Combining Ability, Protein, Heterosis, and Prediction of F_1 Performance with RFLPs in a Diallel of Maize

by

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(ABSTRACT)

Improving protein quality and identifying superior inbreds and hybrids are significant challenges in commercial maize breeding programs. These two problems were addressed in separate studies on inbreds and hybrids from a complete diallel cross of 12 elite proprietary inbred lines of maize evaluated in field trials in two locations for two years. One of the inbreds (W1) was a novel source of high quality protein obtained from Wilson Seeds, Inc. in Harlan, Iowa. In the first study, diallel analyses were used to study combining ability and types of gene action important in the inheritance of protein content, grain yield, grain moisture at harvest, time to silk, kernel hardness, and density. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant for all traits indicating presence of both additive and non-additive effects, respectively. Reciprocal effects (REC), often assumed to be absent in maize diallel studies, were significant for grain yield and protein concentration, suggesting that choice of female parent may be important for these traits. Ratios expressing the relative importance of GCA and SCA indicated that protein concentration is controlled primarily by additive gene action. In the second study, restriction fragment length polymorphism

(RFLP) data were obtained for the 12 inbreds using 42 genomic clones each with four restriction enzymes. Modified Roger's distances were calculated and used in cluster analyses for heterotic grouping of the inbreds. Two measures of level of heterozygosity and hybrid value were evaluated as means of predicting F_1 performance of hybrids in the complete diallel set of hybrids and in groups of hybrids representing crosses between and within heterotic groups. Results from this study confirm those of previous investigations with respect to prediction of hybrid performance when comparable groupings of crosses between related and unrelated lines were evaluated. This study further indicates that RFLPs may also be useful for prediction of hybrid performance in situations typical of early generations of many maize breeding programs where recombinant inbreds are testcrossed to a common tester inbred.

Dedicated to :

my wife Sarah, our sons Nathan and Steven,

our Moms, the loving memories of our Fathers,

and to efforts to advance Science in order to

feed the ever burgeoning world population

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INTRODUCTION

Maize (*Zea mays L.* commonly called corn) holds a dominant place in world agriculture because it produces high grain yields, is highly digestible, and is an important source of energy in the diets of both humans and domestic animals. However, normal corn has a low concentration of protein with an unfavorable amino acid composition for the diets of humans and monogastric animals, and must be supplemented with other, usually more expensive protein sources. Steady improvement of corn hybrid grain yields (Russel, 1993) has made the corn hybrid industry a success story. But development of commercially acceptable corn hybrids with favorable protein quality has not been successful due to industry's emphasis on grain yield and existence of inverse relationships between grain yield and protein in some maize germplasm (Lambert et al., 1969; Ahmadi et al., 1993).

Heterosis is the basis for the successful improvement of maize grain yields. Early maize breeders observed that crosses between diverse lines resulted in more heterosis than crosses between related lines. The concept of heterotic groups was established by relating levels of heterosis with pedigrees (Anderson, 1944; Hallauer and Miranda, 1981). Maize breeders have relied on the recognition and exploitation of heterotic groups for the development of superior hybrids (Gerdes and Tracy, 1993). Continued success of hybrid maize breeding programs depends on efficient procedures to identify maize inbreds that produce superior hybrids.

A novel source of maize germplasm with elevated levels of high-quality protein has been recently discovered in a collection of tropical maize varieties owned by Wilson Seeds Inc., of Harlan Iowa (Donahue and Strissel, 1990, personal communication). As this is apparently the first source of normal-endosperm maize with high-quality protein reported, no data exists on the type of gene action for protein in this germplasm or on combining ability and heterotic patterns. Even though the high-quality protein germplasm in this study is proprietary to Wilson Seeds, Inc., knowledge of the type of gene action for protein in this novel germplasm will be useful to other breeding efforts to improve maize protein quality or identify new sources.

The diallel mating design is an important scheme used for crop breeding. Analysis of diallel experiments is used to determine the type of gene action responsible for traits of interest such as protein, estimate combining abilities in a set of inbred lines, and estimate heterosis in crosses between the lines. A diallel study was conducted in order to assess the combining ability of the novel germplasm.

Restriction fragment length polymorphisms (RFLPs) are a type of molecular marker used to study genetic variation in population biology. They represent a powerful tool for detailed assessment of genetic diversity in cultivated species because they detect differences between individuals directly at the molecular level (DNA). In recent years, several studies have investigated the usefulness of RFLP-based genetic distance between inbreds in diallel crossing schemes for prediction of hybrid

performance of single crosses in maize (for review see: Dudley, 1993; Melchinger 1993).

Two of the above mentioned studies were conducted with European maize inbreds and the remainder were conducted with inbreds from the United States. There are no reports of similar studies conducted with tropical germplasm or with elite maize inbreds developed by private companies to this author's knowledge. The present study was conducted using 12 elite inbreds from three private companies. Four of the inbreds used in the present study have pedigrees with tropical background. The percentage tropical background ranged from 25 to 75% (Donahue, 1994 personal communication).

This dissertation will consist of two separate literature reviews and two manuscripts covering the research. The first literature review covers efforts to improve protein quality in maize, and the second review focuses on hybrid performance and heterosis. Results of this study are reported in separate manuscripts. The first is "Combining ability and heterosis of novel maize germplasm with elevated protein", and the second is "Grouping of parents and prediction of single-cross performance using RFLPs".

Results from the diallel analysis of this study provides information which can be used to address questions on the improvement of protein quality in maize. In addition, this study extends the evaluation of the usefulness of RFLP-based distances for prediction of hybrid performance to germplasm not previously studied.

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LITERATURE REVIEW OF EFFORTS TO IMPROVE PROTEIN QUALITY IN MAIZE

Maize is an important source of energy and dietary protein in human and animal diets in much of the world. Ordinary corn, however, has poor-quality protein because it is low in two amino acids (lysine and tryptophan) and has an undesirable ratio of leucine to isoleucine. Normal corn must be supplemented with higher quality protein sources in the diets of humans and monogastric animals.

Evidence that protein quantity can be modified by breeding has been accumulating in a long-term experiment in Illinois since 1896 (Hopkins, 1899). Progress in this long term experiment was reported after three generations by Hopkins (1899); after ten generations by Smith (1908); after twenty-eight generations by Winter (1929); after forty-seven generations by Woodworth and Jungenheimer (1948); after fifty generations by Woodworth et al. (1952); after seventy-six generations by Dudley (1977); and after ninety generations by Dudley and Lambert (1992).

While the Illinois experiment has proven that protein quantity in maize can be changed by breeding, little was accomplished with respect to protein quality until the discovery of the opaque-2 mutant gene by Mertz et al. (1964). Their report that the opaque-2 gene nearly doubled the lysine content of maize stimulated research efforts to improve the quality of protein in maize and several other cereals. Nelson et al. (1965) reported the discovery of a second mutant gene that could improve the amino

acid composition of maize protein. In 1968 Swedish scientists reported variation for the amino acid composition in a primitive Ethiopian barley obtained from the World Barley Collection (Munck et al., 1969). A few years later, Singh and Axtell (1973) reported the first high lysine sorghum mutant.

Hopes and enthusiasm for overcoming the shortcomings of corn protein were high in the late 1960s and scientists at the International Maize and Wheat Improvement Center (CIMMYT) initiated the conversion of normal maize to opaque-2 (Villegas et al., 1992). By the early 1970s, these efforts had produced an assortment of opaque-2 varieties and hybrids and some of these were grown commercially in Brazil, Colombia, United States, India, Yugoslavia, Hungary, and South Africa (Villegas et al., 1992). Serious objections to the performance of opaque-2 hybrids were soon raised. First among these was low grain yield as compared to normal corn (Lambert et al., 1969; Nass and Crane, 1970; Paez et al., 1970). Lower grain yields in opaque-2 maize were associated with reduced kernel weight and density (Glover and Tosello, 1973; Lambert et al., 1969; Makonnen, 1973; Makonnen and Bauman, 1976; Nass and Crane, 1970; Nelson, 1966; Salamini and Ekpenyong, 1967; Sreeramula and Bauman, 1970). Makonnen (1973) also determined that dry matter accumulation in the ears of opaque-2 maize was generally slower than for normal corn. In his study, some of the opaque-2 hybrids accumulated dry matter as rapidly as normal hybrids; however, they ceased dry matter accumulation seven days before physiological maturity as determined by black layer formation. Lower grain yield was not the only undesirable characteristic of opaque-2

maize. Researchers also reported soft chalky appearing kernels that were more susceptible to ear rot, insects in the field and in storage, and to damage during machine harvest and handling than normal corn (Harpstead, 1969; Francis et al., 1972; Arnold et al., 1977). Efforts to improve the grain quality of opaque-2 maize has involved the exploitation of genetic modifiers to the *o2* locus (Paez et al., 1970; Bauman and Aycock, 1970; Ribeiral, 1974) as well as research on the interactions of double mutants with opaque-2 (Nelson, 1966; Barbosa, 1971; Glover and Tosello, 1973; Glover et al., 1975; Roundy and Glover, 1975). These efforts have been successful, and today an array of improved opaque-2 maize populations referred to as Quality Protein Maize (QPM) are available that do not differ from normal corn in appearance of kernels (Vasal et al., 1980; Wessel-Beaver and Lambert, 1982; Wessel-Beaver et al., 1985; Ortega and Bates, 1983; Kelly, 1985; Vasal et al., 1980; Bjarnason and Vasal, 1992). Pixley and Bjarnason (1993) stated that QPM germplasm is now competitive with normal corn hybrids. However, the best yields in their study (7.87 Mg ha^{-1}) are much lower than the yields obtained from the best hybrids grown in Virginia, USA (Ball et al., 1993a). Today, the greatest disadvantage of QPM continues to be reduced yields. Bockholt and Rooney (1992) speculated that yield of QPM hybrids would always lag behind normal hybrids by 10 to 12 generations, due to the time required to convert inbreds to opaque-2 and then to QPM using conventional techniques.

Improving protein quality in normal dent maize without the use of endosperm mutants, as an alternative to QPM has been the subject of several studies. Zuber and

Helm (1975) reported the successful increase of lysine content in three maize populations by recurrent selection. Significant variation between races and strains of maize for protein and several amino acids, including lysine, tryptophan, and methionine, has been reported by Frey et al., 1949; Miller et al., 1952; Aguirre et al., 1953; Tello et al., 1965; Paez et al., 1969; and numerous others.

Rapidly increasing use of corn grain for human consumption and industrial uses has increased demand for corn with specific grain characteristics. However, inverse relationships exist in some germplasm between yield and grain characteristics required for specific uses and may be points of disadvantage for breeding programs for higher grain quality (Ahmadi et al., 1993; Gupta et al., 1975). In addition, increasing planting populations, a production practice aimed at maximizing yield, has been reported by several researchers to be negatively correlated with grain protein content (Zuber et al., 1954; Genter et al., 1956; Stickler, 1964). Ahmadi et al. (1993) suggested end-users requiring high grain quality may need to provide incentives to growers in order to compensate for lower yields.

A novel source of maize germplasm with elevated levels of high-quality protein and superior grain characteristics has recently been discovered in a collection of tropical maize varieties by a private company (Donahue and Strissel, 1990, personal communication, Ball et al., 1993b). Elite maize inbreds and hybrids with superior grain characteristics have subsequently been developed with this germplasm and are currently being studied by a number of public and private scientists.

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LITERATURE REVIEW OF HYBRID PERFORMANCE AND HETEROSIS

Heterosis may be defined as the increased vigor of hybrid plants with respect to the mid-parent average for yield, size, resistance to diseases or insects, or adaptability. Extensive exploitation of heterosis in hybrid corn has revolutionized the production of corn in the United States and other parts of the world (Mangelsdorf, 1974 and Hallauer and Miranda, 1981).

Heterosis has been the subject of extensive research in the twentieth century because it is of paramount importance in the improvement of both plants and animals. Papers presented in two comprehensive symposia on heterosis in the 1950's and elsewhere now make a complete review an overwhelming task beyond the scope of this paper. My purpose in this manuscript is threefold: 1) to summarize the history of the development of the heterosis concept; 2) to summarize the supporting and contradicting evidence for the most important theories on the genetic basis of heterosis in maize; and 3) to review recent methods of predicting hybrid performance and choosing maize germplasm with favorable alleles for improvement of hybrids.

Pre-Mendelian Observations

Josef Koelreuter was the first to conduct intensive and systematic investigations of plant hybridization. From 1761 to 1766 he published descriptions of hybrid vigor in interspecific crosses in *Nicotiana, Dianthus, Verbascum, Mirabilis,*

Datura, and other genera. Many other scientists noted exceptional vigor in hybrids until by the nineteenth century reports of hybrid vigor were becoming commonplace (Zirkle 1964). Darwin in 1877 was most careful in recording both the ill effects of inbreeding and the beneficial effects of outbreeding but failed to recognize that inbreeding and outbreeding were opposite expressions of the same phenomena (Hallauer and Miranda 1981). In 1880, Beal reported that hybrid corn increased his yield "by as much as 151 exceeds 100 ". Sanborn in 1890 confirmed Beals results reporting a ratio of 131 to 100 (Zirkle 1964).

Genetic Basis

Although studied extensively, the phenomena of heterosis was not put in its proper perspective until the studies of Shull, East, and Jones were completed early this century (Zirkle, 1964; Mangelsdorf. 1974; Hallauer and Miranda, 1981). Shull in 1908, presented the first theory of heterosis known as the heterozygosity per se hypothesis. He believed that a physiological stimulation arising from the different gametic union would cause an excess of development. This theory was non-Mendelian in nature and because of its vagueness was never accepted.

In 1910, Keeble and Pellew followed Bruce (1910) in presenting the first evidence for the dominance theory which is one of two theories that persist until the present. They believed that an accumulation of favorable dominant growth factors or decrease in number of deleterious homozygous recessives was the explanation for hybrid vigor. Two principle objections to this explanation were immediately

forthcoming from Shull in 1911 and again from East and Hayes in 1912. These objections were: 1) inability to recover in one inbred all of the favorable dominant factors and, 2) absence of a skewed distribution of quantitative factors in the F2. Jones in 1917 effectively refuted these objections when he extended the earlier idea of accumulated dominant growth factors to include linkage. The fact is also that significant progress has been made in the yield of some inbred lines which supports the dominance theory (Genter, 1967; Genter, 1971). This theory remains one of the most satisfactory explanations of heterosis to date with the main question remaining being that it does not adequately account for all of the expression of heterosis since the yield of a hybrid often exceeds the sum of its parents (Genter, 1967; Mangelsdorf, 1974; Hallauer and Miranda, 1981).

Hull in 1946 was first to use the term overdominance for the theory of allelic interaction as an explanation of heterosis. This theory simply put says that the heterozygous genotype is superior to either homozygote. Overdominance was first applied to the case of a single locus, but has been also used to describe the case of blocks of closely linked genes. Hull in 1952 called this phenomenon pseudo overdominance. Mangelsdorf (1974) stated that the distinction between the two has become meaningless since the effects of closely linked genes cannot be distinguished from those of alleles at a single locus and since many compound loci were once thought to be single gene loci. However, this may no longer be true because the two phenomena, linkage and compound loci, can now be separated due to the advance of molecular markers.

Non-allelic interaction or epistasis was the last major theory proposed as an explanation of heterosis. Fisher (1949) and Mather (1955) both considered epistasis to be an important factor, if not the major one, in heterosis and inbreeding. Mangelsdorf (1974) stated that maybe the best evidence for non-allelic interaction in heterosis is the vigor of allopolyploids. The important point being that allopolyploids originating from different species are often more vigorous than their parents for numerous measurable traits. This type of heterosis then must be the product of interaction between genes in different genomes.

Since understanding the nature of heterosis is of such paramount importance to corn breeders much research has been conducted to distinguish between the different theories explaining heterosis. Mangelsdorf (1974) concluded that all three currently recognized theories: linked favorable dominants, overdominance and epistasis occur in maize and the problem is then to determine which theory or theories is operating in a given circumstance. Hallauer and Miranda (1981) concluded that there is more evidence for dominant linked factors than overdominance but conceded that definite proof for either will be difficult to establish. Whether this is true remains to be seen as we approach the 21st century and the explosion of information, that has already begun and certainly will continue, from biotechnology.

Stuber et al., 1992 in a recent paper on using molecular markers to study the inheritance of quantitative traits contributing to heterosis and genotype by

environment (G x E) interaction, reported mapping quantitative trait loci (QTLs) and evaluating their phenotypic effect on seven major traits in a cross between two elite inbred lines of maize (B73 and Mo17). Nine isozyme and 67 RFLP loci were used to determine the genotypes of 264 F_4 families. Phenotypic evaluation of the seven traits were done on 528 backcross families produced by backcrossing each of 264 F_3 lines to each of the parental lines. They reported finding QTLs affecting grain yield on all 10 maize chromosomes. They found six QTLs that showed an overall phenotypic effect of 46.7 bushels per acre, and accounted for 60 percent of the phenotypic variation for yield. They also showed that, with one exception, whenever a QTL for yield was found the heterozygote had the superior phenotype. Thus they concluded the majority of QTLs for yield were associated with overdominance, but conceded that their results could not distinguish between overdominance and the possibility of pseudo-overdominance. They reported no evidence for epistasis in heterosis, which is in contrast to a study by Zehr et al. (1992) in which they reported that digenic epistasis appeared to be important in grain yield expression.

