# ADAPTATION OF QUALITY PROTEIN MAIZE (*ZEA MAYS* L.) TO NORTHERN U.S. CORN BELT

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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

There is a need to increase the value of crops and develop the next generation of healthier products. Quality protein maize (*Zea mays* L.) is an option but has never been adapted to short – season environments. Quality protein maize (QPM) with homozygous embryo and endosperm for mutant allele *o2* at the α-zeins regulatory gene *opaque-2* shows about 60 to 100% increase in lysine and tryptophan essential amino acids when comparing with non-QPM maize. The objectives of this research were to adapt QPM genotypes to the northern U.S. through the NDSU Early QPM Program, and to evaluate the agronomic potential of early generation QPM lines and hybrids developed by the NDSU maize breeding program for the northern USA. Fifty-four inbred lines, including 47 QPM donor lines from the Iowa State University (ISU) maize breeding program, six experimental lines from the NDSU maize breeding program and one ex-PVP line from industry, were selected to produce 94 early-QPM backcross populations. Based on the earliness, protein content, and amino acid levels of lysine, 218 $BC_1S_2$ lines were selected for testcrosses with industry testers. Experiments evaluating testcrosses were arranged in 12 x 12 and 10 x 10 partially balanced lattice designs across three environments in 2013 and 2014. Based on this evaluation, totally 48 $S_2$ lines were selected for further development, 17 of them representing the Stiff Stalk (SS) heterotic group and 31 representing the non-SS-group. Selected lines provided unique advanced inbred lines with hybrid combinations showing not only above average grain yield, dry down, and protein content but also, high levels of lysine, tryptophan, and methionine. The results of this research show, for the first time, the successful adaptation of QPM genotypes to short-season environments. The NDSU maize-breeding program has developed the first high quality maize products through the EarlyQPM and EarlyQPMF (for feedstock) national programs.

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## DEDICATION

I would like to dedicate this research dissertation to my wife, Hao Li, and my daughter,

Emma Z. Dong.

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# INTRODUCTION

Maize (*Zea mays* L.) is an important crop for food, animal feed, and industrial raw materials. Maize is also the leading world cereal in both total yield (720 million tons) and in per unit area yield (FAO, 2009). As a C4 plant, maize is more efficient using solar radiation than other cereals. With such a significant yield potential, it is known as the "Queen of Cereals". The progenitor of maize has been identified as a form of teosinte (*Zea mays ssp. parviglumis*) by different studies utilizing molecular techniques (Doebley, 2004). Maize also has an extensive geographical distribution, and could be planted from the equator to above 50° on both hemispheres, which is well beyond the tropical and subtropical areas, including extreme conditions. The top maize producing country is the United States of America (U.S.), and then followed China, Mexico, Brazil and Argentina (USDA, ERS, 2015).

The U.S. is the largest maize producer with approximately 32 million hectares planted to maize. Approximately 20 percent of the maize being exported to other countries makes U.S. the major player in the world trade market. Maize is also the primary U.S. feed grain, approximately 43 % of total maize production was used as animal feed, accounting for more than 90 percent of total feed grain production and use (Figure 1.1). Maize is planted in most of U.S. states, but heartland region including Illinois, Iowa, Indiana, eastern South Dakota and Nebraska, western Kentucky and Ohio, and the northern two-thirds of Missouri are the main production area. Among those states, Iowa and Illinois are the top maize-producing states, typically account for one-third of the U.S. crop (USDA, ERS, 2015)

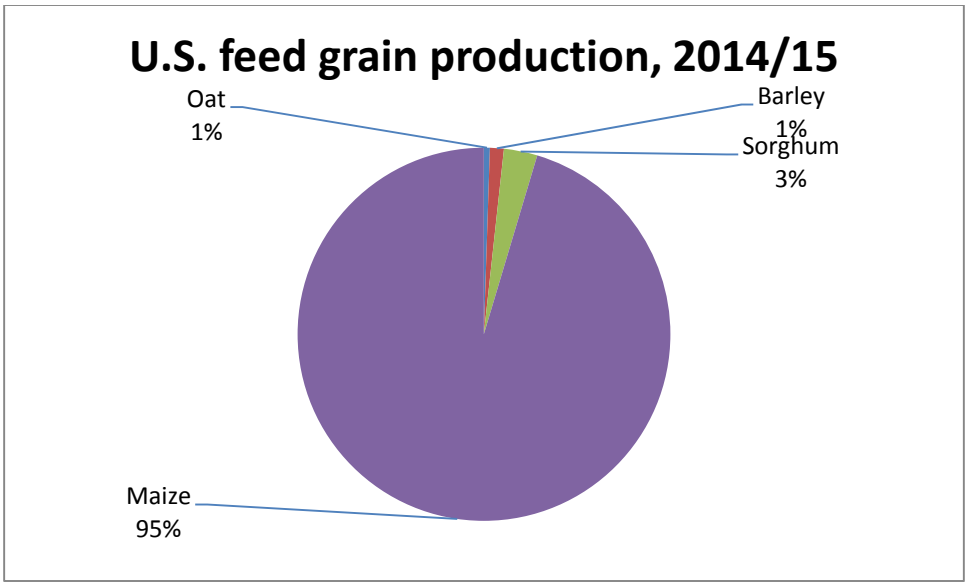


Figure 1.1. U.S. feed grain production during 2014 – 2015 season. (USDA, ERS, 2015)

Globally, approximately 15 % of the total protein and 20 % of the total calories derived from food crops came from maize (National Research Council, 1988). However, the common maize currently lacks the full range of amino acids, especially essential ones like lysine and tryptophan, which human and other animals (e.g. pig and chick) cannot synthesize by their own metabolic systems and playing an important role in synthesizing protein completely.

Quality protein maize (QPM) with homozygous recessive *o2* gene shows about 60 to 100% increase in lysine and tryptophan. Because of the increasing content of these two essential amino acids, digestibility and nitrogen uptake are also increased compared to normal maize, and therefore, the biological value of QPM is about 80%, whereas that of regular maize is 40 to 57% (Bressani, 1992).

The content and quality of protein in maize has been increased due to genetic improvement. However, adaptation of QPM genotypes to various environments has been challenging because of the complex genetic background of QPM. One of the long-term goals of

the NDSU maize-breeding program is to develop the next generation of short-season healthier products through the NDSU EarlyQPM and NDSU EarlyQPMF national programs.

In our research, 47 QPM lines were crossed with NDSU early-maturing lines and hybrids to produce NDSU early-maturing QPM lines adapted to the northern USA. The NDSU early-maturing QPM lines will meet the environmental requirements of northern U.S. and Canada for high quality of protein. Meanwhile, the research is designed to move U.S. Corn Belt dent QPM unique germplasm northward. This research is also the first to assess the incorporation of exotic QPM germplasm as the donor for high protein quality with the target to enhance the nutrition and healthy diet in the northern U.S. Corn Belt.

The specific objectives of this research are to:

1) Adapt QPM genotypes to the northern U.S. through the NDSU Early QPM Program;

2) Determine if derived QPM lines are new competitive sources of short-season elite and healthier hybrids

# LITERATURE REVIEW

## Need of Quality Protein Maize(QPM)

Improving nutritional quality of agricultural crops for a healthy diet is a global goal. This is particularly important in cereal crops since the benefits can be applied to millions of people in a rapid, efficient and effective manner without changing traditional food habits.

Maize (*Zea mays* L.) is planted on more than 177 million hectares all over the world (FAOSTAT, 2015), and hundreds of millions of people in developing countries are dependent on maize as a main food. Maize provides 15 to 56% of the total daily calories of people in about 25 developing countries mainly in Africa (Prasanna et al., 2001). As estimated by FAO food balance sheets (Krivanek et al., 2007), in Africa, 20 % of the total calories and 17 to 60% of people's total daily protein is supplied by maize. These values were estimated only based on average per capita, so specific groups, like infants, are even more dependent on maize within these countries. Maize is their major source of dietary protein and studies already proved there is direct relationship between low protein quality of maize and children and adults becoming sick. For countries where maize is the primary food, rapid growth rate of children does need protein sufficient foods (Millward and River, 1989).

Maize grain with poor amino acid balance is well known and the need for improving its nutritional value was acknowledged long time ago (Osborne and Mendel, 1914). Dependence of maize as a primary protein source puts people at risk for dietary protein deficiency since maize proteins, like most of the cereal crops proteins, lack two essential amino acids, lysine and tryptophan. These amino acids cannot be synthesized by the metabolism of monogastric animals (e.g., pigs and chickens) and humans to complete protein synthesis. Protein deficiency, especially in children, causes ‘kwasshiorkor’, a potentially fatal syndrome characterized by

initial growth failure, irritability, skin lesions, edema, and fatty liver. So for people who rely heavily on maize as their main food, maize cultivars with improved amino acid profile are necessary.

**Development of QPM**

In the maize grain, both the endosperm and embryo contain protein but the embryo protein is superior in quality. However, since the endosperm constitutes the bulk of the grain, it is estimated it may contribute approximately 80 % of the total kernel protein (Zuber and Helm, 1975). The bulk of maize endosperm protein is comprised of zein which is composed of a class of alcohol soluble proteins that are specific to the maize endosperm (Prassana et al., 2001). The zeins contain one major class (α-zeins) and three minor classes (β, γ, δ-zeins). Zeins are the most abundant proteins in the grain endosperm and, particularly, the α-zein (Fraction II in Table 2.2), which are poor in lysine and tryptophan (Gibbon and Larkins, 2005).The ideal approach to improve the nutrition value of maize grain could be suppression of lysine-deficient zein fraction without changing the contribution of other fractions (Vasal, 2002). Decreasing the zein protein proportion causes an elevation of non-zein protein fractions rich in lysine and tryptophan.

Improving the nutritional quality of the maize endosperm protein can be dated back to 1960s. Several natural maize mutants containing higher lysine and tryptophan were identified during the 1960s and 1970s: *opaque-2 (o2), floury-2 (fl2), opaque-7 (o7), opaque-6 (o6)*, and *floury-3 (fl3)* (Table 2.1).

Table 2.1. High lysine/tryptophan maize mutants

| Mutant gene | Allele | Researchers | Year of discovery |
|---|---|---|---|
| opaque-2 | *o2* | Mertz, Bates and Nelson | 1964 |
| floury-2 | *fl2* | Nelson, Mertz and Bates | 1965 |
| opaque-7 | *o7* | McWhirter | 1971 |
| opaque-6 | *o6* | Ma and Nelson | 1975 |
| floury-3 | *fl2* | Ma and Nelson | 1975 |

Table 2.2. Protein fraction distribution of endosperm samples of normal and soft maize endosperm (*o2*)

| Protein fraction | | Percentage of total protein(g/100 g protein) | |
|---|---|---|---|
| Number | Name | Tuxpeno-1 normal endosperm | Tuxpeno-*o2* soft endosperm |
| I | Albumins, globulins and soluble nitrogen | 6.6 | 17.0 |
| II | Zeins (alpha, beta, delta, gamma) | 48.7 | 9.7 |
| III | Zein like | 14.0 | 13.4 |
| IV | Glutelin like | 9.2 | 17.2 |
| V | Glutelin | 17.0 | 34.5 |
| | Residue | 4.5 | 8.1 |

Source: Cited by Bjarnason and Vasal (1992)

Among all these protein maize mutants, the *o2* mutation was the first discovered and identified in a maize field located in Connecticut, USA (Vietmeyer, 2000). It was also proved to be the most appropriate genetic material for breeding programs aimed to develop high quality maize. At the beginning stage of developing QPM, both *o2* and *fl2* genes were used separately or together. However, the utilization of *fl2* was eventually discontinued (Bjarnason and Vasal, 1992; Vasal, 2000; Vasal, 2001).

Although *o*2 was the main gene used for increasing the levels of lysine and tryptophan in the maize endosperm protein, undesirable effects associated with the *o2* gene were identified. Still, major emphasis was placed on utilization of the *o2* mutant (NRC, 1988; Glover, 1992; Villegas et al., 1992). Maize grain with homozygous recessive *o2* (*o2o2*) was shown to have higher lysine and tryptophan levels than maize grain with heterozygous *o2* (*O2o2*) or

homozygous dominant (*O2O2*) at the opaque-2 locus (Crow and Kermicle, 2002). Bressani (1992) showed that by increasing the level of lysine and tryptophan in the maize endosperm, the biological value of maize protein was doubled. The different amount of protein content between high lysine and common maize was approximate 10 %. Therefore, to achieve the ideal amino acid level, people would need to approximately consume more than two times common maize (FAO, 1992).

After learning the nutritional benefits of the *o2* mutation, maize breeding programs all over the world started transforming their normal endosperm inbred lines and populations to *o2* versions by applying direct backcross approach (Gevers, 1995; Prasanna et al., 2001). However, severely negative pleiotropic effects associated with *o2* allele challenged its the practical utilization when direct transform was involved in breeding programs (Bjarnason and Vasal, 1992; Prasanna et al., 2001). These included, compared to common maize, reduction of grain yield, low grain density, greater susceptibility to ear rot, slow rate of grain dry-down, soft and chalky kernel phenotype vulnerable to insect attack and fungal infection; which resulted in reduced germination rate and greater kernel breakage (Vasal et al., 1984a; Bjarnason and Vasal, 1992; Glover, 1992; Villegas et al., 1992; Moro et al., 1995; Lin et al., 1997; Prasanna et al.,2001; Vasal, 2001). In the food market, where most of consumers and producers preferred maize products hard endosperm type, soft endosperm texture is not acceptable (Krivanek et al., 2007). As a consequence, most of the breeding program gave up the pactical use of *o2* mutation in the field.

In order to overcome the pleiotropic effects of *o2* gene, specific selection strategy for hard endosperm was emphasized within *o2* breeding processes. Many reports showed challenges in breeding QPM processes with inheritance of *o2* gene (Bjarnason and Vasal, 1992; Lopes and

Larkins, 1996). QPM breeding programs in CIMMYT were initially focused on converting normal endosperm populations from tropical and subtropical lowland areas to *o2* versions by using backcross-cum-recurrent selection methodology (Vasal et al., 1980, 1984). This modified backcross method (Figure 1.) focused on accumulating hard endosperm types while maintaining protein quality and quantity (NRC, 1988; Bjarnason and Vasal, 1992; Villegas et al., 1992; Prasanna et al., 2001; Vasal, 2001).

