Genetic analysis of the IBM2Syn10-DH maize population for response to low and high nitrogen input

by

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ABSTRACT

Maize growth and development depends highly in the capacity of the plants to absorb Nitrogen (N) from the soil. Producing a high-yielding maize crop that requires less N input is currently one important goal of maize breeding programs. In order to understand the dynamics of N use in maize, the study of phenotypic and genetic response to N deficiency must be performed. Using lines from the high resolution IBM2Syn10-DH population, the goals of this study were: 1) to identify the phenotypic response of the root system architecture (RSA) of 14-day old maize seedlings grown under contrasting levels of N; 2) to discover Quantitative Trait Loci (QTL) that are associated with the RSA response to N variation; 3) to analyze the agronomic response of DH lines grown in 4 environments under contrasting N treatments; and 4) detect QTL associated with the variation of this agronomic response.

A subset of IBM2Syn10-DH lines grown in a cigar roll culture under controlled growth chamber conditions was used to gather phenotypic data to perform a QTL analysis of the RSA traits. A Low N (LN) treatment increased primary root length (PRL), lateral root length (LRL), and lateral root number (LRN) by 8.5%, 31% and 20%, respectively. Alternatively, crown root number (CRN) increased 6.4% and shoot length (SL) grew 12.9% longer under HN treatment. A total of 57 QTL among 8 traits were identified using composite interval mapping (CIM) and a high density genetic map. The results suggest that genomic regions are triggered by N deficiency stress, and control the root system growth for better nutrient acquisition and remobilization.

Several agronomic traits and grain quality traits were measured at independent environments in two locations in Iowa and two consecutive years. Overall, the data showed that

effective LN treatments reduced the DH-lines performance significantly. Grain yield decreased up to 63% at one environment. Grain protein (GPRT) was significantly reduced by 10% under LN conditions. A total of 302 QTL were identified across all trait/environment/N-level combinations. Important QTL clusters located in chromosomes 1, 4, 5, 8 and 10 harbored QTL detected under LN or HN treatments. These clusters are located near loci *gln4* and *gln5*, which regulate the activity of glutamine synthetase; an enzyme involved in N-assimilation and N-remobilization for the production protein in the grain of maize.

CHAPTER 1: GENERAL INTRODUCTION

Maize (*Zea mays* L.) is one of the most widely grown crops in agriculture around the world. The use of Nitrogen (N) application, along with higher planting densities over the last decades have been the key elements for maize yields increase, especially in the United States. Today, the rising cost of producing nitrogen will determine an extra economic load on the farmers (Hirel et al., 2007a). This will necessarily lead to a change in agriculture management, especially in the industrialized counties as well as some developing countries; demanding among other things, a greater productivity of new genotypes under poorer soils conditions. Thus, farmers must be able to optimize the usage of N fertilizer to reduce the contamination with nitrates and to preserve their net income (Bertin and Gallais, 2001). On the other hand, new interesting alternatives have appeared in the market for farmers; being one of those the production of biofuels. Farmers can expand their market to new business, which helps to overcome the difficulties with the rising prices; but it becomes another economic and environmental challenge to the world. The production of biofuels from plant biomass requires the same extensive use of N fertilizers for several species (Hirel et al., 2001).

Overall, the use of N fertilizer will be critical for the production of high yields in all of the main crops that contribute to the global supply of food (FAO, 2012). The required worldwide production for the next century of rice, wheat and maize among other crops, currently cannot be achieved while reducing the amount of N fertilizers at the same time. It is estimated that the demand for 2016 of nitrogen fertilizer will be of 116.0 million tones worldwide (FAO, 2012). Then, it is critical to identify main factors controlling plant nitrogen use efficiency (NUE) from the physiological and genetic points of view; in order to maintain a positive balance between the

worldwide food requirements and an economically viable supply of resources from agriculture (Hirel et al., 2007b).

Thus, to understand better NUE it is necessary to conduct genetic studies that can explain the relationship between N metabolism and agronomical and physiological traits that have been widely studied as part of maize breeding programs. QTL mapping can be a useful tool that can identify genomic regions that control specific traits as well as to determine possible breeding strategies. Consequently, there is the need to study the coincidences among the QTL for several traits associated to N metabolism and specific QTL for NUE (Coque et al., 2008). Furthermore, it is important to determine genomic regions that are associated with maize traits that measure N-assimilation (i.e. root system architecture) and N-utilization (i.e. grain yield). The difficulty for measuring physiological traits, such as root system N absorption and its components, at a large scale represents a disadvantage to determine if QTL are associated with these traits. Thus, it is important to focus the efforts in the identification of QTL for agronomic traits with significant associations to N remobilization throughout the development of a maize plant (Coque et al., 2008; Hirel et al., 2007b).

The maize bi-parental IBMSyn10-DH population was used in this study to perform a genetic analysis associated to N response. Two contrasting N treatments were set up in various different experiments to provide a stressful environment (i.e. Low N) and a normal growing environment (i.e. High N). The general objectives of this study were to: i) identify QTL for grain yield and related traits and grain quality traits that are associated with N response in field assays, ii) identify QTL for root system architecture traits associated with N response under *in vitro* conditions in 14-day old maize seedlings.

Literature Review

Nitrogen budget in maize

The input of nutrients, but especially N is essential for plant growth and development in agriculture in the US. Nevertheless, excess N use in farm systems has negative impacts on aquatic life and limits the use of water bodies for recreation and as drinking water sources. There are several sources of N input to the agriculture system: commercial fertilizer, legume fixation, N mineralization of organic matter, manure from livestock waste, and atmospheric deposition; which are collectively known as budget inputs (Libra et al., 2004). It has been estimated that these N inputs total nearly 4 million tons in the state of Iowa, and over 90% of it is used in agriculture, especially for maize production. Of that total, around 200,000 tons of N will be lost to stream and water resources (Libra et al., 2004). Thus there is a need to improve the efficient use of N by improving management practices (i.e. reducing N rate, improving N supply systems), and by breeding for more NUE crops.

For maize, N fertilizer is one of the largest expenses, and producers need to balance between an economically profitable harvest and controlling environmental harms (Sawyer et al., 2006). It is also the most limiting nutrient for production of maize in the Midwest region of the US. Thus, it is important to understand how N is accumulated during maize growth to identify key stages to improve NUE. A report of N use guidelines summarizes key information of N budget in maize throughout the cycle of development (Figure 1.1; (Sawyer et al., 2006)). Thus, for high-yielding maize crop, the N accumulation starts at about 1 lb. N/acre, until the V4 growth stage. Then, until the tasseling stage maize will produce around 9,000 lb. /acre of aboveground dry matter, and will accumulate around 200 lb. N/acre. During this time, the majority of N will be accumulated in the leaf tissue (75%), with the stalk (20%) as a second source of storage for

the nutrient. From then on, N-uptake rate will reduce during reproductive stages, but available N in plant tissue will begin to remobilize for grain formation and protein and starch synthesis. At physiological maturity, the high-yielding maize crop would have accumulated around 275 lb. N/acre to generate more than 20,000 lb. /acre of aboveground dry matter. Thus, by the end of maize development more that 50% of the acquired N will be in the grain portion of the crop.

NUE and related traits

Nitrogen use efficiency can be defined in several ways with slight differences in the concepts. Agrama et al. (1999) defines NUE as grain produced per unit of N supplied. Another definition is the amount of grain yield per unit of available N in the soil (including residual N present in the soil and the fertilizer) (Moll et al., 1982). Also, from more of a breeding perspective, NUE can be defined as the superior ability of a given individual to produce higher grain yields at low soil N conditions, in comparison with other individuals of the same population (Presterl et al., 2002).

NUE is divided into two primary components: N-uptake efficiency (NUpE) and N-utilization efficiency (NUtE) (Moll et al., 1982). N-uptake efficiency measures the amount of N (as nitrates and ammonium ions) absorbed by the plant compared to that available in the soil (Presterl et al., 2002). Given optimal N conditions, N-uptake is important to supply enough N as it is demanded by the maize plant for growth and development, meanwhile at sub-optimal N levels it is dependent on the capacity of the root system characteristics to acquire and remobilize the scarcely available N from the soil (Presterl et al., 2002). N-utilization efficiency measures the use of available N stored in the plant to produce grain in the ear. NUtE is influenced by the

proficient remobilization of N from the root system to source tissues (i.e. leaves and stalk) of the plant (Nichols, 2008).

To be able to reduce the amount of N used to produce equal or greater yields as the ones currently acquired worldwide, a better understanding of NUE components is needed, which could lead to diminish costs of production and environmental hazards related with maize agriculture (Nichols, 2008). However, measuring and analyzing the components previously discussed is difficult due to the labor intensive techniques needed to physiologically assess the variability within each component. Thus, it is important to establish a set of N responsive agronomical traits that can be phenotyped in a high throughput manner which are related to NUE in maize. Previous studies, have reported significant phenotypic and genotypic variation for NUE-related traits (Bertin and Gallais, 2000; Coque and Gallais, 2007; Gallais et al., 2005). In order to determine N-remobilization efficiency focus can be directed to grain yield and related traits to search for existing correlations with nitrogen input. It has been reported that significant reduction in grain yield (23%) and kernel number (13%) can be associated with low N input (Coque and Gallais, 2007). Moreover, the authors determined that to improve grain yield, an increased N-remobilization and post-silking N-uptake were key factor during the grain filling stage. Consequently, the demand of greater grain yield on breeding programs has pointed selection towards maize germplasm that will perform well under high N conditions due to the management practices of agriculture in developed countries (Moose and Below, 2009); which represent the main market for the major seed companies.

Likewise, studies have focused in the analysis of grain composition to better understand N-utilization efficiency by ears and seeds (Nichols, 2008). The concentration of grain protein has shown to be responsive to the increment of N supply, providing evidence of an inverse

relationship grain yield-protein controlled by N variation (Uribelarrea et al., 2004). However, the authors concluded that high grain yield can be maintained by increasing the N-utilization of the plant to produce higher kernel number while selecting high grain protein concentration.

Hence, significant research in NUE has focused in finding differences in genetic variation, productivity and physiological responses of maize hybrids between contrasting N conditions (Moose and Below, 2009), based on agronomic traits like grain yield and kernel composition, among others.

Root system architecture

One of the main components of NUE is the N-uptake efficiency. It has a close relationship with the capacity that the root system of plant has to assimilate the nutrients available in the soil. Thus, it is important to understand the changing aspects of nutrient availability and how plants can acquire these at each key developmental stage (Shen et al., 2013). Under low N conditions, plants have adapted their response by the alteration of root system architecture (RSA) to increase N acquisition from the soil at minimum metabolic cost (Lynch and Brown, 2001). Given the mobile nature of N in the soil, and that it is one of the limiting factors for plant growth, it is highly important to analyze the RSA of maize to improve overall NUE (Lynch, 2013).

In maize, the root system of young seedlings constitutes of two set of root types that develop during and after seed germination. The embryonic roots which are the primary and seminal roots, and the postembryonic roots that are the crown and lateral roots (Hochholdinger and Tuberosa, 2009). During the earlier stages of development, the embryonic roots make up the majority of the root system, and the number and volume will vary depending on specific genetic

background. However, later in development the crown roots and especially lateral root will make up the majority of the RSA. The first ones will be responsible of initial transportation of nutrient to the shoot and in adult stages will maintain the plants erect. The latter roots are the main structure in the RSA that are responsible of nutrient absorption and assimilation for the plant (Hochholdinger, 2009).

Several studies under depleted mineral nutrient conditions have been performed in order to determine the variation of the root architecture of plants due to this type of stress (Lynch and Brown, 2001; Wang et al., 2005; Zhu et al., 2005a, b). The effect of the nutrient depletion in root morphology can be complex, but some patterns have been found. First, root elongation increases under low N and low P levels, resulting in longer seminal roots, crown roots and primary roots (Liu et al., 2008; Zhu et al., 2005b; Zhu et al., 2006). Longer primary roots, will provide deeper exploration for the root system to reach N and water which are mobile nutrients. On the other hand, longer seminal roots will enable the root system to be swallower and thus explore for P and K availability. Second, an increase number and length of lateral roots was observed (Liu et al., 2008; Zhu et al., 2005a). More lateral root biomass provides a better assimilation capacity, which will be highly beneficial under low nutrient conditions. And third, a higher root-to-shoot ratio (R:S) was observed in young maize seedlings (Abdel-Ghani et al., 2013; Zhu and Lynch, 2004). Nutrient stress prompts the seedling to develop more root biomass in order to absorb the limited available N or P, saving energy in shoot development at least in early developmental stages. Overall, it is well documented the relationship of nutrient (i.e. N and P) depletion and the effect on root system morphology (Lynch, 2013; Mackay and Barber, 1986; Mi et al., 2010; Wang et al., 2005). Moreover, the results indicate that the adaptation of the RSA is important for effective N-uptake at different stages of development (Cai et al., 2012).

QTL mapping

Several studies have demonstrated the presence of genotypic variability across different maize population for traits associated to N-response and the components of NUE (Agrama et al., 1999; Bertin and Gallais, 2001; Cai et al., 2012; Coque and Gallais, 2007; Ribaut et al., 2007). These works used mainly QTL mapping as the primary genetic analysis NUE-related traits. Authors were able to map QTL for NUE-related traits to different genotypic regions where loci controlling factors of N metabolism were found. At low nitrogen input, QTL for traits related with N-utilization were found. Meanwhile, at high N-input QTL for traits related with N-uptake were detected (Bertin and Gallais, 2001; Gallais and Hirel, 2004). Across the studies, authors identified clusters of QTL that were co-localized in specific regions throughout the maize chromosomes. These clusters usually contained QTL for agronomic traits related to NUE (i.e. grain yield, anthesis-silking interval (ASI), etc.), and physiological traits that determined N status (i.e. leaf N content, N-remobilization, etc.) which were not specific for either N level (Gallais and Hirel, 2004; Hirel et al., 2001). However, these earlier studies had a low genetic resolution for QTL mapping (99 molecular markers by Agrama et al., 1999 and 152 markers by Hirel et al., 2001) given by the reduced number of marker loci used. More recently, QTL studies in response to N have increased the resolution 662 SSRs markers (Cai et al., 2012) and more than 2,000 marker loci (Nichols, 2008); which make the results more useful since smaller genomic intervals can be targeted.

The prior information has characterized the genetic complexity of NUE and related traits. Similar to grain yield or drought tolerance, NUE is controlled by several loci that have varying effect according to the stress level (Nichols, 2008). Some of these loci contain genes that have being described as genes of interest for N metabolism, and that are involved in N-assimilation

and N-utilization (Hirel et al., 2001). A few of these genes are controlling nitrogen reductase (NR), glutamine synthetase (GS) and cytokinin oxidase (CKO) activities, which are involved in signal transduction in the N pathway as well as amino acids reallocation from source to sink organs (Hirel et al., 2001; Nichols, 2008).

Similar QTL analysis have been reported for nutrient response in RSA traits in maize presenting high genotypic variability (Bohn et al., 2006; Cai et al., 2012; Hund et al., 2011; Liu et al., 2008; Wang et al., 2005; Zhu et al., 2005a, b; Zhu et al., 2006). Results showed that QTL identified for specific traits of RSA can be co-localized independent of the nutrient stress that the plants have been submitted to. There are some genomic regions that appear to be more involved in RSA control under different abiotic stress conditions. Evidence of this is that results of analysis performed across QTL studies can be co-localized throughout a consensus genetic map to form cluster of QTL with specific physiological functions (Hund et al., 2011). Authors identified at least six candidate genomic regions (bins 1.07, 2.04, 2.08, 3.06, 6.05 and 7.04) that harbored several QTL for root length. More importantly, the number seminal roots were found to be continuously associated with grain yield and related traits genomic regions. This can present a great potential for further exploration of these QTL collocations, which could lead to develop better breeding strategies. Furthermore, genes for root development in maize have been described (Hochholdinger and Tuberosa, 2009; Taramino et al., 2007; Woll et al., 2005), which highlight potential genotypic regions to be studied more in depth. Genes such as *Rtcs, Rum, Rth1* and *Rth3* are some of the genes which are involved in crown, seminal and lateral roots development that could be affected by the exposure to N deficiency conditions.

Organization of the Dissertation

This dissertation is aimed to address the response to N deficiency at two stages of development of maize doubled haploid lines of the IBMSyn10-DH population. The first chapter is a general introduction and a literature review of the main topics concerning research described in the following chapters. The second chapter focuses on the development of the root system architecture of 14-*day* old seedlings grown under controlled conditions. The main objectives were to assess the phenotypic and genotypic variation present in a subset of the bi-parental population lines exposed to normal and low N growing conditions. The third chapter will instead focus in quantifying phenotypic and genotypic variability of the response to high and low N growing conditions measured in field experiments for the same bi-parental population. RSA traits, grain yield and related traits, as well as grain composition traits were used to identify QTL associated to the N response in maize. The final chapter is used to do a general conclusion of the studies described throughput the dissertation.

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Figures

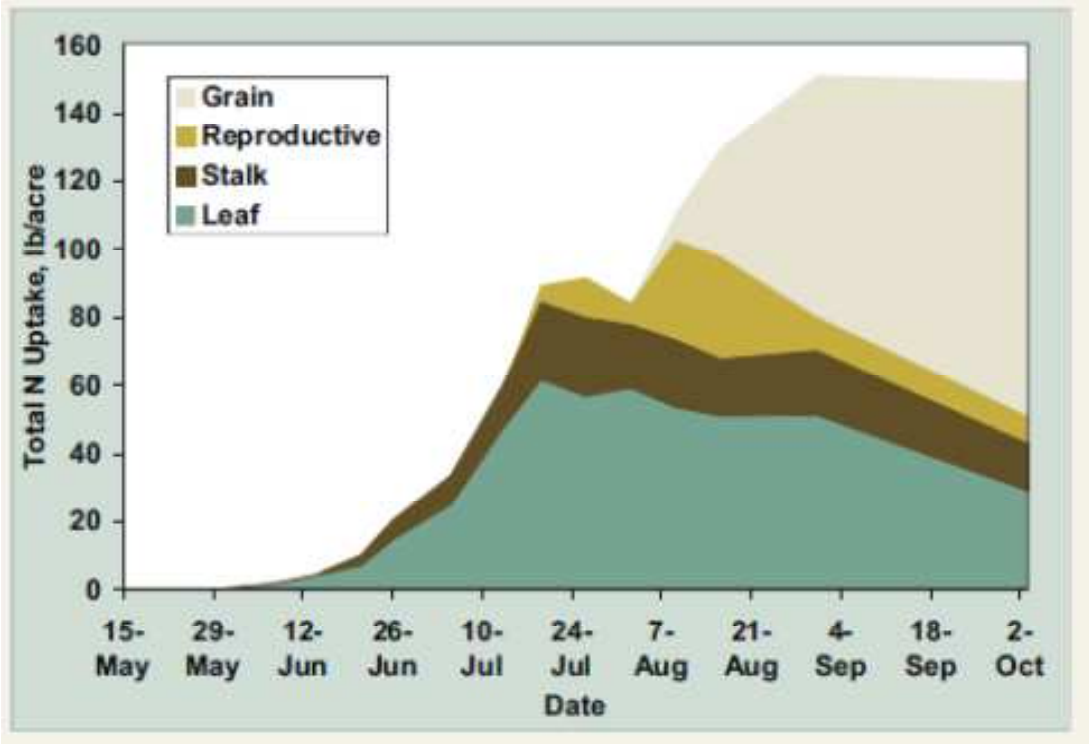

Figure 1.1. Maize aboveground nitrogen accumulation

Partition of N-uptake accumulation into the different plant components during maize growth and development cycle**.** The figure is borrowed from (Sawyer et al., 2006).

CHAPTER 2: GENOTYPIC ANALYSIS OF THE ROOT SYSTEM ARCHITECTURE OF MAIZE IN RESPONSE TO LOW AND HIGH NITROGEN INPUT

A paper to be submitted to Experimental Botany

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Abstract

Maize growth and development depends highly in the capacity of the plants to absorb Nitrogen (N) from the soil. Low N (LN) availability can become a key limitation to improve maize performance. The root system is the primary component for plant adaptation to environments that contain reduced amounts of N. The objective of this study was to identify the response of the root system architecture (RSA) of 14-*day* old maize seedlings to contrasting levels of N, from a phenotypic and genotypic point of view. A subset of IBM2Syn10-DH lines grown in a cigar roll culture under controlled conditions were used to gather phenotypic data to perform a QTL analysis of the traits. A LN treatment increased primary root length (PRL), lateral root length (LRL), and lateral root number (LRN) by 8.5%, 31% and 20%, respectively. Alternatively, crown root number (CRN) increased 6.4% and shoot length (SL) grew 12.9% longer under HN treatment. A total of 57 QTL among 8 traits were identified using composite interval mapping (CIM) with specific LOD thresholds for each trait-N treatment combination. An individual QTL could explain 5.9% to 16.5% of the phenotypic variation. QTL formed clusters in chromosomes 3 and 10, which suggest being genomic regions associated with response of RSA traits to LN environments. Our results suggest that genomic regions are triggered by N deficiency stress, and control the root system growth for better nutrient acquisition and remobilization. It should be possible to exploit genetic variation available to

develop maize varieties that absorb and remobilize efficiently N for the final goal of grain production.

Introduction

Over the past few decades the production of food around the world was doubled, which is closely related with the seven times increase in nitrogen (N) fertilizer utilization (Hirel et al., 2007a). As the worldwide population increases, the rising demand for food will continue to cause an effect on how N and other fertilizers are used. However, since extensive use of N represents higher cost for farmers and higher pressure to the environment (Lynch, 2013), there is the need to develop more efficient cultivars which can produce higher yields with less nutrient supplementation.

Plants adapt their response to nutrient stress conditions in different ways. The alteration of the root system architecture (RSA) increases nutrient acquisition from the soil at minimum metabolic cost (Lynch and Brown, 2001). Thus, RSA is a potential target to improve N uptake. Several efforts have been made to develop suitable ideotypes of RSA to improve the performance of maize under different N requirements (Lynch, 2013; Mi et al., 2010; Shen et al., 2013). An efficient ideotype of maize for improved N acquisition would have: i)steep and deep embryonic roots that can reach N moving down the soil; ii) swallow and thin seminal roots; iii) numerous and highly active lateral roots for maximum absorbance of available N; and iv) long and steep post-embryonic crown roots that support the plant as well as absorb N and water from surface to deep soil (Lynch, 2013; Mi et al., 2010).

Roots become essential to uptake the small amount of N available in N-depleted soils (Gallais et al., 2005; Kamara et al., 2003). Despite of its biological importance, there is a

limitation to study root characteristics in the soil due to the need to extract the whole system intact (Guingo, 1998). To solve this problem, different types of growing techniques have been used such as germination paper culture system (Zhu et al., 2005a), artificial soil (Wang et al., 2005), agar-like gel systems (Iyer-Pascuzzi et al., 2010), among others. These methods provide the ability to examine unbroken roots and also help to examine the amount and the timing of nutrient input (Liu et al., 2008; Zhu et al., 2005b). A disadvantage of using artificial growing methods is that results may not be directly correlated to the expected results when growing in soil. However, with the increasing amount of studies performed for RSA analysis and increased capacity of statistical analysis across experiments and populations (Hund et al., 2011), it may be possible to have stronger extrapolations of results among different platforms and the field trials. New phenotyping methodologies can improve the amount of samples that can be analyzed at the time and improving the resolution of the analyses (Hund et al., 2009; Iyer-Pascuzzi et al., 2010).

Several studies point out the abundant phenotypic variability among different maize populations subjected to various levels of nutrient availability (Abdel-Ghani et al., 2013; Liu et al., 2008; Mackay and Barber, 1986; Wang et al., 2005; Zhu et al., 2005a; Zhu et al., 2006). The RSA is constantly adapting to the changing conditions in the growth substrate. It has been shown that the embryonic roots tend to develop more when nutrient availability is low (Liu et al., 2008; Zhu et al., 2005b). More specifically, primary root length (PRL) increases under low N (LN) treatments (Abdel-Ghani et al., 2013); longer axial roots (ARL) were reported to be important for efficient N acquisition (Liu et al., 2008); and seminal root length (SRL) and number (SRN), also increased as response to P depletion (Zhu et al., 2006).

In addition to the reports on phenotypic variability, there are many reports of high genotypic variability analyzed in QTL and association mapping studies (Abdel-Ghani et al.,

2013; Bohn et al., 2006; Cai et al., 2012; Hund et al., 2011; Liu et al., 2008; Wang et al., 2005; Zhu et al., 2005a, b; Zhu et al., 2006). QTL analysis is a powerful tool to determine the genetic principles of complex traits like RSA that are highly affected by environmental conditions (Hochholdinger and Tuberosa, 2009; Shen et al., 2013). Some QTL for RSA traits measured under high (HN) and low N conditions have been reported (Liu et al., 2008). The study identified 17 QTL across eight maize chromosomes in 94 recombinant inbred lines (RILs). The QTL were detected on 5 root traits that measured root length and number of different sections of the RSA of 20 *day*-old seedlings. Overall, seven, four and six QTL were found for LN, HN treatments and LN/HN ratio, respectively. A major QTL was identified for average axial root length that explained 43.7% of the phenotypic variability. It was detected in chromosome 1 under LN conditions, and co-localized with QTL for N-uptake and grain yield. Furthermore, similar QTL were reported for root traits in response to high and low P conditions (Zhu et al., 2005a, b; Zhu et al., 2006). From all QTL identified in these studies, only few QTL showed a major effect, suggesting that RSA could be controlled by groups of small-effect loci that are activated according to the environmental conditions (de Dorlodot et al., 2007). A recent QTL meta-analysis was performed to target the control of root length in maize across several QTL studies (Hund et al., 2011). In this study, QTL associated with traits related to root length were grouped together at specific genetic positions throughout a consensus genetic map, also some root length QTL collocated with QTL for grain yield and drought response. The evidence of phenotypic and genotypic variability mentioned above suggest that more research is needed to analyze the RSA phenotypic variability using various maize populations and that phenotyping methods in combination with genetic and statistical analysis in different maize populations can help to determine the genetic basis for RSA response to nutrient depletion.

In this study a set of 153 doubled haploid lines of the IBM2Syn10-DH (Hussain et al., 2007) population was evaluated, which provides a higher genetic resolution for QTL mapping given the amount of recombination accumulated by intermating. The experiments were grown under low and high N conditions in a germination paper type culture, and carried under controlled conditions in growth chamber. To our knowledge, there are no studies reported for the analysis of the genotypic variation of the RSA in the IBM maize population under different N treatments. The objectives were to i) analyze the phenotypic variation of the DH-lines at 14-*day* old seedling stage grown under LN and HN conditions, ii) determine significant phenotypic correlations and the repeatability of the experiments, and iii) identify QTL that control traits of the RSA under two N treatments.

Materials & Methods

Plant material

The IBM2Syn10 Doubled Haploid (DH) mapping population of maize (*Zea mays* L.) was used for this study. The population was developed by Pioneer Hi-Bred, and it consists of a set of 360 doubled haploid lines (Hussain et al., 2007). These DH lines were produced from a randomly mated population derived from the cross between B73 x Mo17 after 10 generations of inter-mating, which was obtained from A.R. Hallauer at the Department of Agronomy, Iowa State University, Ames, Iowa. The amount of recombination accumulated after 10 generations of random mating provides the possibility of higher resolution genetic mapping. The germplasm combines important genotypic variability that could be representative of some of the current U.S. maize gene pool. Moreover, the population contains a significant amount of phenotypic variability between the lines (Hussain et al., 2007), which makes it useful for QTL mapping. A subset of 153 DH lines was chosen at random from the entire population.

In a previous study the parental inbred lines of the IBM2Syn10-DH population were compared under different nitrogen levels (Balko and Russell, 1980). B73 was found to have a significant increase in yield and other related traits in response to higher supply of N fertilizer when compared to Mo17. This difference in N response provides evidence of possible phenotypic and genotypic variation in the DH population for the objectives of this study.

Root development study in young maize seedlings

The root development analyses were performed in a growth chamber given the difficulties of carrying controlled experiments in the field, and because of the need of having intact root systems to measure. The protocol followed for germination and root imaging has been previously described (Abdel-Ghani et al., 2013), and was used with minor modifications.

First, the kernels were sterilized with a 6% sodium hypochlorite (Clorox®) solution for 10 minutes, and then washed three times with deionized and sterile water. Sterile kernels of each DH line were placed on germination paper (Anchor Paper, St. Paul, MN, USA), previously treated with Captan® fungicide (1.5 g/l), and rolled-up vertically. 11 to 12 rolls were placed per 2 L glass beaker containing Hoagland solution (Hoagland and Arnon, 1950). Two separate sets of Hoagland solution were used, HN containing 15mM of NO_3^- and LN containing 1.5mM of NO_3^-. The pH of each solution was adjusted to 6.0 using NaOH. The sets of HN and LN glass beakers were moved into growth chamber with controlled conditions. The photoperiod was 16 hours light (200 µmol photons m^{-2} s^{-1}) to 8 hours dark at 23 °C and 55 – 60% relative humidity.

Experimental design

Or this study, 153 DH lines were separated into two sets and were replicated twice within the growth chamber. Each replication consisted of HN and LN treatments. The parental lines B73 and Mo17 were included with each set of DH lines and each set was replicated in time. For each DH line contained in a paper roll, the three most homogeneous and healthiest seedlings were selected for further measurements. This design of the study allowed having 6 data points for a genotype per N treatment in a given experiment.

Phenotypic measurements

Seedlings were grown for 14 days in controlled conditions, and then placed in 30% ethanol in a cold chamber to prevent further development of shoots and roots. Several root and shoot measurements were recorded. Primary root length (PRL), crown root number (CRN), seminal root number (SRN), and shoot length (SL) were either manually measured with a metric tape or counted. Lateral root number (LRN), and lateral root length (LRL) were estimated using the scanner-based root analysis software WinRhizo (WinRhizo Pro 2009, Regent Instruments, Quebec, Canada). Once the phenotypes were measured, roots and shoots were oven-dried at 48°C for 60 hours in separate envelopes. Root dry-weight (RDW) and shoot dry-weight (SDW) were measured using an analytical scale (Sartorius Research R300S, Germany). Four phenotypes were calculated. Total root length (TRL) was calculated by adding PRL and LRL, total plant biomass (TPB) by adding RDW and SDW, root to shoot ratio (R:S) by dividing RDW by SDW, and root to shoot length ratio (PRL:SL) by dividing PRL by SL. All values were averaged over three seedlings per genotype, except for RDW and SDW, where all the roots from the three

seedlings were bulked together in an envelope, as well as the shoots in a separate envelope per genotype.

Data analysis

The means for each of the 12 traits were analyzed separately under HN and LN treatments. LSmeans, minimums, and maximums were used to establish phenotypic differences between the respective N levels. The percentage of variation of the means due to the N stress was calculated by 100 - ((LN/HN)*100). An analysis of variance (ANOVA) was used to establish significant statistical differences between the applied N treatment as well as the genotypic variation and corresponding interactions. A mixed model procedure (PROC MIXED method = type3) was chosen to run the SAS software (SAS, 9.3). The linear model used was the following: $Y_{ijkl} = \mu + E_i + R_{(i)j} + N_k + G_l + N*G_{kl} + E*N*G_{ikl} + e_{(i)jkl}$; where observation Y_{ijkl} is the phenotype given by μ which is the population mean, E_i is the effect of the *ith* experiment, $R_{(i)j}$ is the effect of the *jth* replication within the *ith* experiment, N_k is the effect of the *kth* nitrogen level, G_l is the effect of the *lth* genotype, $N*G_{kl}$ is the N level-by-genotype interaction, $E*N*G_{ikl}$ is the interaction of each experiment-N level-genotype combination, and $e_{(i)jkl}$ which is the error term of the model. Experiments and N levels were considered as fixed effects, while genotypes, replications and the interactions were treated as random effects.

