# USEFULNESS OF EXPIRED PROPRIETARY (EX-PVP) MAIZE (ZEA MAYS L.) GERMPLASM FOR U.S. NORTHERN BREEDING PROGRAMS

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**DOCTOR OF PHILOSOPHY**

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# ABSTRACT

Maize (*Zea mays* L.) inbred lines and hybrids are protected by U.S. Patent and/or Plant Variety Protection Act (PVPA). Protection lasts 20 years and it affects breeding access in a highly confidential and competitive market. This research assessed the usefulness of patent expired short-season maize inbred lines. The study was conducted i) to understand the nature of gene action of a short-season maize breeding sample for agronomy and grain quality traits ii) to identify ex-PVP inbred lines and hybrids as potential breeding sources for short-season maize breeding programs for agronomic, grain quality, and nutritional traits iii) to identify and validate heterotic groups of ex-PVP inbreds and NDSU inbred lines, and iv) to identify desirable top heterotic patterns among ex-PVP, industry testers, and NDSU lines. Three North Carolina Mating Design II (NCII) crosses were made including NDSU lines, ex-PVP lines, and top industry testers in the 2010 North Dakota State University (NDSU) Fargo summer nursery and in the 2010 - 2011 NDSU New Zealand winter nursery. Hybrids were planted across six different ND environments in 2011 and 2012 following partially balanced lattice experimental designs. Combining ability analyses were performed following NCII design. Additive and non-additive genetic variances were important for regulating the expression of most traits with the preponderance of additive genetic variance. Our research identified ex-PVP inbred lines PH207, Q381, PHP02, S8324, PHK76, CR1Ht, PHT77, LH205, LH54, and PHJ40 that could be used as breeding sources to increase mostly grain yield. Most of the inbred lines belong to Stiff Stalk (SS), non-SS, or Lancaster backgrounds, although some belong to both SS/non-SS genetic backgrounds. The top heterotic patterns, from our trials, were represented in the following combinations: SS x non-SS, Iodent x SS, SS x Lancaster, Iodent x Lancaster, and SS/non-SS x SS. Our trials suggest most ex-PVP lines are not useful directly in immediate hybrid production

for agronomic and grain quality traits. In such a context, improvements in intellectual property and re-thinking of breeding rights access are encouraged to explore more suitable hybrids for short-season maize breeding programs.

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Special thanks to my wife, Afsana Noor for her unfailing love, constant company, understanding my busy schedules, inspiration, guiding this manuscript formatting, and caring our son, Asnud while I was compiling this dissertation.

# DEDICATION

I would like to dedicate my research dissertation to my Father Md. Afaz Uddin Shah (late), my Mother, Hasina Begum.

This work is also dedicated to our son Asnud Anahid Shah.

# TABLE OF CONTENTS

# LIST OF TABLES

# DISSERTATION ORGANIZATION

There are three different papers that have been compiled in this dissertation to be submitted for publication. Each paper (Chapter II, III, & IV) has an abstract, an introduction, material and methods, results and discussion, and references. Chapter I includes general introduction, review of literature, and objectives. A general conclusion was added in Chapter V.

# CHAPTER I: GENERAL INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, particularly in the U.S. in terms of area, production, multipurpose uses, and mostly for profitability. Maize was planted on 39.3 million hectares in the U.S. in 2012, giving a production of 273.8 million metric tons (USDA, NASS, 2013). The crop is mostly produced with hybrid cultivars often developed by crossing only two parental inbred lines. These inbred lines are the secret formula for seed companies. Inbred lines and hybrids are protected by U.S. Patent/or U.S. Plant Variety Protection Act (PVPA), normally for 20 years; as a consequence, their access remains restricted and confidential.

Maize was the first agricultural crop grown in North Dakota (ND) (Olson *et al.,* 1927), but remained a minor crop until the late 1990s. Maize hybrids need to withstand challenging environmental conditions with cooler and dry climates, strong winds, and very short growing seasons. Early maturing maize, along with desirable agronomic and quality traits, is required for its cultivation in the northern U.S. Corn Belt, especially in ND, where short growing seasons and frequent early frost injury are permanent crop threats. Breeding for early maturity, fast dry down, and stress tolerance are some of the most important reasons maize acreage is increasing in ND (Carena and Wanner, 2009), and their importance increases gradually in the region. Maize was planted on 316 thousand hectares in 1997 producing 267 thousand metric tons, in 2002 area under maize planting was 498 thousand hectares producing 2.8 million metric tons, while the planted area went up to 1.5 million hectares in 2012 producing 10.7 million metric tons (USDA, NASS, 2013).

The availability of adequate genetic diversity is essential for attaining significant genetic progress in any breeding program (Carena, 2008). Early maturing germplasm is always a scarcity due to its limited sources. Protection expired inbreds from U.S. Patents or PVPA are transferred to the North Central Regional Plant Introduction Station (NCRPIS) at Ames, Iowa, for distribution and use (Mikel, 2006). Incorporating them into university programs could provide unique combining ability for desirable traits not identified before. However, 20 years of protection might limit their current usefulness. Due to the concealed nature of the maize hybrid breeding business and the lack of access to proprietary lines, little is known about the potential of these lines in hybrid combinations with current public and private lines. Assigning heterotic group/s to a particular maize inbred line is useful in order to exploit desirable heterotic patterns. Heterotic groups consist of similar germplasm sources, which when crossed with each other produce consistently better hybrids than when crossed within (Hallauer and Carena, 2009). Heterotic patterns are performances of hybrids resulting from crossing between inbreds across or among heterotic groups (Troyer, 2006). Maize breeding programs for developing inbred lines of potential hybrids are mostly based on the identification and utilization of heterotic groups and heterotic patterns (Melani and Carena, 2005).

This research intends to evaluate the usefulness of expired industry lines that had been commercially used for 20 years before embarking on an effort toward their incorporation, and to validate heterotic groups of NDSU and ex-PVP inbreds. We chose to use the North Carolina Mating Design II (NCII) (Comstock and Robinson, 1948) in order to generate the genetic information needed to determine the potential usefulness of older industry lines for agronomic and quality traits.

## General Literature Review

### Nomenclature

Columbus first saw maize plants on the Island of Cuba, and described it as 'Panizo' or 'panic grass', comparing it to a type of millet. After two explorers went onto the land and saw maize close up they named it 'mahiz' or 'mayz' from the local 'Taino' Indian name, which eventually become maize (Indiana State Museum, 650 W. Washington St. visited on June 6, 2012). Swedish plant classifier Linnaeus had classified and named it *Zea mays* with 'zea' meaning life giving (Linnaeus, 1748). The English word 'Corn', used to describe maize, means fine particles or cereal seed. The term corn is used to describe the staple cereal of a country; corn in England means wheat (*Triticum aestivum*) while in Scotland and Ireland, it refers to oat (*Avena sativa*) (Gibson and Benson, 2002).

### Taxonomic relationship

Maize belongs to genus Zea which is composed of a group of annual and perennial grasses native to Mexico and Central America. The genus includes the wild taxa, 'teosinte' (*Zea* ssp.) and the cultigen, maize (*Z. mays* L. ssp. *mays*). The crop is under the Poaceae or grass family. Teosinte, known as the Mexican annual form (now called *Z. mays* ssp. *mexicana*), has chromosomes that are cytologically similar to those of maize, and its hybrids with maize exhibit complete chromosomal pairing and full fertility. Beadle (1932) showed that crossing-over between maize and teosinte chromosomes occur at frequencies similar to those observed in hybrids of two varieties of maize. The findings convinced scientists that maize is the domesticated annual teosinte and both were members of the same species. Deobley et al. (1984) examined isozyme variation in maize and teosinte populations. The results were in agreement with previous cytological analyses with some additional detail. The allele frequencies of one

Mexican annual teosinte, *Z. mays* ssp. *mexicana*, are more maize like, although still distinct. But archeological and molecular data indicate that modern maize was domesticated from annual Balsas teosinte (*Z. mays* spp. *parviglumis*) in southern Mexico in the state of Jalisco (Piperno and Flannery, 2001). Molecular data also offered the opportunity to apply a molecular clock and estimate the date of the maize teosinte divergence. The allele frequencies Mexican annual teosinte, *Z. mays* ssp. *parviglumis* or Balsas teosinte, are not distinguishable from those of maize. These data suggest that Balsas teosinte is the teosinte most closely related to maize, and therefore, the likely progenitor of maize. Matsuoka et al. (2002) studied microsatellite diversity in maize and teosinte. These authors investigated whether maize was the product of a single or multiple domestication(s) from teosinte as maize land races exhibit a high level of diversity that could result from multiple domestications. Phylogenetic analyses based on the microsatellite data strongly favor a single domestication. Their findings were in agreement with the isozyme data in which the single domestication of maize is derived from Balsas teosinte. The microsatellite data go a bit further and imply that the populations of Balsas teosinte in the central portion of its distribution are ancestral to maize. The group also estimated the time of maize-teosinte divergence with microsatellite data. The molecular dating indicate that maize and Balsas teosinte diverged about 9000 years ago, a date that agrees well with archaeological evidence provided by Piperno and Flannery ( 2001). The same microsatellite data by another group of investigators found that the ssp. *parviglumis* of the Balsas River drainage below 1800 m is the maize ancestor (Matsuoka et al., 2002) which was supported by archaeological evidence. However, previous cytogenetic analysis pointed out that the ssp. *mexicana* of the high lands above 1800 m is the most primitive form of maize (Beadle, 1972).

**Domestication of the species**

About 9000 years ago, people in the South Central part of today's Mexico began to cultivate teosinte. Gradual careful selection of the primitive crop with more desirable traits across generations resulted today's maize. Maize and the teosintes exhibit extreme differences in their adult morphologies and teosinte plants are taller and broader-leaved than most grasses. Their general growth form is similar to that of maize, although they have much longer lateral branches. Maize and teosinte female inflorescences or ears are strikingly different. The teosinte ear possesses only about 5 to 12 kernels, each sealed tightly in a stony casing. The kernels and its stony casing collectively are known as a fruit case. The cupule is formed from an invaginated rachis segment (internode) and a glume (modified bract) that covers over the kernel sitting in the cupule (Doebley, 2004). The cupule and glume are present in maize, but they are reduced in size such that they do not surround the kernel. In maize, these organs form the cob. Thus, maize domestication involved a change in ear development, such that the rachis segments and glumes formed a cob rather than fruit cases. At maturity, the teosinte ear disarticulates and the individual fruitcases become the dispersal units. Protected within its casing, the teosinte kernel can survive in the digestive tracts of birds and grazing mammals, enabling the seed to be easily dispersed. On the other hand, the massive maize ear can bear 500 or more kernels, each of which is attached to the central axis of the ear or cob. Since the kernels are firmly attached to the cob and the ear does not disarticulate, a maize ear left on the plant will eventually fall to ground with its full suite of kernels. When hundreds of maize kernels germinate the next season so close to one another, the emerging plants are unable to obtain adequate light and soil to grow and reproduce. Thus, maize is completely dependent on humans for its survival after domestication (Doebley, 2004).

**Dissemination of maize**

Because teosinte is considered the ancestor of maize, the center of origin of maize was determined to be Mexico at Jalisco state (Piperno and Flannery, 2001). The southward route of maize dispersion took the Central American hybridized maize to South America some 4000 years ago. Maize spread out from the highlands to the western and southern lowlands of Mexico into Guatemala, the Caribbean Islands, the lowlands of South America, and finally into the Andes Mountains (Matsuoka et al., 2002). The northward route of maize dispersal followed through western and northern Mexico into the southwestern U.S., and then through the eastern U.S. and Canada. The first maize race to reach the southwestern United States was Chapalote, some 3000 to 3500 years ago (Fagan, 1995). Maize spread into the eastern Woodlands of North America and appeared as a food stuff in what now are the states of New England and eastern New York during the 12$^{th}$ century A. D. For the western civilization, the story of maize began in 1492 when Columbus's men discovered this new grain in Cuba (Gibson and Benson, 2002). Maize went back to Spain with Columbus. Maize was initially only a garden curiosity in Europe, but it soon began to be recognized as a valuable staple crop. By the end of the 1500s maize was widely grown in Italy, Spain, and southern France. The spread of maize continued to other countries of the 'old world' (Paliwal, 2000). It is believed that Portuguese traders introduced maize to Africa in the early 1500s across the Sahara. Maize spread into the Asian continent via three routes in the 16$^{th}$ century: the Mediterranean trade route, the Atlantic, and Indian Ocean route (Taba, 1997). Taba (1997) also pointed out that after Magellan's voyage, the crop eventually travelled to the Philippines, and eastern Indonesia, and Thailand. Maize was introduced to China in the early 16$^{th}$ century, arriving by land and marine routes (Paliwal, 2000), and maize was first introduced into Japan around 1580 by Portuguese sailors.

**Maize in ND**

Women of agricultural tribes of native Indians, the Mandan, Arikara, and Hidatsas of the upper Missouri valley, are believed to be the first maize growers of ND, around the 1630s (Olson et al., 1927). Thus, maize is considered the oldest agricultural crop of the state. These Indians raised mostly three types of maize named as flints, flour, and sweet corn. They developed several varieties of these species, through careful selection. Olson et al. (1927) indicates USDA compiled maize area and production data in early years. They described maize area in 1891, 1900, 1909, and 1925 were 16, 10, 27, 74, 283, and 427 thousand hectares, respectively, with production of 17.8, 9.7, 38.6, 125.5, 248.9, and 630.4 thousand metric tons.

Maize has been a marginal crop in ND, and has encountered several challenges, over the years, including the short growing season, cool temperatures, low precipitation, and limited growing degree days (Carena et al., 2009). However, this has been an advantage for maize breeding research. NDSU maize breeding research was initiated in early the 1930's under the supervision of Professor Hayes. The NDSU maize breeding program has been developing early maturing products since 1933, and the first official maize breeder, William Wiidakas, who joined the ND Agricultural College (now NDSU) in 1934 (Carena, 2007). In the previous decade, maize area, and production have been escalated at a rapid pace and have expanded very fast in recent years. Contemporary maize area and production indicate a progressive maize cultivation trend (mentioned in the introduction) in this northern U.S. state. This is due to by several factors including changes in farm policies, a fast growing ethanol sector, expansion of the export market, and mostly because of the development and adoption of well suited varieties and technologies (Wilson, 2012), which have that turned maize into one of the most profit oriented crops of ND.

**Maize genome**

The maize genome is composed of 2.3-gigabase of nucleotides. There are about 32,540 genes on just 10 chromosomes. Nearly 85% of the maize genome is comprised of transposable elements which are dispersed non-uniformly across the genome (Patrick et al., 2009). The 10 chromosomes of the maize genome are structurally diverse and have undergone variant changes in chromatin composition. The large size of the maize genome is due to the proliferation of long terminal repeat retrotransposons (LTR retrotransposons). Maize breeders need to work mostly with quantitative traits expressed by hundreds of genes with small cumulative effects. These represent the most economically important traits. Moreover, multi-trait and multi-state selection, and, the complex genetics of most desirable agronomic traits, molecular markers are difficult to employ in applied maize breeding (Hallauer and Carena, 2009), even though these traits were the first ones to be targeted with newer technologies.

**U.S. maize diversity**

Maize was indigenous to the western Hemisphere. Accidental mixtures and cross-pollination would contribute to the wide variation of maize varieties that was present before Columbus arrived in the western Hemisphere (Hallauer and Carena, 2009). Troyer (2006) stated that ancestors of Northern Flint races arrived in present day Arizona and New Mexico about 1000 B.C. and slowly preceded and arrived in New England about 1000 A.D. On the contrary, Spanish Conquistadors introduced Southern Dent races from Mexico to present day Florida, South Carolina, and Virginia via Cuba, from 1539 to 1570. The Northern Flints and Southern Dents are distinct complexes, but the expansion of maize cultivation southward (for the Northern Flints) and northward (for the Southern Dents) resulted in a reciprocal introgression of the two complexes (Hudson, 1994), producing diverse gene pools. European settlers were introduced to

maize and depended on it for their survival. Movement of the people and exchange of maize germplasm permitted the introgression of divergent varieties and complexes of the western Hemisphere. In the northern Hemisphere, expansion of the cultivation of the Northern Flint complex and the Southern Dent complex led to the development of the highly productive U.S. Corn Belt Dent varieties (Anderson and Brown, 1952). Seeds of the two distinct landrace complexes were carried by people, but the crossing between the two complexes probably occurred more by contamination than by planned crosses. Crossing of the two distinct complexes created a vast reservoir of genetic variability for plant and ear traits. Simple mass selection based on individual plants was an effective breeding methodology for developing varieties that possessed traits appealing to growers and early colonial maize breeders. The hybrids arising from crossing the Northern Flint and Southern Dent complexes would not have been as extensive as in pre-Columbian times, but the range in genetic variability was great enough for the development of varieties having distinctive plant and ear traits. As the interior of the U.S. was developed, seeds of settlers' varieties were brought westward from the Atlantic seacoast. Each individual seed lot would have been subjected to different selection pressures, depending on the individuals selecting seed to propagate the crop in following seasons. In some instances, careful selection was given to ear traits, whereas others selected for early maturity, shorter plants, freedom from tillers, and plant type. Varieties with distinctive traits and adapted to specific environments were developed in pre-Columbian times. As cultivation of maize became more extensive, crosses among the selected strains occurred because of reduced isolation. The amount of mixtures among selected strains depended on the topography of the areas and the amount of interchange of seed among pioneer settlers. Troyer (1999) mentioned there were around 250 open-pollinated varieties in the USA in 1840, which was increased to nearly 1000 by the end of 19th century.

Genetic divergence among selected strains was sufficient enough that resulting crosses among them suggested variety hybrids were superior to grow as selected varieties per se. Controlled crosses between selected varieties improved grain yield performance. In the early 1900s, the inbred-hybrid concept (Shull, 1908; Shull, 1909; East, 1908) was developed. Open-pollinated varieties were replaced by hybrid maize (double-cross hybrids) in the 1930s (Mikel, 2008), as an outcome of inbred-hybrid research and since the 1960s, single-cross hybrid cultivars have been commercialized; the method to develop them still exists today. Initially, more diversity existed in the breeding programs, when open pollinated varieties were extensively used, but eventually double-cross hybrids, and finally, single-cross hybrids have been exploited for producing highly productive maize throughout the USA and most of the developed world. With increasing yield and productivity, breeders tried to boost yield and other primary agronomic traits using genetically narrow-based germplasm, and farmers gradually moved to grow available hybrids. Eventually genetic variability was reduced in maize breeding programs, as well as, in farm fields. Such tremendous genetic erosion needed to be addressed through different means. There are many ways a program can increase, or open up its genetic diversity. One alternative way would be to incorporate lines from industry, in which their intellectual property protection has expired.

**Inbred-hybrid concept**

Modern maize breeding programs are largely based upon the inbred-hybrid concept that was developed by public breeders over 100 years ago, during the early 1900s (Carena, 2007). E. M. East, and later H. K. Hayes, and D. F. Jones, explored the potential of maize hybrids by crossing two inbred lines. The most well known public scientist in early hybrid maize research is G.H. Shull whose maize research was recorded as early as 1905. Shull (1908) explained that the

theoretical importance of an isolation method faced difficulties in varietal improvement of Indian maize, as inbreeding or self-fertilization results severe deterioration. The deleterious effect of inbreeding, as a result of isolation, could be due to inharmonious or unbalanced constitution produced by the accumulation of disadvantageous individual variations. However, a very large number of plants normally self-fertilize entirely without lessening their physiological vigor. In tobacco (*Nicotiana tabacum*), cross pollination within the limits of a single strain produces inferior offspring, while on the converse self-fertilization gives the highest vigor. Shull concluded that an ordinary maize field has very complex hybrids and the deterioration resulting from self-fertilization is due to the gradual reduction of strain to a homozygous condition. He also added, plant breeders are responsible not only to find the best inbreds, but also to find and maintain best hybrid combinations. In another publication, Shull (1909) outlined ways to develop inbred lines and find ways to desirable hybrid combinations. He narrated "In finding the best pure-lines it will be necessary to make as many self-fertilizations as practicable, and to continue these year after year until the homozygous state is nearly or quite attained. Then all possible crosses are to be made among these different pure strains and the $F_1$ plants coming from each such cross are to be grown in the form of an ear-to-the-row test, each row being the product of a different cross. These cross-bred rows are then studied as to yield and the possession of other desirable qualities". Shull discontinued his research considering poor seed set on inbred parents making hybrid production costly and impractical from commercial view point. However, researchers like E. M. East and G. H. Shull provided the framework for maize breeding that is still used today with high success (Hallauer and Carena, 2009). Jones (1918) suggested suitable female and male parents to produce adequate maize hybrid seeds that made hybrids feasible for the industry and farmers. Eventually double-cross hybrids evolved to obtain more kernels in

hybrid parents. Around the 1950's, nearly 100 per cent of U.S. lands were covered by double-cross hybrids (Hallauer and Carena, 2009). Careful and adequate selection would allow selecting parents with better performances for single-cross hybrids to exploit highest heterosis. Double-cross hybrids were replaced by single-cross hybrids in the 1960s (Troyer, 2006), which increased yield potential, making hybrids more profitable and a successful way of production by keeping the inbred parents concealed.

**Expired PVP lines**

The U.S. Plant Variety Protection Act (PVPA) was approved in 1970 by the U.S. Congress. It states "the breeder (or the successor), has the right, during the term of the plant variety protection, to exclude others from selling the variety, offering it for sale, reproducing, importing, exporting, or using it in producing a hybrid or different variety there from, to the extent provided by this Act" (Nelson et al., 2008). The PVPA originally granted protection of registered germplasm for 18 years from the date certificate was issued. Maize breeders rarely used it before the 1980s. The U.S. Patent and Trademark Office allowed patenting of maize inbred lines and hybrids in 1985. Consequently, most proprietary maize inbred lines and some commercial hybrids have been patented (Janis and Kesan, 2001). The U.S. Patent grants protection for 20 years from the date of application and unlike PVPA, it does not allow breeding rights (Evenson, 1999). Even though, the PVPA does allow breeding of the protected lines, PVPA protected inbreds are not directly traceable because maize is commercially marketed as the hybrid ($F_1$) of two genetically compatible inbreds. The originator, or genetic provider, controls access and the use of these inbred lines. Companies, at their discretion, can allow use of PVPA protected maize lines through licensing, but, otherwise, in most cases there is no legal access to these inbreds for breeding purposes. Since 1985, inbreds can be either, or both, PVPA

and U.S. patent protected, and these dual protected lines are not practically acquirable until both sources of protection have expired. The PVPA certificates of the registered protected inbred lines that have expired are now available and potentially represent new germplasm sources for many public and private breeding programs (Nelson et al., 2008) for use with additional lines added yearly. Expired lines are deposited in the National Plant Germplasm System and held at the National Center for Germplasm Resource Preservation in Ft. Collins, CO. Upon PVPA certificate expiration, lines are transferred to the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, for maintenance and distribution. These lines are available for research and commercial applications. Although they date back 20 years, they may offer genetic potential to programs that previously did not have access (Mikel, 2006). These lines are assumed to have potential as substantial resources were devoted over years to improve these lines through informed crossing and intense selection (Jason et al., 2010), but extensive research of new combinations with public and contemporary private lines across environments is needed to evaluate their real potential. Mikel (2006) stated that the progenitors of current elite germplasm, as well as, a wide assortment of lines of many backgrounds, are becoming available. This is a continuous process, as more lines become available each year from the growing queue of registered lines with time-dependent expiration of protection. These lines can be valuable sources of knowledge for geneticists, and unique sources for breeders to develop useful products if they still are economically competitive. The challenges to breeders who have not previously had access to these lines is to find new ways to use this germplasm to generate additional genetic diversity and/or specific new hybrid combinations that will, within a cycle or two of recombination and selection, create competitive, genetically diverse commercially successful hybrids. Tapping exotic germplasm, exchange of proprietary maize lines between programs

through licensing, and the use of wide breeding crosses and/or diverse germplasm sources of unique inbred lines, are some of the options to add valued germplasm sources to breeding programs. To effectively breed with these lines there is a need to gain a working understanding on how they combine with each other (e.g., lines representing different backgrounds and companies), as well as, with today's elite public and private maize lines.

**Maturity**

In the northern U.S., particularly in ND, where maize is moving north and west, the availability of very early maturing maize hybrids is essential for successful maize production. Otherwise, severe yield losses frequently occur due to frost damage before maturity. Therefore, investing extensive resources in screening large samples of genotypes for the identification of hybrids with early maturity associated with high yield potential is required.

Hallauer and Russel (1962) defined maturity as the time at which maximum dry weight of the grain was first attained, and was calculated as the number of days from silking. The NDSU maize breeding program has developed new and genetically diverse early maturing products that are not available in industry. Even though multi-million dollar projects have studied the genetic architecture of maize flowering (Buckler et al., 2009), that have emphasized the genetic complexity of this trait, significant genetic progress can be obtained across breeding populations, at a rate of 2 to 4 days earlier per year, with a very simple and inexpensive approach (Carena et al., 2008; Hallauer and Carena, 2009).

Most commercially available maize hybrids in ND are late maturing, lack stress tolerance, and often have poor grain quality because of the short growing season and due to the presence of retail companies without breeding programs. Products purchased by these companies and sold to North Dakota farmers are mostly bred in southern Minnesota and provided to

Foundation Seed Companies (NDSU also acts as a genetic provider) which seldom suit ND environments. Maize grain value decreases when harvested at high moisture levels and the reported maturity classification by industry often does not agree with actual grain moisture at harvest. Developing new, locally early maturing maize hybrids is a long-term solution for maintaining a profitable maize production under the challenging environmental conditions present in northern U.S. states (Carena et al., 2010).

The availability of useful genetic diversity is essential to achieve significant genetic progress and adaptation in marginal and short-season environments. We need to explore more suitable genotypes for extending maize production to the cooler conditions of northern U.S. Expired- PVP lines could complement adapted northern germplasm, but evaluating their usefulness is essential before using them.

