3	6.87	0
30026	2010	11	30	24.42	9.22	0
30026	2010	11	31	--	--	--
30026	2010	11	**Average**	26.89	11.17	1.24
30026	2010	11	**Total**	806.68	334.97	37.34
30026	2010	11	**Highest**	36.32	18.48	9.65
30026	2010	11	**Lowest**	20.81	6.37	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2010	12	1	24.85	10.64	0
30026	2010	12	2	27.25	18.39	9.91
30026	2010	12	3	25.54	14.01	0
30026	2010	12	4	26.12	11.67	0.25
30026	2010	12	5	26.4	13.43	0
30026	2010	12	6	29.63	16.26	0
30026	2010	12	7	37.39	18.34	0
30026	2010	12	8	35.86	11.87	0
30026	2010	12	9	30.89	11.03	0
30026	2010	12	10	31.53	13.58	0
30026	2010	12	11	25.36	18.42	0
30026	2010	12	12	33.03	18.3	0
30026	2010	12	13	32.63	11.22	0
30026	2010	12	14	31.69	19.43	0
30026	2010	12	15	27.02	19.02	0.25
30026	2010	12	16	28.22	18.19	0
30026	2010	12	17	31.49	13.37	0
30026	2010	12	18	33.16	13.01	0
30026	2010	12	19	33.5	13.46	0
30026	2010	12	20	35.01	22.08	0
30026	2010	12	21	33.79	16.6	0.51
30026	2010	12	22	22.06	17.03	0.25
30026	2010	12	23	28.9	15.1	0
30026	2010	12	24	28.5	14.08	0
30026	2010	12	25	32.55	18.03	0
30026	2010	12	26	35.49	20.35	0
30026	2010	12	27	32.18	16.67	0
30026	2010	12	28	30.36	19.86	0
30026	2010	12	29	31.01	16.86	0
30026	2010	12	30	26.94	18.69	0
30026	2010	12	31	31.22	20.07	0.25
30026	2010	12	**Average**	30.2	16.18	0.37
30026	2010	12	**Total**	966.53	517.78	11.43
30026	2010	12	**Highest**	37.39	22.08	9.91
30026	2010	12	**Lowest**	22.06	10.64	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	5	1	34.95	6.94	0
30026	2011	5	2	27.7	15.43	3.56
30026	2011	5	3	26.52	11.66	0
30026	2011	5	4	33.08	15.7	0
30026	2011	5	5	22.69	13.96	6.6
30026	2011	5	6	22.15	14.51	0.25
30026	2011	5	7	21.16	15.64	0
30026	2011	5	8	24.34	17.97	0
30026	2011	5	9	28.03	13.51	0
30026	2011	5	10	21.89	12.01	0
30026	2011	5	11	24.31	9.62	0
30026	2011	5	12	24.75	7.65	0
30026	2011	5	13	20.15	6.74	0
30026	2011	5	14	16.71	9.58	0.25
30026	2011	5	15	21.86	5.55	0
30026	2011	5	16	20.11	8.73	0
30026	2011	5	17	22.89	6.67	0.25
30026	2011	5	18	23.79	6.65	0
30026	2011	5	19	26.25	5.23	0
30026	2011	5	20	27.64	5.29	0
30026	2011	5	21	27.68	7.77	0
30026	2011	5	22	22.95	9.5	9.14
30026	2011	5	23	23.04	13.9	13.46
30026	2011	5	24	18.39	11.16	5.84
30026	2011	5	25	20.21	7.41	0
30026	2011	5	26	21.79	5.12	0
30026	2011	5	27	24.27	5.11	0
30026	2011	5	28	14.7	5.27	1.27
30026	2011	5	29	14.44	5.44	10.41
30026	2011	5	30	16.94	7.74	13.46
30026	2011	5	31	15.16	6.3	2.79
30026	2011	5	**Average**	22.92	9.48	2.17
30026	2011	5	**Total**	710.54	293.79	67.31
30026	2011	5	**Highest**	34.95	17.97	13.46
30026	2011	5	**Lowest**	14.44	5.11	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	6	1	14.97	8.18	0
30026	2011	6	2	21.72	4.89	0
30026	2011	6	3	21.33	5.23	0
30026	2011	6	4	21.69	7.57	1.52
30026	2011	6	5	18.88	5.89	1.52
30026	2011	6	6	18.25	4.61	0.76
30026	2011	6	7	19.88	8.69	0
30026	2011	6	8	21.03	13.63	1.78
30026	2011	6	9	22.71	12.46	0
30026	2011	6	10	24.07	8.41	0
30026	2011	6	11	23.3	11.01	0
30026	2011	6	12	23.23	7.5	0
30026	2011	6	13	21.88	6.11	0.25
30026	2011	6	14	30.39	5.78	1.27