When working with *o*2 maize it was essential to overcome not only the negative pleiotropic effects to improve the endosperm protein quality but also to improve the negative agronomic characteristics at the same time. Several research groups had independently succeeded in developing *o2*/*o2* germplasm with acceptable agronomic characteristics, and had designated those as Quality Protein Maize (QPM) (Gevers and Lake, 1992; Prasanna et al., 2001; Vasal et al., 2002). The term QPM now is referred to maize with the homozygous recessive *o2* alleles, high levels of lysine and tryptophan without the negative pleiotropic effects, and hard endosperm (Vasal, 2001). Modern QPM's appearance and performance is now similar to regular maize without the mutation and can only be reliably differentiated through laboratory tests (Villegas et al., 1992). In addition, QPM is non-GMO, and all the breeding process does not involve genetic engineering (Pixley and Bjarnason, 1993).

In the process of developing QPM, the role of CIMMYT was essential. QPM populations developed by CIMMYT were largely used directly as open pollinated varieties (OPV's) or single plants were selected from these genetically broad-based populations to develop new inbred lines for hybrid production (Vasal et al., 1980; 1984b; Villegas et al., 1992). Since then, adapted (tropical and subtropical genotypes) QPM populations, inbreds, and hybrids were widely used all

over the world for developing high-lysine/tryptophan maize (Bjarnason and Vasal, 1992; Villegas et al., 1992; Vasal, 2001).

In order to fulfill the increasing needs, especially for developing countries, QPM hybrid programs at CIMMYT were founded in 1985(Bjarnason and Vasal, 1992; Vasal et al., 1993b; Vasal, 2001). Several advantages were identified in QPM hybrids over the OPV's: i) increased grain yield performance through hybrids' heterosis effect; ii) relatively easier to purify and maintenance of inbreds with respect to agronomic traits, genetic modifiers and protein quality; iii) decrease the cost on laboratory facilities for monitoring the protein quality of inbreds; iv) uniformity and stability of the final hybrid products with respect to kernel modification; v) involvement of the private seed industry in the QPM adaptation and utilization (Gevers and Lake, 1992; Pixley and Bjarnason, 1993; Vasal et al., 1993a; 1993b; CIMMYT, 2000; Vasal, 2001; Hadji, 2004). Therefore, developing inbred lines and hybrid products were becoming the priority of CIMMYT (Bhatnagar et al., 2004; Hadji, 2004; Xingming et al., 2004).

University of Kwazulu-Natal, South Africa and the Crow's Hybrid Seed Company at Milford, Illinois, USA were the institutions that continued to improve the protein quality of maize other than CIMMYT (Vasal, 2000; Prasanna et al., 2001). The maize-breeding programs in South Africa put great efforts to the development of QPM and had developed high-lysine maize inbred lines, hybrids, and OPVs with excellent agronomic and quality performance (Gevers and Lake, 1992; Hohls et al., 1996; Bhatnagar et al., 2004). The *o2* hybrid with good yield and quality for animal feed developed from Crow's Hybrid Seed Company was reported (Mertz, 1995). Another company in the USA, Texas A&M, had successfully adapted QPM to southern USA, and inbreds and hybrids products with hard endosperm, competitive yield and high protein quality were reported (Betran et al., 2003a; b; c). With continuous efforts, the

geographical distribution of QPM was extending. Cultivars (both OPV's and hybrids) with improved protein quality have been adapted to various environmental conditions like temperate, tropical highland, and subtropical and tropical lowland. However, no research was conducted to develop short-season QPM genotypes.

**Genetics of QPM**

Generally, high lysine and tryptophan maize (QPM) involves three distinct genetic systems (Vasal, 2002; Vivek et al, 2008): 1)*o2* gene in a homozygous recessive state; 2) modifiers/enhancers of *o2o2*-containing endosperm to maintain higher lysine and tryptophan; 3) genes that modify the endosperm type of *o2o2* genotypes from soft to hard.

Study revealed that the possible function of *o2* gene was encoding a transcription factor involved in zein synthesis (Schmidt et al., 1990). The *o2* gene that in homozygous recessive state causes a decrease synthesis of the α-zein fraction of the endosperm protein and a corresponding increase in the proportion of non-zein proteins (Vivek et al., 2008). These non-zein proteins naturally have higher levels of lysine and tryptophan (Gibbon and Lakins, 2005). Therefore, in QPM, the proportion of non-zein is higher, which predisposes *o2* maize to have higher levels of lysine and tryptophan (Vasal, 1992). However, Vivek (2008) concluded that the recessive condition (*o2o2*) alone did not ensure the quality of maize protein as it just predisposed maize to have them.

The second essential genetic system, *o2* modifiers/enhancers, is needed. Across genetically diverse backgrounds, the levels of lysine range from 1.5 % to 2.8 % in common maize and 2.6 % to 5.0 % in *o2* maize (Moro et al., 1996). Therefore, if there are no modifiers/enhancers selected in the breeding process (e.g., if there is no monitoring during the breeding processes), the developed cultivars will end up with *o2o2* genotype and

lysine/tryptophan levels equivalent to those in common maize. This is because lower limits of lysine and tryptophan in *o2o2* maize overlapping the upper limits in normal maize (Vivek et al., 2008). Lysine and tryptophan levels are highly correlated (Hernandez and Bates, 1969; Vivek et al., 2008). Therefore, monitoring for either of the amino acids can be used for analyzing protein quality, to reduce laboratory costs (Krivanek et al., 2007; Vivek et al., 2008). Multi-genic effects controlling amino acid content have been reported (Wang et al., 2001; Wu et al., 2002). Eventually, it becomes apparent that the simple genetic nature of *o2* maize has been converted to a classic polygenic QPM system and must be manipulated as such in breeding programs.

Previous study shown (Wallace et al., 1990) that the *o2*-modified (hard endosperm) grains contained approximately twice amount of γ-zein compared to the *o2*-original mutants, which implied an increased level of γ-zein proteins likely put added value to the recovery of a hard endosperm from a soft endosperm phenotype. These modifying loci that control γ-zein production can easily be selected by the light-table method (Vivek et al., 2008).

**Methods to develop QPM**

By utilizing recurrent selection techniques to accumulate genetic modifiers in *o2* backgrounds (Lonnquist, 1964; Bjarnason and Vasal, 1992) and by recombining of superior hard endosperm *o2* families, CIMMYT had successfully developed new QPM cultivars, mainly for tropical and subtropical regions. These materials were similar in grain yield and other agronomic properties to common maize (Villegas et al., 1980; Ortega et al., 1991; Bjarnason and Vasal, 1992; Villegas et al., 1992) and used as QPM donor stocks and populations for further improvement (Vasal, 2000; Prasanna et al., 2001). The development of QPM donor stocks then led to QPM germplasm development in different genetic backgrounds using an innovative breeding procedure, termed as “modified backcrossing-cum-recurrent selection” (Figure 2.1).

Consequently, a substantial amount of QPM populations and pools possessing different ecological adaptation, maturity, grain color and texture were developed (Vasal et al., 1984b; Vasal, 2001).

Normal maize genotypes that can resist major biotic and abiotic stresses of the region were converted to QPM. For instance, considerable efforts were put in the formation of maize streak virus resistant varieties by converting resistant genotypes (Vivek et al, 2008). Pedigree breeding is commonly used, whereby the best performing inbred lines, complementary in different traits, are crossed to establish new segregating families. Three types of crosses provided a choice of breeding strategies (Krivanek et al., 2007): QPM x QPM, QPM x normal, and QPM x normal backcross conversion. Within each of these segregating populations, successive inbreeding of the material was made to develop new inbred lines while keeping continual selection on the three important QPM genetic systems (recessive mutant allele of *o2*, endosperm modifiers, and amino acid modification).

The history to develop QPM germplasm is relatively short. Most breeding programs considered starting with conventional approaches by converting their non-QPM inbred lines to QPM versions through backcrossing or pedigree crosses within elite non-QPM germplasm and elite QPM donors. An alternative or supplementary approach could be the utilization of molecular markers to assist in the selection of *o2* genotypes. No matter which basic approach is chosen, the following are two unique and essential steps involved in developing QPM germplasm (Vivek et al, 2008): 1) Identification of homozygous recessive (*o2o2*) condition and hard endorsperm simultaneously through F2 to F5 generation in a family or population; 2) Confirmation of QPM quality and quantity through the process.

To identify both homozygous recessives and hard endosperm, Vivek (2008) suggested the use of the light table to help in the selection of genotypes. Several studies also reported the use of marker-assisted selection (MAS) to help selection (Dreher et al., 2003; Babu et al., 2005; Krivanek et al.,2007). However, the use of MAS has shown many challenges, among them, cost and lack of efficient markers for indirect selection for the traits of interest. For conventional breeding, Vivek (2008) reported the use of QPM inbred lines as *o2* donor parents and non-QPM elite lines as recurrent parents (Figure 2.1). The time to convert non-QPM lines is estimated at 4.5 years if two seasons per year are possible. Less time would be possible with winter nurseries offering more than one season per year, especially if short-season maize genotypes are available.

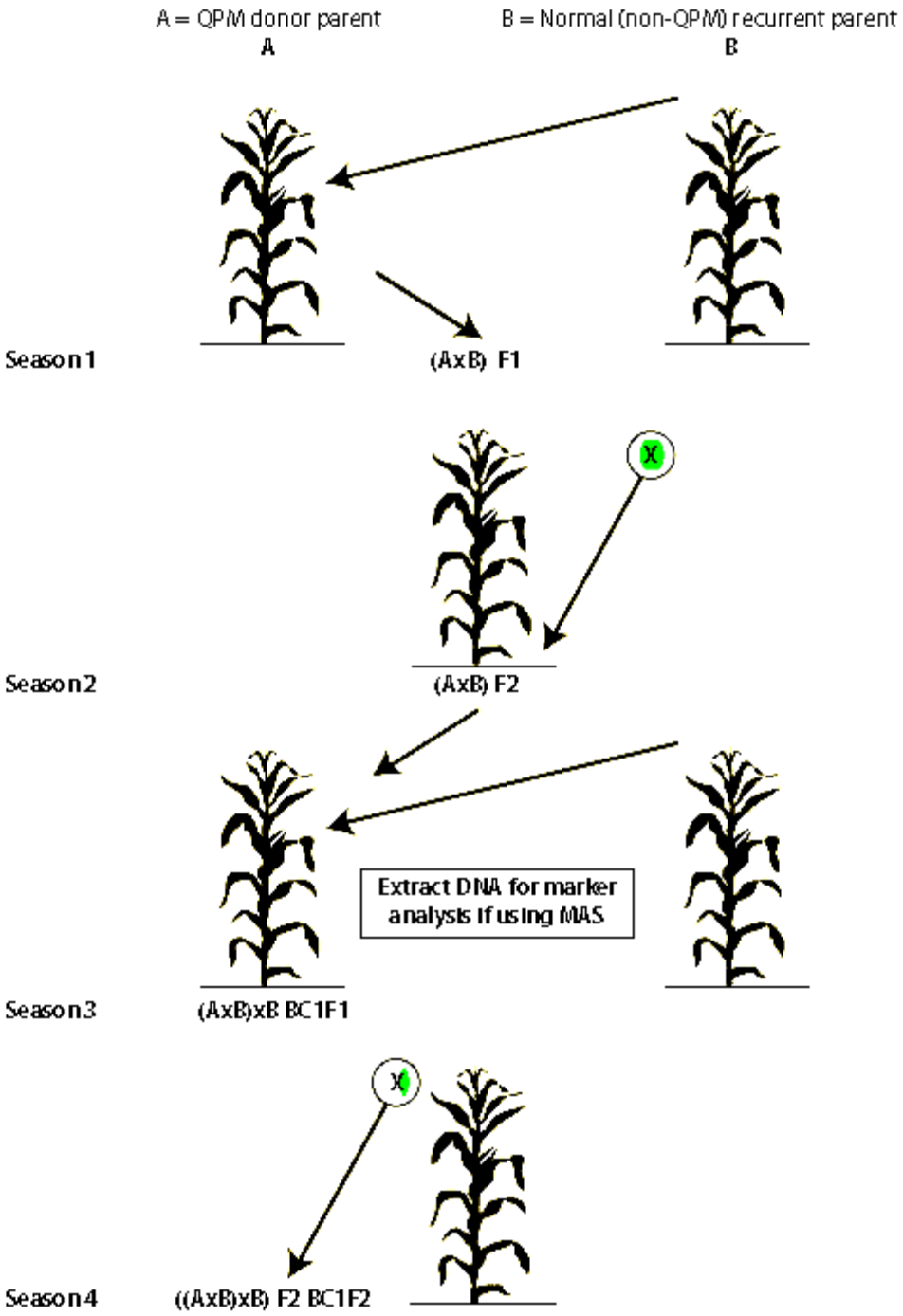


Figure 2.1. Breeding methods used to develop QPM
Source: Vivek B.S., A.F. Krivanek, N. Palacios-Rojas, S. Twumasi-Afriyie, and A.O. Diallo. 2008. *Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars*. Mexico, D.F.: CIMMYT