**North Carolina mating design II (NCII)**

Mating designs develop progenies for evaluation and estimation of components of variance, ultimately creating knowledge on the genetic structure of cultivars. These progenies include relationships among relatives having known genetic components of variance (Hallauer et al., 2010). The NCII accommodate more parents in the crossing program with fewer numbers of crosses, compared to the diallel mating design. The North Carolina mating design II (NCII) analysis provides estimates of males and females expectations equivalent to general combining ability (GCA) and the male by female interaction expectations equivalent to specific combining ability (SCA) variances (Hallauer et al., 2010). These combining ability variances and effects are very important genetic parameters to be considered in breeding programs. Little is known about the genetic basis of combining ability which is complex and polygenetic in nature (Qu et al., 2012). The performance of a hybrid is related to the GCA and SCA of the inbred lines involved

in the cross (Sprague and Tatum, 1942). GCA is associated with additive effects of the genes, while SCA is related to dominance and epistatic effects (non-additive effects) of the genes. Aguiar et al. (2003) analyzed combining abilities of maize inbreds and observed that both additive (GCA) and non-additive (SCA) effects were important for grain yield, while additive effects were important for plant height, ear height, ear placement, and prolificacy. Similar results have been found in other crops. Lan-Ying et al. (2009) analyzed combining ability and heritability of walnut (*Juglans regia*) quality by the NCII design, and found significant additive and non-additive variation for diameter, weight, thickness, and rate of kernel, with predominantly additive genetic variation. An inheritance study of tocopherol content and composition in winter rapeseed using the NCII, revealed that tocopherol content and composition are controlled by GCA effects, and that SCA effects were only detected for α-tocopherol content of rapeseed (*Brassica napus*) (Goffman and Becker, 2000). On the other hand, Kamau et al. (2010) used NCII to estimate combining ability for cassava (*Manihot esculenta*) genotypes, and found that non-additive gene action was more important than the additive gene action in influencing yield and most of its associated traits.

Combining abilities vary, depending on environmental stresses. In high and low stress conditions, SCA effects were significant, showing that the non-additive genetic effects were the most important (Souza et al., 2009). SCA and grain yield showed significant correlations and the genetic control of grain yield differed under contrasting environment.

Musila et al. (2010) estimated the combining ability of early maturing quality protein maize inbred lines, and observed significant GCA effects, indicating additive genetic effects for governing the traits. They also identified some potential inbred lines with higher GCA effects for grain yield, in both well-watered and managed drought stress conditions.

Qu et al. (2012) conducted a basic research study after crossing rice pure lines, following the NCII, in order to dissect their combining ability effects through QTL analysis. They identified several QTLs for all studied traits for combining ability. Some of the QTL's had pleiotropic effects and some of them were linked tightly to each other. The identified QTL's could provide valuable information on the genetics of combining ability.

**Heterotic groups and patterns**

Heterotic groups represent groups of germplasm sources that when crossed with other groups of germplasm sources produce consistently better crosses than when crosses are made within heterotic groups. The concept of heterotic groups for breeding purposes was first recognized by the 9$^{th}$ Corn Improvement Conference of the North Central Region of the United States (Hallauer and Carena, 2009). The North American dent maize germplasm is composed of multiple heterotic groups, that when crossed to each other, can optimize hybrid performance (Mikel and Dubley, 2006). Searching out the best combination among heterotic groups, heterotic pattern, is crucial to the development of successful maize (*Zea mays* L.) hybrids (Barata and Carena, 2006). Heterotic groups in dent maize have been subdivided into Iowa Stiff Stalk Synthetic (BSSS) and non-BSSS (Lu and Bernardo, 2001). A similar grouping consists of Reid Yellow Dent (includes BSSS), Lancaster, and miscellaneous heterotic groups (Gethi et al., 2002). Troyer (1999) divided maize into five genetic backgrounds: Reid Yellow Dent (Iodent Reid and BSSS), Minnesota 13 (W153R and SD105), Northwestern Dent (A48, A509, and A78), Lancaster Sure Crop (Mo17 and Oh43), and Leaming Corn (Oh07). Mikel and Dubley (2006) indicated that the Reid Yellow Dent group is the largest group, and that it has made significant contributions to commercial hybrids.

There are several methods to classify inbreds to heterotic groups. Two major classification methods are widely used across the world to distribute inbreds into heterotic groups (Fan et al., 2009). Firstly, the traditional method uses specific combining ability with some line-pedigree information and/or field hybrid-yield information to assign maize lines to a heterotic group. Secondly, different molecular markers can also be used to compute genetic similarity (GS), or genetic distance (GD), estimates to assign maize lines to a particular heterotic group. Barata and Carena (2006), and others, observed large inconsistencies between molecular marker and field data, and concluded that groups of similar germplasm could not be identified accurately and reliably with molecular markers. They recommend extensive field evaluation to classify inbred lines to heterotic groups.

## Objectives

The major goal of this research is to evaluate the usefulness of ex-PVP industry lines to the NDSU breeding program for short season environments, to ensure their direct or indirect use. The specific objectives were:

i) To study the nature of gene action of yield, yield associated traits, grain quality and nutritional traits of short-season industry and NDSU lines

ii) To identify the potential of ex-PVP lines for agronomy and grain quality traits as breeding sources for short-season maize breeding programs

iii) To identify unique hybrid combinations not tested before

iv) To determine and validate heterotic groups of ex-PVP and NDSU lines

v) To identify promising heterotic patterns among ex-PVP, NDSU, and current industry lines

## References


Anderson, E., and W.L. Brown. 1952. Origin of Corn Belt maize and its genetic significance. p. 124–148. *In* J.W. Gowen (ed.) Heterosis. Iowa State College, Ames, IA.

Anguiar, A.M., L.A. Carlini-Garcia, A.R.D. Silva, M.F. Santos, A.A.F. Garcia, and C.L.D. Souza Jr. 2003. Combining ability of inbred lines of maize and stability of their respective single crosses. *Scientia Agricola* 60 (1): 83-89.

Barata, C., and M.J. Carena. 2006. Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. Euphytica 151:339-349.

Beadle, G.W. 1932. The relation of crossing over to chromosome association in *Zea-euchlaena* hybrids. Genetics 17:481- 501.

Beadle, G.W. 1972. The mystery of maize. *Field Museum Nat. Hist. Bull.* 43:2-11.

Buckler, E.S., J.B. Holland, P.J. Bradbury, C.B. Acharya, P.J. Brown, C. Brown, E. Ersoz, S. Flint- Garcia, A. Garcia, J.C. Glaubitz, M.M. Goodman, C. Harjes, K. Guill, D.E. Kroon, S. Larsson, M.K. Lepak, H. Li, S.E. Mitchell, G. Pressoir, J.A. Peiffer, M.O. Rosas, T.R. Rocheford, M.C. Romay, S. Romero, S. Salvo, H.S. Villeda, H.S. da Silva, Q. Sun, F. Tian, N. Upadyayula, D. Ware, H. Yates, J. Yu, Z. Zhang, S. Kresovich, and M.D. McMullen. 2009. The genetic architecture of maize flowering time. Science 325:714-718.

Carena, M.J. 2007. Maize population hybrids: successful genetic resources for breeding programs and potential alternatives to single-cross hybrids. Acta Agronomica Hung. 55:27-36.

Carena, M.J. 2008. Increasing the genetic diversity or northern U.S. maize hybrids: Integrating pre-breeding with cultivar development. *In* Conventional and Molecular Breeding of Field and Vegetable Crops: Conference Proc. Novi Sad, Serbia. 24-27 Nov. 2008.

Carena, M.J., and D.W. Wanner. 2009. Development of genetically broad-based inbred lines of maize for early- maturing (70-80 RM) hybrids. J. Plant Reg. 3:107-111.

Carena, M.J., C. Eno, and D.W. Wanner. 2008. Registration of NDBS11 (FR-M)C3, NDBS1011, and NDBSK(HI-M)C3 maize germplasm. J. Plant Reg. 2:132-136.

Carena, M.J., D.W. Wanner, and J. Yang. 2010. Linking pre-breeding for local germplasm improvement with cultivar development in maize breeding for short-season (85-95 RM) Hybrids. J. Plant Reg. 4:86-92.

Carena, M.J., L. Pollak, W. Salhuana, and M. Denuc. 2009. Development of unique and novel lines for early-maturing hybrids: moving GEM germplasm northward and westward. Euphytica 170:87-97.

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

Doebley, J. 2004. The genetics of maize evolution. Annu. Rev. Genet. 38:37-58.

Doebley, J.F., M.M. Goodman, and C.W. Stuber. 1984. Isozymic variation in *Zea* (Gramineae). *Syst. Bot.* 9:203–18.

East, E.M. 1908. Inbreeding in corn. p. 419-428. In Connecticut Agricultural Experiment Station Report 1907.

Evanson, R.E. 1999. Intellectual property rights, access to plant germplasm, and crop production scenarios in 2020. Crop Sci. 39:1630-1635.

Fagan, B.M. 1995. Ancient North America: The archaeology of a continent. 2nd ed. Thames and Hudson, New Cork. N.Y.

Fan, X.M., Y.M. Zhang, W.H. Yao, H.M. Chen, J. Tan, C.X. Xu, X.L. Han, L.M. Luo, and M.S. Kang. 2009. Classifying maize inbred lines into heterotic groups using a factorial mating design. Agron. J. 101:106-112.

Gethi, J.G., J.A. Labate, K.R. Lamkey, M.E. Smith, and S. Kresovich. 2002. SSR variation in important US maize inbred lines. Crop Sci. 42:951–957.

Gibson, L., and G. Benson. 2002. Origin, history and uses of corn ( *Zea mays* ). Department of Agronomy, Iowa State University. [On line] available at http://agron-ww.agron.iastate.edu/Courses/agron212/readings/corn_history.htm (accessed on May 7, 2013).

Goffman, F.D., and H.C. Becker. 2000. Genetic analysis of tocopherol content and composition in winter rapeseed. Plant Breeding 120: 182-184.

Hallauer, A. R., and M. J. Carena. 2009. Maize Breeding. pp. 3-98. *In* M. J. Carena (ed.). Handbook of plant breeding: Cereals. *Springer*, New York.

Hallauer, A.R., and W.A. Russel. 1962. Estimates of maturity and its inheritance in maize. Crop Sci. 2:289-294.

Hallauer, A.R., M.J. Carena, and J.B. Miranda Fo. 2010. Hand Book of Plant Breeding: Quantitative Genetics in Maize Breeding. 3rd ed. *Springer,* New York. pp. 103-113.

Hudson, J.C. 1994. Making the corn belt. Indiana University Press, Bloomington and Indianapolis, IN.

Janis, M.D., and J.P. Kesan. 2001. Designing an optimal intellectual property system for plants: A U.S. Supreme Court debate. Nat. Biotechnol. 19:981-983.

Jason, M.A., A.L. Hauck, G.R. Johson, M.O. Bohn, and T.R. Rocheford. 2010. Diallel analysis of elite EX-PVP commercial inbreds. p. 185. 52nd Annual Maize Genetics Conference Abstract. Italy.

Jones, D.F. 1918. The effects of inbreeding and cross breeding upon development. Connecticut Agric. Exp. Stn. Bull. 207: 5-100.

Kamau, J., R. Melis, M. Laing, J. Derera, P. Shanahan, and E. Ngugi. 2010. Combining the yield ability and secondary traits of selected cassava genotypes in the semi-arid areas of Eastern Kenya. J. Plant Breed. & Crop Sci. 2:181-191.

Lan-Ying, Z., X. Qian-Wen, Z. Li, Z. Shang-Jie, and W. Ping-Bang. 2009. Analysis of combining ability and heritability about nut quality of walnut. Botany Res. J. 2(1):24-27.

Linnaeus C. 1748. Systema Naturae. Estocolmo, Suecia: http://gdz.sub.uni-goettingen.de/no_cache/dms/load/img/?IDDOC=233236.

Lu, H., and R. Bernardo. 2001. Molecular marker diversity among current and historical maize inbreds. Theor. Appl. Genet. 103:613-617.

Matsuoka, Y, Y. Vigouroux, M.M. Goodman, J. Sanchez, E.S. Buckler, and J.F. Doebley. 2002. A single domestication for maize shown by multi-locus microsatellite genotyping. *Proc. Natl. Acad. Sci.* USA. 99:6080–84.

Melani, M.D., and M.J. Carena. 2005. Alternative maize heterotic patterns for northern corn belt. Crop Sci. 45:2186-2194.

Mikel, M.A. 2006. Availability and analysis of proprietary dent corn inbred lines with expired U.S. plant variety protection. Crop Sci. 46:2555-2560.

Mikel, M.A. 2008. Genetic diversity and improvement of contemporary proprietary North American dent corn. Crop Sci. 48:1686-1695.

Mikel, M.A. and J.W. Dubley. 2006. Evolution of North American dent corn from public to proprietary germplasm. Crop Sci. 46:1193-1205.

Musila, R.N., A.O. Diallo, D. Makumbi, K. Njoroge. 2010. Combining ability of early maturing quality protein maize inbred lines adapted to eastern Africa. Field Crops Research 119:231-237.

Nelson, P.T., N.D. Coles, J.B. Holland, D.M. Bubeck, S. Smith, and M.M. Goodman. 2008. Molecular characterization of maize inbreds with expired U.S. plant variety protection. Crop Sci. 48:1673-1685.

Olson, P.J., H.L. Walster, and T.H. Hooper. 1927. Corn for North Dakota. North Dakota Agric. Exp. Stn. Bull. No. 207. North Dakota State Univ. Fargo, ND.

Paliwal, R.L. 2000. Origin, evolution, and spread of maize. p. 5-11. Tropical maize improvement and production. Food and Agriculture Organization (FAO) of the United Nations, Rome.

Patrick, S.S., D. Ware, R.S. Fulton, J.C. Stein, F. Wei, S. Pasternak, C. Liang, J. Zhang, L. Fulton, T.A. Graves, P.Minx, A.D. Reily, L. Courtney, S.S. Kruchowski, C. Tomlinson, C. Strong, K. Delehaunty, C. Fronick, B. Courtney, S.M. Rock, E. Belter, F. Du, K.

Kim, R.M. Abbott, M. Cotton, A. Levy, P. Marchetto, K. Ochoa, S.M. Jackson, B. Gillam, W. Chen, L. Yan, J. Higginbotham, M. Cardenas, J. Waligorski, E. Applebaum, L. Phelps, J. Falcone, K. Kanchi, T. Thane, A. Scimone, N. Thane, J. Henke, T. Wang, J. Ruppert, N. Shah, K. Rotter, J. Hodges, E. Ingenthron, M. Cordes, S. Kohlberg, J. Sgro, B. Delgado, K. Mead, A. Chinwalla, S. Leonard, K. Crouse, K. Collura, D. Kudrna, J. Currie, R. He, A. Angelova, S. Rajasekar, T. Mueller, R. Lomeli, G. Scara, A. Ko, K. Delaney, M. Wissotski, G. Lopez, D. Campos, M. Braidotti, E. Ashley, W. Golser, H. Kim, S. Lee, J. Lin, Z. Dujmic, W. Kim, J. Talag, A. Zuccolo, C. Fan, A. Sebastian, M. Kramer, L. Spiegel, L. Nascimento, T. Zutavern, B. Miller, C. Ambroise, S. Muller, W. Spooner, A. Narechania, L. Ren, S. Wei, S. Kumari, B. Faga, M.J. Levy, L. McMahan, P.V. Buren, M.W. Vaughn, K. Ying, C.T. Yeh, S.J. Emrich, Y. Jia, A. Kalyanaraman, A. Hsia, W.B. Barbazuk, R.S. Baucom, T.P. Brutnell, N.C. Carpita, C. Chaparro, J.M. Chia, J.M. Deragon, J.C. Estill, Y. Fu, J.A. Jeddeloh, Y. Han, H. Lee, P. Li, D.R. Lisch, S. Liu, Z. Liu, D.H. Nagel, M.C. McCann, P. SanMiguel, A.M. Myers, D. Nettleton, J. Nguyen, B.W. Penning, L. Ponnala, K.L. Schneider, D.C. Schwartz, A. Sharma, C. Soderlund, N.M. Springer, Q. Sun, H. Wang, M. Waterman, R. Westerman, T.K. Wolfgruber, L. Yang, Y. Yu, L. Zhang, S. Zhou, Q. Zhu, J.L. Bennetzen, R.K. Dawe, J. Jiang, N. Jiang, G.G. Presting,S.R. Wessler, S. Aluru, R.A. Martienssen, S.W. Clifton, W.R. McCombie, R.A. Wing, R.K. Wilson2009. The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112-1115.

Piperno, D., and K. Flannery. 2001. The earliest archaeological maize (*Zea mays* L.) from high land Mexico: new accelerator mass spectrometry dates and their implications. *Proc. Natl. Acad. Sci.* USA. 98:2101-2103.

Qu, Z., L. Lanzhi, J. Luo, P. Wang, S. Yu. 2012. QTL mapping of combining ability and heterosis of agronomic traits in rice backcross recombinant inbred lines and hybrid crosses. PLOS ONE 7(1):e28463. doi:10.1371/journal.pone.0028463.

Shull, G.H. 1908. The composition of a field of maize. Am. Breeders' Assoc. Rep. 4:296-301.

Shull, G.H. 1909. A pure-line method in corn breeding. Am. Breeders' Assoc. Rep. 5:51-59.

Souza, L.V.D., G.V. Miranda, J.C.C. Galvao, L.J.M. Guimaraes, and I.C.D. Santos. 2009. Combining ability of maize grain yield under different levels of environmental stress. Pesq. Agropec. Bras., Brasilia. 44 (10):1297-1303.

Sprague, G.F., and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. Agron. J. 34:923-932.

Taba, S. 1997. Maize. *In* D. Fuccillo, L. Sears, and P. Stapleton (eds.). Biodiversity in trust. Cambridge University Press. Cambridge, UK, 213–226.

Troyer, A.F. 1999. Background of US hybrid corn. Crop Sci. 39:601426.

Troyer, A.F. 2006. Adaptedness and heterosis in Corn and Mule hybrid. Crop Sci. 46:528-543.

United States Department of Agriculture (USDA). 2013. National Agricultural Statistics Service. [On line] available at http://quickstats.nass.usda.gov (accessed on 06 May 2013).

Wilson, W.W. 2012. Northern corn logistics study. P. 13-15. Annual Report. ND Corn Growers Association and ND Corn Council. Available

@http://www.ndcorn.org/uploads/useruploads/ndcg_annualreport2_lr.pdf (verified June 03, 2013).

# CHAPTER II: CAN EXPIRED PROPRIETARY MAIZE (ZEA MAYS L.) INDUSTRY LINES BE USEFUL FOR SHORT-SEASON BREEDING PROGRAMS? I. AGRONOMIC TRAITS


## Abstract

Maize (*Zea mays* L.) inbreds and hybrids are protected by U.S. Patent and/or Plant Variety Protection Act (PVPA) for 20 years. The overall objective of this research was to assess the usefulness of patent expired inbreds. The study was conducted to understand trait gene action and to identify ex-PVP inbreds and hybrids were useful as potential breeding sources for short-season maize breeding programs if useful. Three North Carolina Mating Design II (NCII) crosses were made including NDSU lines, ex-PVP lines, and top industry testers in the 2010 North Dakota State University (NDSU) Fargo summer nursery, and in the 2010 - 2011 NDSU winter nursery in New Zealand. Hybrids were planted across six different ND environments in 2011 and 2012 following partially balanced lattice experimental designs. Combining ability analyses were performed following the NCII design. Additive and non-additive genetic variances were important for regulating most traits studied, with a preponderance of additive genetic variance. Our research identified ex-PVP inbreds PH207, Q381, PHP02, S8324, PHK76, CR1Ht, PHT77, LH205, LH54, and PHJ40, as above average lines in hybrids to increase yield. Our trials suggest most ex-PVP lines may not be useful directly in immediate hybrid formulas. In such a context, improvements in intellectual property and re-thinking of breeding rights access are encouraged to explore more suitable hybrids for short-season maize breeding programs.



**Keywords:** *Zea mays* L., ex-PVP inbreds, hybrids, combining ability analysis, additive, non-additive

## Introduction

Maize (*Zea mays* L.) is mostly produced as hybrids by crossing two inbred parents. Inbred lines are the secret formula of hybrids with very restricted use. Inbred lines and hybrids are protected by U.S. Patent/or U.S. Plant Variety Protection Act (PVPA) for 20 years (Mikel, 2006, Janis and Kesan, 2001). The maize public and private sectors currently suffer from their own intellectual property limitations and controlling systems, especially by limiting breeding access. However, after 20 years, protection expired inbred lines could provide unique combining ability for desirable traits.

Even though maize was the first agricultural crop in ND (Olson et al., 1927), it remained of minor economic importance because of challenging environmental conditions with cooler and drier climates, strong winds, and very short growing seasons. Breeding for early maturity is one of the most important reasons maize is becoming adapted to ND (Carena and Wanner, 2009), and its importance increases gradually in the region as maize expands towards the north and west. Therefore, investing extensive resources for screening large and diverse samples, and identifying hybrids with early maturity associated with high yield potential, is required for maintaining profitable maize production under the challenging environmental conditions present, in northern U.S. states, like ND (Carena et al*.,* 2010).

Protection expired inbreds from U.S. Patents or PVPA are transferred to the North Central Plant Introduction Station (NCPIS) at Ames, Iowa, for distribution and use (Mikel, 2006). Incorporating such off protected industry lines, to university programs could provide unique hybrids for desirable traits not identified before. However, 20 years of protection might limit their current usefulness. Due to the concealed nature of the maize hybrid breeding business and lack of access to proprietary lines, little is known about the potential of these lines. These

lines are assumed to have potential, as substantial resources were devoted over the years to improve these lines through informed crossing and intense selection (Jason et al., 2010). Extensive evaluation of new combinations with public and contemporary private lines across environments is needed to evaluate their real, current economic and breeding potential. To effectively breed with these lines there is a need to understand how well they combine with each other and with today's elite maize lines in hybrid formulas.

The North Carolina mating design II (NCII) analysis provides expectations estimation of males and females equivalent to general combining ability (GCA) and expectations of the male by female sources equivalent to specific combining ability (SCA) variances (Hallauer et al., 2010). The performance of a hybrid is related to the GCA and SCA of the inbred lines involved in the cross (Sprague and Tatum, 1942). GCA is associated with additive effects of the genes, while SCA is related to dominance and epistatic effects (non-additive effects) of the genes. Aguiar et al. (2003) analyzed combining abilities of maize inbreds and observed that both additive (GCA) and non-additive (SCA) effects were important for grain yield, while additive effects were important for plant height, ear height, ear placement, and prolificacy. Similar results have been found in other crops. Lan-Ying et al. (2009) analyzed combining ability and heritability of walnut (*Juglans regia*) quality by NCII design and found significant additive and non-additive variation for diameter, weight, thickness, and rate of kernel with predominantly additive genetic variation. On the other hand, Kamau et al. (2010) used NCII design to estimate combining ability for cassava (*Manihot esculenta*) genotypes and found that non-additive gene action was more important, than the additive gene action, in influencing yield and most of its associated traits.

The major goal of this research was to evaluate the usefulness of ex-PVP industry lines to maize breeding programs for agronomic traits in short-season environments. The specific objectives were: i) to study the nature of gene action of yield and related traits in short-season industry and NDSU lines; ii) to identify potential of ex-PVP lines for yield and associated traits as breeding sources for short-season maize breeding programs; and iii) to identify unique hybrid combinations between ex-PVP, NDSU, and current industry lines not tested before.

## Materials and Methods

### Plant materials

Twelve NDSU released and experimental elite early maturing inbred lines, 24 ex-PVP lines, and seven top industry testers for the northern U.S. Corn Belt were used in this research. NDSU and ex-PVP lines were selected mostly based on earliness (fewer days to silking and growing degree days). NDSU inbreds are ND08-343, ND291, ND2000, ND2001, ND2002, ND2003, ND2004, ND2005, ND2006, ND2007, ND2010, and ND2011. Ex-PVP inbreds are Lp5(8700031), Q381(8500098), NK807(8700151), CR1Ht(8400042), DK78010(8500126), PH207(8300144), FAPW(8200152), LH52(8700020), NK794(8700046), LH54(8600128), DJ7(8500086), NK779(8700041), PHJ40(8600133), PHK05(8800001), PHR25(8800002), PHK76(8800036), PHT77(8800038), OQ603(8800150), S8324(8800153), PHP02(8800212), CR14(8900095), L127(8900201), LH205(9000049), RS710(9000129). PVP numbers, indicated above within parenthesis, can be used in the following USDA web address to explore more information on respective inbreds (http://www.ars-grin.gov/cgi-bin/npgs/html/pvplist.pl). Industry testers were T1 (Oh43 derived Iodent type), T2 (B14 derived), T3 (W153R derived Iodent), T4 (B14 and B73 derived), T5 (LH82 derived), T6 (B14 derived), and T7 (B14 and B73

derived). Testers were provided by our exclusive partner with restricted use so coded names were utilized.

**Crossing procedure**

Three NCII Mating Design crosses (Comstock and Robinson, 1948) were made for this study. In the first one, 12 NDSU lines were used as females and 12 ex-PVP lines were used as male parents in the 2010 NDSU maize breeding nursery in Fargo, ND. Inbreds were planted in paired rows, 7 m long with 0.76 m between rows. All possible pair-row crosses were made. Crosses were harvested and shelled in bulk by cross. In the second set of crosses, the same 12 NDSU lines were used as females and a second set of 12 ex-PVP lines were used as male parents following the same mating design (NCII) at the 2010-2011 winter nursery in Pukekohe, New Zealand. In the third cross combination, seven industry testers were used as females and all 24 ex-PVP lines (used in the first and second sets of crosses) were again used as males following the same design at the 2010-2011 New Zealand winter nursery.

**Field trials**

Hybrids from the three sets of crosses were tested in the four different trials. Each trial includes five industry hybrid checks and evaluated at six ND environments in 2011 and 2012 (one of the trials had five environments) with two replications. Experiments were arranged in an 11 by 11 partially balance lattice design (first trial: hybrids from the 1$^{st}$ set of crosses), a 9 by 9 partially balanced lattice design (second trial: hybrids from the 2$^{nd}$ set of crosses), a 8 by 9 rectangular lattice design (third trial: hybrids from the 3$^{rd}$ set of crosses) and a 8 by 8 partially balanced design (fourth trial: also hybrids from the 3$^{rd}$ set of crosses). All environments targeted the eastern ND region. Checks were DKC 33-54 (83RM), Pioneer 39D85 (85RM), TR2015+TR1099*TR3622 CBLL (87RM), Pioneer 38N88 (92RM), and TR3622 x TR4010

(100RM). In 2012, DKC 38-89 (88RM) was used as a check instead of TR2015+TR1099*TR3622 CBLL, as the previous source was depleted. Plot size was 6.10m by 0.76m. Plots were over planted and thinned back to approximately 70,000 plants $ha^{-1}$. Plots were planted and harvested by machines adapted for small experimental plots. Fertilizer and field management practices were used at each location for optimum maize production.