Involvement of Gibberellins (GAs) in the Regulation of Heterosis

Possible involvement of endogenous plant hormones in heterosis was reported as early as 1940 (Robbins, W.J., 1940). Sarkissian et al., 1964 and Paleg, 1965 independently proposed that the control of α-amylase synthesis and subsequent hydrolysis of seed reserves infers a link between GAs and heterosis in maize, because heterosis is observed for seed germination and early seedling growth. Rood et al.,

1988 studied the role of GAs in regulating heterosis in a diallel study of elite inbreds and their hybrids. They observed significant genotypic differences for GA concentration in apical meristems and surrounding leaf tissue. In 11 of 12 comparisons, mean concentrations of GAs in hybrids were higher than in their parental inbreds. They reasoned that if GA level is responsible for heterosis of shoot growth, then exogenous application of GA should preferentially increase the growth of inbreds relative to hybrids. Application of exogenous GAs in their study confirmed this; in all cases inbreds were more responsive than hybrids. In addition, the inbreds approached hybrids in terms of shoot height after the application of exogenous GAs. Rood et al., 1990 extended the experimentation to include additional genotypes in order to determine whether the relationship between GA and heterosis was general in maize. Recently, this same relationship has been shown to apply to shoot growth in sorghum (*Sorghum bicolor* L.) (Rood et al., 1992). Rood et al., 1988 reasoned that the enhanced production of GAs in hybrids relative to inbreds is probally the result of an enhancement of overall GA biosynthesis in the hybrids. They suggested that enhanced biosynthesis of GAs in hybrids could result from enzymic polymorphism, known to occur in maize, and that the heterozygous condition of hybrids may confer biosynthetic superiority.

Prediction of F1 Performance and Heterosis

As Sprague (1983) aptly stated: "The plant breeder's interest in this controversy is secondary: he is more interested in the question of whether heterosis

can be manipulated to produce more productive forms". Recognition of inbreds with high combining ability for yield is the most costly phase in maize breeding efforts today. Generally this requires extensive field testing of cross performance between literally thousands of inbred lines. From a breeders standpoint then, a more appropriate question becomes: How can we predict the expression of heterosis in crosses between two inbred lines ?

From a study of maize from different geographical regions, Moll et al. (1962) concluded that heterosis in maize appears to increase with increased genetic divergence of the parent populations over a rather wide range of diversity. Realizing that this experiment could not account for differences in adaptation since only one location was used, Moll et al. (1965) studied six of the same varieties in four different geographic regions. They concluded that heterosis increased with increasing divergence within a restricted range, but that extremely divergent crosses result in a decrease in heterosis.

Genetic relatedness between inbreds is determined from estimating the probability of inheriting identical alleles by descent. Most of these estimates are indirect and require the assumption that such a relationship exists. Using geographic divergence as estimates of genetic divergence requires such an assumption and must be tested on a case-by-case basis (Price et al. 1986).

Malecot's coancestry coefficient has been used to estimate genetic diversity in many autogamous crops, but coancestry coefficients of maize have been difficult

to determine because of incomplete or unreliable pedigree data. For example, Smith et al. (1990) reported a "default" coefficient of parentage of zero between several inbred lines because no pedigree relationships between those lines were available. They conceded that the assumption of zero for a coefficient of parentage between these lines did not imply lack of relationship. Furthermore, relatedness based on coancestry coefficients may be inaccurate because of over simplified models assuming equal parental contribution and no selection (Melchinger et al. 1991).

Morphological characters have been used for description and identity of inbreds and to assign inbreds to heterotic groups. These characters have been recognized as being unreliable because of environmental interactions and unknown genetic control of these traits (Smith and Smith 1989). More recently molecular markers such as isozymes and restriction fragment length polymorphism have been proposed as potential tools to predict heterosis of single crosses (Kannenberg 1971; Stuber and Moll 1972; Burr et al. 1983). Molecular markers make superior descriptors of genotype since they are not environmentally affected and their genetic basis is generally well understood (Melchinger et al. 1991). This approach involves identification of superior inbred combinations from molecular data to restrict field testing to only the most promising hybrids (Boppenmaier et al. 1992).

Hunter and Kannenberg (1971) attempted to relate heterosis in maize with diversity at enzyme loci using 15 inbred lines of maize and six enzyme systems. They reported a non-significant correlation of $r = 0.09$ between yield and diversity

at enzyme loci for the 104 hybrids produced from the 15 inbred lines. However, when grouped as "high" or "low" diversity, the high diversity group's mean yield exceeded the mean yield of the low diversity group. They also concluded that 11 enzyme loci represent a small fraction of a genome and might not be expected to predict hybrid performance consistently. Several studies during the last two decades have been published relating diversity at enzyme loci with hybrid performance. In general, studies with unrelated lines have shown that diversity at enzyme loci is not a good predictor of hybrid performance (Hunter and Kannenberg, 1971; Heidrich-Sobrinho and Cordeiro, 1975; Stuber et al., 1980; Price et al., 1986; Lamkey et al., 1987; Smith and Smith, 1989). One exception was reported by Frei et al., 1986, who found a significant correlation between isozyme diversity and grain yield when analysis was restricted to crosses with similar pedigree background. Several reasons for the poor ability of isozyme diversity to predict hybrid yield have been given in addition to that mentioned above by Hunter and Kannenberg. They are: 1) absence of a correlation between enzyme diversity and combining ability, 2) enzyme diversity, as measured by enzyme loci, may not be linked to heterosis, and 3) other factors in heterosis may be involved (Hadjinov et al., 1980 Lamkey et al., 1987).

Burr et al., in 1983 suggested that use of restriction fragment length polymorphisms (RFLPs) might overcome the limitations of isozyme studies. Nearly unlimited numbers of RFLP loci and large numbers of polymorphisms in maize have facilitated the construction of extensive RFLP linkage maps (Helentjaris, 1987; Burr

et al., 1988). These RFLP loci allow calculation of genetic distances based on more complete genome sampling than was possible with isozyme markers (Smith et al., 1990).

Association of RFLPs among eight maize inbreds crossed in diallel with yield of their single crosses was investigated by Lee et al., 1989. Restriction fragment length polymorphism data were obtained on the eight inbreds using five restriction enzymes, five cDNA and 28 genomic clones distributed over the maize genome. Banding patterns of the single crosses were predicted from analysis of the inbred parents. Analysis of variance and principle component analysis was used to detect association of genetic distance, calculated as modified Rogers' distance (MRD), with yield. Genetic distances calculated for inbreds and hybrids from RFLP data agreed with known pedigree information and they suggested that RFLP analysis may be used as an alternative to field testing when assigning maize inbreds to heterotic groups. In contrast, Godshalk et al., 1990 found no association between genetic distance calculated from RFLP data and agronomic performance of 47 single crosses produced from inbred lines from different heterotic groups.

Melchinger et al., 1990 concluded that RFLPs may be useful for studying pedigree relationships among inbred lines but genetic distances computed from RFLP data are of limited value in predicting heterotic performance of single crosses between unrelated lines. In 1991, Melchinger et al. supported the proposal of Lee et al., 1989 that genetic distance estimates form RFLP data are useful for assigning

maize inbreds to heterotic groups. However, they suggested that a larger number of probe-enzyme combinations are necessary to accurately measure genetic distance between inbred lines.

Smith et al. (1990) used 257 probe-restriction enzyme combinations and cluster analysis to calculate genetic distances between 37 inbred lines representing both related and divergent elite maize germplasm. They reported high correlation for genetic distances calculated from coefficient of parentage and a measure of similarity based on RFLP data. Although genetic distances calculated from RFLP data were nearly the same as those from pedigree data, the RFLP data were more informative since they included both a measure of identity by descent and identity in state. Smith et al., 1990 also concluded that RFLP-based genetic distances provide better prediction of F_1, yield than any other predictors that they had examined. They suggested that additional information on quantitative trait loci for yield may provide a weighting of markers that would improve the predictive power of genetic markers.

Dudley et al. (1991) used modified Roger's distance (MRD) values and data from a 14 line diallel of maize in an attempt to predict yield potential of hybrids and assign inbreds to heterotic groups. They concluded that MRD values were not significantly correlated with hybrid yields, but that a hybrid value based on the number of marker loci having the highest yielding genotype was significantly associated with hybrid yield. They further reported that a measure of relationship based on marker data was significantly correlated with a measure of relationship

based on yield if the parents of the hybrids were distantly related. Other researchers recently have supported this conclusion that RFLP-based measures of genetic distance are of limited value in predicting hybrid performance of single crosses between unrelated lines from different heterotic groups (Melchinger et al., 1992; Boppenmaier et al., 1992).

Bernardo (1994) has proposed the use of best linear unbiased prediction (BLUP) based on RFLP data for parental inbreds and information from a related set of single crosses for prediction of maize single-cross performance. This approach requires 1) RFLP information on the relationships among inbreds within heterotic groups, and 2) hybrid performance of a related set of single crosses. The BLUP method facilitates the analysis of unbalanced yield performance data such as is commonly available in maize breeding programs. For example, yield performance information may be available for a large number of testcrosses that were not all grown in the same locations or years. One of the weaknesses of the BLUP method has been the requirement for precise estimates of genetic variances. Bernardo, 1994 discussed a method of obtaining these variances from unbalanced information in breeding programs. First a balanced reference set of inbreds can be selected from each heterotic group. Then crosses can be made between the reference inbreds from different heterotic groups and grown in the same locations for several years. Genetic variances can then be estimated from this balanced data set. The BLUP method can then be used to predict hybrid performance of crosses between future inbreds from the different heterotic groups. Correlations between predicted and

observed single-cross yield ranged from 0.688 to 0.800 where the theoretical maximum correlation was 0.901. He concluded that BLUP may be useful for prediction of superior single-cross combinations but conceded that more research was necessary on data sets with a larger number of crosses than the 54 single crosses in the study.

Methods for Identification of Parental Maize Germplasm with Favorable Alleles

Recognition of parental germplasm with superior cross performance is critical to the success of corn breeding programs (Hallauer and Miranda, 1988). Choice of parental germplasm remains the most costly phase of hybrid corn development after more than eighty years since Shull (1909) proposed hybrid breeding in maize. Traditionally, evaluation of potential germplasm has involved breeders intuition, information on heterotic patterns, and/or assessment of average performance in extensive testcrosses with inbred lines, populations, and various hybrids as well as per se performance of the germplasm (Kramer and Ullstrup, 1959; Abel and Pollak, 1991). This approach has proved successful in increasing grain yield of maize hybrids over the last 40 years (Duvick, 1984). Quantitative genetic theory has been used to predict the probability of success in identifying superior inbreds in segregating populations from a cross between two inbreds (Bailey, 1977), and more recently to develop methods for choosing parental germplasm with favorable alleles in maize pedigree breeding programs (Dudley, 1984a,b; 1987a,b; Gerloff and Smith, 1988a,b). Restriction fragment length polymorphisms (RFLPs) have been used to

determine heterotic patterns (Dudley et al., 1991), to identify quantitative trait loci (QTLs) associated with yield and heterosis (Smith et al., 1990, Stuber et al., 1992) and as a tool to detect favorable alleles in donor populations of maize (Dudley et al., 1992, Zehr et al., 1992). These latter approaches should achieve better results because sources of new favorable alleles can be more accurately identified with fewer resources and in a shorter time period.

Dudley, 1984a proposed a method for estimating the relative number of favorable alleles for any given trait (μG) present in a potential donor inbred (I_w) but not present in the single cross to be improved ($I_1 \times I_2$). This method requires performance evaluation of the inbred parents (I_1,I_2), the potential donor inbreds (I_w), the single crosses of I_1 and I_2 with each of the donor lines ($I_1 \times I_w$, $I_2 \times I_w$ ...), and the elite single cross $I_1 \times I_2$ per se. Also, the method provides an estimate of the relative relationship (identity in state) of the I_w lines to I_1 and I_2. A summary of the theoretical basis of this method follows. Only eight classes of loci exist for any three inbred lines they are :

Example 1. (From Dudley, 1984a)

Class of loci	I_1	I_2	I_w
A	+	+	+
B	+	+	-
C	+	-	+
D	+	-	-
E	-	+	+
F	-	+	-
G	-	-	+
H	-	-	-

Where (+) represents loci homozygous for favorable allele, and (-) loci homozygous for unfavorable allele. Of the eight classes, class G is of most interest since I_w contains favorable alleles not present in either I_1 or I_2. Let A, B, C, D, E, F, G, and H be the number of loci in their respective classes. Furthermore, let μ, aμ, and -μ be the genotypic values of the three possible genotypes at a locus: ++, +-, and --, respectively. Now, four assumptions are necessary : 1) μ is constant for all loci, 2)a = 1 (complete dominance), 3) there is no epistasis, and 4) $\mu A = \mu H$. Under these assumptions, the genotypic values for each line and the possible F_1's and F_2's between the lines can be written in terms of the classes of loci and μ.

Example 2. (From Table 2. Dudley, 1984a)

$$I_1 = (B + C + D - E - F - G)\mu$$

$$I_2 = (B - C - D + E + F - G)\mu$$

$$I_1 \times I_w = (B + C + D + E - F + G)\mu$$

Now substitution of a measurement of interest like yield for I_1, I_2, I_w, $I_1 \times I_2$, $I_1 \times I_w$, and $I_2 \times I_w$ gives six equations with seven unknowns. Dudley (1984a) showed that, since μ is a constant multiplier, these equations can be resolved in terms of μB, μC ... μG which are estimates of relative number of loci in each class.

Example 3. (From Table 3. Dudley, 1984a)

$$\mu G = [(I_2 \times I_w) + (I_1 \times I_w) - I_w - I_2 - I_1 - (I_1 \times I_2)]/4$$

Positive estimates of μG are an indication that I_w has favorable alleles not present in either of the other parents. Dudley (1984a) discussed application of his method to a maize breeding program. A summary of his discussion follows:

> Suppose that your objective is to find an inbred line that can be used to improve one of the parents of an elite single cross $I_1 \times I_2$ and you have 10 donor lines (I_w lines) which might be used for improvement of I_1 or I_2. First, cross the 10 donor lines to both I_1 and I_2. The following season conduct a performance trial using the 20 F_1's, $I_1 \times I_2$, the 10 I_w lines per se, I_1 and I_2. In addition produce F_2 seed from each of the F_1's. After evaluation of performance trial data, begin selection in the F_2's with the largest μG values. Consideration should be given to the relative values of $\mu C + \mu F$ and $\mu D + \mu E$ to determine which of the F_2's should be used to maintain the original heterotic pattern.

Dudley (1984b) in a similar manner developed an estimator $\overline{p}_{\mu}$ for identifying populations with favorable alleles not present in elite germplasm. The theoretical basis for $\overline{p}_{\mu}$ is essentially the same as for μG and will not be repeated.

Gerloff (1985) showed that failure of Dudley's assumptions: $\mu A = \mu H$ (where A and H represent the number of loci ++ and -- respectively in I_1, I_2, and I_w) and $z = -\mu$ could lead to erroneous estimates of the relative value of populations as donors of favorable alleles. Dudley (1987a,b) presented modified, less biased

estimators of favorable alleles μG' and $\bar{p}_{\mu}$' in which these assumptions were removed. Estimates of $\bar{p}_{\mu}$' are calculated from one of four equations (cases) on the basis of the frequency of the alleles at Classes *j* and *k* in the donor population. The criteria for determining the proper case depends on the fact that the F_1 performance must be either greater or lesser than the performance per se of both inbred parents and is given in detail in Dudley (1987b). It seems that this dependency might make determination of the proper case difficult for traits (like grain moisture, maturity, and lodging) that show little heterosis. Gerloff and Smith (1988a) proposed another estimator UBND and used computer simulation to compare test-crossing to a single cross (TCSC), UBND, and $\bar{p}_{\mu}$ as methods of identifying favorable alleles (Gerloff and Smith, 1988b). Their results from computer simulation, assuming complete dominance, no epistasis, and no restrictions on allelic effects at each locus, indicated that the TCSC method was more favorable than $\bar{p}_{\mu}$ and UBND because of the correlation of TCSC with the actual superiority measure and because of testing resources required.

Comparing estimates of $\bar{p}_{\mu}$, $\bar{p}_{\mu}$', UBND, and TCSC with simulated data, Dudley, 1987b showed that estimates of $\bar{p}_{\mu}$' ranked the populations more closely to the actual performance measure than any of the other three methods. Zanoni and Dudley, 1989 compared several methods of estimating the value of inbred lines as donors of favorable alleles to improve an elite single cross. Their data indicated that μG' was highly correlated with TCSC and UBND estimates for most traits and with GCA for traits where additive genetic effects are important. They also showed that

estimates of relative genetic distance of I_w to I_1 or I_2 was in good agreement with known pedigree information. Their conclusions were supported by Mišević (1989) who also reported that the highest values for all estimators of new favorable alleles were found for donor lines that were in different heterotic groups than the single cross to be improved. Mišević (1990) using Dudley's minimally biased estimate of new favorable alleles ('$\bar{p}_{\mid}\mu$') reported significant differences among donor hybrids when twenty-four commercial hybrids were considered as target single crosses. However, he reported that ranking hybrids for their value as a direct source of inbred lines to be crossed to a parent of a target single cross did not always agree with their ranking as a source of new favorable alleles for improving a single cross. Hogan and Dudley (1991) concluded that '$\bar{p}_{\mid}\mu$' precisely ranks populations or inbreds relative to frequencies of favorable alleles and that '$\bar{p}_{\mid}\mu$' should be useful in choosing parents to improve elite single crosses. Pfarr and Lamkey (1992a) also demonstrated that the '$\bar{p}_{\mid}\mu$' and UBND estimators were equally effective for identifying populations with the greatest frequency of favorable alleles not present in an elite single cross. Pfarr and Lamkey (1992b) evaluated the methods proposed by Dudley (1984a, 1987b) by applying the methods to populations of known composition and relationship. They showed that '$\bar{p}_{\mid}\mu$' correctly identified the population expected to have the highest frequency of favorable alleles in three of five single crosses for grain yield and four of five single crosses for ear height. Their conclusions differed from those of Hogan and Dudley (1991) because they concluded that the successful application of '$\bar{p}_{\mid}\mu$' to exotic populations of uncertain merit is uncertain because of low frequencies of

favorable dominant alleles in exotics for economically important traits.

Dudley et al. (1992) calculated a donor value (DV), using genotypic means for loci that showed significant associations with grain yield, which was highly correlated with μG. They suggested that the utility of DV in a maize breeding program was not clear due to the relatively expensive molecular marker data even though high correlations with μG were encouraging. They further suggested that the use of DV might be justified if molecular data could also be used to follow favorable QTLs for yield in segregating generations.