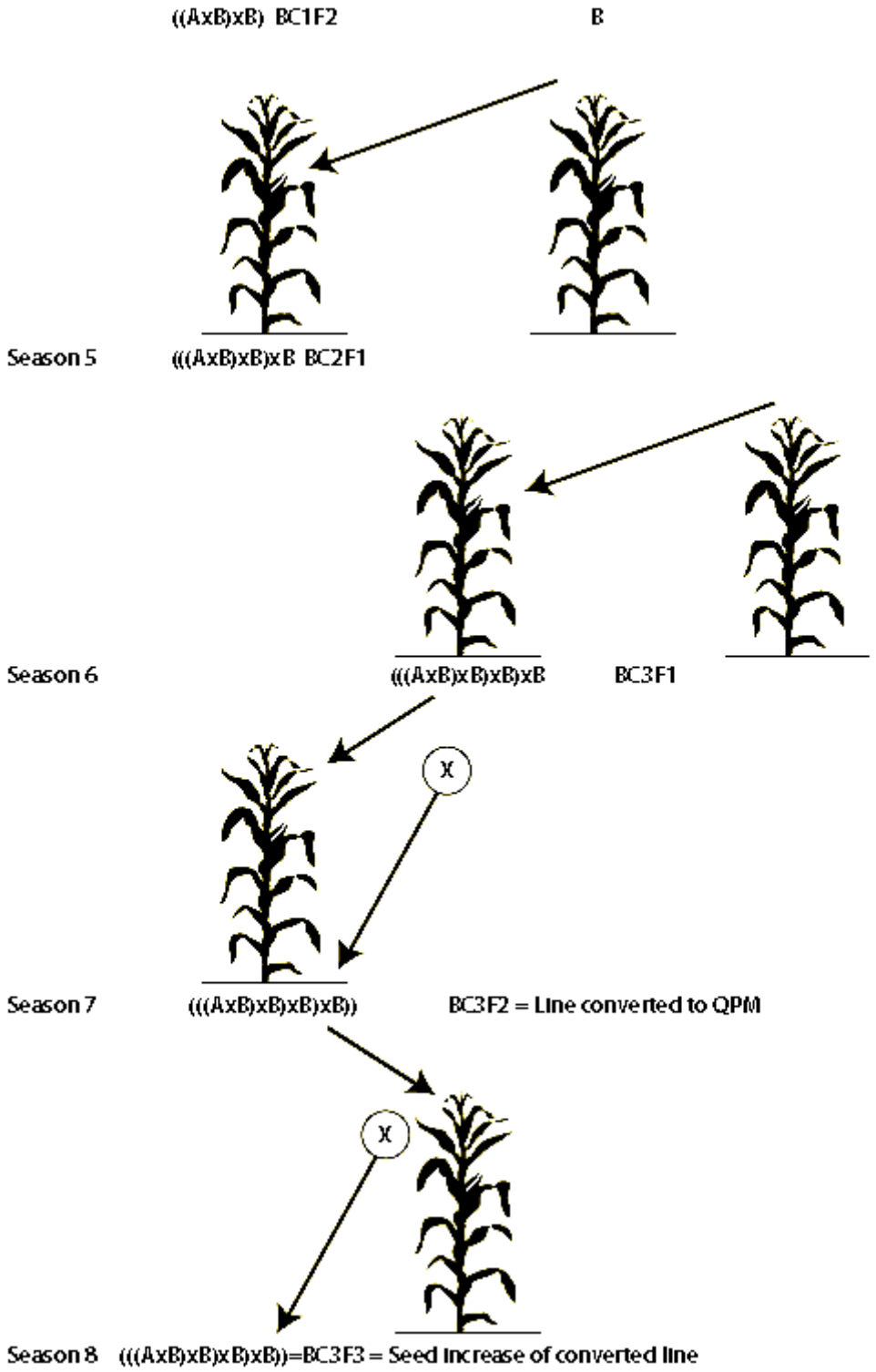


Figure 2.1. Breeding methods used to develop QPM (continued)
Source: Vivek B.S., A.F. Krivanek, N. Palacios-Rojas, S. Twumasi-Afriyie, and A.O. Diallo. 2008. *Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars*. Mexico, D.F.: CIMMYT

**Utility of QPM**

Maize with high lysine and tryptophan has been applied to feeding studies in both animals and humans.

Pigs fed with a high lysine/tryptophan maize diet gained weight roughly at twice the rate of pigs fed only on a common maize diet without additional protein supplements. An equal amount of QPM substituted for normal maize in pig feed diets can maintain the amino acid balance and decrease the use of synthetic lysine (Burgoon et al., 1992)


Figure 2.2. Pig fed high lysine/tryptophan maize (larger animal labeled QPM or Q4) compared with its sibling fed normal maize (labeled normal or N4)
Source: Crops Research Institute, Kumasi, Ghana; Animal Science Department, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, and Sasakawa Global 2000

Human nutrition studies found that: children fed with QPM had less sick days, a better chance to escape death from diarrhea and other infections, reduced stunting and better growth-enhancing capabilities than those fed with normal maize (Akuamoa-Boateng, 2002).

The main goal of our research was to develop the next generation of healthier products by adapting QPM genotypes to the northern U.S. through the NDSU Early QPM Program

## MATERIALS AND METHODS

### Materials

Fifty-three inbred lines, including 47 QPM donor lines from the ISU maize breeding program, 5 experimental lines from the NDSU maize breeding program and one ex-PVP line from industry , were selected to produce 94 elite x elite single-cross hybrids. The target was to develop NDSU short-season-QPM lines. NDSU lines developed from improved genetically broad-based populations with above average grain quality performance and earliness. QPM donor lines were originally developed at CIMMYT and adapted to central U.S. Corn Belt conditions by crossing CIMMYT genetic materials with elite Iowa lines (Tables 3.1 and 3.2). Elite and current industry testers were used in this study representing Stiff Stalk (SS) and non-SS heterotic groups: were T1 (SS type), and T2 (non-SS type).

Table 3.1. Genetic background of QPM maize lines utilized in this study

| Inbred | Recurrent Parent | Source of QPM† | Origin of Iowa maize lines |
|---|---|---|---|
| QPM 1 | B91 | CLQ06901 | |
| QPM 2 | B91 | CLQ06901 | Iowa Corn Borer Synthetic No. 1(R)C8 |
| QPM 3 | B95 | CLQ06901 | |
| QPM 4 | B95 | CLQ06901 | Iowa Corn Borer Synthetic No. 1(R)C8 |
| QPM 5 | B95 | CLQ06901 | |
| QPM 6 | B97 | CLQ06901 | |
| QPM 7 | B97 | CLQ06901 | Iowa Corn Borer Synthetic No. 1(R)C9 |
| QPM 8 | B97 | CLQ06901 | |
| QPM 9 | B97 | CLQ06901 | |
| QPM 10 | B98 | CLQ06901 | |
| QPM 11 | B98 | CLQ06901 | |
| QPM 12 | B98 | CLQ06901 | BS11(FR)C5 |
| QPM 13 | B98 | CLQ06901 | |
| QPM 14 | B98 | CLQ06901 | |

Table 3.1. Genetic background of QPM maize lines utilized in this study (continued)

| Inbred | Recurrent Parent | Source of QPM | Origin of Iowa maize lines |
|---|---|---|---|
| QPM 15 | B98 | CLQ06901 | BS11(FR)C5 |
| QPM 16 | B98 | CLQ06901 | |
| QPM 17 | B98 | CLQ06901 | |
| QPM 18 | B99 | CLQ06901 | Iowa Corn Borer Synthetic No. 1(R)C10 |
| QPM 19 | B99 | CLQ06901 | |
| QPM 20 | B99 | CLQ06901 | |
| QPM 21 | B99 | CLRQ00502 | |
| QPM 22 | B99 | CLRQ00502 | |
| QPM 23 | B100 | CLQ06901 | B85 x H99 |
| QPM 24 | B100 | CLRQ00502 | |
| QPM 25 | B100 | CLRQ00502 | |
| QPM 26 | B113 | CLQ06901 | BS11(FR)9 |
| QPM 27 | B113 | CLQ06901 | |
| QPM 28 | B113 | CLQ06901 | |
| QPM 29 | B113 | CLQ06901 | |
| QPM 30 | B113 | CLQ06901 | |
| QPM 31 | B113 | CLQ06901 | |
| QPM 32 | B113 | CLQ06901 | |
| QPM 33 | B113 | CLQ06901 | |
| QPM 34 | B113 | CLQ06901 | |
| QPM 35 | B104 | CLQ06901 | BS13 (S)C5 |
| QPM 36 | B104 | CLQ06901 | |
| QPM 37 | B109 | CLRQ00502 | Recovered B73 |
| QPM 38 | B109 | CLRQ00502 | |
| QPM 39 | B110 | CLQ06901 | BS13(S)C5 |
| QPM 40 | B110 | CLQ06901 | |
| QPM 41 | B110 | CLQ06901 | |
| QPM 42 | B111 | CLQ06901 | BSSS(R)C9 |
| QPM 43 | B111 | CLQ06901 | |
| QPM 44 | B114 | CLQ06901 | Pool 41, developed by CIMMYT |
| QPM 45 | B114 | CLQ06901 | |
| QPM 46 | B114 | CLQ06901 | |
| QPM 47 | B114 | CLRQ00502 | |

†:CLQ06901 is a direct derivative of QPM population 69 (Templado Amarillo QPM). It has intermediate maturity and a yellow flint grain type. CLRQ00502 is a recycled QPM line representing subtropical population 502. There is little genetic relationship between the two lines. Both of them were developed by CIMMYT

Table 3.2. Genetic background of non-QPM lines utilized in this study

| Inbred | Genetic background | Heterotic Group† | Source |
|---|---|---|---|
| ND2000 | [NDSCD(M)C8-3-2-1-1-1-1] | SS | NDSU |
| ND2006 | [NDSBF(LM)C7(HGR)C4-1-1-2-1-2] | SS | NDSU |
| ND06-36 | [(B73xMo17)]-87-1-1-1-1-1 derived] | SS | NDSU |
| ND05-73 | NDCG(FS)C0 | SS | NDSU |
| ND291 | [NDSM(M)C1-1-1-1-1-1-2 | Non-SS | NDSU |
| LH162 | Mo17, ND246 | Non-SS | Ex-PVP |

†: SS, Stiff stalk group; Non-SS, non-Stiff stalk group

**Methods**

Field Evaluation

*Mating design and field trials (Early QPM breeding scheme)*

All 53 lines were separated, based on genetic background, into three heterotic groups: stiff stalk group (SS), non-stiff stalk group (Non-SS), and unrelated group (UR). Early x late QPM crosses within heterotic groups were made in the 2009 New Zealand winter production nursery (north island). Backcrosses ($BC_1:S_0$) were produced between QPM crosses and elite early-maturing donors in the 2010 Fargo summer breeding nursery. Genotypes flowering 10 or more days later than the recurrent parent were discarded. $BC_1:S_1$ early-generation lines representing at least two heterotic groups were produced in the 2011 Fargo summer breeding nursery. $BC_1:S_2$ lines were selected and produced in the 2012 Fargo summer breeding nursery. Test-crosses of best early generation lines with industry testers representing the opposite heterotic group were made in the 2012 New Zealand winter nursery while screening of early generation lines for cold tolerance was made in the 2012 New Zealand winter production screening nursery (south island)

All single-cross hybrids made from selected $BC_1:S_2$ high quality lines and industry testers were evaluated in replicated field trials including 14 commercial checks and three NDSU experimental hybrids (Table 3.3 and 3.4).

Table 3.3. Genetic background and heterotic groups of maize backcrosses utilized in this study

| Female | | Male | Heterotic Group[†] |
|---|---|---|---|
| QPM 1 X ND291 | X | ND291 | Non-SS |
| QPM 2 X LH162 | X | LH162 | Non-SS |
| QPM 3 X ND291 | X | ND291 | Non-SS |
| QPM 4 X LH162 | X | LH162 | Non-SS |
| QPM 5 X LH162 | X | LH162 | Non-SS |
| QPM 6 X ND291 | X | ND291 | Non-SS |
| QPM 7 X ND291 | X | ND291 | Non-SS |
| QPM 8 X LH162 | X | LH162 | Non-SS |
| QPM 9 X LH162 | X | LH162 | Non-SS |
| QPM10 X ND291 | X | ND291 | Non-SS |
| QPM11 X ND291 | X | ND291 | Non-SS |
| QPM12 X ND291 | X | ND291 | Non-SS |
| QPM13 X ND291 | X | ND291 | Non-SS |
| QPM14 X LH162 | X | LH162 | Non-SS |
| QPM15 X LH162 | X | LH162 | Non-SS |
| QPM16 X LH162 | X | LH162 | Non-SS |
| QPM17 X LH162 | X | LH162 | Non-SS |
| QPM18 X ND291 | X | ND291 | Non-SS |
| QPM19 X LH162 | X | LH162 | Non-SS |
| QPM20 X LH162 | X | LH162 | Non-SS |
| QPM21 X ND291 | X | ND291 | Non-SS |
| QPM22 X LH162 | X | LH162 | Non-SS |
| QPM23 X LH162 | X | LH162 | Non-SS |
| QPM24 X LH162 | X | LH162 | Non-SS |
| QPM25 X ND291 | X | ND291 | Non-SS |
| QPM26 X ND291 | X | ND291 | Non-SS |
| QPM27 X ND291 | X | ND291 | Non-SS |
| QPM28 X ND291 | X | ND291 | Non-SS |
| QPM29 X ND291 | X | ND291 | Non-SS |
| QPM30 X LH162 | X | LH162 | Non-SS |
| QPM31 X LH162 | X | LH162 | Non-SS |

Table 3.3. Genetic background and heterotic groups of maize backcrosses utilized in this study (continued)

| Female | | Male | Heterotic Group |
|---|---|---|---|
| QPM32 X LH162 | X | LH162 | Non-SS |
| QPM33 X LH162 | X | LH162 | Non-SS |
| QPM34 X LH162 | X | LH162 | Non-SS |
| QPM35 X ND2000 | X | ND2000 | SS |
| QPM36 X ND2006 | X | ND2006 | SS |
| QPM37 X ND2000 | X | ND2000 | SS |
| QPM38 X ND2006 | X | ND2006 | SS |
| QPM39 X ND2000 | X | ND2000 | SS |
| QPM40 X ND2006 | X | ND2006 | SS |
| QPM41 X ND06-36 | X | ND06-36 | SS |
| QPM42 X ND05-73 | X | ND05-73 | SS |
| QPM43 X ND2006 | X | ND2006 | UR |
| QPM44 X ND2006 | X | ND2006 | UR |
| QPM45 X ND2000 | X | ND2000 | UR |
| QPM46 X ND291 | X | ND291 | UR |
| QPM47 X LH162 | X | LH162 | UR |

†: SS, Stiff stalk group; Non-SS, non-Stiff stalk group

Table 3.4. Company and experimental checks utilized in this study

| Company | Hybrid Name | Relative maturity |
|---|---|---|
| Pioneer | Brand 39V07 | 80 |
| | 38N88 | 92 |
| | 39N99 | 89 |
| Monsanto | DKC 35-43 | 85 |
| | DKC 33-54 | 83 |
| | DKC 36-34 | 86 |
| | DKC 30-20 | 80 |
| | DKC 48-12 | 98 |
| | DKC 38-03 | 88 |
| | DKC 31-09 | 95 |
| Syngenta | N29T-3000GT Brand | 92 |
| NDSU | TR3622 x ND2004 | 95 |
| NDSU | ND2004 x LH176 | 98 |
| NDSU | TR3622 x ND2000 | 87 |
| Thurston | TR3622 x TR4010 | 93 |
| Thurston | TR3030 x TR3622 | 87 |
| Thurston | GP2678 X TR3046 | 90 |

Field trials were arranged in 12 x 12 and 10 x 10 partial balanced lattice experimental designs for stiff stalk and non-stiff stalk tester groups respectively, at three North Dakota locations (Fargo, Casselton and Prosper), in 2013 and 2014. Therefore, a total of six environments were utilized for each heterotic group as each location x year combination was considered an environment in this study. The experiment units of this study were one-row plot which were planted by four-row planter (Almaco) with 45 kernels first and then thinned to 40 plants after 6-leaf stage to maintain approximately 80,000 plants per ha. Fertilizer was used to field trials with: 225 kg $ha^{-1}$ of Nitrogen, 55 kg $ha^{-1}$ of Phosphorus, and 55 kg $ha^{-1}$ of Potassium. Herbicide and hand weeding was applied to produce high grain yields in each location. Plots were harvest by one-row combine (Almaco) and by same time, approximately 0.5 kg of grain was sampled from bulked ears per plot for grain quality screen in the lab. A total of 2928 samples were collected in two year.