**Traits evaluated**

Grain yield (Mg $ha^{-1}$), grain moisture at harvest (g $Kg^{-1}$), test weight (Kg $hl^{-1}$), plant height (cm), ear height (cm), root lodging (%), stalk lodging (%), days to anthesis, and days to silking were recorded for this study. Data were collected on each individual plot for the study. Grain weight, grain moisture, and test weight were obtained electronically while harvesting by combine. Grain yield (Mg $ha^{-1}$) was adjusted based on 155 g $kg^{-1}$ (15.5%) grain moisture at harvest. Root lodging was measured as a percentage of plants in a plot leaning at an angle greater than 30° from vertical, while stalk lodging was measured as a percentage of plants in a plot with stalks broken at, or below, the top ear. Lodging was evaluated before harvest and analyzed as percentage to total stand per plot. Plant height was measured as the distance from soil surface to the top leaf node, and ear height was measured as the distance from soil surface to the base of the top ear attachment, both in centimeters. Both plant and ear height data were measured after flowering on 10 randomly selected plants and averaged. Days to anthesis was measured as the number of days from planting to 50% of plants shedding pollen on half of the tassel, and days to silking was measured as the number of days from planting to 50% of plants with emerged silks. In all four trials flowering dates were taken in five out of six environments.

**Statistical procedures**

Location by year combination was considered as environments. General and specific combining ability variances were calculated using SAS software version 9.3 (SAS, 2010) following NCII (model II) as described by Hallauer and Miranda (1988). The GCA is the male and female expectations, and SCA is the male by female interaction expectation. Genetic parameters, including additive ($\sigma^2_A$), dominance ($\sigma^2_D$), level of dominance ($\bar{d}$), and heritability in the narrow sense ($h_n^2$), were calculated using the formula described by Hallauer et al., 2010. Inbred parents were used in our study, thus the, inbreeding coefficient (F) was 1, and $\sigma^2_A = \sigma^2_m + \sigma^2_f$, $\sigma^2_D = \sigma^2_{mf}$, $\bar{d} = \sqrt{\frac{2\,\sigma 2mf}{(\sigma 2m + \sigma 2f)/2}}$, and $h^2_n = \frac{\sigma 2A}{(\frac{\sigma 2e}{r} + \sigma 2A + \sigma 2D)}$. The assumptions were random parents, no epistasis, no linkage disequilibrium, and no maternal effects exist on the material we used. The following linear random model was utilized (Scott et al., 2009) for the analysis:

$$Y_{ijkl} = \mu + \varepsilon_l + r_k(l) + m_i + f_j + mf(ij) + m_i(l) + f_j(l) + mf\varepsilon(ijl) + e_{ijlk}$$

Where,

$Y_{ijkl}$ = observed values

$\mu$ = overall mean of the experiments

$\varepsilon_l$ = $l$ environmental effects

$r_k(l)$ = replicate effects within $l$ environments

$m_i$ = effects of $i^{th}$ male parental lines

$f_j$ = effects of $j^{th}$ female parental lines

$mf(ij)$ = interaction effects of $i^{th}$ male with $j^{th}$ female parents

$m_i$ ($_l$)= interaction effects of $i^{th}$ male with $l$ environments

$f_j$ ($_l$) = interaction effects of $j^{th}$ female with $l$ environments

$mf\varepsilon$ ($_{ijl}$) = interaction effects of $i^{th}$ male by $j^{th}$ female by $l$ environments

$e_{ijlk}$ = residual

Homogeneity of error variances were tested using the 10-fold thumb rule before combining data across environments (Tabachnik and Fidell, 2001) of field trials. If the error mean squares (EMS) were approximately 10-fold, EMSs were considered homogeneous and were combined. Mean comparisons among genotypes were assessed by Fisher's protected least significant difference (LSD) at < 0.05 level of significance, which has been shown to be an appropriate test for detecting differences (Carmer and Swanson, 1971).

## Results and Discussion

### Genetic parameter estimation

A combined ANOVA was computed for all four trials (Table 2.1). The males and females expectations were pooled together to have combined GCA mean square estimate. GCA mean squares were significant ($P$ <0.01) for yield in all trials. SCA mean squares (male by female interaction expectations) were larger ($P$ <0.001) in first, third, and fourth trials (non-significant in second trial), indicating a preponderance of non-additive gene action in most trials for determining yield. Therefore, both additive and non-additive gene action were responsible for grain yield. Similar results were observed by Nass et al. (2000), Aguiar et al. (2003), Melani and Carena (2005), Jumbo and Carena (2008), and Fan et al. (2008, 2009). However, Bhatnagar et al. (2004) observed significant SCA for grain yield in both white and yellow QPM hybrids. Additive ($\sigma^2_A$) and dominance genetic ($\sigma^2_D$) variances were similar for all experiments, except in

the second, where additive genetic variance ($\sigma^2_A$) was larger. Overdominace gene action was present for yield in all four trials as per the estimated degree of dominance ($\overline{d}$) (1.44 to 2.03). Silva et al. (2004) also found overdominance in grain yield of maize. Narrow-sense heritability was moderate (0.31-0.50) in all four trial. Beavis et al. (1994) estimated broad-sense heritability as 0.74 in the $F_4$ generation, and 0.56 in top cross progenies.

Grain moisture was controlled by both GCA and SCA in all trials for this set of short-season corn inbred hybrids (Table 2.1). Melani and Carena (2005) and Jumbo and Carena (2008), however, reported only significant GCA for the same trait. In this case, the germplasm was represented by genetically broad-based populations and not inbred lines in hybrid combinations as it was in the previous two studies. Additive genetic variance ($\sigma^2_A$) was larger than the dominance genetic variance ($\sigma^2_D$) in determining grain moisture content though. The degree of dominance ($\overline{d}$) showed partial to complete dominance (0.38-0.97) in regulating the trait. Narrow-sense heritability was large (72% to 89%) in all trials (Table 2.1). High heritability values were also reported by Beavis et al. (1994) for grain moisture content, but the estimates were broad sense in their case.

For test weight, GCA variances were highly significant ($P$ <0.001) in all trials, but different magnitudes of non-additive gene effects were observed. In trial one, SCA was non-significant, while in the rest of the trials the SCA mean squares were significant at different levels (Table 2.1). Overall, we can conclude that for this set of genotypes, GCA was more important than SCA in governing this character, even though Jumbo and Carena (2008) reported non-significant GCA and SCA for test weight in genetically broad-based populations. Estimates of additive genetic variance ($\sigma^2_A$) were higher than the dominance genetic variance ($\sigma^2_D$) for test weight in all of the trials. Different levels of partial dominance were observed in test weight in

trial one, two, and three, but over-dominance was found to be important in the fourth trial. Heritability in the narrow-sense was moderate (50%- 87%) to high for test weight. Beavis et al. (1994) reported large broad-sense heritability of 73% in the $F_4$ generation and 85% with top cross progenies.

Both root and stalk lodging are very important agronomic traits in ND, where short-growing seasons and strong winds are prevalent. GCA variance was significant for percent root lodging in trials one, two, and four, explaining the prevalence of additive genetic variance for the trait. The additive genetic variance ($\sigma^2_A$) was larger than the dominance genetic variance ($\sigma^2_D$) for this trait in all trials studied. Degree of dominance ranged from 0.57 to 0.97, indicating presence of partial to complete dominance in the expression of this trait. Heritability ranged from low to moderate (19% to 56%). In percent stalk lodging irregular variances were observed. In trial one, two, and four, GCA variances were significant at different levels, but SCA variances were significant (at $P < 0.05$) only in trial two. Additive genetic variance was greater than the dominance genetic variance present for stalk lodging in all trials. Melani and Carena (2005) reported significant GCA for both root and stalk lodging, while significant GCA was observed for stalk lodging by Jumbo and Carena (2008). The estimate of degree of dominance reflected overdominance in the expression of stalk lodging. Heritability in the narrow-sense was low to moderate (35%-55%) for percent stalk lodging.

GCA and SCA mean squares were significant for plant height, ear height (except SCA in trial three), days to anthesis, and days to silking (except SCA in trial two). For all four traits, additive genetic variances were more important than dominant genetic variances for regulating the mentioned traits. Overdominance, though partial dominance in trials two and four, was important for plant height. The remainder of the traits varied from partial to complete

dominance. Heritability ranged from moderate to large values. In agreement with our results, Aguiar et al. (2003) reported significant GCA and SCA for plant and ear height.

Based on presence of inbreds in hybrid combinations and additionally their GCA effects (data not shown) were used to identify desirable inbreds for different traits of interest. Selected inbreds for yield are CR1Ht, Q381, PH207, LH54, PHP02, S8324, PHK76, PHT77, LH205, PHJ40, ND2002, ND2003, T1, and T6.

Negative significant GCA effects are preferable for grain moisture because it indicates the general capacity of parent to transmit this trait to progeny in cross combinations, with other parents, resulting in a low moisture containing hybrids. NK779, PHK05, PHJ40, ND2000, ND2006, T2, and T3 were desirable parents to decrease moisture. Significant desirable GCA effects for test weight were found in PHK05, PHK76, RS710, ND291, ND2011, and T6. Inbred lines LH52, CR1Ht, L127, Lp5, ND2004, ND2003, T5, and T7 can be used to develop root lodge resistance genotypes. For stalk lodging resistances selected parents are DK78010, L127, PHP02, PHR25, PH207, LH205, OQ603, Q381, ND2004, ND2007, ND2010, and ND2011. Desirable and significantly negative GCA effects were found in inbreds: LH52, NK807, NK779, PHK05, PHR25, RS710, ND291, ND2006, ND2011, and T6 for days to anthesis and days to silking. Selected inbreds for a particular trait could be intercrossed to develop new population with increased favorable alleles to develop more competent inbreds.

**Analysis of variance and selected hybrids**

Significant differences were observed among genotypes (data not shown) for all the agronomic traits we studied in trials one and four. In trials two and three, genotypes were significantly different for all traits, except for root and stalk lodging. We also found significant genotype by environmental interaction for yield and most agronomic characters. Mean values of

selected top hybrids in all trials are presented in Table 2.2 to Table 2.5. The basis of selection was higher yield, test weight, relatively lower moisture, lodging, days to anthesis, and silking. None of the selected hybrids yielded higher than the highest yielding check, but all were in the same group as the checks mean for yield in trial one (Table 2.2). Highest and lowest yield was observed in ND2002 x Lp5 (6.34 Mg $ha^{-1}$) and ND291 x Q381 (5.05 Mg $ha^{-1}$) among the selected hybrids; the checks mean was 6.04 Mg $ha^{-1}$. In trial two, ND2002 x PHP02 (6.64 Mg $ha^{-1}$) was the selected hybrid with largest yield, while ND291 x PHP02 (5.79 Mg $ha^{-1}$) was the lowest yielding hybrid, and the checks mean was 6.43 Mg $ha^{-1}$. In the third trial, hybrids T4 x Q381 (9.36 Mg $ha^{-1}$) and T1 x CR1Ht (9.29 Mg $ha^{-1}$) had significantly higher yield than the mean of the five checks (8.14 Mg $ha^{-1}$) (Table 2.4). All the selected hybrids had comparable yield with the checks mean (Table 2.4). All 12 selected hybrids yielded higher than the mean of the checks (6.49 Mg $ha^{-1}$), even though they were not statistically different (Table 2.5).

Harvest grain moisture is as important as grain yield for short-season maize breeding programs. Grain moisture ranged from 175.57 g $Kg^{-1}$ in hybrid ND2011 x CR1Ht, to 215.79 g $Kg^{-1}$ in hybrid Lp5 x ND2002, among the selected hybrids. Eleven of the selected hybrids had comparable moisture with the checks mean (181.97 g $Kg^{-1}$) in trial one (Table 2.2). Among the selected hybrids, only three had, statistically the same grain moisture as the checks mean (174 g $Kg^{-1}$) in the second trial (Table 2.3). In the third trial, the mean of checks for grain moisture at harvest was 146.65 g $Kg^{-1}$, and among the selected hybrids, seven had a similar moisture range as the checks (Table 2.4); eight of the 10 selected hybrids exhibited moisture percentages similar to the checks. In the fourth trial (Table 2.5), moisture ranged from 142.30 to 201.82 g $Kg^{-1}$ among the selected hybrids, and only two of them comparable with the checks mean.

All the selected hybrids had similar test weight compared to the checks mean (63.94 Kg $hL^{-1}$) in trial one (Table 2.2). In trial two, three hybrids out of the selected ten were found to have significantly higher test weight values when compared to the mean of the checks at 69.29 Kg $hL^{-1}$(Table 2.3). In the third trial, the test weight of selected hybrids ranged from 66.28 to 73.19 Kg $hL^{-1}$, while the checks ranged from 68.35 to 72.59 Kg $hL^{-1}$, (Table 2.4). In the fourth trial, test weight ranged from 66.82 to 72.79 Kg $hL^{-1}$ for the selected hybrids (Table 2.5), and two of them were statistically higher than the checks mean (69.79 Kg $hL^{-1}$).

Percent root lodging ranged from 0% to12.5% for the selected hybrids, while percent stalk lodging ranged from 1.83 to11.85% in trial one (Table 2.2). Thirteen of the selected hybrids were statistically similar to the checks for percent root lodging. All the selected hybrids were statistically similar to the checks mean (5.50%) for per cent stalk lodging. For trial two (Table 2.3), the selected hybrids had a root lodging percentage range from 0% to 8.1 %, which was statistically similar to the checks. Selected hybrids for trial three (Table 2.4) were more competitive for lodging resistance. The same pattern was also observed in trial four (Table 2.5), where mean root and stalk lodging of the selected hybrids was 1.20% and 3.06%, respectively, and similar statistically to the checks. Plant and ear heights were in suitable ranges for all the selected hybrids in all the trails (Table 2.2 to Table 2.5). Days to anthesis ranged from 61 to 65 days in the selected hybrids, and the checks ranged from 60 to 67 days (Table 2.2).

Our findings suggest both additive and dominance gene action were important in regulating yield and most yield attributing traits, with a preponderance of additive genetic variance in this selective set of northern U.S. corn hybrids. Ex-PVP inbreds with higher GCA could be used as potential parents but further testing is needed to confirm their potential usefulness. Overdominance expression found in our study could be exploited in hybrid breeding.

Our trials indicated that ex-PVP material is not directly useful in immediate commercial hybrids. It is clear from our results that most, if not all, ex-PVP lines need breeding work. Pre-breeding efforts could complement local development efforts. Selected ex-PVP lines could be inter-mated to develop new populations to increase favorable alleles and maximizing improvement, before considering new inbred line development with these materials. Rethinking breeding right access could be a timely approach to provide improved hybrids when maize is expanding north and west in ND.

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## References

Aguiar AM, Carlini-Garcia LA, Silva ARD, Santos MF, Garcia AAF, Souza Jr. CLD, 2003. Combining ability of inbred lines of maize and stability of their respective single crosses. *Scientia Agricola* 60: 83-89

Beavis WD, Smith OS, Grant D, Fincher R, 1994. Identification of quantitative trait loci using a small sample top crossed and $F_4$ progeny from maize. Crop Sci. 34: 882-896

Bhatnagar S, Betrán FJ, Rooney LW, 2004. Combining abilities of quality protein maize inbreds. Crop Sci. 44: 1997-2005

Carena MJ, Wanner DW, 2009. Development of genetically broad-based inbred lines of maize for early- maturing (70-80 RM) hybrids. J. Plant Reg. 3: 107-111

Carena MJ, Wanner DW, Yang J, 2010. Linking pre-breeding for local germplasm improvement with cultivar development in maize breeding for short-season (85-95 RM) Hybrids. J. Plant Reg. 4: 86-92

Carmer SG, Swanson MR, 1971. Detection of differences between means: A Monte Carlo study of five pair wise multiple comparison procedures. Agron J. 63: 940-945

Comstock RE, Robinson HF, 1948. The components of genetic variance in the populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics 4: 254-266

Fan XM, Chen HM, Tan J, Xu CX, Zhang YM, Huang YX, Kang MS, 2008. A new maize heterotic pattern between temperate and tropical germplasms. Agron. J. 100: 917-923

Fan XM, Zhang YM, Yao WH, Chen HM, Tan J, Xu CX, Han XL, Luo LM, Kang MS, 2009. Classifying maize inbred lines into heterotic groups using a factorial mating design. Agron. J. 101: 106-112

Hallauer AR, Carena MJ, Miranda Fo JB, 2010. Hand Book of Plant Breeding: Quantitative genetics in maize breeding. 3rd ed. Springer, New York. pp 103-113

Hallauer AR, Miranda Fo JB, 1988. Quantitative genetics in Maize Breeding. 2nd ed. Iowa State University Press, Ames. pp 64-72

Janis MD, Kesan JP, 2001. Designing an optimal intellectual property system for plants: A U.S. Supreme Court debate. Nat. Biotechnol. 19: 981–983

Jason MA, Hauck AL, Johson GR, Bohn MO, Rocheford TR, 2010. Diallel analysis of elite ex-PVP commercial inbreds. p. 185. 52nd Annual Maize Genetics Conference Abstract. Italy

Jumbo MB, Carena MJ, 2008. Combining ability, maternal, and reciprocal effects of elite-early maturing maize population hybrids. Euphytica 162: 325-333

Kamau J, Melis R, Laing M, Derera J, Shanahan P, Ngugi E, 2010. Combining the yield ability and secondary traits of selected cassava genotypes in the semi-arid areas of Eastern Kenya. J. Plant Breed. & Crop Sci. 2: 181-191

Lan-Ying Z, Qian-Wen X, Li Z, Shang-Jie Z, Ping-Bang W, 2009. Analysis of combining ability and heritability about nut quality of walnut. Botany Res. J. 2: 24-27

Melani MD, Carena MJ, 2005. Alternative maize heterotic patterns for the northern corn belt. Crop Sci. 45: 2186-2194

Mikel MA, 2006. Availability and analysis of proprietary dent corn inbred lines with expired U.S. plant variety protection. Crop Sci. 46: 2555-2560

Nass LL, Lima M, Vencovsky R, Gallo PB, 2000. Combining ability of maize inbred lines evaluated in three environments in Brazil. Scientia Agricola 57: 129-134

Olson PJ, Walster HL, Hooper TH, 1927. Corn for North Dakota. North Dakota Agric. Exp. Stn. Bull. No. 207. North Dakota State Univ. Fargo, ND

SAS Institute. 2010. SAS user's guide guide: Statistics. 4th ed. SAS Inst., Cary, NC

Silva AR, Souza Jr. CL, Aguiar AM, Souza APD, 2004. Estimates of genetic variance and level of dominance in a tropical maize population. I. grain yield and plant traits. Maydica 49: 65-71

Sprague GF, Tatum LA, 1942. General vs. specific combining ability in single crosses of corn. Agron. J. 34: 923-932

Tabachnik B, Fidell L, 2001. Computer –Assisted Research Design and Analysis. Boston: Allyn and Bacon

Table 2.1. North Caroline design II derived variances, on selected sources of variation, in four short-season maize trials across six environments (Environments are years by locations combinations: Casselton, Prosper in 2011 and Casselton, Prosper, Fargo, Barney in 2012)

| SOV | Yield (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT[†] (Kg $hL^{-1}$) | PRL[‡] (%) | PSL[§] (%) | PH[¶] (cm) | EH[#] (cm) | DA[††] (days) | DS[‡‡] (days) |
|---|---|---|---|---|---|---|---|---|---|
| Trial I: Maize (*Zea mays* L.) hybrids, derived from crosses between 12 NDSU lines by 1st set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | |
| GCA(M+F)[§§] | 13.27** | 71248*** | 459*** | 449.9*** | 590.2** | 4555*** | 4622*** | 491*** | 438*** |
| SCA(M*F)[¶¶] | 3.71*** | 3912*** | 78.05 | 75.07 | 143.98 | 405*** | 187*** | 20.85*** | 26.67*** |
| Residual | 2.06 | 891.92 | 57.1 | 79.27 | 96.32 | 124.98 | 62.58 | 7.87 | 14.06 |
| $\sigma^2A$[##] | 0.14 | 1434.14 | 6.57 | 5.91 | 6.38 | 70.04 | 76.98 | 10.01 | 8.67 |
| $\sigma^2D$[†††] | 0.15 | 266.39 | 1.17 | 0.50 | 2.36 | 24.87 | 10.31 | 1.22 | 1.23 |
| $\overline{d}$[‡‡‡] | 2.03 | 0.86 | 0.84 | 0.58 | 1.22 | 1.19 | 0.73 | 0.70 | 0.75 |
| $h_n^2$[§§§] | 0.31 | 0.80 | 0.53 | 0.41 | 0.35 | 0.66 | 0.83 | 0.83 | 0.77 |
| Trial II: Maize hybrids, derived from crosses between 12 NDSU lines by 2nd set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | |
| GCA(M+F)[§§] | 15.38** | 31947*** | 326*** | 61.2** | 965.8** | 3563*** | 3554*** | 247.34*** | 253.61*** |
| SCA(M*F)[¶¶] | 3.79 | 1588*** | 15.10* | 14.17 | 166.45* | 196*** | 114*** | 3.82** | 6.12 |
| Residual | 2.06 | 335.58 | 8.88 | 19.96 | 93.84 | 62.61 | 42.28 | 2.56 | 5.26 |
| $\sigma^2A$[##] | 0.32 | 1224.86 | 10.43 | 1.97 | 18.79 | 118.36 | 122.08 | 10.27 | 10.43 |
| $\sigma^2D$[†††] | 0.11 | 116.98 | 0.62 | 0.00 | 5.77 | 12.27 | 7.45 | 0.23 | 0.23 |
| $\overline{d}$[‡‡‡] | 1.44 | 0.38 | 0.24 | 0.00 | 1.23 | 0.41 | 0.24 | 0.09 | 0.09 |
| $h_n^2$[§§§] | 0.50 | 0.89 | 0.87 | 0.56 | 0.55 | 0.86 | 0.91 | 0.95 | 0.92 |

Table 2.1. NCII derived variances, on selected sources of variation, in four short-season maize trials across six environments (continued)

| SOV | Yield (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT[†] (Kg $hL^{-1}$) | PRL[‡] (%) | PSL[§] (%) | PH[¶] (cm) | EH[#] (cm) | DA[††] (days) | DS[‡‡] (days) |
|---|---|---|---|---|---|---|---|---|---|
| Trial III: Maize hybrids, derived from crosses between seven industry testers by 1st set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | |
| GCA(M+F)[§§] | 24.23** | 6105*** | 200*** | 187.6 | 180.8 | 1682*** | 1505*** | 132.47*** | 140.23*** |
| SCA(M*F)[¶¶] | 7.82*** | 343*** | 10.11*** | 87.36 | 61.76 | 322*** | 104 | 7.75*** | 9.74*** |
| Residual | 2.43 | 89.27 | 3.31 | 56.8 | 37.6 | 125.21 | 73.16 | 2.34 | 2.65 |
| $\sigma^2A$[##] | 0.56 | 200.63 | 4.99 | 1.44 | 2.57 | 34.86 | 36.16 | 4.09 | 4.29 |
| $\sigma^2D$[†††] | 0.57 | 27.14 | 0.62 | 0.57 | 0.00 | 14.60 | 1.56 | 0.52 | 0.72 |
| $\overline{d}$[‡‡‡] | 2.03 | 0.74 | 0.70 | 1.26 | 1.10 | 1.29 | 0.42 | 0.71 | 0.82 |
| $h_n^2$[§§§] | 0.40 | 0.84 | 0.84 | 0.19 | 0.46 | 0.58 | 0.83 | 0.85 | 0.81 |
| Trial IV: Maize hybrids, derived from crosses between seven industry testers by 2nd set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | |
| GCA(M+F)[§§] | 17.21** | 11959** | 187*** | 139.6** | 292.8* | 2292*** | 2120*** | 114*** | 107*** |
| SCA(M*F)[¶¶] | 5.48*** | 1226*** | 41.53** | 32.91 | 60.06 | 147** | 75.78* | 9.05*** | 9.49*** |
| Residual | 2.45 | 453.25 | 27.99 | 25.55 | 65.97 | 79.9 | 39.35 | 2.84 | 3.33 |
| $\sigma^2A$[##] | 0.27 | 255.45 | 3.95 | 2.79 | 4.14 | 57.23 | 56.62 | 4.08 | 3.77 |
| $\sigma^2D$[†††] | 0.24 | 60.43 | 1.55 | 0.68 | 0.00 | 5.04 | 2.41 | 0.72 | 0.78 |
| $\overline{d}$[‡‡‡] | 1.91 | 0.97 | 1.25 | 0.99 | 1.11 | 0.59 | 0.41 | 0.84 | 0.91 |
| $h_n^2$[§§§] | 0.38 | 0.72 | 0.50 | 0.50 | 0.43 | 0.46 | 0.83 | 0.91 | 0.81 |

*, **, *** significance at $P$ <0.05, <0.01, and <0.001; [†] test weight, [‡] per cent root lodging, [§]per cent stalk lodging, [¶] plant height, [#]ear height, [††]days to anthesis, [‡‡] days to silking; [§§] GCA (M+F) is the male and female variances pooled together, [¶¶] SCA (M*F), is Male by female interaction variance, [##] $\sigma^2A$, is the additive genetic variance, [†††]$\sigma^2D$, is the dominance genetic variance, [‡‡‡]$\overline{d}$, is the degree of dominance, [§§§] $h_n^2$, is the narrow- sense heritability, for derived equation of these please check statistical procedure section

Table 2.2. Selected maize hybrids, from combined analysis across six environments (Casselton, Prosper in 2011 and Casselton, Prosper, Fargo, Barney in 2012), based on a relative combination of higher yield, test weight (twt), and lower grain moisture (MSTR), percent root lodging (PRL), percent shoot lodging (PSL), days to anthesis (DA), days to silking (DS) of trial I