Stuber et al. (1992) used molecular markers to study quantitative traits contributing to heterosis and genotype by environment interaction. They reported mapping QTLs affecting grain yield on all 10 chromosomes, six of which accounted for 60 percent of the phenotypic variation for yield. They also showed that whenever a QTL for yield was found the heterozygote had the superior phenotype with one exception. Thus, they concluded that the majority of QTLs for yield were associated with overdominance, but conceded that their results could not distinguish between overdominance and the possibility of pseudo-overdominance.

Beavis et al. (1994) used the same statistical techniques, progeny from the same parents, and similar effective sample sizes as Stuber et al. (1992) to search for QTLs for yield. They reported yield QTLs on chromosomes 1, 2, 4, 5 and 9. Only one QTL (located on chromosome 5) identified by Beavis et al. (1994) was in common with those identified by Stuber et al. (1992). Beavis et al. (1994) suggested

that the most likely explanation for this incongruity was the small number of independently sampled lines for each study. They concluded that this lack of congruency suggests that the number of yield QTL is large and that two breeders selecting for the same traits in the same environments on independent samples of progeny will select for different sets of QTLs. They suggested that this would result in different lines that could be crossed for additional genetic gains.

Zehr et al., 1992 used RFLP's as markers to search for favorable alleles in a maize population [BS11(FR)C7] not present in an elite single cross hybrid (FRMO17 × FRB73). They used linear contrasts to determine statistical associations between segregation of RFLP markers among F_2 individuals and QTL's in F_2 × FRB73 progeny. They found RFLP bands unique to BS11(FR)C7 that were favorable over those from one of the parents for several traits. Multiple bands at a marker locus for most of the markers presented problems in determining statistical associations between markers and QTL's. They addressed this problem by using linear contrasts to test the effect of substitution of each band at a marker locus while pooling the others. A marker was considered associated with a QTL if any one of its linear contrasts was significant. Then a t-test was used, for each marker with multiple bands associated with a QTL, to group bands with similar effects. They interpreted bands within the same t-group to be associated with QTL alleles with similar effects. Application to marker-assisted selection was discussed. They suggested that when bands associated with the most favorable effects were in the same t-group, selection should be against bands not in the t-group and conversely for

a band only when the favorable band was not in the t-group. In contrast to Stuber et al. (1992), they reported that digenic epistasis seemed to be important for yield as indicated by a 12 percent increase in the proportion of variation for yield which was accounted for when di-marker interactions were added to the linear model. They finally concluded that their work may be useful in selecting F_2-derived lines or backcross progeny with increased grain yield over FRMO17 in crosses to FRB73, but that more information would be needed to determine whether these affects are consistent for other inbred testers.

Simple sequence repeats (SSRs) have recently been described as having potential for detecting the precise targets of both natural and man-directed selection (Saghai Maroof et al., 1994). Also called microsatellites, SSRs are short tandem repeats in repetitive DNA and offer some advantage over RFLPs because of their abundance and the fact that polymorphisms between them can be detected using polymerase chain reaction (PCR) (for details see Saghai Maroof et al., 1994). Another recent study reports the use of RFLP and SSR markers for analysis and prediction of heterosis in rice (*Oryza sativa*) (Zhang et al., 1994). Correlations between hybrid rice yield and molecular marker based heterozygosity in this study were significant for mid-parent heterosis of yield, seeds/panicle, and F_1 kernel weight.

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Combining Ability and Heterosis of Novel Maize Germplasm With Elevated Protein

ABSTRACT

Improving protein quality and quantity in normal dent maize without the use of endosperm mutants is an alternative to Quality Protein Maize (QPM). The objectives of this study were to (i) determine the combining ability and heterotic relationships for protein concentration, grain yield, maturity, and kernel hardness of a novel high-protein maize inbred W1 with 11 normal inbreds; and (ii) assess the potential of W1 in the development of acceptable high-protein maize hybrids. Twelve maize inbreds were used in a diallel study in four environments. Protein concentration, grain yield, grain moisture at harvest, time to silk, and kernel hardness and density were measured. Significant genotype × environment interactions for nearly all effects suggest that breeding efforts and the evaluation of cross performance for these traits should be conducted in the environment of interest. General combining ability (GCA) and specific combining ability (SCA) effects were highly significant for all traits indicating both additive and non-additive effects, respectively. The orthogonal contrast parents vs. crosses was significant for all traits, confirming the presence of nonadditive gene effects. Reciprocal effects (REC), often assumed to be absent in maize diallel studies, were significant for grain yield and protein concentration suggesting that choice of female parent may be important for these traits. Ratios expressing the relative importance of GCA and SCA indicate that protein concentration is controlled primarily by additive gene action. Negative mid-parent heterosis for protein concentration gave evidence for partial dominance for low protein. A theoretical genetic model is presented that shows additive gene action for protein concentration in W1 crosses. While we believe this model indicates the true genetic control of protein concentration in W1 crosses, it does not alter the significance of an effective partial dominance for low protein to breeding programs. The development of high-protein hybrids from W1 should be straightforward since W1 had high positive GCA estimates for protein concentration in all four environments. This study demonstrates the existence of novel high-protein germplasm with new promise of commercially acceptable high-protein hybrids.

Maize has sometimes been referred to as the "King of feeds" and holds a dominate place in world agriculture because it produces high grain yields, is highly digestible, is among the highest in net energy content and lowest in fiber. Normal corn protein, however, is deficient in the essential amino acids lysine and tryptophan. Because of this unfavorable amino acid composition, normal corn must be supplemented with more expensive protein sources in both human and monogastric animal diets.

Evidence that the protein content and quality of corn can be modified by breeding has been well established. Past work to improve the quality of corn protein has centered around the discovery of the opaque-2 mutant gene, reported by Mertz et al. in 1964. Their report that the opaque-2 gene nearly doubled the lysine content of maize endosperm tissue stimulated much research interest in improving the protein quality of maize and several other cereals. Great hopes for opaque-2 corn were never brought to fruition because the grain had many undesirable characteristics: reduced yields and soft chalky appearing kernels with lower resistance to fungi and insects in the field and in storage (Nelson, 1966; Harpstead, 1969; Lambert et al., 1969; Sreeramulu and Bauman, 1970; Francis et al., 1970; Singh and Asnani, 1975; Arnold et al., 1977; Vasal, 1975; Vasal et al., 1980).

Breeders at the International Maize and Wheat Improvement Center (CIMMYT) have subsequently developed opaque-2 materials with normal appearing kernels known as Quality Protein Maize (QPM) by exploiting genetic modifiers of

the *o*2 locus (Vasal, 1975; Vasal et al., 1980). Quality Protein Maize materials with varying proportions of translucent and opaque fractions have been studied by a number of researchers (Bauman and Aycock, 1970; Gentinetta et al., 1975; Bjarnason et al., 1976; Vasal, 1975). Today, the biggest disadvantage of QPM continues to be reduced yields. Bockholt and Rooney (1992) speculated that yield of QPM hybrids would always lag behind normal hybrids by 10 to 12 generations, due to the time required to convert inbreds to opaque-2 and then to QPM using conventional techniques.

Improving protein quality in normal dent maize without the use of endosperm mutants, as an alternative to QPM has been the subject of several studies. Zuber and Helm (1975) reported the successful increase of lysine content in three maize populations by recurrent selection. Significant variation between races and strains of maize for protein and several amino acids, including lysine, tryptophan, and methionine, has been reported by Frey et al., 1949; Miller et al., 1952; Aguirre et al., 1953; Tello et al., 1965; Paez et al., 1969; and numerous others.

We learned of W1, a privately owned normal-endosperm inbred with an elevated level of high-quality protein in the grain, in 1990. Company data showed that W1 had concentrations of 166 g kg^{-1} for protein and 4.2 g kg^{-1} for lysine. These concentrations are considerably higher than typical of normal maize inbreds which have average concentrations of 90 to 110 g kg^{-1} for protein and approximately 2.1 g kg^{-1} for lysine. Recent studies have confirmed the favorable amino acid composition

of hybrids containing W1 as one of the parental inbreds (Bond et al., 1991; Burgoon et al., 1992; Dove, 1994). Bond et al. (1991) concluded that W1 hybrids have the potential to spare soybean meal or other protein supplements in poultry, and to improve performance. Dove, (1994) compared a W1 hybrid and a normal elite commercial hybrid in a feeding trial with finishing pigs. He reported that use of the W1 hybrid in the diet reduced the need for soybean meal by 56 % which resulted in a $14-16 per ton decrease in diet costs.

Information on the combining ability and heterotic relationships of the W1 inbred with other commercial inbreds is essential for development of new competitive high-protein hybrids. The objectives of this study were to: (i) determine the combining ability and heterotic patterns for protein concentration, grain yield, maturity, and kernel hardness of W1 with 11 normal inbreds; and (ii) assess the potential of W1 as a parent in the development of acceptable high-protein maize hybrids by comparing grain yield, protein concentration, and physical kernel characteristics of W1 hybrids with normal hybrids.

MATERIALS AND METHODS

Twelve normal yellow maize inbreds including W1, and representing a wide genetic base were crossed in a diallel mating design in Hawaii during the 1990 winter season. Pedigree, origin, and days to relative maturity for the inbreds are given in Table 1. The parents and their crosses, including reciprocals, totaling 144 entries were planted with a cone-type plot planter in two locations during both the 1991 and 1992 seasons (Table 2). One-hundred-twelve kernels from each entry were planted in four 6.1-m rows spaced 76 cm apart to achieve a final population of $\approx$ 54 361 plants ha^{-1}. The experimental design was a randomized complete block, with three replications in each environment. The experimental unit consisted of the two center rows of a four-row plot.

Data were recorded for time to silk (days from planting to 50% extruded silks), grain weight, total Kjeldahl N, percentage floaters on a 1.25 specific gravity $NaNO_3$ solution, percentage grain moisture at harvest, and weight of 500 kernels. Avoiding row ends, a subsample of five random ears from each plot (taken before machine harvest for grain weight, dried to constant moisture in a forced-air oven, and hand shelled) was used to measure protein concentration, percentage floaters, and weight of 500 kernels. Percentage floaters was measured following the method of Wichser (1961) and is considered a measure of kernel hardness. Samples used for Kjeldahl N were ground to pass a 1-mm screen and digested with H_2SO_4 in the presence of a Se catalyst. Protein concentration was calculated as total Kjeldahl N

Table 1. Background, origin, and days to relative maturity of the twelve inbred lines used in this diallel study.

Inbred line	Background	Origin	Days to relative maturity
H1	Mo17	Holdens[1]	115
H2	LH7, W153R	Holdens	106
H3	Pioneer 3535	Holdens	117
H4	B37, B73	Holdens	115
H5	B73	Holdens	115
H6	A662, B73	Holdens	111
O1	Private B73	Orsan[2]	110
W1	Private unrelated Tropical	Wilson Seeds[3]	125
W2	Private B73, Tuxpeno	Wilson Seeds	118
W3	Private Lancaster, Tuxpeno	Wilson Seeds	116
W4	Private Lancaster, Tuxpeno	Wilson Seeds	116
W5	Private Mo17, R177, H99	Wilson Seeds	115

[1]Holdens Foundation Seed Company, Williamsburg, IA.
[2]Orsan Inc., Les Ulis Cedex, France.
[3]Wilson Seeds Inc., Harlan IA.

Table 2. Location, year, and characteristics of the four environments used in this study.

Location	Year	Altitude above sea level	Latitude	Longitude	Precipitation†	Irrigation water added
		m			cm	cm
Blacksburg, Virginia	1991	610	37°12′ N	80°32′ W	34	-
	1992				77	-
Mount Holly, Virginia	1991	43	38°06′ N	76°42′ W	49	14
	1992				62	12

†Total precipitation for the months April, May, June, July, and August.

times 6.25. Percentage dry matter was determined using subsamples of the ground material according to the Association of Official Analytical Chemists (1975) method. Grain moisture at harvest was measured in the field with a commercial moisture tester. Yield was calculated from the sum of the grain weight from machine harvest plus the grain weight of the 5-ear sample. Yields were adjusted to 155 g kg^{-1} grain moisture.

Statistical Analysis

Analyses of variance were conducted, using individual plot data, for grain yield, time to silk, protein concentration, percentage floaters, and 500-kernel weight for each environment separately (data not shown) and then combined across environments. Environments were considered random effects and genotypes fixed effects. Analysis III of Gardner and Eberhart (1966) was used to obtain estimates of general (GCA) and specific combining ability (SCA) and reciprocal effects (REC). Orthogonal partitioning of the total entry variance by least-squares was used to estimate the effect of parents vs. crosses, a test of average heterosis. Variation among crosses was further subdivided into that due to GCA, SCA, and REC. The model used to predict hybrid performance is as follows:

$$x_{ijkl} = \mu + g_i + g_j + s_{ij} + r_{ij} + e_{ijkl}$$

where x_{ijkl} is the performance of the cross between the *i*th and *j*th genotypes in the *k*th replication in the *l*th environment; μ is the overall mean; g_i and g_j are GCA effects for the *i*th and *j*th parents, respectively; s_{ij} is the SCA effect for the cross

between the *i*th and *j*th genotypes; r_{ij} is the REC effect for the cross between the *i*th and *j*th genotypes; and e_{ijkl} is the error effect associated with the *ijkl*th observation. The relative importance of GCA and SCA was studied by calculating ratios of GCA and SCA mean squares and sums of squares, as suggested by Baker (1978) and Auld et al. (1983):

Baker: $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$

Auld et al.: $SS_{GCA} / (SS_{GCA} + SS_{SCA})$.

Proximity of these numbers to unity indicates primarily additive gene effects.

Analyses of variance tests of significance for main effects and their partitions were conducted with their respective mean squares for interaction with environment used as the denominator of the *F*-test. Interactions with environment were tested against the pooled error. Percentage heterosis for grain yield and protein concentration was calculated relative to the high-parent and low-parent values, respectively.

RESULTS AND DISCUSSION

Analyses of Variance

Mean squares from the analyses of variance, combined across environments, for six traits (Table 3) revealed significant differences ($P \leq 0.01$) among environments for all traits. The differences can be largely attributed to differences in soil water (Table 2) and temperature (data not shown) between environments. Blacksburg, VA in 1992 and Mount Holly, VA in both 1991 and 1992 had favorable environments for yield throughout the growing season. Hot and dry conditions in the Blacksburg 1991 environment resulted in a 3 Mg ha^{-1} reduction in mean grain yield and a significant increase in protein concentration and kernel hardness. Large differences in mean performance due to differences in environment are favorable for expression of genotype × environment (G × E) interactions. Significant G × E interactions for nearly all effects in this study suggest that when differences between environments can be predicted in advance; breeding efforts and the evaluation of the performance of crosses for these traits should be conducted in the environment of interest.

Entry mean squares were highly significant ($P \leq 0.001$) for all traits as were their subdivisions into differences among parents and among crosses (Table 3). The orthogonal contrast parents vs. crosses was also significant for all traits, indicating the presence of nonadditive gene effects for these traits. Partitions of the sum of squares due to crosses revealed significant GCA and SCA for all traits, indicating

Table 3. Combined analyses of variance of diallel crosses among 12 normal maize inbreds for grain yield, protein concentration, grain moisture at harvest, time to silk, percentage floaters, and weight of 500 kernels.

Source	df	Mean squares					
		Grain yield	Protein	Grain moisture at harvest	Time to silk	Floaters	500 kernel weight
		Mg ha^{-1}	g kg^{-1}	g kg^{-1}	d	%	g
Environment (E)	3	728.93***	17781.2***	38805.4***	16770.7***	48721.6***	17679.2**
Replication/E	8	2.87**	205.8***	471.3***	30.5***	1118.9***	1698.5***
Entry (G)	143	73.70***	1101.5***	508.4***	70.6***	3394.1***	5103.8***
Parents vs. Crosses	1	4643.38***	13659.4**	1914.0*	1570.1**	23218.4*	112648.7**
Parents	11	10.13***	3655.0***	619.3***	105.0***	5158.0***	5916.5***
Crosses (C)	131	44.15***	791.2***	488.4***	56.2***	3094.6***	4214.6***
GCA	11	108.06***	8266.1***	449.3***	535.0***	27736.8***	34644.4***
SCA	54	82.42***	161.8***	247.4***	24.8***	1641.0***	3015.6***
REC	66	2.19**	60.3***	18.2	2.0	176.9	124.0
G × E	429	3.24***	71.1***	74.9***	2.9***	534.5***	312.3***
Parents vs. C × E	3	35.75***	203.9***	88.5***	32.2***	1211.1***	2575.6***
Parents × E	33	1.78**	91.8***	160.8***	3.3***	708.6***	391.8***
Crosses × E	393	3.12***	68.4***	67.6***	2.7***	514.7***	288.3***
GCA × E	33	16.48***	337.8***	505.6***	6.8***	3057.9***	1341.4***
SCA × E	162	2.63***	62.1***	44.8***	2.9***	423.3***	293.7***
REC × E	198	1.28**	28.7	13.3	1.8***	165.7	108.4
Error	1144	0.97	26.5	12.9	0.9	153.9	110.5
Relative importance of GCA and SCA:							
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.72	0.99	0.97	0.98	0.97	0.96
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.21	0.91	0.79	0.81	0.77	0.70

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

both additive gene effects and deviations from additivity, respectively. Reciprocal effects were significant ($P \leq 0.01$) for grain yield and protein concentration. Reciprocal differences, often assumed to be absent in diallel studies, have been found in maize for tassel date, silk date, plant height, ear height, ear diameter, ear length, ears per plant, number of kernel rows, kernel weight, number of erect plants, number of leaves, moisture content at harvest, and grain yield (Abdalla 1974, Baynes and Brawn 1973, Fleming et al. 1960, and Singh 1966). Reciprocal differences originate from differences in cytoplasm, usually involving DNA in replicating organelles such as mitochondria, or from differences in the maternal environment provided to the developing embryo by the female parent. A thorough discussion of the causes for REC differences was given by Jinks in 1964. Regardless of their origin, REC differences may influence decisions in plant breeding programs. This is especially significant in a crop like maize in which F_1 hybrid seed is produced each year by crossing two parental inbreds, and where reversal of the parents in the F_1 hybrid may result in improvement of a character.