*Traits evaluated*

The following traits were measured:

1) Grain yield (YIELD): grain weight harvested by combine for each plot, adjusted to Mg $ha^{-1}$ with constant moisture of 15.5%;

2) Grain moisture at harvest (MOIST): harvest moisture in percentage, collected directly by combine;

3) Test weight (TWT): wet test weight, collected directly by combine;

4) Stand (STAN): plant counting for each plot, conducted before harvest, final plant density of each plot was adjusted to plants $ha^{-1}$;

5) Root lodging (PRL): Percentage of root lodged plants per plot relative to stand. Root lodging meant when the angle between ground and stalk equal or less the 70 $^{\circ}$;

6) Stalk lodging (PSL): Percentage of stalk lodged plants per plot relative to stand. Stalk lodging meant when stalk was broken underneath the first ear above the ground;

7) Days to anthesis (DA): the number of the days from planting till 50% of the plants from the plot were shedding pollen;

8) Days to silking (DS): the number of the days from planting till 50% of the plants from the plot had silk emerged;

9) Grain quality: grain quality traits included: protein content and quality (lysine, methionine, cysteine), oil content, starch content. These traits were measured by the Near-Infrared machine, Dickey John OmegaAnalyzerG (Bruins Instrument). The calibrations for protein content, oil content, and starch content used the Iowa State database from crop years 1990-2003. The calibrations for lysine, cysteine, and methionine amino acid used the Iowa State database from crop years 2006-2009. All the measurements and calibrations were developed under the spectral range 850-1048 nm and no National Type Evaluation Program (NTEP) data were involved in this study.

*Data Analyses*

Data from each environment were analyzed using SAS version 9.3 (SAS Institute, 2009). Genotypes were considered as a fixed factor whereas all other sources of variation (replication, blocks within replication, and environment) were treated as random factors. Missing values were estimated based on the method described by Cochran and Cox (Cochran and Cox, 1957). The linear model of the lattice design for this study was:

$$Y_{ijk} = \mu + \beta_i + \delta_j(\beta_i) + \tau_k + \varepsilon_{ijk}$$

in which $Y_{ijk}$ is the observation of the $k^{th}$ treatment in $j^{th}$ incomplete block at the $i^{th}$ replicate, $\beta_i$ is the effect of $i^{th}$ replicate, $\delta_j(\beta_i)$ is the effect of $j^{th}$ block at the $i^{th}$ replicate, $\tau_k$ is the effect of the $k^{th}$ treatment, and $\varepsilon_{ijk}$ is the intra-block error of the $Y_{ijk}$ observation.

Analysis of variance for each location was performed using the PROC LATTICE procedure from SAS. If the relative efficiency of the lattice design was greater than 105 % comparing it to a randomized complete block design (RCBD) then means adjusted by incomplete blocks were used. If efficiency was lower than 105 %, then unadjusted means were utilized in analyses. The PROC GLM procedure from SAS was used for combined analyses across environments with use of adjusted or unadjusted means from each trait and location. The "10x rule of thumb" procedure for homogeneity of variances was used, in which combined analyses across environments were applied if the largest error variance was not 10 times greater than the smallest one (Patterson and Silvey, 1980). The combined error mean square (pooled error) was calculated by pooling the correspondent individual error mean square weighed by their degrees of freedom (Gomez and Gomez, 1984):

$$\text{Pooled EMS} = \frac{\sum f_i s_i^2}{\sum f_i}$$

where $f_i$ is the degree of freedom for the correspondent EMS at $i^{th}$ location, $s_i^2$ is the EMS at $i^{th}$ location.

Fisher's Protected Least Significant Difference (LSD) was used to compare the differences among genotype means.

## RESULTS AND DISCUSSION

### $S_0$ to $S_2$ selection

According to previous studies on developing QPM (Vasal 2002; Vivek et al., 2008), the quality level of each generation was monitored to develop inbred lines having high level of lysine (Table 4.1). Based on average pollination dates (data not shown) and quality data, the earliest 11 backcross populations ($BC_1:S_0$) with high level of lysine were selected. These populations were sent to the Iowa State University Grain Quality Laboratory to determine the amino acid level with the chemical method with two objectives in mind: 1) to compare the quality data from our NIR machine and the chemical method to determine possible rank changes for selection; 2) to determine the populations' quality level for further development. The results (data not shown) showed that there were no significant differences of ranking within the 11 populations and among populations and checks. In addition, the lysine and tryptophan levels of backcross populations were almost two times (2x) higher when compared with checks.

The ears from the selected populations were planted as ear-to row in 2011 and 2012 Fargo summer breeding nurseries to produce $S_2$ early generations for testcrosses and inbreeding. At this point, two backcross populations were discarded due to above average stalk-lodging leaving the total number of selected backcross population to nine. $S_2$ bulk seed from each early generation line from all backcross populations was screened by NIR machine and, based on the results, top $BC_1S_2$ with high level of lysine and protein content, which were list in Table 4.1, were grown in our 2012 New Zealand winter nursery for testcrossing (Table 4.1) with elite industry testers.

**Correlation among traits**

Positive correlation coefficients were observed between protein content and cysteine and methionine levels for both non-Stiff Stalk (Trial 1) and Stiff Stalk groups (Trial 2). Weak and negative correlations were present between grain protein and yield (Table 4.2 and 4.3). As a consequence, most of selected $S_2$ testcrosses did not show higher yield than the mean of the checks. Large negative correlation between starch and protein content were determined. This is in agreement with studies indicating a significant negative relationship between starch content and crude protein yield (Harrelson et al., 2008; Idikut et al., 2009).

From the result, the whole-grain protein content and quality had weak relationship with other agronomic traits. This is consistent with previous studies indicating that whole-grain protein content and quality are generally negatively correlated or having lack of relationship with other agronomic traits (Hall, 1972; Hilliard and Daynard, 1974; Bhatia and Rabson, 1987; Leeson et al., 1993; Reed et al., 1993; Dale, 1994; Pixley and Bjarnason, 2002). This conclusion was also supported by animal feeding studies as well (Patterson et al., 1993; Birkelo et al., 1994; Johnston, 1995; Weichenthal et al., 1998). Knowledge on relationships between economic important traits and lysine and tryptophan content is limited. Our results showed that lysine had a weak relationship with all other quality and agronomic traits evaluated except in trial 1 (non-stiff stalk group) where lysine and methionine levels had a strong correlation among them ($\rho$=0.75, $P<0.001$). This is encouraging in order to combine, during selection, favorable traits without much concern about undesirable linkages.

**Analysis of variance and selections among $S_2$ lines**

The selection intensity was 20 % for both trials to maintain enough genetic variability for further development and keeping program manageable. Significantly differences were observed

among hybrids for all agronomic and quality traits in both experiments. We also found significant genotype by environmental interaction (G x E) for yield and most agronomic and quality traits. Trial mean values of selected $S_2$ lines are shown in Table 4.4 and 4.5. The basis of selection used to keep top lines for further inbreeding and testcrossing was based on above average grain yield, test weight, protein, lysine, and methionine means; as well as below average grain moisture, root and stalk lodging, days to anthesis, and days to silking, relative to the commercial checks.

*Agronomic traits*

Grain yield is, often, inversely related to most grain quality traits. Our purpose was to find $S_2$ testcrosses with reasonably higher yield, along with elevated grain quality traits when compared to commercial checks. None of the selected $S_2$ testcrosses yielded higher than the top yielding checks in both trials (Table 4.4 and 4.5). In trial 1, the highest and lowest grain yield was observed in [(QPM19 X LH162) x (LH162)-15]-2-2 x T1 (8.6 Mg $ha^{-1}$) and [(QPM19 X LH162) x (LH162)-4]-1-3 x T1 (5.2 Mg $ha^{-1}$), respectively, while the mean of the checks was 7.5 Mg $ha^{-1}$ (Table 4.4). There were eight $S_2$ testcrosses out of the 31 selected testcrosses that had statistically similar grain yield to the highest yielding check (Table 4.4). In trial 2, [(QPM38 X ND2006) x (ND2006)-5]-5-2 x T2 (7.3 Mg $ha^{-1}$) was the highest grain yield observed among all 17 selected testcrosses, while [(QPM43 X ND2006) x (ND2006)-18]-3-3 x T2 (6.2 Mg $ha^{-1}$) was the lowest, while the mean of the checks was 6.6 Mg $ha^{-1}$ (Table 4.5). All the selected hybrids had statistically similar yield to the mean of the checks (Table 4.5). However, eight of the 17 $S_2$ selected testcrosses had the potential to out yield checks.

Harvest grain moisture is as important as grain yield for short-season maize breeding programs, especially as this is the first attempt to adapt QPM germplasm to North Dakota. In

trial 1, grain moisture ranged from 11.8 % in [(QPM19 X LH162) x (LH162)-13]-7-2 x T1 to 14.4 % in [(QPM19 X LH162) x (LH162)-15]-2-2 x T1 (Table 4.4). Out of the 31 selected $S_2$ testcrosses, 19 $S_2$ testcrosses had comparable moisture content to the mean of the checks (12.8 %). In trial 2, grain moisture ranged from 13.4 % to 16.2 % (Table 4.5). [(QPM38 X ND2006) x (ND2006)-5]-5-3 x T2 had the lowest harvest moisture, while [(QPM43 X ND2006) x (ND2006)-18]-1-1 x T2 had the highest (Table 4.5). In this case, seven of the 17 selected $S_2$ testcrosses had comparable moisture content to the average shown in checks (13.4 %) (Table 4.5). The data showed, after adaptation, original late QPM lines were well adapted to short-season environment with relative low harvest moisture.

Percent root lodging ranged from 0 % to 13.1 %, and percent stalk lodging ranged from 0.8 % to 6.3 % for trial 1 (Table 4.4). For trial 2, percent root lodging ranged from 0 % to 9.9 %, and percent stalk lodging ranged from 8.1 % to 23.4 % (Table 4.5). These values were not statistically different between $S_2$ testcrosses and checks. Plant and ear height were in suitable ranges for all selected $S_2$ testcrosses in both trials (Table 4.4 and 4.5). However, for days to anthesis and days to silking, only a few selected $S_2$ testcrosses were found to have statistically similar values when compared to the earliest checks.

*Grain quality traits*

All the selected $S_2$ testcrosses and trial checks had statistically similar test weight values in both trials. In trial 1, grain protein content ranged from 106.0 g $kg^{-1}$ to 88.8 g $kg^{-1}$ for selected $S_2$ testcrosses. The mean of selected $S_2$ testcrosses was 98.5 g $kg^{-1}$, while the mean of the checks was 91.6 g $kg^{-1}$. As a consequence, 23 out of 31 selected testcrosses had significantly higher protein content than checks. In trial 2, grain protein content ranged from 102.2 g $kg^{-1}$ to 94.4 g $kg^{-1}$ for selected $S_2$ testcrosses. The mean of the selected $S_2$ testcrosses was 98.4 g $kg^{-1}$, while the

mean of the checks was 88.9 g $kg^{-1}$. All selected testcrosses had significantly higher protein content then the checks consequence of our EarlyQPM breeding scheme.

For lysine content, 25 of the selected $S_2$ testcrosses had statistically higher values than checks in trial 1, in which [(QPM14 X LH162) x (LH162)-2]-5-3 x T1 had the greatest lysine content (3.44 g kg-1), while the mean of checks was 3.07 g kg-1 (Table 4.4). In trial 2, lysine content ranged from 3.14 g kg-1 to 3.05 g kg-1, the mean of selected S2 testcrosses was 3.08 g kg-1, while the mean of checks was 3.00 g kg-1. In this trial, there were not statistically differences between selected $S_2$ testcrosses and checks (Table 4.5).

Methionine is the third limiting amino acid in maize used in non-ruminant diets after lysine and tryptophan, and it is the first limiting amino acid in legumes (Scott et al., 2004). In trial 1, 18 out of 31 selected $S_2$ testcrosses showed statistically higher methionine content than checks, while in trial 2, all the selected $S_2$ testcrosses had statistically higher methionine content then checks (Table 4.4 and 4.5). These results showed the potential of these unique and new short-season products for high quality maize feeding diets.

For cysteine, the overall experimental mean of EarlyQPM testcrosses was higher than the mean of the checks in both trials (2.14 vs. 2.10 g $Kg^{-1}$,for trial 1 and 2.30 vs. 2.16 g $Kg^{-1}$ for trial 2)..Seventeen of the selected $S_2$ testcrosses had higher cysteine content than the checks (Table 4.4) in trial 1, while all the selected $S_2$ testcrosses had higher cysteine content than checks (Table 4.5) in trial 2.

In trial 1, grain oil content ranged from 44.00 g $Kg^{-1}$ to 41.2 g $Kg^{-1}$. The mean of the selected $S_2$ testcrosses was higher than the mean of the checks (44.74 vs. 43.58 g $Kg^{-1}$). [(QPM19 X LH162) x (LH162)-13]-1-1 x T1, showed the top experiment value of 46.86 g $Kg^{-1}$. In trial 2, 13 selected $S_2$ testcrosses had statistically higher values than the mean of the checks.

The top hybrid for oil content was [(QPM38 X ND2006) x (ND2006)-5]-2-2 x T2 with 50.50 g $Kg^{-1}$ oil content.

By comparing two heterotic group trials (trial 1 vs. trial 2), non-stiff stalk group had relatively higher potential to develop high lysine products and stiff stalk group had relatively higher potential to develop high methionine and cysteine products. The reasons should due to their different genetic background. The observed potential trend will be confirmed in the future inbred line development process. Since this was only $S_2$ stage, there was no soft-endosperm kernels were observed, but kernel hardiness will be another concern with monitoring levels of lysine and earliness during the further early-QPM development. All the selected $S_2$ lines will be in next cycle of self-pollination in Fargo nursery, and testcross with more testers for each heterotic group, cross between heterotic group will be evaluated in future development. New lines and populations (created from top lines) will be released as a consequence of this research and numerous publications are expected to be published as well. Populations will follow recurrent selection for improving grain quality protein further.