Variances obtained with this procedure were used to estimate the repeatability of the process on an entry-mean basis (Fehr, 1987). The formula used was: $\frac{\sigma_G^2}{\frac{\sigma_e^2}{RNE} + \frac{\sigma_{GNE}^2}{NE} + \frac{\sigma_{GN}^2}{N} + \frac{\sigma_R^2}{R} + \sigma_G^2}$; where σ_G^2 is the genotypic variance, σ_R^2 is the replication variance, σ_{GN}^2 is the variance of the genotype *x* N level interaction, σ_{GNE}^2 is the variance of the triple interaction of genotype *x* N level

x experiment, and σ_e^2 is the residual variance. The denominator factors R, N, and E represent the number of replications, N levels and experiments respectively. Besides, the repeatability was calculated within each N level using the variance component obtained with a simplified model. Thus, the formula used was: $\frac{\sigma_G^2}{\frac{\sigma_e^2}{RE}+\frac{\sigma_{GE}^2}{E}+\frac{\sigma_R^2}{R}+\sigma_G^2}$; where σ_{GE}^2 is the variance of the genotype *x* experiment interaction, and the other terms in the formula are the same as previously described. Also, Pearson correlation estimates were calculated using PROC CORR in SAS for each N level separately.

QTL analysis

Of the 153 DH lines included in the study, 142 produced high quality genotypic data. Due to this factor, 142 DH lines were used for the QTL analysis. This analysis was carried out with QTL Cartographer version 1.7 (Basten et al., 2005) using the model composite interval mapping (CIM). The cofactors were set to the 10 more significant, and were identified with forward and backward regression. 1 cM intervals were used to scan within each analyzed QTL (walking speed); and the window size was set to 10 cM to block out regions around the test interval. In order to determine the experiment-wise levels of significance and control the comparison-wise probabilities 1000 permutation tests were conducted in each analysis performed independently for each trait. Given the permutations results, significant thresholds were determined for each trait and under each N level (Table A2.1). These thresholds ranged among all traits for HN 4.09 to 4.25 LOD. For LN the range was from 4.10 to 4.25 LOD.

Eight traits out of the twelve originally measured were used for the QTL analysis. SRN and RDW were excluded due to the lack of significant differences at the N level found after the

analysis of variances. Also, R:S and PRL:SL ratios are phenotypic calculations to simplify the quantitative comparison between the roots and shoot development influenced by N stress. However, ratios are discarded from the QTL analysis due to the interdependence of the traits used to calculate them.

A high-density genetic map was used for QTL mapping. The map developed by (Liu et al., in preparation) at the Beijing Genomics Institute (Beijing, China); consist of 6,618 recombination bins developed by genotyping-by-sequencing (GBS). The IBM2Syn10-DH population was re-sequenced to search for SNPs among the DH lines. A 15-SNP sliding window was used to determine the recombination break points (Huang et al., 2009), which were used to create recombination maps, or so-called bins maps. All DH lines were aligned and compared to intervals of at least 100kb. This comparison yielded the 6,618 recombination bins, which captured the majority of recombination events among the DH lines.

The resulting GBS generated map of the IBM2Syn10-DH had a genetic distance of 11,198.5cM. The average genetic distance among the bin markers was 1.7cM. Additionally, the map length was adjusted to a F2-based map comparable length to run the QTL analysis. The equation used to calculate the expansion factor is, $\alpha = \frac{j}{2} + \frac{2^i - 1}{2^i}$, where j is the number of generations of inter-mating, counting the two generations for creating the F2 segregating population, and i is the number of generations of inbreeding after inter-mating (Teuscher et al., 2005). In the case of IBM2Syn10-DH, j=12 and i=1, due to only one generation for the DH process after inter-mating. The resulting expansion factor was 6.5, which was directly used to adjust the new map to 1,722.9cM.

Results

Phenotypic results

Significant statistical differences over the two nitrogen levels were found for 10 of the 12 traits (Table 2.1). Only SRN and RDW did not show response to the N treatment, however significant genotypic variation was observed for both traits (Table 2.1). Highly significant differences among DH lines were observed for all traits.

LSmeans were estimated across all experiments to capture the variation of the means given by the N treatments (Table 2.1). Root development was more prominent under LN levels. The main evidence is given by the increase in PRL (8.6%), LRL (30.5%), and TRL (~26%). Another important root trait that showed higher values under LN levels was LRN. The development of lateral was 20% greater at LN than at HN. Weight and length ratios were consistently higher in the LN treatment (R:S 16.6%, PRL:SL 23.6%).

However, traits like SL and SDW increase 12.9% and 11.6% respectively in the HN treatments. Also, CRN was higher under HN levels by 6.4% compared to LN. Overall shoot biomass influenced in the TPB positively in HN, which was 7% higher than LN.

Variance components and repeatability

The analysis of variances was made across the Experiments and N levels (Table 2.2). The variance components calculated showed significant statistical differences among all genotypes for all traits. Even though the N effect was significant for almost all the traits, there were no significant N*Gen interactions. Using the values of the variance components (Table 2.2), repeatability for each trait was calculated on an entre-mean basis. The results showed that repeatability ranged between 0.70 (RDW) and 0.88 (SRN). Furthermore, repeatability was also calculated within each N level to assess the quality of the date for QTL mapping. In HN level

(Table 2.3) the repeatability values ranged from 0.50 (RDW) to 0.79 (CRN); while in LN level (Table 2.4), the repeatability ranged between 0.40 (LRN) to 0.78 (SRN).

Phenotypic correlations

The phenotypic correlations were calculated separately for each N level (Table 2.5). The majority of correlations were statistically significant. The range of the magnitudes of the correlations varied widely within each N level. For Low N the range went from -0.64 to 0.99. In the case of High N, these values ranged from -0.57 to 0.99.

Under LN level, SL has high correlation with SDW and TPB as expected, but it was also highly correlated to the TRL and LRL (r = 0.57). This value was even higher than the correlation among PRL with TRL (r = 0.48) and with LRL (r = 0.44). LRN, TRL and LRL are highly correlated as a group, as well as SDW, RDW and TPB that are highly correlated as another group of traits. R:S is the traits with more non-significant correlations or with weak correlations with other traits.

For HN level, the pattern of correlations was similar as in LN (Table 2.5). The magnitudes of the correlations were also similar among all traits, and a few traits formed high correlation groups among them. One important difference to notice is how CRN and LRN were not significantly correlated in HN but were correlated at LN. A comparison of the genotype means shows that the dispersion of the data at HN treatment is greater than in LN (Figures A2.1 & A2.2). The loss of correlation could be due to the preferential development of SL and CRN in HN, rather than the rest of component of the RSA as shown in the phenotypic results above. This is a significant datum given that these traits share QTL in common genetic positions as presented below.

Genotypic results

A total of 57 QTL were associated with the 8 roots and shoots traits (Table 2.6). The QTL analysis was performed separately by N level; 25 and 32 QTL were detected under HN and LN levels respectively. The QTL were distributed among the 10 chromosomes of maize (Figure 2.1).

For SL, 9 QTL were detected in total. Of those, four were under HN and 5 under LN levels. An individual QTL could explain from 8.1% to 12% of the phenotypic variation. One region in chromosome 4, less that 10cM apart (*qSLh-4a*: 20.3cM and *qSLl*-4: 13,7cM), was detected in both N levels. The total phenotypic contribution of the QTL was 39.5% and 51.4% for HN and LN levels respectively.

For PRL, 6 QTL were detected in total, three at each of the N levels. The range of phenotypic variation explained by a single QTL varied from 6.6% to 10.4%. Two QTL, *qPRLh-9* and *qPRLl-9*, were located at position 14.9 cM in chromosome 9; explaining 9.6% and 10.4% of the phenotypic variation for HN and LN respectively. The cumulative contribution of the QTL was 26.4% for HN and 25.7% for LN.

Eight QTL were observed for CRN under the N levels, three for HN and five for LN. On chromosome 3, QTL *qCRNh-3* and *qCRNl-3* were found at the same position (19.8cM) in HN and LN levels. The QTL contributed 7.5% to the phenotypic variation in HN and 12.8% in LN. On chromosome 8 another two QTL (*qCRNh-8* and *qCRNl-8*) were detected at a common position (75.1cM) under both N levels. In total, the QTL at HN explained for 19.4% of the phenotypic variation, and 48% was explained by the QTL at LN level.

A total of 9 QTL were detected for LRN, four under HN and five under LN levels. A single QTL explained 6.6% to 14.5% of the phenotypic variation. Some QTL were located in

chromosomes 1, 3 and 8 under both N levels. *qLRNh*-1 was the second major QTL found in this study and explained 14.5% of the phenotypic variation. At chromosome 8, QTL *qLRNh-8* 75.4cM and *qLRNl-8* 75.5 cM were detected at almost the same position. These QTL are also collocated with *qCRNh-8* and *qCRNl-8*. The QTL detected under HN explained 40.8% of the total phenotypic variation, while the ones under LN explained 41.4%.

Five QTL were detected for LRL, two for HN and three for LN levels. Both QTL for HN were located at chromosome 1, and explained a total of 17.2% of the phenotypic variation. The first QTL, *qLRLh-1* (34cM), was located in a common region to *qLRNh-1* (37.7cM). The three QTL under LN explained a total of 25.6% of the phenotypic variation of the trait.

For TRL, a total of 9 QTL were found in the analysis. Six QTL were detected for HN and 3 for LN. Two of the QTL *qTRLh-1a* and *qTRLl-1b* were collocated with *qLRLh-1a* and *qLRLl-1b* respectively; differing in less than 1cM apart. Also, *qTRLl-3* collocated with *qLRLl-3*. It was located at position 181.8cM for TRL and 181.9cM for LRL. Besides, *qTRLh-5a* and *qTRLl-5* were located at the same position under both N levels.

Seven QTL were detected for SDW, in which two were in HN and five in LN level. Under HN, *qSDWh-5* (97.7cM) was located in a common region with *qSLh-5* (94.8cM). Besides, three of the five QTL at LN (*qSDWl-2, qSDWl-4a,* and *qSDWl*-6) were located at common regions with QTL *qSLl-2, qSLl-4* and *qSLl-6*. Furthermore, in chromosome 9 a common genetic region was detected for *qSDWh-9* and *qSDWl-9* in positions 97.7cM and 98cM respectively. It is important to mention that the major QTL of the entire study was *qSDWl-2*, which explained 16.5% of the phenotypic variation. The total phenotypic variation explained by QTL was 22.5% at HN and 51% at LN.

Only 4 QTL were detected for TPB, one at HN and three at LN level. All the QTL at LN were located at a common region with *qSDWl-2, qSDWl-4b* and *qSDWl-9* as well as *qSLl-2* (Table 2.6). These QTL for TPB were detected at chromosomes 2, 4, and 9, which together explained for 24.1% of the phenotypic variability of the trait.

Discussion

A general observation of this study is that root development was greater under N-limiting conditions. The lengths of the primary root and the lateral roots, as well as the number of lateral roots were greater under LN level by 8.5%, 31% and 20%, respectively. The effect of these root components, added to the increase of the TRL by 26% under N-limiting conditions. Similarly, this increase in TRL in LN treatments has been observed using five maize inbred lines (Wang et al., 2005) and a set of 94 RIL from China (Liu et al., 2008), respectively. It has been previously reported that the increase in total length of the RSA is one of the main components of an ideal maize root ideotype for effective N acquisition (Lynch, 2013; Mi et al., 2010; Shen et al., 2013). Also, it was found that TRL is highly correlated with LRL (0.99) and LRN (0.63) under LN, and moderately correlated to PRL (0.48). It is important to notice that 14-*day* old seedlings RSA is mainly composed by a primary root (PR), seminal roots (SR), lateral roots (LR) and crown roots (CR) (Hochholdinger and Tuberosa, 2009). Even though at early stages of development the PR is the thicker and usually longer component of the root system, the SR and LR are of high importance at the moment of nutrient acquisition and surface exploration (Hochholdinger and Tuberosa, 2009; Shen et al., 2013; Zhu et al., 2005b; Zhu et al., 2006).

There has been some controversy on the response of LRL to N limitation. Wang et al., (2005) found that the total length of LR increased with the increment of N concentration in the culture solution, and at the same time increased the N accumulation in the root tissue. On the

contrary, Liu et al., (2008) found that LRL increased with low N stress. The results in the present study concur with an increase in LRL under LN conditions, which was also observed using a diverse panel of maize inbred lines (Abdel-Ghani et al., 2013). Reasons for these discrepancies may be attributable to the concentration of NO_3^- in HN solution was 15mM in this study versus 4mM (Wang et al., 2005). Root development can be inhibited when N input is high enough (Shen et al., 2013), thus it could be argued HN level actually reduced the elongation of LR. However, in Liu et al., (2008) the NO_3^- concentration at HN level was 2mM, which is lower than in Wang et al., (2005), and they were still able to observe longer LR in LN conditions. Another reason can be the stage of development at which the seedlings where harvested for measurements. Older seedlings have higher root biomass and will develop longer roots in either N level. Wang et al., (2005) used 25-*day* old seedlings versus 14-*day* old in the present study. The nutrient requirements vary by developmental stage, thus the needs of older plantlets will be higher because of the higher biomass been produce than the needs of younger seedlings (Cai et al., 2012). .

Overall, the pronounced development of the RSA under LN conditions increases the capabilities of a seedling to capture more of the scarce N in the culture medium or in the field if that is the case (Shen et al., 2013). In this study the effect of N stress increased R:S, meaning that root mass was higher under LN; this is supported by higher RDW means under LN. These results are in agreement to previous reports (Abdel-Ghani et al., 2013).

The influence of HN over the maize seedlings was especially obvious in the development of the shoot and the shoot-borne roots. SL, SDW and CRN were all greater in the HN medium. Similar results were observed by (Abdel-Ghani et al., 2013) for SL and SDW, who also found that crown root length (CRL) was positively increased under HN. This suggests that at optimum

N conditions in the growth medium, maize seedlings are able to prioritize remobilization of nutrients for development of the shoot and shoot-borne roots (Mi et al., 2010; Shen et al., 2013).

The repeatability of the study was relatively high for all the traits when calculated across experiments and N treatments. In general, repeatabilities for all traits were higher than 0.70 (Table 2.2), and are comparable to previous results (Abdel-Ghani et al., 2013). Interpretation suggests that the majority of the variation observed in the study is due to the genetic variability among the DH lines that were used. Furthermore, when repeatability was determined within each N treatment the lowest repeatability values were 0.59 and 0.46 for LRN under HN (Table 2.3) and LN (Table 3.4), respectively. High repeatability values suggest that the quality of the data that was used for QTL mapping is acceptable for determining genomic regions associated with the traits. These results diverge from the lower repeatabilities obtained when RSA traits were evaluated in field conditions; suggesting that effects of the environment are a factor when measuring RSA in the field (Cai et al., 2012; Trachsel et al., 2011). Thus, it seems important to increase the amount measurements performed in field experiments, whether that is by incrementing the number of individuals, replications, locations or years where and when the experiments are performed.

Significant genotypic variation was detected among the lines used in this study. This is comparable with previous reports of genetic variation found in maize lines that were subjected to different forms of abiotic stress. IBM recombinant inbred lines (RILs) were tested under contrasting levels of phosphorus (P) to study LR traits and root hair traits, among others (Zhu et al., 2005a, b). Several QTL were found in these studies, providing evidence of significant genotypic variation for RSA within a population of a similar genetic makeup to the one used in the present experiments.

Furthermore, there is a similar study to the one presented here in which the RSA of 94 RILs was analyzed. They analyzed traits such as length of lateral roots, as well as length and number of axial roots, which include crown roots and seminal roots as used in this study. QTL for some RSA related traits in chromosomes 1, 3, 5, 6, 8, and 10 were detected in both studies. For instance, QTL for axial root number (ARN) for low N tolerance and average axial root length (AARL) in HN, were detected in chromosome 3 (Liu et al., 2008); whereas in the present study QTL for CRN, LRN and TRL under high and low N were found in chromosome 3. Therefore, it is possible that the same genomic regions in chromosome 3 may control the development of the number of post-embryonic roots and total length of the root system independently of the N effect. Liu et al., (2008) identified QTL for LRL under HN (Chr. 8), and LN (Chr. 10). In the present study, QTL for LRN and CRN were detected in chromosome 8 but these were independent of the nitrogen treatment (Table 2.6, Figure 2.1). However, there were other QTL for LRN and CRN detected in chromosome 10 that were present only under LN. The results of these two studies suggest that these regions in chromosome 8 and 10 could harbor important loci responsible of post-embryonic development of the RSA, by controlling the lateral rooting specifically. In addition, QTL that control the length of the RSA were detected in chromosome 5 in both studies. Liu et al., (2008) identified QTL for LRL, AARL and maximum ARL (MARL) near genomic regions where QTL for TRL, and PRL were identified in this study. A total of 57 QTL were identified, which were divided among 8 RSA traits related with the early development of seedlings under contrasting N levels. The locations of these QTL were spread throughout the almost all the chromosomes, only chromosome 7 did not presented a QTL. It was found at least one QTL in each N level for every trait analyzed (Figure 2.1). Several of the QTL were located near or in exact chromosomal regions among different traits or N levels (Table 2.6).

Some QTL collocated for two different traits and at both N levels (e.g. *qCRNh-8, qCRNl-8, qLRNh-8, qLRNl-8*; Table 2.6, Figure 2.1 (Chr. 8)), forming clusters of QTL in what appears to be important genomic regions for RSA. These types of clusters have been previously reported for different root traits (Cai et al., 2012; Liu et al., 2008). Clusters that included QTL for ARN have been found in chromosomes 6 and 10 at early stages of development (Cai et al., 2012), which are similar to the QTL identified in this study for CRN and LRN in the same chromosomes. Besides, clusters of QTL in chromosomes 1, 2, 3 and 6 that have been reported in QTL meta-analysis for different types of abiotic stress (Hund et al., 2011) have also been described to carry important loci for RSA in response to P deficiency (Zhu et al., 2005b). These observations coincide with the genomic regions were QTL have been identified in the present study. Interestingly, Zhu et al., (2005b) identified QTL for LRL under low P levels that collocate with the ones in this study associated to the same trait under LN. Furthermore, Liu et al., (2008) also found QTL collocated at chromosome 8 under HN compared to QTL under high P (Zhu et al., 2005b). These comparable results of QTL analyses made under different abiotic stresses, in different populations of maize, and using different set of molecular markers are a good indication that several important loci are located at the stated chromosomal regions that control the early development of the RSA. In the meta-QTL analysis performed using several reports of QTL for root traits in diverse mapping populations (Hund et al., 2011); important chromosomal regions, which contained multiple QTL each (MQTL), were identified as central for further analysis. Some of the QTL for CRN, LRN and LRL, found in the present study, were located near the regions containing MQTL; which were described as key loci that regulate the number of axial roots, and that control the lateral rooting among other functions. Thus, it seems reasonable to address the importance of these putative genomic regions with further and deeper analysis to

start employing these loci in marker assisted selection programs in breeding for nutrient-use efficiency.

The genotypic information developed by GBS (Liu et al. in publication) that was used in this study, was subjected to an adjustment of the expanded genetic map so it could be compared to F2-based maps used in previous studies. An expanded map reflects the observed recombination that is accumulated by meiosis in each generation of crossovers (Winkler et al., 2003). The IBM2Syn10-DH was adjusted after the formulas and theory developed by Teuscher et al., (2005), considering that the marker density used was high enough to directly apply the expansion factor to adjust the original extended map. This procedure yielded an adjusted map of 1,722.9 cM. This allowed the comparison of genetic positions of the QTL observed in this study to the ones of other studies performed with populations of lesser resolution or lower marker densities.

In conclusion, it has been observed that the development of RSA in maize seedlings is positively influenced under limiting N conditions. The length and number of embryonic roots and lateral roots increases under LN conditions. Instead, high N conditions favor the development of the shoot length and biomass, as well as the number of shoot-borne roots like crown roots. Given the significant genotypic variation among the DH lines used in the study, several QTL were identified for the RSA traits analyzed. Moreover, many of the QTL that were found can be collocated with QTL that have been previously reported. Thus, there is evidence of important genomic regions that control the development of the RSA under contrasting N treatments of 14-day old maize seedlings. This is one of the few reports available that analyzes the genotypic variation for RSA traits using the IBMSyn10-DH population under contrasting N treatments. This information can be utilize in conjunction with the one in previous reports to

identify loci with large effects over the phenotypic variability found in response to nutrient deficiency in maize. This could lead to the determine candidate loci to be used in marker assisted selection for nutrient-use efficiency in the future.

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Figures

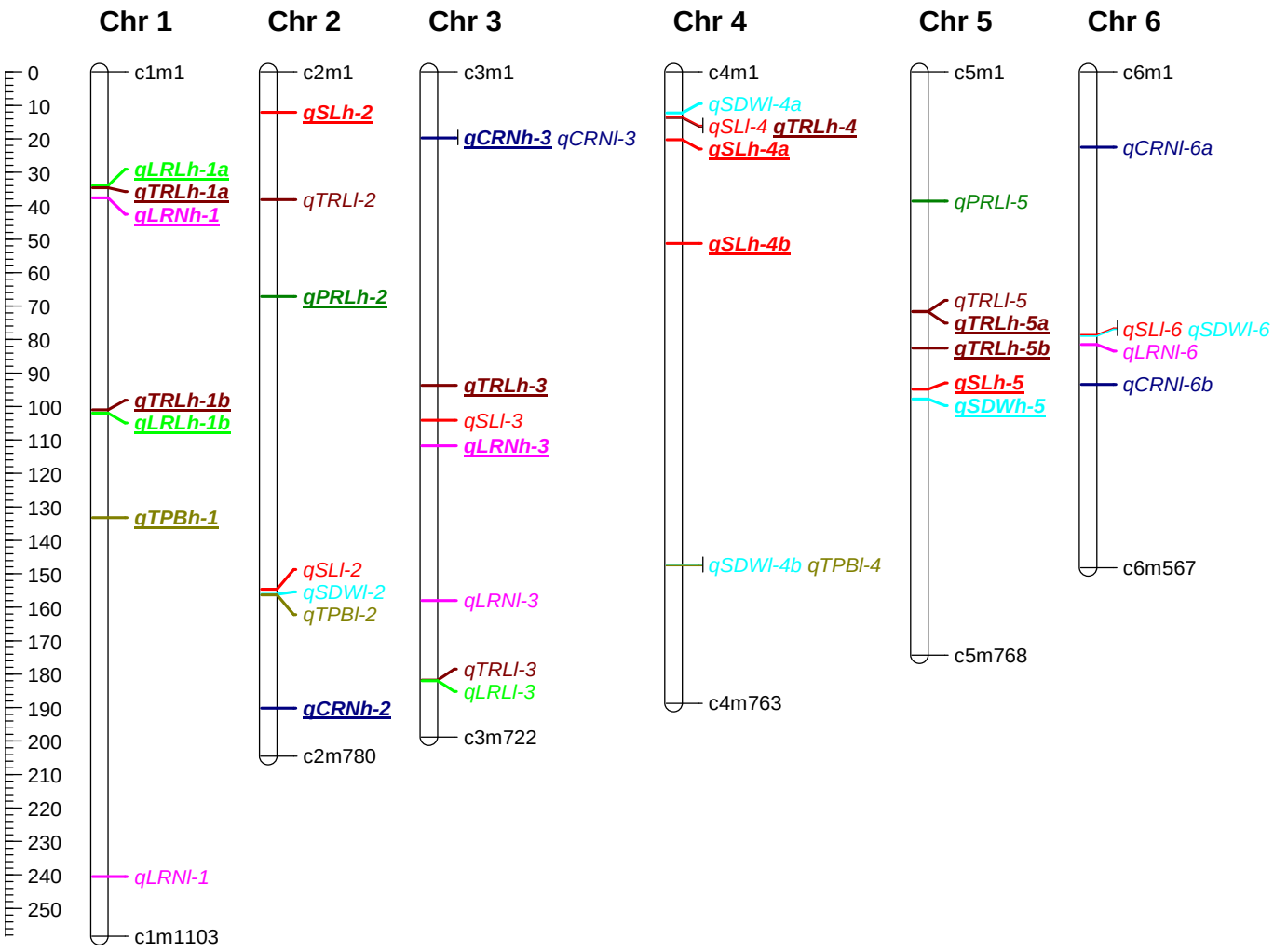

Figure 2.1. QTL identified for RSA traits under low and high N treatments.

Maize GBS map containing QTL for several RSA traits measured under high and low N levels. The scale to the left of the figure is given in centiMorgan. The start and end markers were placed to show the genetic length for each chromosome (Chr.). QTL for traits under HN are underlined and in bold letters. QTL for 8 traits are shown: SL (red), PRL (green), CRN (blue), LRN (pink), LRL (light green), TRL (brown), SDW (turquoise), TPB (olive).

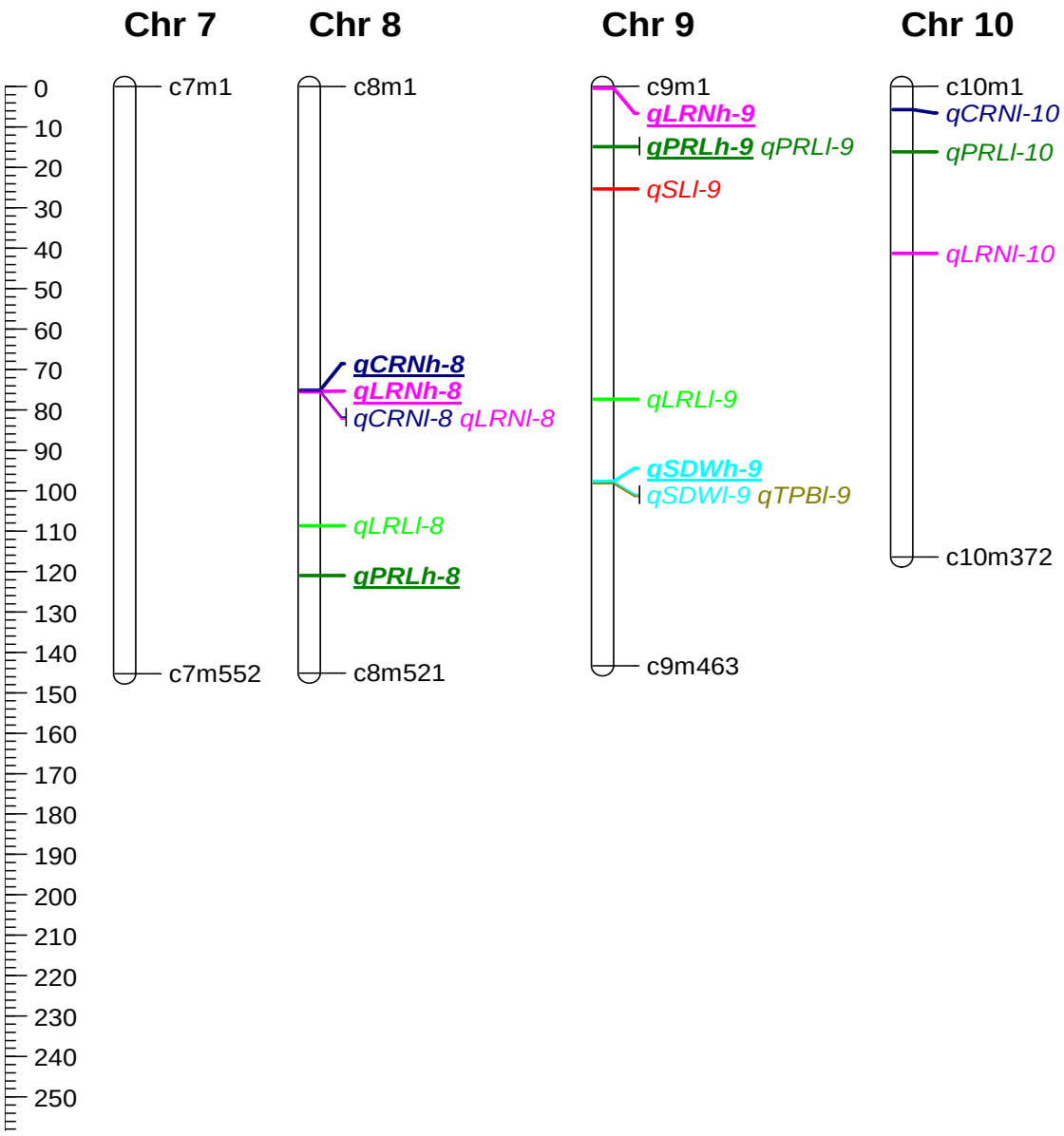

Figure 2.1 Continued

Tables

Table 2.1 Estimates of means and ranges of seedling root traits under high and low nitrogen treatments; and ANOVA for 153 IBM-10 DH lines.

Trait	N	Mean	Min	Max	Relative Difference (%)	Analysis of Variance			
						N level	Genotype	Genotype X N level	Exp*N*Gen
SL (cm)	**HN**	30.4	14.8	44.7	12.89	***	***	ns	**
	LN	26.5	9.3	39.3					
PRL (cm)	**HN**	22.2	8.5	31.0	-8.55	***	***	ns	***
	LN	24.1	11.2	35.0					
CRN	**HN**	4.1	1.0	8.3	6.38	***	***	ns	ns
	LN	3.8	0.0	7.0					
SRN	**HN**	2.4	0.0	6.7	1.39	ns	***	ns	***
	LN	2.3	0.0	5.7					
LRN	**HN**	109.4	30.3	207.7	-20.29	***	***	ns	***
	LN	131.7	30.0	281.3					
TRL (cm)	**HN**	93.5	24.9	245.3	-26.17	***	***	ns	***
	LN	118.0	32.7	325.8					
LRL (cm)	**HN**	71.5	8.2	219.3	-30.45	***	***	ns	***
	LN	93.3	12.9	296.7					
SDW (mg)	**HN**	347.9	83.2	842.7	11.56	***	***	ns	***
	LN	307.7	52.1	654.6					
RDW (mg)	**HN**	155.9	42.0	354.3	-2.43	ns	***	ns	*
	LN	159.7	17.3	375.7					
TPB (mg)	**HN**	501.3	115.0	1171.9	7.33	***	***	ns	***
	LN	464.6	69.4	926.9					
R:S	**HN**	0.45	0.19	0.97	-16.60	***	***	*	***
	LN	0.52	0.21	0.99					
PRL:SL	**HN**	0.75	0.39	1.35	-23.61	***	***	ns	***
	LN	0.92	0.52	1.75					

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant; N Nitrogen levels; HN High Nitrogen; LN Low Nitrogen

Table 2.2 Variance component estimates and repeatability for seedling root traits calculated across experiments and nitrogen levels for IBM-10 DH lines.