The ratios expressing the relative importance of GCA and SCA (Table 3) indicate that additive gene effects were of primary importance in inheritance of protein concentration, harvest moisture, time to silk, percentage floaters and 500-kernel weight. Additive as well as nonadditive genetic effects were important in controlling grain yield. This indicates that improvement of traits controlled primarily by additive gene effects could be made while simultaneously seeking crosses heterotic for grain yield.

Performance of Parents

Grain yield of the parents was low, ranging from 1.23 to 3.85 Mg ha^{-1} (Table 4). The highest and lowest grain yields for parents were recorded for H2 and H1, respectively. W1 was among the lowest but was not statistically different from the mean of the parents for grain yield. Protein concentration of W1 was 168 g kg^{-1}. This was clearly higher than the normal inbreds which fell in the narrow range 98 to 126 g kg^{-1}. H3 and W1 had the highest grain moisture at harvest (252 and 251 g kg^{-1}, respectively) followed by W4 (228 g kg^{-1}). The lowest grain moisture at harvest was recorded for H6 (188 g kg^{-1}). Harvest moisture of the remaining eight parents ranged from 191 to 215 g kg^{-1}. For time to silk, W1 and W2 were the latest at 79 d and H2 and O1 were the earliest at 71 d. The remainder of the parents ranged from 75 to 78 days for time to silk. Percentage floaters is an index of kernel hardness used by industry in commercial grain trade. H1, W2, and W4 had medium kernels ($>$ 60 and $<$ 81) whereas the remainder of the parents had hard kernels ($\leq$ 60). Weight of 500 kernels, another measure of kernel density, ranged from 99 to 164 g.

Mean Performance and Heterosis of Crosses

Grain yield among crosses ranged from 3.28 to 11.04 Mg ha^{-1} and high-parent heterosis for grain yield ranged from 16 to 469 percent (Table 4). Grain yield ranged from 7.93 to 9.64 Mg ha^{-1} for crosses involving W1 compared to the range 3.28 to 11.04 Mg ha^{-1} for normal crosses (Tables 4 and 5). Percentage high-parent heterosis

Table 4. Means of 12 maize inbreds and their crosses across four environments for grain yield, high-parent heterosis for grain yield, protein concentration, grain moisture at harvest, time to silk, percentage floaters, and 500 kernel weight.

Pedigree	Grain yield	Heterosis for grain yield	Protein	Grain moisture at harvest	Time to silk	Floaters	500 kernel weight
	Mg ha^{-1}	%	g kg^{-1}	g kg^{-1}	d	%	g
			Crosses				
H1 × H2	8.48	120.2	105	197	69	55	153
H1 × H3	9.21	160.9	107	228	73	23	188
H1 × H4	9.61	249.4	108	225	73	16	186
H1 × H5	9.99	356.2	98	234	73	23	182
H1 × H6	9.14	218.5	97	203	70	49	161
H1 × O1	10.35	176.0	98	218	70	48	178
H1 × W1	9.38	424.0	124	242	74	11	198
H1 × W2	10.87	469.1	98	227	73	22	181
H1 × W3	4.03	25.1	111	221	76	35	157
H1 × W4	3.28	16.3	113	205	77	57	154
H1 × W5	6.71	441.1	111	205	74	42	148
H2 × H3	9.95	158.4	109	216	70	33	143
H2 × H4	9.34	142.6	110	211	69	25	139
H2 × H5	9.48	146.2	107	204	70	30	141
H2 × H6	8.47	120.0	106	189	68	40	128
H2 × O1	9.32	142.1	102	194	68	49	128
H2 × W1	7.93	105.9	125	208	70	15	153
H2 × W2	8.94	132.2	109	201	72	32	135
H2 × W3	8.01	108.0	113	206	70	39	144
H2 × W4	8.74	127.0	111	208	71	59	152
H2 × W5	8.31	115.8	107	203	69	38	110
H3 × H4	10.07	185.3	112	233	73	9	170
H3 × H5	9.87	179.6	115	238	72	9	155
H3 × H6	9.07	156.9	105	215	72	20	152
H3 × O1	11.04	194.4	100	215	70	25	155
H3 × W1	9.55	170.5	129	257	74	11	176
H3 × W2	10.89	208.5	104	229	74	19	166
H3 × W3	10.05	184.7	113	246	74	12	179
H3 × W4	9.83	178.5	108	245	73	48	175
H3 × W5	9.89	180.2	113	231	73	11	157
H4 × H5	4.15	50.9	105	190	75	27	136
H4 × H6	5.30	84.7	107	194	74	27	132
H4 × O1	6.06	61.6	111	208	71	22	145
H4 × W1	9.22	235.3	128	248	73	11	169
H4 × W2	5.19	88.7	107	206	77	45	141
H4 × W3	9.92	208.1	110	246	75	25	186
H4 × W4	9.82	248.2	110	250	75	47	182
H4 × W5	9.01	227.6	110	224	73	14	150
H5 × H6	5.24	82.6	100	195	73	39	132
H5 × O1	5.34	42.4	101	200	71	45	145
H5 × W1	8.79	301.4	124	244	73	16	164
H5 × W2	5.64	157.5	103	209	75	52	149
H5 × W3	10.51	226.4	106	242	73	42	179

continued next page

Table 4. cont.

Pedigree	Grain yield	Heterosis for grain yield	Protein	Grain moisture at harvest	Time to silk	Floaters	500 kernel weight
	Mg ha^{-1}	%	g kg^{-1}	g kg^{-1}	d	%	g
			Crosses				
H5 × W4	10.41	269.1	107	245	73	50	181
H5 × W5	8.15	272.1	104	219	74	29	146
H6 × O1	6.06	61.6	96	187	70	41	125
H6 × W1	9.09	216.7	121	223	72	20	158
H6 × W2	5.42	88.8	100	194	76	64	132
H6 × W3	8.99	179.2	108	220	71	41	164
H6 × W4	9.70	237.9	100	213	71	68	157
H6 × W5	7.98	178.0	105	209	72	28	139
O1 × W1	9.01	140.3	116	231	71	27	162
O1 × W2	6.69	78.4	98	203	73	62	141
O1 × W3	10.25	173.3	101	228	71	55	174
O1 × W4	8.64	130.4	104	230	71	53	162
O1 × W5	9.13	143.5	101	203	70	46	137
W1 × W2	9.51	397.9	126	241	74	6	177
W1 × W3	9.64	199.4	126	263	74	14	185
W1 × W4	8.88	214.9	122	271	75	16	182
W1 × W5	8.51	375.4	129	250	73	5	159
W2 × W3	11.00	241.6	102	255	75	36	173
W2 × W4	10.68	278.7	101	261	75	59	179
W2 × W5	9.67	406.3	105	224	76	19	150
W3 × W4	3.20	-0.6	116	222	77	62	138
W3 × W5	6.76	109.9	114	234	74	37	140
W4 × W5	7.81	177.0	111	228	74	40	147
			Parents				
H1	1.23	-	112	191	78	66	153
H2	3.85	-	126	193	71	35	102
H3	3.53	-	125	252	78	23	134
H4	2.75	-	117	196	75	15	136
H5	2.19	-	105	200	76	45	121
H6	2.87	-	107	188	73	50	104
O1	3.75	-	98	190	71	60	114
W1	1.79	-	168	251	79	28	151
W2	1.91	-	124	201	79	80	110
W3	3.22	-	122	213	78	35	164
W4	2.82	-	113	228	77	76	142
W5	1.24	-	116	215	78	46	99
SE of a difference	0.80		4.2	2.9	0.8	10.1	8.6

Table 5. Range and mean for grain yield, protein concentration, grain moisture at harvest, time to silk, percentage floaters, and 500 kernel weight; and heterosis for grain yield and protein concentration across environments of W1 crosses compared to normal crosses.

Crosses	Grain yield	High-parent heterosis for grain yield	Protein	Low-parent heterosis for protein	Grain moisture at harvest	Time to silk	Floaters	500 kernel weight
	Mg ha^{-1}	%	g kg^{-1}	%	g kg^{-1}	d	%	g
W1								
Range	7.93 to 9.64	106 to 424	116 to 129	-0.8 to 18.4	208 to 271	70 to 75	5 to 27	153 to 198
Mean	9.05a†	253	125a	8.7	243a	73a	14b	171a
Normal								
Range	3.28 to 11.04	16 to 469	96 to 115	-16.4 to 13.3	187 to 261	68 to 76	9 to 68	110 to 186
Mean	8.48b	171	106b	-3.0	220b	72b	35a	156b

† Within columns, means not followed by the same letter are significantly different at probability 0.05 based on Student Newman Keuls test.

ranged from 106 to 424 and from 16 to 469 for W1 and normal crosses, respectively. The high heterosis exhibited by all W1 crosses is noteworthy, and suggests that W1 may belong to a separate heterotic class. This conclusion is in general agreement with pedigree information given in Table 1. Mean grain yield was higher for W1 crosses than for normal crosses, however the highest yielding W1 cross (W1 × W3) yielded only 87 percent as much as the highest normal cross (H3 × O1). One of the tenets in selection of lines for superior cross performance is that heterosis in maize increases with increasing divergence of the parents over a wide range of diversity. Moll et al. 1965, Melchinger et al. 1990, Boppenmaier et al. 1992, and others, however, have shown that this relationship does not hold over an unlimited range and that extremely divergent crosses result in decreased grain yields and heterosis. Since the occurrence of heterosis is dependent on directional dominance, and since cumulative differences in gene frequencies between isolated populations may become large (Falconer 1989), it seems logical that heterosis in crosses between two extremely divergent inbreds may be limited by lack of complementary gene combinations. Failure of the highest W1 cross to match the yield of the higher normal crosses may be the result of unharmonious gene combinations due to the wide genetic distance between W1 and the normal inbreds.

Protein concentration among crosses ranged from 96 to 129 g kg^{-1} (Table 4). Crosses involving W1 resulted in higher protein concentrations than normal crosses with one exception, O1 × W1, whose protein concentration was not significantly different from the highest normal cross, H3 × H5 (Tables 4 and 5). Mean protein

concentration was significantly higher for crosses involving W1 (125 g kg^{-1}) than for normal crosses (106 g kg^{-1}). Mid-parent heterosis was always negative for protein concentration (data not shown) suggesting dominance in the direction of low protein. Mean low-parent heterosis for W1 crosses was 8.7 % but was -3.0 % for normal crosses.

Negative low parent heterosis for protein concentration of normal crosses is noteworthy because if low protein was completely dominant then the crosses are expected to equal the low parent. East and Jones (1919) and Hayes (1922) suggested that there is only a semblance of dominance for low protein due to the immediate effect on protein of heterosis for grain yield. Protein concentration tends to decline with increasing yield of grain, so heterosis for grain yield could lead to a corresponding reduction in protein content and to apparent negative heterosis for protein content. Considering the previous indication that additive gene effects were of overriding importance for protein concentration (Table 3), a theoretical genetic model was constructed showing an adjustment for the effect of heterosis for grain yield on protein concentration (Fig. 1). To achieve this adjustment, the protein concentration of each entry was adjusted by linear regression to the mean grain yield of all entries. The regression coefficient of -0.04 $\pm$ 0.01 (g kg^{-1}) / (Mg ha^{-1}) was obtained from an analysis of plot means with a model including effects of entry and grain yield. This resulted in a negative adjustment for protein concentration in low yielding inbreds and a positive adjustment for W1 crosses associated with their above-average grain yields. Adjusted protein concentration for the mean of W1 crosses was

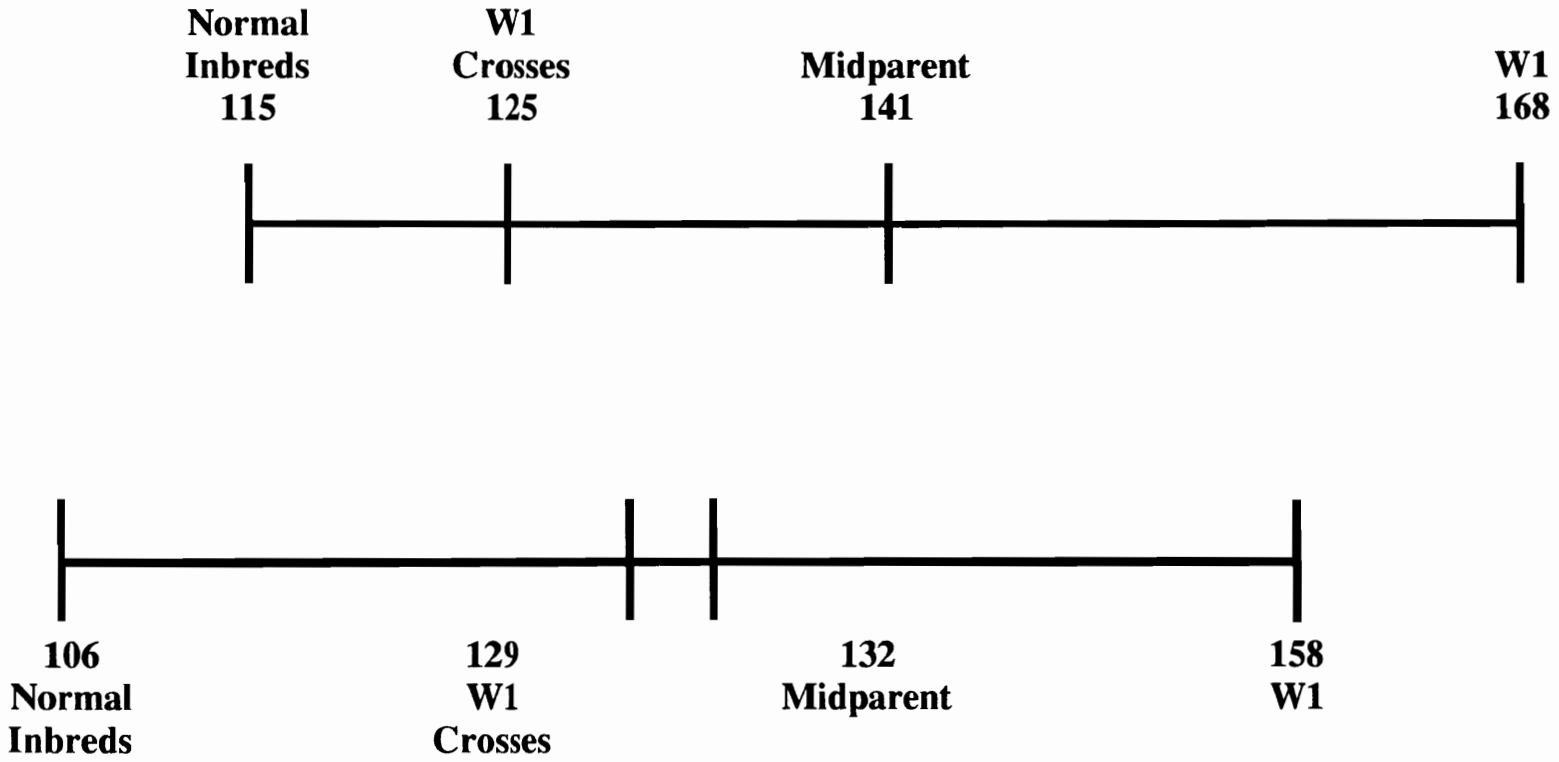

Fig 1. Theoretical genetic model for protein concentration with adjustment for the effect of grain yield. Protein concentration means (g kg^{-1}) of normal inbreds, the Wil500 inbred, and Wil500 crosses are shown on the top line relative to the midparent value of the inbreds. Comparable means, adjusted by linear regression for the effect of grain yield are shown on the bottom line.

2.3 % below the midparent value compared to the unadjusted value which was 14.4 % below the midparent. This model suggests that dominance is not very important in the genetic control of protein concentration in W1 crosses and is in agreement with ratios expressing the relative importance of GCA and SCA in Table 3. While we believe this model represents the true genetic control of protein in W1, it in no way detracts from the significance of an effective partial dominance for low protein concentration in breeding programs.

Harvest moisture ranged from 187 to 271 g kg^{-1} for crosses (Table 4) and was significantly correlated ($P < 0.001$) with grain yield, $r = 0.33$. Time to silk fell in the narrow range 68 to 76 days for crosses and was also significantly ($P < 0.001$) correlated with grain yield, $r = 0.22$. Percentage floaters for crosses ranged from 5 to 68 % indicating a distribution of kernel hardness from hard to medium by industry standards. All W1 crosses had hard kernels (Table 5) and this is noteworthy because kernel hardness is of commercial interest to producers and processors in the grain trade. Kernel hardness has been associated with kernel breakage, attack by storage insects, dust formation during handling, starch processing, and value in specialty packaged foods (Wichser, 1961; Thompson and Isaacs, 1967; Paulsen et al., 1983; Pomeranz et al., 1984). Correlations for percentage floaters with grain yield and protein were significant ($P < 0.001$), $r = -0.16$ and $r = -0.51$, respectively; indicating that both higher yielding crosses and those with higher protein concentration had harder kernels. This is in contrast to the report of Ahmadi et al. (1993) who reported that kernel hardness, although difficult to quantify, was

inversely related to yield and may be influenced by agronomic practices that increase yield. Although not equal to the higher yielding normal crosses, W1 crosses in general had higher yields, more protein, higher grain moisture at harvest, harder kernels, higher 500 kernel weight, and were slightly later in time to silk than normal crosses (Table 5).