Table 4.1. Lysine (LYS), methionine (MET), protein (PRO), and cysteine (CYS) levels of each selected maize filial generation. ($BC_1S_1$ was not included because of environmental stress in 2011 summer nursery and there were not enough seeds for the NIR screen)

| Filial Generation | Pedigree | LYS (g $Kg^{-1}$) | MET (g $Kg^{-1}$) | PRO (g $Kg^{-1}$) | CYS (g $Kg^{-1}$) |
|---|---|---|---|---|---|
| $F_1$ | QPM14 x LH162 | 4.31 | 4.16 | 180.89 | 3.05 |
| | QPM15 x LH162 | 4.36 | 4.10 | 178.05 | 3.06 |
| | QPM19 x LH162 | 3.90 | 3.32 | 139.21 | 2.49 |
| | QPM20 x LH162 | 3.89 | 3.68 | 146.58 | 2.74 |
| | QPM21 x ND291 | 3.27 | 3.07 | 131.14 | 2.27 |
| | QPM22 x LH162 | 3.73 | 3.42 | 142.35 | 2.57 |
| | QPM24 x LH162 | 3.56 | 3.32 | 142.02 | 2.59 |
| | QPM38 x ND2006 | 3.77 | 3.40 | 141.03 | 2.64 |
| | QPM43 x ND2006 | 3.76 | 3.33 | 130.82 | 2.53 |
| $BC_1S_0$ | (QPM14 x LH162) x LH162 | 3.15 | 2.85 | 144.82 | 2.59 |
| | (QPM15 x LH162) x LH162 | 3.23 | 2.88 | 144.71 | 2.63 |
| | (QPM19 x LH162) x LH162 | 3.09 | 2.63 | 132.97 | 2.44 |
| | (QPM20 x LH162) x LH162 | 3.04 | 2.63 | 132.70 | 2.46 |
| | (QPM21 x ND291) x ND291 | 3.20 | 2.86 | 142.93 | 2.61 |
| | (QPM22 x LH162) x LH162 | 3.13 | 2.78 | 135.89 | 2.53 |
| | (QPM24 x LH162) x LH162 | 3.18 | 2.83 | 141.54 | 2.55 |
| | (QPM38 x ND2006) x ND2006 | 3.14 | 2.78 | 135.94 | 2.54 |
| | (QPM43 x ND2006) x ND2006 | 3.39 | 2.89 | 140.75 | 2.57 |
| $BC_1S_2$ | [(QPM14 X LH162) x (LH162)-1]-1 | 4.61 | 3.74 | 165.46 | 2.86 |
| | [(QPM14 X LH162) x (LH162)-1]-3 | 3.88 | 3.33 | 160.57 | 2.81 |
| | [(QPM14 X LH162) x (LH162)-14]-1 | 3.89 | 3.36 | 164.25 | 2.83 |
| | [(QPM14 X LH162) x (LH162)-14]-2 | 3.81 | 3.37 | 164.67 | 2.83 |
| | [(QPM14 X LH162) x (LH162)-2]-5 | 4.21 | 3.50 | 162.66 | 2.81 |
| | [(QPM14 X LH162) x (LH162)-2]-8 | 4.68 | 3.82 | 164.01 | 2.91 |
| | [(QPM14 X LH162) x (LH162)-3]-4 | 3.77 | 3.54 | 166.83 | 2.95 |
| | [(QPM14 X LH162) x (LH162)-3]-7 | 3.75 | 3.24 | 158.74 | 2.74 |
| | [(QPM14 X LH162) x (LH162)-6]-3 | 3.96 | 3.40 | 161.10 | 2.83 |
| | [(QPM14 X LH162) x (LH162)-7]-9 | 3.92 | 3.28 | 163.22 | 2.82 |
| | [(QPM14 X LH162) x (LH162)-8]-2 | 3.98 | 3.41 | 164.23 | 2.91 |
| | [(QPM14 X LH162) x (LH162)-9]-1 | 3.84 | 3.34 | 161.15 | 2.82 |
| | [(QPM14 X LH162) x (LH162)-9]-2 | 4.32 | 3.65 | 165.69 | 2.88 |
| | [(QPM14 X LH162) x (LH162)-9]-4 | 4.00 | 3.50 | 162.31 | 2.89 |
| | [(QPM15 X LH162) x (LH162)-11]-6 | 4.11 | 3.49 | 163.80 | 2.89 |
| | [(QPM15 X LH162) x (LH162)-12]-6 | 3.70 | 3.27 | 165.10 | 2.86 |

Table 4.1. Lysine (LYS), methionine (MET), protein (PRO), and cysteine (CYS) level of each selected maize filial generation. ($BC_1S_1$ was not included because of environmental stress in 2011 summer nursery and there were not enough seeds for the NIR screen) (continued)

| Filial Generation | Pedigree | LYS (g $Kg^{-1}$) | MET (g $Kg^{-1}$) | PRO (g $Kg^{-1}$) | CYS (g $Kg^{-1}$) |
|---|---|---|---|---|---|
| $BC_1S_2$ | [(QPM15 X LH162) x (LH162)-15]-7 | 3.93 | 3.28 | 161.25 | 2.79 |
| | [(QPM15 X LH162) x (LH162)-16]-2 | 4.21 | 3.53 | 161.28 | 2.83 |
| | [(QPM15 X LH162) x (LH162)-19]-1 | 3.90 | 3.42 | 165.44 | 2.90 |
| | [(QPM15 X LH162) x (LH162)-19]-2 | 3.97 | 3.46 | 165.07 | 2.91 |
| | [(QPM15 X LH162) x (LH162)-19]-7 | 4.03 | 3.36 | 166.29 | 2.92 |
| | [(QPM15 X LH162) x (LH162)-7]-1 | 3.87 | 3.29 | 160.60 | 2.77 |
| | [(QPM19 X LH162) x (LH162)-13]-1 | 4.05 | 3.41 | 160.34 | 2.84 |
| | [(QPM19 X LH162) x (LH162)-13]-7 | 4.07 | 3.43 | 164.72 | 2.82 |
| | [(QPM19 X LH162) x (LH162)-15]-2 | 3.80 | 3.28 | 158.97 | 2.8 |
| | [(QPM19 X LH162) x (LH162)-15]-7 | 3.92 | 3.37 | 164.14 | 2.87 |
| | [(QPM19 X LH162) x (LH162)-4]-1 | 3.99 | 3.16 | 159.28 | 2.66 |
| | [(QPM19 X LH162) x (LH162)-4]-5 | 3.95 | 3.50 | 167.94 | 2.95 |
| | [(QPM19 X LH162) x (LH162)-9]-1 | 3.81 | 3.30 | 165.07 | 2.86 |
| | [(QPM20 X LH162) x (LH162)-1]-6 | 4.01 | 3.37 | 162.38 | 2.83 |
| | [(QPM20 X LH162) x (LH162)-12]-8 | 4.04 | 3.56 | 166.21 | 2.96 |
| | [(QPM20 X LH162) x (LH162)-4]-9 | 3.75 | 3.19 | 158.59 | 2.75 |
| | [(QPM20 X LH162) x (LH162)-7]-5 | 3.81 | 3.18 | 165.79 | 2.79 |
| | [(QPM20 X LH162) x (LH162)-9]-1 | 4.01 | 3.45 | 163.92 | 2.92 |
| | [(QPM20 X LH162) x (LH162)-9]-4 | 3.75 | 3.36 | 159.23 | 2.85 |
| | [(QPM20 X LH162) x (LH162)-9]-5 | 3.99 | 3.26 | 158.62 | 2.75 |
| | [(QPM21 X ND291) x (ND291)-13]-5 | 3.65 | 3.02 | 162.59 | 2.77 |
| | [(QPM21 X ND291) x (ND291)-2]-3 | 3.73 | 3.19 | 161.58 | 2.73 |
| | [(QPM21 X ND291) x (ND291)-4]-4 | 3.94 | 3.08 | 161.12 | 2.72 |
| | [(QPM21 X ND291) x (ND291)-5]-7 | 3.66 | 3.05 | 160.72 | 2.73 |
| | [(QPM21 X ND291) x (ND291)-7]-5 | 3.46 | 3.00 | 159.91 | 2.75 |
| | [(QPM21 X ND291) x (ND291)-8]-1 | 3.67 | 3.24 | 166.59 | 2.87 |
| | [(QPM22 X LH162) x (LH162)-2]-2 | 3.85 | 3.24 | 165.07 | 2.82 |
| | [(QPM22 X LH162) x (LH162)-8]-1 | 4.44 | 3.30 | 159.44 | 2.72 |
| | [(QPM24 X LH162) x (LH162)-2]-3 | 4.05 | 3.36 | 164.81 | 2.9 |
| | [(QPM24 X LH162) x (LH162)-3]-1 | 3.79 | 3.26 | 163.07 | 2.84 |
| | [(QPM38 X ND2006) x (ND2006)-1]-3 | 3.81 | 3.21 | 159.04 | 2.82 |
| | [(QPM38 X ND2006) x (ND2006)-10]-2 | 3.78 | 3.15 | 150.64 | 2.70 |
| | [(QPM38 X ND2006) x (ND2006)-12]-3 | 3.91 | 3.44 | 164.90 | 2.96 |
| | [(QPM38 X ND2006) x (ND2006)-2]-1 | 3.75 | 3.27 | 157.68 | 2.83 |
| | [(QPM38 X ND2006) x (ND2006)-3]-1 | 3.82 | 3.30 | 162.60 | 2.81 |
| | [(QPM38 X ND2006) x (ND2006)-3]-2 | 4.05 | 3.12 | 150.38 | 2.58 |

Table 4.1. Lysine (LYS), methionine (MET), protein (PRO), and cysteine (CYS) level of each selected maize filial generation. ($BC_1S_1$ was not included because of environmental stress in 2011 summer nursery and there were not enough seeds for the NIR screen) (continued)

| Filial Generation | Pedigree | LYS (g $Kg^{-1}$) | MET (g $Kg^{-1}$) | PRO (g $Kg^{-1}$) | CYS (g $Kg^{-1}$) |
|---|---|---|---|---|---|
| $BC_1S_2$ | [(QPM38 X ND2006) x (ND2006)-3]-3 | 3.89 | 3.19 | 154.07 | 2.83 |
| | [(QPM38 X ND2006) x (ND2006)-5]-5 | 3.81 | 3.37 | 162.73 | 2.87 |
| | [(QPM38 X ND2006) x (ND2006)-6]-1 | 3.96 | 3.68 | 165.50 | 3.11 |
| | [(QPM38 X ND2006) x (ND2006)-6]-3 | 3.70 | 3.25 | 154.33 | 2.75 |
| | [(QPM38 X ND2006) x (ND2006)-6]-5 | 3.89 | 3.28 | 156.76 | 2.81 |
| | [(QPM43 X ND2006) x (ND2006)-1]-1 | 3.91 | 3.39 | 158.94 | 2.87 |
| | [(QPM43 X ND2006) x (ND2006)-1]-3 | 3.98 | 3.37 | 159.59 | 2.84 |
| | [(QPM43 X ND2006) x (ND2006)-10]-5 | 3.89 | 3.25 | 155.15 | 2.71 |
| | [(QPM43 X ND2006) x (ND2006)-11]-3 | 3.70 | 3.29 | 157.39 | 2.80 |
| | [(QPM43 X ND2006) x (ND2006)-16]-6 | 3.93 | 3.18 | 154.38 | 2.72 |
| | [(QPM43 X ND2006) x (ND2006)-16]-7 | 3.84 | 3.15 | 150.16 | 2.62 |
| | [(QPM43 X ND2006) x (ND2006)-17]-4 | 3.73 | 3.15 | 150.51 | 2.75 |
| | [(QPM43 X ND2006) x (ND2006)-18]-1 | 4.08 | 3.41 | 159.17 | 2.82 |
| | [(QPM43 X ND2006) x (ND2006)-18]-3 | 3.92 | 3.28 | 157.18 | 2.76 |
| | [(QPM43 X ND2006) x (ND2006)-18]-4 | 3.97 | 3.34 | 158.6 | 2.81 |
| | [(QPM43 X ND2006) x (ND2006)-2]-5 | 3.67 | 3.25 | 157.94 | 2.82 |
| | [(QPM43 X ND2006) x (ND2006)-4]-1 | 3.91 | 3.38 | 157.39 | 2.78 |
| | [(QPM43 X ND2006) x (ND2006)-8]-1 | 3.75 | 3.13 | 151.83 | 2.69 |
| | [(QPM43 X ND2006) x (ND2006)-8]-2 | 3.93 | 3.44 | 162.95 | 2.90 |
| | [(QPM43 X ND2006) x (ND2006)-8]-3 | 4.07 | 3.30 | 154.95 | 2.74 |
| | [(QPM38 X ND2006) x (ND2006)-5]-2 | 3.89 | 3.13 | 151.6 | 2.68 |

Table 4.2. Phenotypic correlation coefficients among maize traits measured on 127 test-crosses across 6 environments for trial 1 of non-Stiff Stalk group (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper[†])

| | YIELD[‡] | MOIST | TWT | DA | DS | STAN | PRL | PSL | MET | PRO | OIL | STARCH | CYS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MOIST | 0.21*[§] | | | | | | | | | | | | |
| TWT | 0.26*** | 0.05 | | | | | | | | | | | |
| DA | 0.13 | 0.35*** | -0.12 | | | | | | | | | | |
| DS | 0.12 | 0.38*** | -0.13 | 0.97*** | | | | | | | | | |
| STAN | 0.34*** | 0.03 | -0.02 | -0.07 | -0.03 | | | | | | | | |
| PRL | -0.06 | 0.16 | -0.07 | 0.31*** | 0.30*** | -0.13 | | | | | | | |
| PSL | -0.27** | -0.01 | -0.09 | 0.09 | 0.08 | -0.28*** | 0.39*** | | | | | | |
| MET | -0.26** | 0.04 | -0.10 | -0.02 | 0.03 | 0.11 | -0.18* | -0.35*** | | | | | |
| PRO | -0.43*** | -0.03 | -0.04 | -0.04 | 0.00 | -0.05 | -0.06 | -0.15 | 0.85*** | | | | |
| OIL | 0.15* | 0.07* | -0.01 | 0.08* | 0.13* | 0.25** | -0.09* | -0.35*** | 0.32*** | 0.10 | | | |
| STARCH | 0.25** | 0.02 | 0.09 | -0.04 | -0.09 | -0.12 | 0.09 | 0.35*** | -0.87*** | -0.83*** | -0.61*** | | |
| CYS | -0.40*** | 0.02 | 0.03 | -0.18* | -0.17* | -0.02 | -0.09* | -0.07* | 0.73*** | 0.86*** | -0.02 | -0.63*** | |
| LYS | -0.17* | -0.11* | -0.27*** | 0.11* | 0.15** | 0.15* | -0.08 | -0.32*** | 0.75*** | 0.59*** | 0.43*** | -0.79*** | 0.32** * |

[†]: MOIST data were from 5 environments except 2013 Prosper; TWT data were from 5 environments except 2014 Casselton; RL data were from 5 environments except 2014 Fargo; DA and DS data were from 2013 Fargo, 2013 Prosper, 2014 Fargo, 2014 Prosper.