30026	2011	6	15	15.3	10.21	24.89
30026	2011	6	16	16.05	10.4	10.16
30026	2011	6	17	20.62	6.34	0
30026	2011	6	18	21.98	4.36	4.32
30026	2011	6	19	18.83	7.51	8.38
30026	2011	6	20	19.35	4.59	0.25
30026	2011	6	21	28.88	3.17	0
30026	2011	6	22	19.19	8.41	17.02
30026	2011	6	23	15.54	8.21	11.43
30026	2011	6	24	15.7	8.35	4.32
30026	2011	6	25	16.67	9.83	3.81
30026	2011	6	26	18.36	6.44	0
30026	2011	6	27	21.74	2.72	0
30026	2011	6	28	18.13	3.57	0
30026	2011	6	29	17.71	8.62	9.14
30026	2011	6	30	15.61	4.28	0.76
30026	2011	6	31	--	--	--
30026	2011	6	**Average**	20.1	7.23	3.39
30026	2011	6	**Total**	603.03	216.93	101.6
30026	2011	6	**Highest**	30.39	13.63	24.89
30026	2011	6	**Lowest**	14.97	2.72	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	7	1	18.97	2.68	12.19
30026	2011	7	2	17.02	7.68	4.06
30026	2011	7	3	18.02	5.83	0.25
30026	2011	7	4	16.25	5.58	0
30026	2011	7	5	18.85	3.2	0
30026	2011	7	6	20.84	1.85	0
30026	2011	7	7	24.43	1.74	0
30026	2011	7	8	25.39	3.22	0
30026	2011	7	9	26.02	6.57	0
30026	2011	7	10	27.93	6.68	0
30026	2011	7	11	27.11	7.7	0
30026	2011	7	12	26.1	6.26	0
30026	2011	7	13	23.83	5.66	0.25
30026	2011	7	14	25.74	4.36	0
30026	2011	7	15	28.08	6.79	0
30026	2011	7	16	29.59	6.65	0
30026	2011	7	17	24.98	6.48	0
30026	2011	7	18	20.58	5.24	0
30026	2011	7	19	20.78	3.41	0.25
30026	2011	7	20	23.05	3.39	0
30026	2011	7	21	22.52	3.86	0
30026	2011	7	22	22.91	8.86	0
30026	2011	7	23	19.82	10.77	0
30026	2011	7	24	17.42	11.49	0.25
30026	2011	7	25	15.66	9.2	0.51
30026	2011	7	26	23.85	4.76	0
30026	2011	7	27	23.18	4.9	4.57
30026	2011	7	28	14.37	5.32	14.99
30026	2011	7	29	17.21	6.76	0
30026	2011	7	30	19.23	4.47	0.25
30026	2011	7	31	20.63	2.88	0
30026	2011	7	**Average**	21.93	5.5	1.21
30026	2011	7	**Total**	723.66	181.51	37.59
30026	2011	7	**Highest**	29.59	11.49	14.99
30026	2011	7	**Lowest**	14.37	1.74	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	8	1	26.98	2.39	0
30026	2011	8	2	29.2	8.16	0
30026	2011	8	3	24.45	8.28	0
30026	2011	8	4	15.8	9.36	22.61
30026	2011	8	5	16.19	3.63	2.03
30026	2011	8	6	19.21	0.8	0
30026	2011	8	7	23.96	2.08	0
30026	2011	8	8	21.5	2.95	0
30026	2011	8	9	25.36	5.93	0
30026	2011	8	10	28.29	4.17	0
30026	2011	8	11	20.78	4.23	0
30026	2011	8	12	15.73	6.68	18.03
30026	2011	8	13	16.5	4.36	0
30026	2011	8	14	18.12	1.65	0.25
30026	2011	8	15	22.29	4.78	0.25
30026	2011	8	16	29.94	3.21	0
30026	2011	8	17	29.19	7.37	0
30026	2011	8	18	19.77	6.69	6.1
30026	2011	8	19	16.92	6.16	7.62
30026	2011	8	20	22.66	3.17	2.79
30026	2011	8	21	13.52	9.84	12.19
30026	2011	8	22	18.6	7.33	0
30026	2011	8	23	18.32	8.59	4.83
30026	2011	8	24	20.47	5.18	0
30026	2011	8	25	23.45	4.44	1.52
30026	2011	8	26	18.32	3.63	2.03
30026	2011	8	27	19.24	1.48	0.25
30026	2011	8	28	24.96	2.76	0
30026	2011	8	29	29.56	5.34	0
30026	2011	8	30	22.28	6.95	4.83
30026	2011	8	31	21.01	7.61	0
30026	2011	8	**Average**	21.7	5.14	2.75
30026	2011	8	**Total**	672.56	159.21	85.34
30026	2011	8	**Highest**	29.94	9.84	22.61
30026	2011	8	**Lowest**	13.52	0.8	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	9	1	18.35	5.18	16.26