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| ND2002 x Lp5 | 6.34 | 215.79 | 63.05 | 12.46 | 3.78 | 198 | 97 | 65 | 66 |
| ND2002 x CR1Ht | 6.26 | 186.41 | 64.73 | 6.46 | 1.83 | 196 | 85 | 63 | 64 |
| ND2002 x PH207 | 6.02 | 193.27 | 63.01 | 6.98 | 7.43 | 191 | 95 | 64 | 65 |
| ND2002 x DKFAPW | 5.87 | 204.95 | 62.32 | 5.94 | 8.24 | 207 | 99 | 66 | 67 |
| ND2010 x PH207 | 5.78 | 215.91 | 60.46 | 2.05 | 6.09 | 211 | 117 | 66 | 68 |
| ND2011 x PH207 | 5.64 | 201.79 | 68.32 | 1.06 | 2.88 | 196 | 97 | 62 | 62 |
| ND291 x DK78010 | 5.48 | 207.53 | 66.32 | 3.17 | 6.81 | 192 | 100 | 63 | 64 |
| ND2004 x NK807 | 5.46 | 204.86 | 65.15 | 1.75 | 7.06 | 197 | 94 | 64 | 64 |
| ND2010 x NK807 | 5.45 | 177.73 | 65.49 | 2.12 | 7.01 | 216 | 114 | 64 | 65 |
| NK779 x ND 2005 | 5.42 | 195.05 | 63.39 | 2.01 | 2.84 | 186 | 90 | 65 | 68 |
| ND2004 x DKFAPW | 5.21 | 192.24 | 62.62 | 0.00 | 5.16 | 199 | 95 | 67 | 69 |
| ND 291 x PH207 | 5.17 | 191.55 | 64.19 | 1.63 | 7.58 | 196 | 96 | 62 | 63 |
| ND2011 x CR1Ht | 5.11 | 175.57 | 65.34 | 4.50 | 6.32 | 201 | 94 | 61 | 62 |
| ND291 x Q381 | 5.05 | 185.40 | 66.83 | 3.86 | 11.85 | 191 | 95 | 61 | 63 |
| Checks: | | | | | | | | | |
| DKC 33-54 | 5.71 | 149.97 | 64.04 | 0.17 | 7.75 | 190 | 88 | 60 | 61 |
| Pioneer 39D85 | 5.93 | 162.41 | 63.46 | 0.45 | 0.74 | 199 | 96 | 62 | 64 |
| DKC 38-89 | 5.46 | 161.68 | 63.49 | 1.48 | 3.68 | 202 | 89 | 64 | 64 |

Table 2.2. Selected maize hybrids, from combined analysis across six environments, based on combination of different desirable traits of trial I (continued)

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| Checks: | | | | | | | | | |
| Pioneer 38N88 | 6.50 | 203.94 | 65.33 | 0.00 | 4.89 | 190 | 98 | 64 | 63 |
| TR3622 xTR4010 | 6.58 | 231.83 | 63.38 | 0.25 | 10.45 | 201 | 98 | 67 | 67 |
| Mean of selections§ | 5.59 | 196.29 | 64.37 | 3.86 | 6.06 | 198 | 98 | 64 | 65 |
| Checks mean¶ | 6.04 | 181.97 | 63.94 | 0.47 | 5.50 | 196 | 94 | 63 | 64 |
| Mean# | 4.53 | 207.10 | 60.94 | 3.93 | 8.18 | 196 | 96 | 64 | 65 |
| EMS | 1.84 | 807.09 | 46.18 | 65.15 | 82.55 | 58 | 37 | 3 | 4 |
| LSD, 0.05 | 1.10 | 23.01 | 5.50 | 6.54 | 7.36 | 6 | 5 | 1 | 2 |
| CV,% | 29.95 | 13.72 | 11.15 | 205.29 | 111.03 | 4 | 6 | 3 | 3 |

† Plant height (PH), ‡ ear height (EH), § Mean of selected 14 entries, ¶ mean of five checks, # mean of 121 entries; Selection were carried out by yield sort in descending order and around 60 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher test weight, hybrids were further selected for lower percent root and stalk lodging, and lower days to anthesis and silking; always basis of selection was best check and average values of industry checks

Table 2.3. Selected maize hybrids, from combined analysis, across five environments (Casselton, Prosper in 2011 and Casselton, Prosper, Barney in 2012), based on relative combination of higher yield, test weight (twt), and lower grain moisture (MSTR), percent root lodging (PRL), percent shoot lodging (PSL), days to anthesis (DA), days to silking (DS) of trial II

| Hybrids | YIELD ($Mg\ ha^{-1}$) | Moisture ($g\ Kg^{-1}$) | TWT ($Kg\ hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| ND2002 x PHP02 | 6.64 | 205.16 | 69.19 | 8.05 | 5.09 | 192 | 90 | 64 | 64 |
| ND2000 x S8324 | 6.45 | 175.84 | 68.47 | 6.76 | 10.79 | 191 | 92 | 62 | 63 |
| ND2010 x PHP02 | 6.44 | 218.90 | 64.41 | 2.25 | 3.61 | 213 | 111 | 66 | 68 |
| ND2003 x PHK76 | 6.36 | 204.78 | 72.07 | 1.50 | 8.01 | 191 | 87 | 67 | 69 |
| ND2011 x PHK76 | 6.29 | 190.43 | 75.84 | 3.61 | 10.36 | 206 | 94 | 62 | 63 |
| ND2011 x OQ603 | 6.25 | 217.31 | 71.29 | 0.32 | 5.49 | 198 | 93 | 64 | 65 |
| ND2011 x PHP02 | 6.15 | 214.82 | 70.41 | 2.27 | 1.60 | 195 | 87 | 62 | 63 |
| ND2011 x L127 | 6.12 | 194.99 | 73.01 | 2.57 | 4.20 | 184 | 82 | 60 | 63 |
| ND2011 x PHT77 | 5.83 | 178.30 | 73.84 | 1.50 | 3.48 | 200 | 96 | 63 | 65 |
| ND291 x PHP02 | 5.79 | 181.91 | 71.75 | 0.00 | 12.68 | 197 | 93 | 62 | 63 |
| Checks: | | | | | | | | | |
| DKC 33-54 | 5.09 | 158.09 | 71.80 | 0.00 | 27.35 | 185 | 83 | 62 | 63 |
| Pioneer 39D85 | 6.98 | 168.43 | 69.04 | 1.03 | 5.26 | 194 | 89 | 62 | 64 |
| DKC 38-89 | 6.77 | 187.38 | 67.76 | 1.77 | 6.68 | 196 | 82 | 63 | 65 |
| Pioneer 38N88 | 6.92 | 159.72 | 69.61 | 1.16 | 1.36 | 190 | 99 | 63 | 63 |
| TR3622 x TR4010 | 6.38 | 195.81 | 68.23 | 0.00 | 40.46 | 200 | 95 | 67 | 68 |

Table 2.3. Selected maize hybrids, from combined analysis across five environments based on relative combination of different desirable traits of trial II (continued)

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| Mean of selections§ | 6.23 | 198.24 | 71.03 | 2.88 | 6.53 | 197 | 92 | 63 | 65 |
| Checks mean¶ | 6.43 | 173.89 | 69.29 | 0.79 | 16.22 | 193 | 90 | 63 | 65 |
| Mean# | 5.13 | 201.06 | 69.64 | 5.00 | 10.84 | 195 | 91 | 64 | 65 |
| MSE | 1.87 | 243 | 12.66 | 413.68 | 801.36 | 34 | 24 | 2 | 4 |
| LSD, 0.05 | 1.21 | 13.84 | 3.16 | 18.02 | 25.12 | 5 | 4 | 1 | 2 |
| % CV, | 26.67 | 7.76 | 5.11 | 406.14 | 261.19 | 3 | 5 | 2 | 3 |

† Plant height (PH), ‡ ear height (EH), § Mean of selected 10 entries, ¶ mean of five checks, # mean of 81 entries; selected entries were subjectively selected relative to the top and average values of industry checks; Selection were carried out by yield sort in descending order and around 40 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher test weight, hybrids were further selected for lower percent root and stalk lodging, and lower days to anthesis and silking; always basis of selection was best check and average values of industry checks

Table 2.4. Selected maize hybrids, from combined analysis across six environments (Casselton, Prosper in 2011 and Casselton, Prosper, Fargo, Barney in 2012), based on relative combination of higher yield, test weight (twt), and lower grain moisture (MSTR), percent root lodging (PRL), percent shoot lodging (PSL), days to anthesis (DA), days to silking (DS) of trial III

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| T4 x Q381 | 9.36 | 150.95 | 68.39 | 1.32 | 1.41 | 193 | 92 | 64 | 65 |
| T1 x CR1Ht | 9.29 | 150.34 | 66.28 | 1.18 | 4.18 | 191 | 84 | 64 | 66 |
| T4 x PH207 | 9.22 | 142.44 | 69.81 | 1.85 | 1.74 | 191 | 94 | 64 | 65 |
| T1 x 794 | 8.75 | 167.29 | 66.83 | 0.00 | 3.36 | 189 | 90 | 65 | 67 |
| T6 x Q381 | 8.72 | 145.64 | 73.44 | 4.39 | 0.28 | 190 | 82 | 61 | 62 |
| T1 x LH52 | 8.61 | 150.96 | 67.94 | 3.03 | 1.72 | 192 | 89 | 64 | 66 |
| T6 x CR1Ht | 8.49 | 150.06 | 73.19 | 0.71 | 0.18 | 193 | 81 | 60 | 61 |
| T7 x Q381 | 7.95 | 155.91 | 70.70 | 1.47 | 3.19 | 183 | 81 | 65 | 67 |
| T3 x LH52 | 7.87 | 137.68 | 69.78 | 0.73 | 2.28 | 188 | 87 | 63 | 65 |
| Checks | | | | | | | | | |
| DKC 33-54 | 7.32 | 135.84 | 72.59 | 1.39 | 5.24 | 182 | 84 | 61 | 61 |
| Pioneer 39D85 | 8.15 | 139.16 | 69.78 | 0.78 | 2.32 | 192 | 91 | 62 | 64 |
| DKC38-89 | 7.15 | 142.79 | 68.88 | 0.68 | 4.99 | 193 | 83 | 63 | 65 |
| Pioneer 38N88 | 9.41 | 143.97 | 70.86 | 0.33 | 0.33 | 186 | 97 | 62 | 63 |
| TR3622xTR4010 | 8.65 | 171.51 | 68.35 | 0.59 | 1.13 | 197 | 95 | 66 | 68 |

Table 2.4. Selected maize hybrids, from combined analysis across six environments, based on relative combination of different desirable traits of trial III (continued)

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| Mean of selections§ | 8.70 | 150.14 | 69.60 | 1.63 | 2.04 | 190 | 87 | 63 | 65 |
| Checks mean¶ | 8.14 | 146.65 | 70.09 | 0.75 | 2.80 | 190 | 90 | 63 | 64 |
| Mean# | 6.77 | 153 | 69.92 | 3.18 | 3.78 | 188 | 89 | 64 | 65 |
| MSE | 2.06 | 65.87 | 2.99 | 39.29 | 27.97 | 54 | 42 | 2 | 2 |
| LSD, 0.05 | 1.15 | 6.53 | 1.39 | 5.04 | 4.25 | 6 | 5 | 1 | 1 |
| CV,% | 21.17 | 5.29 | 2.47 | 197.11 | 140.08 | 4 | 7 | 2 | 2 |

† Plant height (PH), ‡ ear height (EH), § Mean of selected 9 entries, ¶ mean of five checks, # mean of 72 entries; selected entries were subjectively selected relative to the top and average values of industry checks; Selection were carried out by yield sort in descending order and around 35 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher test weight, hybrids were further selected for lower per cent root and stalk lodging, and lower days to anthesis and silking; always basis of selection was best check and average values of industry checks

Table 2.5. Selected maize hybrids, from combined analysis across six environments (Casselton, Prosper in 2011 and Casselton, Prosper, Fargo, Barney in 2012), based on a relative combination of higher yield, test weight (twt), and lower grain moisture (MSTR), percent root lodging (PRL), percent shoot lodging (PSL), days to anthesis (DA), days to silking (DS) of trial IV

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| T1 x LH205 | 7.62 | 197.83 | 66.97 | 2.24 | 1.51 | 204 | 99 | 66 | 67 |
| T7 x PHP02 | 7.55 | 188.91 | 70.56 | 0.07 | 1.10 | 194 | 87 | 64 | 65 |
| T6 x PHP02 | 7.47 | 179.39 | 69.87 | 4.57 | 1.95 | 202 | 90 | 60 | 61 |
| T7 x PHT77 | 7.08 | 185.21 | 68.45 | 0.30 | 1.45 | 197 | 92 | 66 | 68 |
| T1 x S8324 | 7.01 | 187.07 | 67.56 | 1.13 | 2.11 | 193 | 87 | 65 | 67 |
| T4 x PHP02 | 6.93 | 181.70 | 66.82 | 1.42 | 3.23 | 198 | 97 | 64 | 65 |
| T1 x PHT77 | 6.90 | 177.63 | 67.52 | 0.01 | 5.51 | 195 | 94 | 67 | 69 |
| T4 x L127 | 6.88 | 181.80 | 69.41 | 0.43 | 8.11 | 194 | 91 | 64 | 66 |
| T7 x OQ603 | 6.75 | 195.42 | 69.45 | 0.63 | 0.74 | 198 | 93 | 66 | 67 |
| T1 x RS710 | 6.72 | 157.76 | 69.68 | 0.55 | 4.34 | 178 | 75 | 62 | 64 |
| T6 x PHK76 | 6.64 | 190.43 | 72.79 | 1.41 | 2.77 | 204 | 92 | 63 | 64 |
| T1 x PHJ40 | 6.58 | 156.65 | 71.96 | 1.61 | 3.94 | 194 | 93 | 61 | 62 |
| Checks | | | | | | | | | |
| DKC 33-54 | 5.93 | 142.30 | 71.14 | 0.00 | 10.76 | 191 | 89 | 62 | 63 |
| Pioneer 39D85 | 6.12 | 158.31 | 70.66 | 0.25 | 1.31 | 194 | 94 | 62 | 64 |
| DKC38-89 | 7.47 | 159.83 | 70.10 | 0.67 | 2.77 | 197 | 99 | 63 | 64 |
| Pioneer 38N88 | 7.22 | 163.94 | 68.96 | 1.38 | 1.82 | 192 | 101 | 63 | 63 |
| TR3622xTR4010 | 5.70 | 201.82 | 68.09 | 0.22 | 2.77 | 203 | 100 | 67 | 68 |

Table 2.5. Selected maize hybrids, from combined analysis across six environments, based on a relative combination of different desirable traits of trial IV (continued)

| Hybrids | YIELD (Mg $ha^{-1}$) | Moisture (g $Kg^{-1}$) | TWT (Kg $hL^{-1}$) | PRL (%) | PSL (%) | PH† (cm) | EH‡ (cm) | DA (days) | DS (days) |
|---|---|---|---|---|---|---|---|---|---|
| Mean of selections§ | 7.02 | 180.95 | 68.86 | 1.30 | 2.93 | 197 | 92 | 64 | 66 |
| Checks mean¶ | 6.49 | 165.24 | 69.79 | 0.50 | 3.89 | 196 | 97 | 63 | 64 |
| Mean# | 5.93 | 173.56 | 69.91 | 2.36 | 4.74 | 195 | 92 | 63 | 65 |
| MSE | 2.33 | 188.73 | 4.53 | 22.64 | 65.21 | 36.62 | 21 | 2 | 3 |
| LSD, 0.05 | 3.03 | 27.26 | 4.22 | 9.44 | 16.02 | 12.01 | 9 | 3 | 3 |
| CV,% | 25.73 | 7.92 | 3.05 | 201.30 | 170.25 | 3.10 | 5 | 2 | 3 |

† Plant height (PH), ‡ ear height (EH), § Mean of selected 12 entries, ¶ mean of five checks, # mean of 64 entries; selected entries were subjectively selected relative to the top and average values of industry checks; Selection were carried out by yield sort in descending order and around 30 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher test weight, hybrids were further selected for lower per cent root and stalk lodging, and lower days to anthesis and silking; always basis of selection was best check and average values of industry checks

# CHAPTER III: CAN EXPIRED PROPRIETARY MAIZE (ZEA MAYS L.) INDUSTRY LINES BE USEFUL FOR SHORT-SEASON BREEDING PROGRAMS? II. GRAIN QUALITY AND NUTRITIONAL TRAITS


## Abstract

Protection expired (ex-PVP) maize (*Zea mays* L.) inbred lines are publicly available to utilize, after being restricted through the U.S. Patent and/or Plant Variety Protection Act (PVPA) for 20 years. The purpose of this study was to assess the grain quality properties of ex-PVP maize short-season inbred lines. Our specific research objectives were to understand the nature of gene action and to select ex-PVP inbreds and hybrids for grain quality traits targeting short-season maize breeding programs. Three sets of North Carolina Mating Design II (NCII) crosses were made with 12 NDSU lines, 24 ex-PVP lines, and 7 top industry testers in the 2010 North Dakota State University (NDSU) Fargo, ND summer nursery and in the 2010 - 2011 NDSU New Zealand winter nursery. Hybrids were arranged in four different partially balanced lattice trials across six ND environments in 2011 and 2012. Trials included five commercial checks and were analyzed with SAS 9.3 software. The NCII design was used to analyze combining ability. Both GCA and SCA were important for regulating most quality traits with the preponderance of additive genetic variance. However, our research showed the lack of interest seed companies had for developing inbred lines and hybrids with top grain quality traits. Ex-PVP inbreds with good GCA for grain quality could be intermated with public high quality lines in order to develop new synthetic varieties that could improve grain quality. Public breeding programs have the opportunity to increase the value of this particular commodity and complement industry efforts for a better and more profitable crop for U.S. farmers.

**Keywords:** *Zea mays* L., ex-PVP inbred lines, hybrids, quality traits, combining ability analysis, GCA, SCA.

## Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, particularly in terms of area, production, multi-purpose uses, and mostly for profitability. In the USA, maize was planted on 39.3 million hectares in 2012, producing 273.8 million metric tons (USDA, NASS, 2013). The crop is mostly produced with hybrid cultivars, often developed by crossing two inbred lines. In the USA, patents and/or Plant Variety Protection Acts (PVPA) protect inbred lines and hybrids for 20 years. As a consequence, access of maize inbred lines and hybrids often remains restricted and confidential. Maize inbreds and hybrids are not practically acquirable until both sources of protection have expired. Expired lines are transferred to the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, and potentially represent new germplasm sources (Nelson et al. 2008) for use with additional lines added yearly. Although they date back 20 years, NCRPIS offers lines with relatively different genetic backgrounds to programs that previously did not have access (Mikel 2006).

Most commercially available maize hybrids in North Dakota (ND) are late maturing, lack stress tolerance, and often have poor grain quality because of lack of stability and adaptation to northern U.S. environmental challenges, and the narrow genetics present in them, due in part to the presence of retailer companies without breeding programs. Therefore, short-season maize products with improved grain quality are necessary for extending maize into these areas.

Maize is an important source of food, feed, fiber, and fuel, globally. In many countries including Africa, Asia, and Latin America, maize is the staple food crop (Musila et al. 2010). Maize breeding for improved yield in genetically narrow germplasm has contributed to reduce of

grain quality, in some cases, representing a nutritional challenge. Ethanol plants have been established in ND, and need higher grain proportions of extractable and fermentable starch to have profitable ethanol production. Maize consumption through ethanol and livestock have been the number one priority in recent years for ND maize growers (Skunes-Arther 2012). As a consequence, increasing the amount of extractable starch under ND's challenging environments is one of the important breeding objectives for grain quality (Carena 2013). To address growers' demands for a high value crop, we need to breed not only for high yielding hybrids, but also for hybrids carrying improved levels of grain quality. Moreover, dietary improvements through higher quality maize would result in decreased feed consumption, ultimately reducing the cost of meat production (Scott et al. 2008).

A series of QPM lines were developed after crossing between U.S. Corn Belt adapted public lines and CIMMYT developed QPM lines by Scott et al. (2009). They found both additive and non-additive genetic effects for determining their yield. They also observed significantly higher yield for hybrids derived from different QPM donor lines. Bhatnagar et al. (2004) evaluated seven white, and nine yellow, high lysine inbred lines (from CIMMYT, Texas A & M, and the University of Natal, South Africa) in two different diallel experiments tested in five southern U.S. environments. The purpose was to estimate general (GCA) and specific combining abilities (SCA) for grain yield and secondary traits, and also to identify potential heterotic relationships among them. They noted non-significant GCA effects for yield, while highly significant GCA effects for agronomic and kernel quality traits. Usually lines with higher GCA for protein concentration would result in the highest protein containing hybrids (Pixley and Bjornason 1993). These authors also reported that simultaneous improvement of yield and protein could be attainable for various tropical QPM lines. Contrary to their findings, Scott et al.

(2004) reported that improved maize quality is often associated with poor agronomic performances. These could be minimized through appropriate breeding methodologies and large sample sizes. Musila et al. (2010) crossed 14 early-maturing QPM inbred lines using the diallel mating design to assess the yield potential of QPM hybrids, and estimated GCA and SCA for grain yield and other agronomic traits. They reported additive genetic effects were much more important in the set of genotypes they used.

Quality traits of ex-PVP hybrids are mostly undetermined. Therefore, the uniqueness of our study relies on the assessment of grain quality traits in hybrids of ex-PVP, NDSU, and commercially available short-season maize inbred lines. The overall objective of this study was to assess the utility of short-season expired industry lines to the NDSU, and other short-season maize breeding programs, for grain quality traits. The specific objectives were: i) to understand the nature of gene action for grain quality traits in short-season industry and NDSU lines, ii) to identify potential ex-PVP inbreds for quality traits, and iii) to explore the next generation of suitable short-season hybrids for grain quality and nutritional traits.

## Materials and Methods

### Plant materials

Twenty four ex-PVP lines, 12 NDSU inbred lines, and seven top industry testers for the northern U.S. Corn Belt were used in this study. The 12 NDSU lines included ND08-343, ND291, ND2000, ND2001, ND2002, ND2003, ND2004, ND2005, ND2006, ND2007, ND2010, and ND2011. Ex-PVP inbreds were represented by Lp5(8700031), Q381(8500098), NK807(8700151), CR1Ht(8400042), DK78010(8500126), PH207 (8300144), FAPW(8200152), LH52(8700020), NK794(8700046), LH54(8600128), DJ7(8500086), NK779(8700041), PHJ40(8600133), PHK05(8800001), PHR25(8800002), PHK76(8800036), PHT77(8800038),

OQ603(8800150), S8324(8800153), PHP02(8800212), CR14(8900095), L127(8900201), LH205(9000049), RS710(9000129). PVP numbers within parentheses can be used to find more information for each ex-PVP line at the Germplasm Resources Information Network web page of the United States Department of Agriculture (USDA) (http://www.ars-grin.gov/cgi-bin/npgs/html/pvplist.pl). Ex-PVP materials were requested and collected from the USDA National Plant Germplasm System through the North Central Regional Plant Introduction Station (NCRPIS) at Ames, Iowa. Our exclusive partner provided restricted testers, so coded names were utilized to designate them. The testers used in our study were T1 (Iodent type, Oh43), T2 (SS type), T3 (Iodent type), T4 (SS type), T5 (non-SS type), T6 (SS type), and T7 (SS type).

**Crossing procedure**

Three different crosses were executed following the NCII Mating Design (Comstock and Robinson 1948). The first set of crosses were carried out in the 2010 NDSU maize breeding nursery in Fargo, ND, where 12 NDSU lines were used as females to cross with 12 ex-PVP lines used as male parents. NDSU and ex-PVP inbreds were grown in paired rows (around 20 plants in each row). Rows were 7m long with 0.76m between rows. All possible crosses were made within paired rows. All pollinated ears in a row were harvested in bulk, dried, shelled, and put in cold storage for later use. The second set of crosses included the same 12 NDSU lines used as females, crossed with another set of 12 ex-PVP lines used as males, following the same mating design (NCII) at a winter nursery at Pukekohe, New Zealand, 2010-11. A third set of crosses included 24 ex-PVP lines (used in the first and second sets of crosses) which were again used as males and crossed with seven current industry testers (used as females) following NCII at the same winter nursery at Pukekohe, New Zealand, in 2010-2011. The advantage of the NCII vs. a diallel is that it accommodates more parents in the crossing program with fewer numbers of

crosses. This is the reason we chose NCII. The analysis provides estimates of GCA for parents and of SCA for the interaction between parents (males and females) (Hallauer et al. 2010). The combining abilities and their effects are important genetic parameters to be considered in breeding programs (Qu et al. 2012).

**Field trials**

Hybrids obtained from the three sets of crosses were planted in four different trials, and each trial included five commercial checks. All the trials except second were tested in six ND environments (Casselton, Prosper, in 2011 and Casselton, Prosper, Fargo, and Barney of ND in 2012). An 11 by 11 partially balanced lattice design was utilized for evaluating hybrids from the first set of crosses. A 9 by 9 partially balanced lattice design was used for the second set of crosses. An 8 by 9 rectangular lattice design, and an 8 by 8 partially balanced lattice design were utilized for the third set of crosses. Checks representing different relative maturities (RM) included DKC 33-54 (83RM), Pioneer 39D85 (85RM), TR2015+TR1099*TR3622 CBLL (87RM), Pioneer 38N88 (92RM), and TR3622 x TR4010 (100RM). In 2012, TR2015+TR1099*TR3622 CBLL was replaced by DKC 38-89 (88RM), as the seed source was depleted. Plots were over planted and thinned back to approximately 30-35 plants per plot, the plot size was 6.10m by 0.76m (70,000 plants $ha^{-1}$). Fertilization and field management practices were carried out as per recommendation at each location for optimum maize production. Experimental plots were planted and harvested by machines adapted for small experimental plots. Approximately 500 g seed samples were kept from each plot for grain quality tests.

**Traits evaluated**

Protein (g $Kg^{-1}$), oil (g $Kg^{-1}$), starch (g $Kg^{-1}$), cysteine (g $Kg^{-1}$), lysine (g $Kg^{-1}$), methionine (g $Kg^{-1}$), fermentable starch (HFC) (g $Kg^{-1}$), and extractable starch (HES) (g $Kg^{-1}$) were measured and presented in this study. Grain yield (Mg $ha^{-1}$) and moisture (g $Kg^{-1}$) were also added in this study, as a reference point, mostly for selecting hybrids. Data were collected on a plot basis. Grain quality traits were assessed by two different machines, an OmegAnalyzer G (Bruins Instruments), and an Infratec© 1241 Grain NIR (Near Infra-red Reflectance) analyzer (provided by Monsanto).

**Statistical procedures**

Analyses followed the linear random model showed below. SAS software version 9.3 (SAS, 2010) was used to carry out ANOVA to estimate GCA and SCA. Combining abilities were split into male and female, and interactions of male and female. The mating design assumes there is no linkage disequilibrium, no epistasis, no maternal inheritance, and parents were random for executing this analysis (Hallauer et al. 2010). The model (Scott et al. 2009) was:

Y= Mean + Env. + Rep. (Env.) + M. + F. + M. x F. + M. x Env. + F. x Env. + M. x F. x Env. + Error

Where,

Y= observed values

Mean = overall mean of experiment

Env. = environmental effects

Rep. (Env.) = replicates within environment

M. = effects of the male parental lines

F.= effects of the female parental lines

M. x F. = interaction effects of male by female

M. x Env. = interaction effects of male by environments

F. x Env. = interaction effects of female by environments

M. x F. x Env. = interaction effects of male by female by environments

Error = residual

Expectations of males and females were pooled together to represent a single GCA as a whole, and interactions of males and females were presented as SCA. Additive ($\sigma^2_A$) and dominance ($\sigma^2_D$) variances, level of dominance ($\bar{d}$), and heritability in narrow- sense were calculated using the formula described by Hallauer and Miranda (1988). In addition we utilize estimated GCA effects, of respective inbred lines, additionally to select potential inbred parents.