[‡]:MOIST, harvest moisture; TWT, harvest test weight; DA, days to anthesis emergence; DS, days to silking; STAN, plant stands per ha; PRL, percentage of root lodging; PSL, percentage of stalk lodging; MET, Methionine; PRO, protein; CYS, Cysteine; LYS, Lysine.

[§]: Green shows positive correlation and red shows negative correlation; color gradient indicates the level of significance.

***, significant at p=0.001 level; **, significant at p=0.01 level; *, significant at p=0.05 level.

Table 4.3. Phenotypic correlation coefficients among maize traits measured on 83 test-crosses across 6 environments for trial 2 of Stiff Stalk group (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper[†])

| | YIELD | MOIST | TWT | DA | DS | STAND | PRL | PSL | MET | PRO | OIL | STARCH | CYS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MOIST | 0.14[‡] | | | | | | | | | | | | |
| TWT | -0.02 | -0.32$^{**}$ | | | | | | | | | | | |
| DA | 0.58$^{***}$ | 0.47$^{***}$ | -0.27$^{**}$ | | | | | | | | | | |
| DS | 0.53$^{***}$ | 0.49$^{***}$ | -0.30$^{**}$ | 0.97$^{***}$ | | | | | | | | | |
| STAND | 0.23$^{*}$ | -0.24$^{*}$ | 0.17 | 0.05 | 0.03 | | | | | | | | |
| PRL | 0.13 | 0.42$^{***}$ | -0.26$^{**}$ | 0.40$^{***}$ | 0.41$^{***}$ | -0.09 | | | | | | | |
| PSL | -0.14 | 0.13 | 0.06 | 0.04 | 0.07$^{*}$ | -0.21$^{*}$ | -0.03 | | | | | | |
| MET | -0.19 | 0.38$^{***}$ | -0.07 | -0.04 | 0.06 | -0.25$^{*}$ | 0.27$^{**}$ | 0.17 | | | | | |
| PRO | -0.28$^{**}$ | 0.27$^{**}$ | 0.00 | -0.20$^{*}$ | -0.10 | -0.28$^{**}$ | 0.24$^{*}$ | 0.22$^{*}$ | 0.92$^{***}$ | | | | |
| OIL | 0.16 | 0.39$^{***}$ | -0.21$^{*}$ | 0.29$^{**}$ | 0.35$^{***}$ | -0.19 | 0.26$^{**}$ | 0.15 | 0.29$^{**}$ | 0.28$^{**}$ | | | |
| STARCH | 0.13 | -0.35$^{***}$ | 0.13 | 0.01 | -0.09 | 0.29$^{**}$ | -0.30$^{**}$ | -0.22$^{*}$ | -0.85$^{***}$ | -0.90$^{***}$ | -0.64$^{***}$ | | |
| CYS | -0.32$^{***}$ | 0.32$^{**}$ | 0.09 | -0.20$^{*}$ | -0.08 | -0.26$^{**}$ | 0.17 | 0.30$^{**}$ | 0.82$^{***}$ | 0.88$^{***}$ | 0.27$^{**}$ | -0.80$^{***}$ | |
| LYS | -0.04 | 0.05 | -0.30$^{**}$ | -0.01 | 0.01 | -0.12 | 0.16 | 0.06 | 0.57$^{***}$ | 0.48$^{***}$ | 0.12 | -0.48$^{***}$ | 0.20$^{*}$ |

[†]: MOIST data were from 5 environments except 2013 Prosper; RL data were from 4 environments except 2013 Prosper and 2014 Prosper; DA and DS data were from 2013 Fargo, 2013 Prosper, 2014 Fargo, 2014 Prosper.

[‡]: Green shows positive correlation and red shows negative correlation; color gradient indicates the level of significance.

$^{***}$, significant at p=0.001 level; $^{**}$, significant at p=0.01 level; $^{*}$, significant at p=0.05 level

Table 4.4. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 1 (non-Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits

| Pedigree | YIELD (Mg ha$^{-1}$) | MOIST$^{\dagger}$ (%) | TWT (Kg hL$^{-1}$) | DA (days) | DS (days) | EH (cm) | PH (cm) | PRL (%) | PSL (%) |
|---|---|---|---|---|---|---|---|---|---|
| [(QPM14 X LH162) x (LH162)-2]-5-3 x T1 | 6.7 | 12.5 | 72.6 | 64.5 | 65.0 | 86.0 | 203.3 | 0.0 | 0.8 |
| [(QPM19 X LH162) x (LH162)-4]-1-2 x T1 | 5.6 | 12.9 | 73.8 | 65.1 | 65.4 | 93.6 | 204.6 | 1.4 | 1.6 |
| [(QPM15 X LH162) x (LH162)-11]-6-2 x T1 | 5.5 | 12.5 | 72.9 | 63.7 | 63.5 | 87.6 | 199.0 | 0.1 | 4.5 |
| [(QPM19 X LH162) x (LH162)-13]-1-1 x T1 | 6.4 | 13.4 | 74.3 | 67.3 | 67.5 | 107.4 | 219.5 | 8.4 | 5.0 |
| [(QPM19 X LH162) x (LH162)-13]-1-2 x T1 | 6.8 | 12.5 | 73.9 | 67.4 | 67.7 | 95.6 | 214.3 | 5.5 | 3.8 |
| [(QPM22 X LH162) x (LH162)-8]-1-2 x T1 | 5.9 | 12.8 | 70.8 | 66.3 | 66.4 | 92.1 | 207.8 | 2.7 | 5.5 |
| [(QPM14 X LH162) x (LH162)-9]-4-2 x T1 | 5.9 | 12.8 | 72.6 | 66.9 | 67.6 | 92.5 | 208.5 | 0.6 | 3.3 |
| [(QPM14 X LH162) x (LH162)-14]-2-2 x T1 | 6.4 | 12.4 | 72.9 | 65.2 | 65.7 | 95.5 | 215.4 | 1.7 | 1.4 |
| [(QPM14 X LH162) x (LH162)-2]-5-1 x T1 | 6.6 | 12.2 | 73.8 | 65.9 | 66.0 | 89.5 | 205.1 | 1.5 | 1.1 |
| [(QPM22 X LH162) x (LH162)-8]-1-1 x T1 | 7.0 | 12.5 | 73.9 | 66.3 | 66.9 | 100.9 | 216.5 | 3.2 | 3.6 |
| [(QPM14 X LH162) x (LH162)-3]-4-3 x T1 | 6.0 | 12.5 | 75.1 | 66.3 | 66.2 | 92.5 | 208.8 | 1.1 | 1.0 |
| [(QPM19 X LH162) x (LH162)-13]-7-1 x T1 | 5.2 | 12.8 | 72.5 | 65.4 | 65.8 | 86.9 | 197.3 | 0.0 | 4.1 |
| [(QPM19 X LH162) x (LH162)-4]-5-2 x T1 | 6.5 | 12.5 | 72.6 | 67.4 | 67.9 | 92.9 | 211.6 | 2.1 | 2.9 |
| [(QPM14 X LH162) x (LH162)-9]-2-2 x T1 | 6.2 | 12.8 | 72.2 | 64.7 | 64.6 | 85.2 | 200.7 | 0.8 | 3.4 |
| [(QPM22 X LH162) x (LH162)-8]-1-3 x T1 | 7.5 | 13.5 | 74.6 | 66.6 | 67.4 | 104.9 | 226.6 | 13.1 | 3.8 |
| [(QPM14 X LH162) x (LH162)-14]-2-1 x T1 | 6.5 | 12.7 | 71.0 | 66.5 | 67.1 | 91.3 | 213.4 | 0.7 | 4.9 |
| [(QPM14 X LH162) x (LH162)-1]-1-1 x T1 | 7.7 | 13.5 | 73.6 | 64.8 | 65.1 | 100.3 | 214.0 | 1.2 | 5.4 |
| [(QPM15 X LH162) x (LH162)-19]-2-1 x T1 | 6.3 | 13.4 | 74.2 | 66.0 | 66.4 | 93.0 | 213.6 | 3.0 | 2.8 |
| [(QPM14 X LH162) x (LH162)-14]-1-1 x T1 | 5.7 | 12.4 | 71.7 | 66.1 | 66.0 | 96.8 | 211.0 | 3.2 | 4.9 |
| [(QPM20 X LH162) x (LH162)-1]-6-3 x T1 | 6.8 | 13.3 | 74.6 | 65.7 | 66.3 | 99.7 | 215.0 | 2.8 | 3.2 |
| [(QPM14 X LH162) x (LH162)-9]-4-1 x T1 | 6.4 | 13.2 | 75.4 | 65.3 | 65.8 | 94.0 | 210.5 | 2.4 | 2.2 |
| [(QPM19 X LH162) x (LH162)-9]-1-2 x T1 | 6.2 | 12.7 | 74.8 | 65.0 | 65.3 | 88.6 | 206.8 | 0.4 | 2.3 |

Table 4.4. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 1 (non-Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | YIELD (Mg ha$^{-1}$) | MOIST (%) | TWT (Kg hL$^{-1}$) | DA (days) | DS (days) | EH (cm) | PH (cm) | PRL (%) | PSL (%) |
|---|---|---|---|---|---|---|---|---|---|
| [(QPM19 X LH162) x (LH162)-4]-1-3 x T1 | 5.2 | 12.7 | 73.0 | 65.9 | 66.5 | 96.6 | 214.0 | 0.4 | 3.9 |
| [(QPM19 X LH162) x (LH162)-13]-7-2 x T1 | 5.7 | 11.8 | 71.7 | 65.3 | 65.6 | 86.1 | 197.2 | 0.0 | 5.4 |
| [(QPM14 X LH162) x (LH162)-2]-8-3 x T1 | 6.9 | 13.0 | 74.9 | 65.6 | 66.5 | 97.7 | 215.3 | 0.3 | 6.3 |
| [(QPM19 X LH162) x (LH162)-15]-2-1 x T1 | 7.8 | 14.3 | 74.8 | 67.5 | 67.3 | 100.2 | 222.9 | 1.1 | 6.7 |
| [(QPM14 X LH162) x (LH162)-7]-9-3 x T1 | 7.5 | 13.8 | 74.3 | 65.9 | 66.1 | 101.6 | 219.4 | 3.3 | 3.3 |
| [(QPM19 X LH162) x (LH162)-15]-2-2 x T1 | 8.6 | 14.4 | 75.0 | 67.7 | 68.0 | 102.6 | 225.4 | 1.9 | 2.9 |
| [(QPM24 X LH162) x (LH162)-2]-3-3 x T1 | 7.3 | 13.6 | 73.6 | 66.2 | 66.4 | 97.6 | 213.1 | 0.2 | 2.7 |
| [(QPM19 X LH162) x (LH162)-15]-2-3 x T1 | 7.2 | 13.4 | 75.6 | 65.9 | 66.3 | 100.9 | 223.1 | 2.9 | 4.9 |
| [(QPM15 X LH162) x (LH162)-19]-7-1 x T1 | 7.6 | 13.2 | 74.1 | 67.1 | 67.3 | 95.7 | 214.3 | 4.0 | 1.6 |
| Checks: | | | | | | | | | |
| Pioneer Brand 39V07 | 5.9 | 11.8 | 70.7 | 62.5 | 62.6 | 97.3 | 202.4 | 0.7 | 5.8 |
| Pioneer 38N88 | 7.9 | 12.8 | 75.3 | 65.4 | 65.0 | 104.7 | 213.9 | 1.5 | 2.0 |
| Pioneer 39N99 | 7.9 | 13.1 | 74.3 | 64.4 | 64.8 | 95.2 | 213.0 | 1.4 | 1.6 |
| Monsanto DKC 35-43 | 6.2 | 12.2 | 78.0 | 66.4 | 66.2 | 94.6 | 212.3 | 0.1 | 3.1 |
| Monsanto DKC 33-54 | 6.3 | 11.5 | 74.8 | 63.3 | 63.1 | 83.1 | 202.2 | 0.3 | 2.2 |
| Monsanto DKC 36-34 | 8.1 | 12.9 | 74.1 | 63.5 | 63.7 | 83.1 | 209.3 | 0.1 | 0.5 |
| Monsanto DKC 30-20 | 5.8 | 11.7 | 72.5 | 62.4 | 62.7 | 88.9 | 212.9 | 0.5 | 0.9 |
| Monsanto DKC 48-12 | 9.4 | 14.5 | 72.0 | 69.0 | 68.8 | 98.5 | 223.9 | 4.1 | 0.7 |
| Monsanto DKC 38-03 | 9.5 | 13.4 | 74.8 | 65.3 | 65.1 | 90.7 | 220.4 | 1.4 | 2.4 |
| Monsanto DKC 31-09 | 7.4 | 11.9 | 74.3 | 62.1 | 62.6 | 81.9 | 211.3 | 0.7 | 0.3 |
| Syngenta N29T-3000GT Brand | 9.3 | 12.8 | 70.1 | 69.0 | 68.7 | 112.6 | 228.5 | 3.9 | 0.3 |
| TR3622 x ND2004 | 7.2 | 13.2 | 76.6 | 63.9 | 64.3 | 87.8 | 207.3 | 0.1 | 1.8 |
| ND2004 x LH176 | 6.0 | 14.5 | 77.8 | 63.0 | 63.3 | 91.7 | 205.5 | 1.9 | 1.1 |