General and Specific Combining Ability

Separation of the GCA effects, combined across environments, by the Student-Newman-Keuls' test are presented in Table 6. Significant differences between GCA estimates for grain yield were observed among the inbreds, however they were small in magnitude. H3 had a higher GCA estimate for yield than H4, H5, and H6. W1 had as high a GCA estimate for grain yield as any line in this study.

For protein concentration, W1 had a clearly higher GCA estimate than any other line and there were no other significant differences between lines. Protein concentration has been shown to be highly sensitive to environment (Norden et al., 1952; Prince, 1954; Genter et al., 1956; Lang et al., 1956; and Ahmadi et al., 1993). Therefore it was not surprising that the magnitude or sign of the GCA estimates for protein changed across environments for most normal lines (data not shown). In contrast, W1 had a high positive GCA estimate for protein in all four environments (17.89, 13.00, 16.68, and 17.05 g kg^{-1}). This indicates W1 was consistent in contributing high protein in crosses with normal corn across the environments in this study.

Table 6. Estimates of general combining ability (GCA) for grain yield, percentage protein, grain moisture at harvest, time to silk, percentage floaters, and 500 kernel weight combined over four environments.

Parents	Grain yield	Protein	Grain moisture at harvest	Time to silk	Floaters	500 kernel weight
	Mg ha^{-1}	g kg^{-1}	g kg^{-1}	d	%	g
H1	-0.23ab†	-3.54b	-3.61ab	0.21bc	1.3abc	15.9abc
H2	0.43ab	0.73b	-20.54b	-3.24f	4.4abc	-20.3d
H3	1.61a	1.22b	10.63ab	-0.14c	-13.6bc	8.3ab
H4	-0.52b	1.42b	-1.42ab	0.80bc	-8.6abc	0.8abc
H5	-0.61b	-3.20b	-0.12ab	0.28bc	-0.1abc	-0.9abc
H6	-0.87b	-4.28b	-20.57b	-1.00d	6.5abc	-14.7cd
O1	-0.13ab	-7.14b	-13.67ab	-2.19e	10.3ab	-7.4bcd
W1	0.75ab	16.16a	22.12a	0.56bc	-20.7c	14.9a
W2	0.01ab	-4.21b	1.82ab	2.13a	4.4abc	-0.1abc
W3	-0.10ab	1.38b	13.90ab	0.97bc	3.5abc	10.0a
W4	-0.04ab	0.08b	14.43ab	1.20b	18.6a	7.5ab
W5	-0.34ab	1.44b	-1.83ab	0.42bc	-6.0abc	-14.1cd

† Within columns, estimates followed by the same letter are not significantly different at probability 0.05 based on Student-Newman-Keuls' test.

General combining ability estimates for grain moisture at harvest fell in the narrow range -20.57 to 22.12 g kg^{-1} (Table 6). W1 had the highest GCA estimate for grain moisture at harvest, which was significantly different from the two lowest estimates. No other differences for GCA estimates of grain moisture at harvest were significant. The high GCA estimate for grain moisture at harvest for W1 was reflected in the mean performance of its crosses (Table 5). General combining ability estimates for time to silk was significantly correlated ($P \leq 0.01$) with grain moisture at harvest, r=0.71. H2, O1, and H6, respectively had the most negative GCA estimates for time to silk and contributed significantly to earliness in their crosses. Conversely, W2 followed by W4 had the highest estimates of GCA for time to silk. The GCA estimates for time to silk of the remainder of the inbreds fell in the range -0.14 to 0.97 days and were not significantly different.

The GCA estimate for percentage floaters of W1 was as low or lower than any line in this study. Negative GCA estimates for percentage floaters indicates a contribution to harder kernels. In addition, W1 had as high or higher estimate of GCA for 500 kernel weight as any line in the study. These are very positive traits for a novel high-protein system, considering the problems associated with the early Quality Protein Maize hybrids.

Specific combining ability estimates for grain yield across environments ranged from -4.43 to 2.59 Mg ha^{-1} with over half being significantly different from zero (data not shown). This indicates that a number of hybrids deviated from

expected values, based on an additive model, and suggests that the parents of hybrid crosses can not always be chosen on the basis of their GCA. However, only three significant SCA estimates involved W1 and they were small in magnitude and variable between environments.

Few SCA estimates were significantly different from zero for protein concentration, grain moisture at harvest, time to silk, percentage floaters, and 500 kernel weight and they rarely involved W1 (data not shown). In general, SCA effects were less important than GCA effects for all traits except grain yield indicating that parents with the best GCA estimates could be used to produce the best crosses for traits other than grain yield.

Reciprocal effects were significant only for grain yield and protein concentration (Table 3). For grain yield, only five REC estimates were significantly different from zero. Significant REC estimates for grain yield across environments ranged from -0.66 to -2.03 Mg ha^{-1}; none of these involved crosses with W1 (data not shown). Only two REC estimates for protein concentration across environments were significant. One of these was for the cross W1 × W2 (3.97 g kg^{-1}), in favor of the female parent, which did not vary between environments. Significant REC estimates for crosses can be an important consideration in breeding plans because they indicate that for these crosses, choice of female parent is important.

Conclusions

This investigation confirms, as other diallel studies have shown, that protein concentration is controlled primarily by additive gene action and this is especially true for W1 crosses. Previous studies on the inheritance of protein concentration, however have assumed the absence of reciprocal differences, showing only the relative importance of additive vs. nonadditive effects. The present study demonstrated that such an assumption may not always be valid. Transferring genes for improved protein concentration from W1 into materials adapted to a specific target environment should be straightforward, since W1 had a very large and significant GCA estimate across and within environments. Considering the yield potential and kernel hardness of W1 crosses, we conclude that there is considerable promise for this novel high-protein system in value-added marketing of high yielding hybrids with exceptional grain quality.

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Grouping of Parents and Prediction of Single-Cross Performance Using RFLPs

ABSTRACT

Identifying superior inbreds and hybrid combinations of parents in maize breeding programs involves extensive field testing of thousands of testcrosses. The frequency of new superior inbreds subsequently identified for use in commercial hybrids is very low. Use of restriction fragment length polymorphisms (RFLPs) has been proposed as an approach to increase efficiency in maize breeding programs by reducing the number lines created in early generations and restricting field testing in later generations to the most promising hybrids. The objectives of this study were to evaluate the use of RFLP information to : 1) assign inbreds to heterotic groups and 2) predict hybrid performance in groups of crosses typical of the situation in traditional maize breeding. One hundred thirty-two single crosses and 12 elite proprietary maize inbreds, resulting from a complete diallel crossing scheme including reciprocals and selfs were evaluated in field trials at two locations for two years. Grain yield, mid and high-parent heterosis were measured. Restriction fragment length polymorphism data for the 12 inbreds were obtained for 99 marker loci resolved by 42 genomic clones each with four restriction enzymes. Cluster analysis based on two measures of modified Rogers' distance values agreed well with available pedigree background information. Cluster analysis based on 23 probe-enzyme combinations (P/Es) associated with published QTLs for grain yield agreed best with clustering based on grain yield. Results from this study confirm those of previous investigations in that the prediction of hybrid performance by RFLP-based level of heterozygosity is strongly dependent on the origin of the parents and is low for groups of crosses between unrelated inbreds from different heterotic groups. However, when groups of crosses involving a common parent were evaluated; high correlations between RFLP-based heterozygosity and F1 performance were obtained when the common parent had low general combining ability. A measure of hybrid value based on the number of highest yielding genotypes at marker loci was also associated with hybrid performance. This association was generally lower than for level of heterozygosity with hybrid performance, but was more stable across the different groups of crosses. In particular, hybrid value was significantly correlated with hybrid performance for

the groups of crosses involving a common inbred with high general combining ability. This study indicates that RFLPs may be useful for prediction of hybrid performance in situations typical of traditional maize breeding programs where recombinant inbreds are testcrossed to a common tester, but only with a tester inbred with low general combining ability.

Traditional development of new commercial maize inbreds and hybrids is based on pedigree selection in which elite inbred lines are crossed and then selfed for several generations in order to produce a large number of recombinant inbreds which after several generations of selfing are then crossed in hybrid combination. This pure-line method of maize breeding has not changed in principle since first described by Shull (1909 and 1910). The basic concepts will not change, but modifications to increase the effectiveness and efficiency of developing inbred lines and identifying superior hybrids will occur as new techniques and tools become available (Hallauer, 1990). Much of the effort in these breeding programs is expended in testing thousands of crosses in order to identify new superior inbred lines and later to identify superior combinations of the selected lines. Selection in the early generations of inbreeding is usually weak "visual" selection or non-existent. Selection (before testcrosses are made) based on molecular markers for quantitative trait loci (QTLs) could increase early selection intensity and permit a large reduction in the number of lines created in the S_1 and S_2 generations (Lande, 1992; Zehr et al., 1992; Lande and Thompson, 1990). In addition, prediction of F_1 performance (before field tests) by use of molecular markers could reduce the number of needless crosses in later generations of inbreeding by restricting field testing to only the most promising hybrids. Thus the cost of the RFLP analysis might well be offset by increased efficiency.

Genetic distance between parents or inferred level of heterozygosity in potential hybrids based on molecular marker assays has been suggested as a potential

tool for predicting hybrid performance of crosses (Hunter and Kannenberg, 1971) Stuber and Moll, 1972; Burr et al., 1983). Theoretical and empirical studies indicating that hybrid performance is, with certain restrictions, positively related to parental genetic divergence has stimulated this approach (see Hallauer and Miranda, 1988 and Schnell and Cockerham, 1992 for recent reviews). Several RFLP studies have been conducted in recent years to evaluate the usefulness of RFLP-based genetic distance for the prediction of F_1 performance. The conclusion from comparison of results of these studies was that the association between molecular marker-based genetic distance and hybrid performance and heterosis is strongly dependent on the origin of the germplasm evaluated. Boppenmaier et al., 1993 compared these studies by classifying them according to the types of crosses evaluated. Correlations of marker-based genetic distance with F_1 performance for yield were: 1) high for crosses between mixtures of lines related by pedigree and unrelated lines, 2) medium for crosses between distantly related lines from the same heterotic group, 3) near zero for crosses between unrelated lines from different heterotic groups, and 4) medium to low for crosses between mixtures of crosses between both (2) and (3). Two recent studies, of sets of crosses grouped according to the various classifications defined above, corroborated the above conclusions (Melchinger et al, 1992; Boppenmaier et al., 1993).

None of the above studies evaluated crosses between groups of inbreds with a common tester inbred typical of the situation in commercial breeding programs where elite recombinant inbreds are testcrossed to common testers. The objectives

of this study were to evaluate the use of RFLP information to 1) assign inbreds to heterotic groups and 2) predict hybrid performance for yield in groups of crosses typical of traditional maize breeding programs.

MATERIALS AND METHODS

Maize Inbred Lines and Crosses Examined

Twelve inbreds and 132 single crosses resulting from a complete diallel crossing scheme including reciprocals and selfs were evaluated in field trials at two locations for two years. Details of the experimental design and cultural practices have been given by Ball et al., (1994). Quantitative traits measured or calculated were grain yield and mid and high-parent heterosis. All of the inbred lines used in this study were elite proprietary commercial lines (Table 1).

RFLP Analysis

Restriction fragment length polymorphism data were obtained for each of the 12 inbred lines for 42 maize genomic clones. The maize clones were obtained from three different sources: University of Missouri, Columbia (umc), Brookhaven National Laboratory (bnl), and Native Plants, Inc. (npi). The clones were chosen to mark all chromosome arms in the maize genome. All of the 42 markers have been previously mapped onto 10 maize chromosomes. Total cellular DNA was extracted from bulked leaf tissue of 10 seedlings for each of the 12 parents. Approximately eight micrograms of total cellular DNA was singly digested with four restriction enzymes (EcoRI, DraI, EcoRV, and HindIII) chosen for their ability to reliably digest maize DNA to completion. DNA fragments were then size-fractionated by electrophoresis in 0.8% agarose gels with a running buffer (1 M Tris, 0.125 M NaAC, 0.01 M EDTA, pH 8.1) at 60 milliamps for 18 hours. DNA fragments in

Table 1. Background, origin, and days to relative maturity of the twelve inbred lines used in this diallel study.

Inbred line	Background	Origin	Days to relative maturity
H1	Mo17	Holdens[1]	115
H2	LH7, W153R	Holdens	106
H3	Pioneer 3535	Holdens	117
H4	B37, B73	Holdens	115
H5	B73	Holdens	115
H6	A662, B73	Holdens	111
O1	Private B73	Orsan[2]	110
W1	Private unrelated Tropical	Wilson Seeds[3]	125
W2	Private B73, Tuxpeno	Wilson Seeds	118
W3	Private Lancaster, Tuxpeno	Wilson Seeds	116
W4	Private Lancaster, Tuxpeno	Wilson Seeds	116
W5	Private Mo17, R177, H99	Wilson Seeds	115

[1]Holdens Foundation Seed Company, Williamsburg, IA.
[2]Orsan Inc., Les Ulis Cedex, France.
[3]Wilson Seeds Inc., Harlan IA.

the gels were transferred to nylon membranes and probed for RFLPs by blot hybridization with radio-labelled clones. DNA extraction, digestion, electrophoresis, Southern-blotting, hybridization, and autoradiography followed previously published procedures (Saghai Maroof et al., 1984; Zhang et al., 1993). Autoradiography of 164 probe enzyme combinations (P/Es) resolved a total of 99 marker-loci that were used in subsequent analyses. RFLP profiles on autoradiograms were visually scored. Each band was assigned a number according to its relative migration distance in the gel and two bands were scored as different only when they were clearly separated from each other across all lanes in which they appeared. Molecular marker genotypes of the inbreds were used to predict marker genotypes of the hybrids.

Statistical Analyses

Modified Rogers' distances (MRDs) were calculated using the equation given by Rogers, 1972 :

$$MRD_{ij} = \left[\sum_{k=1}^{\ell} \left(p_{ik} - p_{jk} \right)^2 \right]^{1/2} (2n)^{-1/2}$$

where p_{ik} and p_{jk} are frequencies of the kth allele for the ith and jth inbreds, respectively; n is the number of loci; and ℓ is the total number of alleles (across all loci). MRD values may range from 0 (no diversity) to 1.0 (no similarity) for any given set of loci. When inbred lines are used, MRDs are equivalent to the square root of the proportion of heterozygous loci in the hybrids.

Relationships among lines were measured by the SAS procedure PROC CLUSTER (SAS, 1988) using Ward's (1963) minimum variance method. Input matrices were the 12 × 12 distance matrices of MRD values based on 99 P/Es (MRD99), 23 P/Es associated with published QTLs for grain yield (Stuber et al., 1992) (MRD23), or grain yield.

Diallel analysis was performed as described in detail in Ball et al. (1994). In addition, combined ANOVAs of F_1 grain yield data were calculated for subsets of the crosses as determined from different groupings obtained from dendograms produced by cluster analysis based on MRD23 (Fig. 1). The inbreds included in the groups were as follows:

Major group 1 (G1):	H1, W3, W4, W5, H2, H3, W1
Major group 2 (G2):	H4, H6, O1, H5, W2
Subgroup 1 (SG1):	H1, W3, W4, W5
Subgroup 2 (SG2):	H2, H3, W1

The eight resulting subsets of crosses included all combinations of parental inbreds including reciprocals, between and within groups and were designated as follows: G1 × G2, SG1 × SG2, SG1 × G2, SG2 × G2, Within G1, Within G2, Within SG1, and Within SG2.

Because one of the objectives of this study was to evaluate usefulness of RFLP data for prediction of F_1 performance in regard to commercial breeding programs,

the complete diallel set of crosses was also subset into groups of crosses involving each individual line with all other lines. These 12 subsets were designated by the inbred name : H1, H2, H3, H4, H5, H6, O1, W1, W2, W3, W4, and W5. These groupings were deemed most like the situation in early generations of commercial maize breeding programs where several new recombinant inbreds are often testcrossed to a common inbred tester.

Two measures of heterozygosity and two measures of hybrid value each was used to calculate pearson product-moment correlation coefficients (r) with F_1 performance for grain yield, and mid- and high-parent heterosis for the complete diallel set of crosses and its 20 subsets. Measures of heterozygosity were the number of heterozygotes for an individual cross summed over marker loci for 99 P/Es (H99) or over the 23 P/Es associated with published QTLs for grain yield (H23) (Stuber et al., 1992). These measures of heterozygosity were chosen instead of the MRDs because while the MRDs have the advantage of representing a Euclidean distance for use in cluster analysis, they represent a non-linear transformation of the heterozygosity data and the correlation between them is not unity.

Hybrid values were calculated as first defined by Dudley et al. (1991). First, RFLP loci showing significant genotypic variation for grain yield were determined by calculating a one-way ANOVA from hybrid yield data, for each of the 99 marker loci, using marker genotypes at a locus as the main effect and hybrids within genotypes as the error term. Mean grain yield was calculated for each of the 71

marker loci showing significant genotypic variation for grain yield. At each marker locus for each hybrid a score of 1 was given if the hybrid had the highest yielding genotype or one that did not differ significantly from the highest at the 0.10 probability level based on a Duncan's multiple range test; otherwise a score of 0 was assigned. The 0.10 probability level was chosen to restrict the number of highest-yielding genotypes at a locus, especially at loci containing a large number of genotypes. Hybrid values were the sum of the scores across all 71 loci showing significant genotypic variation for grain yield in this study (HVAL71) and across the 23 loci associated with QTL regions for yield in a previous study (HVAL23) (Stuber et al., 1992). Values could thus range from 0 to 71 or from 0 to 23 for HVAL71 and HVAL23, respectively.