In the general ANOVA, location by year combination was considered as random environments for field trials. Analyses of variance were performed for all traits at each environment, as well as combined across environments (SAS 2010) for all the studied experiments. Data were collected and summarized on Excel files and then imported to SAS for analysis. Combined ANOVA were carried out using the following linear model:

Y= Mean + Env. + Rep (Env.) + Block (Rep. x Env.) + Trt. + Trt. x Env. + Error

Where,

Y = observed value

Mean = mean values observed in the experiments

Env. = environmental effects

Rep. (Env.) = effect of replicate within environments

Block = effect of block within (Rep.x Env.)

Trt. = effect of treatments or hybrids

Trt. x Env. = effects of genotype by environments interaction

Error = residual

Homogeneity of error variances were tested using the ten-fold thumb rule before combining data across environments. The rule considers that if the error mean squares (EMS) are within ten-fold (Tabachnik and Fidell 2001) then EMSs are considered homogeneous and were combined. Mean comparisons among genotypes were assessed by Fisher's protected least significant difference (LSD) at <0.05 level of significance as it is considered to be an appropriate test for detecting differences (Carmer and Swanson 1971).

## Results and Discussion

### Genetic parameter estimation

A combined ANOVA for combining ability was computed for grain yield, grain moisture at harvest, grain quality and nutritional traits (Table 3.1). Both additive and non-additive gene actions were responsible for grain yield. Estimated degree of dominance ($\bar{d}$) (1.44 to 2.43) showed overdominance gene action determining yield in all trials. Narrow-sense heritability for grain yield was moderate. Grain moisture at harvest was controlled by GCA and SCA in all trials (Table 3.1). The estimated degree of dominance ($\bar{d}$) for this trait was partial to complete, while its narrow sense heritability was high, as expected, ranging from72 % to 89 % in this set of genotypes (Table 3.1).

General grain quality traits behaved similarly. GCA and SCA mean squares were significant for grain starch, grain protein, and grain oil contents in all trials (Table 3.1). Also,

additive genetic variances ($\sigma^2_A$) for these traits were greater than dominance genetic variance ($\sigma^2_D$) across trials.

For maize grain protein, Singh et al. (1977), Motto et al. (1978), and Wessel-Beaver et al. (1985) also reported higher additive genetic variance, compared to dominance genetic variance. The degree of dominance ($\overline{d}$) indicated overdominance gene expression (1.14 to 1.93) for grain protein which may be cause by linkage biases. Narrow-sense heritability ranged from 43 % to 67 %. Motto et al. (1979) observed 68 % narrow-sense heritability for percent grain protein among half-sib families.

Estimated additive genetic variances ($\sigma^2_A$) were greater than dominance genetic variances ($\sigma^2_D$) in all four trials for grain oil content, similar results was also reported by Joshi et al. (1998). However, unlike grain protein, partial to complete dominance was found across trials (0.44 to 1.08) for regulating grain oil content, which may reject the hypothesis that linkage might be present in these sets of crosses. Narrow-sense heritability was very high, ranging from 71 % to 92 %.

Both GCA and SCA variances were significant for starch in our four trials (Table 3.1), and additive genetic variances ($\sigma^2_A$) were greater than dominance genetic variances ($\sigma^2_D$).Contrary to our findings Joshi et al. (1998) reported non-additive gene action for starch content of maize. On average, it seems that complete dominance was present in this trait and narrow-sense heritability was moderate to high (0.61-0.75).

Specific grain quality traits behaved differently. In the case of the amino acid cysteine, GCA and SCA mean squares were significant for trials I and III; GCA variance was significant in trial IV, and SCA variance was significant in trial II. Additive genetic variance ($\sigma^2_A$), however, was greater than dominance genetic variance ($\sigma^2_D$) in all four trials. Overdominance

seemed to be present in trials I and II, while complete dominance was recorded in trials III and IV. Heritability in the narrow sense varied from 28% to 62%.

For lysine amino acid content, GCA and SCA mean squares were significant in trial I, SCA was significant in trial II, and significant GCA variances were observed in trials III and IV. Additive genetic variance ($\sigma^2_A$) was greater than dominance genetic variance ($\sigma^2_D$) in all trials except for trial II where both genetic parameters were similar. Singh et al. (1977), Motto et al. (1978), and Wessel-Beaver et al. (1985) also reported large additive genetic variance when compared to dominance genetic variance for this trait. Trial I and IV exhibited complete dominance, while trials II and III showed overdominance. The narrow-sense heritability for lysine content in these sets of crosses ranged from 41% to 69%.

For methionine content, GCA and SCA variances were significant in all trials, as with the general grain quality traits. Also, estimated additive genetic variance ($\sigma^2_A$) was larger than dominance genetic variance ($\sigma^2_D$). The degree of dominance ($\overline{d}$) was near complete dominance in trials I and III and overdominance was present in trials II and IV. Narrow-sense heritability was moderate to high (41 % to 68 %).

A significant GCA mean square was found for fermentable starch (HFC) across trials. However, only trial III showed a significant SCA mean square for HFC. The additive genetic variance ($\sigma^2_A$) was greater than the dominance genetic variance ($\sigma^2_D$) in all trials and partial dominance regulated grain HFC. Heritability in the narrow-sense, ranged from 62 % to 72 %.

Both GCA and SCA variances were highly significant for extractable starch (HES). We observed higher additive genetic variance ($\sigma^2_A$) than dominance genetic variance ($\sigma^2_D$) in all trials. The degree of dominance for this trait ranged from partial dominance in trials I and II, to

complete dominance in trial III, and overdominance in trial IV. Narrow-sense heritability was moderate to high (53 % to 78 %).

Significant and positive GCA effects were obtained in LH54, ND2005, ND2006, and T5 for grain protein content. NK807, NK779, PHK05, PHT77 ND291, ND2002 and T7 had significant and desirable GCA effects for grain starch content. Significant and desirable GCA effects were found for oil content in CR1Ht, LH52, NK779, S8324, CR14, LH205, ND08-343, ND2005, ND2007, ND2011, and T5. Significant and desirable GCA effects were found in LH54, PH207, and ND2005 for cysteine. CH1Ht, DKFAPW, and ND2006 had significant GCA effects for lysine content. LH54, PH207, ND2005, ND2006, and T5 would be useful to increase methionine content. Inbred lines CR1Ht, NK794, CR14, PHP02, OQ603, ND2000, ND2002, T3 and T4 would be useful to increase HFC considering significant GCA effects. LP5, DJ7, PHT77, ND291, ND2002, ND2010, and T2 had significant desirable GCA effects for extractable starch (data not shown).

**Analysis of variance and hybrids selection**

Significant differences were observed among hybrids for all grain quality traits across trials (date not shown). We also found significant genotype-by-environment interactions for all grain quality traits in trials I and III; in trial II significant genotype-by-environment interactions were found for all traits, except for cysteine and HFC, while in trial IV, all traits, other than grain oil content and lysine, exhibited significant genotype-by-environment interaction. As a consequence, our data show these traits are more genetically complex than originally thought.

Yield is often inversely related to most grain quality traits, especially if sample sizes for evaluation are limited. Our purpose was to find hybrids with reasonably higher yield, along with elevated grain quality traits when compared to commercial checks. In trial I, grain protein

content from selected hybrids ranged from 98 to 114 g $Kg^{-1}$ (Table 3.2). Seven of the selected hybrids had significantly higher grain protein content than the mean (100 g $Kg^{-1}$) for all checks (Table 3.2). In the second trial, four hybrids were significantly higher than the check average mean for grain protein (101 g $Kg^{-1}$ and $LSD_{0.05}$= 7) (Table 3.3). Nine of the selected hybrids were significantly higher than the top check, TR3622 xTR4010, for grain protein (108 g $Kg^{-1}$) in the third trial (Table 3.4). Trial IV was not the exception, as four of the selected hybrids had significantly higher protein content than the mean of the checks for grain protein (104 g $Kg^{-1}$) (Table 3.5).

In trial I, grain oil content ranged from 37 to 50 g $Kg^{-1}$, and the overall experimental mean was 43 g $Kg^{-1}$. In this trial, among the selected hybrids, ND2011 x CR1Ht had the highest oil content (49 g $Kg^{-1}$), PH207 x ND2003 had 46 g $Kg^{-1}$, ND2011 x PH207 had 45 g $Kg^{-1}$, and ND2002 x CR1Ht had 43 g $Kg^{-1}$ grain oil. All these hybrids were significantly higher than the mean of commercial checks for grain oil (41 g $Kg^{-1}$) (Table 3.2). In the second trial, five hybrids had significantly higher oil content than the mean checks (39 g $Kg^{-1}$) (Table 3.3). Oil content of the checks ranged from 38 to 40 g $Kg^{-1}$, and it varied from 38 to 45 g $Kg^{-1}$ among the selected hybrids in trial III. Among the selected hybrids, 11 had statistically higher oil content than the checks mean in this trial (Table 3.4). Four hybrids had significantly higher oil content than the top check for grain oil content (42 g $Kg^{-1}$) in the fourth trial (Table 3.5).

Often, grain starch content is correlated to grain yield, in the most breeding effects in genetically narrow hybrids. Therefore, commercial checks are normally found among the top hybrids. In trial I, ND2002 x NK807 (711 g $Kg^{-1}$) and ND2002 x Q381 (710 g $Kg^{-1}$), had significantly higher starch content than the mean of the five commercial checks for grain starch content (705 g $Kg^{-1}$); eight more hybrids had similar starch content compared with the checks

mean for grain starch (Table 3.2). ND2002 x PHP02 (716 g $Kg^{-1}$) had significantly higher starch than the checks mean for grain starch (707 g $Kg^{-1}$) in the second trial (Table 3.3); 12 more hybrids had similar starch when compared with the checks mean. In the third trial, only one hybrid (T7 x Q381; 702 g $Kg^{-1}$) had similar starch content to the mean of the checks (706 g $Kg^{-1}$) ($LSD_{0.05}$= 5) (Table 3.4). In trial IV, eight hybrids had similar starch content as the mean of the checks (703 g $Kg^{-1}$) (Table 3.5).

For cysteine, the overall experimental mean was higher than the mean of the checks (2.21 vs. 2.09 g $Kg^{-1}$). Six of the hybrids had higher cysteine content than the checks mean (Table 3.2) in the first trial. All of the selected hybrids had similar cysteine content in trial II (Table 3.3). In the third trial, all the selected entries had significantly higher cysteine, than the checks mean of 2.14 g $Kg^{-1}$(Table 3.4). In the fourth trial, selected entries had similar cysteine content as the checks (Table 3.5).

Two hybrids from trial I, and one hybrid in trial IV, had statistically higher lysine content than the checks mean (Tables 3.2 and 3.5), while the rest of the selected hybrids had similar lysine content as the commercial checks in both trials. In the second trial, 14 out of 17 selected hybrids had similar lysine content as the checks mean (3.26 g $Kg^{-1}$) (Table 3.3). However, in trial III, 13 of the 18 hybrids had significantly higher lysine compared to the checks mean (3.28 g $Kg^{-1}$) (Table 3.4).

In trial I, five hybrids had significantly higher methionine content than the checks average (2.29 g $Kg^{-1}$) (Table 3.2). The rest of the selected hybrids had comparable values for methionine. In trial II, 13 hybrids had similar methionine as the checks mean, and one was statistically higher (Table 3.3). In trial III, 16 of 18 hybrids had significantly higher methionine than the average of the checks (Table 3.4). All of the selected hybrids had similar methionine

content as the checks mean (2.33 g $Kg^{-1}$) and two of them were statistically higher than the checks mean (Table 3.5) in trial IV.

Three of the selected hybrids had statistically more HFC compared to the checks mean (490 g $Kg^{-1}$; Table 3.2) in trial I. In the same trial, 16 out of 17 selected hybrids had comparable HFC to the checks average. Similar findings were also observed in trials II and IV, where all but one of the selected hybrids had similar HFC as the commercial checks (Tables 3.3 and 3.5). In the third trial, five of the selected 17 hybrids had higher HFC compared to the checks mean (484 g $Kg^{-1}$; Table 3.4).

In trial I, four hybrids had significantly higher HES than the checks mean (617 g $Kg^{-1}$) (Table 3.2), and in trial II six hybrids had statistically higher HES than checks mean (616 g $Kg^{-1}$) (Table 3.3). None of the selected hybrids were reported to be statistically higher than the checks mean (616 g $Kg^{-1}$) in trial III, but three of them were similar to the checks for HES content (Table 3.4). One selected hybrid, T1 x PHT77, had significantly higher HES content compared to the checks mean, and 12 of them were comparable to the checks (Table 3.5) in the fourth trial.

Additive and dominance gene action were important in regulating all grain quality traits evaluated in this particular set of short-season hybrids. Our short-season representative sample for the northern U.S. Corn Belt indicated that additive genetic variances were more important compared to dominance genetic variance for most traits. However, the dominance and overdominance inheritance observed in our study could be exploited through hybrid breeding. A limited amount of hybrids had better quality traits than commercially available checks at similar yield and moisture ranges. Ex-PVP genetic materials might not be directly useful as immediate commercial hybrids for grain quality traits. Few ex-PVP inbreds were selected with higher GCA effects that would provide unique alleles for different desirable traits. It seems genetically broad

materials would be more desirable than ex-PVP genetically narrow materials. Top ex-PVP lines for certain desirable traits could be inter-mated to develop new populations. However, there would be a need to maximize genetic improvement before initiating new inbred line development with these materials. Farmers still do not have access to potentially better hybrids. These are still missing for the northern U.S. Corn Belt due to lack of breeding access in PVPA/patent protected materials. Breeding right access could provide improved hybrids for ND, and surrounding areas, even before expiration of the protected material.

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**References**

Bhatnagar S, Betrán FJ, Rooney LW, 2004. Combining abilities of quality protein maize inbreds. Crop Sci. 44: 1997-2005

Carena MJ, 2013. Development of cold and drought tolerant short-season maize germplasm for fuel and feed utilization. Crop Breeding and Applied Biotechnology 13: 1-8

Carmer SG, Swanson MR, 1971. Detection of differences between means: A Monte Carlo study of five pair wise multiple comparison procedures. Agron J. 63: 940-945

Comstock RE, Robinson HF, 1948. The components of genetic variance in the populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics 4: 254-266

Hallauer AR, Carena MJ, Miranda Fo JB, 2010. Hand Book of Plant Breeding: Quantitative Genetics in Maize Breeding. 3rd ed. Springer, New York. pp 103-113

Hallauer AR, Miranda Fo JB, 1988. Quantitative Genetics in Maize Breeding. 2nd ed. Iowa State University Press, Ames. pp 64-72

Joshi VN, Pandiya NK, Dubey RB, 1998. Heterosis and combining ability for quality and yield in early maturing single cross hybrids of maize (*Zea mays* L.). Indian J. Genet. 58(4): 519-524

Mikel MA, 2006. Availability and analysis of proprietary dent corn inbred lines with expired U.S. plant variety protection. Crop Sci. 46: 2555-2560

Motto M, 1979. Heritability and interrelationships of seed quality and agronomic traits in a modified opaque-2 synthetic variety on maize (*Zea mays* L.). Maydica 24: 193-202

Motto M, Lorenzoni C, Gentinetta E, Maggiore T, Salamini F, 1978. Expected genetic gain for protein related traits and allocation of resources in a modified opaque-2 synthetic. Maydica 23: 35-44

Musila RN, Diallo AO, Makumbi D, Njoroge K, 2010. Combining ability of early maturing quality protein maize inbred lines adapted to eastern Africa. Field Crops Research 119: 231-237

Nelson PT, Coles ND, Holland JB, Bubeck DM, Smith S, Goodman MM, 2008. Molecular characterization of maize inbreds with expired U.S. plant variety protection. Crop Sci. 48: 1673-1685

Pixley KV, Bjarnason MS, 1993. Combining ability for yield and protein quality among modified endosperm *opaque-2* tropical maize inbreds. Crop Sci. 33: 1229-1234

Qu Z, Lanzhi L, Luo J, Wang P, Yu S, 2012. QTL mapping of combining ability and heterosis of agronomic traits in rice backcross recombinant inbred lines and hybrid crosses. PLOS ONE 7(1):e28463. doi:10.1371/journal.pone.0028463

SAS Institute. 2010. SAS user's guide guide: Statistics. 4th ed. SAS Inst., Cary, NC

Scott MP, Bhatnagar S, Betran J, 2004. Tryptophan and methionine levels in quality protein maize breeding germplasm. Maydica 49: 303-311

Scott MP, Darrigues A, Stahly TS, Lamkey K, 2008. Recurrent selection to alter grain methionine concentration and improve nutritional value of maize. Crop Sci. 48:1705-1713

Scott MP, Peterson JM, Hallauer AR, 2009. Evaluation of combining ability of quality protein maize derived from U.S. public inbred lines. Maydica 49: 303-311

Singh SV, Singh J, Singh D, Bahl DK, 1977. Genetic-environmental interactions in a diallel cross of opaque-2 maize. Maydica 22: 89-95

Skunes-Arther K, 2012. Chairman's report. p. 2. Annual Report. ND Corn Growers Association and ND Corn Council. Available @ http://www.ndcorn.org/uploads/useruploads/ndcg_annualreport2_lr.pdf (verified September 20, 2013)

Tabachnik B, Fidell L, 2001. Computer –Assisted Research Design and Analysis. Boston: Allyn and Bacon

United States Department of Agriculture (USDA), 2013. National Agricultural Statistics Service. [On line] available at http://quickstats.nass.usda.gov (accessed on 06 May 2013)

Wessel-Beaver L, Lambert RJ, Dudley JW, 1985. Genetic variability and correlations in a modified endosperm texture opaque-2 maize population. Crop Sci. 25: 129-132

Table 3.1. North Carolina design II derived variances, on selected sources of variation, in four short-season maize trials across six environments (Environments are year by location combinations: Trials were planted at Casselton, Prosper in 2011, and were planted at Casselton, Prosper, Fargo, Barney in 2012)

| SOV | Yield (Mg ha$^{-1}$) | Moisture (g Kg$^{-1}$) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | CYS† (g Kg$^{-1}$) | LYS‡ (g Kg$^{-1}$) | MET§ (g Kg$^{-1}$) | HFC¶ (g Kg$^{-1}$) | HES# (g Kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial I: Maize (*Zea mays* L.) hybrids, derived from crosses between 12 NDSU lines by 1st set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | | |
| GCA (M+F) †† | 13.27** | 71248*** | 1989*** | 334*** | 2654*** | 0.34*** | 0.25*** | 0.67*** | 1117*** | 6340*** |
| SCA (M*F) ‡‡ | 3.71*** | 3912*** | 193*** | 19*** | 180*** | 0.041*** | 0.023*** | 0.06*** | 130.18 | 430*** |
| Residual | 2.06 | 891.92 | 54.8 | 3.27 | 55.05 | 0.015 | 0.012 | 0.026 | 103.49 | 185.29 |
| $\sigma^2A$§§ | 0.14 | 1434.14 | 31.77 | 6.05 | 44.04 | 0.01 | 0.01 | 0.01 | 18.33 | 106.39 |
| $\sigma^2D$¶¶ | 0.15 | 266.39 | 10.93 | 1.26 | 10.02 | 0.002 | 0.002 | 0.003 | 0.13 | 15.40 |
| $\bar{d}$## | 2.03 | 0.86 | 1.17 | 0.91 | 0.95 | 1.25 | 1.11 | 1.04 | 0.17 | 0.76 |
| $h_n^2$††† | 0.31 | 0.80 | 0.67 | 0.80 | 0.75 | 0.62 | 0.69 | 0.68 | 0.68 | 0.78 |
| Trial II: Maize hybrids, derived from crosses between 12 NDSU lines by 2nd set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | | |
| GCA (M+F) †† | 15.38** | 31947*** | 1013** | 210*** | 1488*** | 0.26 | 0.19 | 0.39* | 561*** | 3949*** |
| SCA (M*F) ‡‡ | 3.79 | 1588*** | 169** | 15*** | 146*** | 0.11** | 0.11* | 0.08*** | 89.42 | 412*** |
| Residual | 2.06 | 335.58 | 108.57 | 4.89 | 98.82 | 0.101 | 0.042 | 0.046 | 90.03 | 246.13 |
| $\sigma^2A$§§ | 0.32 | 1224.86 | 19.56 | 6.49 | 38.49 | 0.01 | 0.01 | 0.01 | 15.08 | 88.06 |
| $\sigma^2D$¶¶ | 0.11 | 116.98 | 8.34 | 0.84 | 7.25 | 0.005 | 0.01 | 0.005 | 0.30 | 23.03 |
| $\bar{d}$## | 1.44 | 0.38 | 1.31 | 0.72 | 0.87 | 1.88 | 1.85 | 1.73 | 0.28 | 1.02 |
| $h_n^2$††† | 0.50 | 0.89 | 0.50 | 0.83 | 0.69 | 0.28 | 0.41 | 0.41 | 0.62 | 0.65 |
| $h_n^2$††† | 0.40 | 0.84 | 0.56 | 0.71 | 0.61 | 0.56 | 0.50 | 0.57 | 0.72 | 0.64 |

Table 3.1. NCII derived variances, on selected sources of variation, in four short-season maize trials across six environments (continued)

| SOV | Yield (Mg ha$^{-1}$) | Moisture (g Kg$^{-1}$) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | CYS$^{\dagger}$ (g Kg$^{-1}$) | LYS$^{\ddagger}$ (g Kg$^{-1}$) | MET$^{\S}$ (g Kg$^{-1}$) | HFC$^{\P}$ (g Kg$^{-1}$) | HES$^{\#}$ (g Kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial III: Maize hybrids, derived from crosses between seven industry testers by 1st set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | | |
| GCA (M+F) $^{\dagger\dagger}$ | 24.23** | 6105*** | 1113** | 91.53*** | 1042*** | 0.23** | 0.22** | 0.45*** | 1437*** | 3542*** |
| SCA (M*F) $^{\ddagger\ddagger}$ | 7.82*** | 343*** | 182** | 11*** | 153** | 0.037** | 0.053 | 0.071** | 149** | 445*** |
| Residual | 2.43 | 89.27 | 109 | 2.78 | 85.4 | 0.02 | 0.03 | 0.042 | 92.02 | 237.63 |
| $\sigma^2A^{\S\S}$ | 0.56 | 200.63 | 19.34 | 2.00 | 21.02 | 0.004 | 0.004 | 0.01 | 35.92 | 75.94 |
| $\sigma^2D^{\P\P}$ | 0.57 | 27.14 | 6.33 | 0.58 | 6.20 | 0.001 | 0.002 | 0.002 | 6.16 | 23.04 |
| $\bar{d}^{\#\#}$ | 2.03 | 0.74 | 1.14 | 1.08 | 1.09 | 1.15 | 1.21 | 1.11 | 0.83 | 1.10 |
| $h_n^{2\dagger\dagger\dagger}$ | 0.40 | 0.84 | 0.56 | 0.71 | 0.61 | 0.56 | 0.50 | 0.57 | 0.72 | 0.64 |
| Trial IV: Maize hybrids, derived from crossing between seven industry testers by 2nd set of 12 ex-PVP inbreds, were used in this analysis | | | | | | | | | | |
| GCA (M+F) $^{\dagger\dagger}$ | 17.21** | 11959** | 696** | 287*** | 1211*** | 0.71* | 0.20** | 0.37** | 572*** | 2746*** |
| SCA (M*F) $^{\ddagger\ddagger}$ | 5.48*** | 1226*** | 189*** | 9** | 192*** | 0.19 | 0.07 | 0.09*** | 108 | 612*** |
| Residual | 2.45 | 453.25 | 59.66 | 3.54 | 56.25 | 0.106 | 0.058 | 0.036 | 71.42 | 173.11 |
| $\sigma^2A^{\S\S}$ | 0.27 | 255.45 | 14.45 | 7.82 | 27.34 | 0.01 | 0.01 | 0.01 | 14.83 | 61.98 |
| $\sigma^2D^{\P\P}$ | 0.24 | 60.43 | 11.96 | 0.38 | 11.30 | 0.002 | 0.002 | 0.005 | 2.15 | 39.63 |
| $\bar{d}^{\#\#}$ | 1.91 | 0.97 | 1.93 | 0.44 | 1.29 | 0.94 | 1.10 | 1.54 | 0.76 | 1.60 |
| $h_n^{2\dagger\dagger\dagger}$ | 0.38 | 0.72 | 0.43 | 0.92 | 0.63 | 0.49 | 0.45 | 0.51 | 0.65 | 0.53 |

*, **, *** significance at $P$ <0.05, <0.01, and <0.001; $^{\dagger}$ Cysteine, $^{\ddagger}$ lysine, § methionine, $^{\P}$ high fermentable corn, $^{\#}$ extractable starch. $^{\dagger\dagger}$GCA (M+F), is male and female expectations pooled together, $^{\ddagger\ddagger}$ SCA (M*F), is male by female interaction expectation, $^{\S\S}$ $\sigma^2A$, is the additive genetic variance, $^{\P\P}$ $\sigma^2D$, is the dominance genetic variance, $^{\#\#}$ $\bar{d}$, is the degree of dominance, $^{\dagger\dagger\dagger}$ $h_n^2$is the narrow- sense heritability, for derived equation please check statistical procedure section