Trait	Variance Components					Repeatability
	Rep (Exp)	Gen	N*Gen	Exp*N*Gen	Residual	
SL	0.02 ns	6.53 ***	0.0 ns	1.75 **	9.75	0.82
PRL	0.04 ns	2.78 ***	0.0 ns	1.83 ***	5.16	0.73
CRN	0.01 **	0.40 ***	0.02 ns	0.02 ns	0.48	0.83
SRN	0.01 **	0.51 ***	0.0 ns	0.1 ***	0.41	0.88
LRN	5.67 *	314.8 ***	0.0 ns	181.1 ***	574.29	0.82
TRL	13.17 **	517.7 ***	0.0 ns	249.1 ***	790.20	0.78
LRL	12.15 **	492.2 ***	0.0 ns	246.7 ***	725.85	0.77
SDW	234.8 ***	3576.7 ***	0.0 ns	1305 ***	4485.10	0.81
RDW	260.5 ***	795.9 ***	0.0 ns	191.4 *	1468.62	0.70
TPB	364.9 ***	8264.1 ***	0.0 ns	2346.2 ***	9369.32	0.82

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 2.3. Variance component estimates and repeatability for seedling root traits calculated at high nitrogen level; for IBM-10 DH lines.

Trait	Variance Components				Repeatability
	Rep(Exp)	Gen	Exp*Gen	Residual	
SL	0.0 ns	12.96 ***	3.31 **	11.84	0.74
PRL	0.02 ns	3.74 ***	2.03 ***	5.68	0.60
CRN	0.004 ns	0.48 ***	0.0 ns	0.53	0.79
SRN	0.002 ns	0.44 ***	0.13 **	0.41	0.72
LRN	53.4 ***	255 ***	155.9 ***	392.9	0.56
TRL	30.1 **	420.9 ***	111.3 *	652.6	0.64
LRL	28.6 **	407.2 ***	114.3 *	602.6	0.65
SDW	85.5 *	5876 ***	939.2 *	5513.6	0.76
RDW	1000.9 ***	959.1 ***	395.7 ***	1080.6	0.50
TPB	961.6 ***	12663 ***	2462.9 **	10382	0.75

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 2.4. Variance component estimates and repeatability for seedling root traits calculated at low nitrogen level; for IBM-10 DH lines.

Trait	Variance Components				Repeatability
	Rep (Exp)	Gen	Exp*Gen	Residual	
SL	0.0 ns	7.26 ***	1.95 **	7.84	0.71
PRL	0.0 ns	3.09 ***	1.63 ***	4.71	0.61
CRN	0.01 **	0.40 ***	0.02 ns	0.44	0.76
SRN	0.001 ns	0.56 ***	0.10 **	0.42	0.78
LRN	133.8 ***	220 ***	229.8 ***	572.8	0.40
TRL	13.2 *	734.7 ***	401 ***	905.4	0.63
LRL	12.3 *	707.6 ***	398.3 ***	829.4	0.63
SDW	147.02 **	2897 ***	2022 ***	3702.4	0.59
RDW	1.6 ns	856 ***	289.5 **	1370.7	0.64
TPB	126.3 *	6959 ***	3238.1 ***	7988.3	0.65

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 2.5. Phenotypic correlations among seedling root traits estimated across experiments; for 153 IBM-10 DH lines

Correlations calculated for low N treatment are found above the diagonal. Correlations results for high N treatment are below the diagonal in the table.

N Level = Low

	SL (cm)	**PRL(cm)**	**CRN**	**LRN**	**TRL(cm)**	**LRL(cm)**	**SDW (mg)**	**RDW (mg)**	**TPB (mg)**	**R:S**	**PRL:SL**
SL (cm)		0.35 ***	0.31 ***	0.35 ***	0.57 ***	0.57 ***	0.71 ***	0.54 ***	0.70 ***	-0.09 *	-0.64 ***
PRL(cm)	0.41 ***		0.05 ns	0.34 ***	0.48 ***	0.44 ***	0.28 ***	0.40 ***	0.34 ***	0.17 ***	0.43 ***
CRN	0.35 ***	0.03 ns		0.12 **	0.16 ***	0.15 **	0.20 ***	0.25 ***	0.26 ***	0.06 ns	-0.22 ***
LRN	0.25 ***	0.32 ***	0.03 ns		0.63 ***	0.62 ***	0.31 ***	0.35 ***	0.34 ***	0.11 **	-0.05 ns
TRL(cm)	0.51 ***	0.59 ***	0.11 **	0.55 ***		0.99 ***	0.49 ***	0.43 ***	0.49 ***	-0.0 ns	-0.14 **
LRL(cm)	0.49 ***	0.54 ***	0.11 **	0.55 ***	0.99 ***		0.49 ***	0.41 ***	0.49 ***	-0.03 ns	-0.17 ***
SDW (mg)	0.73 ***	0.32 ***	0.26 ***	0.17 ***	0.40 ***	0.43 ***		0.68 ***	0.96 ***	-0.21 ***	-0.41 ***
RDW (mg)	0.48 ***	0.33 ***	0.23 ***	0.27 ***	0.28 ***	0.30 ***	0.61 ***		0.86 ***	0.50 ***	-0.18 ***
TPB (mg)	0.70 ***	0.35 ***	0.27 ***	0.22 ***	0.39 ***	0.41 ***	0.96 ***	0.79 ***		0.07 ns	-0.35 ***
R:S	-0.16 ***	0.13 **	-0.01 ns	0.18 ***	-0.04 ns	-0.05 ns	-0.25 ***	0.52 ***	-0.01 ns		0.21 ***
PRL:SL	-0.57 ***	0.39 ***	-0.31 ***	0.06 ns	-0.0 ns	-0.03 ns	-0.42 ***	-0.14 **	-0.35 ***	0.22 ***	

N Level = High

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 2.6. QTL detected for various root and shoot traits under two contrasting N levels

Summary of the QTL identified for 8 traits measured in 14-day old maize seedlings. The traits are shoot length (SL), primary root length (PRL), crown root number (CRN), lateral root number (LRN), lateral root length (LRL), total root length (TRL), shoot dry weight (SDW), and total plant biomass (TPB).

Trait	N level	Chr.	QTL	Position (cM) *a*	LOD *b*	Add. *c*	R^2 (%) *d*
SL	High N	2	*qSLh-2*	12.1	5.4	-1.4	8.5
		4	*qSLh-4a*	20.3	7.2	-1.7	11.6
		4	*qSLh-4b*	51.3	7.0	1.5	11.3
		5	*qSLh-5*	94.8	5.1	1.3	8.1
	Low N	2	*qSLl-2*	156.2	6.6	1.1	10.8
		3	*qSLl-3*	104.1	7.3	-1.2	12.0
		4	*qSLl-4*	13.7	6.3	-1.2	10.3
		6	*qSLl-6*	78.8	5.6	1.1	9.1
		9	*qSLl-9*	25.3	5.7	-1.0	9.2
PRL	High N	2	*qPRLh-2*	67.1	5.8	0.8	9.7
		8	*qPRLh-8*	121.0	4.4	-0.7	7.1
		9	*qPRLh-9*	14.9	5.7	-0.8	9.6
	Low N	5	*qPRLl-5*	38.6	4.4	-0.6	6.6
		9	*qPRLl-9*	14.9	6.7	-0.8	10.4
		10	*qPRLl-10*	16.2	5.7	-0.7	8.7
CRN	High N	2	*qCRNh-2*	190.1	4.4	0.2	6.0
		3	*qCRNh-3*	19.8	5.4	0.2	7.5
		8	*qCRNh-8*	75.1	4.4	0.2	5.9
	Low N	3	*qCRNl-3*	19.8	7.5	0.3	12.8
		6	*qCRNl-6a*	22.5	4.9	-0.2	8.0
		6	*qCRNl-6b*	93.4	6.0	0.3	10.0
		8	*qCRNl-8*	75.5	5.5	0.2	9.1
		10	*qCRNl-10*	5.7	4.9	0.2	8.1
LRN	High N	1	*qLRNh-1*	37.7	9.6	8.4	14.5
		3	*qLRNh-3*	111.8	4.9	5.9	6.9
		8	*qLRNh-8*	75.4	8.3	7.6	12.2
		9	*qLRNh-9*	0.4	5.2	-6.0	7.2
	Low N	1	*qLRNl-1*	240.5	6.1	-7.4	9.3
		3	*qLRNl-3*	158.0	4.9	-7.0	7.9
		6	*qLRNl-6*	81.5	4.8	6.2	7.3
		8	*qLRNl-8*	75.5	6.7	7.6	10.3
		10	*qLRNl-10*	41.3	4.4	6.4	6.6
LRL	High N	1	*qLRLh-1a*	34.0	5.3	7.2	8.0
		1	*qLRLh-1b*	102.0	6.0	7.9	9.2

Table 2.6. Continued

	Low N	3	*qLRLl-3*	181.9	5.7	10.5	9.2
		8	*qLRLl-8*	108.6	5.5	-11.5	8.8
		9	*qLRLl-9*	77.4	4.8	-9.5	7.6
TRL	High N	1	*qTRLh-1a*	34.6	6.9	8.3	10.1
		1	*qTRLh-1b*	101.0	8.8	9.8	13.3
		3	*qTRLh-3*	93.7	4.4	-6.6	6.1
		4	*qTRLh-4*	13.7	5.9	-7.7	8.3
		5	*qTRLh-5a*	71.7	6.2	8.4	8.8
		5	*qTRLh-5b*	82.6	5.4	-7.6	7.5
	Low N	2	*qTRLl-2*	38.2	4.9	10.3	7.5
		3	*qTRLl-3*	181.8	7.6	12.4	12.2
		5	*qTRLl-5*	71.6	5.8	11.4	9.2
SDW	High N	5	*qSDWh-5*	97.8	5.8	35.4	10.8
		9	*qSDWh-9*	97.7	6.6	-33.4	11.7
	Low N	2	*qSDWl-2*	156.1	9.7	30.5	16.5
		4	*qSDWl-4a*	12.2	4.3	-19.5	6.8
		4	*qSDWl-4b*	147.4	6.2	23.7	10.0
		6	*qSDWl-6*	78.8	5.7	23.5	10.0
		9	*qSDWl-9*	98.0	4.9	-21.5	7.7
TPB	High N	1	*qTPBh-1*	133.2	4.9	-40.0	8.7
	Low N	2	*qTPBl-2*	156.3	5.9	34.4	9.7
		4	*qTPBl-4*	147.4	4.6	29.5	7.3
		9	*qTPBl-9*	98.0	4.5	-29.9	7.1

a Position in cM from the top of the chromosome calculated by QTL Cartographer v.1.7
b LOD value corresponding to the position of the QTL calculated by QTL Cartographer v.1.7
c Additive effects values calculated as the average from the difference between homozygotes for each parental allele at a locus. (-) is the direction of the additive effect for Mo17 inbred parent.
d Part of the phenotypic variance explained by each QTL by composite interval mapping

Appendix

Table A2.1. Thresholds for QTL mapping calculated for each trait under each N treatment

LR (Likelihood ratio), and LOD (logarithm base 10 of odds) calculated after 1000 permutations

		Threshold	
Trait	**N level**	**LR**	**LOD**
SL	High N	19.16	4.16
	Low N	18.88	4.10
PRL	High N	18.97	4.12
	Low N	19.03	4.13
CRN	High N	19.30	4.19
	Low N	19.00	4.12
LRN	High N	18.97	4.11
	Low N	19.60	4.25
LRL	High N	18.96	4.11
	Low N	19.20	4.16
TRL	High N	19.61	4.25
	Low N	19.20	4.16
SDW	High N	19.30	4.19
	Low N	18.96	4.11
TPB	High N	18.88	4.09
	Low N	19.26	4.18

Formula A2.1 Repeatability: calculated on an entry-mean basis across N-levels

$$H^2 = \frac{{\sigma^2}_G}{{\sigma^2}_e/_{RNE} + {\sigma^2}_{GNE}/_{NE} + {\sigma^2}_{GN}/_{N} + {\sigma^2}_R/_{R} + {\sigma^2}_G}$$

Formula A2.2. Repeatability, calculated on an entry-mean basis within N-levels

$$H^2 = \frac{{\sigma^2}_G}{{\sigma^2}_e/_{RE} + {\sigma^2}_{GE}/_{E} + {\sigma^2}_R/_{R} + {\sigma^2}_G}$$

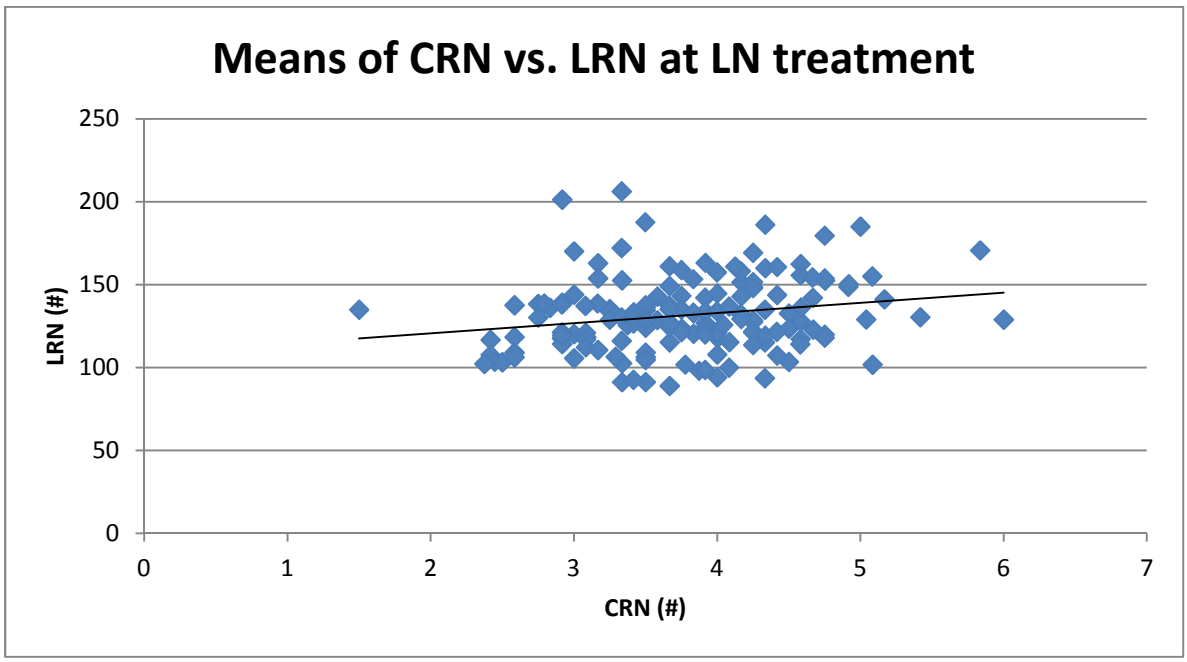

Figure A2.1. Comparison of the genotype means of CRN and LRN under LN treatment.

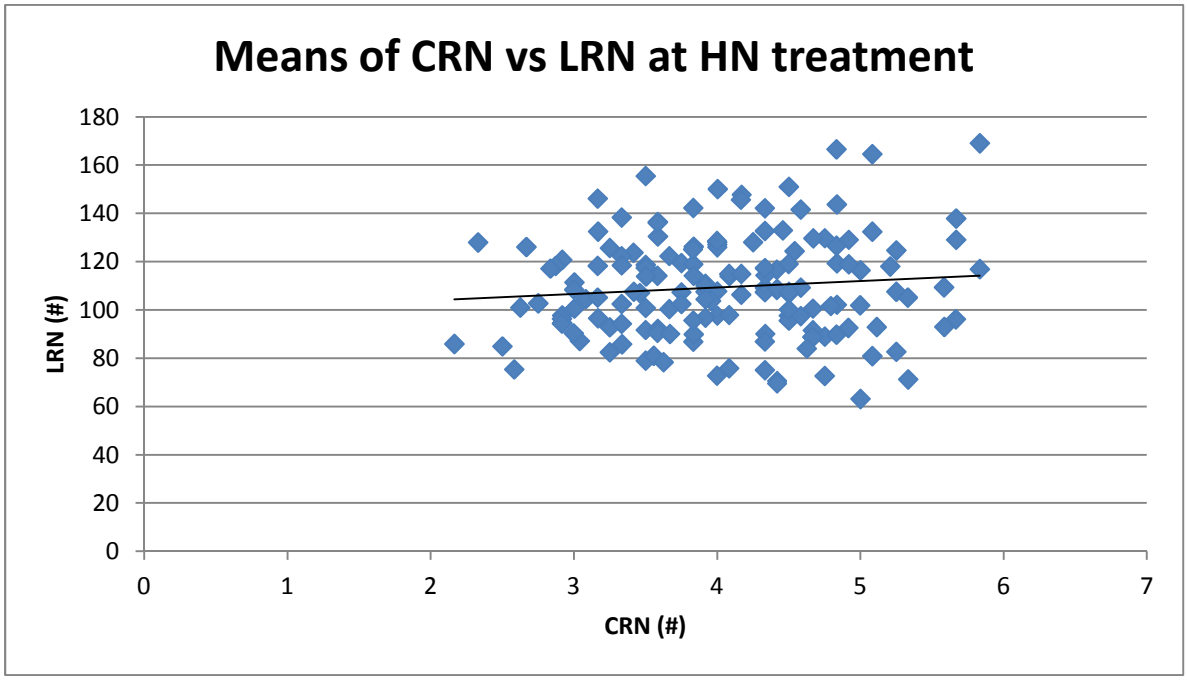

Figure A2.2. Comparison of the genotype means of CRN and LRN under HN treatment.

CHAPTER 3: GENOTYPIC ANALYSIS OF GRAIN YIELD/YIELD RELATED, AND GRAIN QUALITY TRAITS OF MAIZE IN RESPONSE TO LOW AND HIGH NITROGEN INPUTS

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Abstract

Producing a high-yielding maize crop that requires less Nitrogen (N) input is currently one important goal of maize breeding programs. Understanding the genetic mechanism that control agronomic traits response to N is key for improving maize varieties. In this study, a QTL mapping approach was used to analyze a set of doubled-haploid (DH) lines that were evaluated in different environments using contrasting levels of N. Several agronomic traits and grain quality traits were measured at independent environments. Significant environmental effects were found in the study, which conditioned the analysis to be carried separately for each environment. Overall, the data showed that effective low N (LN) treatments reduced the DH-lines performance significantly. Grain yield decreased up to 63% at one environment. Plant height and ears per plant, among other traits, were also affected by around 16% each under LN, when compared to experiments grown under high N (HN) treatments. Grain protein (GPRT) was significantly reduced by 10% under LN conditions, while grain oil (GO) increased by around 3% only at one of the environment tested. A total of 302 QTL were identified across all trait/environment/N-level combinations. Important QTL clusters located in chromosomes 1, 4, 5,

8 and 10 harbored QTL detected under LN or HN treatments. These clusters are located near loci *gln4* and *gln5*, which regulate the activity of glutamine synthetase; an enzyme involved in N-assimilation and N-remobilization for the production protein in the grain of maize.

Introduction

The progressively growing worldwide population demands higher yields of cultivated crops. As a response, the industry has addressed that demand by breeding better producing cultivars, which take up more nutrients. In maize and other cereals, yields have been actively improved by providing higher amount of Nitrogen (N) (Cardwell, 1982; Mueller et al., 2012; Raun and Johnson, 1999). As an elevated amount of N represents higher cost for farmers and higher pressure to the environment, there is the need to develop more efficient plants which can produce high yields with less N supplementation.

Hence, nitrogen use efficiency (NUE) is important for agriculture as it addresses these current economic and ecological problems. NUE can be achieved by more efficient farming techniques and by using plant cultivars with improved response to low N supply (Bertin et al., 2000). Reports propose that there is considerable genetic variation for N response in maize in US, European and tropical germplasm (Presterl et al., 2003; Uribelarrea et al., 2004; Worku et al., 2007); which can be exploited towards the production of more efficient cultivars. Nonetheless, critical steps need to be associated with N metabolism during the vegetative growth phase of the plant and its seed formation (Hirel et al., 2007b).

NUE is divided into two primary physiological components: N-uptake efficiency (NUpE) and N-utilization efficiency (NUtE) (Moll et al., 1982). N-uptake represents the amount of N (as nitrates and ammonium ions) absorbed by the plant compared to that available in the soil

(Presterl et al., 2002). N-utilization efficiency measures the use of available N stored in the plant to produce grain in the ear. NUtE can be influenced by the proficient remobilization of N from the root system to source tissues (i.e. leaves and stalk) of the plant (Presterl et al., 2002). This study focuses on the agronomic performance of maize NUE, rather than a physiological assessment of N-absorption and accumulation through plant development. The variation of grain yield and yield related traits were analyzed when subjected to extreme differences in N application rates, Bertin and Gallais (2000) showed that genetic variation in N metabolism differs between low N and high N input. At low N, genes associated with senescence, anthesis-silking interval (ASI); and N-utilization efficiency may be responsible of the adaptation to stress. On the other hand, when nitrogen input is high enough, N-uptake efficiency is more important, and is associated with traits like grain yield and kernel weight and nutritional composition. It has been shown that N availability for protein and oil synthesis balance will impact final nutritional composition (Tsai et al., 1978).

Little is known regarding the genetic architecture responsible for the response to N. Various genetic studies of NUE (Bertin et al., 2001; Coque et al., 2008; Gallais and Hirel, 2004; Hirel et al., 2001; Nichols, 2008) have identified QTL in maize populations grown under low and high N rates; and the impact of genes involved in N metabolism has been proposed (Gallais and Hirel, 2004; Hirel et al., 2001; Nichols, 2008).

After determining QTLs for grain yield and related traits influenced by N availability, the identification of genomic regions controlling these traits must be determined. For the N metabolic pathways, some loci are already known and mapped which encode for the enzymes involved in the N assimilation and remobilization within a plant (Bertin et al., 2001; Hirel et al., 2001). Overall, finding loci involved in grain yield through NUE and producing functional

markers for these genomic regions will be the ultimate goal of researchers in order to provide useful tools for current breeding programs in maize and other cereals.

Progeny derived from the cross between B73 x Mo17 inbred lines (IBM) were randomly mated several generations with the goal of improving the resolution of genetic analysis of quantitative traits (Lee et al., 2002). Double haploid (DH) lines were derived from the IBM2SYN10 population (Hussain et al., 2007), which provides a high-resolution bi-parental population for QTL analysis. The IBM2SYN10-DH population insures accurate mapping of genetic positions that can be co-localized within the intervals of candidate loci for N metabolism and N response. Since the physical map of B73 is available, a map-based approach for identification of genomic regions correlated with NUE is possible. Furthermore, a previous study of N response demonstrated that the parental inbred lines B73 and Mo17 showed phenotypic variability when grown under different N levels (Balko and Russell, 1980). B73 was found to have a significant increase in grain yield and other related traits due to higher supply of nitrogen (N) fertilizer when compared to the behavior of Mo17. This is an important difference in N response, which provides evidence of significant phenotypic and genotypic variation in the DH population which is critical for discriminating QTL analyses.

The main objectives of this study were to: 1) analyze the phenotypic variation of the DH-lines for grain yield, related traits and grain quality traits grown under low N and high N treatments, 2) determine significant phenotypic correlations and the repeatability of the traits within the experiments, and 3) identify QTL for the agronomic and quality traits that are associated to N response.

Materials & Methods

Plant material

The mapping population utilized for QTL analysis experiments was a subset of 243 doubled haploid (DH) lines from the IBM2SYN10-DH mapping population of maize (*Zea mays* L.), which consists of a set of 360 individuals. This DH population was developed by DuPont Pioneer (Hussain et al., 2007) from the previously produced population derived from the cross between B73 x Mo17 plus 10 generations of random inter-mating, Iowa State University. This population was selected for mapping for three main reasons: 1) The amount of recombination accumulated after 10 generations of random mating provides the possibility of higher resolution genetic mapping; 2) the germplasm combines important genotypic variability that could be representative of some of the current U.S. maize gene pool, and 3) the population was reported to contain a significant amount of phenotypic variability between the lines (Hussain et al., 2007).

Experimental design and field management

The field experiments were grown in two locations in Iowa. The first location (Burkey) was Burkey Farm, at the ISU Agronomy Research Station, near Boone, Iowa. The second location (Marion) was the Pioneer Research Center at Marion, Iowa. The later was managed by DuPont Pioneer, but access was granted to make all possible phenotypic measurements. The experiments were grown in two consecutive years at each location. Each combination of year and location was considered an environment (E) for our design, with a total of four. Thus, E1 corresponds to growing season 2010 at Burkey, E2 to 2010 at Marion, E3 to 2011 at Burkey, and E4 to 2011 at Marion. Within each E, two nitrogen treatments were applied which represented low N (LN) and high N (HN) conditions for the study. Given the different historical management conditions of the two locations, different soil types, and changing environmental conditions from

year to year, the treatments were established by applying different levels of N at each environment. Thus, for E1, Urea 46-0-0 was the commercial product used to target 250 kg N ha^{-} for HN and no N was applied for LN. At E2, N form was 32 UAN and the HN and LN areas received 269 kg N ha^{-} and 56 kg N ha^{-}, respectively. E3 received 250 kg N ha^{-} at HN in the form of a blend of the commercial product ESN® (Agrium) and AMS (ammonium sulfate). The LN area in E3 received 67.2 kg N ha^{-} in the same form as in HN. At the E4, 269 kg N ha^{-} and 67.2 kg N ha^{-} were applied to HN and LN, respectively. All applications of N were done pre-planting. Weed control at both locations was made with application of herbicides (Dual II Magnum) and insecticides (Lorsban, or Force 3G, Marion) done before planting. That was followed by cultivation and continuous manual control as needed. In E3, a more intensive weed control had to be done due to higher than normal weed presence. Basagran (bentazon), Laudis (Tembotrione) and Impact (Topramezone) were applied post emerge to kill broadleaf-type and grass-type weeds. These procedures proved to be effective to control weed impact over the maize inbreds.

The experiments were grown in a randomized complete block design (RCBD). Each N treatment was a block within an experiment. The genotypes were randomly assigned at two replications, which were nested within each N treatment (block). All plots were planted at a high seed rate and thinned to a stand density of 67,760 plants ha^{-}. Each plot consisted of two 5.64 m rows spaced by 0.76 m per row at Burkey. At Marion the two-row plots were 5.3 m long with 0.76 m spacing between rows. The 243 DH lines plus the two parental inbred lines were grown in each replication of the experiment, adding up to 16 unique observations for each genotype across all the environments and N treatments.

Phenotypic measurements

Several agronomic traits were measured in all four environments used in the experiments. Plant height (PHT) was measured after anthesis from the soil surface to the node of the flag leaf. Growing degree units to silking (GDUSLK) and GDU to anthesis (GDUSHD) were determined when 50% of the plants within the plot showed silk visible from the shoot and pollen shed from the tassel, respectively. GDUs were calculated as: $\sum_{i=1}^{n}\left(\frac{T_{max}+T_{min}}{2}\right) - T_{base}$; where i=1, …, n is the number of days from planting to 50% silking or anthesis, T_{max} is maximum daily temperature and is set equal to 86°F when temperatures exceed 86°F, T_{min} is the minimum daily temperature and is set equal to 50°F when temperatures fall below 50°F, and T_{base} is the base temperature for the organism, which in the case of maize is 50°F. Anthesis - silking interval (ASI) was recorded as the difference between GDUSLK and GDUSHD. At Burkey (E1 and E3), grain yield was measured on all plots by hand harvesting, and drying the ears for four days to constant weight at 37.8°C in an air-blown commercial dryer. The ears were shelled using single and bulk-shelling machines. Harvest weight and grain moisture was measured for each plot, then GY was corrected to 15.5% moisture content reported in metric tons per ha (T/ha). In Marion (E2 and E4), all plots were harvested using a research plot combine where grain yield and moisture were measured on the machine at harvest.

Near Infrared spectroscopy (NIR) was used to determine grain protein (GPRT), grain oil (GO), grain starch (GSTH), and grain density (GD) contents only from samples collected at Burkey (E1 and E3). At this location, the number of ears per plants (EPP) and 300 kernel weight (KW) were estimated as well. In contrast, grain-related phenotypes were not measured at Marion due to a regulatory limitation that restricted access to grain harvested at DuPont Pioneer

locations. Furthermore, since the plots at this location were not hand harvested, thus there were not available ears to determine EPP and KW.

In 2010 the nitrogen status of the plots at E1 and E2, was evaluated by measuring leaf chlorophyll content through the use of a chlorophyll meter SPAD - 502 (Minolta Camera Co., Osaka, Japan). Chlorophyll measurements (CHLO) were taken from the ear leaf 15 days after 50% of the plants in a plot showed silks. Ten representative plants within a plot were randomly selected and a plot average was calculated by averaging three readings made per selected plant.

In 2011, nitrogen status of plots at E3 and E4 was evaluated by estimating the nitrogen percentage (N %) 20 days after 50% of the plant per plot showed silks. Four representative plants were tagged within the middle of the plot. Leaf samples were taken from the selected plants by a 7/8" leaf puncher. Two 2.4 in^2 leaf punches per plant from the leaf immediately above the ear leaf were collected. The samples were bulked by plot, dried, weighed and sent to the laboratory managed by DuPont Pioneer for nitrogen percentage measurements.

Statistical analysis

The analysis of the phenotypic data was performed by a mixed model procedure (PROC MIXED method = type3) using SAS software (SAS, 9.3). Given the significant heterogeneity of the four environments, all traits were analyzed separately by environments and by nitrogen treatments. The linear model used was the following: $Y_{ijk} = \mu + N_i + R_{(i)j} + G_k + N*G_{ik} + e_{(i)jk}$; where observation Y_{ijk} is the phenotype given by μ which is the population mean, N_i is the effect of the *ith* nitrogen treatment, $R_{(i)j}$ is the effect of the *jth* replication within the *ith* nitrogen treatment, G_k is the effect of the *kth* genotype, $N*G_{jk}$ is the N treatment-by-genotype interaction, and $e_{(i)jk}$ which is the error term of the model. N treatment was considered as fixed effect, while

genotypes, replications and the interactions were treated as random effects. Best linear unbiased predictions (BLUPs) were used to estimate the phenotypic value of each DH line for each trait in the experiments. These values were calculated separate by N treatment.

Based on the estimated BLUPs for the DH lines, the means of each one of the traits were used to establish phenotypic differences between the N treatments. The percentage of variation of the means due to the N stress was calculated by 100 - ((LN/HN)*100). An analysis of variance (ANOVA) was used to establish significant statistical differences between the applied N treatment as well as the genotypic variation and corresponding interaction with the environment.

Variances components estimates obtained with PROC MIXED method=type3 were used to estimate the repeatability of the process on an entry-mean basis (Fehr, 1987). The formula used was: $H^2 = \frac{\sigma_G^2}{\frac{\sigma_e^2}{RN} + \frac{\sigma_{GN}^2}{N} + \frac{\sigma_R^2}{R} + \sigma_G^2}$; where σ_G^2 is the genotypic variance, σ_R^2 is the replication variance, σ_{GN}^2 is the variance of the genotype *x* N treatment interaction, and σ_e^2 is the residual variance. The denominator factors R and N number of replications and N treatments, respectively. Also, Pearson correlation estimates were calculated using PROC CORR in SAS for each N treatment separately, as well as for each environment.