RESULTS

Restriction fragment length polymorphisms

Large differences were observed for RFLPs among the inbred lines and within lines from one restriction enzyme to another. All 42 probes revealed polymorphism between the 12 inbreds with at least two of the four restriction enzymes. Six (3.6 %) out of 168 P/Es were monomorphic. One hundred thirty eight (82 %) of the P/Es yielded single-band RFLP patterns while the remaining 30 P/Es gave multi-band RFLP patterns with up to 16 bands per line. Multiple-band patterns cannot be resolved into their respective loci in a diallel study and so were dropped from further analyses. Each inbred had a unique RFLP profile and even the most closely related lines (H4 and H6) differed at 16 % of the loci assayed (data not shown). Two P/Es (*UMC*10 and *UMC*12 with EcoRI) each were able to distinguish between 11 of the 12 inbreds when scored phenotypically and thus would be very useful for DNA fingerprinting and legal purposes.

Single-locus banding patterns resolved by different restriction enzymes classified the 12 inbreds exactly the same for 33 P/Es thus producing redundant information. Only non-redundant P/Es were used in the analyses and consequently the amounts of information contributed by different restriction enzymes were not equal (Table 2). In all, a total of 99 P/Es yielded unique information and were used in the analyses.

Table 2. Number of clones and unique RFLP banding patterns for each restriction enzyme associated with each of the ten chromosomes in the maize genome for the diallel set of lines.

Chromosome	No. of clones	Number of RFLP variants				
		EcoRI	DraI	EcoRV	HindIII	All Enzymes
1	5	3	2	5	4	14
2	4	3	2	1	3	9
3	4	0	2	1	2	5
4	6	4	2	4	4	14
5	6	5	1	3	2	11
6	3	3	1	3	2	9
7	3	2	2	3	1	8
8	3	3	2	2	1	8
9	4	4	2	3	1	10
10	3	3	2	3	3	11
Total	41	30	18	28	23	99 †

†Total out of 168 possible probe enzyme combinations (P/E).

Genetic distances between inbreds and cluster analyses based on RFLP data

Modified Rogers' distances between parental inbreds of the complete diallel set of 132 F_1 crosses ranged from 0.35 to 0.86 with a mean of 0.744 based on the full set of 99 marker loci. Based on the reduced set of 23 P/Es linked to published QTLs for grain yield, MRDs ranged from 0.0 to 0.96 with a mean of 0.758 (Table 3). Values were generally higher for MRDs based on 23 P/Es than for those based on 99 P/Es.

Mean MRD99 values for groups of individual inbred line crosses were in the narrow range from 0.658 to 0.845. However, there were large differences between the ranges of MRD99 values for different groups of inbred line crosses. This was expected because these groups represent mixtures of crosses between 1) related lines, 2) unrelated lines from the same heterotic group, and 3) unrelated lines from different heterotic groups.

Dendograms obtained from clustering based on MRDs for 99 P/Es, 23 P/Es and grain yield all resulted in a clear separation of stiff-stalk (G2) from non-stiff-stalk lines (G1) (Fig. 1). Based on pedigree background information and in agreement with cluster analyses, crosses between G1 and G2 lines consisted exclusively of crosses between unrelated lines from different heterotic groups. The mean MRD value for group G1 × G2 crosses was high (0.824) and the range was narrow (0.74 to 0.86) indicating very little similarity (Table 3). These values also agree well with pedigree background information.

Table 3. Mean, minimum, maximum, and standard deviation (SD) of modified Rogers' distance coefficients calculated from RFLP data for 99 and 23 probe enzyme combinations (P/E); for the complete diallel set of crosses and different subsets of crosses representing crosses between related and unrelated lines.

		Modified Rogers' distance							
		99 P/E combinations				23 P/E combinations			
Cross group†	Number	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
Complete Diallel	132	0.744	0.35	0.86	0.154	0.758	0.00	0.96	0.208
H1	22	0.770	0.44	0.86	0.141	0.833	0.55	0.96	0.152
H2	22	0.820	0.74	0.85	0.029	0.854	0.69	0.88	0.057
H3	22	0.777	0.74	0.84	0.035	0.801	0.69	0.88	0.056
H4	22	0.676	0.35	0.86	0.205	0.650	0.00	0.93	0.317
H5	22	0.688	0.40	0.86	0.190	0.704	0.36	0.96	0.221
H6	22	0.658	0.35	0.86	0.221	0.650	0.00	0.93	0.317
O1	22	0.700	0.39	0.86	0.184	0.681	0.30	0.93	0.256
W1	22	0.845	0.82	0.86	0.014	0.873	0.83	0.91	0.030
W2	22	0.683	0.36	0.85	0.190	0.663	0.30	0.93	0.254
W3	22	0.781	0.53	0.85	0.119	0.823	0.55	0.91	0.126
W4	22	0.729	0.44	0.84	0.127	0.761	0.51	0.88	0.135
W5	22	0.798	0.67	0.84	0.051	0.805	0.51	0.91	0.111
G1 × G2	70	0.824	0.74	0.86	0.031	0.863	0.75	0.96	0.047
SG1 × SG2	24	0.815	0.74	0.86	0.038	0.874	0.78	0.91	0.039
SG1 × G2	40	0.828	0.78	0.86	0.026	0.880	0.83	0.96	0.040
SG2 × G2	30	0.820	0.74	0.86	0.038	0.840	0.75	0.88	0.046
Within G1	42	0.759	0.44	0.86	0.116	0.787	0.51	0.91	0.132
Within G2	20	0.430	0.35	0.51	0.055	0.331	0.00	0.47	0.132
Within SG1	12	0.627	0.44	0.82	0.140	0.613	0.51	0.78	0.098
Within SG2	6	0.799	0.74	0.84	0.047	0.787	0.69	0.83	0.073

†Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

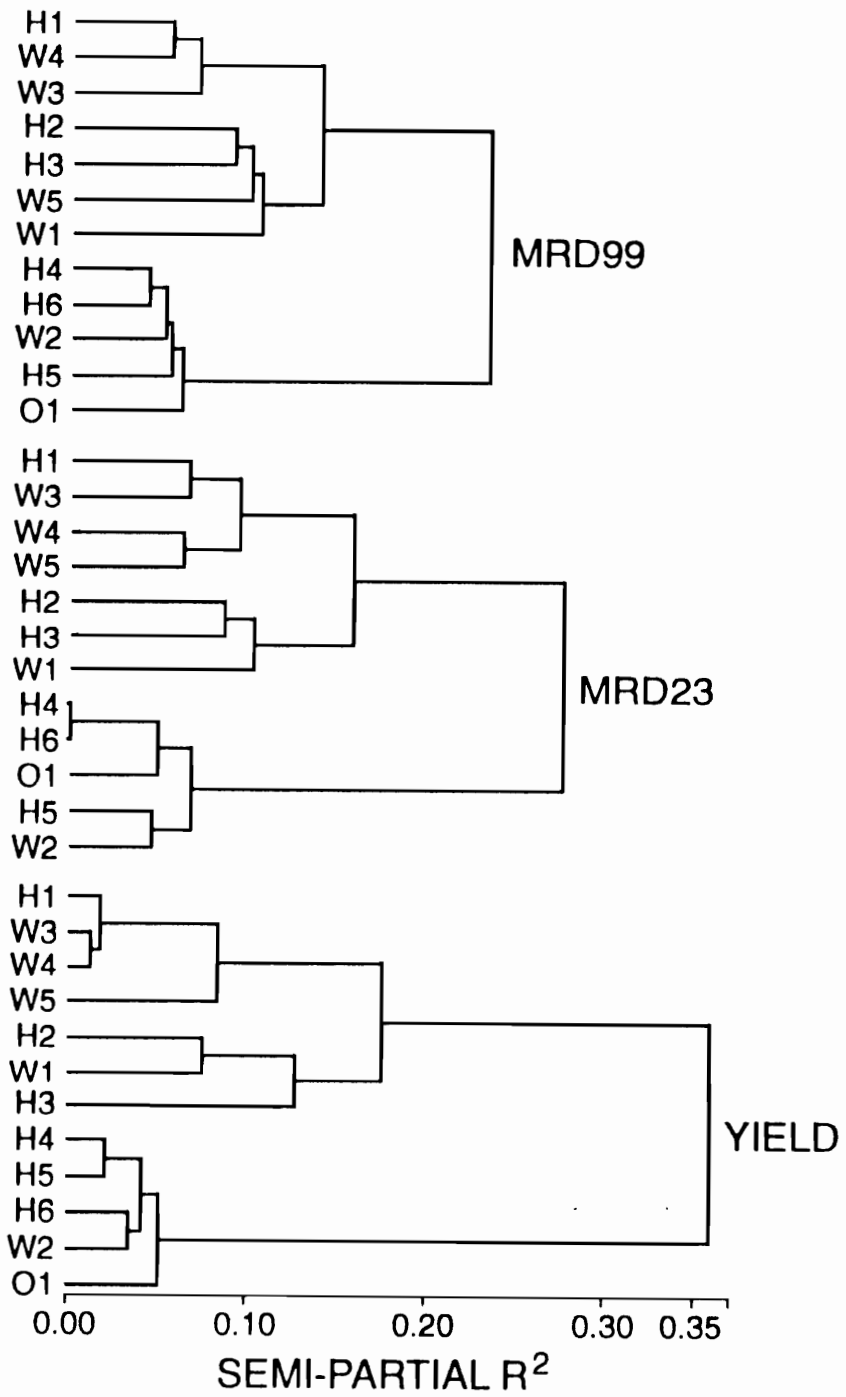

Fig. 1. Dendograms from Ward's minimum variance cluster analyses of 12 maize inbred lines based on modified Roger's distances calculated from 99 loci (MRD99), 23 loci selected for their proximity to published QTLs for grain yield (MRD23), and grain yield. Semipartial R-squared is the decrease in the proportion of variance accounted for due to joining two clusters.

Cluster analyses based on the three different criteria generally agreed well with available pedigree background information as well as with common perceptions of maize breeders in grouping stiff-stalk and non-stiff-stalk lines separately. In addition, all three dendograms grouped inbred lines H1 (C103 derivative) and H2 (W153R derivative) in separate subgroups within non-stiff-stalk lines. However, pedigree details of the 12 elite proprietary lines are not publicly available and thus detailed comparison of the agreement of cluster analyses with pedigree data was not possible. Clustering based on yield resulted in differences in minor groupings from either clustering based on 99 P/Es or based on the 23 P/Es associated with yield QTLs, but was in closest agreement with the latter.

Variation for hybrid value

Hybrid value (number of best genotypes) for grain yield based on the 71 P/Es associated with grain yield in this study (HVAL71) ranged from 0 to 31 with a mean of 13 for the complete diallel set of hybrids (Table 4). Based on the 23 P/Es linked to published QTLs for grain yield, hybrid value (HVAL23) for the complete diallel set of hybrids ranged from a minimum of one best genotype to a maximum of nine with a mean of three.

Number of best genotypes for grain yield based on 99 P/Es was generally highly variable within groups of individual line crosses and within different group × group crosses as indicated by ranges greater than 14 for all cases except the two groups of crosses W1 and W5 with ranges of 12 and 8, respectively (Table 4).

Table 4. Mean, minimum, maximum, and standard deviation (SD) of number of best genotypes for grain yield (HVAL71 and HVAL23) summed over marker loci for 71 and 23 probe enzyme combinations (P/E); calculated for the complete diallel set of crosses and different subsets of crosses representing crosses between related and unrelated lines.

		Number of best genotypes summed over marker loci							
		HVAL71				HVAL23			
Cross group†	Number‡	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
Complete Diallel	132	13	0	31	10	3	1	9	2
H1	22	16	1	29	11	3	1	5	2
H2	22	10	3	20	4	3	1	9	2
H3	22	18	7	25	5	5	2	9	2
H4	22	13	0	31	12	2	1	6	2
H5	22	13	1	31	11	4	1	8	2
H6	22	13	0	31	12	2	1	6	2
O1	22	11	1	26	10	2	1	6	2
W1	22	10	6	18	3	3	1	6	2
W2	22	14	1	31	12	3	1	7	2
W3	22	18	2	31	12	4	1	8	2
W4	22	15	1	28	11	3	1	6	2
W5	22	7	3	11	3	2	1	3	1
G1 × G2	70	19	7	31	8	4	1	8	2
SG1 × SG2	24	10	3	17	4	3	1	5	1
SG1 × G2	40	23	9	31	8	4	2	8	2
SG2 × G2	30	14	7	25	6	3	1	6	2
Within G1	42	9	1	20	6	3	1	9	2
Within G2	20	1	0	4	1	1	1	3	1
Within SG1	12	2	1	3	1	1	1	2	0
Within SG2	6	15	8	20	6	6	4	9	2

†Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡Each observation is the mean of three reps in each of four environments.

Consistent with common perceptions of maize breeders, the two most closely related groups of hybrids (Within G2 and Within SG1) had low means and narrow ranges of HVAL71.

Variation for grain yield, mid-parent, and high-parent heterosis

Genotypic differences among crosses for grain yield were highly significant ($p < 0.01$) for the complete diallel and for each subset of crosses (combined ANOVAs not shown). Grain yield was generally highly variable within individual groups of line crosses as indicated by ranges exceeding 5 Mg ha^{-1} for 8 of the 12 groups (Table 5). The exceptions were groups of crosses involving inbreds H2, H3, W1, and W5 which all had high positive general combining ability (Ball et al. 1994) and high mean MRD estimates (Table 3).

Grain yield means for the different sets of group × group crosses ranged from 9.14 to 9.65 Mg ha^{-1}. These high means are consistent with common expectations of maize breeders since all of these hybrids represent crosses between unrelated lines from different heterotic groups or crosses between unrelated lines from the same heterotic group. As expected, all within group crosses had significantly lower ($p < 0.01$) mean grain yield than their respective group × group crosses with the exception of the Within SG2 crosses (data not shown). The high mean grain yield of the Within SG2 crosses is consistent with its high mean MRD value (Table 3).

Mid-parent heterosis ranged from 6 to 592% and high-parent heterosis ranged from 0 to 482% in the complete diallel set of crosses. Both mid-parent and high-

Table 5. Mean, minimum, maximum, and standard deviation of grain yield in a diallel set of crosses and in subsets representing crosses between related and unrelated maize lines.

Cross group†	Number‡	Grain Yield			
		Mean	Min.	Max.	SD
		Mg ha^{-1}			
Complete diallel	132	8.53	3.20	11.04	1.92
H1	22	8.32	3.28	10.87	2.41
H2	22	8.92	7.93	10.36	0.70
H3	22	9.99	8.78	11.04	0.55
H4	22	8.06	4.15	10.63	2.23
H5	22	7.97	4.15	10.51	2.27
H6	22	7.73	4.94	10.40	1.88
O1	22	8.40	5.34	11.04	1.76
W1	22	9.20	7.93	10.43	0.55
W2	22	8.56	4.98	11.00	2.27
W3	22	8.43	3.20	11.00	2.45
W4	22	8.49	3.20	11.00	2.41
W5	22	8.22	6.66	9.89	0.99
G1 × G2	70	9.59	7.14	11.04	0.82
SG1 × SG2	24	9.14	8.01	10.43	0.71
SG1 × G2	40	9.65	7.14	11.00	0.89
SG2 × G2	30	9.52	8.19	11.04	0.72
Within G1	42	8.13	3.20	10.43	2.04
Within G2	20	5.62	4.15	7.04	0.73
Within SG1	12	5.45	3.20	7.81	1.72
Within SG2	6	9.48	7.93	10.36	0.88

† Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡Means of grain yields were across three replications and four environments.

parent heterosis were highly variable within groups of crosses and, like grain yield, were less variable within more diverse groups as indicated by MRDs (Table 3 and Table 6). However, among groups of crosses, high mean MRD values were not indicative of high heterosis because some groups of crosses with almost identical mean MRD values differed substantially in average heterosis (Table 3 and Table 6).

Correlation of heterozygosity with F_1 performance for grain yield and heterosis

Two measures of heterozygosity were used for assessing the association of heterozygosity with F1 performance for grain yield and heterosis: the number of heterozygotes summed over all 99 marker loci (H99) and the number of heterozygotes summed over the 23 marker loci associated with Stuber's QTLs for grain yield (H23). Simple correlations in the complete diallel set of crosses of H99 and H23 with grain yield were highly significant r=0.82 and 0.78, respectively indicating that heterozygosity contributes an important component to grain yield in this diallel set of crosses (Table 7).

One striking result of this study is the extremely high correlations of H99 and H23 with grain yield obtained when the diallel set of crosses was divided into groups of hybrids involving a single inbred line (Table 7). Correlation coefficients of H99 with grain yield among individual groups of line crosses ranged from -0.64 to 0.96 with 7 out of 12 above 0.90. These results are in agreement with those of Smith et al. (1990) who reported extremely high correlations r=0.93 for association of proportion of heterozygotes based on 257 RFLP loci with grain yield in a study that

Table 6. Mean, minimum, maximum, and standard deviation (SD) of mid-parent and high-parent heterosis for grain yield calculated for the complete diallel set of crosses and different subsets of crosses representing crosses between related and unrelated lines.

		Heterosis for grain yield							
		Mid-parent				High-parent			
Cross group†	Number‡	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
Complete Diallel	132	247	6	592	118.9	186	0	482	101.6
H1	22	344	62	592	167.2	245	14	483	160.4
H2	22	186	127	254	35.2	132	106	169	18.1
H3	22	237	170	316	44.7	179	148	208	14.8
H4	22	212	68	427	107.4	165	51	282	77.7
H5	22	244	68	484	121.4	189	42	356	97.4
H6	22	193	83	365	92.2	149	62	238	64.7
O1	22	174	80	316	69.5	124	42	194	46.6
W1	22	332	181	591	103.8	259	106	483	107.0
W2	22	295	108	592	153.1	230	74	469	134.4
W3	22	195	6	328	85.2	151	0	242	73.6
W4	22	219	6	364	96.5	178	0	290	87.8
W5	22	332	199	512	99.0	234	107	460	117.8
G1 × G2	70	288	137	592	99.9	222	113	469	81.3
SG1 × SG2	24	279	127	591	118.1	202	108	483	103.9
SG1 × G2	40	321	163	592	104.5	243	130	469	81.6
SG2 × G2	30	244	137	422	74.4	195	113	405	73.5
Within G1	42	243	6	591	126.6	176	0	483	117.8
Within G2	20	111	68	177	31.3	83	42	159	29.4
Within SG1	12	186	6	460	151.0	134	0	460	158.6
Within SG2	6	215	169	278	45.5	154	106	185	28.8

†Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡Each observation is the mean of three reps in each of four environments.