Table 3.2. Selected maize hybrids, from combined analysis across six environments (six environments are year by location combination: 2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney), of trial I, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits

| Hybrids | Yield (Mg $ha^{-1}$) | Moist. (g $Kg^{-1}$) | Protein (g $Kg^{-1}$) | Oil (g $Kg^{-1}$) | Starch (g $Kg^{-1}$) | CYS‡ (g $Kg^{-1}$) | LYS§ (g $Kg^{-1}$) | MET¶ (g $Kg^{-1}$) | HFC# (g $Kg^{-1}$) | HES†† (g $Kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| ND2002 x CR1Ht | 6.26 | 186 | 108 | 43 | 697 | 2.16 | 3.39 | 2.31 | 492 | 617 |
| ND2002 x PH207 | 6.02 | 193 | 104 | 38 | 708 | 2.15 | 3.26 | 2.30 | 490 | 623 |
| ND2002 x DKFAPW | 5.87 | 205 | 100 | 37 | 707 | 2.12 | 3.32 | 2.30 | 488 | 610 |
| ND2011 x PH207 | 5.64 | 202 | 101 | 45 | 701 | 2.17 | 3.18 | 2.33 | 476 | 611 |
| ND2002 x LH54 | 5.63 | 233 | 101 | 40 | 708 | 2.13 | 3.25 | 2.28 | 487 | 627 |
| ND2002 x Q381 | 5.53 | 202 | 99 | 39 | 710 | 2.10 | 3.21 | 2.21 | 496 | 629 |
| ND291 x DK78010 | 5.48 | 208 | 106 | 39 | 702 | 2.18 | 3.23 | 2.27 | 490 | 628 |
| ND2005 x NK779 | 5.42 | 195 | 111 | 42 | 694 | 2.25 | 3.35 | 2.46 | 491 | 602 |
| ND291 x PH207 | 5.17 | 192 | 104 | 38 | 706 | 2.12 | 3.24 | 2.33 | 499 | 618 |
| ND2002 x NK807 | 5.14 | 173 | 98 | 39 | 711 | 2.11 | 3.18 | 2.21 | 483 | 628 |
| ND2011 x CR1Ht | 5.11 | 176 | 98 | 49 | 698 | 2.07 | 3.30 | 2.20 | 484 | 618 |
| ND08-343 x LH54 | 5.06 | 213 | 114 | 44 | 691 | 2.30 | 3.34 | 2.54 | 484 | 606 |
| ND291 x Q381 | 5.05 | 185 | 104 | 39 | 705 | 2.16 | 3.22 | 2.28 | 491 | 624 |
| ND2000 x PH207 | 5.03 | 161 | 114 | 42 | 695 | 2.25 | 3.33 | 2.46 | 500 | 614 |
| Lp5 x ND291 | 5.03 | 207 | 101 | 41 | 705 | 2.11 | 3.18 | 2.13 | 482 | 637 |
| PH207 x ND2003 | 4.96 | 207 | 111 | 46 | 691 | 2.34 | 3.25 | 2.44 | 489 | 613 |
| ND2000 x Q381 | 4.96 | 164 | 112 | 41 | 698 | 2.24 | 3.27 | 2.41 | 499 | 618 |

Table 3.2. Selected maize hybrids, from combined analysis across six environments, of trial I, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits (continued)

| Hybrids | Yield (Mg ha$^{-1}$) | Moist. (g Kg$^{-1}$) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | CYS[‡] (g Kg$^{-1}$) | LYS[§] (g Kg$^{-1}$) | MET[¶] (g Kg$^{-1}$) | HFC[#] (g Kg$^{-1}$) | HES[††] (g Kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Checks: | | | | | | | | | | |
| DKC 33-54 | 5.71 | 150 | 100 | 41 | 706 | 2.06 | 3.20 | 2.19 | 494 | 631 |
| Pioneer 39D85 | 5.93 | 162 | 99 | 39 | 709 | 2.09 | 3.36 | 2.41 | 495 | 602 |
| DKC 38-89 | 5.46 | 162 | 96 | 41 | 708 | 2.01 | 3.26 | 2.22 | 488 | 624 |
| Pioneer 38N88 | 6.50 | 204 | 100 | 42 | 704 | 2.08 | 3.29 | 2.28 | 493 | 611 |
| TR3622 xTR4010 | 6.58 | 232 | 106 | 41 | 700 | 2.22 | 3.18 | 2.33 | 480 | 618 |
| | | | | | | | | | | |
| Mean of selections[‡‡] | 5.37 | 194 | 105 | 41 | 702 | 2.17 | 3.27 | 2.32 | 489 | 619 |
| Checks mean[§§] | 6.04 | 182 | 100 | 41 | 705 | 2.09 | 3.26 | 2.29 | 490 | 617 |
| Exp. Mean[¶¶] | 4.53 | 207 | 107 | 43 | 698 | 2.21 | 3.28 | 2.35 | 487 | 614 |
| | | | | | | | | | | |
| EMS | 1.84 | 807 | 32 | 3 | 39 | 0.01 | 0.01 | 0.02 | 103 | 149 |
| LSD, 0.05 | 1.10 | 23 | 5 | 1 | 5 | 0.08 | 0.08 | 0.10 | 8 | 10 |
| CV,% | 29.95 | 14 | 5 | 4 | 0.79 | 4.53 | 2.92 | 5.43 | 2 | 2 |

[†] Grain moisture, [‡] cysteine, [§] lysine, [¶] methionine, [#] high fermentable corn, [††] extractable starch, [‡‡] mean of selected 17 hybrids, [§§]mean of five checks, [¶¶]mean of 121 entries; Selection were carried out by yield sort in descending order and around 60 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher protein, hybrids were further selected for higher oil, starch, and other traits mentioned in the table; The basis of selection was best check and average values of industry checks for respective traits

Table 3.3. Selected maize hybrids, from combined analysis across five environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Barney), of trial II, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits

| Hybrids | Yield (Mg $ha^{-1}$) | Moist† (g $Kg^{-1}$) | Protein (g $Kg^{-1}$) | Oil (g $Kg^{-1}$) | Starch (g $Kg^{-1}$) | CYS‡ (g $Kg^{-1}$) | LYS§ (g $Kg^{-1}$) | MET¶ (g $Kg^{-1}$) | HFC# (g $Kg^{-1}$) | HES†† (g $Kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| ND2002 x PHP02 | 6.64 | 205 | 96 | 37 | 716 | 2.06 | 3.06 | 2.10 | 492 | 637 |
| ND2000 x S8324 | 6.45 | 176 | 106 | 37 | 707 | 2.18 | 3.20 | 2.36 | 494 | 626 |
| ND2010 x PHP02 | 6.44 | 219 | 104 | 40 | 702 | 2.24 | 3.29 | 2.30 | 482 | 627 |
| ND2003 x S8324 | 6.32 | 201 | 105 | 41 | 694 | 2.32 | 3.26 | 2.40 | 484 | 618 |
| ND2011 x OQ603 | 6.25 | 217 | 100 | 42 | 706 | 2.12 | 3.09 | 2.18 | 484 | 620 |
| ND2011 x PHP02 | 6.15 | 215 | 100 | 42 | 705 | 2.13 | 3.25 | 2.22 | 484 | 621 |
| ND08-343 x PHP02 | 5.89 | 203 | 108 | 42 | 698 | 2.17 | 3.24 | 2.33 | 483 | 613 |
| ND291 x PHP02 | 5.79 | 182 | 98 | 39 | 711 | 2.09 | 3.15 | 2.10 | 483 | 644 |
| ND2011 x PHR25 | 5.77 | 172 | 100 | 44 | 703 | 2.20 | 3.17 | 2.26 | 482 | 618 |
| ND08-343 x S8324 | 5.73 | 209 | 102 | 46 | 700 | 2.16 | 3.19 | 2.17 | 487 | 625 |
| ND2010 x PHJ40 | 5.72 | 191 | 101 | 37 | 709 | 2.24 | 3.06 | 2.23 | 482 | 632 |
| ND291 x OQ603 | 5.66 | 181 | 100 | 39 | 709 | 2.10 | 3.15 | 2.11 | 489 | 644 |
| ND2010 x PHK76 | 5.63 | 217 | 102 | 38 | 707 | 2.21 | 3.16 | 2.34 | 488 | 619 |
| ND2003 x PHJ40 | 5.44 | 168 | 108 | 37 | 704 | 2.32 | 3.22 | 2.38 | 489 | 622 |
| ND08-343 x PHT77 | 5.38 | 194 | 108 | 38 | 703 | 2.18 | 3.25 | 2.32 | 486 | 619 |
| ND2003 x RS710 | 5.31 | 165 | 105 | 40 | 701 | 2.34 | 3.29 | 2.28 | 479 | 628 |
| ND2003 x CR14 | 5.30 | 209 | 117 | 39 | 693 | 2.03 | 3.47 | 2.52 | 494 | 600 |
| Checks: | | | | | | | | | | |
| DKC 33-54 | 5.09 | 158 | 98 | 39 | 709 | 2.10 | 3.21 | 2.16 | 491 | 634 |
| Pioneer 39D85 | 6.98 | 168 | 99 | 38 | 711 | 2.16 | 3.31 | 2.38 | 493 | 604 |
| DKC 38-89 | 6.77 | 187 | 101 | 38 | 705 | 2.18 | 3.27 | 2.39 | 488 | 616 |
| Pioneer 38N88 | 6.92 | 160 | 99 | 40 | 707 | 2.10 | 3.26 | 2.23 | 487 | 616 |
| TR3622 xTR4010 | 6.38 | 196 | 107 | 40 | 701 | 2.27 | 3.23 | 2.36 | 482 | 610 |

Table 3.3. Selected maize hybrids, from combined analysis across five environments, of trial II, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits (continued)

| Hybrids | Yield (Mg $ha^{-1}$) | Moist† (g $Kg^{-1}$) | Protein (g $Kg^{-1}$) | Oil (g $Kg^{-1}$) | Starch (g $Kg^{-1}$) | CYS‡ (g $Kg^{-1}$) | LYS§ (g $Kg^{-1}$) | MET¶ (g $Kg^{-1}$) | HFC# (g $Kg^{-1}$) | HES†† (g $Kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean of selections‡‡ | 5.87 | 196 | 104 | 40 | 704 | 2.18 | 3.21 | 2.27 | 486 | 624 |
| Checks mean§§ | 6.43 | 174 | 101 | 39 | 707 | 2.16 | 3.26 | 2.31 | 488 | 616 |
| Exp. Mean¶¶ | 5.13 | 201 | 104 | 39 | 704 | 2.20 | 3.20 | 2.28 | 485 | 622 |
| | | | | | | | | | | |
| EMS | 1.87 | 243 | 59 | 4 | 60 | 0.09 | 0.03 | 0.03 | 90 | 127 |
| LSD, 0.05 | 1.21 | 14 | 7 | 2 | 7 | 0.26 | 0.16 | 0.14 | 8 | 10 |
| CV,% | 26.67 | 8 | 7 | 5 | 1 | 13.57 | 5.76 | 7.07 | 2 | 2 |

† Grain moisture, ‡ cysteine, § lysine, ¶ methionine, # high fermentable corn, †† extractable starch, ‡‡ mean of selected 17 hybrids, §§mean of five checks, ¶¶mean of 81 entries; selected entries were subjectively selected relative to the top and average values of industry checks; Selection were carried out by yield sort in descending order and around 40 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher protein, hybrids were further selected for higher oil, starch, and for other traits mentioned in the table; The basis of selection was best check and average values of industry checks for respective traits

Table 3.4. Selected maize hybrids, from combined analysis across six environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney), of trial III, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits

| Hybrids | Yield (Mg $ha^{-1}$) | Moist[†] (g $Kg^{-1}$) | Protein (g $Kg^{-1}$) | Oil (g $Kg^{-1}$) | Starch (g $Kg^{-1}$) | CYS[‡] (g $Kg^{-1}$) | LYS[§] (g $Kg^{-1}$) | MET[¶] (g $Kg^{-1}$) | HFC[#] (g $Kg^{-1}$) | HES[††] (g $Kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| T4 x Q381 | 9.36 | 151 | 113 | 42 | 694 | 2.28 | 3.42 | 2.42 | 492 | 606 |
| T1 x CR1Ht | 9.29 | 150 | 114 | 44 | 684 | 2.36 | 3.56 | 2.55 | 486 | 600 |
| T4 x PH207 | 9.22 | 142 | 112 | 42 | 695 | 2.23 | 3.46 | 2.40 | 493 | 604 |
| T1 x Lp5 | 8.75 | 173 | 110 | 42 | 694 | 2.28 | 3.43 | 2.36 | 487 | 611 |
| T1 x NK794 | 8.75 | 167 | 112 | 41 | 693 | 2.28 | 3.34 | 2.36 | 483 | 613 |
| T6 x Q381 | 8.72 | 146 | 113 | 40 | 695 | 2.37 | 3.41 | 2.48 | 480 | 599 |
| T1 x LH52 | 8.61 | 151 | 114 | 43 | 691 | 2.27 | 3.36 | 2.45 | 491 | 605 |
| T1 x LH54 | 8.50 | 169 | 116 | 42 | 690 | 2.35 | 3.49 | 2.58 | 480 | 594 |
| T6 x CR1Ht | 8.49 | 150 | 116 | 42 | 690 | 2.29 | 3.40 | 2.49 | 486 | 599 |
| T7 x Q381 | 7.95 | 156 | 107 | 38 | 702 | 2.33 | 3.32 | 2.50 | 482 | 595 |
| T3 x LH52 | 7.87 | 138 | 120 | 43 | 686 | 2.38 | 3.52 | 2.58 | 494 | 589 |
| T1 x DJ7 | 7.80 | 185 | 112 | 43 | 692 | 2.34 | 3.35 | 2.40 | 477 | 614 |
| T6 x LH52 | 7.76 | 147 | 113 | 41 | 694 | 2.34 | 3.30 | 2.49 | 478 | 599 |
| T3 x LH54 | 7.57 | 149 | 116 | 42 | 691 | 2.37 | 3.54 | 2.57 | 488 | 589 |
| T3 x NK794 | 7.46 | 160 | 123 | 40 | 688 | 2.39 | 3.48 | 2.54 | 496 | 602 |
| T1 x DKFAPW | 7.39 | 161 | 116 | 38 | 692 | 2.43 | 3.50 | 2.58 | 480 | 586 |
| T3 x Q381 | 7.36 | 136 | 119 | 41 | 689 | 2.43 | 3.46 | 2.58 | 487 | 593 |
| T4 x CR1Ht | 7.32 | 146 | 118 | 45 | 684 | 2.23 | 3.67 | 2.47 | 507 | 602 |
| Checks: | | | | | | | | | | |
| DKC 33-54 | 7.32 | 136 | 101 | 40 | 706 | 2.13 | 3.17 | 2.17 | 486 | 632 |
| Pioneer 39D85 | 8.15 | 139 | 97 | 38 | 712 | 2.09 | 3.39 | 2.32 | 486 | 608 |
| DKC 38-89 | 7.15 | 143 | 97 | 40 | 708 | 2.07 | 3.19 | 2.17 | 485 | 619 |
| Pioneer 38N88 | 9.41 | 144 | 103 | 40 | 704 | 2.12 | 3.36 | 2.33 | 486 | 613 |
| TR3622 xTR4010 | 8.65 | 172 | 108 | 40 | 699 | 2.29 | 3.27 | 2.37 | 475 | 607 |

Table 3.4. Selected maize hybrids, from combined analysis across six environments, of trial III, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits

| Hybrids | Yield (Mg ha$^{-1}$) | Moist$^{\dagger}$ (g Kg$^{-1}$) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | CYS$^{\ddagger}$ (g Kg$^{-1}$) | LYS$^{\S}$ (g Kg$^{-1}$) | MET$^{\P}$ (g Kg$^{-1}$) | HFC$^{\#}$ (g Kg$^{-1}$) | HES$^{\dagger\dagger}$ (g Kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Checks: | | | | | | | | | | |
| DKC 33-54 | 7.32 | 136 | 101 | 40 | 706 | 2.13 | 3.17 | 2.17 | 486 | 632 |
| Pioneer 39D85 | 8.15 | 139 | 97 | 38 | 712 | 2.09 | 3.39 | 2.32 | 486 | 608 |
| DKC 38-89 | 7.15 | 143 | 97 | 40 | 708 | 2.07 | 3.19 | 2.17 | 485 | 619 |
| Pioneer 38N88 | 9.41 | 144 | 103 | 40 | 704 | 2.12 | 3.36 | 2.33 | 486 | 613 |
| TR3622 xTR4010 | 8.65 | 172 | 108 | 40 | 699 | 2.29 | 3.27 | 2.37 | 475 | 607 |
| | | | | | | | | | | |
| Mean of selections$^{\ddagger\ddagger}$ | 8.23 | 154 | 115 | 42 | 691 | 2.33 | 3.45 | 2.49 | 487 | 600 |
| Checks mean$^{\S\S}$ | 8.14 | 147 | 101 | 40 | 706 | 2.14 | 3.28 | 2.27 | 484 | 616 |
| Exp. Mean$^{\P\P}$ | 6.78 | 153 | 111 | 42 | 694 | 2.30 | 3.38 | 2.43 | 484 | 604 |
| | | | | | | | | | | |
| EMS | 2.06 | 66 | 37 | 2 | 33 | 0.01 | 0.02 | 0.02 | 80 | 93 |
| LSD, 0.05 | 1.15 | 7 | 5 | 1 | 5 | 0.08 | 0.11 | 0.10 | 7 | 8 |
| CV,% | 21.15 | 5 | 5 | 4 | 0.82 | 4.21 | 4.18 | 5.20 | 2 | 2 |

$^{\dagger}$ Grain moisture, $^{\ddagger}$ cysteine, $^{\S}$ lysine, $^{\P}$ methionine, $^{\#}$ high fermentable corn, $^{\dagger\dagger}$ extractable starch, $^{\ddagger\ddagger}$ mean of selected 18 hybrids, $^{\S\S}$mean of five checks, $^{\P\P}$mean of 72 entries; selected entries were subjectively selected relative to the top and average values of industry checks; Selection were carried out by yield sort in descending order and around 35 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher protein, hybrids were further selected for higher oil, starch, and for other traits mentioned in the table; The basis of selection was best check and average values of industry checks for respective traits

Table 3.5. Selected maize hybrids, from combined analysis across six environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney), of trial IV, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits

| Hybrids | Yield (Mg ha$^{-1}$) | Moist$^{\dagger}$ (g Kg$^{-1}$) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | CYS$^{\ddagger}$ (g Kg$^{-1}$) | LYS$^{\S}$ (g Kg$^{-1}$) | MET$^{\P}$ (g Kg$^{-1}$) | HFC$^{\#}$ (g Kg$^{-1}$) | HES$^{\dagger\dagger}$ (g Kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| T1 x LH205 | 7.62 | 198 | 111 | 45 | 690 | 2.18 | 3.31 | 2.40 | 491 | 618 |
| T7 x PHP02 | 7.55 | 189 | 102 | 38 | 707 | 2.07 | 3.20 | 2.31 | 492 | 614 |
| T6 x PHP02 | 7.47 | 179 | 106 | 39 | 704 | 2.00 | 3.22 | 2.32 | 493 | 619 |
| T4 x PHT77 | 7.19 | 173 | 107 | 38 | 698 | 2.14 | 3.51 | 2.44 | 496 | 615 |
| T7 x PHT77 | 7.08 | 185 | 106 | 37 | 705 | 2.21 | 3.34 | 2.42 | 492 | 610 |
| T1 x S8324 | 7.01 | 187 | 111 | 47 | 689 | 1.87 | 3.33 | 2.40 | 492 | 618 |
| T4 x PHP02 | 6.93 | 182 | 108 | 41 | 699 | 1.87 | 3.44 | 2.37 | 499 | 609 |
| T1 x PHT77 | 6.90 | 178 | 104 | 40 | 703 | 2.11 | 3.28 | 2.28 | 495 | 623 |
| T4 x L 127 | 6.88 | 182 | 107 | 44 | 697 | 1.93 | 3.38 | 2.29 | 497 | 613 |
| T7 x OQ603 | 6.75 | 195 | 103 | 39 | 702 | 2.24 | 3.30 | 2.45 | 487 | 604 |
| T1 x RS710 | 6.72 | 158 | 105 | 45 | 697 | 2.15 | 3.20 | 2.37 | 491 | 613 |
| T1 x PHP02 | 6.68 | 188 | 111 | 42 | 693 | 1.98 | 3.42 | 2.51 | 496 | 596 |
| T6 x PHK76 | 6.64 | 190 | 107 | 39 | 704 | 2.09 | 3.14 | 2.42 | 482 | 611 |
| T1 x PHJ40 | 6.58 | 157 | 105 | 43 | 700 | 2.13 | 3.14 | 2.31 | 490 | 621 |
| T1 x CR14 | 6.53 | 175 | 114 | 44 | 689 | 2.18 | 3.39 | 2.49 | 496 | 601 |
| Checks: | | | | | | | | | | |
| DKC 33-54 | 5.93 | 142 | 103 | 41 | 704 | 2.04 | 3.23 | 2.22 | 498 | 629 |
| Pioneer 39D85 | 6.12 | 158 | 102 | 39 | 708 | 2.07 | 3.35 | 2.38 | 497 | 604 |
| DKC 38-89 | 7.47 | 160 | 106 | 40 | 701 | 2.14 | 3.21 | 2.35 | 491 | 610 |
| Pioneer 38N88 | 7.22 | 164 | 103 | 42 | 703 | 2.06 | 3.28 | 2.32 | 495 | 617 |
| TR3622 xTR4010 | 5.70 | 202 | 107 | 42 | 699 | 2.00 | 3.22 | 2.38 | 483 | 613 |

Table 3.5. Selected maize hybrids, from combined analysis across six environments, of trial IV, based on a relative combination of lower grain moisture and higher yield, grain quality, and nutritional traits (continued)

| Hybrids | Yield (Mg $ha^{-1}$) | Moist† (g $Kg^{-1}$) | Protein (g $Kg^{-1}$) | Oil (g $Kg^{-1}$) | Starch (g $Kg^{-1}$) | CYS‡ (g $Kg^{-1}$) | LYS§ (g $Kg^{-1}$) | MET¶ (g $Kg^{-1}$) | HFC# (g $Kg^{-1}$) | HES†† (g $Kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean of selections‡‡ | 6.97 | 181 | 107 | 41 | 699 | 2.08 | 3.31 | 2.38 | 492 | 612 |
| Checks mean§§ | 6.49 | 165 | 104 | 41 | 703 | 2.06 | 3.26 | 2.33 | 493 | 615 |
| Exp. Mean¶¶ | 5.93 | 174 | 110 | 42 | 697 | 2.10 | 3.28 | 2.41 | 492 | 609 |
| | | | | | | | | | | |
| EMS | 2.33 | 189 | 29 | 3 | 26 | 0.09 | 0.05 | 0.02 | 68 | 1 |
| LSD, 0.05 | 1.24 | 11 | 4 | 1 | 4 | 0.24 | 0.18 | 0.12 | 7 | 8 |
| CV,% | 25.73 | 8 | 5 | 4 | 0.73 | 13.95 | 6.75 | 6.16 | 2 | 0.15 |

† Grain moisture, ‡ cysteine, § lysine, ¶ methionine, # high fermentable corn, †† extractable starch, ‡‡ mean of selected 15 hybrids, §§mean of five checks, ¶¶mean of 64 entries; Selection were carried out by yield sort in descending order and around 30 hybrids were selected, then sorted by moisture in ascending order and removed higher grain moisture containing hybrids, hybrids were then screened for higher protein, hybrids were further selected for higher oil, starch, and for other traits mentioned in the table; The basis of selection was best check and average values of industry checks for respective traits

# CHAPTER IV: VALIDATION OF HETEROTIC GROUPS AND PATTERNS IN A SHORT-SEASON ELITE MAIZE (ZEA MAYS L.) SAMPLE


## Abstract

Maize (*Zea mays* L.) is mostly produced as hybrids which are developed by crossing two inbred lines. Maize inbreds are under restricted use because they are protected by U.S. Patent and/or the U.S. Plant Variety Protection Act (PVPA). The patents-expired inbred lines could serve as alternative breeding sources for cultivar development. The objectives of this research were i) to identify and validate heterotic groups of ex-PVP inbreds and NDSU inbred lines, and ii) to identify desirable top heterotic patterns among ex-PVP, industry testers, and NDSU lines. . Three groups of crosses were made for the study following North Carolina Mating Design II (NCII) including 12 NDSU, 24 ex-PVP lines, and seven top industry testers in the 2010 North Dakota State University (NDSU) summer nursery, Fargo, ND, and in the 2010-2011 NDSU winter nursery in New Zealand. Hybrids were planted in four different experiments at six ND environments following partially balanced lattice experimental design in 2011 and 2012. The NCII design (model II) derived SCA effects were additionally used. Highest grain yield and SCA effects across environments were used to determine and validate heterotic groups from the known heterotic groups of industry testers. Top heterotic patterns were selected based upon grain yield and other favorable traits. Most of the inbred lines belong to SS, non-SS, Iodent, and Lancaster groups, while some of the inbreds belong to both SS/non-SS backgrounds. Top heterotic patterns, in our trials were SS x non-SS, Iodent x Lancaster, SS x Lancaster, and SS/non-SS x SS.



**Key words:** *Zea mays* L., ex-PVP heterotic groups, heterotic patterns, SCA.

## Introduction

Maize breeding is a confidential and highly profitable business. Therefore, inbred parents and hybrids are protected by the U.S. Patent/or U.S. Plant Variety Protection Act (PVPA). Expired-PVP (ex-PVP) inbred lines, after being protected for 20 years, are maintained at the North Central Regional Plant Introduction Station (NCRPIS) at Ames, IA. These lines could be available and potentially represent new germplasm sources for many public and private breeding programs for research and use (Nelson et al. 2008). However, many breeders doubt their usefulness due to their original development date.

Maize breeding programs focused on developing inbred lines for hybrids are mostly dependent on the identification and utilization of heterotic groups and heterotic patterns (Melani and Carena 2005). Assigning and validating ex-PVP inbred lines to heterotic groups could be useful to exploit desirable heterotic patterns. Heterotic groups represent groups of germplasm sources that when crossed with each other produce consistently better crosses than when crosses are made within those groups (Hallauer and Carena 2009). Identifying heterotic patterns, which are crosses between known genotypes (from different heterotic groups) expressing a high level of heterosis (Carena and Hallauer 2001), is key to the development of successful maize (*Zea mays* L.) hybrids (Eyherabide and Hallauer 1991; Barata and Carena 2006). Heterotic patterns can also be termed as good performances of hybrids resulting from crossing between inbreds across or within heterotic groups (Troyer 2006). The North American dent maize germplasm is composed of multiple heterotic groups that when crossed to each other can optimize hybrid performance (Mikel and Dubley 2006). Such groups may not be distinct, but their identification still helps exploit suitable heterotic patterns. Dubreuil et al. (1996) emphasized that the accurate

assignment of inbred lines to heterotic groups is a prerequisite for efficient utilization of germplasm.

Heterotic groups in dent maize have been subdivided into Iowa Stiff Stalk Synthetic (BSSS) and non-BSSS (Lu and Bernardo 2001). A similar grouping consists of Reid Yellow Dent (related to BSSS), Lancaster, Iodent, and miscellaneous heterotic groups (Gethi et al. 2002). Troyer (1999) divided maize into five genetic backgrounds: Reid Yellow Dent (Iodent Reid and BSSS), Minnesota 13 (W153R and SD105), Northwestern Dent (A48, A509, and A78), Lancaster Sure Crop (Mo17 and Oh43), and Leaming Corn (Oh07). Mikel and Dubley (2006) indicated that the Reid Yellow Dent is the largest group, and has made significant contributions to commercial hybrids.