QTL analysis

A high-density genetic map was used for QTL mapping. The map developed by (Liu et al., submitted for publication) at the Beijing Genomics Institute (Beijing, China) consists of 6,618 recombination bins developed by genotyping-by-sequencing (GBS). Around 280 DH lines of the IBM2SYN10-DH population were re-sequenced to search for SNPs. A 15 SNP sliding window was used to determine the recombination break points (Huang et al., 2009), which were

used to create recombination maps, or so called bins maps. All the sequenced DH lines where then aligned and the genotypes were called based on the comparison of 100kb minimum intervals. This comparison yielded the 6,618 recombination bin markers, which captured the majority of recombination events among the DH lines.

The resulting GBS-generated map of the IBM2SYN10-DH had a genetic distance of 11,198.5cM; averaging 1.7cM between bin markers. The map length was adjusted to a F2-based map to run the QTL analysis in order to do extra comparisons. The expansion-reduction factor was calculated using the equation: $\alpha = \frac{j}{2} + \frac{2^i - 1}{2^i}$ (Teuscher et al., 2005), where *j* is the number of generations of inter-mating, counting the two generations for creating the F2 segregating population, and *i* is the number of generations of inbreeding after inter-mating. In the case of IBM2SYN10-DH, *j*=12 and *i*=1, due to only one generation for the DH process after inter-mating. The resulting expansion factor was 6.5, which was directly used to adjust the new map to 1,722.9cM.

The agronomic traits were analyzed in 243 DH lines in the field experiments. Of those, 209 samples produced high quality genotypic data after the GBS procedure. Thus, the phenotypic and genotypic information of the 209 DH lines was used for the QTL analysis. This analysis was carried out with QTL Cartographer version 1.7 (Basten et al., 2005) using the model composite interval mapping (CIM). The ten more significant cofactors were identified with forward and backward stepwise regression. Cofactors increase the power of detection of a given QTL effect by reducing for genetic background variability due to other QTL. Intervals of 1 cM were used to scan within each analyzed QTL (walking speed); and the window size was set to 10 cM to block out regions around the test interval. In order to determine the experiment-wise

significant levels and control the comparison-wise probabilities 1000 permutation tests were ran in each analysis performed independently for each trait, environments, and nitrogen treatment combination. Given the permutations results, significant thresholds were determined for each one of the combinations (Table A3.1). Thus, under the HN treatment the range of values was from 3.50 to 4.14 LOD across all traits and environments. Under LN treatment the range was from 2.90 up to 4.15 LOD.

Results

Phenotypic results

Four environments were used to measure the N response of grain yield and related traits. The overall analysis of the data showed that there was a significant effect of the environments over the performance of the DH population across N treatments for the majority of the agronomic traits (Table 3.1). Moreover, the ANOVA showed that the magnitude of the effect of environments in the model was big compared to other variance components. Also, in the cases of KW and GMST, the combined ANOVA shows that there was no significant N and G effect, respectively. Interestingly, N% which was evaluated only in E3 and E4, showed no E effect and highly significant N and G effect. It appears to be a stable phenotype to assess nitrogen status in a maize population.

The response of the DH population to the N treatments was heterogeneous across environments. At E1 and E2, the agronomic performance of the individuals decreased under LN treatments (Tables 3.2 & 3.3). However, at E3 and E4 the performance under LN either improved or equaled the one under HN treatment (Tables 3.4 & 3.5). Thus, the analysis of this study was performed separate by environments. This enables a better assessment of the nature of

the data within each environment to establish the efficiency of the N treatments, as well as the repeatability of the data to determine putative QTL. The ANOVA at E1 showed significant statistical differences between the two N treatments at the majority of the traits (Table 3.2). Also, all the traits showed significant genotypic variation. At E1 a higher variation of the means for the majority of the agronomic traits was observed when compared to the other environments. For example for GY, a 63% reduction was observed under LN conditions (Table 3.2). In general, reducing the N input at E1 decreased the development (PHT: 16.2%) and performance (EPP: 16.2%, KW: 16.9%) of the maize plants and increased the time to reach maturity (ASI: -52.4%). With respect to the grain quality analysis, HN treatment primarily favored the synthesis of proteins (GPRT) in the kernels by more than 10%. Grain density was significantly increased by HN (1.8%), with not much variation in the other phenotypes. On average the DH lines observed 30% less chlorophyll content at LN (Table 3.2). The analysis for E2 showed a similar pattern as E1. Though the variation of the means followed the same direction, the magnitude was reduced by around half in E2 except for GDUSLK (Table 3.3). Grain quality assessment was not performed at this E due to its location. On the contrary, the response to N of the DH lines in E3 and E4 was different to what was observed previously. The analysis at E3 showed that although there was significant N and G variation, the direction and magnitude of the means clearly shifted for the majority of the traits (Table 3.4). In general, lines at the LN treatment performed better than at HN conditions. GY at E3 was 25% higher under LN, and was almost 4X higher than the observed yield at E1. Nonetheless, GPRT and N% after flowering were still higher in HN treatment by around 11% and 8%, respectively (Table 3.4). Finally, the ANOVA at E4 showed low or nonexistent significant differences at the N treatments for the majority of the traits, except for ASI, which was 16% higher in LN treatment (Table 3.5).

Variance components

The analysis of variance was performed across N treatments to estimate the genotypic variability as well as the repeatability within each E. Significant genotypic variation was estimated for all traits and across all E (Tables 3.6 – 3.9). Repeatability, on an entry-mean basis, was calculated using the variance components estimated in the analysis; ranging from 0.35 (GMST) to 0.88 (GO) in E1 (Table 3.6). In the other environments, the estimates of repeatability were maintained. Furthermore, at E3 the majority of traits showed higher repeatability (Table 3.8), where GY reached its highest H^2=0.90. These repeatability values indicate that the majority of the variation observed was due to the genetic variation of the DH lines within environments.

Phenotypic correlation

BLUPs were used to estimate the genotypic effects in order to perform a correlation among all the traits in the study. Pairwise Pearson's correlations performed across all environments showed that GY was highly correlated mainly with EPP, grain moisture and chlorophyll content under LN conditions (Table 3.10). GY was not found to be highly correlated with grain quality traits, and it was uncorrelated to GPRT in LN levels. CHLO and N%, which were used to evaluate N status in the plants after flowering, showed different correlation patterns between GY and PHT (Table 3.10). While CHLO presents positive and strong correlations (GY: 0.84; PHT: 0.76), N% showed weaker (GY: 0.24) and even negative correlation with PHT (-0.21). Among the grain quality traits, the higher positive correlation under LN was found between GPRT and GD (0.50). Comparing traits under HN showed that GY is correlated with EPP (0.71) and CHLO (0.68). Also, the trends for CHLO and N% were similar as before when

compared to GY and PHT (Table 3.10). However at HN, the correlation between GY and GPRT (-0.41) is negative and highly significant in contrast to the lack of correlation found at LN.

Given that there is a strong environmental component over the phenotypic variation (Table 3.1), Pairwise correlation analysis were performed within each E*N treatment combination to further examine the previous correlations. The results of E1 and LN combination are shown in Table 3.11. GY showed positive correlations with EPP (0.50), GO (0.31), and CHLO (0.41). As expected the correlations of GY and flowering traits (GDUs, ASI) were significant and negative. However, this negative correlation between GY and ASI has a positive agronomic outcome since higher GY is positively influenced by shorter interval between pollen shed and silking. In contrast to the overall correlations, at E1 a negative correlation is observed between GY and GPRT (-0.64), and GD (-0.21). Also, positive correlation with GSTH (0.55) was found at E1. PHT was the trait that showed more variation with respect to the overall analysis. Almost all correlations decreased within E1, and some even changed direction (GDUSLK, GDUSHD). The comparison made under HN levels showed a pretty similar pattern of correlations among traits as in LN.

The results are presented for E1, E2, E3, and E4 in Tables 3.11, 3.12, 3.13, and 3.14, respectively. The majority of correlations were maintained across environments. The description of the correlations for E1 shown above can be extrapolated to the other environments. Thus, in general GY is positively correlated mainly with EPP, GO, GSTH, CHLO and N% under both N treatments. It is interesting to notice that GY and GPRT are negatively correlated, especially at LN (-0.72, E3). Finally, among the grain quality traits the higher positive correlations were found between GPRT and GD at both HN and LN treatments. On the other hand, GPRT and GSTH showed the highest negative correlations under both N treatments at E1 and E3.

Genotypic results

The genotypic analysis identified QTL associated with every trait under high N and low N treatments. In total, 302 QTL were identified across all trait/environment/N-level combination. Also, a similar number of QTL were found in HN (162) and LN (140) treatments across all traits and environments. Furthermore, the average number of QTL identified for each trait ranged from 3.5 for GMST up to 14 for GO. A complete list of the QTL identified in the study that contains the position, LOD peak, additive effect, R^2 value, and Total R^2 value is presented (Table 3.15).

The QTL found were distributed across the 10 chromosomes of maize. Only chromosome 6 at E2 did not present any QTL (Figure 3.2). QTL were either found in the exact same genomic position or very close even at different environments or N treatments. For example, GD showed consistency at Chr. 1, 33.2 cM; where 1 QTL at E1 and 2 QTL for E3were identified for HN and LN (Figures 3.1 & 3.3). The same type of consistencies were determined for GY, PHT, EPP, SLK, SHD, ASI, PRT, GO, STH, CHLO, and N% (Figures 3.1 – 3.4). Furthermore, it was observed that QTL formed clusters within chromosomes. One clear example is chromosome 8, where several QTL were identified in a region covering positions ~81 cM to ~91 cM across the four environments (Figure 3.5).

For GY, 29 QTL were found across all E*N combinations (Table 3.15). A single QTL could explain from 5.2% to 10.7% of the phenotypic variation. The total explained phenotypic variation ranged from 19.4% (E3-LN) to 38.5% (E1-HN). The QTL with a major effect for GY was identified under LN treatment, and located in chromosome 7 at 85.3cM. This QTL presented a negative effect of 0.175 t/ha. One cluster of 5 QTL was determined for GY in chromosome 7

between positions 83.7cM to 101.6cM. Also, some pairs of QTL were identified in the exact or very close positions in other chromosomes (Figure 3.5).

For PHT, a total of 25 QTL were found throughout the environments and N treatments (Table 3.15). The total phenotypic variation explained by QTL varied from 28.1% (E2-HN) to 36.8% (E1-HN). An individual QTL could explain from 4.7% up to 17.4% of the phenotypic variation. The latter is a QTL that had a positive effect of 9.7 cm and was collocated with several other QTL in chromosome 8 between positions 84.6cM and 85.2cM (Figure 3.5). This genomic region grouped the QTL for PHT with the higher effect over the phenotypic variation. Besides, in chromosome 1 a total of 11 QTL for PHT were identified and were grouped into two clusters. All of these clusters had QTL found in HN and LN treatments.

The flowering traits were among the traits that had more QTL in the study (Table 3.15). QTL for SLK (33), SHD (38), and ASI (28) were distributed along the 10 chromosomes (Figure 3.5). For GDUSLK, the QTL with the highest R^2 (17.6%) was identified at chromosome 8 in position 85.2cM. This QTL was collocated with several other QTL for GDUs as well as PHT, N% and GPRT. The total phenotypic variation explained by the sum of the QTL effects ranged from 23% (E3-LN) to 46.4% (E2-HN). Four major clusters of QTL were observed for GDUSLK at chromosomes 1, 5, 8 and 9. The QTL with higher effect grouped at the cluster in chromosome 8. For GDUSHD, the results of the positioning of the QTL were similar to GDUSLK (Table 3.15). Again, four main clusters were determined in chromosomes 1, 3, 8 and 9 (Figure 3.5). The two QTL with higher R^2 of the entire study were identified for this trait. Both QTL were collocated at position 84.8cM in chromosome 8, and explained 25.5% and 23.9% of the phenotypic variation, respectively. The cumulative phenotypic variance explained by the QTL ranged from 34.4% (E1-LN) to 47.7% (E2-LN). The QTL associated with ASI were located in

two main clusters, the first in chromosome 1 and the second in chromosome 7 (Figure 3.5). Both of these clusters collocated QTL of ASI with some of GY, and where usually not associated with GDUs QTL. Single QTL could explain from 5.1% to 9.3% of the phenotypic variability (Table 3.15). The total phenotypic contribution of QTL varied from 15.2% (E4-HN) to 31% (E1-LN).

Several traits were measured at fewer environments (Table A3.3). QTL for EPP (13), KW (18), and MST (7) were identified at E1 and E3 (Table 3.15). For EPP, QTL were found in pairs closely located in chromosomes 4 and 5 (Figures 3.1 & 3.3). QTL with major effect were not observed, and cumulatively, the QTL explained from 9% (E1-HN) to 31.4% (E3-LN). For KW, the QTL with the R^2 (16.1%) was located at chromosome 8 in position 103.4cM. The total phenotypic variation explained by the QTL ranged from 9.7% (E3-LN) to 41.8% (E1-HN). Although many QTL were located in pairs or associated with other traits, two almost exclusive locations in chromosomes 2 and 10 were observed for KW (Figure 3.1). Grain moisture was the trait that had fewer QTL in the study. Only one QTL was identified in E3 (Table 3.15). The QTL explaining more phenotypic variance (R^2: 10.7%) was located at chromosome 5 in position 125.9cM for LN treatment. Only one QTL was found for HN treatment and it was associated with QTL for N% at chromosome 10 (Figure 3.5).

For the grain quality traits, several QTL were located across the 10 maize chromosomes (Figure 3.5). Thus, for GPRT (13), GO (28), GSTH (16), and GD (14) QTL were identified in clusters associated with other traits. However, GO presented some unique positions in chromosome 6 (Figures 3.1 & 3.3), and chromosomes 9 and 10 (Figure 3.3). The QTL with the highest R^2 (11.6%) was located at chromosome 4 in position 134.8cM (Table 3.15) forming a cluster with 3 other GO QTL. The total phenotypic variation explained by the QTL ranged from 31% (E1-LN) to 51.5% (E3-HN) which was the highest in the study. For GPRT, a single QTL

could explain from 5.1% to 10.1% of the phenotypic variation. Some of these QTL were located close to GY and GSTH QTL. GPRT presented the highest negative correlations to those two traits, which is an important factor to notice for further marker-assisted selection strategies. For GSTH, the QTL with highest R^2 (11.5%) was located in chromosome 10 in position 66.8cM. The total phenotypic variation explained by the QTL ranged from 11.8% (E1-LN) to 42.2% (E3-LN). The majority of GSTH QTL formed clusters with the QTL for GO and GPRT (Figure 3.5). Of the QTL found for GD, the only one at E1-LN showed the highest R^2 (10.6%) that explained the phenotypic variability (Table 3.15). In general, the QTL identified for GD formed close association with QTL found under LN for GMST (Figure 3.5).

Finally, several QTL were found for the traits used to evaluate nitrogen status of the DH lines after flowering (Table 3.15). Under E1 and E2, 18 QTL were identified for CHLO across N treatments. An individual QTL could explain from 4.8% to 9.7% of the phenotypic variation. The QTL for were spread into the majority of chromosomes, and usually pairs of CHLO QTL were located in clusters in chromosomes 1, 5, 8 and 10 (Figure 3.5). For N%, 22 QTL were found in E3 and E4 (Table 3.15). The QTL with the highest R^2 (12%) was located in chromosome 3 in position 156.cM. The total phenotypic variance explained by the QTL ranged from 30.5% (E3-LN) to 41.8% (E4-HN). Some of these QTL were located close to the ones of GY in chromosomes 1 and 3 at position where QTL were located at higher densities (Figure 3.5). However, QTL for N% and CHLO were also located closely but in less denser genomic regions. Clusters at chromosomes 6 and 10 were observed for QTL identified at HN treatments especially.

Discussion

Phenotypic analysis

One of the starting premises of this study was to create environments with contrasting N levels to analyze the development of the IBM2SYN10-DH maize population. Since two locations with different management history were used for two years, it was difficult to predict that the outcome of the N conditions was going to be similar at both sites and over the years. The results showed an important effect of the environments for the analysis of the data (Table 3.1). The weather conditions varied between growing seasons 2010 and 2011. Given that N is a mobile nutrient in the soil (Lynch, 2013), the availability of the nutrient could have been impacted by the extreme amount of precipitation observed in 2010 compared to 2011. Soil samples were analyzed in both years at Burkey (E1 and E3). Results showed that the available amount of Nitrate (NO_3^-) between tasseling and silking stages was 2X to almost 4X higher at the LN areas of the field in 2011 (Table A3.2). This suggests that enough N was available at LN areas for the DH lines to fully develop and reproduce at E3eliminating a contrasting N treatment for the study in 2011 at Burkey. Due to these events, and the results of the statistical analyses, it makes sense to interpret the outcomes with focus in each environment and N treatment.

The focus of this study was on grain yield and related traits, as well as grain quality traits; more than a total plant physiological approach. Significant variation of the means was observed for the majority of traits at different environments. A severe 63% reduction of GY at E1 under LN treatment was the most extreme variation of all the study. Similar or even more drastic reduction have been reported in maize (Ribaut et al., 2007) in reduced N experiments. At E2 the reduction was moderate (31%), which according to some reports is a preferred condition in order

to ensure the evaluation of GY and other GY related traits (Bertin and Gallais, 2000). To be able to target certain percentage of reduction in yield, previous knowledge must be available about yield scores in a specific location. Since it was the first time that the IBM2SYN10-DH population was grown at Burkey under the field conditions described before, there was no clear expectation of a specific GY difference. Furthermore, the poor performance of the DH lines (μ=0.93 t/ha) and the low amount of residual N measured in soil tests at mid-season in E1-LN (Table A3.2), suggested that additional N fertilizer was needed prior to the next growing season at the LN area in E3. The results for GY were not the expected ones compared to what was observed in E1 and E2, although there was still a significant difference of the means at E3 due to the N treatment (Table 3.4). Other traits like PHT, flowering traits (GDUs, ASI), and yield related traits (EPP, KW) were affected in a similar manner as GY at E3 and E4. These results show the plasticity of the set of maize DH lines to a low increment of N in the soil from 0 (E1) to 67.2 kg N ha^{-} (E3). It could be argued that N-uptake and remobilization of the available N in the source tissues were effective for the inbred lines at this N level. Moreover, it has been reported negative correlations at LN levels between N-remobilization and post-silking N-uptake in lines *per se* compared to testcross progeny (Coque and Gallais, 2008). This supports the observed data that an inbred plant can perform well even if a reduced amount of N is available in the soil when the plant reaches maturity. However it was not a result that was expected in the study, which did not allow for a differentiation of the desired environments.

Results showed a reduction of GY, PHT, EPP, and KW values at LN levels in E1 and E2, while flowering traits increased (GDUs, ASI). These results were expected based on previous studies performed using different maize mapping populations or at the testcross level (Bertin and Gallais, 2000; Cai et al., 2012; Ribaut et al., 2007). Interestingly, the correlations observed

throughout the study were generally maintained despite the variation in N levels as well as the E effect. Pairwise correlation between GY and PHT were not significant at any level (Tables 3.11-3.13). Significant positive correlation have been reported between the two traits under HN and LN conditions (Ribaut et al., 2007). The authors showed that PHT had a stable performance across the environments, even when the LN treatments where in wet or dry environments. Hence, in is possible that at LN conditions a maize plant will have more difficulty to remobilize scarce N for grain production. It could be likely that small plants would have less of this latter problem due to the reduce biomass demanding for N, thus increasing GY. In HN conditions, the balance of N is instead important since there is no limiting N factor in the soil. A taller plant could continue to uptake and remobilize N for biomass development. However, taller plants are also more susceptible to stem lodging and green-snap due to environmental pressure at flowering stages (Blackmer et al., 1996). Moreover, it has been reported that increase N rate increase stalk breakage (Elmore and Ferguson, 1999), which could certainly correlate to lower GY of tall plants. It was suggested that PHT should be used as part of an index for selection for plant performance in LN levels (Ribaut et al., 2007), however these results do not support this argument.

Focusing on the grain quality traits, the analysis of the means showed that grain protein and density are a positively influenced by the increase N levels (Tables 3.2, 3.4). Even at E3, where no positive effect of HN for the agronomic traits was observed. It was reported that selection for grain protein in the Illinois High Protein (IHP) maize material transformed N use by the maize plant by increasing the ability of IHP lines to uptake N (Uribelarrea et al., 2007). This could lead breeders to develop varieties with better grain quality and that have higher N use efficiency. However, results in the present study also show a significantly negative correlation

between GY and GPRT across both environments and N treatments. This is evidence that although plants could have a better uptake of N, the remobilization of it is not adequate. Other report also found significant negative phenotypic correlation between GY and %N in the grain at HN conditions (Bertin and Gallais, 2000). Furthermore, strong phenotypic correlations among grain quality characteristics based in a complex genotypic system have been previously discussed (Cook et al., 2012; Dudley et al., 2004). N status in the plants was estimated based in the analysis of two physiological traits; leaf chlorophyll content (CHLO) and leaf N percentage at 20 DAF (N %). Even though these traits were not analyzed in the same environments, both showed significant response to the N treatments. Besides, both traits showed positive correlations with GY in the respective environment and N treatment combinations. Reports of using SPAD-meter and N% have shown that there is a positive correlation on the detection of N status of the plants at varying rates of N applied (Bullock and Anderson, 1998). Also their results showed that correlations increased and became more significant after R1 stage in maize, which is when it was measured in the present study. Furthermore, leaf N% and chlorophyll measurements can determine prolonged photosynthesis and leaf senescence activity, which has positive effect in N-assimilation and N-remobilization in maize, and thus in yield (Hirel et al., 2007a). However, there is still the need to understand the regulation behind the balance between keeping N in the leaves for increasing photosynthesis, and the remobilization for producing grain.

Genotypic analysis

It was found that the IBM2SYN10-DH population had significant genetic variability for the objectives of the study (Tables 3.2-3.5). The amount of variation is essential for QTL mapping to be effective. The analysis of variances was used to estimate variance components and repeatability of the study (Tables 3.6-3.9). Repeatability ranged from 0.28 (GMST-E3) to

0.95 (SLK-E4). High repeatability is the result of high genetic variance components estimated within the model. This supports the QTL analysis procedures to better detect genomic regions associated with the traits of interest. Within each environment, significant genotype * N interactions were observed for almost all the traits; that lead to perform QTL analysis for each N treatment. Furthermore, given that significant spatial variability was observed between the replications of the experiments (Table 3.1), BLUPs were used to estimate the trait values for the analysis. This in order to reduce the influence of spatial variation and maximize the influence of the genetic variation observed.

A total of 302 QTL were identified in this study. These QTL were located across the 10 maize chromosomes, and were found for almost all trait/environment/N treatment combination that was analyzed. In general, the QTL showed a tendency to form clusters across the chromosomes. In E1, such clusters can be observed in chromosomes 1, 5, 7 and 8, where QTL for HN and LN can be found interchangeably (Figure 3.5). Also, some of the clusters seemed to group QTL primarily for agronomic traits (Chr.7, Figure 3.2) and others grouped QTL for grain quality traits (Chr. 2 & 4, Figure 3.2). This phenomenon can be due to associations of traits that explain a physiological action or due to a genetic linkage in the population (Bertin and Gallais, 2001). Previous studies have presented the formation of clusters in N-related experiments (Cai et al., 2012; Gallais and Hirel, 2004; Liu et al., 2012; Nichols, 2008). These groups of QTL identified for several agronomic traits can be target regions in the genome to search for loci associated with N response. However, it is important to determine the stability of the QTL especially across N treatments in order to specify the effect of these putative loci in the N metabolism and response.

In this study, significant environmental effects were detected, which reduced the possibilities to do a QTL analysis across all environments. Hence, QTL identified at E1 were not always identified at E3 (Figures 3.1, 3.3). For chromosome 1, QTL associated with GY and KW were located in E1 but QTL for the same traits were not located in E3. The same can be observed for E2 and E4, where QTL for GY identified in chromosomes 7 and 9 were not found at both environments (Figures 3.2, 3.4). On the other hand, even though significant N * Gen interactions were observed for almost all the traits (Tables 3.6-3.9), the QTL * N interaction observed was not substantial across the environments. Some QTL were identified only at specific N treatments, but the majority of clusters grouped QTL for traits for both HN and LN as observed in Figure 3.5. Furthermore, through all E, only one small cluster for N% and CHLO is observed in chromosome 6, which groups 3 QTL for HN identified at 3 different environments. A partial explanation to the collocation of QTL found for both N treatments in similar or close genetic position can be repeatability. It was measured in an entry-mean basis, which means that the values for repeatability (Tables 3.6-3.9) are greater if the genetic component is higher. So even if N * Gen interaction is significant, it only account for a small percentage of the variability. Traits with higher repeatability values (PHT, GDUs, ASI, GO) usually group QTL for HN and LN treatments close together, even at E1 and E2 where the N treatments were effective. So, it is better to focus in specific QTL for each E in other to compare to reported studies.

The genotypic information developed by GBS (Liu et al. in publication) was subjected to an adjustment of the expanded genetic map so it could be compared to F2-based maps used in previous studies. Then, QTL identified from environments E1 and E2 such as *GYHN-1a, GYLN-1, GYLN-3a, GYHN-7, GYLN-7, and GYLN-9* (Table 3.15), co-localized with QTL intervals previously reported for grain yield (Liu et al., 2012). Moreover, some of the clusters of QTL for

agronomic traits (Figures 3.1 & 3.2), co-localize with clusters of similar traits in chromosomes 1, 4, 5, 8, and 10 (Bertin and Gallais, 2001; Gallais and Hirel, 2004) which are known to carry loci associated with N metabolism (Liu et al., 2012). One of those loci is the *glutamine synthetase4* (*gln4*) that maps between 205,237 and 205,240 kb (Locus Lookup tool;(Andorf et al., 2010) in chromosome 5 (Figure A3.1). At this locus, the active GS enzyme is one of the main involved with N assimilation and glutamine conversion in mature plants (Hirel et al., 2001). This GS activity had a positive correlation with GY at low N levels, and can have a direct impact in KW as well due the control in N-remobilization after flowering (Gallais and Hirel, 2004). Results of the present study showed QTL for GY, EPP and CHLO at this region in chromosome 5 (Figure 3.5); and also for ASI and SLK under both N treatments. This could mean that ASI and SLK have a physiological importance to the response to nutrient stress tolerance. Although ASI and SLK had a negative correlation with GY, it is interesting to note that shorter ASI and earlier SLK are actually beneficial for individuals under stress due to the ability to compensate and guarantee effective pollination earlier in the season (Gallais et al., 2007). Another interesting cluster of QTL is the one identified in chromosome 10 (Figure 3.5). Results showed that QTL for CHLO, N%, and ASI were collocated with QTL intervals reported for leaf senescence and ASI (Bertin and Gallais, 2001; Gallais and Hirel, 2004). CHLO and N% readings are determinants of the senescence stage of the leaves, and therefore the N-uptake capacity of the plant for grain filling. The longer the leaf tissue can hold the source of N for remobilization, the higher possible influence over GY and KW it will have. The activity of GS enzyme is determined at this genomic region too (Gallais and Hirel, 2004). The locus *gln5* has been reported to have a post-transcriptional control of N-assimilation mediated by the GS enzyme, which could accumulate

amino-acid in the leaves for further remobilization if necessary (Hirel et al., 2007a; Migge et al., 2000).

QTL identified for grain quality traits also showed the formation of clusters in some chromosomal regions. These clusters co-localize with some previously reported using the NAM maize population (Cook et al., 2012). The most important regions were located in chromosomes 1, 2, 5, 6 and 10, where QTL for grain protein (PRT), oil (GO) and starch (STH) were grouped (Figures 3.1 & 3.3). It could be expected to find similar genomic regions that control these quality traits due to correlations (Tables 3.11 & 3.13). These traits make almost the entire maize kernel composition; meaning that the increase in protein percentage will reduce the percentage of the other two traits. Besides, the study in the NAM population suggested a high level of pleiotropy for these traits due to the high correlation between allele effects (Cook et al., 2012). Results in the IBM2SYN10-DH population showed five specific genetic positions for QTL only associated with GO, and no other QTL for grain quality traits in chromosome 6. QTL for HN and LN (*GOHN-6a*, *GOLN*-6b) co-localized very close to the mapped position of locus *ln1*: 102,191 to 104391 kb (Figure A3.2) of chromosome 6 (Locus Lookup tool, (Andorf et al., 2010). Actually, the QTL analysis in the NAM population identified the QTL with highest LOD located in chromosome 6 for grain oil (Cook et al., 2012). The authors of the study found that this QTL was overlapping with locus *ln1*, where the high oil allele *DGAT1-2* was located. Furthermore, they were able to find some alleles with additive genetic effect up to 0.21% for high oil content. Thus, QTL at this chromosome region can give valuable information to detect more loci associated with increase grain quality in maize. However, due to negative correlations and possible pleiotropy reported, it becomes a challenge to improve protein and oil content in maize varieties.

In conclusion, environmental effects are determinant in N related studies and probably even more critical when studying inbred lines. The IBM2SYN10-DH population showed to be significantly responsive to the increase of N from one year to another. If results are not stable across environments, it becomes difficult to predict the performance of maize lines for breeding purposes. Yet, experiments using the IBM2SYN10-DH in a testcross population are in progress to be able to determine the response to contrasting N treatments at a hybrid level. Nonetheless, significant phenotypic and genotypic variation was observed across the study for grain yield and related traits, as well as for grain quality traits.

Several QTL were identified for specific E and some were consistent across environments. This lead to the formation of clusters, which included QTL for traits such as GY, EPP, ASI, CHLO, N% among others, and were located near important loci that are responsible of the N metabolism control. Even though the traits were affected by the variation in N supply, only a few QTL specific for each N treatment were identified. This could mean that the population lacks the variation of alleles responsible to low or high N levels, or that more quantitative genetic approaches are needed to clearly determine alleles for N response. Nonetheless, given the genetic resolution provided by the IBM2SYN10-DH population, many QTL or clusters of QTL were co-localized with previously described N related loci. It is important to understand, that these QTL have to be further analyzed and validated in order to obtain a better knowledge of the genetics behind N response.

Finally, the genetic analysis showed that many of the clusters of QTL identified in this study grouped traits that are negatively correlated. One of the goals of maize breeders will be to develop varieties with improved response to N stress, but also with enhanced agronomic and qualitative characteristics. These represents a great challenge according to the results presented

here and in previous N related studies. Although GY will still be the main trait to breed for, increased demand in high protein grain and more efficient N use keeps adding pressure to find the underlying genetic basis to be able to improve the selection indexes in a positive way without having to give up on one of the traits listed above. The identification of NUE related traits that present a high correlation with yield, will be a key factor to developing varieties responsive to N variation (Agrama et al., 1999). Genetic studies can help to understand the dynamics of grain yield and related traits under varying environmental pressure, and could help to make better breeding decision in a near future. Assessing the response to N supply with different strategies will help breeders to improve their maize germplasm for efficient N response.