Table 4.4. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 1 (non-Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | Yield (Mg $ha^{-1}$) | Moisture (%) | Weight (Kg $hL^{-1}$) | DA (days) | DS (days) | EH (cm) | PH (cm) | PRL (%) | PSL (%) |
|---|---|---|---|---|---|---|---|---|---|
| Checks: | | | | | | | | | |
| TR3622 x ND2000 | 7.1 | 12.6 | 73.1 | 62.8 | 63.4 | 91.9 | 202.5 | 0.8 | 11.0 |
| TR3622 x TR4010 | 7.3 | 12.8 | 76.1 | 65.9 | 66.2 | 95.4 | 219.2 | 1.9 | 2.3 |
| TR3030 x TR3622 | 8.5 | 13.5 | 73.5 | 66.1 | 66.5 | 100.0 | 215.5 | 2.6 | 1.1 |
| GP2678 X TR3046 | 7.9 | 12.4 | 73.9 | 66.2 | 66.5 | 102.3 | 228.0 | 0.3 | 0.8 |
| | | | | | | | | | |
| Mean of slections§ | 6.6 | 13.0 | 73.6 | 66.0 | 66.3 | 95.0 | 211.9 | 2.3 | 3.5 |
| Checks mean¶ | 7.5 | 12.8 | 74.2 | 64.8 | 64.9 | 94.1 | 213.4 | 1.3 | 2.2 |
| Exp. Mean# | 6.38 | 13.04 | 73.77 | 65.85 | 66.06 | 94.75 | 211.52 | 2.58 | 4.28 |
| | | | | | | | | | |
| EMS | 1.49 | 0.62 | 11.36 | 1.85 | 2.36 | 28.58 | 57.36 | 23.16 | 27.41 |
| LSD, 0.05 | 2.39 | 1.55 | 6.61 | 2.67 | 3.01 | 10.48 | 14.84 | 9.43 | 10.26 |
| CV,% | 19.13 | 6.05 | 4.57 | 2.07 | 2.33 | 5.64 | 3.58 | 186.31 | 122.35 |

Table 4.4. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 1 (non-Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | PRO ($g\ Kg^{-1}$) | LYS ($g\ Kg^{-1}$) | MET ($g\ Kg^{-1}$) | CYS ($g\ Kg^{-1}$) | OIL ($g\ Kg^{-1}$) | STARCH ($g\ Kg^{-1}$) |
|---|---|---|---|---|---|---|
| [(QPM14 X LH162) x (LH162)-2]-5-3 x T1 | 104.058 | 3.436 | 2.380 | 2.219 | 46.183 | 694.770 |
| [(QPM19 X LH162) x (LH162)-4]-1-2 x T1 | 106.000 | 3.393 | 2.319 | 2.247 | 43.567 | 697.503 |
| [(QPM15 X LH162) x (LH162)-11]-6-2 x T1 | 105.874 | 3.379 | 2.379 | 2.337 | 42.671 | 697.523 |
| [(QPM19 X LH162) x (LH162)-13]-1-1 x T1 | 98.500 | 3.369 | 2.216 | 2.139 | 49.004 | 696.830 |
| [(QPM19 X LH162) x (LH162)-13]-1-2 x T1 | 97.844 | 3.338 | 2.191 | 2.110 | 47.069 | 700.315 |
| [(QPM22 X LH162) x (LH162)-8]-1-2 x T1 | 99.199 | 3.334 | 2.159 | 2.159 | 44.431 | 702.182 |
| [(QPM14 X LH162) x (LH162)-9]-4-2 x T1 | 104.977 | 3.331 | 2.358 | 2.233 | 44.516 | 697.445 |
| [(QPM14 X LH162) x (LH162)-14]-2-2 x T1 | 101.231 | 3.327 | 2.256 | 2.156 | 44.744 | 700.085 |
| [(QPM14 X LH162) x (LH162)-2]-5-1 x T1 | 97.184 | 3.323 | 2.184 | 2.132 | 45.887 | 702.043 |
| [(QPM22 X LH162) x (LH162)-8]-1-1 x T1 | 98.712 | 3.320 | 2.240 | 2.158 | 44.014 | 703.205 |
| [(QPM14 X LH162) x (LH162)-3]-4-3 x T1 | 102.452 | 3.319 | 2.302 | 2.232 | 44.638 | 699.112 |
| [(QPM19 X LH162) x (LH162)-13]-7-1 x T1 | 94.074 | 3.316 | 2.140 | 2.142 | 45.932 | 704.259 |
| [(QPM19 X LH162) x (LH162)-4]-5-2 x T1 | 99.751 | 3.307 | 2.196 | 2.161 | 44.788 | 701.127 |
| [(QPM14 X LH162) x (LH162)-9]-2-2 x T1 | 99.769 | 3.304 | 2.194 | 2.197 | 44.038 | 702.222 |
| [(QPM22 X LH162) x (LH162)-8]-1-3 x T1 | 102.495 | 3.283 | 2.209 | 2.213 | 44.233 | 699.231 |
| [(QPM14 X LH162) x (LH162)-14]-2-1 x T1 | 91.454 | 3.281 | 2.093 | 2.021 | 46.622 | 706.259 |
| [(QPM14 X LH162) x (LH162)-1]-1-1 x T1 | 97.656 | 3.272 | 2.186 | 2.132 | 45.085 | 702.618 |
| [(QPM15 X LH162) x (LH162)-19]-2-1 x T1 | 99.071 | 3.272 | 2.205 | 2.115 | 46.125 | 700.233 |
| [(QPM14 X LH162) x (LH162)-14]-1-1 x T1 | 101.803 | 3.272 | 2.253 | 2.203 | 45.857 | 697.656 |
| [(QPM20 X LH162) x (LH162)-1]-6-3 x T1 | 96.106 | 3.264 | 2.169 | 2.104 | 45.249 | 704.262 |
| [(QPM14 X LH162) x (LH162)-9]-4-1 x T1 | 99.931 | 3.264 | 2.194 | 2.160 | 45.366 | 700.972 |
| [(QPM19 X LH162) x (LH162)-9]-1-2 x T1 | 100.411 | 3.263 | 2.221 | 2.177 | 43.839 | 701.899 |

Table 4.4. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 1 (non-Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | PRO ($g\ Kg^{-1}$) | LYS ($g\ Kg^{-1}$) | MET ($g\ Kg^{-1}$) | CYS ($g\ Kg^{-1}$) | OIL ($g\ Kg^{-1}$) | STARCH ($g\ Kg^{-1}$) |
|---|---|---|---|---|---|---|
| [(QPM19 X LH162) x (LH162)-4]-1-3 x T1 | 99.576 | 3.263 | 2.213 | 2.176 | 43.235 | 703.406 |
| [(QPM19 X LH162) x (LH162)-13]-7-2 x T1 | 95.031 | 3.262 | 2.143 | 2.130 | 45.177 | 705.109 |
| [(QPM14 X LH162) x (LH162)-2]-8-3 x T1 | 97.877 | 3.260 | 2.178 | 2.113 | 43.544 | 704.546 |
| [(QPM19 X LH162) x (LH162)-15]-2-1 x T1 | 92.154 | 3.218 | 2.112 | 2.066 | 44.485 | 708.236 |
| [(QPM14 X LH162) x (LH162)-7]-9-3 x T1 | 93.522 | 3.180 | 2.093 | 2.096 | 43.476 | 708.042 |
| [(QPM19 X LH162) x (LH162)-15]-2-2 x T1 | 88.738 | 3.142 | 2.005 | 2.045 | 43.609 | 712.068 |
| [(QPM24 X LH162) x (LH162)-2]-3-3 x T1 | 95.680 | 3.122 | 2.117 | 2.140 | 44.670 | 704.801 |
| [(QPM19 X LH162) x (LH162)-15]-2-3 x T1 | 93.165 | 3.118 | 2.075 | 2.082 | 43.766 | 708.403 |
| [(QPM15 X LH162) x (LH162)-19]-7-1 x T1 | 98.331 | 3.105 | 2.144 | 2.219 | 41.240 | 707.332 |
| Checks: | | | | | | |
| Pioneer Brand 39V07 | 91.935 | 3.169 | 2.099 | 2.164 | 41.245 | 711.530 |
| Pioneer 38N88 | 88.130 | 3.174 | 2.059 | 2.019 | 44.319 | 712.840 |
| Pioneer 39N99 | 87.739 | 2.974 | 2.029 | 2.065 | 46.864 | 710.269 |
| Monsanto DKC 35-43 | 90.961 | 3.075 | 2.079 | 2.108 | 41.706 | 713.108 |
| Monsanto DKC 33-54 | 94.492 | 3.104 | 2.044 | 2.120 | 42.117 | 709.463 |
| Monsanto DKC 36-34 | 92.317 | 3.035 | 2.098 | 2.110 | 43.285 | 710.039 |
| Monsanto DKC 30-20 | 96.950 | 3.116 | 2.140 | 2.182 | 41.486 | 709.842 |
| Monsanto DKC 48-12 | 84.248 | 3.046 | 1.947 | 1.962 | 46.080 | 714.417 |
| Monsanto DKC 38-03 | 87.130 | 3.147 | 1.989 | 2.039 | 42.662 | 715.612 |
| Monsanto DKC 31-09 | 91.845 | 3.055 | 2.015 | 2.095 | 45.279 | 709.326 |
| Syngenta N29T-3000GT Brand | 90.165 | 3.232 | 2.065 | 2.007 | 44.064 | 710.683 |
| TR3622 x ND2004 | 99.695 | 3.013 | 2.128 | 2.226 | 42.301 | 706.519 |
| ND2004 x LH176 | 93.998 | 3.011 | 2.184 | 2.220 | 45.320 | 708.430 |

Table 4.4. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 1 (non-Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | PRO ($g\ Kg^{-1}$) | LYS ($g\ Kg^{-1}$) | MET ($g\ Kg^{-1}$) | CYS ($g\ Kg^{-1}$) | OIL ($g\ Kg^{-1}$) | STARCH ($g\ Kg^{-1}$) |
|---|---|---|---|---|---|---|
| Checks: | | | | | | |
| TR3622 x ND2000 | 95.519 | 2.971 | 2.058 | 2.174 | 45.482 | 708.082 |
| TR3622 x TR4010 | 87.672 | 2.893 | 1.996 | 2.082 | 43.489 | 715.896 |
| TR3030 x TR3622 | 92.977 | 3.048 | 2.044 | 2.091 | 45.152 | 708.461 |
| GP2678 X TR3046 | 92.202 | 3.104 | 2.113 | 2.110 | 39.980 | 712.272 |
| | | | | | | |
| Mean of selections | 98.472 | 3.279 | 2.198 | 2.155 | 44.744 | 702.377 |
| Checks mean | 91.646 | 3.069 | 2.064 | 2.104 | 43.578 | 710.988 |
| Exp. Mean | 96.447 | 3.182 | 2.137 | 2.143 | 43.865 | 705.810 |
| | | | | | | |
| EMS | 0.2641 | 0.0002 | 0.0001 | 0.0001 | 0.0415 | 0.259 |
| LSD, 0.05 | 10.073 | 0.246 | 0.235 | 0.183 | 3.992 | 9.971 |
| CV,% | 0.53 | 0.39 | 0.56 | 0.44 | 0.46 | 0.07 |

†:MOIST, harvest moisture; TWT, harvest test weight; DA, days to anthesis emergence; DS, days to silking; PRL, percentage of root lodging; PSL, percentage of stalk lodging; PRO, protein; LYS, Lysine; MET, Methionine; CYS, Cysteine.

§ mean of selected entries, ¶mean of checks, # mean of experiment.

Selection were carried out by sorting yield in descending order and then sorting by lysine level in descending order, then selected $S_2$ testcrosses were screened for lower moisture, higher test weight, lower per cent root and stalk lodging, and lower days to anthesis and silking, higher protein, and methionine. The basis of selection was comparing the selected $S_2$ testcrosses with best check and mean values of checks for respective traits.

Table 4.5. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 2 (Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits

| Pedigree | Yield (Mg $ha^{-1}$) | MOIST† (%) | Weight (Kg $hL^{-1}$) | DA (days) | DS (days) | EH (cm) | PH (cm) | PRL (%) | PSL (%) |
|---|---|---|---|---|---|---|---|---|---|
| [(QPM43 X ND2006) x (ND2006)-10]-5-2 x T2 | 7.2 | 13.7 | 73.5 | 65.7 | 66.7 | 104.6 | 226.2 | 3.4 | 15.6 |
| [(QPM43 X ND2006) x (ND2006)-18]-3-1 x T2 | 6.3 | 13.5 | 71.0 | 65.0 | 66.6 | 103.2 | 222.5 | 3.4 | 17.6 |
| [(QPM43 X ND2006) x (ND2006)-8]-1-3 x T2 | 7.2 | 15.9 | 72.4 | 66.7 | 67.0 | 106.3 | 231.9 | 7.3 | 13.8 |
| [(QPM38 X ND2006) x (ND2006)-8]-7-2 x T2 | 6.9 | 15.6 | 74.4 | 65.5 | 66.2 | 97.3 | 216.8 | 4.7 | 14.2 |
| [(QPM38 X ND2006) x (ND2006)-5]-2-2 x T2 | 6.3 | 15.1 | 71.7 | 66.8 | 67.2 | 101.7 | 217.4 | 3.6 | 13.6 |
| [(QPM43 X ND2006) x (ND2006)-8]-2-1 x T2 | 6.4 | 14.0 | 71.7 | 65.2 | 66.3 | 99.6 | 221.3 | 9.9 | 13.5 |
| [(QPM43 X ND2006) x (ND2006)-17]-4-2 x T2 | 6.3 | 13.9 | 72.6 | 64.4 | 65.0 | 108.1 | 225.4 | 0.0 | 23.4 |
| [(QPM43 X ND2006) x (ND2006)-1]-3-3 x T2 | 6.9 | 13.7 | 74.9 | 64.7 | 65.4 | 95.0 | 220.7 | 4.7 | 8.1 |
| [(QPM38 X ND2006) x (ND2006)-5]-5-3 x T2 | 7.2 | 13.4 | 72.8 | 65.4 | 66.6 | 95.9 | 217.8 | 2.3 | 15.0 |
| [(QPM38 X ND2006) x (ND2006)-3]-3-2 x T2 | 7.1 | 15.4 | 71.3 | 65.9 | 66.6 | 94.1 | 217.0 | 9.7 | 14.9 |
| [(QPM43 X ND2006) x (ND2006)-18]-3-3 x T2 | 6.2 | 14.2 | 72.7 | 63.1 | 64.3 | 90.4 | 205.9 | 5.6 | 13.0 |
| [(QPM43 X ND2006) x (ND2006)-11]-3-3 x T2 | 6.5 | 15.9 | 71.2 | 66.2 | 66.6 | 103.3 | 227.4 | 5.5 | 11.2 |
| [(QPM43 X ND2006) x (ND2006)-10]-5-3 x T2 | 6.7 | 13.9 | 72.9 | 65.5 | 66.2 | 94.3 | 212.5 | 9.6 | 11.7 |
| [(QPM43 X ND2006) x (ND2006)-17]-4-1 x T2 | 7.2 | 14.4 | 72.1 | 65.5 | 66.1 | 107.4 | 227.1 | 3.2 | 16.9 |
| [(QPM38 X ND2006) x (ND2006)-5]-5-2 x T2 | 7.3 | 15.4 | 72.2 | 67.7 | 68.6 | 105.8 | 230.2 | 9.5 | 11.4 |
| [(QPM43 X ND2006) x (ND2006)-18]-1-1 x T2 | 6.6 | 16.2 | 72.4 | 66.3 | 66.7 | 93.6 | 220.1 | 5.9 | 11.1 |
| [(QPM38 X ND2006) x (ND2006)-3]-1-1 x T2 | 6.5 | 14.2 | 74.9 | 65.7 | 66.7 | 90.4 | 213.3 | 5.5 | 13.7 |
| Checks: | | | | | | | | | |
| Pioneer Brand 39V07 | 5.8 | 12.2 | 72.7 | 63.3 | 64.2 | 100.4 | 210.7 | 0.0 | 9.1 |
| Pioneer 38N88 | 7.9 | 13.7 | 73.1 | 67.3 | 67.3 | 103.9 | 217.5 | 0.0 | 8.5 |
| Pioneer 39N99 | 6.8 | 14.0 | 73.8 | 65.3 | 65.9 | 97.4 | 218.0 | 3.0 | 8.3 |
| Monsanto DKC 35-43 | 5.9 | 12.6 | 76.4 | 66.4 | 66.2 | 93.8 | 215.1 | 0.9 | 10.2 |