There are several methods to classify maize inbreds into heterotic groups. Two major classification methods are widely used across the world (Fan et al. 2009). The traditional method uses specific combining ability with line-pedigree information, and/or field hybrid-yield information, to assign maize lines to a heterotic group. A more challenging method is to use different molecular markers to compute genetic similarity (GS) or genetic distance (GD) estimates to assign maize lines to a particular heterotic group, which is not always accurate. Fan et al. (2009) executed a third approach, by using heterotic group's specific and general combining ability (HSGCA) to classify inbreds into heterotic groups. They claimed their way is efficient compared to SSR markers and yield-based specific combining abilities. Menkir et al. (2004) classified inbred lines into heterotic groups by yield-based specific combining ability and molecular marker based approaches. They reported that yield-based combining ability derived heterotic groups did not match with groups established using molecular markers. Melchinger (1999) extensively discussed the potentials of DNA markers in assigning inbreds of unknown

genetic origin to established heterotic groups. However, he concluded that if a large number of genotypes are available and proven testers exist, the testcross performances should be the main criteria for classifying materials into heterotic groups. In addition, Barata and Carena (2006) observed large inconsistencies between molecular marker based classification and field trial based classification (e.g., testcross and diallel data) of a diverse set of inbreds. They concluded that groups of similar germplasm and heterosis properties could not be identified accurately and reliably with molecular markers. Consequently, they recommended extensive field evaluation across environments to classify inbred lines into heterotic groups. Alternatively, the North Carolina Mating Design II (NCII) (Comstock and Robinson 1948) can be used to test a larger set of progenies, extensively over locations and years to classify inbreds to heterotic groups. Many ex-PVP lines do not have assigned heterotic groups yet; an approximation can be deduced based on PVP documents and their genesis. Moreover, reported heterotic groups may not be stable in different situations. The objectives of the study were i) to identify and validate heterotic groups of ex-PVP inbreds and NDSU inbred lines, and ii) to identify desirable top heterotic patterns among ex-PVP-, industry testers, and NDSU lines.

## Materials and Methods

### Plant materials

Twenty four ex-PVP lines (Table 4.1), 12 NDSU inbred lines (Table 4.2.), and seven top industry testers (Table 4.2.) for the northern U.S. Corn Belt were used in this study. Ex-PVP and NDSU lines were selected because they had the fewest number of silking days and growing degree days. This germplasm partially represents earliness pools for northern U.S. maize breeding.

Table 4.1. Sets of short- season ex-PVP lines, used in our study along with their PVP number and approximate heterotic groups

| Line | PVP number | Heterotic group[†] |
|---|---|---|
| First set of 12 ex-PVP lines | | |
| Lp5 | 8700031 | Stiff Stalk (SS) & Unrelated (UR) |
| Q381 | 8500098 | Unrelated (UR) |
| NK807 | 8700151 | Stiff Stalk & MN13 |
| CR1Ht | 8400042 | Lancaster (Lan) & MN13 |
| DK78010 | 8500126 | Stiff Stalk |
| PH207 | 8300144 | Iodent (IO) |
| DKFAPW | 8200152 | Stiff Stalk |
| LH52 | 8700020 | Lancaster |
| NK794 | 8700046 | Stiff Stalk |
| LH54 | 8600128 | Lancaster |
| DJ7 | 8500086 | Stiff Stalk (SS) &Unrelated (UR) |
| NK779 | 8700041 | MN13 & Unrelated (UR) |
| Second set of 12 ex-PVP lines | | |
| PHJ40 | 8600133 | Stiff Stalk (SS) |
| PHK05 | 8800001 | Not defined |
| PHR25 | 8800002 | Iodent (IO) |
| PHK76 | 8800036 | Not defined |
| PHT77 | 8800038 | Lancaster (Lan) & Unrelated (UR) |
| OQ603 | 8800150 | Not defined |
| NKS8324 | 8800153 | Stiff Stalk (SS) |
| PHP02 | 8800212 | Not defined |
| CR14 | 8900095 | Stiff Stalk (SS) |
| L127 | 8900201 | Not defined |
| LH205 | 9000049 | Not defined |
| RS710 | 9000129 | Not defined |

Heterotic groups were adopted from Mikel (2006) and from GRIN website, [†] background, MN13 refers to Minnesota 13, not defined means not clearly indicated or identified in the PVP documents

Respective PVP numbers mentioned in Table 4.1 can be used at Agricultural Research Services of Germplasm Resources Information Network (GRIN) web page of United States Department of Agriculture (USDA) (http://www.ars-grin.gov/cgi-bin/npgs/html/pvplist.pl?) to explore more information about the off-protected inbreds we used in our study. Ex-PVP

materials were requested and obtained from National Plant Germplasm System of the USDA at the North Central Regional Plant Introduction Station (NCRPIS) at Ames, IA. The twelve NDSU lines and industry testers are presented (Table 4.2.) below.

Table 4.2. NDSU lines and testers, used in our study along with their respective heterotic groups

| Entries | Heterotic background |
|---|---|
| NDSU lines | |
| ND08-343 | Stiff Stalk (SS) |
| ND291 | SS/non-SS |
| ND2000 | SS |
| ND2001 | Lancaster |
| ND2002 | SS |
| ND2003 | SS |
| ND2004 | SS |
| ND2005 | non-SS |
| ND2006 | SS |
| ND2007 | Non-SS |
| ND2010 | SS |
| ND2011 | Non-SS |
| Industry testers | |
| T1 | Iodent |
| T2 | SS |
| T3 | Iodent |
| T4 | SS |
| T5 | Non-SS |
| T6 | SS |
| T7 | SS |

These contemporary industry testers represent major heterotic groups available in the northern U.S. Corn Belt. T1 is an Iodent line, T2 is B14 derived, T3 is another Iodent line, T4 is B14 and B73 derived, T5 is an LH82 derived non-SS line, T6 is B14 derived, and T7 is a B14 and B73 derived lines; heterotic groups are indicated in Table 4.2.

**Crossing procedure**

Twelve NDSU lines were crossed with 12 ex-PVP lines in the 2010 NDSU Fargo maize breeding nursery following the NCII mating design (Comstock and Robinson, 1948). The same 12 NDSU lines were crossed with another set of 12 ex-PVP lines, following another NCII mating design at the 2010-2011 New Zealand NDSU winter nursery. All 24 ex-PVP lines (i.e. the lines used in the first and second sets of crosses) were also crossed in the winter with seven current industry testers following the NCII design. Inbred lines were planted in paired rows, 7 m long with 0.76 m between rows. All possible pair-row crosses were made during pollination. Crosses were harvested and shelled in bulk for each cross combination.

**Field trials**

Hybrids obtained from the three sets of crosses were planted as four different trials along with five hybrids, which are currently widely grown in ND, and were used as checks in this study. Trial I included 121 hybrids from the first set of crosses and was arranged in an 11 x 11 partially balance lattice design planted at six ND environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney). Trial II included 81 hybrids from the second set of crosses arranged in a 9 x 9 partially balanced lattice design, planted in five ND environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Barney), due to seed shortage. Trial III included 72 hybrids from the third set of crosses, arranged in an 8 x 9 rectangular lattice design, planted in six ND environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney). Trial IV included 64 entries, from the third set of crosses, arranged in an 8 x 8 partially balanced lattice design, planted in the same six ND environments. Experiment checks representing a wide maturity range for ND were: DKC 33-54 (83RM), Pioneer 39D85 (85RM), TR2015+TR1099*TR3622 CBLL (87RM), Pioneer 38N88 (92RM), TR3622 x TR4010

(100RM). In 2012, TR2015+TR1099*TR3622 CBLL was replaced by DKC 38-89 (88RM) because the previous seed sources was depleted. Experiment plots were machine planted with 50 kernels and thinned back to 35 plants at about 30 days after planting, which resulting in plant populations of approximately 70,000 plants $ha^{-1}$. Fertilization and field management practices were as recommended for ensuring optimum maize production. Experimental plots were planted and harvested using machines that had been modified for small experimental plots. While harvesting, approximately 500 g seed samples were kept from each plot for grain quality assessment.

**Traits evaluated**

The following agronomic traits were collected for this study: grain yield (Mg $ha^{-1}$), test weight (Kg $hl^{-1}$), grain moisture at harvest (g $Kg^{-1}$), per cent root lodging (%), and per cent stalk lodging (%). Data were recorded on an individual plot basis. Grain weight, grain moisture, and test weight were measured electronically on the combine while harvesting. Grain yield (Mg $ha^{-1}$) was adjusted to 155 g $kg^{-1}$ grain moisture. Root lodging was measured as percentage of plants in a plot leaning at an angle greater than 30° from vertical while stalk lodging was measured as a percentage of plants in a plot with stalks broken at or below top ear. Lodging were counted before harvest and analyzed as percentages to total stands per plot. In addition, grain quality traits like protein (g $Kg^{-1}$), starch (g $Kg^{-1}$), and oil (g $Kg^{-1}$) were assessed by the OmegAnalyzer G (Bruins Instruments) NIR machine.

**Statistical procedures**

Plot means of all phenotypes were used for statistical analyses. Analyses of variance were performed for all traits at each location, as well as combined across locations and years using SAS 9.3 software (SAS 2010), for the four experiments. Date were collected and summarized in

Excel files and then exported to SAS for analyses. General and specific combining ability effects and variances of parental inbreds, and respective hybrids were estimated through ANOVA considering year by location as environments. Combining abilities were further partitioned into male and female, and interactions of male and female (Scott et al. 2009). The used random model was:

$Y_{ijkl} = \mu + \varepsilon_{l} + r_{k}(l) + m_i + f_j + mf(ij) + m_i(l) + f_j(l) + mf\varepsilon(ijl) + e_{ijlk}$

Where,

$Y_{ijkl}$ = observed values

$\mu$ = overall mean of the experiments

$\varepsilon_{l}$ = $l$ environmental effects

$r_{k}(l)$ = replicate effects within $l$ environments

$m_i$ = effects of $i^{th}$ male parental lines

$f_j$ = effects of $j^{th}$ female parental lines

$mf(ij)$ = interaction effects of $i^{th}$ male with $j^{th}$ female parents

$m_i(l)$= interaction effects of $i^{th}$ male with $l$ environments

$f_j(l)$ = interaction effects of $j^{th}$ female with $l$ environments

$mf\varepsilon(ijl)$ = interaction effects of $i^{th}$ male by $j^{th}$ female by $l$ environments

$e_{ijlk}$ = residual

General and specific combining ability effects and variances were also calculated using SAS software version 9.3 (SAS 2010). General combined ANOVA were carried out using the following linear model:

$Y = \mu + \varepsilon_l + r_j(l) + b_k(jl) + t_i + t\varepsilon(il) + e_{ijkl}$

Where,

Y = observed value

$\mu$ = mean values observed in the experiments

$\varepsilon_l$ = environmental effects

$r_j(l)$ = effect of $j^{th}$ replicates within $l$ environments

$b_k(jl)$ = effect of $k^{th}$ block within $j^{th}$ rep and $l^{th}$ environments

$t_i$ = effect of $i^{th}$ treatments or hybrids

$t\varepsilon(il)$ = interaction effects of $i^{th}$ treatment by $l^{th}$ environments

$e_{ijkl}$ = residual

Homogeneity of error variances were tested using the ten-fold thumb rule (Tabachnik and Fidell 2001) before combining data across environments. If the error mean squares (EMS) were within ten-fold, EMSs were considered homogeneous and were combined. Mean comparisons among genotypes were assessed by Fisher's protected least significant difference (LSD) at <0.05 level of significance, which has been shown to be an appropriate test for detecting differences (Carmer and Swanson 1971).

## Results and Discussion

A combined ANOVA for combining ability was computed for grain yield (Mg $ha^{-1}$), moisture (g $Kg^{-1}$), test weight (Kg $hL^{-1}$), percent root lodging (%), percent stalk lodging (%), protein (g $Kg^{-1}$), starch (g $Kg^{-1}$), and oil (g $Kg^{-1}$) in this paper. SCA effects derived from NCII

(random) for yield, along with mean grain yield were utilized to estimate and validate heterotic groups of short season ex-PVP inbreds, and NDSU lines from the known heterotic groups of testers. Pedigree and composition of a particular inbred line was also used to determine heterotic groups when inbreds combined well with contrasting heterotic testers.

**Heterotic group determination for ex-PVP lines**

SCA effects and mean grain yield have been widely used to classify maize heterotic groups (Menkir et al. 2004; Melani and Carena 2005; Fan et al. 2008). Therefore, we used SCA effects and mean grain yield of research trial III to determine and validate heterotic groups of our first set of 12 ex-PVP inbred lines. We arranged SCA effects in descending order and selected the lines with top SCA effects (Table 4.3), unless we obtained all the 12 ex-PVP inbreds in the hybrid combinations. Inbreds that were combining well with two contrasting testers (belonging to different heterotic groups) are also presented in Table 4.3.

**Classification of the first set of 12 ex-PVP maize inbreds**

**SS inbreds:** Lp5 combined well with Iodent tester T1, resulting in a grain yield average value of 8.75 Mg $ha^{-1}$ and SCA effect of 1.03 Mg $ha^{-1}$ (Table 4.3). Therefore, Lp5 belongs to the SS heterotic group, which is in agreement with the PVP documents as presented in Table 4.1 (Mikel 2006). DK78010 combined well with both Iodent type tester T1 and SS testerT7 (Table 4.3). This inbred had above average combining ability with testers of the same and opposite heterotic groups' testers. Barata and Carena (2006) also found ND278 and ND282 combined very well across testers. So, the reported heterotic group, SS of DK78010 is an accurate classification. Inbred, NK807 combined well with non-SS tester T5, and therefore, the line belongs to the SS group; the line also has a Lancaster background (Table 4.1) (Mikel 2006). We found that the SS background of DKFAPW was correct, as it combined well with the Iodent

tester T3 (with a yield of 7.17 Mg ha$^{-1}$ and SCA effect of 0.44 Mg ha$^{-1}$) (Table 4.3). Inbred NK794 also belongs to the SS, as it combined well with Iodent tester T1.

The combining ability of DJ7 is quite good with SS tester T2 (with a yield of 6.79 Mg ha$^{-1}$ and SCA effect of 0.41 Mg ha$^{-1}$) (Table 4.3), so the inbred could be SS as mentioned by Mikel (2006) and presented in Table 4.2. The unrelated proportion (Table 4.1) of DJ7 could combine with SS testers.

**Non-SS inbreds:** The heterotic background of Q381 is unrelated (Table 4.1). In our extensive evaluation, Q381 combined very well with SS tester T4 (with a yield of 9.36 Mg ha$^{-1}$ and SCA effect of 1.20 Mg ha$^{-1}$) (Table 4.3). So, Q381 belongs, in our opinion, to a non-SS group. Our evaluation infers that PH207 had a non-SS background, possibly Iodent, and combined very well with SS tester T4 (with a yield of 9.22 Mg ha$^{-1}$ and SCA effect of 1.31 Mg ha$^{-1}$) (Table 4.3); NK779 combined well with the SS tester T4 (with a yield of 6.16 Mg ha$^{-1}$ and SCA effect of 0.34 Mg ha$^{-1}$) (Table 4.3), and belongs to the non-SS heterotic group, which supported the line's MN13 and unrelated composition as mentioned by Mikel (2006).

**Lancaster inbreds:** CR1Ht has been reported to have Lancaster and MN13 backgrounds (Table 4.1), and combined very well with Iodent tester T1 (with a yield of 9.29 Mg ha$^{-1}$and SCA effect of 0.96 Mg ha$^{-1}$) (Table 4.3). Thus, our trials revealed that the inbred line CR1Ht belongs to the Lancaster group. Previous research suggested that LH52 belonged to the Lancaster group (Table 4.1). Our trials supported this finding; in our evaluation, LH52 combined well with Iodent tester T1 (with a yield of 8.61 Mg ha$^{-1}$ and SCA effect of 0.71 Mg ha$^{-1}$) (Table 4.3). Similarly LH54, showed good combining ability (with a yield of 8.50 Mg ha$^{-1}$and SCA effect of 0.52 Mg ha$^{-1}$) (Table 4.3) with an Iodent tester, and belongs to the Lancaster heterotic group.

**Classification of the second sets of 12 ex-PVP inbreds**

**SS inbreds:** We found that PHJ40 belongs to the SS group because it combined (with a yield of 6.58 Mg $ha^{-1}$ and SCA effect of 0.12 Mg $ha^{-1}$) (Table 4.4) well with Iodent tester T1. This is in agreement with the PVP documented report (Table 4.1). The combining ability of NKS8324 was good with Iodent tester T1 (with a yield of 7.01 Mg $ha^{-1}$and SCA effect of 0.33 Mg $ha^{-1}$) (Table 4.4). The results validated the reported SS group (Table 4.1) of the line (Mikel 2006). CR 14, LH205, and RS710 also belong to the SS group because they combined well with Iodent tester T1 (Table 4.4).

**Non-SS inbreds:** We observed that PHK05 combined well (with a yield of 5.57 Mg $ha^{-1}$ and SCA effect of 0.17 Mg $ha^{-1}$) (Table 4.4) with SS tester T4, confirming that the inbred belongs to the non-SS group. In our evaluation, PHK76 combined well (with a yield of 6.64 Mg $ha^{-1}$ and SCA effect of 0.35 Mg $ha^{-1}$) (Table 4.4) with SS tester T6. Therefore, PHK76 belongs to the non-SS group, as previously reported (Table 4.1). Similarly, OQ603, PHP02, and L127 belong to the non-SS group, because they combined well with SS testers (Table 4.4).

PHR25 could be grouped as Iodent, because it combined well (with yield of 6.43 Mg $ha^{-1}$ and SCA effect of 0.31 Mg $ha^{-1}$) (Table 4.4) with SS tester T4. PHT77 combined well with SS tester T7 (with a yield of 7.08 Mg $ha^{-1}$ and SCA effect of 0.24 Mg $ha^{-1}$) (Table 4.4), which was supported by Mikel (2006), who reported that the line belongs to Lancaster and unrelated groups.

**Heterotic group determination and validation of NDSU inbreds**

From trials three and four (Table 4.3 & 4.4), where we had hybrids between known testers with unknown ex-PVP inbreds, we found heterotic group/s of respective ex-PVP inbreds. The first set of 12 ex-PVP lines was crossed to 12 NDSU lines and the resulting hybrids were

evaluated in trial I. The SCA effects and mean grain yield were used to validate heterotic group/s of NDSU inbreds.

**SS inbreds:** ND2004 combined well with non-SS inbred NK779 and SS inbred NK807 (Table 4.5). The testcross results from Carena and Wanner (2009) infer that ND2004 has a SS heterotic group (Table 4.2). Therefore, we can conclude that ND2004 belongs to SS, but has unique combining ability with both heterotic groups based on our current results. In our trial, ND2002 also combined well with SS and unrelated line Lp5 (Table 4.1). The unrelated proportion of Lp5 might have contributed to combine well with SS part of the line ND2002 (Table 4.5). ND2003 has a SS background (Table 4.2) and combined well with Lancaster line LH54 in trial I (Table 4.5). Testcross data from Carena and Wanner, (2009) also supported SS background for ND2003. ND2006 has a SS heterotic background as reported in Table 4.2. ND2006 combined well with a SS and an unrelated inbred Lp5. ND08-383 combined well with Lancaster inbred LH54 (with a yield of 5.06 Mg $ha^{-1}$and a SCA effect of 0.32 Mg $ha^{-1}$) (Table 4.5). These results indicate ND08-383 belongs to the SS heterotic group, which is in agreement with PVP documents as presented in Table 4.2. ND2010 combined well with the Lancaster inbred PH207 and SS inbred NK807 (Table 4.5). From current and previous findings, we could categorize ND2010 as a member of the SS group. ND2007 could have a SS background because it combined well (with a yield of 5.20 Mg $ha^{-1}$ and SCA effect of 0.39 Mg $ha^{-1}$) (Table 4.5) with Iodent inbred PH207.

**Non-SS inbreds:** The highest SCA effect was observed in hybrids between ND2005 and NK779 (with a yield of 5.42 Mg $ha^{-1}$ and SCA effect of 0.75* Mg $ha^{-1}$) (Table 4.5). We knew the heterotic group of ND2005 as non-SS (Table 4.2). ND2005 combined well with testers from both heterotic groups though (Carena and Wanner 2009). Barata and Carena (2006) reported that

inbreds could combine well with other inbreds from the same group. Therefore, even though NK779 belongs to the non-SS heterotic group, ND2005 could also belong to a non-SS group. In our trial, ND2011 combined well (with a yield of 5.25 Mg $ha^{-1}$ and SCA effect of 0.37 Mg $ha^{-1}$) (Table 4.5) with DJ7, so ND2011 belongs to non-SS background.

We observed good combining ability between ND2001 and SS line DK78010. Therefore, ND2001 should belong to the Lancaster heterotic group. Our findings supported the previous findings that ND2001 was derived from MN13 (Carena et al. 2010), and belonged to Lancaster.

**SS/non-SS lines:** Inbred ND2000 has unique capabilities to combine with both SS and non-SS inbreds (Table 4.5). So, the heterotic group of ND2000 is SS/non-SS, but Carena and Wanner (2003) indicated that molecular marker analysis and yield data to SS background. ND291 showed a broad heterotic base of SS/non-SS groups, as exhibited by its good combining ability with DJ7 (SCA effect of 0.45 Mg $ha^{-1}$ and yield of 5.54 Mg $ha^{-1}$) (Table 4.5), which is in agreement with the previous molecular marker analysis and yield trial data (Carena et al. 2003).

**Heterotic pattern detection**

In the US, the most used heterotic pattern is BSSS x Lancaster (Melani and Carena 2005). In our third trial, including hybrids between the first set of ex-PVP inbreds and industry testers, we were able to identify some promising hybrid combinations. T4 x Q381, which has SS x non-SS heterotic patterns, had significantly ($P \leq 0.05$) higher yield (9.36 Mg $ha^{-1}$) than the checks mean (8.05 Mg $ha^{-1}$) across environments, and statistically similar yield with the best check Pioneer 38N88 (9.41 Mg $ha^{-1}$) of the trial (Table 4.6). T4 x Q381 had similar moisture (151 vs. 147 g $Kg^{-1}$), root (1.32 vs. 0.75%) and stalk (1.41 vs. 2.80%) lodging resistance when compared to the checks means. The hybrid T4 x Q381 also had similar moisture, stalk, and root lodging as the top check (Table 4.6). The hybrids had significantly higher ($P \leq 0.05$) protein and

oil content, but statistically lower starch than the top check (Table 4.6). The Iodent x Lancaster heterotic pattern was represented in the hybrid T1 x CR1Ht (yield is 9.29 Mg ha$^{-1}$). This hybrid had statistically ($P \leq 0.05$) higher yield than the checks mean (8.05 Mg ha$^{-1}$), and had comparable yield with the top check (9.41 Mgha$^{-1}$). The hybrid also had significantly higher protein and oil content compared to the top check and checks mean, and significantly lower starch content compared to the checks mean and top check. The rest of the desirable traits of the hybrid were comparable with the checks mean, as well as the top check (Table 4.6). Hybrid T4 x PH207, which is another SS x Iodent heterotic pattern, yielded (9.22 Mg ha$^{-1}$) statistically ($P \leq 0.05$) higher than the checks mean (8.05 Mg ha$^{-1}$). The other promising hybrids in this trial are mostly Iodent x SS, SS x non-SS, and Iodent x Lancaster. We also found, surprisingly, one good SS x SS combination.

In the fourth trial, which includes hybrids between the second set of ex-PVP inbreds and industry testers, also provided a few promising heterotic patterns. The highest yielding (7.62 Mg ha$^{-1}$) hybrid was T1 x LH205, an Iodent x SS heterotic pattern, and it had comparable yield with best check (7.22 Mg ha$^{-1}$) (Table 4.7). The hybrid had similar test weight, root and stalk lodging, but statistically higher moisture, protein and oil content, than the best check, Pioneer 38N88. Hybrid T7 x PHP02 has the typical SS x non-SS heterotic pattern (Table 4.7). Moreover, SS x non-SS, Iodent x SS, SS x Lancaster type of heterotic patterns are also prevalent in this trial (Table 4.7).

In the first trial, ND2002 x CR1Ht, an SS x Lancaster heterotic pattern, had the highest yield (6.26 Mg ha$^{-1}$) (Table 4.8). Most of the selected hybrids in this trial had statistically similar yield and other desirable traits compared with the best performing check, and exhibited mostly SS x non-SS or SS x Lancaster type of heterotic patterns. Most NDSU lines are genetically broad

based and have unique capabilities to combine well with a wide range of inbred lines, which is reflected in the heterotic patterns (Table 4.8) of this trial. The universal combination of SS x non-SS is prevalent in the second trial (Table 4.9). In this trial, we found many inbred lines, belong to the same heterotic groups, provided outstanding hybrids.

Our short- season maize samples were categorized as SS or non-SS, Lancaster, and Iodent; some of them belonged to both SS and non-SS (because same inbreds showed above average combining ability with testers from different backgrounds). Heterotic groups are conceptual (Hallauer and Carena 2009) and often are not straight forward. A specific line from one heterotic group may fall in to another heterotic group based on a particular hybrid combination. Pedigree information is a good reference. Still, heterotic groups and patterns are useful to exploit and use as inbred parents in hybrid combination. We found SS x non-SS heterotic patterns were most frequent in our trials. Mikel and Dubley (2006) also described BSSS x non-BSSS as one of most prevalent heterotic patterns in the U.S. Our observed Iodent x Lancaster pattern in our trials could be considered as a useful alternative heterotic pattern. The lines showing good combining ability within heterotic groups, such heterotic patterns, like SS x SS, Non-SS x Non-SS, hybrids could be useful to develop inbred lines. Our research indicates many of these lines may not be immediately useful when comparing their hybrids with top checks, therefore, inbred, pre-breeding could be valuable. GCA effects with empirically determined heterotic groups could be used together to develop new inbred lines by crossing across and within heterotic groups.