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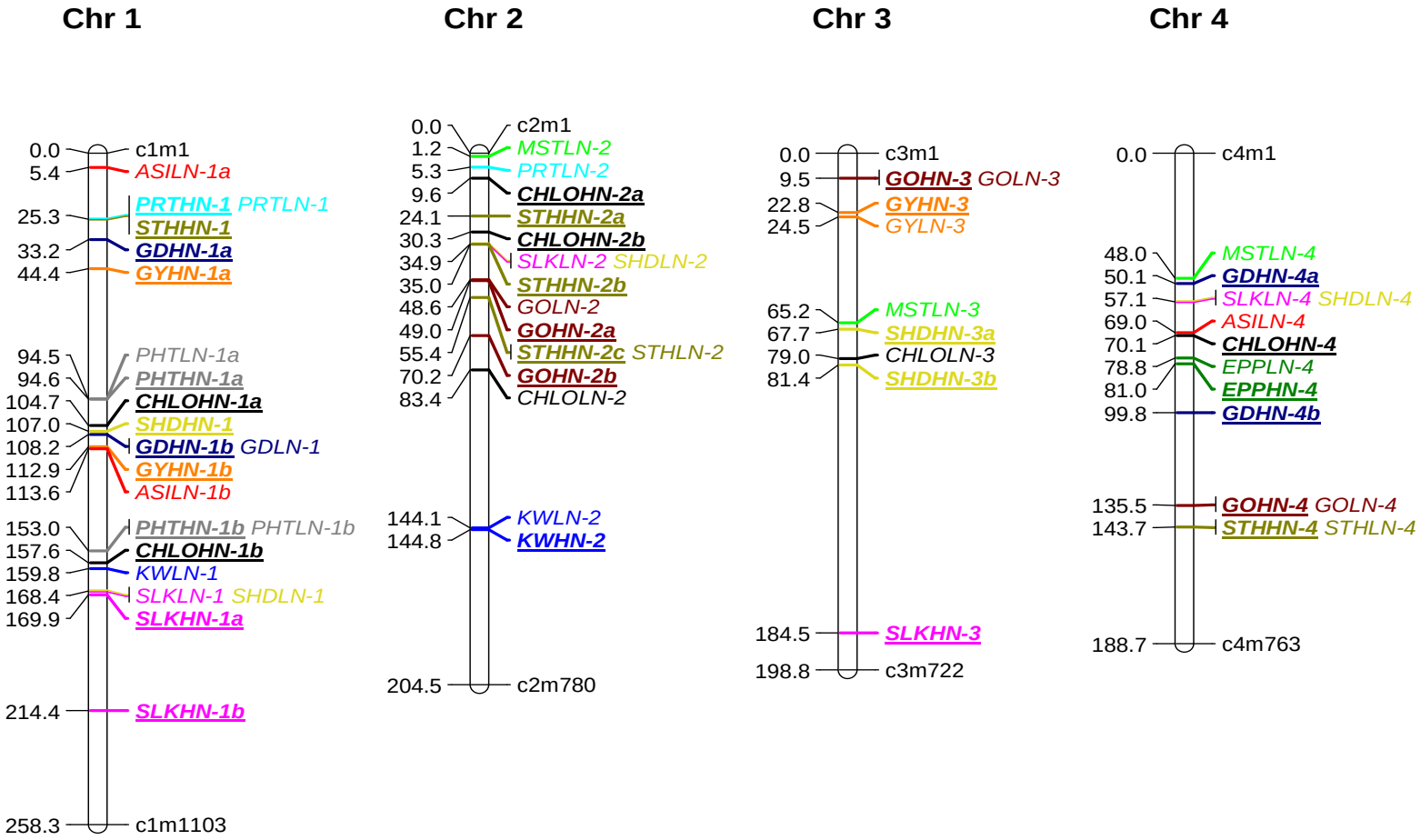


Figure 3.1. Results of QTL analysis of agronomic, grain quality and physiological traits measured in High and Low N treatments on the IBM2SYN10-DH population at environment 1 (Burkey-2010).

The traits measured include grain yield (GY, orange), plant height (PHT, grey), growing degree units to silking (SLK, pink), growing degree units to anthesis (SHD, yellow), anthesis-silking interval (ASI, red), ears per plant (EPP, green), kernel weight (KW, blue), grain moisture (MST, light green), grain protein (PRT, turquoise), grain oil (GO, dark red), grain starch (STH, mustard), grain density (GD, dark blue), and leaf chlorophyll content (CHLO, black). Flanking markers were placed for each chromosome (Chr). QTL for traits under HN are underlined and in bold letters.

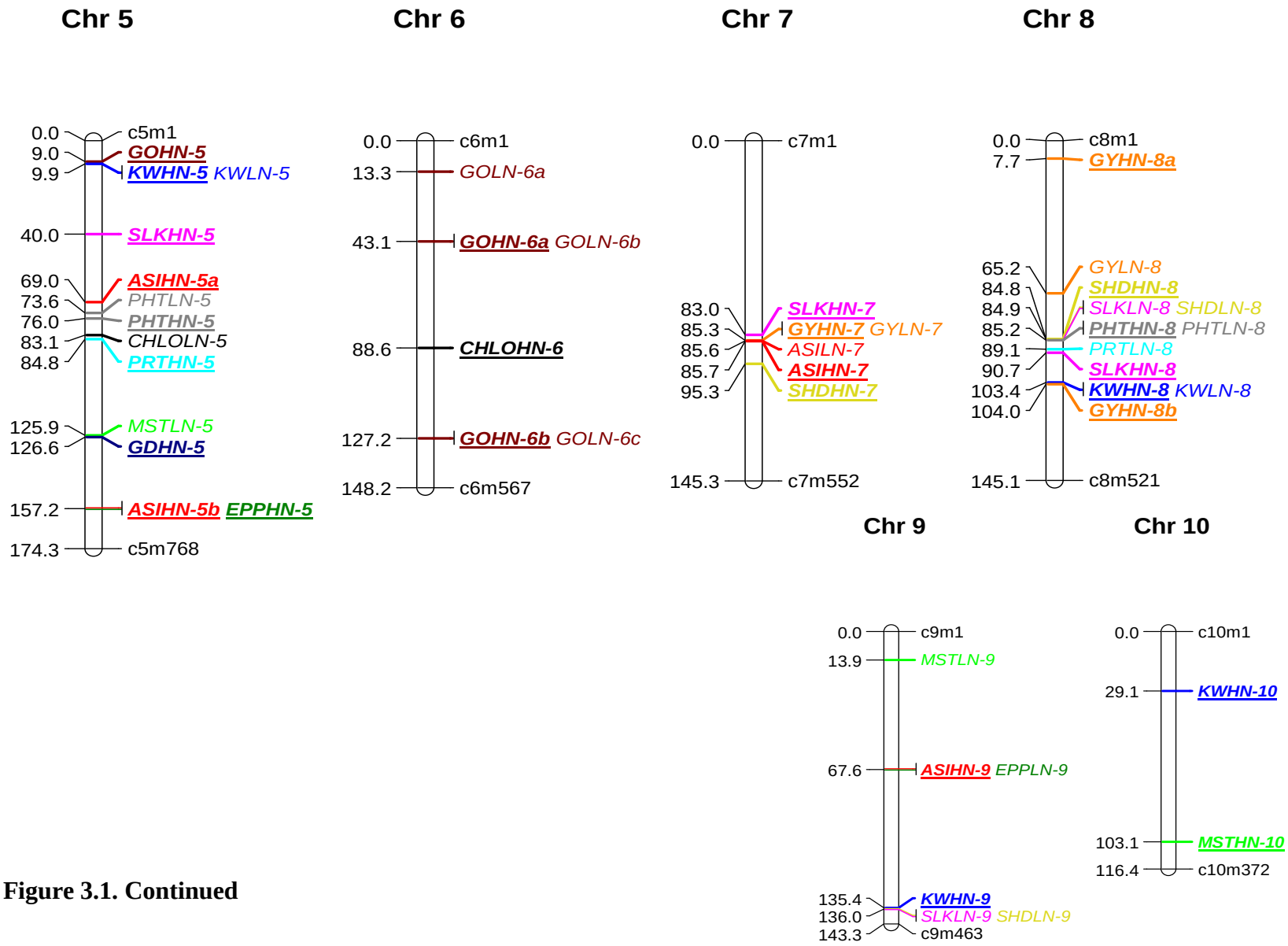


Figure 3.1. Continued

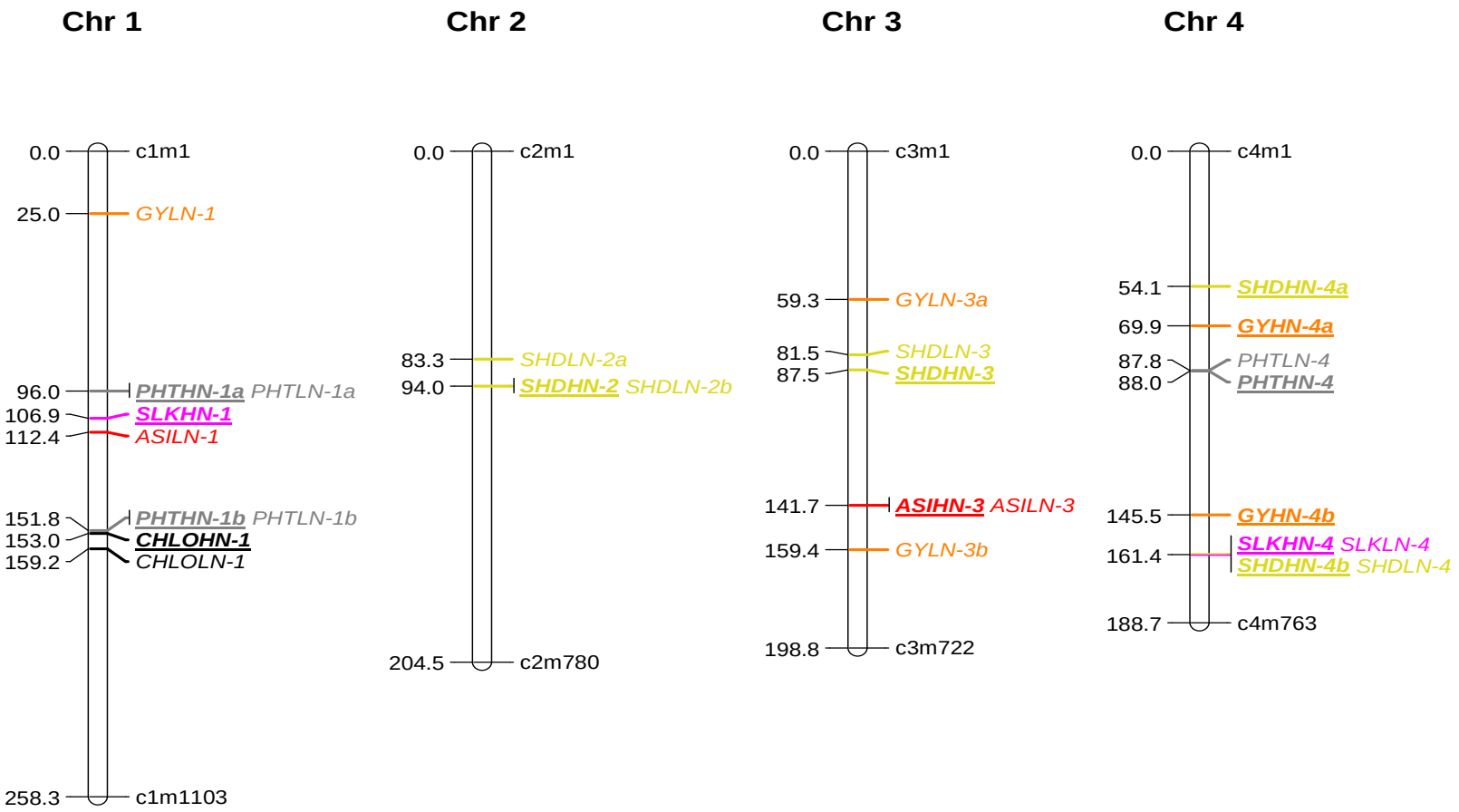


Figure 3.2. Results of QTL analysis of agronomic, grain quality and physiological traits measured in high and low N treatments on the IBM2SYN10-DH population at environment 2 (Marion-2010).
The traits measured include grain yield (GY, orange), plant height (PHT, grey), growing degree units to silking (SLK, pink), growing degree units to anthesis (SHD, yellow), anthesis-silking interval (ASI, red), and leaf chlorophyll content (CHLO, black). Flanking markers were placed for each chromosome (Chr.). QTL for traits under HN are underlined and in bold letters.

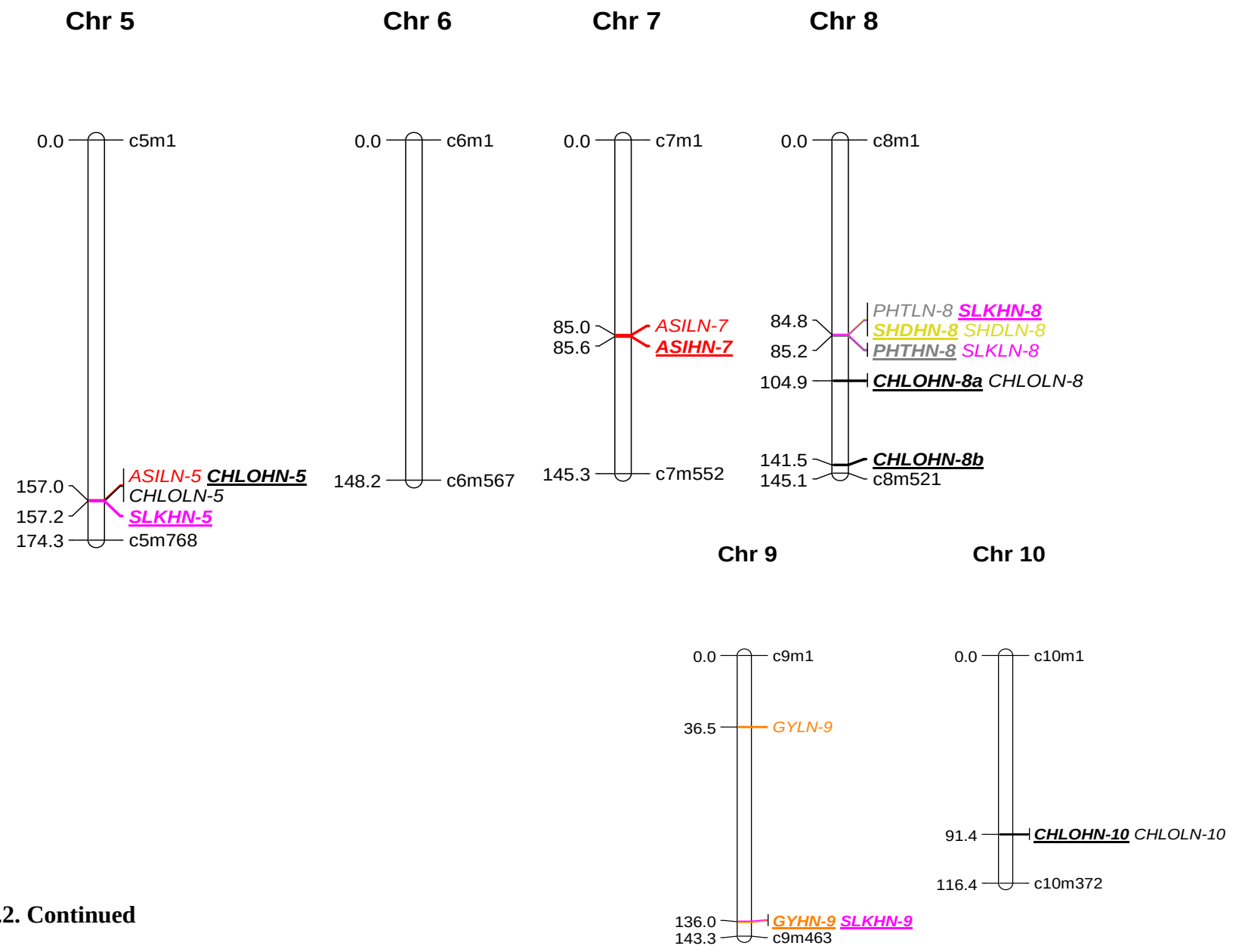


Figure 3.2. Continued

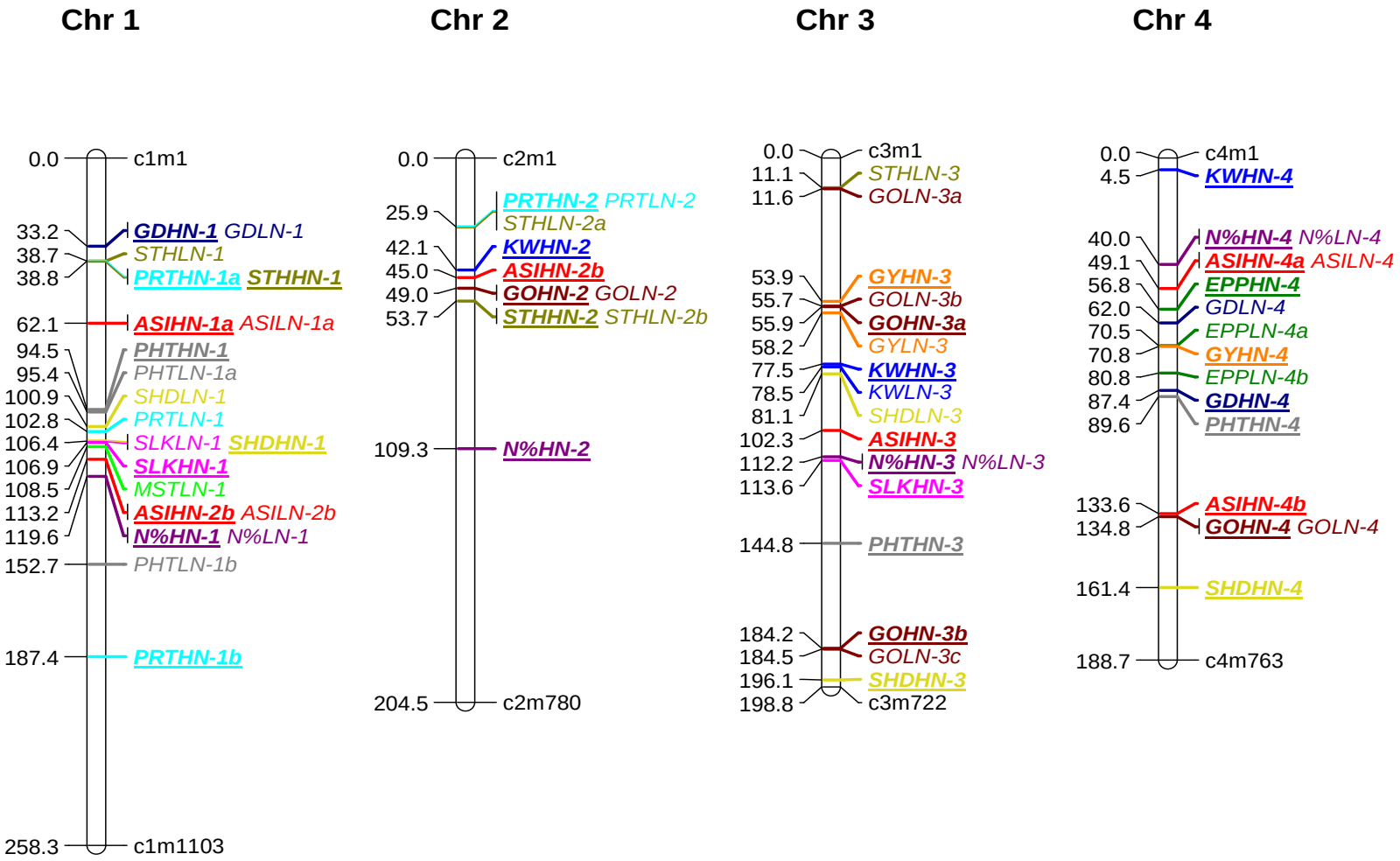
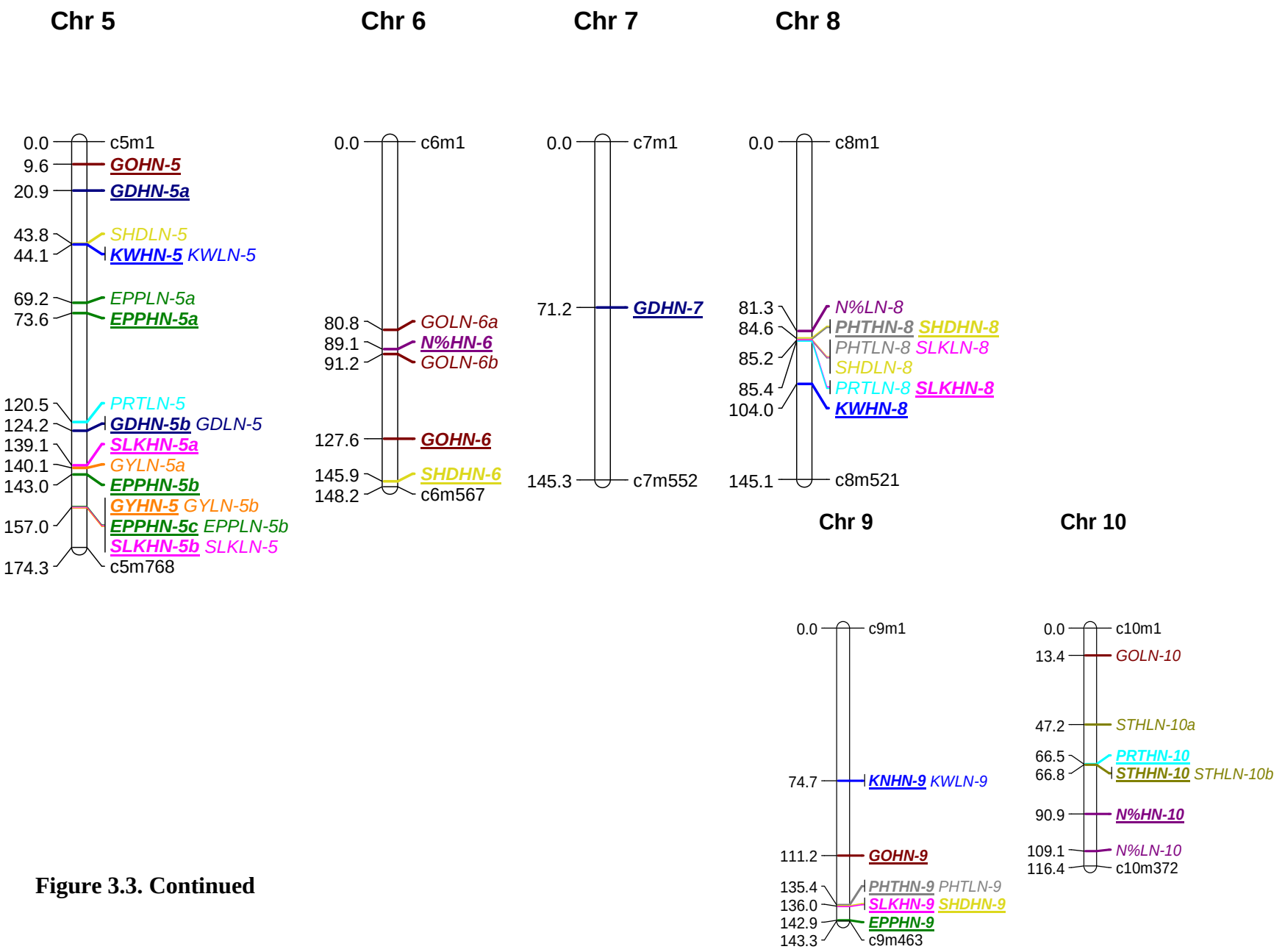


Figure 3.3. Results of QTL analysis of agronomic, grain quality and physiological traits measured in High and Low N treatments on the IBM2SYN10-DH population at environment 3 (Burkey-2011).

The traits measured include grain yield (GY, orange), plant height (PHT, grey), growing degree units to silking (SLK, pink), growing degree units to anthesis (SHD, yellow), anthesis-silking interval (ASI, red), ears per plant (EPP, green), kernel weight (KW, blue), grain moisture (MST, light green), grain protein (PRT, turquoise), grain oil (GO, dark red), grain starch (STH, mustard), grain density (GD, dark blue), and leaf nitrogen percentage 20 DAF (N%, lilac). Flanking markers were placed for each chromosome (Chr). QTL for traits under HN are underlined and in bold letters.



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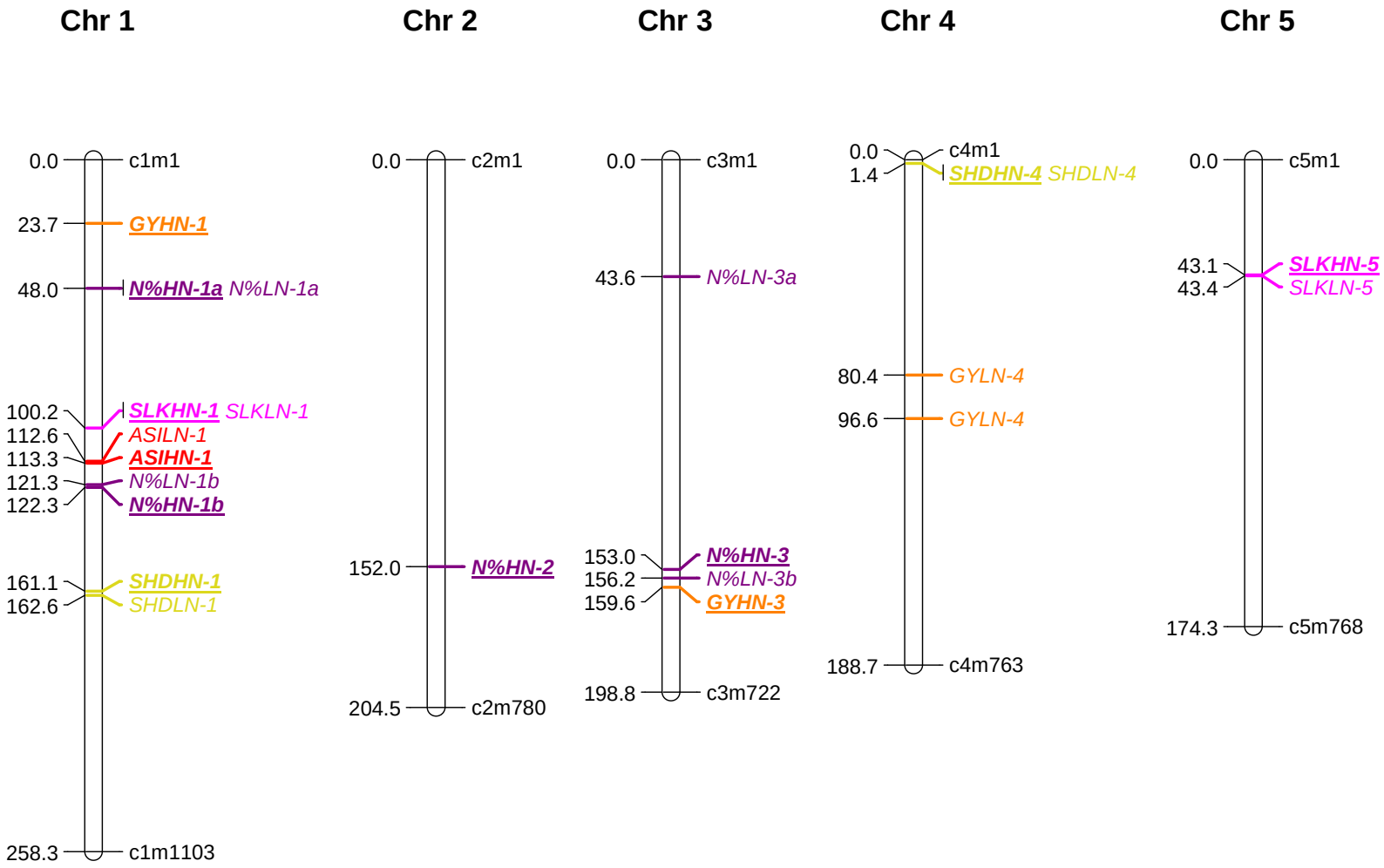
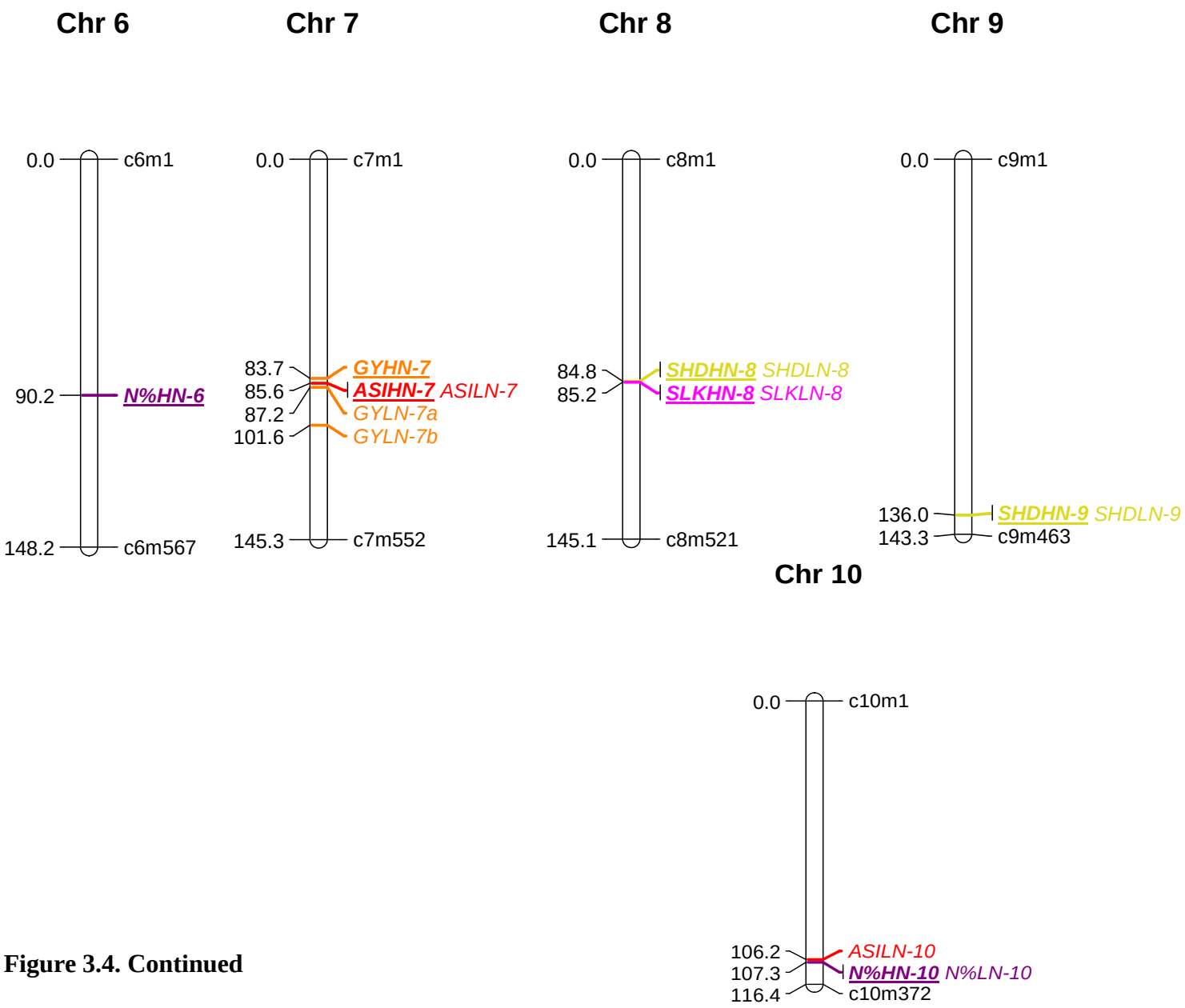


Figure 3.4. Results of QTL analysis of agronomic, grain quality and physiological traits measured in high and low N treatments on the IBM2SYN10-DH population at environment 4 (Marion-2011).
The traits measured include grain yield (GY, orange), growing degree units to silking (SLK, pink), growing degree units to anthesis (SHD, yellow), anthesis-silking interval (ASI, red)), and leaf nitrogen percentage 20 DAF (N%, lilac). Flanking markers were placed for each chromosome (Chr.). QTL for traits under HN are underlined and in bold letters.