30026	2011	9	2	15.62	8.33	2.54
30026	2011	9	3	19.54	6.07	0
30026	2011	9	4	21.69	7.46	0
30026	2011	9	5	25.6	4.96	0
30026	2011	9	6	26.4	5.43	0.25
30026	2011	9	7	36.11	6.41	0
30026	2011	9	8	16.5	12.21	4.32
30026	2011	9	9	20.49	11.36	1.78
30026	2011	9	10	19.18	9.91	0.25
30026	2011	9	11	23.08	7.94	0
30026	2011	9	12	19.8	7.41	0.25
30026	2011	9	13	17.39	10.28	4.06
30026	2011	9	14	22.78	7.85	0
30026	2011	9	15	20.4	5.78	1.52
30026	2011	9	16	24.97	2.93	0
30026	2011	9	17	26.14	4.49	0
30026	2011	9	18	16.98	8.17	5.59
30026	2011	9	19	18.27	5.65	0
30026	2011	9	20	19.73	5.13	0
30026	2011	9	21	22.5	4.71	0
30026	2011	9	22	20.18	4.49	0
30026	2011	9	23	21.76	4.98	0
30026	2011	9	24	29.12	4.34	0
30026	2011	9	25	32.37	6.62	0
30026	2011	9	26	22.01	9.02	0
30026	2011	9	27	22.45	6.59	0
30026	2011	9	28	30.32	7.6	0
30026	2011	9	29	20.47	7.56	0
30026	2011	9	30	22.09	4.39	0
30026	2011	9	31	--	--	--
30026	2011	9	**Average**	22.41	6.77	1.23
30026	2011	9	**Total**	672.28	203.22	36.83
30026	2011	9	**Highest**	36.11	12.21	16.26
30026	2011	9	**Lowest**	15.62	2.93	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	10	1	20.2	4.81	0
30026	2011	10	2	19.46	12.63	0
30026	2011	10	3	25.22	5.73	1.52
30026	2011	10	4	20.56	7.48	0
30026	2011	10	5	27.44	4.77	0
30026	2011	10	6	33.61	4.47	0
30026	2011	10	7	29.79	6.2	0
30026	2011	10	8	31.98	6.95	0
30026	2011	10	9	37.51	9.69	0
30026	2011	10	10	28.82	11.76	0
30026	2011	10	11	27.25	10.03	0
30026	2011	10	12	30.46	9.78	0
30026	2011	10	13	26.37	13.1	0
30026	2011	10	14	23.95	12.79	0.25
30026	2011	10	15	24.67	9.9	0
30026	2011	10	16	26.86	9.41	0
30026	2011	10	17	32.19	13.63	0.76
30026	2011	10	18	20.97	12.9	17.27
30026	2011	10	19	22.14	10.35	0.25
30026	2011	10	20	24.42	7.73	0
30026	2011	10	21	27.31	7.65	0
30026	2011	10	22	25.5	9.01	0.76
30026	2011	10	23	25.08	12.39	0
30026	2011	10	24	23.34	12.25	0
30026	2011	10	25	22.64	9.47	0
30026	2011	10	26	22.2	7.95	3.3
30026	2011	10	27	20.83	7.45	1.27
30026	2011	10	28	21.83	6.21	0
30026	2011	10	29	21.43	5.76	1.02
30026	2011	10	30	19.22	4.62	1.78
30026	2011	10	31	19.31	5.17	2.03
30026	2011	10	**Average**	25.24	8.78	0.98
30026	2011	10	**Total**	782.55	272.06	30.23
30026	2011	10	**Highest**	37.51	13.63	17.27
30026	2011	10	**Lowest**	19.22	4.47	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	11	1	23.52	9.08	0
30026	2011	11	2	27.54	7.13	0
30026	2011	11	3	22.61	8.11	0.25
30026	2011	11	4	22.33	11.39	0.76
30026	2011	11	5	21.37	10.99	0
30026	2011	11	6	27.32	7.93	0
30026	2011	11	7	32.57	5.36	0
30026	2011	11	8	24.44	9.27	2.03
30026	2011	11	9	20.02	9.63	1.02
30026	2011	11	10	20.48	6.62	0
30026	2011	11	11	24.62	4.93	0
30026	2011	11	12	23.01	5.58	16.26
30026	2011	11	13	20.63	6.52	3.05
30026	2011	11	14	24.05	5.15	0
30026	2011	11	15	24.33	6.64	0
30026	2011	11	16	27.33	8.76	0
30026	2011	11	17	30.01	9.42	0
30026	2011	11	18	31.07	18.13	0
30026	2011	11	19	35.82	13.47	0.51
30026	2011	11	20	22.73	12.19	10.67
30026	2011	11	21	21.37	10.23	0
30026	2011	11	22	22.42	7.81	0.25