Table 4.5. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 2 (Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | Yield (Mg $ha^{-1}$) | MOIST (%) | Weight (Kg $hL^{-1}$) | DA (days) | DS (days) | EH (cm) | PH (cm) | PRL (%) | PSL (%) |
|---|---|---|---|---|---|---|---|---|---|
| Checks: | | | | | | | | | |
| Monsanto DKC 33-54 | 5.5 | 12.5 | 73.9 | 61.8 | 62.5 | 85.3 | 202.2 | 0.0 | 9.9 |
| Monsanto DKC 36-34 | 7.2 | 13.2 | 75.2 | 63.9 | 64.2 | 83.5 | 210.1 | 0.7 | 9.2 |
| Monsanto DKC 30-20 | 5.7 | 12.1 | 76.5 | 62.1 | 62.3 | 90.4 | 217.8 | 0.2 | 7.6 |
| Monsanto DKC 48-12 | 7.6 | 15.4 | 70.6 | 69.3 | 69.7 | 99.2 | 227.8 | 9.0 | 10.7 |
| Monsanto DKC 38-03 | 7.6 | 13.8 | 72.7 | 67.0 | 67.5 | 93.5 | 223.6 | 1.1 | 13.3 |
| Monsanto DKC 31-09 | 6.4 | 12.3 | 72.8 | 62.3 | 62.8 | 85.9 | 216.3 | 2.0 | 7.9 |
| Syngenta N29T-3000GT Brand | 8.9 | 13.7 | 71.1 | 69.0 | 69.8 | 110.4 | 233.3 | 6.0 | 7.7 |
| TR3622 x ND2004 | 5.5 | 13.7 | 74.1 | 63.9 | 64.3 | 88.6 | 211.0 | 5.0 | 15.6 |
| ND2004 x LH176 | 5.2 | 15.2 | 75.4 | 63.3 | 63.7 | 90.6 | 207.6 | 2.0 | 13.5 |
| TR3622 x ND2000 | 5.4 | 12.7 | 73.0 | 63.9 | 65.1 | 91.5 | 204.1 | 1.0 | 13.9 |
| TR3622 x TR4010 | 6.8 | 14.1 | 75.2 | 66.2 | 66.7 | 96.8 | 221.5 | 7.0 | 10.2 |
| TR3030 x TR3622 | 7.5 | 14.0 | 72.4 | 65.8 | 66.3 | 99.9 | 220.0 | 1.1 | 10.0 |
| GP2678 X TR3046 | 7.3 | 13.1 | 72.3 | 66.8 | 66.8 | 105.0 | 236.1 | 2.6 | 8.7 |
| | | | | | | | | | |
| Mean of selections[§] | 6.75 | 14.60 | 72.62 | 65.60 | 66.40 | 99.48 | 220.78 | 5.51 | 14.05 |
| Checks mean[¶] | 6.64 | 13.43 | 73.60 | 65.14 | 65.59 | 95.06 | 217.20 | 2.45 | 10.25 |
| Exp. Mean[#] | 6.47 | 14.34 | 73.04 | 65.33 | 66.07 | 96.01 | 217.21 | 4.44 | 13.06 |
| | | | | | | | | | |
| EMS | 1.72 | 1.00 | 7.87 | 1.52 | 1.53 | 26.42 | 29.23 | 41.57 | 65.78 |
| LSD, 0.05 | 2.57 | 1.96 | 5.50 | 2.41 | 2.43 | 10.08 | 10.60 | 12.64 | 15.90 |
| CV,% | 20.28 | 6.99 | 3.84 | 1.89 | 1.87 | 5.35 | 2.49 | 145.25 | 62.11 |

Table 4.5. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 2 (Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | PRO ($g\ Kg^{-1}$) | LYS ($g\ Kg^{-1}$) | MET ($g\ Kg^{-1}$) | CYS ($g\ Kg^{-1}$) | OIL ($g\ Kg^{-1}$) | STARCH ($g\ Kg^{-1}$) |
|---|---|---|---|---|---|---|
| [(QPM43 X ND2006) x (ND2006)-10]-5-2 x T2 | 101.377 | 3.141 | 2.352 | 2.360 | 48.523 | 696.770 |
| [(QPM43 X ND2006) x (ND2006)-18]-3-1 x T2 | 100.324 | 3.123 | 2.253 | 2.360 | 46.758 | 699.785 |
| [(QPM43 X ND2006) x (ND2006)-8]-1-3 x T2 | 100.694 | 3.118 | 2.268 | 2.358 | 48.414 | 697.765 |
| [(QPM38 X ND2006) x (ND2006)-8]-7-2 x T2 | 101.731 | 3.108 | 2.259 | 2.393 | 48.476 | 696.767 |
| [(QPM38 X ND2006) x (ND2006)-5]-2-2 x T2 | 96.213 | 3.107 | 2.226 | 2.304 | 50.506 | 698.068 |
| [(QPM43 X ND2006) x (ND2006)-8]-2-1 x T2 | 96.349 | 3.100 | 2.215 | 2.291 | 46.623 | 703.846 |
| [(QPM43 X ND2006) x (ND2006)-17]-4-2 x T2 | 96.721 | 3.087 | 2.192 | 2.295 | 47.688 | 702.343 |
| [(QPM43 X ND2006) x (ND2006)-1]-3-3 x T2 | 102.202 | 3.084 | 2.275 | 2.390 | 47.682 | 697.638 |
| [(QPM38 X ND2006) x (ND2006)-5]-5-3 x T2 | 97.392 | 3.073 | 2.181 | 2.352 | 48.258 | 700.187 |
| [(QPM38 X ND2006) x (ND2006)-3]-3-2 x T2 | 94.373 | 3.070 | 2.133 | 2.286 | 48.406 | 702.629 |
| [(QPM43 X ND2006) x (ND2006)-18]-3-3 x T2 | 100.016 | 3.070 | 2.206 | 2.346 | 48.169 | 698.945 |
| [(QPM43 X ND2006) x (ND2006)-11]-3-3 x T2 | 100.597 | 3.068 | 2.235 | 2.354 | 48.826 | 697.505 |
| [(QPM43 X ND2006) x (ND2006)-10]-5-3 x T2 | 96.145 | 3.064 | 2.185 | 2.279 | 48.204 | 702.605 |
| [(QPM43 X ND2006) x (ND2006)-17]-4-1 x T2 | 98.437 | 3.059 | 2.185 | 2.294 | 48.774 | 699.892 |
| [(QPM38 X ND2006) x (ND2006)-5]-5-2 x T2 | 95.114 | 3.054 | 2.174 | 2.334 | 50.253 | 699.471 |
| [(QPM43 X ND2006) x (ND2006)-18]-1-1 x T2 | 95.190 | 3.053 | 2.145 | 2.258 | 49.394 | 701.401 |
| [(QPM38 X ND2006) x (ND2006)-3]-1-1 x T2 | 99.916 | 3.053 | 2.196 | 2.369 | 49.200 | 697.793 |
| Checks: | | | | | | |
| Pioneer Brand 39V07 | 91.908 | 3.170 | 2.116 | 2.203 | 43.692 | 708.974 |
| Pioneer 38N88 | 85.162 | 3.079 | 2.007 | 2.062 | 47.019 | 713.009 |
| Pioneer 39N99 | 85.166 | 2.941 | 1.973 | 2.118 | 48.938 | 710.146 |
| Monsanto DKC 35-43 | 85.807 | 2.992 | 1.958 | 2.098 | 43.129 | 715.992 |

Table 4.5. Selected $S_2$ x T maize hybrids, from combined analyses across six environments (2013 Fargo, 2013 Casselton, 2013 Prosper, 2014 Fargo, 2014 Casselton, 2014 Prosper), of trial 2 (Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | PRO ($g\ Kg^{-1}$) | LYS ($g\ Kg^{-1}$) | MET ($g\ Kg^{-1}$) | CYS ($g\ Kg^{-1}$) | OIL ($g\ Kg^{-1}$) | STARCH ($g\ Kg^{-1}$) |
|---|---|---|---|---|---|---|
| Checks: | | | | | | |
| Monsanto DKC 33-54 | 90.835 | 3.019 | 1.989 | 2.163 | 44.078 | 710.299 |
| Monsanto DKC 36-34 | 88.593 | 2.954 | 2.008 | 2.135 | 45.524 | 710.559 |
| Monsanto DKC 30-20 | 94.446 | 3.049 | 2.100 | 2.258 | 43.712 | 708.634 |
| Monsanto DKC 48-12 | 82.360 | 3.022 | 1.930 | 2.016 | 48.239 | 713.461 |
| Monsanto DKC 38-03 | 83.134 | 3.017 | 1.979 | 2.110 | 46.409 | 714.272 |
| Monsanto DKC 31-09 | 91.980 | 3.052 | 2.067 | 2.140 | 48.229 | 705.442 |
| Syngenta N29T-3000GT Brand | 85.512 | 3.003 | 2.030 | 2.038 | 45.961 | 712.255 |
| TR3622 x ND2004 | 93.968 | 2.929 | 2.066 | 2.240 | 45.288 | 708.281 |
| ND2004 x LH176 | 93.173 | 2.995 | 2.199 | 2.324 | 46.457 | 707.214 |
| TR3622 x ND2000 | 94.769 | 2.997 | 2.064 | 2.268 | 47.564 | 706.057 |
| TR3622 x TR4010 | 85.637 | 2.886 | 1.964 | 2.174 | 46.595 | 713.870 |
| TR3030 x TR3622 | 90.340 | 3.029 | 2.112 | 2.195 | 47.519 | 707.002 |
| GP2678 X TR3046 | 88.236 | 2.972 | 2.007 | 2.178 | 41.800 | 713.007 |

Table 4.5. Selected $S_2$ x T maize hybrids, from combined analyses across six environments, of trial 2 (Stiff Stalk group), based on a relative combination of lower grain moisture, stalk/root-lodging and higher yield, grain quality, and nutritional traits (continued)

| Pedigree | PRO ($g\ Kg^{-1}$) | LYS ($g\ Kg^{-1}$) | MET ($g\ Kg^{-1}$) | CYS ($g\ Kg^{-1}$) | OIL ($g\ Kg^{-1}$) | STARCH ($g\ Kg^{-1}$) |
|---|---|---|---|---|---|---|
| Mean of selections | 98.40 | 3.084 | 2.217 | 2.331 | 48.480 | 699.612 |
| Checks mean | 88.88 | 3.006 | 2.034 | 2.160 | 45.891 | 710.499 |
| Exp. Mean | 95.25 | 3.027 | 2.152 | 2.300 | 47.488 | 703.561 |
| | | | | | | |
| EMS | 0.2212 | 0.0001 | 0.0001 | 0.0001 | 0.0318 | 0.1851 |
| LSD, 0.05 | 9.218 | 0.210 | 0.218 | 0.183 | 3.494 | 8.432 |
| CV,% | 0.49 | 0.35 | 0.52 | 0.41 | 0.38 | 0.06 |

†:MOIST, harvest moisture; TWT, harvest test weight; DA, days to anthesis emergence; DS, days to silking; PRL, percentage of root lodging; PSL, percentage of stalk lodging; PRO, protein; LYS, Lysine; MET, Methionine; CYS, Cysteine.

§ mean of selected entries, ¶mean of checks, # mean of experiment.

Selection were carried out by sorting yield in descending order and then sorting by lysine level in descending order, then selected $S_2$ testcrosses were screened for lower moisture, higher test weight, lower per cent root and stalk lodging, and lower days to anthesis and silking, higher protein, and methionine. The basis of selection was comparing the selected $S_2$ testcrosses with best check and mean values of checks for respective traits.

## GENERAL CONCLUSION

Based on the early-generation hybrid yield trials results, a totall of 48 $S_2$ lines were selected for further development (31 from the non-SS group and 17 from the SS-group). All the selected lines have potential to develop short season inbred lines and hybrids with high grain yield and protein content, high levels of lysine, tryptophan, and methionine.

The results of this research show, for the first time, the successful adaptation of QPM genotypes to short-season environments. NDSU, through its maize breeding program and the uniqueness of this particular project, lead the first national program to adapt Quality Protein Maize (QPM) genotypes to the northern U.S. and to extensively evaluate elite and adapted populations, lines, and hybrids for amino acid composition. As a consequence, we are providing added value to U.S. northern farmers and ranchers by developing NDSU corn hybrids for not only ethanol utilization but also for high quality protein products, the next generation of healthier hybrids through the NDSU EarlyQPM and EarlyQPMF programs.

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