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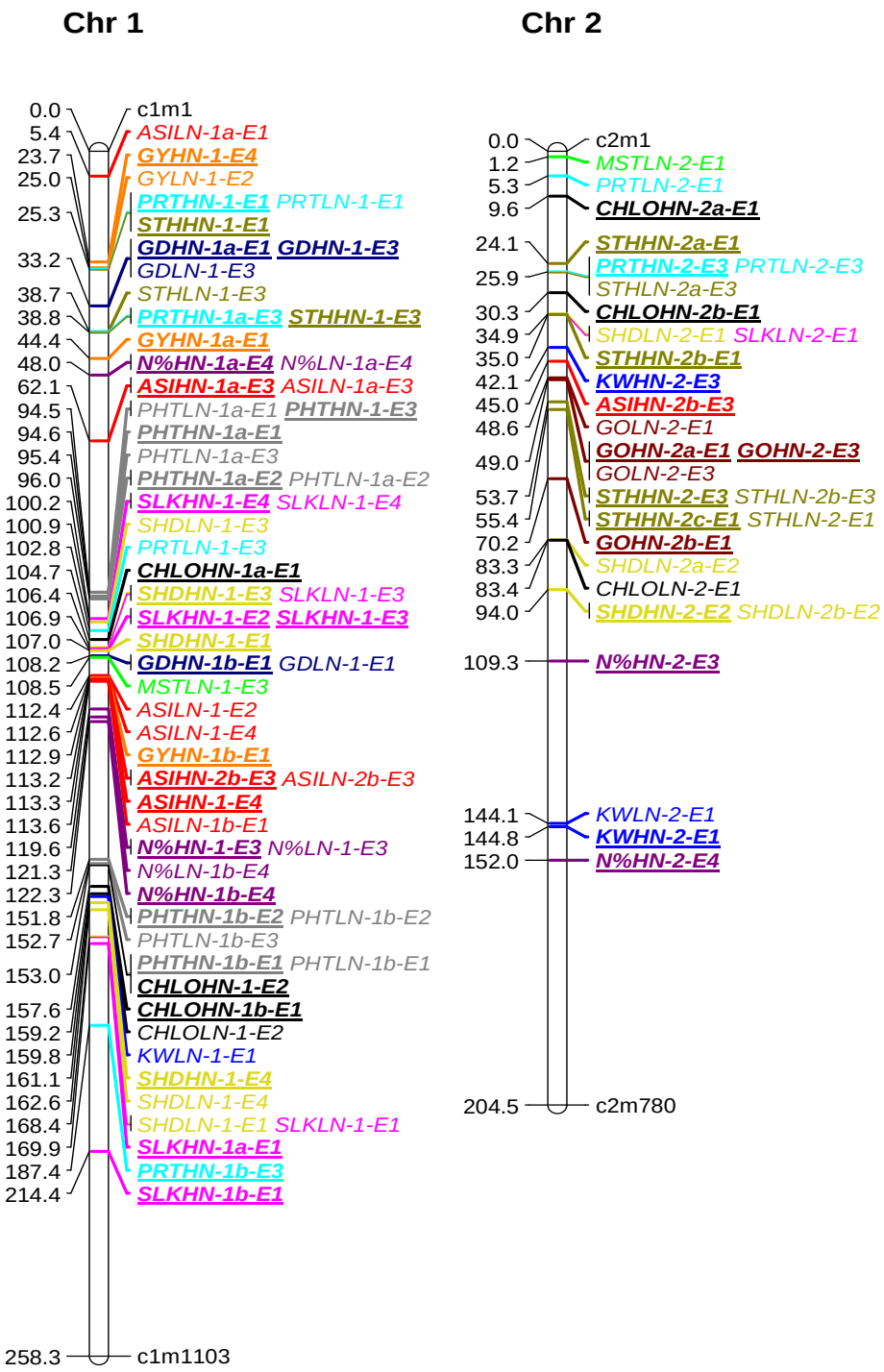
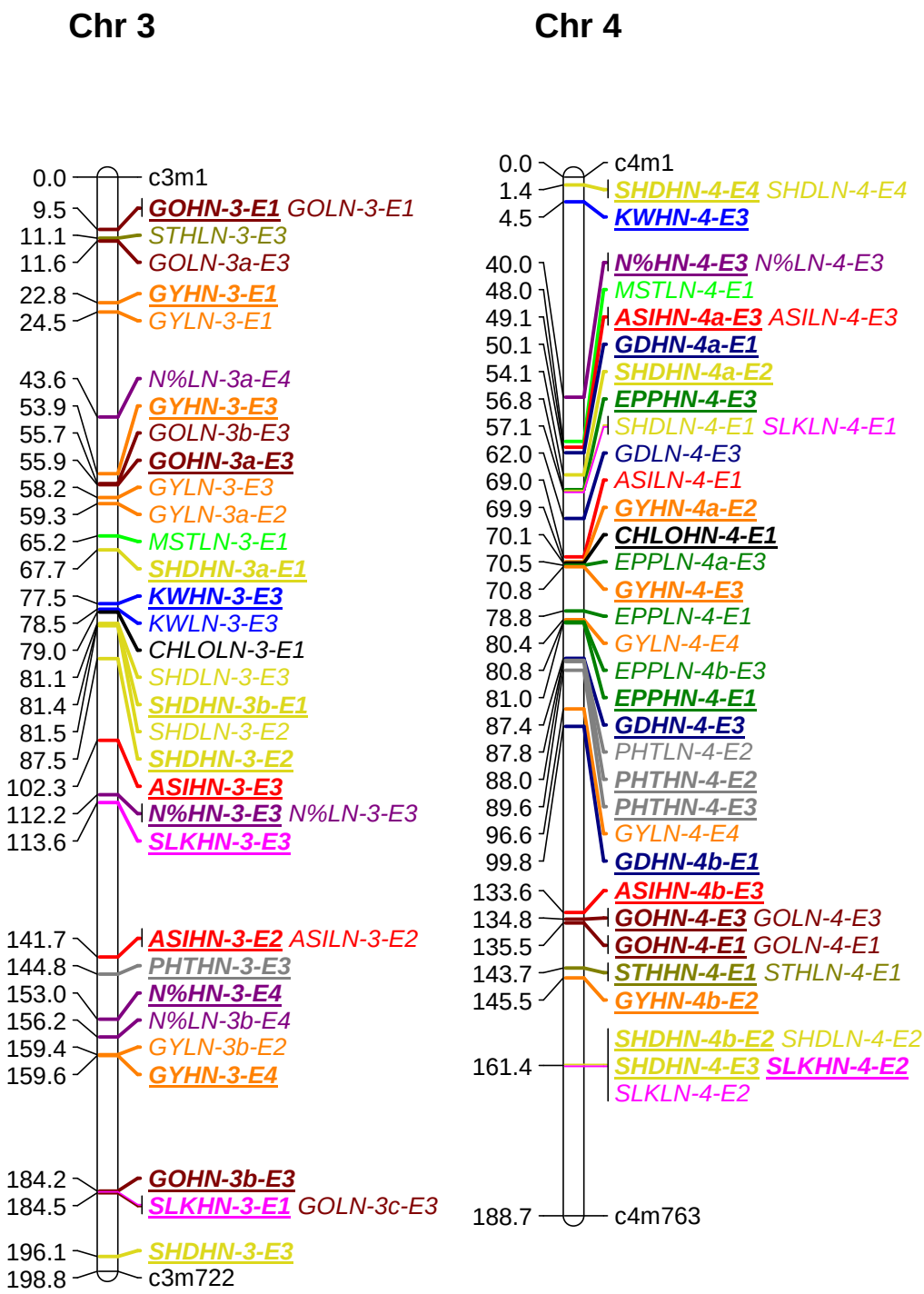


Figure 3.5. Results of QTL analysis of agronomic, grain quality and physiological traits measured in high and low N treatments on the IBM2SYN10-DH population.

The traits measured include grain yield (GY, orange), plant height (PHT, grey), growing degree units to silking (SLK, pink), growing degree units to anthesis (SHD, yellow), anthesis-silking interval (ASI, red), ears per plant (EPP, green), kernel weight (KW, blue), grain moisture (MST, light green), grain protein (PRT, turquoise), grain oil (GO, dark red), grain starch (STH, mustard), grain density (GD, dark blue), leaf chlorophyll content (CHLO, black), and leaf nitrogen percentage 20 DAF (N%, lilac). Flanking markers were placed for each chromosome (Chr.). QTL for traits under HN are underlined and in bold letters. QTL for each environment is marked by the corresponding E number.



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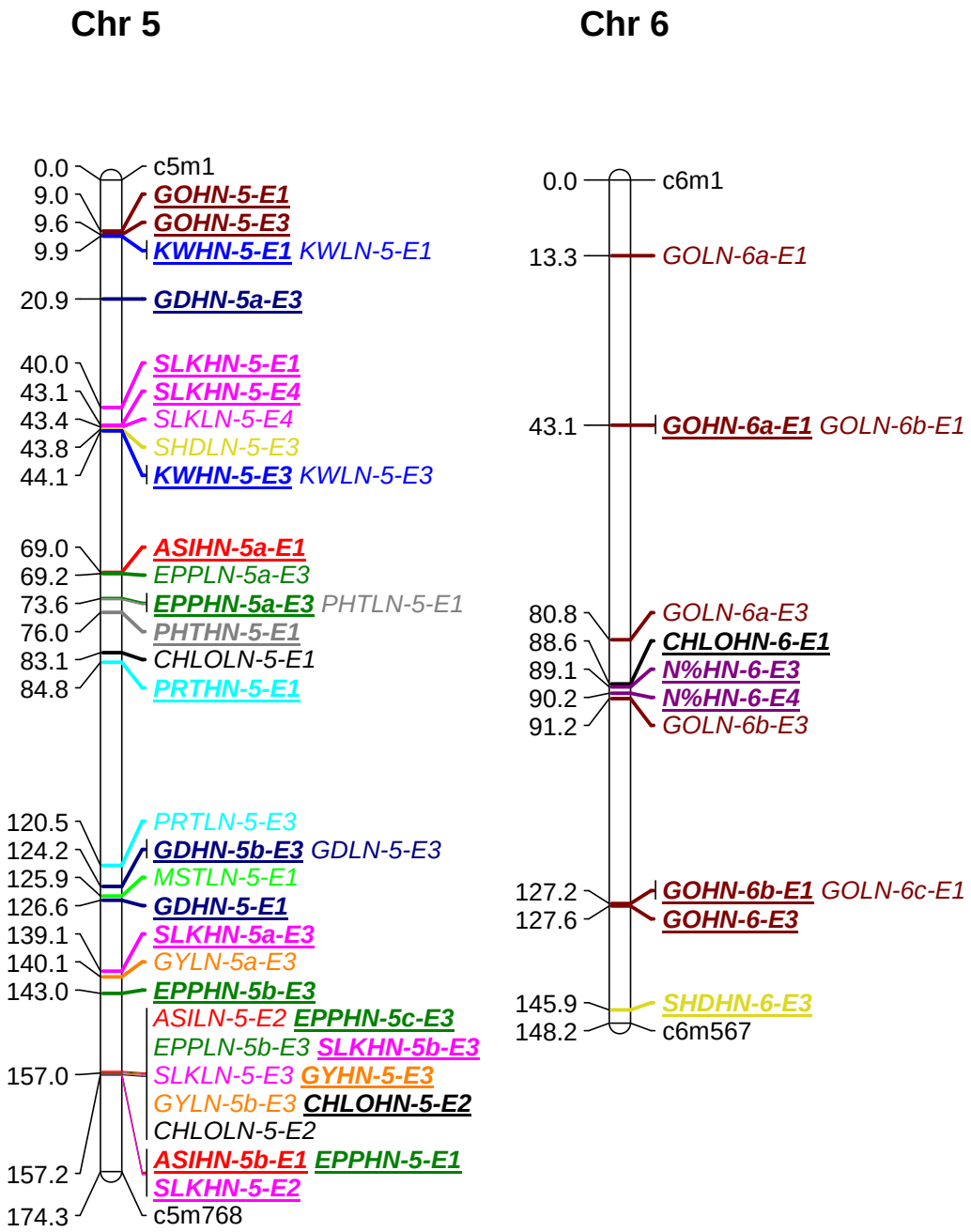

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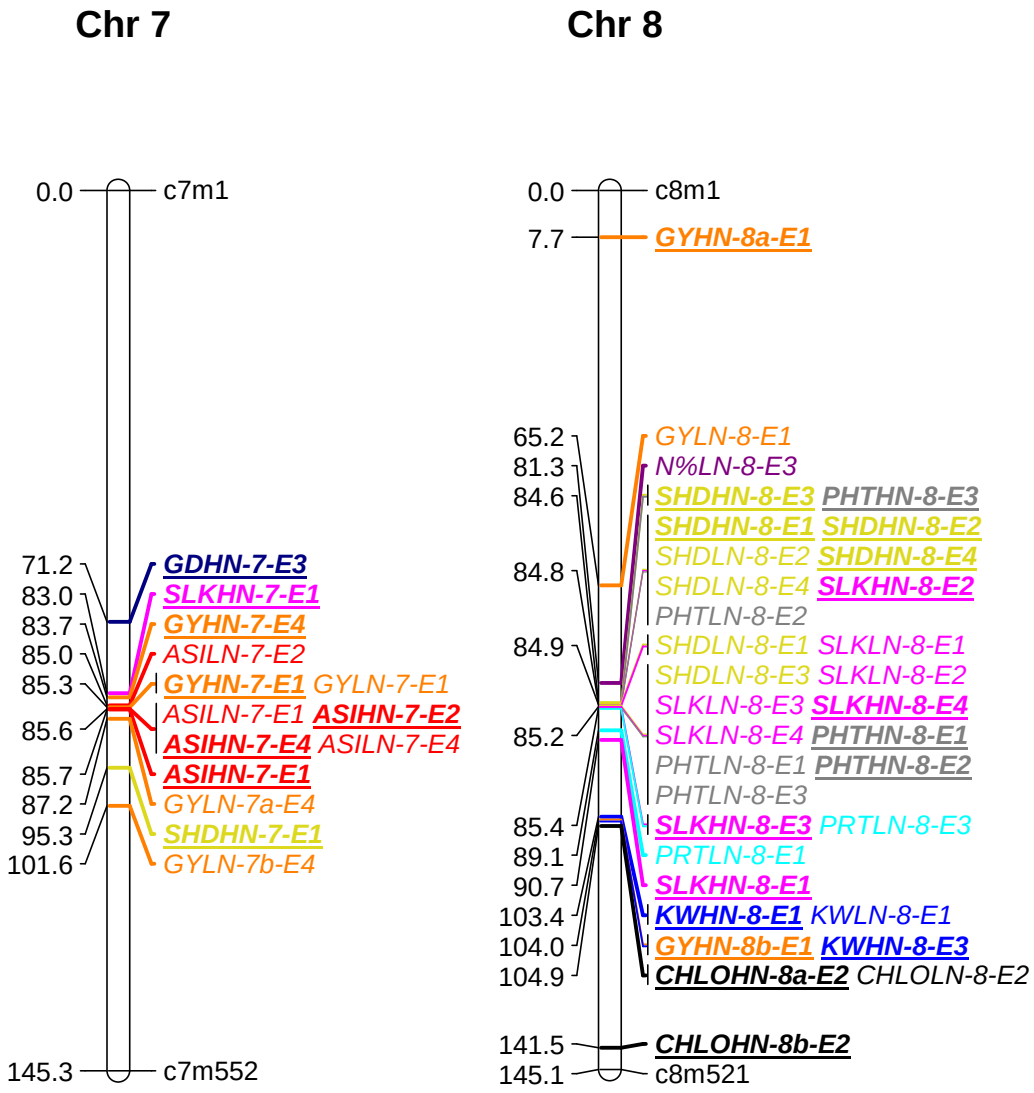

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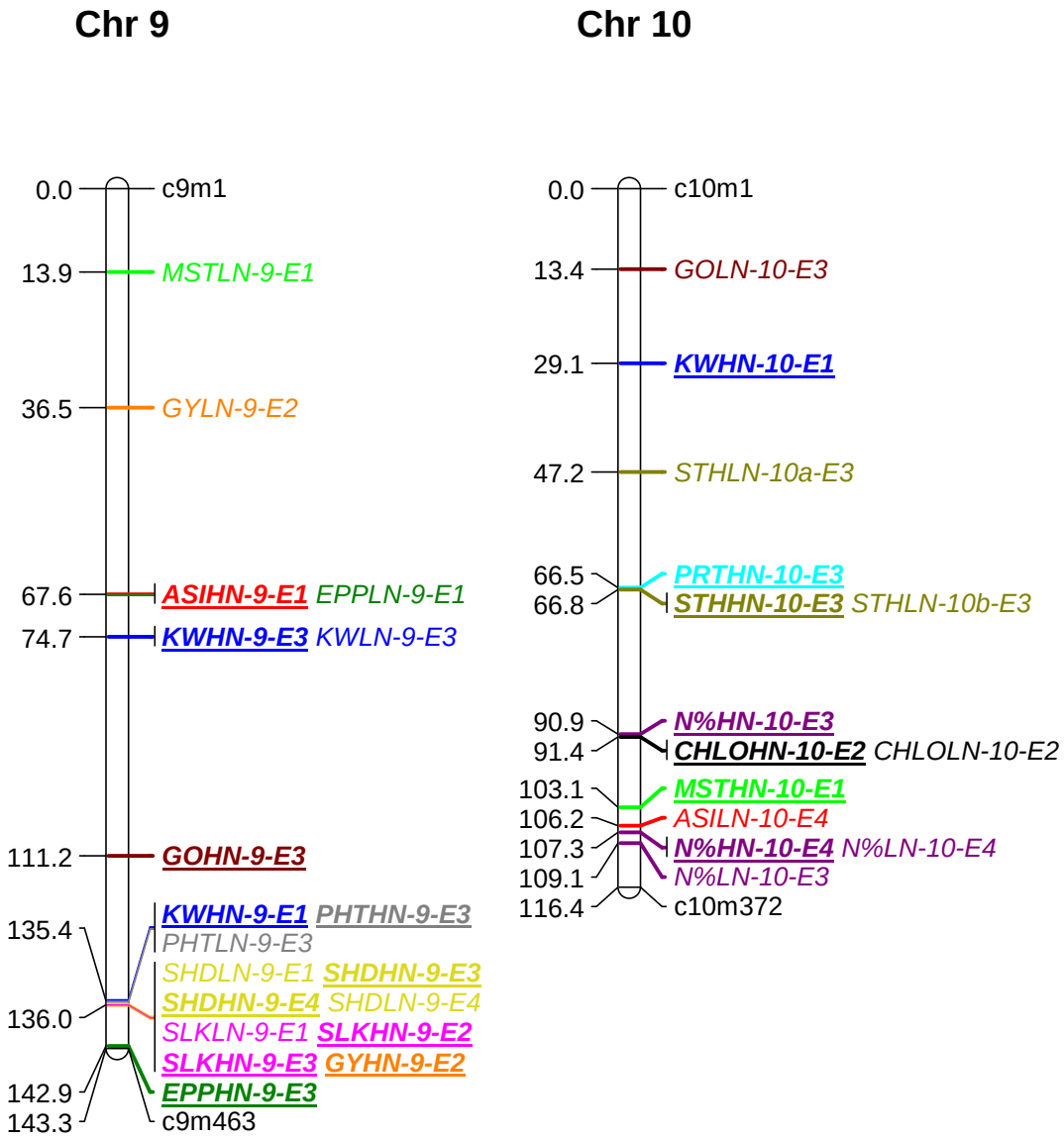


Figure 3.5. Continued

Tables

Table 3.1. Combined ANOVA shows effects of environment (Env), nitrogen (N), replications within nitrogen (R(N)), genotype (Gen) and the interactions on agronomic and grain quality traits grown under high and low N levels in the IBM2SYN10-DH population.
The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), leaf chlorophyll content (CHLO), and nitrogen percentage 20 days after flowering (N%).

Trait		Env	N	Env*N	R(N)	Gen	Env*Gen	N*Gen	Env*N*Gen
GY	**F Value**	34.66	183.05	381.52	41.72	6.08	2.44	1.67	1.64
	Pr > F	0.0014	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
PHT	**F Value**	4.54	0	39	19.98	0.02	5557.2	1.13	1.45
	Pr > F	0.0111	0.9992	<.0001	<.0001	1	<.0001	0.1284	<.0001
GDUSLK	**F Value**	161.57	1070.49	495.92	36.67	13.13	1.53	1.45	1.67
	Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
GDUSHD	**F Value**	204.3	1858.49	520.22	37.63	20.9	1.58	1.13	1.51
	Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1109	<.0001
ASI	**F Value**	42.14	69.36	101.43	7.7	6.86	1.3	1.14	1.47
	Pr > F	0.0006	<.0001	<.0001	<.0001	<.0001	0.0002	0.1056	<.0001
EPP	**F Value**	143.35	6.99	56.68	1.47	3.14	1.09	0.91	1.29
	Pr > F	0.0008	0.0087	<.0001	0.2312	<.0001	0.2636	0.7703	0.0059
KW	**F Value**	0	0	0	1.69	3.58	1.15	1.06	1.2
	Pr > F	0.9996	0.9998	0.9999	0.1855	<.0001	0.1355	0.3262	0.035
GMST	**F Value**	96.05	110.05	54.53	28.41	1.19	1.34	1.15	0.86
	Pr > F	0.0098	<.0001	<.0001	<.0001	0.1319	0.0124	0.1335	0.9235
GPRT	**F Value**	250.98	793.89	9.03	4.31	6.76	1.83	1.42	1.69
	Pr > F	<.0001	<.0001	0.0029	0.0136	<.0001	<.0001	0.0035	<.0001
GO	**F Value**	29.77	46.96	13.31	0.92	14.47	1.4	1.05	1.59
	Pr > F	0.0002	<.0001	0.0003	0.4007	<.0001	0.0049	0.3428	<.0001
GSTH	**F Value**	156.6	279.83	70.72	4.67	7.85	1.21	1.15	1.14
	Pr > F	0.0033	<.0001	<.0001	0.0096	<.0001	0.0713	0.147	0.0935
GD	**F Value**	369.46	361.28	35.71	1.94	9.61	1.45	1.03	1.9
	Pr > F	<.0001	<.0001	<.0001	0.1443	<.0001	0.0022	0.4034	<.0001
CHLO	**F Value**	208.37	3197.49	672	21.68	2.48	1.61	1.05	1.44
	Pr > F	0.0029	<.0001	<.0001	<.0001	<.0001	0.0001	0.351	<.0001
N%	**F Value**	0.2	528.96	0.1	14.48	7.54	1.1	1.06	1.07
	Pr > F	0.6993	<.0001	0.7495	<.0001	<.0001	0.2419	0.3169	0.2517

Table 3.2. Estimates of means, its variations, and ANOVA of agronomic and grain quality traits under high and low nitrogen levels for IBM-10 DH lines in environment 1 (Burkey-2010).

The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), and leaf chlorophyll content (CHLO).

					Analysis of Variance		
Trait	**N**	**Mean**	**Std. Error**	**Relative Difference (%)**	**N**	**Gen**	**Gen * N**
GY (T/ha)	**HN**	2.52	0.97	63.0	**	***	***
	LN	0.93	0.51				
PHT (cm)	**HN**	194.9	22.36	16.2	**	***	***
	LN	163.3	19.09				
GDUSLK	**HN**	1657.0	91.31	-9.0	**	***	***
	LN	1806.4	97.77				
GDUSHD	**HN**	1560.3	61.61	-5.5	**	***	***
	LN	1645.5	66.41				
ASI	**HN**	97.5	44.29	-52.4	**	***	***
	LN	148.6	43.54				
EPP	**HN**	0.74	0.10	16.2	**	***	ns
	LN	0.62	0.13				
KW (kg)	**HN**	0.058	0.01	16.9	***	***	***
	LN	0.048	0.01				
GMST (%)	**HN**	7.43	0.11	-7.0	*	**	ns
	LN	7.96	0.09				
GPRT (%)	**HN**	11.37	0.99	10.1	**	***	***
	LN	10.22	1.01				
GO (%)	**HN**	3.90	0.31	-0.8	ns	***	***
	LN	3.93	0.31				
GSTH (%)	**HN**	70.5	0.76	-0.6	ns	***	ns
	LN	70.9	0.74				
GD (%)	**HN**	1.27	0.03	1.8	**	***	***
	LN	1.25	0.03				
CHLO	**HN**	48.8	2.47	33.2	***	***	***
	LN	32.6	3.50				

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant; N Nitrogen levels; HN High Nitrogen; LN Low Nitrogen

Table 3.3. Estimates of means, its variations, and ANOVA of agronomic under high and low nitrogen levels for IBM-10 DH lines in environment 2 (Marion-2010).
The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), and leaf chlorophyll content (CHLO).

Trait	N	Mean	Std. Error	Relative Difference (%)	Analysis of Variance		
					N	Gen	Gen * N
GY (T/ha)	**HN**	4.11	0.96	31.1	*	***	***
	LN	2.83	0.78				
PHT (cm)	**HN**	244.0	19.64	8.4	**	***	**
	LN	223.4	19.44				
GDUSLK	**HN**	1394.1	53.89	-9.7	**	***	***
	LN	1528.9	60.23				
GDUSHD	**HN**	1326.9	46.88	-8.6	**	***	ns
	LN	1441.4	47.63				
ASI	**HN**	67.1	22.51	-28.9	ns	***	*
	LN	86.5	22.99				
CHLO	**HN**	54.9	2.85	11.3	*	***	ns
	LN	48.7	2.81				

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant; N Nitrogen levels; HN High Nitrogen; LN Low Nitrogen

Table 3.4. Estimates of means, its variations, and ANOVA of agronomic and grain quality traits under high and low nitrogen levels for IBM-10 DH lines in environment 3 (Burkey-2011).

The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), and leaf nitrogen percentage 20 DAF (N%).

Trait	N	Mean	Std. Error	Relative Difference (%)	Analysis of Variance		
					N	Gen	Gen * N
GY (T/ha)	**HN**	3.20	1.44	-25.2	**	***	***
	LN	4.01	1.40				
PHT (cm)	**HN**	198.8	23.89	-4.9	***	***	**
	LN	208.6	23.28				
GDUSLK	**HN**	1634.4	80.96	1.4	*	***	***
	LN	1611.8	77.91				
GDUSHD	**HN**	1530.6	65.45	1.0	ns	***	***
	LN	1515.0	61.59				
ASI	**HN**	105.5	40.11	7.3	**	***	**
	LN	97.8	38.63				
EPP	**HN**	0.82	0.15	-6.8	**	***	***
	LN	0.88	0.13				
KW (kg)	**HN**	0.069	0.01	1.4	ns	***	ns
	LN	0.068	0.01				
GMST (%)	**HN**	9.15	0.10	-1.2	ns	**	ns
	LN	9.27	0.10				
GPRT (%)	**HN**	12.62	1.23	10.7	***	***	***
	LN	11.27	1.30				
GO (%)	**HN**	3.92	0.33	-2.6	**	***	**
	LN	4.02	0.33				
GSTH (%)	**HN**	69.1	1.08	-1.5	***	***	***
	LN	70.1	1.11				
GD (%)	**HN**	1.29	0.02	0.9	**	***	***
	LN	1.28	0.03				
N%	**HN**	3.50	0.21	7.9	**	***	**
	LN	3.23	0.21				

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant; N Nitrogen levels; HN High Nitrogen; LN Low Nitrogen

Table 3.5. Estimates of means, its variations, and ANOVA of agronomic under high and low nitrogen levels for IBM-10 DH lines in environment 4 (Marion-2011).
The traits measured include grain yield (GY), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), and leaf nitrogen percentage 20 DAF (N%)

Trait	N	Mean	Std. Error	Relative Difference (%)	Analysis of Variance		
					N	Gen	Gen * N
GY (T/ha)	**HN**	3.35	1.17	-0.5	ns	***	***
	LN	3.36	1.11				
GDUSLK	**HN**	1530.2	72.91	-2.2	*	***	***
	LN	1563.6	74.39				
GDUSHD	**HN**	1451.3	62.93	-3.2	**	***	**
	LN	1497.3	61.57				
ASI	**HN**	79.4	35.21	16.3	*	***	***
	LN	66.4	34.39				
N%	**HN**	3.51	0.25	7.4	*	***	**
	LN	3.25	0.26				

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant; N Nitrogen levels; HN High Nitrogen; LN Low Nitrogen

Table 3.6. Variance component estimates and repeatability of agronomic and grain quality traits under high and low nitrogen levels for IBM-10 DH lines in environment 1 (Burkey-2010).

The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), and leaf chlorophyll content (CHLO).

Trait	Variance Components				Repeatability
	R(N)	Gen	N*Gen	Residual	
GY	0.01 **	0.4 ***	0.29 ***	0.21	0.67
PHT	7.02 ***	396.1 ***	72.7 ***	104.9	0.86
GDUSLK	47.2 **	7866.3 ***	2191.8 ***	3965.5	0.79
GDUSHD	10.4 ns	3755.5 ***	586.1 ***	1283.5	0.86
ASI	6.2 ns	1609.9 ***	987.9 ***	2205.8	0.61
EPP	0.0 ns	0.022 ***	0.007 ns	0.096	0.45
KW	0.0 ns	0.0001 ***	0.00003 ***	0.00004	0.81
GMST	0.03 ***	0.045 **	0.0 ns	0.36	0.35
GPRT	0.02 ***	0.94 ***	0.21 ***	0.42	0.82
GO	0.001 **	0.094 ***	0.01 ***	0.029	0.88
GSTH	0.02 **	0.74 ***	0.05 ns	1.09	0.71
GD	0.0 ns	0.0007 ***	0.0001 ***	0.0003	0.84
SPAD	0.1 **	6.5 ***	5.6 ***	8.9	0.56

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 3.7. Variance component estimates and repeatability of agronomic and grain quality traits under high and low nitrogen levels for IBM-10 DH lines in environment 2 (Marion-2010).

The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), and leaf chlorophyll content (CHLO).

Trait	Variance Components				Repeatability
	R(N)	Gen	N*Gen	Residual	
GY	0.23 ***	0.69 ***	0.27 ***	0.56	0.71
PHT	16.7 ***	394.6 ***	12.4 **	89.83	0.93
GDUSLK	610.2 ***	3056.7 ***	558.1 ***	1443.15	0.83
GDUSHD	226.5 ***	2401.2 ***	54.7 ns	989.27	0.90
ASI	86.4 ***	700.6 ***	114.9 *	1317.09	0.64
SPAD	2.45 ***	10.1 ***	1.1 ns	14.85	0.70

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 3. 8. Variance component estimates and repeatability of agronomic and grain quality traits under high and low nitrogen levels for IBM-10 DH lines in environment 3 (Burkey-2011).

The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), and leaf nitrogen percentage 20 DAF (N%).