## References

Barata C, Carena MJ (2006) Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. Euphytica 151:339-349

Carmer SG, Swanson MR (1971) Detection of differences between means: A Monte Carlo study of five pair wise multiple comparison procedures. Agron J. 63:940-945

Carena MJ, Hallauer AR (2001) Response to inbred progeny recurrent selection in leaming and midland yellow dent populations. Maydica 46:1-10

Carena MJ, Wanner DW, Cross HZ (2003) Registration of ND291 inbred line of maize. Crop Sci. 43:1568

Carena MJ, Wanner DW, Yang J (2010) Linking prebreeding for local germplasm improvement with cultivar development in maize breeding for short- season (85-95 RM) hybrids. J. of Plant Registrations 4:86-92

Carena MJ, Wanner DW (2003) Registration of ND2002 inbred line of maize. Crop Sci. 43:1568-1569

Carena MJ, Wanner DW (2009) Development of genetically broad-based inbred lines of maize for early-maturing (70-80 RM) hybrids. J. of Plant Registrations 3(1):107-111

Comstock RE, Robinson HF (1948) The components of genetic variance in the populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics 4:254-266

Debreuil P, Dufour P, Krejci E, Causse M, Vienne Dd, Gallais A, Charcosset A (1996) Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci. 36:796-799

Eyherabide GH, Hallauer AR (1991) Reciprocal full-sib recurrent selection in maize: II. Contribution of additive, dominance, and genetic drift effects. Crop Sci. 31:1442-1448

Fan XM, Chen HM, Tan J, Xu CX, Zhang YM, Huang YX, Kang MS (2008) A new maize heterotic pattern between temperate and tropical germplasms. Agron. J. 100: 917-923

Fan XM, Zhang YM, Yao WH, Chen HM, Tan J, Xu CX, Han XL, Luo LM, Kang MS (2009) Classifying maize inbred lines into heterotic groups using a factorial mating design. Agron. J. 101:106-112

Gethi JG, Labate JA, Lamkey KR, Smith ME, Kresovich S (2002) SSR variation in important US maize inbred lines. Crop Sci. 42:951–957

Hallauer AR, Carena MJ (2009) Maize Breeding. In: Carena MJ (ed) Handbook of plant breeding: Cereals. *Springer*, New York, pp 3-98

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor. Appl. Genet. 103:613-617

Melani MD, Carena MJ (2005) Alternative maize heterotic patterns for northern Corn Belt. Crop Sci. 45:2186-2194

Melchinger AE (1999) Genetic diversity and heterosis. In: Coors JG, Pandey S, Gender JT (eds) The genetics and exploitation of heterosis in crops. CSSA-SP, Madison, Wisconsin, pp 99-118

Menkir A, Melake-Berhan A, The C, Ingelbrecht I, Adepoju A (2004) Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. Theor Appl Genet. 108:1582-1590

Mikel MA, Dubley JW (2006) Evolution of North American dent corn from public to proprietary germplasm. Crop Sci. 46:1193-1205

Nelson PT, Coles ND, Holland JB, Bubeck DM, Smith S, Goodman MM (2008) Molecular characterization of maize inbreds with expired U.S. plant variety protection. Crop Sci. 48:1673-1685

SAS Institute. 2010. SAS user's guide guide: Statistics. 4th ed. SAS Inst., Cary, NC

Scott MP, Peterson JM, Hallauer AR (2009) Evaluation of combining ability of quality protein maize derived from U.S. public inbred lines. Maydica 49:303-311

Tabachnik B, Fidell L, 2001. Computer –Assisted Research Design and Analysis. Boston: Allyn and Bacon

Troyer AF (1999) Background of US hybrid corn. Crop Sci. 39:601426

Troyer AF (2006) Adaptedness and heterosis in Corn and Mule hybrid. Crop Sci. 46:528-543

Table 4.3. SCA effects for yield, and grain yield, utilized in trial III to determine heterotic group/s of first set of 12 ex-PVP maize inbreds

| Hybrids | SCA Effects (Mg ha$^{-1}$) | Yield (Mg ha$^{-1}$) | Testers | Testers HG† | Ex-PVP inbreds | Ex-PVP inbreds HG |
|---|---|---|---|---|---|---|
| T4 x PH207 | 1.31 | 9.22 | T4 | SS | PH207 | Iodent |
| T4 x Q381 | 1.20 | 9.36 | T4 | SS | Q381 | Non-SS |
| T1 x Lp5 | 1.03 | 8.75 | T1 | Iodent | Lp5 | SS |
| T1 x CR1Ht | 0.96 | 9.29 | T1 | Iodent | CR1Ht | Lancaster |
| T1 x NK794 | 0.77 | 8.75 | T1 | Iodent | NK794 | SS |
| T1 x LH52 | 0.71 | 8.61 | T1 | Iodent | LH52 | Lancaster |
| T1 x DK78010 | 0.56 | 7.68 | T1 | Iodent | DK78010 | SS |
| T7 x DK78010 | 0.47 | 7.38 | T7 | SS | DK78010 | SS |
| T1 x LH54 | 0.52 | 8.50 | T1 | Iodent | LH54 | Lancaster |
| T3 x DKFAPW | 0.44 | 7.17 | T3 | Iodent | DKFAPW | SS |
| T2 x DJ7 | 0.41 | 6.79 | T2 | SS | DJ7 | SS |
| T4 x NK779 | 0.34 | 6.16 | T4 | SS | NK779 | Non-SS |
| T5 x NK807 | 0.21 | 6.01 | T5 | Non-SS | NK807 | SS |

†testers' heterotic group (HG) were used to derive ex-PVP inbreds' heterotic group; SS refers Stiff Stalk, non-SS refers non-Stiff Stalk

Table 4.4. SCA effects for yield and grain yield, utilized in trial IV to determine heterotic group/s of second set of 12 ex-PVP maize inbreds

| Hybrids | SCA effects (Mg ha$^{-1}$) | Yield ( Mg ha$^{-1}$) | Testers | Testers HG$^{\dagger}$ | Ex-PVP | Ex-PVP inbreds HG |
|---|---|---|---|---|---|---|
| T1 x LH205 | 0.77* | 7.62 | T1 | Iodent | LH205 | SS |
| T1 x RS710 | 0.72 | 6.72 | T1 | Iodent | RS710 | SS |
| T6 x PHP02 | 0.54 | 7.47 | T6 | SS | PHP02 | Non-SS |
| T1 x CR14 | 0.39 | 6.53 | T1 | Iodent | CR14 | SS |
| T7 x OQ603 | 0.35 | 6.75 | T7 | SS | OQ603 | Non-SS |
| T6 x PHK76 | 0.35 | 6.64 | T6 | SS | PHK76 | Non-SS |
| T1 x NKS8324 | 0.33 | 7.01 | T1 | Iodent | NKS8324 | SS |
| T4 x L127 | 0.33 | 6.88 | T4 | SS | L127 | Non-SS |
| T4 x PHR25 | 0.31 | 6.43 | T4 | SS | PHR25 | Iodent |
| T7 x PHT77 | 0.24 | 7.08 | T7 | SS | PHT77 | Lancaster |
| T1 x PHJ40 | 0.12 | 6.58 | T1 | Iodent | PHJ40 | SS |
| T4 x PHK05 | 0.17 | 5.57 | T4 | SS | PHK05 | Non-SS |

* Significance at $P$ <0.05; $^{\dagger}$testers' heterotic group (HG) were used to derive ex-PVP inbreds' heterotic group; SS refers Stiff Stalk, non-SS refers non-Stiff Stalk

Table 4.5. SCA effects for yield and grain yield, utilized in trial I to determine heterotic group/s of NDSU lines from known heterotic group of first set of ex-PVP inbreds

| Hybrids | SCA effects (Mg $ha^{-1}$) | Yield ( Mg $ha^{-1}$) | Ex-PVP Inbreds | Ex-PVP's HG† | NDSU Inbreds | NDSU's HG |
|---|---|---|---|---|---|---|
| ND2005 x NK779 | 0.75* | 5.42 | NK779 | Non-SS | ND2005 | Non-SS |
| ND2004 x NK779 | 0.52 | 5.45 | NK779 | Non-SS | ND2004 | SS |
| ND2004 x NK807 | 0.39 | 5.46 | NK807 | SS | ND2004 | SS |
| Lp5 x ND2002 | 0.46 | 6.34 | Lp5 | SS & UR | ND2002 | SS |
| DJ7 x ND291 | 0.45 | 5.54 | DJ7 | SS & UR | ND291 | SS/non-SS |
| ND2003 x LH54 | 0.41 | 5.54 | LH54 | Lancaster | ND2003 | SS |
| ND2007 x PH207 | 0.39 | 5.20 | PH207 | Iodent | ND2007 | SS |
| ND2011 x DJ7 | 0.37 | 5.25 | DJ7 | SS & UR | ND2011 | Non-SS |
| ND08-343 x LH54 | 0.32 | 5.06 | LH54 | Lancaster | ND08-343 | Non-SS |
| ND2010 x PH207 | 0.31 | 5.78 | PH207 | Iodent | ND2010 | SS |
| ND2010 x NK807 | 0.32 | 5.45 | NK807 | SS | ND2010 | SS |
| ND2006 x Lp5 | 0.27 | 4.92 | Lp5 | SS & UR | ND2006 | SS |
| DK78010 x ND2001 | 0.25 | 4.90 | DK78010 | SS | ND2001 | SS/non-SS |
| ND2000 x Q381 | 0.17 | 4.96 | Q381 | Non-SS | ND2000 | SS |
| ND2000 x 794 | 0.16 | 4.68 | NK794 | SS | ND2000 | SS |

* Significance at $P < 0.05$; †Ex-PVP inbreds' heterotic group (HG) were used to derive NDSU inbreds' heterotic group; SS refers Stiff Stalk, non-SS refers non-Stiff Stalk;

Table 4.6. Heterotic patterns of selected hybrids, among industry testers and first set of ex-PVP inbreds, from combined analysis across six environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney), of trial III

| Hybrids | Yield (Mg ha$^{-1}$) | MSTR[†] (g Kg$^{-1}$) | TWT[‡] (Kg hL$^{-1}$) | PRL[§] (%) | PSL[¶] (%) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | Heterotic Pattern[#] |
|---|---|---|---|---|---|---|---|---|---|
| T4 x Q381 | 9.36 | 151 | 68.39 | 1.32 | 1.41 | 113 | 41.67 | 694 | SS x non-SS |
| T1 x CR1Ht | 9.29 | 150 | 66.28 | 1.18 | 4.18 | 114 | 44.01 | 684 | Iodent x Lan |
| T4 x PH207 | 9.22 | 142 | 69.81 | 1.85 | 1.74 | 112 | 41.73 | 695 | SS x non-SS |
| T1 x Lp5 | 8.75 | 173 | 65.55 | 6.64 | 5.19 | 110 | 41.80 | 694 | Iodent x SS |
| T1 x NK794 | 8.75 | 167 | 66.83 | 0.00 | 3.36 | 112 | 41.31 | 693 | Iodent x SS |
| T6 x Q381 | 8.72 | 146 | 73.44 | 4.39 | 0.28 | 113 | 39.63 | 695 | SS x non-SS |
| T1 x LH52 | 8.61 | 151 | 67.94 | 3.03 | 1.72 | 114 | 42.95 | 691 | Iodent x Lan |
| T1 x LH54 | 8.50 | 169 | 65.13 | 0.00 | 0.81 | 116 | 41.78 | 690 | Iodent x Lan |
| T6 x CR1Ht | 8.49 | 150 | 73.19 | 0.71 | 0.18 | 116 | 42.35 | 690 | SS x Lan |
| T7 x Q381 | 7.95 | 156 | 70.70 | 1.47 | 3.19 | 107 | 37.86 | 702 | SS x non-SS |
| T3 x LH52 | 7.87 | 138 | 69.78 | 0.73 | 2.28 | 120 | 42.93 | 686 | Iodent x Lan |
| T1 x DJ7 | 7.80 | 185 | 65.45 | 1.85 | 3.58 | 112 | 43.17 | 692 | Iodent x Lan |
| T6 x DKFAPW | 7.70 | 143 | 71.28 | 0.67 | 1.95 | 113 | 39.68 | 694 | SS x SS |
| T1 x DK78010 | 7.68 | 151 | 65.98 | 1.85 | 3.49 | 111 | 40.51 | 694 | Iodent x SS |
| | | | | | | | | | |
| Pioneer 38N88[††] | 9.41 | 144 | 70.86 | 0.33 | 0.33 | 103 | 39.58 | 704 | |
| Mean of Checks[‡‡] | 8.05 | 147 | 70.09 | 0.75 | 2.80 | 101 | 39.58 | 706 | |
| Exp. Mean[§§] | 6.78 | 153 | 69.92 | 3.18 | 3.78 | 111 | 41.58 | 694 | |
| MSE | 2.06 | 66 | 2.99 | 39.29 | 27.97 | 37 | 2.35 | 33 | |
| LSD, 0.05 | 1.15 | 7 | 1.39 | 5.04 | 4.25 | 5 | 1.24 | 5 | |
| CV,% | 21.15 | 5 | 2.47 | 197.11 | 140.08 | 5 | 3.69 | 1 | |

[†] Moisture, [‡] test weight, [§] percent root lodging, [¶] percent stalk lodging, [#] Lan refers Lancaster, SS refers Stiff stalk, [††] best performing check of the trial, [‡‡] mean of five checks, [§§] Experimental mean of 72 entries; hybrids were selected based on a combination of higher yield, lower grain moisture, higher test weight, lower per cent root and stalk lodging, higher grain quality traits

Table 4.7. Heterotic patterns of selected hybrids, derived by crossing between industry testers and second set of ex-PVP inbreds, from combined analysis across six environments (2011: Casselton, Prosper; 2012: Casselton, Proper, Fargo, Barney), of trial IV

| Hybrids | Yield (Mg ha$^{-1}$) | MSTR[†] (g Kg$^{-1}$) | TWT[‡] (Kg hL$^{-1}$) | PRL[§] (%) | PSL[¶] (%) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | Heterotic Pattern[#] |
|---|---|---|---|---|---|---|---|---|---|
| T1 x LH205 | 7.62 | 198 | 66.97 | 2.24 | 1.51 | 111 | 45.11 | 690 | Iodent x SS |
| T7 x PHP02 | 7.55 | 189 | 70.56 | 0.07 | 1.10 | 102 | 38.04 | 707 | SS x non-SS |
| T6 x PHP02 | 7.47 | 179 | 69.87 | 4.57 | 1.95 | 106 | 38.75 | 704 | SS x non-SS |
| T7 x PHT77 | 7.08 | 185 | 68.45 | 0.30 | 1.45 | 106 | 36.63 | 705 | SS x Lan |
| T1 x S8324 | 7.01 | 187 | 67.56 | 1.13 | 2.11 | 111 | 47.33 | 689 | Iodent x SS |
| T4 x PHP02 | 6.93 | 182 | 66.82 | 1.42 | 3.23 | 108 | 41.20 | 699 | SS x non-SS |
| T1 x PHT77 | 6.90 | 178 | 67.52 | 0.01 | 5.51 | 104 | 39.80 | 703 | Iodent x Lan |
| T4 x L127 | 6.88 | 182 | 69.41 | 0.43 | 8.11 | 107 | 44.16 | 697 | SS x non-SS |
| T7 x OQ603 | 6.75 | 195 | 69.45 | 0.63 | 0.74 | 103 | 38.85 | 702 | SS x non-SS |
| T1 x RS710 | 6.72 | 158 | 69.68 | 0.55 | 4.34 | 105 | 44.75 | 697 | Iodent x SS |
| T6 x PHK76 | 6.64 | 190 | 72.79 | 1.41 | 2.77 | 107 | 39.13 | 704 | Non-SS x SS |
| T1 x PHJ40 | 6.58 | 157 | 71.96 | 1.61 | 3.94 | 105 | 42.57 | 700 | Iodent x SS |
| T4 x PHR25 | 6.43 | 158 | 69.07 | 2.19 | 0.02 | 108 | 41.88 | 698 | SS x Iodent |
| T6 x L127 | 6.24 | 184 | 73.15 | 0.41 | 1.54 | 113 | 41.33 | 695 | SS x non-SS |
| T6 x PHR25 | 6.16 | 154 | 71.64 | 0.12 | 2.09 | 110 | 39.01 | 699 | SS x Iodent |
| | | | | | | | | | |
| Pioneer 38N88[††] | 7.22 | 164 | 68.96 | 1.38 | 1.82 | 103 | 41.97 | 703 | |
| Checks mean[‡‡] | 6.49 | 165 | 69.79 | 0.50 | 3.89 | 104 | 40.77 | 703 | |
| Exp. Mean[§§] | 5.93 | 174 | 69.91 | 2.36 | 4.74 | 109 | 42.00 | 697 | |
| MSE | 2.33 | 189 | 4.53 | 22.64 | 65.21 | 29 | 3.14 | 26 | |
| LSD, 0.05 | 1.24 | 11 | 1.72 | 3.85 | 6.54 | 4 | 1.44 | 4 | |
| CV,% | 25.73 | 8 | 3.05 | 201.30 | 170.25 | 5 | 4.25 | 0.73 | |

[†] Moisture, [‡] test weight, [§] per cent root lodging, [¶] per cent stalk lodging, [#] Lan refers Lancaster, SS refers Stiff stalk, [††] best performing check of the trial, [‡‡] mean of five checks, [§§] Experimental mean of 64 entries; hybrids were selected based on a combination of higher yield, lower grain moisture, higher test weight, lower per cent root and stalk lodging, and higher grain quality traits

Table 4.8. Heterotic patterns of selected hybrids, derived by crossing between NDSU and first set of ex-PVP inbreds, from combined analysis across six environments (2011: Casselton, Prosper; 2012: Casselton, Prosper, Fargo, Barney), of trial I

| Hybrids | Yield (Mg ha$^{-1}$) | MSTR[†] (g Kg$^{-1}$) | TWT[‡] (Kg hL$^{-1}$) | PRL[§] (%) | PSL[¶] (%) | Protein (g Kg$^{-1}$) | Oil (g Kg$^{-1}$) | Starch (g Kg$^{-1}$) | Heterotic Pattern[#] |
|---|---|---|---|---|---|---|---|---|---|
| ND2002 x CR1Ht | 6.26 | 186 | 64.73 | 6.46 | 1.83 | 108 | 42.63 | 697 | SS x Lan |
| ND2002 x PH207 | 6.02 | 193 | 63.01 | 6.98 | 7.43 | 104 | 37.91 | 708 | SS x Iodent |
| ND2002 x DKFAPW | 5.87 | 205 | 62.32 | 5.94 | 8.24 | 100 | 37.28 | 707 | SS x SS |
| ND2010 x PH207 | 5.78 | 216 | 60.46 | 2.05 | 6.09 | 102 | 42.15 | 697 | SS x non-SS |
| ND2011 x PH207 | 5.64 | 202 | 68.32 | 1.06 | 2.88 | 101 | 44.97 | 701 | SS x non-SS |
| ND2002 x LH54 | 5.63 | 233 | 60.55 | 1.21 | 7.76 | 101 | 39.77 | 708 | SS x Lan |
| ND2003 x LH54 | 5.54 | 234 | 58.81 | 0.61 | 4.43 | 103 | 43.00 | 700 | SS x Lan |
| ND291 x DK78010 | 5.48 | 208 | 66.32 | 3.17 | 6.81 | 106 | 39.17 | 702 | SS/non-SS x SS |
| ND2004 x NK807 | 5.46 | 205 | 65.15 | 1.75 | 7.06 | 95 | 37.73 | 714 | SS x Lan |
| ND2004 x NK779 | 5.45 | 254 | 60.53 | 3.11 | 4.00 | 110 | 41.34 | 695 | SS x non-SS |
| ND2010 x NK807 | 5.45 | 178 | 65.49 | 2.12 | 7.01 | 96 | 40.80 | 708 | SS x Lan |
| ND2005 x NK779 | 5.42 | 195 | 63.39 | 2.01 | 2.84 | 111 | 42.34 | 694 | SS x non-SS |
| Pioneer 38N88[††] | 6.50 | 204 | 65.33 | 0.00 | 4.89 | 100 | 42.31 | 704 | |
| Checks mean[‡‡] | 6.04 | 182 | 63.94 | 0.47 | 5.50 | 100 | 40.84 | 705 | |
| Exp. Mean[§§] | 4.53 | 207 | 60.94 | 3.93 | 8.18 | 107 | 42.59 | 698 | |
| EMS | 1.84 | 807 | 46.18 | 65.15 | 82.55 | 32 | 3.08 | 31 | |
| LSD, 0.05 | 1.10 | 23 | 5.50 | 6.54 | 7.36 | 5 | 1.42 | 5 | |
| CV,% | 29.95 | 14 | 11.15 | 205.29 | 111.03 | 5 | 4.12 | 1 | |

[†] Moisture, [‡] test weight, [§] percent root lodging, [¶] percent stalk lodging, [#] Lan refers Lancaster, SS refers Stiff stalk, [††] best performing check of the trial, [‡‡] mean of five checks, [§§] Experimental mean of 121 entries; hybrids were selected based on a combination of higher yield, lower grain moisture, higher test weight, lower per cent root and stalk lodging, higher grain quality traits

Table 4.9. Heterotic patterns of selected hybrids, derived by crossing between NDSU lines and second set of ex-PVP inbreds, from combined analysis across six environments (2011: Casselton, Prosper; 2012: Casselton, Proper, Fargo, Barney), of trial II

| Hybrids | YIELD (Mg ha $^{-1}$) | MSTR[†] (g Kg $^{-1}$) | TWT[‡] (Kg hL $^{-1}$) | PRL[§] (%) | PSL[¶] (%) | Protein (g Kg $^{-1}$) | Oil (g Kg $^{-1}$) | Starch (g Kg $^{-1}$) | Heterotic Pattern |
|---|---|---|---|---|---|---|---|---|---|
| ND2002 x PHP02 | 6.64 | 205 | 69.19 | 8.05 | 5.09 | 96 | 37.12 | 716 | SS x non-SS |
| ND2000 x S8324 | 6.45 | 176 | 68.47 | 6.76 | 10.79 | 106 | 37.41 | 707 | SS/non-SS x SS |
| ND2010 x PHP02 | 6.44 | 219 | 64.41 | 2.25 | 3.61 | 104 | 40.27 | 702 | SS x Non-SS |
| ND2003 x PHK76 | 6.36 | 205 | 72.07 | 1.50 | 8.01 | 105 | 38.04 | 704 | SS x non-SS |
| ND2003 x S8324 | 6.32 | 201 | 68.22 | 0.75 | 5.46 | 105 | 41.34 | 694 | SS x SS |
| ND2002 x L127 | 6.31 | 225 | 69.95 | 4.64 | 7.00 | 99 | 39.58 | 709 | SS x non-SS |
| ND2003 x PHT77 | 6.30 | 223 | 68.65 | 0.00 | 2.77 | 99 | 35.96 | 712 | SS x Lan |
| ND2011 x PHK76 | 6.29 | 190 | 75.84 | 3.61 | 10.36 | 95 | 39.38 | 713 | non-SS x non-SS |
| ND2002 x S8324 | 6.27 | 229 | 67.07 | 2.43 | 4.07 | 97 | 37.28 | 713 | SS x SS |
| ND2011 x OQ603 | 6.25 | 217 | 71.29 | 0.32 | 5.49 | 100 | 42.11 | 706 | non-SS x non-SS |
| ND2011 x L127 | 6.12 | 195 | 73.01 | 2.57 | 4.20 | 97 | 41.38 | 709 | non-SS x non-SS |
| ND2011 x PHT77 | 5.83 | 178 | 73.84 | 1.50 | 3.48 | 102 | 38.00 | 709 | non-SS x Lan |
| ND2011 x PHR25 | 5.77 | 172 | 70.73 | 2.24 | 0.00 | 100 | 43.56 | 703 | non-SS x non-SS |
| ND2003 x PHJ40 | 5.44 | 168 | 69.10 | 0.00 | 5.61 | 108 | 36.82 | 704 | SS x SS |
| | | | | | | | | | |
| Pioneer 38N88[††] | 6.92 | 160 | 69.61 | 1.16 | 1.36 | 99 | 40.20 | 707 | |
| Checks mean[‡‡] | 6.43 | 174 | 69.29 | 0.79 | 16.22 | 101 | 38.99 | 707 | |
| Exp. Mean[§§] | 5.13 | 201 | 69.64 | 5.00 | 10.84 | 104 | 39.68 | 704 | |
| MSE | 1.87 | 243 | 12.66 | 412.68 | 801.36 | 59 | 4.39 | 60 | |
| LSD, 0.05 | 1.21 | 14 | 3.16 | 18.02 | 25.12 | 7 | 1.86 | 7 | |
| CV%, | 26.67 | 8 | 5.11 | 406.14 | 261.19 | 7 | 5.28 | 1 | |

[†] Moisture, [‡] test weight, [§] percent root lodging, [¶] percent stalk lodging, [#] Lan refers Lancaster, SS refers Stiff stalk, [††] best performing check of the trial, [‡‡] mean of five checks, [§§] Experimental mean of 81 entries; hybrids were selected based on a combination of higher yield, lower grain moisture, higher test weight, lower per cent root and stalk lodging, higher grain quality traits

# CHAPTER V: GENERAL CONCLUSIONS

Our findings suggest that both additive and dominance gene action were important in regulating grain yield and related traits, with a preponderance of additive genetic variance in this selective set of northern U.S. corn hybrids. Similar gene action was also reported for grain quality and nutritional traits. Few ex-PVP inbreds with higher breeding values could be used as potential parents, but further testing is needed to confirm their potential usefulness. Our trials indicated that ex-PVP material is not directly useful in immediate commercial hybrids. It is clear from our results that most, if not all, ex-PVP lines need breeding work. Pre-breeding efforts could complement local development efforts. Our short-season maize samples were categorized as SS, non-SS, Lancaster, and Iodent; some of them belong to both the SS and non-SS heterotic groups, because the same inbreds showed above average combining ability with testers from different backgrounds. Heterotic groups are conceptual (Hallauer and Carena 2009), and often are not straight forward. A specific line from one heterotic group may fall in to another heterotic group based on a particular hybrid combination. Pedigree information is a very good reference in such situations. Heterotic groups and patterns are useful to exploit and select inbred parents in hybrid combinations. We found SS x non-SS heterotic patterns were most frequent in this set of genotypes. Mikel and Dubley (2006) also described SS x non-SS as one of most prevalent heterotic patterns in the U.S. Our observed Iodent x Lancaster pattern in our trials could be considered as a useful heterotic pattern too. We should not discard alternative combinations not tested before within and among company genotypes. Top ex-PVP lines could be inter-mated to develop new populations to maximize genetic improvement before considering new inbred line development with these materials. Farmers still do not have access to potentially better hybrids. They are missing for the northern U.S. Corn Belt due to a lack of access to the protected

materials. Rethinking breeding right access could be a timely approach to provide improved hybrids as maize is expands north and west in short-season regions of U.S. and Canada.

## References

Hallauer AR, Carena MJ (2009) Maize Breeding. In: Carena MJ (ed) Handbook of Plant Breeding: Cereals. *Springer*, New York, pp 3-98

Mikel MA, Dubley JW (2006) Evolution of North American dent corn from public to proprietary germplasm. Crop Sci. 46:1193-1205