Table 7. Correlation coefficients and significance of two measures of heterozygosity (H) and two measures of hybrid value (HVAL) with F_1 performance for grain yield (Mg ha^{-1}) in a diallel set of crosses and in subsets representing crosses between related and unrelated maize lines for grain yield.

		F_1 performance for grain yield (Mg ha^{-1}) versus			
Cross group†	Number‡	H99§	H23	HVAL71	HVAL23
Complete diallel	132	0.82***	0.78***	0.77***	0.67***
H1	22	0.96***	0.96***	0.82***	0.89***
H2	22	-0.64**	-0.63**	0.67***	0.56**
H3	22	0.03ns	-0.41ns	0.54**	0.27ns
H4	22	0.94***	0.91***	0.87***	0.71***
H5	22	0.89***	0.87***	0.89***	0.86***
H6	22	0.93***	0.91***	0.84***	0.68***
O1	22	0.93***	0.91***	0.83***	0.71***
W1	22	0.14ns	0.06ns	0.66***	0.50*
W2	22	0.93***	0.94***	0.88***	0.80***
W3	22	0.91***	0.96***	0.82***	0.86***
W4	22	0.91***	0.81***	0.79***	0.80***
W5	22	0.20ns	0.45*	0.64**	0.52*
G1 × G2	70	-0.18ns	-0.10ns	0.64***	0.63***
SG1 × SG2	24	-0.32ns	-0.27ns	0.69***	0.73***
SG1 × G2	40	0.18ns	0.24ns	0.70***	0.63***
SG2 × G2	30	-0.65***	-0.76***	0.73***	0.73***
Within G1	42	0.79***	0.72***	0.78***	0.64***
Within G2	20	0.59**	-0.11ns	0.02ns	0.29ns
Within SG1	12	0.82**	0.39ns	0.86***	-0.40ns
Within SG2	6	-0.50ns	-0.59ns	0.88*	0.80ns

*,**, and *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

† Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡ Means of grain yields were across three replications and four environments.

§ H99 and H23 are the number of heterozygotes summed over marker loci for 99 and 23 P/E combinations. HVAL71 and HVAL23 are the number of best genotypes summed over 71 and 23 P/E combinations.

included crosses of elite inbreds from the same as well as different heterotic groups. In this study, three of the correlation coefficients for H99 versus grain yield among groups of individual line crosses were not significant and one was significant but negative r=-0.64. These same four groups of crosses are the groups noted earlier involving inbred lines with high general combining ability. Correlation of the predictive ability of H99 for grain yield in individual line crosses with the general combining ability of their respective inbred line was highly significant r=-0.71 (data not shown).

For the complete diallel set of crosses, correlations of H99 and H23 with mid-parent heterosis were much lower (r=0.63 and r=-.61) than those for grain yield and lowest for high-parent heterosis (r=0.58 and r=0.54) (Tables 8 and 9). For the different groups of crosses, correlations of H99 and H23 with both mid and high-parent heterosis were generally lower than for correlations with grain yield but otherwise followed the same pattern as for grain yield. However, level of heterozygosity predicted high-parent heterosis better than mid-parent heterosis for 6 out of 12 groups of individual line crosses.

Table 8. Correlation coefficients and significance of two measures of heterozygosity (H) and two measures of hybrid value (HVAL) with mid-parent heterosis for grain yield (Mg ha^{-1}) in a diallel set of crosses and in subsets representing crosses between related and unrelated maize lines for grain yield.

		Mid-parent heterosis for grain yield (Mg ha^{-1}) versus			
Cross group†	Number‡	H99§	H23	HVAL71	HVAL23
Complete diallel	132	0.63***	0.61***	0.42***	0.32***
H1	22	0.78***	0.68***	0.59**	0.72***
H2	22	-0.19ns	-0.12ns	-0.06ns	0.02ns
H3	22	-0.13ns	0.18ns	-0.33ns	-0.33ns
H4	22	0.84***	0.85***	0.70***	0.52*
H5	22	0.86***	0.88***	0.72***	0.63**
H6	22	0.82***	0.83***	0.63**	0.46*
O1	22	0.82***	0.81***	0.62**	0.48*
W1	22	0.55**	0.43*	0.01ns	0.06ns
W2	22	0.84***	0.85***	0.61**	0.52*
W3	22	0.82***	0.82***	0.70***	0.75***
W4	22	0.72***	0.52*	0.66***	0.60**
W5	22	0.12ns	0.16ns	0.32ns	0.30ns
G1 × G2	70	0.30*	0.36**	0.13ns	0.16ns
SG1 × SG2	24	0.28ns	0.31ns	0.07ns	0.06ns
SG1 × G2	40	0.35*	0.47**	-0.09ns	-0.15ns
SG2 × G2	30	0.21ns	-0.16ns	-0.12ns	0.22ns
Within G1	42	0.56***	0.44**	0.21ns	0.05ns
Within G2	20	0.46*	0.06ns	0.77***	0.71***
Within SG1	12	0.72**	0.38ns	0.71**	-0.48ns
Within SG2	6	0.75*	0.67ns	0.09ns	-0.36ns

*,**, and *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

† Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡ Means of grain yields were across three replications and four environments.

§ H99 and H23 are the number of heterozygotes summed over marker loci for 99 and 23 P/E combinations. HVAL71 and HVAL23 are the number of best genotypes summed over 71 and 23 P/E combinations.

Table 9. Correlation coefficients and significance of two measures of heterozygosity (H) and two measures of hybrid value (HVAL) with high-parent heterosis for grain yield (Mg ha^{-1}) in a diallel set of crosses and in subsets representing crosses between related and unrelated maize lines for grain yield.

		High-parent heterosis for grain yield (Mg ha^{-1}) versus			
Cross group†	Number‡	H99§	H23	HVAL71	HVAL23
Complete diallel	132	0.58***	0.54***	0.44***	0.36***
H1	22	0.63**	0.46*	0.40ns	0.53*
H2	22	-0.64**	-0.63**	0.67***	0.56**
H3	22	0.05ns	-0.16ns	0.44*	-0.01ns
H4	22	0.88***	0.87***	0.83***	0.66***
H5	22	0.83***	0.84***	0.70***	0.63**
H6	22	0.85***	0.85***	0.79***	0.64**
O1	22	0.93***	0.91***	0.84***	0.72***
W1	22	0.62**	0.30ns	-0.04ns	0.01ns
W2	22	0.80***	0.79***	0.52*	0.46*
W3	22	0.87***	0.92***	0.85***	0.88***
W4	22	0.78***	0.67***	0.82***	0.75***
W5	22	0.05ns	0.04ns	0.14ns	0.15ns
G1 × G2	70	0.24*	0.23ns	0.18ns	0.16ns
SG1 × SG2	24	0.36ns	0.32ns	0.22ns	0.20ns
SG1 × G2	40	0.14ns	0.30ns	0.11ns	0.06ns
SG2 × G2	30	0.30ns	-0.11ns	-0.12ns	0.17ns
Within G1	42	0.50***	0.36*	0.25ns	0.12ns
Within G2	20	0.15ns	-0.13ns	0.70***	0.88***
Within SG1	12	0.62*	0.37ns	0.62*	-0.33ns
Within SG2	6	-0.15ns	-0.26ns	0.86*	0.59ns

*,**, and *** indicate significance at the 0.05, 0.01 and 0.001 probability levels, respectively.

† Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡ Means of grain yields were across three replications and four environments.

§ H99 and H23 are the number of heterozygotes summed over marker loci for 99 and 23 P/E combinations. HVAL71 and HVAL23 are the number of best genotypes summed over 71 and 23 P/E combinations.

Correlation of number of best genotypes at yield-associated RFLP loci with F_1 performance for grain yield and heterosis

Two measures of the hybrid value first defined by Dudley et al. 1991 were also used to assess the value of RFLP data for prediction of F1 performance for grain yield and heterosis: number of best genotypes at RFLP loci associated with grain yield in this study (HVAL71) and at RFLP loci associated with previously published QTL regions for grain yield (HVAL23) . Simple correlations of HVAL71 with grain yield were significant for the complete diallel set of crosses and for all subsets of crosses with the exception of one group representing crosses between related and unrelated parents from the stiff-stalk heterotic group (Table 7). Significant correlation coefficients for prediction of grain yield for HVAL71 ranged from 0.54 to 0.89 and were more stable across the groups than those of H99 or H23. In general, correlations of HVAL23 with grain yield were lower than those of HVAL71 but were the same or higher for four groups of crosses.

Correlations of either HVAL71 or HVAL23 with mid-parent heterosis were generally lower than for either measure of heterozygosity and were often too low to be of any practical value in breeding programs (Table 8). One notable exception was for the group of hybrids representing crosses between both unrelated and related lines from the same heterotic group (Within G2) where the correlation with mid and high-parent heterosis was much higher for both measures of hybrid value than for heterozygosity.

DISCUSSION

The level of polymorphism revealed by RFLP assay of 12 elite proprietary maize inbreds in the present study was high and in close agreement with results from recent RFLP studies (Smith et al., 1990; Dudley et al., 1991; Melchinger et al., 1991; Livini et al., 1992; Melchinger et al., 1992; Messmer et al., 1992; Boppenmaier et al., 1993). Probe enzyme combinations showing multi-band RFLP patterns were more powerful for discrimination between genotypes of inbreds than P/Es yielding single-band patterns; thus they would be valuable for DNA fingerprinting and plant variety protection.

Modified Rogers' distance values based on either 99 or 23 P/Es demonstrated that, as expected, line combinations from different heterotic groups are genetically more dissimilar at the DNA level than those originating from the same heterotic group. The average relative increase in the mean MRD99 (28%) and MRD23 (35%) observed for between heterotic group crosses compared to within heterotic group crosses was substantially greater than reported in other recent RFLP studies with maize inbreds (Melchinger et al., 1991, Boppenmaier et al., 1993). However, these findings are consistent with the relative increase in F_1 performance for grain yield (28%) observed for between heterotic group crosses as opposed to within heterotic group crosses. In contrast, Melchinger et al., 1991 reported smaller mean relative increases in genetic distances (8 %) for between heterotic groups of crosses over those within heterotic groups than expected considering the 20 to 30 % increase in

grain yield observed for the same groups of crosses. However, they suggest that the 166 P/Es employed in their study may represent a lower limit. Messmer et al., 1991 and Smith et al., 1992 have also indicated that at least 100 loci are required to provide acceptable estimates of true genetic relationships among maize inbred lines. As a measure of true genetic relationship these suggestions are reasonable since molecular markers representing a sample of a plant's genome are used to infer similarities of the entire genome among a set of lines. However, true genetic distances based on the entire genome may not be associated with F_1 performance for grain yield or heterosis. Instead, results from this study indicate that genetic distances based on a few P/Es selected for their association with QTLs for yield may be better associated with grain yield and heterosis; and thus may more accurately represent genetic distance in the context of heterotic grouping. Reinforcement of this conclusion is given by the better agreement, in this study, of clustering based on MRD23 than on MRD99 with both pedigree background information and clustering based on grain yield. These conclusions may help explain the differences between grouping based on genetic distance versus grouping based on yield reported in some studies. It should not be difficult to verify the validity of these conclusions by reanalyzing some of the previously published data.

Correlations of heterozygosity with grain yield observed in this study corroborate the findings of Dudley et al. 1991, Melchinger et al. 1992, and Boppenmaier et al. 1993 in that the correlation of heterozygosity with F1 performance strongly depends on the origin of the parents. In addition, findings of

the present study, like theirs, indicate the prediction of F_1 performance for grain yield by RFLP data for level of heterozygosity is promising for crosses of lines within the same heterotic group, but is non-existent for those between heterotic groups.

One striking result of this study not reported heretofore is the extremely high correlations obtained for several different groups of crosses when the complete set of crosses was divided into groups of crosses involving an individual inbred line in combination with all other lines. This result indicates that RFLP-based heterozygosity may be useful for prediction of hybrid performance when hybrids are to be produced from parents with a related as well as unrelated pedigree background. This situation arises in practical maize breeding programs when recombinant inbreds of unknown heterotic pattern developed from crosses between parents from different heterotic groups are to be tested in hybrid combination with a common tester. Also, the observation that for every case where an inbred line had high general combining ability the correlation of level of heterozygosity with hybrid grain yield was non-significant has implications regarding choice of inbred tester. This finding suggests that when RFLP data are to be used to predict hybrid performance the inbred tester needs to have low general combining ability. This is in agreement with the theoretical calculations of Bernardo (1992) who showed that a high level of dominance in a group of crosses is necessary for prediction of hybrid performance with molecular marker heterozygosity.

The correlation of hybrid value (based on highest yielding genotypes at marker loci) with grain yield was generally lower than the correlation of heterozygosity with yield, but was more stable across the different groups of crosses. In particular, they were significant in the individual line crosses when the individual line had high GCA effects and also in the group × group crosses where the correlation of heterozygosity with yield was not significant. Dudley et al. (1991) recommended the identification of specific best genotypes for loci associated with traits of interest. Questions that remain to be answered are : 1) whether enough markers to explain a sufficiently large amount of genotypic variation for yield can be found with reasonable expenditures and 2) whether identified specific markers for yield hold across a wide range of germplasm. A large amount of research effort is currently in progress to answer these questions at several private and public institutions (S. V. Evola and K. R. Lamkey, 1994, personal communications).

In summary, the results of this study corroborate the findings of recent RFLP studies in that the association of RFLP-based genetic distances or heterozygosity with F_1 performance for grain yield depends on the types of crosses examined (Melchinger et al., 1992; Boppenmaier et al., 1993). The results of this study are also in agreement with theoretical expectations elucidated by R. Bernardo (1992) in that prediction of F_1 performance for grain yield by RFLP based genetic distance depends on a strong level of dominance and in that testcross performance of elite recombinant inbred lines is low when the GCA of the inbred tester is high.

This study differs from previously published studies in the definition of groups of crosses that represent the typical maize breeding situation. Instead of large sets of crosses between lines from different heterotic groups, we have subset the diallel set of crosses into crosses involving a single inbred line. The extremely high correlations found for level of heterozygosity with F_1 performance for grain yield in these crosses when the individual line had low GCA indicate that RFLP-based prediction of grain yield may be possible for testcross performance in maize breeding programs when the inbred tester has low general combining ability. More research is necessary to determine if these relationships hold across a wide range of maize germplasm and it may be possible to verify these conclusions by regrouping and reanalyzing some previously published studies.

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SUMMARY and FUTURE PROSPECTS

Combining ability for protein concentration and other agronomic traits, heterosis and prediction of F1 performance for yield by RFLPs were studied in a diallel cross of 12 diverse maize inbreds. The source of germplasm in this study was different from that used in any previously reported investigations. All of the inbred lines were elite proprietary commercial lines, four of which contained tropical germplasm in their pedigree. In addition, one of the inbreds (W1) is a novel source of high quality protein.

Diallel analysis showed that general combining ability (GCA) and specific combining ability (SCA) effects were highly significant for all traits (protein concentration, grain yield, grain moisture at harvest, days to mid-silk, and kernel hardness) indicating both additive and non-additive effects, respectively. The orthogonal contrast parents vs. crosses was significant for all traits, confirming the presence of nonadditive gene effects. Reciprocal effects (REC), often assumed to be absent in maize diallel studies, were significant for grain yield and protein concentration suggesting that choice of female parent may be important for these traits.

Ratios expressing the relative importance of GCA and SCA indicate that protein concentration is controlled primarily by additive gene action. However, negative mid-parent heterosis for protein concentration gave evidence for partial dominance for low protein. A theoretical genetic model is presented that shows

additive gene action for protein concentration in crosses involving the high-protein inbred (W1). While I believe this model indicates the true genetic control of protein concentration in W1 crosses, it does not alter the significance of an effective partial dominance for low protein to breeding programs.

This investigation confirms, as other diallel studies have shown, that protein concentration in maize is controlled primarily by additive gene action and this is especially true for W1 crosses. Grain yields are encouraging for crosses involving (W1) as the mean grain yield was higher for W1 crosses than for normal crosses. However the highest yielding W1 cross (W1 × W3) yielded only 87 percent as much as the highest normal cross (H3 × O1). This indicates that these high-protein hybrids still need improvement for grain yield. However, transferring genes for improved protein concentration from W1 while selecting for high yield should be possible since W1 had a very large significant GCA estimate across and within environments.

Restriction fragment length polymorphism data for the 12 inbreds were obtained for 99 marker loci resolved by 42 genomic clones each with four restriction enzymes. Cluster analysis based on two measures of modified Rogers' distance values agreed well with available pedigree background information. Cluster analysis based on 23 probe-enzyme combinations (P/Es) associated with published QTLs for grain yield agreed best with clustering based on grain yield.

Results from this study confirm those of previous investigations in that the prediction of hybrid performance by RFLP-based level of heterozygosity is strongly dependent on the origin of the parents, and is low for groups of crosses between unrelated inbreds from different heterotic groups. However, when groups of crosses involving a common parent were evaluated, high correlations between RFLP-based heterozygosity and F1 performance were obtained when the common parent had low general combining ability. Results of this study are especially important because conclusions from previous studies have been extended to additional sources of maize germplasm.

A measure of hybrid value based on the number of highest yielding genotypes at marker loci was associated with hybrid performance. This association was generally lower than for level of heterozygosity with hybrid performance, but was more stable across the different groups of crosses. In particular, hybrid value was significantly correlated with hybrid performance for the groups of crosses involving a common inbred with high general combining ability. This indicates that this measure of hybrid value could be used to predict grain yield of crosses involving the high-protein parent (W1) used in this study.