Trait	Variance Components				Repeatability
	R(N)	Gen	N*Gen	Residual	
GY	0.01 **	1.94 ***	0.16 ***	0.54	0.90
PHT	0.0 ns	586.5 ***	25.7 **	210.87	0.90
GDUSLK	31.5 **	6079.7 ***	566.7 ***	1450.22	0.90
GDUSHD	30.6 ***	3924.2 ***	353.2 ***	797.52	0.91
ASI	0.0 ns	1686.1 ***	180.1 **	1185.38	0.81
EPP	0.0001 *	0.017 ***	0.004 ***	0.007	0.83
KW	0.0 ns	0.0001 ***	0.00003 ns	0.0004	0.53
GMST	0.04 ***	0.04 **	0.0 ns	0.42	0.28
GPRT	0.0 ns	1.47 ***	0.19 ***	0.33	0.89
GO	0.001 **	0.11 ***	0.006 **	0.019	0.94
GSTH	0.0 ns	1.14 ***	0.11 ***	0.22	0.91
GD	0.0 ns	0.0006 ***	0.00007 ***	0.0001	0.92
N%	0.003 **	0.05 ***	0.007 **	0.06	0.73

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001; *ns* non-significant

Table 3.9. Variance component estimates and repeatability of agronomic and grain quality traits under high and low nitrogen levels for IBM-10 DH lines in environment 4 (Marion-2011).

The traits measured include grain yield (GY), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), and leaf nitrogen percentage 20 DAF (N%).

Trait	Variance Components				Repeatability
	R(N)	Gen	N*Gen	Residual	
GY	0.004 *	1.25 ***	0.21 ***	0.45	0.85
GDUSLK	83.9 ***	5502.9 ***	241.5 ***	682.83	0.95
GDUSHD	150.7 ***	3929.8 ***	151.6 **	657.49	0.94
ASI	15.6 **	1208.3 ***	282 ***	810.64	0.78
N%	0.02 ***	0.07 ***	0.007 **	0.03	0.85

* significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.0001.

Table 3.10. Pairwise Pearson's correlations among agronomic, grain quality and physiological traits measured in high (below the diagonal) and low (above the diagonal) N treatments on the IBM2SYN10-DH population.

The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), leaf chlorophyll content (CHLO), and leaf nitrogen percentage 20 DAF (N%).

N=Low

TRAIT	GY	PHT	GDUSLK	GDUSHD	ASI	EPP	KW	GMST	GPRT	GO	GSTH	GD	CHLO	N%
GY		0.51	-0.68	-0.58	-0.62	0.80	0.59	0.83	-0.01	0.29	0.02	0.33	0.84	0.24
		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.80	<.0001	0.70	<.0001	<.0001	<.0001
PHT	0.38		-0.54	-0.50	-0.48	0.54	0.67	0.71	0.35	0.14	-0.32	0.40	0.76	-0.21
	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.005	<.0001	<.0001	<.0001	0.00
GDUSLK	-0.63	-0.53		0.93	0.76	-0.70	-0.44	-0.74	0.04	-0.26	-0.03	-0.23	-0.86	-0.33
	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	0.42	<.0001	0.57	<.0001	<.0001	<.0001
GDUSHD	-0.54	-0.52	0.94		0.49	-0.59	-0.41	-0.71	0.02	-0.22	-0.01	-0.21	-0.86	-0.37
	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	0.71	<.0001	0.85	<.0001	<.0001	<.0001
ASI	-0.53	-0.37	0.65	0.37		-0.65	-0.37	-0.52	0.08	-0.23	-0.07	-0.18	-0.66	-0.07
	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.10	<.0001	0.17	0.00	<.0001	0.16
EPP	0.71	0.12	-0.50	-0.34	-0.48		0.47	0.69	0.07	0.25	-0.05	0.31	0.17	0.20
	<.0001	0.02	<.0001	<.0001	<.0001		<.0001	<.0001	0.16	<.0001	0.28	<.0001	0.02	0.004
KW	0.06	0.35	0.07	0.06	0.03	0.05		0.71	0.34	0.03	-0.28	0.46	0.16	-0.08
	0.21	<.0001	0.17	0.22	0.60	0.30		<.0001	<.0001	0.48	<.0001	<.0001	0.02	0.25
GMST	0.28	0.08	-0.14	-0.24	0.09	0.29	0.46		0.38	0.11	-0.34	0.44	0.07	0.09
	<.0001	0.12	0.00	<.0001	0.07	<.0001	<.0001		<.0001	0.02	<.0001	<.0001	0.34	0.21
GPRT	-0.41	0.08	0.40	0.28	0.37	-0.18	0.31	0.46		-0.15	-0.92	0.50	-0.29	-0.25
	<.0001	0.11	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001		0.002	<.0001	<.0001	<.0001	0.0003
GO	0.31	0.08	-0.24	-0.17	-0.21	0.23	-0.08	0.01	-0.14		-0.08	0.18	0.06	0.07
	<.0001	0.11	<.0001	0.00	<.0001	<.0001	0.12	0.87	0.004		0.11	0.0003	0.39	0.30
GSTH	0.26	-0.10	-0.28	-0.16	-0.30	0.08	-0.32	-0.57	-0.93	-0.10		-0.34	0.30	0.25
	<.0001	0.04	<.0001	0.00	<.0001	0.10	<.0001	<.0001	<.0001	0.05		<.0001	<.0001	0.0003
GD	-0.04	0.06	0.14	0.11	0.13	0.07	0.29	0.31	0.44	0.15	-0.33		-0.06	0.06
	0.36	0.25	0.00	0.03	0.01	0.16	<.0001	<.0001	<.0001	0.003	<.0001		0.41	0.36
CHLO	0.68	0.55	-0.79	-0.79	-0.51	0.32	0.20	0.06	-0.40	0.17	0.34	-0.10		-
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.004	0.37	<.0001	0.02	<.0001	0.15		-
N%	0.28	-0.15	-0.27	-0.29	-0.07	0.26	-0.09	0.11	-0.32	0.07	0.31	0.02	-	
	<.0001	0.03	<.0001	<.0001	0.18	0.00	0.21	0.11	<.0001	0.33	<.0001	0.79	-	

N=High

Table 3.11. Pairwise Pearson's correlations among agronomic, grain quality and physiological traits measured in high (below the diagonal) and low (above the diagonal) N treatments on the IBM2SYN10-DH population at environment 1 (Burkey-2010).
The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), and leaf chlorophyll content (CHLO).

Env=E1, N=Low

TRAIT	GY	PHT	GDUSLK	GDUSHD	ASI	EPP	KW	GMST	GPRT	GO	GSTH	GD	CHLO
GY		-0.09	-0.65	-0.46	-0.58	0.50	-0.02	0.09	-0.64	0.31	0.55	-0.21	0.41
		0.31	<.0001	<.0001	<.0001	<.0001	0.80	0.38	<.0001	<.0001	<.0001	0.002	<.0001
PHT	0.01		0.27	0.39	-0.06	0.03	0.36	-0.03	0.20	0.04	-0.18	0.18	-0.07
	0.54		<.0001	<.0001	0.49	0.92	<.0001	0.55	0.002	0.37	0.004	0.01	0.58
GDUSLK	-0.62	0.08		0.81	0.63	-0.31	0.20	-0.06	0.56	-0.21	-0.43	0.34	-0.29
	<.0001	0.20		<.0001	<.0001	<.0001	0.001	0.72	<.0001	0.0004	<.0001	<.0001	<.0001
GDUSHD	-0.46	0.25	0.84		0.17	-0.09	0.25	0.00	0.50	-0.14	-0.38	0.35	-0.29
	<.0001	<.0001	<.0001		0.004	0.06	<.0001	0.61	<.0001	0.01	<.0001	<.0001	<.0001
ASI	-0.55	-0.18	0.77	0.33		-0.42	-0.06	-0.08	0.34	-0.19	-0.26	0.15	-0.19
	<.0001	0.01	<.0001	<.0001		<.0001	0.72	0.44	<.0001	0.003	<.0001	0.07	0.001
EPP	0.57	0.04	-0.39	-0.16	-0.48		0.05	0.00	-0.19	0.18	0.19	0.01	0.17
	<.0001	0.71	<.0001	0.00	<.0001		0.81	0.92	0.001	0.004	0.002	0.97	0.002
KW	-0.17	0.37	0.17	0.23	-0.03	-0.05		0.05	0.20	-0.12	-0.13	0.30	0.16
	0.01	<.0001	0.00	<.0001	0.94	0.24		0.31	0.005	0.08	0.03	<.0001	0.02
GMST	-0.03	-0.08	-0.05	-0.06	-0.02	-0.01	0.001		-0.19	-0.28	0.10	-0.41	0.07
	0.52	0.29	0.72	0.68	0.95	0.73	0.68		0.003	<.0001	0.15	<.0001	0.58
GPRT	-0.63	0.11	0.54	0.47	0.39	-0.27	0.21	-0.18		-0.18	-0.86	0.49	-0.29
	<.0001	0.06	<.0001	<.0001	<.0001	<.0001	0.001	0.01		0.01	<.0001	<.0001	<.0001
GO	0.35	0.11	-0.25	-0.16	-0.23	0.19	-0.11	-0.24	-0.11		-0.05	0.15	0.06
	<.0001	0.07	<.0001	0.01	0.0003	0.003	0.05	0.0002	0.18		0.30	0.01	0.11
GSTH	0.50	-0.16	-0.42	-0.38	-0.30	0.25	-0.18	0.08	-0.86	-0.13		-0.25	0.30
	<.0001	0.01	<.0001	<.0001	<.0001	<.0001	0.003	0.28	<.0001	0.02		<.0001	<.0001
GD	-0.21	0.09	0.25	0.26	0.14	-0.03	0.26	-0.41	0.42	0.16	-0.15		-0.06
	0.0006	0.14	0.0002	0.0001	0.03	0.54	0.0001	<.0001	<.0001	0.01	0.01		0.30
CHLO	0.45	0.09	-0.52	-0.43	-0.48	0.32	0.20	0.06	-0.40	0.17	0.34	-0.10	
	<.0001	0.08	<.0001	<.0001	<.0001	<.0001	0.004	0.37	<.0001	0.004	<.0001	0.05	

Env=E1, N=High

Table 3.12. Pairwise Pearson's correlations among agronomic, grain quality and physiological traits measured in high (below the diagonal) and low (above the diagonal) N treatments on the IBM2SYN10-DH population at environment 2 (Marion-2010).
The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), and leaf chlorophyll content (CHLO).

	Env=E2, N=Low					
TRAIT	**GY**	**PHT**	**GDUSLK**	**GDUSHD**	**ASI**	**CHLO**
GY		-0.02	-0.52	-0.41	-0.29	0.36
		0.76	<.0001	<.0001	<.0001	<.0001
PHT	0.01		0.25	0.32	-0.01	-0.11
	0.91		<.0001	<.0001	0.76	0.13
GDUSLK	-0.43	0.29		0.83	0.52	-0.39
	<.0001	<.0001		<.0001	<.0001	<.0001
GDUSHD	-0.33	0.34	0.82		-0.02	-0.31
	<.0001	<.0001	<.0001		0.89	<.0001
ASI	-0.25	-0.02	0.51	-0.05		-0.19
	<.0001	0.66	<.0001	0.66		0.002
CHLO	0.34	-0.18	-0.34	-0.32	-0.15	
	<.0001	0.01	<.0001	<.0001	0.0105	

Env=E2, N=High

Table 3.13. Pairwise Pearson's correlations among agronomic, grain quality and physiological traits measured in high (below the diagonal) and low (above the diagonal) N treatments on the IBM2SYN10-DH population at environment 3 (Burkey-2011).
The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain starch (GSTH), grain density (GD), and leaf nitrogen percentage 20 DAF (N%).

Env=E3, N=Low

TRAIT	GY	PHT	GDUSLK	GDUSHD	ASI	EPP	KW	GMST	GPRT	GO	GSTH	GD	N%
GY		0.12	-0.65	-0.49	-0.56	0.68	0.02	0.24	-0.72	0.34	0.61	-0.15	0.27
		0.10	<.0001	<.0001	<.0001	<.0001	0.61	0.0004	<.0001	<.0001	<.0001	0.01	<.0001
PHT	0.15		0.26	0.35	-0.09	0.14	0.28	0.04	0.02	0.07	-0.05	-0.01	-0.21
	0.04		<.0001	<.0001	0.10	0.10	<.0001	0.31	0.54	0.15	0.19	0.71	0.0002
GDUSLK	-0.68	0.17		0.84	0.64	-0.51	0.13	-0.13	0.59	-0.26	-0.53	0.12	-0.30
	<.0001	0.01		<.0001	<.0001	<.0001	0.01	0.08	<.0001	<.0001	<.0001	0.03	<.0001
GDUSHD	-0.51	0.25	0.82		0.14	-0.32	0.14	-0.06	0.49	-0.22	-0.44	0.13	-0.30
	<.0001	<.0001	<.0001		0.04	<.0001	0.004	0.49	<.0001	0.001	<.0001	0.01	<.0001
ASI	-0.58	-0.13	0.66	0.14		-0.55	0.02	-0.18	0.40	-0.19	-0.36	0.05	-0.10
	<.0001	0.02	<.0001	0.06		<.0001	0.51	0.01	<.0001	0.001	<.0001	0.57	0.57
EPP	0.74	0.14	-0.59	-0.38	-0.60		-0.12	0.19	-0.42	0.24	0.35	-0.08	0.20
	<.0001	0.06	<.0001	<.0001	<.0001		0.02	0.01	<.0001	0.0001	<.0001	0.16	0.003
KW	-0.001	0.35	0.11	0.15	-0.01	-0.14		0.14	-0.01	-0.06	0.02	0.07	-0.08
	0.45	<.0001	0.03	0.0032	0.78	0.01		0.13	0.49	0.30	0.65	0.05	0.22
GMST	0.26	0.05	-0.13	-0.07	-0.18	0.18	0.16		-0.22	-0.04	0.15	-0.29	0.09
	0.0002	0.24	0.09	0.35	0.02	0.01	0.04		0.001	0.59	0.03	<.0001	0.43
GPRT	-0.66	0.004	0.54	0.46	0.36	-0.43	0.01	-0.28		-0.26	-0.93	0.30	-0.25
	<.0001	0.78	<.0001	<.0001	<.0001	<.0001	0.73	<.0001		<.0001	<.0001	<.0001	<.0001
GO	0.30	0.05	-0.24	-0.18	-0.19	0.26	-0.09	-0.05	-0.22		-0.02	0.10	0.07
	<.0001	0.20	0.0002	0.01	0.002	<.0001	0.32	0.52	0.0004		0.63	0.13	0.22
GSTH	0.56	-0.01	-0.48	-0.40	-0.32	0.35	0.02	0.21	-0.93	-0.08		-0.18	0.25
	<.0001	0.54	<.0001	<.0001	<.0001	<.0001	0.99	0.003	<.0001	0.15		0.002	<.0001
GD	-0.12	-0.03	0.15	0.16	0.06	-0.04	0.02	-0.31	0.25	0.13	-0.17		0.06
	0.01	0.88	0.01	0.003	0.61	0.37	0.43	<.0001	<.0001	0.03	0.002		0.48
N%	0.31	-0.15	-0.31	-0.29	-0.12	0.26	-0.09	0.11	-0.32	0.07	0.31	0.02	
	<.0001	0.01	<.0001	<.0001	0.40	0.0002	0.22	0.22	<.0001	0.25	<.0001	0.97	

Env=E3, N=High

Table 3.14. Pairwise Pearson's correlations among agronomic, grain quality and physiological traits measured in high (below the diagonal) and low (above the diagonal) N treatments on the IBM2SYN10-DH population at environment 4 (Marion-2011). The traits measured include grain yield (GY), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), and leaf nitrogen percentage 20 DAF (N%).

	Env=E4, N=Low				
TRAIT	**GY**	**GDUSLK**	**GDUSHD**	**ASI**	**N%**
GY		-0.41	-0.20	-0.47	0.27
		<.0001	0.0004	<.0001	0.0001
GDUSLK	-0.42		0.86	0.55	-0.35
	<.0001		<.0001	<.0001	<.0001
GDUSHD	-0.21	0.85		0.04	-0.42
	0.0001	<.0001		0.48	<.0001
ASI	-0.43	0.50	-0.04		-0.002
	<.0001	<.0001	0.40		0.60
N%	0.25	-0.30	-0.35	-0.001	
	0.001	<.0001	<.0001	0.30	

Env=E4, N=High

Table 3.15. Results of QTL analysis of agronomic, grain quality and physiological traits measured in high and low N treatments on the IBM2SYN10-DH population.
The traits measured include grain yield (GY), plant height (PHT), growing degree units to silking (GDUSLK), growing degree units to anthesis (GDUSHD), anthesis-silking interval (ASI), ears per plant (EPP), kernel weight (KW), grain moisture (GMST), grain protein (GPRT), grain oil (GO), grain density (GD), leaf chlorophyll content (CHLO), and leaf nitrogen percentage 20 DAF (N%).

Trait	Env	N level	Chr.	QTL	Marker	Position (cM) *a*	LOD *b*	Add. *c*	R^2 (%) *d*
GY	E1	High	1	*GYHN-1a*	112	44.41	6.44	0.282	8.1%
			1	*GYHN-1b*	489	112.91	6.29	0.273	7.8%
			3	*GYHN-3*	40	22.81	4.60	-0.247	5.8%
			7	*GYHN-7*	371	85.31	4.95	-0.252	6.1%
			8	*GYHN-8a*	22	7.71	4.43	-0.232	5.4%
			8	*GYHN-8b*	436	104.01	4.26	-0.243	5.2%
									38.5% ***f***
		Low	3	*GYLN-3*	45	24.51	4.65	-0.130	6.0%
			7	*GYLN-7*	371	85.31	7.95	-0.175	10.7%
			8	*GYLN-8*	240	65.21	7.20	-0.157	9.6%
									26.3% ***f***
	E2	High	4	*GYHN-4a*	227	69.91	6.18	0.315	8.0%
			4	*GYHN-4b*	669	145.51	4.21	0.238	5.3%
			9	*GYHN-9*	443	136.01	5.50	-0.283	7.0%
									20.3% ***f***
		Low	1	*GYLN-1*	59	25.01	7.85	0.256	10.5%
			3	*GYLN-3a*	163	59.31	6.11	0.243	8.0%
			3	*GYLN-3b*	618	159.41	5.42	-0.214	7.1%
			9	*GYLN-9*	105	36.51	5.34	0.245	7.0%
									32.6% ***f***
	E3	High	3	*GYHN-3*	133	53.91	6.25	0.430	7.9%
			4	*GYHN-4*	239	70.81	5.14	0.441	6.5%
			5	*GYHN-5*	720	157.01	5.18	0.408	6.4%
									20.8% ***f***
		Low	3	*GYLN-3*	158	58.21	5.48	0.399	7.0%
			5	*GYLN-5a*	666	140.11	4.14	-0.383	6.3%
			5	*GYLN-5b*	720	157.01	4.90	0.389	6.2%
									19.4% ***f***
	E4	High	1	*GYHN-1*	55	23.71	4.46	0.305	6.0%
			3	*GYHN-3*	620	159.61	6.45	-0.385	8.8%
			7	*GYHN-7*	359	83.71	6.22	-0.375	8.5%
									23.3% ***f***
		Low	4	*GYLN-4*	314	80.41	5.08	-0.336	6.5%
			4	*GYLN-4*	421	96.61	5.00	0.320	6.5%
			7	*GYLN-7a*	382	87.21	6.11	-0.356	8.0%

Table 3.15. Continued

			7	*GYLN-7b*	440	101.61	5.34	0.343	6.9%
									27.9% ***f***
PHT	E1	High	1	*PHTHN-1a*	369	94.61	5.40	-6.164	6.0%
			1	*PHTHN-1b*	667	153.01	4.62	-5.726	5.1%
			5	*PHTHN-5*	306	76.01	6.81	-7.049	8.4%
			8	*PHTHN-8*	349	85.21	13.91	9.677	17.4%
									36.8% ***f***
		Low	1	*PHTLN-1a*	368	94.51	5.73	-5.507	6.6%
			1	*PHTLN-1b*	667	153.01	6.76	-6.093	7.9%
			5	*PHTLN-5*	284	73.61	7.06	-6.189	8.3%
			8	*PHTLN-8*	349	85.21	11.20	7.520	14.0%
									36.8% ***f***
	E2	High	1	*PHTHN-1a*	385	96.01	6.51	-6.030	7.9%
			1	*PHTHN-1b*	664	151.81	4.16	-4.693	4.9%
			4	*PHTHN-4*	373	88.01	6.07	5.608	7.3%
			8	*PHTHN-8*	349	85.21	6.60	5.803	8.1%
									28.1% ***f***
		Low	1	*PHTLN-1a*	385	96.01	6.52	-5.984	7.9%
			1	*PHTLN-1b*	664	151.81	4.23	-4.703	5.0%
			4	*PHTLN-4*	372	87.81	5.68	5.339	6.8%
			8	*PHTLN-8*	346	84.81	6.85	5.896	8.6%
									28.3% ***f***
	E3	High	1	*PHTHN-1*	368	94.51	4.88	-6.361	5.8%
			3	*PHTHN-3*	561	144.81	4.08	-5.580	4.8%
			4	*PHTHN-4*	381	89.61	4.05	5.641	4.7%
			8	*PHTHN-8*	344	84.61	9.96	8.841	12.6%
			9	*PHTHN-9*	441	135.41	6.08	7.273	7.3%
									35.2% ***f***
		Low	1	*PHTLN-1a*	380	95.41	5.75	-6.712	6.6%
			1	*PHTLN-1b*	666	152.71	4.57	-5.879	5.2%
			8	*PHTLN-8*	349	85.21	9.60	8.384	11.8%
			9	*PHTLN-9*	441	135.41	5.74	6.828	6.7%
									30.4% ***f***
GDUSLK	E1	High	1	*SLKHN-1a*	754	169.91	4.46	-22.519	5.4%
			1	*SLKHN-1b*	978	214.41	4.68	22.426	5.5%
			3	*SLKHN-3*	674	184.51	5.10	-23.732	6.1%
			5	*SLKHN-5*	85	40.01	6.19	25.620	7.3%
			7	*SLKHN-7*	357	83.01	5.53	25.069	6.5%
			8	*SLKHN-8*	405	90.71	6.12	26.847	7.5%
									38.3% ***f***
		Low	1	*SLKLN-1*	751	168.41	5.69	-26.630	6.6%

Table 3.15. Continued

			2	*SLKLN-2*	79	34.91	4.46	23.440	5.1%
			4	*SLKLN-4*	168	57.11	4.14	-22.576	4.8%
			8	*SLKLN-8*	347	84.91	10.55	36.034	13.0%
			9	*SLKLN-9*	443	136.01	4.21	23.886	4.9%
									34.4% *f*
	E2	High	1	*SLKHN-1*	454	106.91	6.36	-13.993	6.3%
			4	*SLKHN-4*	718	161.41	8.43	-16.301	8.5%
			5	*SLKHN-5*	721	157.21	7.75	-16.690	8.5%
			8	*SLKHN-8*	346	84.81	14.87	21.715	16.2%
			9	*SLKHN-9*	443	136.01	6.89	15.392	6.9%
									46.4% *f*
		Low	4	*SLKLN-4*	718	161.41	5.17	-15.683	6.1%
			8	*SLKLN-8*	349	85.21	13.10	24.667	16.8%
									23.0% *f*
	E3	High	1	*SLKHN-1*	454	106.91	6.84	-24.847	8.0%
			3	*SLKHN-3*	426	113.61	4.58	21.701	5.4%
			5	*SLKHN-5a*	666	139.11	5.37	22.706	6.2%
			5	*SLKHN-5b*	720	157.01	6.70	-25.943	7.9%
			8	*SLKHN-8*	352	85.41	5.41	21.277	6.2%
			9	*SLKHN-9*	443	136.01	8.81	30.145	10.6%
									44.2% *f*
		Low	1	*SLKLN-1*	450	106.41	7.47	-24.343	8.7%
			5	*SLKLN-5*	720	157.01	7.08	-24.683	8.3%
			8	*SLKLN-8*	349	85.21	7.59	24.076	8.8%
									25.8% *f*
	E4	High	1	*SLKHN-1*	412	100.21	4.75	-18.177	5.1%
			5	*SLKHN-5*	98	43.11	6.71	21.107	7.4%
			8	*SLKHN-8*	349	85.21	14.71	31.941	17.6%
									30.1% *f*
		Low	1	*SLKLN-1*	412	100.21	5.65	-20.366	6.3%
			5	*SLKLN-5*	99	43.41	5.25	18.806	5.9%
			8	*SLKLN-8*	349	85.21	13.08	30.126	15.4%
									27.5% *f*
GDUSHD	E1	High	1	*SHDHN-1*	455	107.01	6.20	-17.109	7.0%
			3	*SHDHN-3a*	224	67.71	5.32	-16.415	5.9%
			3	*SHDHN-3b*	308	81.41	4.26	14.987	4.8%
			7	*SHDHN-7*	421	95.31	5.67	19.066	6.4%
			8	*SHDHN-8*	346	84.81	11.29	23.655	13.6%
									37.6% *f*
		Low	1	*SHDLN-1*	751	168.41	5.69	-26.630	6.6%
			2	*SHDLN-2*	79	34.91	4.46	23.440	5.1%

Table 3.15. Continued

			4	*SHDLN-4*	168	57.11	4.14	-22.576	4.8%
			8	*SHDLN-8*	347	84.91	10.55	36.034	13.0%
			9	*SHDLN-9*	443	136.01	4.21	23.886	4.9%
									34.4% *f*
	E2	High	2	*SHDHN-2*	293	94.01	4.43	-10.111	4.3%
			3	*SHDHN-3*	326	87.51	4.48	11.702	4.4%
			4	*SHDHN-4a*	149	54.11	4.20	-10.249	4.1%
			4	*SHDHN-4b*	718	161.41	5.78	-11.693	5.8%
			8	*SHDHN-8*	346	84.81	21.41	24.396	25.5%
									44.1% *f*
		Low	2	*SHDLN-2a*	242	83.31	5.42	11.636	5.5%
			2	*SHDLN-2b*	293	94.01	4.65	-10.754	4.7%
			3	*SHDLN-3*	309	81.51	6.12	13.557	6.3%
			4	*SHDLN-4*	718	161.41	6.95	-13.345	7.2%
			8	*SHDLN-8*	346	84.81	19.86	24.157	23.9%
									47.7% *f*
	E3	High	1	*SHDHN-1*	450	106.41	6.83	-19.874	8.1%
			3	*SHDHN-3*	711	196.11	4.27	-17.714	4.9%
			4	*SHDHN-4*	718	161.41	4.97	-16.821	5.8%
			6	*SHDHN-6*	555	145.91	4.61	-16.025	5.3%
			8	*SHDHN-8*	344	84.61	11.04	26.234	13.5%
			9	*SHDHN-9*	443	136.01	5.70	18.907	6.7%
									44.3% *f*
		Low	1	*SHDLN-1*	415	100.91	8.18	-19.973	9.3%
			3	*SHDLN-3*	305	81.11	6.33	19.020	7.0%
			5	*SHDLN-5*	101	43.81	4.43	14.996	4.8%
			8	*SHDLN-8*	349	85.21	11.29	23.479	13.0%
									34.0% *f*
	E4	High	1	*SHDHN-1*	706	161.11	5.53	-16.035	5.8%
			4	*SHDHN-4*	5	1.41	4.20	-14.697	4.3%
			8	*SHDHN-8*	346	84.81	13.71	25.473	15.5%
			9	*SHDHN-9*	443	136.01	6.11	17.844	6.4%
									32.1% *f*
		Low	1	*SHDLN-1*	717	162.61	7.14	-18.090	7.8%
			4	*SHDLN-4*	5	1.41	6.08	-17.969	6.6%
			8	*SHDLN-8*	346	84.81	12.90	24.580	15.1%
			9	*SHDLN-9*	443	136.01	6.54	18.578	7.2%
									36.7% *f*
ASI	E1	High	5	*ASIHN-5a*	238	69.01	4.89	12.973	6.2%
			5	*ASIHN-5b*	721	157.21	4.54	-11.674	5.8%
			7	*ASIHN-7*	375	85.71	6.23	13.958	8.5%

Table 3.15. Continued

			9	*ASIHN-9*	216	67.61	4.18	10.448	5.3%
									25.8% *f*
		Low	1	*ASILN-1a*	19	5.41	4.95	12.019	6.6%
			1	*ASILN-1b*	492	113.61	6.85	-13.976	9.3%
			4	*ASILN-4*	221	69.01	6.11	-14.541	8.2%
			7	*ASILN-7*	374	85.61	5.17	12.250	6.9%
									31.0% *f*
	E2	High	3	*ASIHN-3*	547	141.71	5.47	-6.135	7.2%
			7	*ASIHN-7*	374	85.61	6.65	7.434	9.0%
									16.2% *f*
		Low	1	*ASILN-1*	482	112.41	5.96	-7.047	8.2%
			3	*ASILN-3*	547	141.71	5.65	-6.556	7.8%
			5	*ASILN-5*	720	157.01	4.45	-5.904	6.0%
			7	*ASILN-7*	369	85.01	4.37	5.905	5.9%
									27.9% *f*
	E3	High	1	*ASIHN-1a*	165	62.11	6.82	12.330	8.6%
			1	*ASIHN-2b*	490	113.21	6.61	-11.951	8.2%
			2	*ASIHN-2b*	108	45.01	4.48	-10.693	5.9%
			3	*ASIHN-3*	388	102.31	6.53	12.235	8.2%
			4	*ASIHN-4a*	129	49.11	5.89	-11.346	7.3%
			4	*ASIHN-4b*	588	133.61	4.33	-12.198	5.3%
									43.6% *f*
		Low	1	*ASILN-1a*	165	62.11	4.72	9.949	6.2%
			1	*ASILN-2b*	490	113.21	6.26	-11.526	8.2%
			4	*ASILN-4*	129	49.11	4.17	-9.314	5.4%
									19.8% *f*
	E4	High	1	*ASIHN-1*	491	113.31	4.84	-9.058	6.4%
			7	*ASIHN-7*	374	85.61	6.50	11.206	8.8%
									15.2% *f*
		Low	1	*ASILN-1*	485	112.61	4.59	-8.502	5.7%
			7	*ASILN-7*	374	85.61	5.86	10.366	7.5%
			10	*ASILN-10*	353	106.21	4.16	-8.009	5.1%
									18.4% *f*
EPP	E1	High	4	*EPPHN-4*	323	81.01	4.32	-0.025	4.1%
			5	*EPPHN-5*	721	157.21	5.07	0.025	4.9%
									9.0% *f*
		Low	4	*EPPLN-4*	303	78.81	4.14	-0.033	5.8%
			9	*EPPLN-9*	216	67.61	3.07	-0.029	4.2%
									10.0% *f*
	E3	High	4	*EPPHN-4*	167	56.81	4.73	0.042	6.2%
			5	*EPPHN-5a*	284	73.61	4.04	-0.038	5.3%