30026	2011	11	23	20.97	10.2	3.56
30026	2011	11	24	23.34	11.22	0
30026	2011	11	25	27.28	7.53	0
30026	2011	11	26	28.21	7.09	0
30026	2011	11	27	22.67	15.42	0
30026	2011	11	28	32.05	13.27	0
30026	2011	11	29	27.89	10.56	0
30026	2011	11	30	28.25	11.31	0
30026	2011	11	31	--	--	--
30026	2011	11	**Average**	25.34	9.37	1.28
30026	2011	11	**Total**	760.24	280.95	38.35
30026	2011	11	**Highest**	35.82	18.13	16.26
30026	2011	11	**Lowest**	20.02	4.93	0

DAILY REPORT: Available Data Shown						
Compno	Year	Month	Day	Tx	Tn	Rain
30026	2011	12	1	27.18	13.99	0
30026	2011	12	2	28.77	12.53	0
30026	2011	12	3	27.68	16.31	0
30026	2011	12	4	37.78	14.65	0
30026	2011	12	5	31.05	13.96	0
30026	2011	12	6	31.7	14.4	0.76
30026	2011	12	7	21.83	12.98	1.78
30026	2011	12	8	22.22	11.92	0
30026	2011	12	9	28.42	13.94	0
30026	2011	12	10	24.68	10	2.54
30026	2011	12	11	26.51	12.45	0
30026	2011	12	12	27.96	10.17	0
30026	2011	12	13	25.49	12.39	6.1
30026	2011	12	14	20.1	10.9	3.3
30026	2011	12	15	25.79	10.07	0
30026	2011	12	16	29.16	8.15	0
30026	2011	12	17	32.84	9.78	0
30026	2011	12	18	32.22	11.34	1.52
30026	2011	12	19	25.99	15.67	0
30026	2011	12	20	30.67	10.22	0
30026	2011	12	21	28.99	12.5	0
30026	2011	12	22	21.88	11.84	7.87
30026	2011	12	23	28.04	9.88	0
30026	2011	12	24	32.22	10.59	0
30026	2011	12	25	35.38	16.34	0
30026	2011	12	26	31.99	16.36	0
30026	2011	12	27	30.97	14.65	0
30026	2011	12	28	33.22	14.38	0
30026	2011	12	29	33	12.71	0
30026	2011	12	30	29.68	12	0
30026	2011	12	31	29.62	11.8	0
30026	2011	12	**Average**	28.54	12.49	0.77
30026	2011	12	**Total**	913.14	399.75	23.88
30026	2011	12	**Highest**	37.78	16.36	7.87
30026	2011	12	**Lowest**	20.1	8.15	0

KEY NOTES FOR DAILY REPORT			
ELEMENT	DESCRIPTION	UNIT	STATION TYPE
Tx	Daily Maximum Temperature	°C	AWS
Tn	Daily Minimum Temperature	°C	AWS
Rain	Total Rainfall [Calculated From Hourly Data]	mm	AWS
Rs	Total Radiation [Calculated From Hourly Data]	MJ/m2	AWS
U2	Average Wind Speed [Calculated From Hourly Data]	ms	AWS
RHx	Daily Maximum Relative Humidity	%	AWS
RHn	Daily Minimum Relative Humidity	%	AWS
ET0	Total Relative Evapotranspiration [Calculated From Hourly Data]	mm	AWS
HU	Total Heat Units [Calculated From Hourly Data]	Unitless	AWS
CU	Total Cold Units [Calculated From Hourly Data]	Unitless	AWS
DPCU	Daily Positive Chilling Units [Calculated From Hourly Data]	Unitless	AWS
VP	Vapour Pressure [Calculated From Hourly Data / 06:00 - 18:00]	~~~	AWS
SVP	Saturated Vapour Pressure [Calculated From Hourly Data]	~~~	AWS
VPD	Vapour Pressure Deficit [Calculated From Hourly Data / 06:00 - 18:00]	~~~	AWS
AveT	Average Temperature [[Tx + Tn] / 2]	°C	AWS
AveRH	Average Relative Humidity [[RHx + RHn] / 2]	%	AWS
Tx	Daily Maximum Temperature	°C	MWS
Tn	Daily Minimum Temperature	°C	MWS
RHx	Average Daily Maximum Relative Humidity	%	MWS
RHn	Average Daily Minimum Relative Humidity	%	MWS
Rain	Total Daily Rainfall	mm	MWS
APan	Total Daily Apan Evaporation	mm	MWS
UTot	Daily Wind Run	KM/day	MWS
Suns	Sunshine Hours	Hours	MWS
AveT	Daily Average Temperature [[Tx + Tn] / 2]	°C	MWS