Future Prospects

There is considerable promise for this novel high-protein system in value-added marketing of high yielding hybrids with exceptional grain quality. Additive gene action for this high-quality protein system indicates that the improved protein

quality of W1 is not the result of a mutant phenotype, and thus offers hope for the discovery of other high-protein maize germplasm. In fact, a very recent study of 28 landraces of maize of tropical origin found protein concentration as high as 141.1 g kg^{-1} (Arnason et al., 1994). In addition, the author of the present study has found protein concentration as high as 136 g kg^{-1} in a population consisting of 25 races of maize from Mexico (data not published). An immediate future goal should be efforts to extract high-protein inbreds from these populations.

This research further indicates that RFLPs may be useful for prediction of hybrid performance in situations typical of traditional maize breeding programs where recombinant inbreds are testcrossed to a common tester, but only with a tester inbred with low general combining ability. A large increase in efficiency of maize breeding programs would result if superior crosses can be predicted reliably (before field tests) by the simple screening of inbreds for RFLPs. An immediate goal should be the verification of these results by reanalysis of some previous RFLP studies using the cross groupings described in this study.

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APPENDIX

Table A1. List of traits measured or calculated, their trait codes, and definitions.

Trait	Trait code	Definition
Grain yield	YLD	Yield of grain at 15.5% moisture (Mg ha^{-1})
Grain moisture	H20	Percentage moisture at harvest measured with a John Deere type 200 moisture meter
Days to midsilk	DMS	Number of days from planting until 50% of plants in a plot had visible silk
Percent protein	PRO	6.25 × % nitrogen in ground ear corn
Plant height	PHT	Distance from ground to base of tassel (cm)
Ear height	EHT	Distance from ground to ear node (cm)
Ear length	ELN	Ear length from butt to end of grain fill (cm)
Ear diameter	EDI	Diameter of ear at widest point (mm)
Ear circumference	ECI	Π X EDI (mm)
Ear weight	EWT	Weight of ear (grams)
Cob weight	CBW	Weight of cob (grams)
Cob diameter	CDI	Diameter of cob at widest point (mm)
Husk cover	HSK	Poor, Intermediate, or Good
Grain weight	GWT	EWT - CBW (grams)
500 Kernel weight	500K	Weight of 500 kernels (grams)
Ear kernel number	KNM	(EWT - CBW) X (500K / 500)
Kernel row number	KRN	Number of rows of kernels per ear
Kernels per row	KPR	KNM / KRN
Kernel depth	KDP	(EDI - CDI) / 2 (mm)
Kernel thickness	KTH	ELN / KPR (mm)
Kernel width	KWD	(KDP + CDI)Π / KRN (mm)
Percent floaters	PCF	Percentage floaters on 1.25 specific gravity solution of $NaNO_3$

Table A2. Combined ANOVA for plant height in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	52293.6	8	461.59***
Replication /E	8	113.3	1144	7.97***
Entry (G)	143	1498.8	429	33.69***
Parents vs. Crosses	1	60177.8	3	88.95**
Parents	11	1520.5	33	21.82***
Crosses (C)	131	1049.0	393	27.94***
GCA	11	7190.0	33	46.47***
SCA	54	1030.7	162	30.96***
REC	66	40.5	198	1.88**
G × E	429	44.5	1144	3.13***
Parents vs. C × E	3	676.5	1144	47.62***
Parents × E	33	69.7	1144	4.90***
Crosses × E	393	37.6	1144	2.64***
GCA × E	33	154.7	1144	10.89***
SCA × E	162	33.3	1144	2.34***
REC × E	198	21.5	1144	1.51***
Error	1144	14.2		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.93		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.59		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A3. Combined ANOVA for ear height in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	20726.6	8	756.37***
Replication /E	8	27.4	1144	3.32***
Entry (G)	143	518.2	429	26.03***
Parents vs. Crosses	1	19665.6	3	64.50**
Parents	11	393.8	33	20.37***
Crosses (C)	131	382.5	393	21.51***
GCA	11	2751.6	33	36.81***
SCA	54	339.4	162	22.43***
REC	66	22.9	198	2.19***
G × E	429	19.9	1144	2.41***
Parents vs. C × E	3	304.9	1144	36.96***
Parents × E	33	19.3	1144	2.34***
Crosses × E	393	17.8	1144	2.15***
GCA × E	33	74.8	1144	9.06***
SCA × E	162	15.1	1144	1.83***
REC × E	198	10.4	1144	1.27**
Error	1144	8.2		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.94		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.62		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A4. Combined ANOVA for ear length in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	36526.9	8	150.58***
Replication /E	8	242.6	1144	0.48ns
Entry (G)	143	4396.2	429	7.26***
Parents vs. Crosses	1	178660.3	3	132.79***
Parents	11	4374.8	33	11.20***
Crosses (C)	131	3067.7	393	4.97***
GCA	11	22441.6	33	18.20***
SCA	54	2235.6	162	3.64***
REC	66	519.6	198	1.00ns
G × E	429	605.3	1144	1.20**
Parents vs. C × E	3	1345.5	1144	2.67*
Parents × E	33	390.8	1144	0.78ns
Crosses × E	393	617.6	1144	1.23**
GCA × E	33	1233.1	1144	2.45***
SCA × E	162	614.4	1144	1.22*
REC × E	198	517.7	1144	1.03ns
Error	1144	503.9		

Relative importance of GCA and SCA:	
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†	0.95
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡	0.67

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A5. Combined ANOVA for ear diameter in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	2361.9	8	157.14***
Replication /E	8	15.0	1144	4.16***
Entry (G)	143	134.8	429	19.69***
Parents vs. Crosses	1	6529.9	3	103.24**
Parents	11	101.9	33	10.66***
Crosses (C)	131	88.8	393	14.34***
GCA	11	489.5	33	21.32***
SCA	54	109.1	162	18.64***
REC	66	5.4	198	1.47*
G × E	429	6.8	1144	1.89***
Parents vs. C × E	3	63.2	1144	17.49***
Parents × E	33	9.6	1144	2.64***
Crosses × E	393	6.2	1144	1.71***
GCA × E	33	22.9	1144	6.35***
SCA × E	162	5.8	1144	1.62***
REC × E	198	3.7	1144	1.02ns
Error	1144	3.6		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.90		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.48		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A6. Combined ANOVA for ear weight in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	3571053.9	8	112.01***
Replication /E	8	31882.2	1144	2.57**
Entry (G)	143	605070.7	429	22.24***
Parents vs. Crosses	1	38016393.3	3	136.94**
Parents	11	107361.6	33	4.26***
Crosses (C)	131	361280.4	393	14.19***
GCA	11	1377138.2	33	15.62***
SCA	54	569023.8	162	20.34***
REC	66	21998.9	198	1.70**
G × E	429	27210.8	1144	2.19***
Parents vs. C × E	3	277622.2	1144	22.38***
Parents × E	33	25193.1	1144	2.03***
Crosses × E	393	25468.7	1144	2.05***
GCA × E	33	88148.7	1144	7.11***
SCA × E	162	27978.2	1144	2.26***
REC × E	198	12968.8	1144	1.05ns
Error	1144	12406.2		

Relative importance of GCA and SCA:	
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†	0.83
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡	0.33

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A7. Combined ANOVA for cob weight in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	26398.8	8	13.53**
Replication /E	8	1951.7	1144	7.66***
Entry (G)	143	11354.0	429	18.98***
Parents vs. Crosses	1	435629.9	3	1.56ns
Parents	11	2214.1	33	3.10**
Crosses (C)	131	8882.7	393	15.74***
GCA	11	74087.9	33	29.93***
SCA	54	5883.4	162	11.67***
REC	66	469.2	198	1.59**
G × E	429	598.1	1144	2.35***
Parents vs. C × E	3	278601.7	1144	1092.81***
Parents × E	33	713.1	1144	2.80***
Crosses × E	393	564.3	1144	2.21***
GCA × E	33	2475.5	1144	9.71***
SCA × E	162	504.2	1144	1.98***
REC × E	198	294.9	1144	1.16ns
Error	1144	254.9		

Relative importance of GCA and SCA:	
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†	0.96
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡	0.72

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A8. Combined ANOVA for cob diameter in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	1058.2	8	173.08***
Replication /E	8	6.1	1144	3.37***
Entry (G)	143	63.1	429	22.89***
Parents vs. Crosses	1	557.4	3	37.77**
Parents	11	90.0	33	24.51***
Crosses (C)	131	57.1	393	22.04***
GCA	11	585.2	33	64.99***
SCA	54	16.5	162	6.77***
REC	66	2.3	198	1.38*
G × E	429	2.8	1144	1.52***
Parents vs. C × E	3	14.7	1144	8.12***
Parents × E	33	3.7	1144	2.02***
Crosses × E	393	2.6	1144	1.43***
GCA × E	33	9.0	1144	4.96***
SCA × E	162	2.4	1144	1.34**
REC × E	198	1.6	1144	0.91ns
Error	1144	1.8		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.99		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.88		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A9. Combined ANOVA for husk cover in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	5.8	8	1.48ns
Replication /E	8	3.9	1144	16.79***
Entry (G)	143	2.9	429	5.15***
Parents vs. Crosses	1	28.5	3	58.46**
Parents	11	3.6	33	8.75***
Crosses (C)	131	2.7	393	4.60***
GCA	11	17.2	33	7.26***
SCA	54	2.4	162	4.33***
REC	66	0.5	198	1.52*
G × E	429	0.6	1144	2.39***
Parents vs. C × E	3	0.5	1144	2.07ns
Parents × E	33	0.4	1144	1.72**
Crosses × E	393	0.6	1144	2.45***
GCA × E	33	2.4	1144	10.05***
SCA × E	162	0.6	1144	2.33***
REC × E	198	0.3	1144	1.29**
Error	1144	0.2		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.94		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.60		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A10. Combined ANOVA for number of rows of kernels per ear in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	99.15	8	28.43***
Replication /E	8	3.49	1144	5.56***
Entry (G)	143	32.72	429	33.47***
Parents vs. Crosses	1	67.09	3	31.51*
Parents	11	80.37	33	77.91***
Crosses (C)	131	28.45	393	29.51***
GCA	11	315.29	33	107.07***
SCA	54	4.04	162	5.08***
REC	66	0.62	198	0.80ns
G × E	429	0.98	1144	1.56***
Parents vs. C × E	3	2.13	1144	3.39*
Parents × E	33	1.03	1144	1.64*
Crosses × E	393	0.96	1144	1.54***
GCA × E	33	2.94	1144	4.69***
SCA × E	162	0.80	1144	1.27*
REC × E	198	0.77	1144	1.23*
Error	1144	0.63		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.99		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.94		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A11. Combined ANOVA for kernel depth in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	113.86	8	35.31***
Replication /E	8	3.23	1144	3.26**
Entry (G)	143	14.02	429	9.12***
Parents vs. Crosses	1	817.10	3	151.13**
Parents	11	10.92	33	6.53ns
Crosses (C)	131	8.15	393	5.45ns
GCA	11	25.57	33	5.88ns
SCA	54	13.36	162	8.56***
REC	66	1.00	198	1.03ns
G × E	429	1.54	1144	1.56***
Parents vs. C × E	3	5.41	1144	5.47***
Parents × E	33	1.67	1144	1.69***
Crosses × E	393	1.50	1144	1.51***
GCA × E	33	4.35	1144	4.40***
SCA × E	162	1.56	1144	1.58***
REC × E	198	0.97	1144	0.98**
Error	1144	0.99		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.79		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.28		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A12. Combined ANOVA for kernel thickness in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	58.01	8	15.18***
Replication /E	8	3.82	1144	2.32***
Entry (G)	143	10.08	429	4.83***
Parents vs. Crosses	1	457.78	3	201.70*
Parents	11	52.59	33	7.90**
Crosses (C)	131	3.09	393	1.82***
GCA	11	11.57	33	3.98***
SCA	54	3.24	162	1.97***
REC	66	1.56	198	1.01ns
G × E	429	2.09	1144	1.27***
Parents vs. C × E	3	2.27	1144	1.38***
Parents × E	33	6.66	1144	4.05***
Crosses × E	393	1.70	1144	1.03***
GCA × E	33	2.91	1144	1.77***
SCA × E	162	1.64	1144	1.00***
REC × E	198	1.55	1144	0.94ns
Error	1144	1.65		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.88		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.42		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table A13. Combined ANOVA for kernel width in a 12 line complete diallel study.

Source	DF	Mean Square	Denominator DF	F Value
Environment (E)	3	37.51	8	59.13***
Replication /E	8	0.63	1144	3.15**
Entry (G)	143	5.48	429	19.72***
Parents vs. Crosses	1	36.41	3	63.79**
Parents	11	8.59	33	19.98***
Crosses (C)	131	4.98	393	18.95***
GCA	11	51.86	33	77.82***
SCA	54	1.34	162	5.45***
REC	66	0.14	198	0.68ns
G × E	429	0.28	1144	1.38***
Parents vs. C × E	3	0.57	1144	2.83*
Parents × E	33	0.43	1144	2.13***
Crosses × E	393	0.26	1144	1.30***
GCA × E	33	0.67	1144	3.30***
SCA × E	162	0.25	1144	1.22*
REC × E	198	0.21	1144	1.04ns
Error	1144	0.20		
Relative importance of GCA and SCA:				
(a) $2MS_{GCA} / (2MS_{GCA} + MS_{SCA})$†		0.99		
(b) $SS_{GCA} / (SS_{GCA} + SS_{SCA})$‡		0.89		

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† Ratio suggested by Baker (1978).
‡ Ratio suggested by Auld et al. (1983).

Table 14. Mean grain yield (Mg ha^{-1}), mid-parent and high-parent heterosis in a diallel set of crosses and in subsets representing crosses between related and unrelated maize lines.

Cross group type†	Number‡	Grain yield	MPHET	HPHET
Line Crosses		Mg ha^{-1}		
H1	264	8.32ab	344	245
H2	264	8.92ab	186	132
H3	264	9.99a	237	179
H4	264	8.06b	212	165
H5	264	7.97b	244	189
H6	264	7.73b	193	149
O1	264	8.40ab	174	123
W1	264	9.20ab	332	258
W2	264	8.56ab	295	230
W3	264	8.43ab	195	151
W4	264	8.49ab	219	178
W5	264	8.22ab	332	234
Major Groups				
G1 × G2	840	9.59a	288	222
Within G1	504	8.13b	243	176
Within G2	240	5.61c	111	83
Subgroups				
SG1 × G2	480	9.65a	321	243
SG2 × G2	360	9.52a	244	195
Within SG2	72	9.48a	215	154
SG1 × SG2	288	9.14b	279	202
Within G2	240	5.61c	111	83
Within SG1	144	5.45c	186	134
Complete diallel	1584	8.53	247	186

† Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡ Means followed by same letter are not different by Student Neuman Keul's $P < 0.05$. Statistics are for comparison of means within sets of crosses. Means between sets are not directly comparable.

Table 15. Mean, minimum, maximum, and standard deviation (SD) of number of heterozygotes summed over marker loci for 99 and 23 probe enzyme combinations (P/E); calculated for the complete diallel set of crosses and different subsets of crosses representing crosses between related and unrelated lines.

		Number of heterozygotes summed over marker loci							
		99 P/E combinations				23 P/E combinations			
Cross group†	Number‡	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
Complete Diallel	132	57	12	74	19	14	0	21	6
H1	22	60	19	74	18	16	7	21	5
H2	22	66	54	71	4	17	11	18	2
H3	22	60	54	69	5	15	11	18	2
H4	22	49	12	73	25	12	0	20	8
H5	22	50	16	74	24	12	3	21	7
H6	22	48	12	73	26	12	0	20	8
O1	22	52	15	74	23	12	2	20	7
W1	22	71	67	74	2	18	16	19	1
W2	22	49	13	72	24	12	2	20	7
W3	22	62	28	72	16	16	7	19	4
W4	22	54	19	70	16	14	6	18	4
W5	22	63	45	70	7	15	6	19	4
G1 × G2	70	67	54	74	5	17	13	21	2
SG1 × SG2	24	66	54	73	6	18	14	19	2
SG1 × G2	40	68	60	74	4	18	16	21	2
SG2 × G2	30	67	54	74	6	16	13	18	2
Within G1	42	58	19	73	15	15	6	19	4
Within G2	20	19	12	26	5	3	0	5	2
Within SG1	12	41	19	66	18	9	6	14	3
Within SG2	6	63	54	69	7	14	11	16	3

†Individual line groups represent a subset of the complete diallel involving all F_1s where the line is a parent. G1, G2, SG1, and SG2 are based on cluster analysis of the 23 P/E combinations (loci) associated with published QTLs for grain yield. G1 and G2 represent non-Stiff-Stalk and Stiff-Stalk lines, respectively and SG1 and SG2 are subgroups of the non-stiff-stalk lines.

‡Each observation is the mean of three reps in each of four environments.

VITA

Dale Warren Ball was born July 8, 1957 in Richmond, Virginia and was raised in the city of Richmond until graduation from George Wythe High School in 1975. The author is married to Sarah Lawson Ball, who is a Registered Nurse certified in College Health. They have two sons, Nathan 14 years old and Steven who is 10.

The author enrolled at Virginia Polytechnic Institute and State University in September 1975. After two years in the Animal Science curriculum Dale left school to manage a commercial swine farm at Bailey, North Carolina. After four years, the author accepted a job as herdsman of a dairy farm in Mount Airy, North Carolina. The author accepted a job as seed manager for Longest Seed Company at St. Stephens Church, Virginia in August 1982. In August 1984, the author returned to Virginia Polytechnic Institute and State University where he received a Bachelor of Science in Agronomy with a minor in Animal Science in June 1987 and a Master of Science in Agronomy in July, 1990. The author has pursued the degree of Doctor of Philosophy in Crop and Soil Environmental Sciences until the present. The author has held a faculty position as Senior Research Associate with the corn program in the Crop and Soil Environmental Sciences Department from August 1989 until the present. Upon graduation the author hopes to work as a research scientist in the application of Biotechnology Techniques to Crop Improvement.

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