Table 3.15. Continued

			5	*EPPHN-5b*	672	143.01	4.70	-0.041	6.2%
			5	*EPPHN-5c*	720	157.01	4.51	0.042	5.9%
			9	*EPPHN-9*	459	142.91	4.18	-0.037	5.4%
									29.0% *f*
		Low	4	*EPPLN-4a*	235	70.51	6.76	0.048	9.1%
			4	*EPPLN-4b*	321	80.81	5.58	-0.043	7.4%
			5	*EPPLN-5a*	241	69.21	6.16	-0.044	8.2%
			5	*EPPLN-5b*	720	157.01	5.13	0.037	6.8%
									31.4% *f*
KW	E1	High	2	*KWHN-2*	581	144.81	4.47	-0.003	5.8%
			5	*KWHN-5*	23	9.91	4.61	-0.003	6.1%
			8	*KWHN-8*	434	103.41	10.73	0.005	16.1%
			9	*KWHN-9*	441	135.41	4.94	0.003	6.5%
			10	*KWHN-10*	71	29.11	5.49	-0.004	7.3%
									41.8% *f*
		Low	1	*KWLN-1*	699	159.81	4.10	-0.003	6.0%
			2	*KWLN-2*	579	144.11	4.75	-0.003	6.5%
			5	*KWLN-5*	23	9.91	4.13	-0.003	5.9%
			8	*KWLN-8*	434	103.41	4.29	0.003	6.1%
									24.5% *f*
	E3	High	2	*KWHN-2*	100	42.11	3.61	0.002	3.4%
			3	*KWHN-3*	287	77.51	5.16	0.002	4.5%
			4	*KWHN-4*	14	4.51	4.77	-0.002	4.1%
			5	*KWHN-5*	105	44.11	4.30	0.002	3.7%
			8	*KWHN-8*	436	104.01	4.10	0.002	3.5%
			9	*KWHN-9*	251	74.71	8.23	-0.002	7.4%
									26.6% *f*
		Low	3	*KWLN-3*	287	78.51	3.91	0.002	2.8%
			5	*KWLN-5*	105	44.11	3.34	0.002	2.2%
			9	*KWLN-9*	251	74.71	6.70	-0.002	4.6%
									9.7% *f*
GMST	E1	High	10	*MSTHN-10*	348	103.11	4.82	0.031	6.7%
									6.7% *f*
		Low	2	*MSTLN-2*	11	1.21	4.32	-0.024	5.7%
			3	*MSTLN-3*	203	65.21	4.11	0.024	5.4%
			4	*MSTLN-4*	124	48.01	5.28	0.028	7.0%
			5	*MSTLN-5*	615	125.91	7.90	-0.034	10.7%
			9	*MSTLN-9*	46	13.91	4.68	0.024	6.2%
									34.9% *f*
	E3	Low	1	*MSTLN-1*	465	108.51	4.12	0.024	5.9%
									5.9% *f*

Table 3.15. Continued

GPRT	E1	High	1	*PRTHN-1*	60	25.31	7.31	-0.318	10.1%
			5	*PRTHN-5*	389	84.81	5.62	-0.281	7.6%
									17.7% ***f***
		Low	1	*PRTLN-1*	60	25.31	6.84	-0.304	8.7%
			2	*PRTLN-2*	16	5.31	4.12	0.237	5.1%
			8	*PRTLN-8*	395	89.11	5.95	0.281	7.5%
									21.4% ***f***
	E3	High	1	*PRTHN-1a*	93	38.81	4.58	-0.309	5.9%
			1	*PRTHN-1b*	851	187.41	4.98	0.340	6.9%
			2	*PRTHN-2*	53	25.91	4.54	-0.355	6.8%
			10	*PRTHN-10*	267	66.51	4.88	0.334	6.3%
									25.8% ***f***
		Low	1	*PRTLN-1*	422	102.81	6.03	-0.414	8.1%
			2	*PRTLN-2*	53	25.91	4.53	-0.382	7.3%
			5	*PRTLN-5*	597	120.51	4.76	-0.338	6.2%
			8	*PRTLN-8*	352	85.41	4.75	0.348	6.2%
									27.8% ***f***
GO	E1	High	2	*GOHN-2a*	124	49.01	5.21	0.079	5.2%
			2	*GOHN-2b*	192	70.21	7.65	0.094	7.8%
			3	*GOHN-3*	21	9.51	6.42	0.082	6.5%
			4	*GOHN-4*	600	135.51	8.39	0.118	8.7%
			5	*GOHN-5*	21	9.01	6.01	-0.081	6.0%
			6	*GOHN-6a*	217	43.11	4.80	0.075	4.7%
			6	*GOHN-6b*	516	127.21	6.21	0.083	6.6%
									45.5% ***f***
		Low	2	*GOLN-2*	120	48.61	4.19	0.070	4.4%
			3	*GOLN-3*	21	9.51	5.66	0.079	6.1%
			4	*GOLN-4*	600	135.51	6.52	0.106	7.1%
			6	*GOLN-6a*	45	13.31	4.17	0.069	4.4%
			6	*GOLN-6b*	217	43.11	4.19	0.072	4.4%
			6	*GOLN-6c*	516	127.21	4.08	0.069	4.6%
									31.0% ***f***
	E3	High	2	*GOHN-2*	124	49.01	10.05	0.116	10.2%
			3	*GOHN-3a*	143	55.91	9.30	0.103	9.3%
			3	*GOHN-3b*	673	184.21	6.23	0.081	6.0%
			4	*GOHN-4*	595	134.81	8.33	0.114	8.2%
			5	*GOHN-5*	22	9.61	5.29	-0.077	5.1%
			6	*GOHN-6*	517	127.61	5.14	0.075	4.9%
			9	*GOHN-9*	383	111.21	4.31	0.073	4.1%
									47.7% ***f***
		Low	2	*GOLN-2*	124	49.01	5.93	0.088	5.7%

Table 3.15. Continued

			3	*GOLN-3a*	27	11.61	4.14	0.069	3.9%
			3	*GOLN-3b*	140	55.71	9.95	0.113	10.6%
			3	*GOLN-3c*	674	184.51	6.47	0.087	6.3%
			4	*GOLN-4*	595	134.81	11.34	0.136	11.6%
			6	*GOLN-6a*	376	80.81	4.96	0.076	4.7%
			6	*GOLN-6b*	427	91.21	4.31	0.072	4.1%
			10	*GOLN-10*	28	13.41	5.02	0.077	4.8%
									51.5% *f*
GSTH	E1	High	1	*STHHN-1*	60	25.31	6.35	0.225	8.3%
			2	*STHHN-2a*	51	24.11	4.09	0.182	5.3%
			2	*STHHN-2b*	80	35.01	4.97	-0.206	6.5%
			2	*STHHN-2c*	145	55.41	5.23	-0.203	6.8%
			4	*STHHN-4*	660	143.71	6.60	-0.246	8.7%
									35.5% *f*
		Low	2	*STHLN-2*	145	55.41	5.14	-0.189	6.3%
			4	*STHLN-4*	660	143.71	4.50	-0.188	5.5%
									11.8% *f*
	E3	High	1	*STHHN-1*	93	38.81	9.05	0.388	11.1%
			2	*STHHN-2*	136	53.71	6.53	-0.323	7.8%
			10	*STHHN-10*	268	66.81	9.34	-0.415	11.5%
									30.4% *f*
		Low	1	*STHLN-1*	92	38.71	6.65	0.321	7.6%
			2	*STHLN-2a*	53	25.91	4.47	0.301	6.2%
			2	*STHLN-2b*	136	53.71	6.77	-0.325	7.6%
			3	*STHLN-3*	26	11.11	4.18	-0.251	4.6%
			10	*STHLN-10a*	187	47.21	5.89	0.318	6.5%
			10	*STHLN-10b*	268	66.81	8.46	-0.399	9.7%
									42.2% *f*
GD	E1	High	1	*GDHN-1a*	81	33.21	5.92	0.007	6.8%
			1	*GDHN-1b*	461	108.21	6.04	-0.007	6.8%
			4	*GDHN-4a*	132	50.11	4.65	-0.007	5.2%
			4	*GDHN-4b*	440	99.81	5.25	-0.007	6.0%
			5	*GDHN-5*	617	126.61	4.82	0.007	5.5%
									30.2% *f*
		Low	1	*GDLN-1*	461	108.21	8.40	-0.009	10.6%
									10.6% *f*
	E3	High	1	*GDHN-1*	81	33.21	6.55	0.007	8.0%
			4	*GDHN-4*	370	87.41	4.25	-0.006	5.1%
			5	*GDHN-5a*	42	20.91	4.79	-0.006	5.7%
			5	*GDHN-5b*	612	124.21	6.65	0.007	8.1%
			7	*GDHN-7*	311	71.21	8.07	0.009	10.0%
									36.9% *f*

Table 3.15. Continued

		Low	1	*GDLN-1*	81	33.21	6.43	0.009	8.3%
			4	*GDLN-4*	193	62.01	5.44	-0.008	7.0%
			5	*GDLN-5*	612	124.21	4.88	0.007	6.2%
									21.5% ***f***
CHLO	E1	High	1	*SPADHN-1a*	434	104.71	3.97	0.582	4.8%
			1	*SPADHN-1b*	697	157.61	7.02	0.880	8.8%
			2	*SPADHN-2a*	22	9.61	7.67	-0.855	9.7%
			2	*SPADHN-2b*	59	30.31	5.46	0.723	6.7%
			4	*SPADHN-4*	229	70.11	4.81	0.709	5.9%
			6	*SPADHN-6*	420	88.61	6.94	0.789	8.7%
									44.5% ***f***
		Low	2	*SPADLN-2*	244	83.41	4.10	-0.821	5.0%
			3	*SPADLN-3*	290	79.01	4.42	-0.871	5.4%
			5	*SPADLN-5*	372	83.11	5.38	0.989	7.4%
									17.8% ***f***
	E2	High	1	*SPADHN-1*	667	153.01	4.95	0.847	6.8%
			5	*SPADHN-5*	720	157.01	5.00	0.824	6.9%
			8	*SPADHN-8a*	438	104.91	4.71	-0.820	6.5%
			8	*SPADHN-8b*	513	141.51	4.13	-0.697	5.6%
			10	*SPADHN-10*	327	91.41	5.09	-0.812	7.0%
									32.7% ***f***
		Low	1	*SPADLN-1*	698	159.21	5.24	0.790	6.8%
			5	*SPADLN-5*	720	157.01	4.59	0.743	6.0%
			8	*SPADLN-8*	438	104.91	4.87	-0.803	6.3%
			10	*SPADLN-10*	327	91.41	4.92	-0.756	6.3%
									25.5% ***f***
N%	E3	High	1	*N%HN-1*	522	119.61	5.58	-0.053	5.9%
			2	*N%HN-2*	413	109.31	6.43	0.059	7.0%
			3	*N%HN-3*	422	112.21	4.96	-0.053	5.9%
			4	*N%HN-4*	97	40.01	5.39	0.051	5.8%
			6	*N%HN-6*	421	89.11	4.72	0.050	5.1%
			10	*N%HN-10*	326	90.91	4.44	-0.049	4.7%
									34.4% ***f***
		Low	1	*N%LN-1*	522	119.61	5.86	-0.056	6.5%
			3	*N%LN-3*	422	112.21	8.30	-0.067	9.5%
			4	*N%LN-4*	97	40.01	4.64	0.049	5.1%
			8	*N%LN-8*	329	81.31	4.11	0.045	4.5%
			10	*N%LN-10*	358	109.11	4.51	-0.048	5.0%
									30.5% ***f***
	E4	High	1	*N%HN-1a*	124	48.01	6.40	0.067	6.8%
			1	*N%HN-1b*	534	122.31	4.60	-0.057	4.8%

Table 3.15. Continued

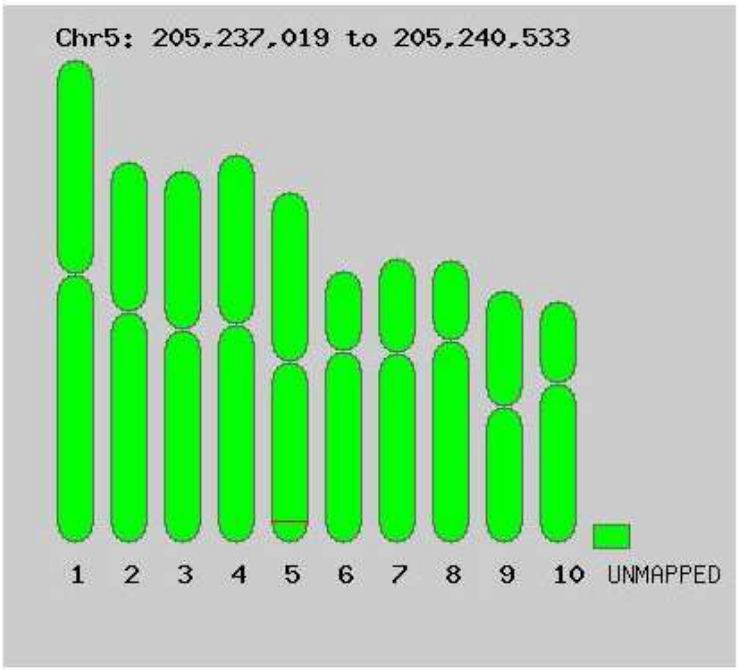
	2	*N%HN-2*	605	152.01	4.29	0.056	4.5%
	3	*N%HN-3*	595	153.01	10.53	0.098	11.8%
	6	*N%HN-6*	425	90.21	5.10	0.064	5.4%
	10	*N%HN-10*	355	107.31	7.96	-0.076	8.6%
							41.8% *f*
Low	1	*N%LN-1a*	124	48.01	5.34	0.064	5.5%
	1	*N%LN-1b*	532	121.31	9.73	-0.092	10.8%
	3	*N%LN-3a*	92	43.61	5.07	-0.064	5.3%
	3	*N%LN-3b*	605	156.21	10.67	0.104	12.0%
	10	*N%LN-10*	355	107.31	4.76	-0.062	5.0%
							38.5% *f*

a Position in cM from the top of the chromosome calculated by QTL Cartographer v.1.7
b LOD value corresponding to the position of the QTL calculated by QTL Cartographer v.1.7
c Additive effects values calculated as the average from the difference between homozygotes for each parental allele at a locus. (+) is the direction of the additive effect for B73, (-) is the direction of the additive effect for Mo17 inbred parent.
d Part of the phenotypic variance explained by each QTL by composite interval mapping
f Total phenotypic variance explained by the sum of the QTL at each environment by N treatment combination.

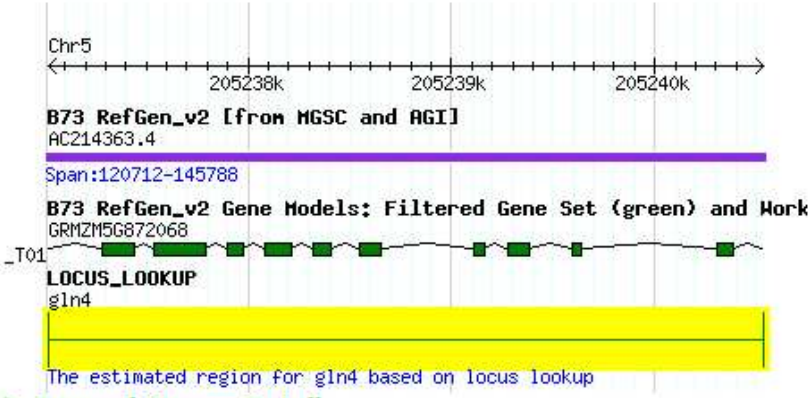
Appendix

The Locus **gln4** is between **205,237,019** and position **205,240,533** on **Chromosome 5**
This information is based on associated gene model positions.

This region is **3,514** base pairs.



(Click on image to go to the Genome Browser)



▶ Expand Image Detail

Figure A3.1. Screen caption of Locus Lookup tool at Maize GDB website.
Locus *gln4* information is shown in physical position, and the characteristics in the genomic region below.

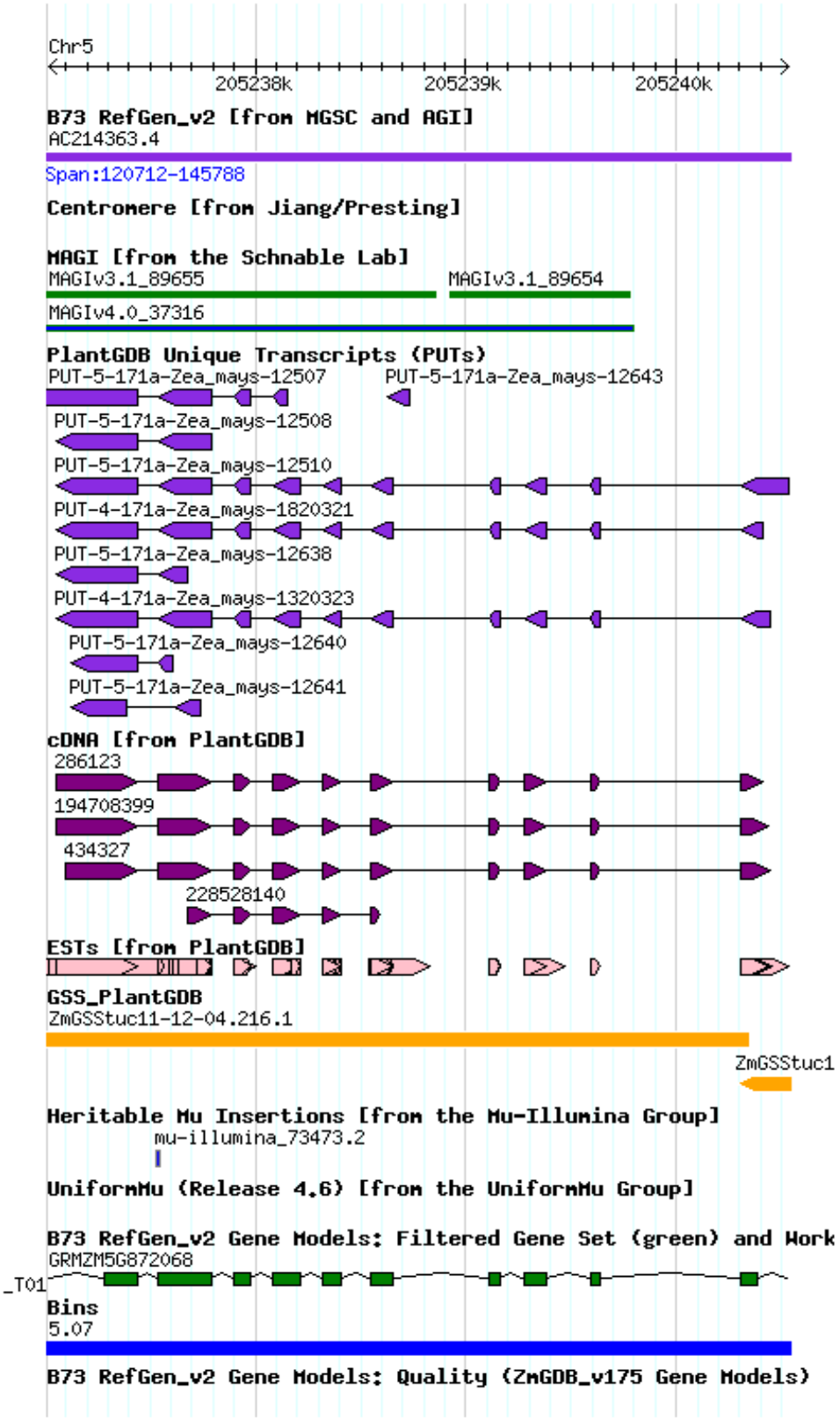
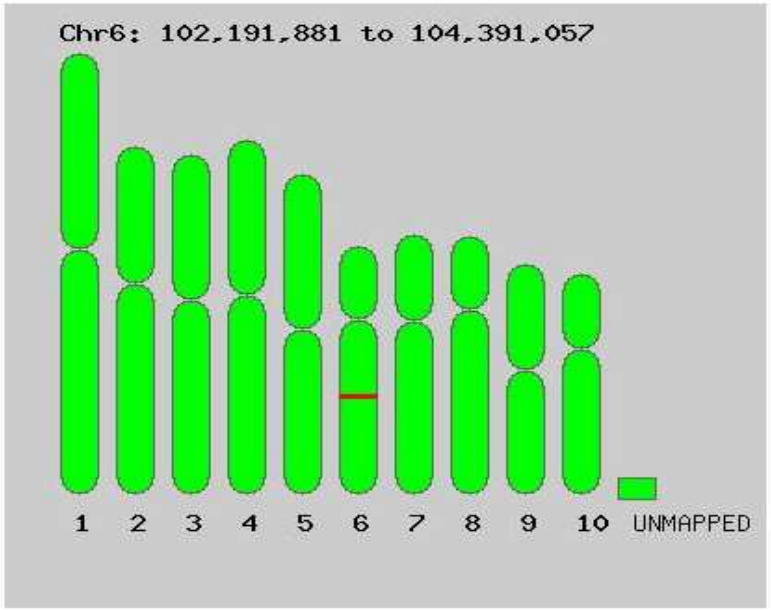


Figure A3.1. Continued

The Locus **ln1** is estimated between position **102,191,881** and position **104,391,057** on **Chromosome 6**

This region is **2,199,176** base pairs long.



(Click on image to go to the Genome Browser)

▶ **Expand Image Detail**

Figure A3.2. Screen caption of Locus Lookup tool at Maize GDB website.
Locus *ln1* information is shown in physical position, and the characteristics in the genomic region below.

Figure A3.2. Continued

Table A3.1. QTL threshold values obtained after 1000 permutations for each trait in each environment and N treatment combination.
LR (Likelihood ratio), and LOD (logarithm base 10 of odds) calculated after 1000 permutations

			Threshold	
Trait	**Env**	**N level**	**LR**	**LOD**
GY	E1	High N	19.1	4.14
		Low N	18.82	4.08
	E2	High N	18.74	4.07
		Low N	19.14	4.15
	E3	High N	18.63	4.04
		Low N	18.6	4.03
	E4	High N	18.47	4.01
		Low N	19.12	4.15
PHT	E1	High N	18.82	4.08
		Low N	18.57	4.03
	E2	High N	18.77	4.07
		Low N	18.28	3.97
	E3	High N	18.37	3.98
		Low N	18.67	4.05
GDUSLK	E1	High N	18.51	4.02
		Low N	18.17	3.94
	E2	High N	18.91	4.10
		Low N	18.45	4.00
	E3	High N	18.46	4.00
		Low N	19.09	4.14
	E4	High N	18.32	3.97
		Low N	18.24	3.96
GDUSHD	E1	High N	18.45	4.00
		Low N	18.43	4.00
	E2	High N	18.86	4.09
		Low N	18.9	4.10
	E3	High N	18.66	4.05
		Low N	18.65	4.05
	E4	High N	18.46	4.00
		Low N	18.89	4.10
ASI	E1	High N	18.48	4.01
		Low N	18.88	4.10
	E2	High N	18.54	4.02
		Low N	18.72	4.06
	E3	High N	18.29	3.97
		Low N	18.71	4.06

Table A3.1. Continued

	E4	High N	18.17	3.94
		Low N	18.6	4.03
EPP	E1	High N	16.61	3.60
		Low N	13.38	2.90
	E3	High N	17.86	3.87
		Low N	18.45	4.00
KW	E1	High N	18.38	3.99
		Low N	18.43	4.00
	E3	High N	16.15	3.50
		Low N	14.14	3.07
GMST	E1	High N	18.15	3.94
		Low N	18.06	3.92
	E3	High N	18.88	4.10
		Low N	18.57	4.03
GPRT	E1	High N	18.87	4.09
		Low N	18.82	4.08
	E3	High N	18.67	4.05
		Low N	18.54	4.02
GO	E1	High N	18.11	3.93
		Low N	18.64	4.04
	E3	High N	18.64	4.04
		Low N	18.79	4.08
GSTH	E1	High N	18.64	4.04
		Low N	18.2	3.95
	E3	High N	18.92	4.10
		Low N	18.46	4.00
GD	E1	High N	18.42	4.00
		Low N	18.28	3.97
	E3	High N	18.96	4.11
		Low N	18.97	4.11
CHLO	E1	High N	17.85	3.87
		Low N	18.55	4.02
	E2	High N	18.13	3.93
		Low N	18.47	4.01
N%	E3	High N	18.51	4.02
		Low N	18.43	4.00
	E4	High N	18.36	3.98
		Low N	18.75	4.07

Table A3.2. Soil analysis results of Bulks collected at random and bulked together within 4 quadrants at each N level in Burkey in 2010 (E1) and 2011(E3).

Samples were collected using a 12 inches soil sampler, and tested at the ISU Soil and Plant Analysis Laboratory.

			Low N				High N			
			Bulk 1	Bulk 2	Bulk 3	Bulk 4	Bulk 1	Bulk 2	Bulk 3	Bulk 4
	Date	Test								
E1	4/19/2010	pH	6.7	7.1	7.0	6.7	7.8	7.6	8.0	8.0
		ppm NH4	4.0	4.0	4.0	4.0	5.0	8.0	4.0	5.0
		ppm NO_3^-	2.0	3.0	6.0	5.0	8.0	8.0	7.0	8.0
	6/22/2010	ppm NH4	4.0	3.0	4.0	4.0	6.0	7.0	7.0	5.0
		ppm NO_3^-	7.0	7.0	8.0	8.0	47.0	54.0	55.0	71.0
E3	5/9/2011	pH	7.1	7.2	7.2	7.0	7.9	7.9	7.9	8.0
		ppm NH4	5.0	3.6	2.7	3.2	5.7	6.0	5.8	5.8
		ppm NO_3^-	7.6	6.6	5.5	6.3	10.4	6.1	4.9	5.7
	6/28/2011	ppm NH4	2.0	2.0	3.0	1.0	5.0	6.0	8.0	5.0
		ppm NO_3^-	26.0	16.0	16.0	17.0	30.0	35.0	36.0	21.0
	10/17/2011	pH	6.5	6.5	6.6	6.6	7.5	7.8	7.5	7.7
		ppm NH4	1.9	1.9	2.0	3.0	3.5	3.9	3.6	3.1
		ppm NO_3^-	2.6	4.1	3.5	5.4	20.3	21.7	19.2	16.1

Table A3.3. List of agronomic and grain quality traits measured under different locations and years combinations.
These combinations, denominated environments, are the following: Burkey-2010 (E1), Marion-2010 (E2), Burke-2011 (E3), and Marion-2011 (E4). The X marks the environments where the trait was measured.

TRAIT	E1	E2	E3	E4
Grain yield (GY)	X	X	X	X
Plant Height (PHT)	X	X	X	
Growing Degree Units to Silk (GDUSLK)	X	X	X	X
Growing Degree Units to Pollen Shed (GDUSHD)	X	X	X	X
Anthesis – Silking Interval (ASI)	X	X	X	X
Ears per plant (EPP)	X		X	
Kernel Weight (KW)	X		X	
Grain Moisture (GMST)	X		X	
Grain Protein (GPRT)	X		X	
Grain Oil (GO)	X		X	
Grain Starch (GSTH)	X		X	
Grain Density (GD)	X		X	
Leaf Chlorophyll content (CHLO)	X	X		
Leaf Nitrogen % 20 DAF (N %)			X	X

CHAPTER 4: GENERAL CONCLUSIONS

The main goal of this study was to have a better understanding of the genetic control associated to maize response to N input. Phenotypic and genotypic analyses were performed in a subset of lines of the IBM2SYN-DH high-resolution mapping population under High and Low N treatments. These analyses focus in the study N effect over the growth of root system architecture in 14-day old seedlings, as well as the performance of adult plants measured by grain yield and related traits.

Significant phenotypic variation was observed in the RSA traits among the lines used in the study. Greater development of some RSA components was observed as an effect of LN treatment. The lengths of the primary root and the lateral roots, as well as the number of lateral roots were greater under LN level by 8.5%, 31% and 20%, respectively. In general, the increased root biomass triggered by the lack of N, suggests that the maize seedlings can adapt rapidly to promote the N-uptake through the root system. Alternatively, under HN treatment the shoot length and crown root number was greater than at limiting conditions. Thus, N-assimilation and N-remobilization are promoted under normal N levels to benefit the growth and development of plant biomass.

57 QTL were identified among 8 RSA traits. Of those, several QTL can be collocated to similar genomic regions to QTL previously reported. Hence, QTL identified for LN for crown root number, lateral root number and total root length in chromosome 3, can be collocated to QTL for axial root number for low N tolerance in a similar region (Liu et al., 2008). In addition, QTL for LRN and CRN were detected only under LN in chromosome 10; similar to QTL for LRL detected in the same genomic region of that chromosome for LN (Liu et al., 2008). This

suggests that specific genomic regions in chromosome 3 and 10 contain loci associated with the development of the RSA grown in LN conditions. Furthermore, QTL in this study grouped at certain regions, which have been previously described as essential genomic regions related to RSA control in meta-QTL analyses across maize populations (Hund et al., 2011). More specifically, a cluster at chromosome 8 that grouped QTL for CRN and LRN under both N treatments; has been described as central for response to low phosphorus (Zhu et al., 2005b). Thus, it seems reasonable to think that these genomic regions are triggered by abiotic stress of nutrient deficiency, and control the root system growth for better nutrient acquisition and remobilization.

There is no evidence available of a comprehensive study for RSA made in the IBM2Syn10-DH population. The results showed here could be used as a starting point for further and more in depth analysis of the genetic factors controlling the root system development under N limiting conditions. In this study moderate to high repeatability was observed, and the high-resolution genetic mapping population was used, reasons that can allow targeting the QTL for fine mapping to determine sequences that can be associated with genes in the maize physical map. Eventually, the goal should be to produce functional markers to improve breeding strategies for the response to N input.

The performance of adult plants was significantly influenced by the effect of the environments were the experiments were conducted. N availability varied between two different years at the same locations, which turns to be determinant for analyses of response to N input. The phenotypic variation observed due to effects other than genetic, makes it difficult to predict the performance of maize lines for breeding purposes. However, the N treatments used in the experiments allowed establishing differences to the response of N input for several traits. Hence,

LN treatment reduced grain yield up to 63% in E1 (Burkey-2010) compared to the lines grown at HN levels. At that level of stress, proper evaluation of other agronomic traits becomes difficult; thus a more moderate reduction as the observed in E2 (Marion-2010) of 31% should be targeted. Overall, results showed that under LN treatments in E1 and E2, the performance of the lines was diminished. Plant grew shorter, produced fewer ears per plant on average per plot, and increased the flowering intervals. Moreover, it was found that leaf N % at maturity and grain protein content, were reduced by LN treatment, even in environments where GY was not negatively affected (E3 and E4). Thus it is likely that under LN conditions, the maize plants have reduced rates of N-remobilization and N-utilization for the production of high yielding and high quality grain.

Significant genotypic variation was observed among the DH-lines in the study. QTL were identified for specific environments as expected by the GxE observed for the majority of the traits; however, a few QTL were found across environments. Besides, QTL for agronomic traits were also found in clusters in chromosomes 1, 4, 5, 8, and 10; which collocated with clusters previously reported to carry loci associated with N metabolism (Bertin and Gallais, 2001; Gallais and Hirel, 2004). A couple of these loci, *gln4* and *gln5*, regulate the activity of glutamine synthetase; which is an enzyme involved in N-assimilation and N-remobilization for the production protein in the grain of maize. Only a few QTL specific for each N treatment were identified, even though the traits analyzed showed significant N treatments effects. This could mean that the population lacks the variation of alleles responsible to low or high N levels, or that more quantitative genetic approaches are needed to clearly determine alleles for N response. Thus, further analyses of these genomic regions should be pursued under more numerous and

stable environments, in order to determine valid loci that can be used to improve the genetics of the response to N input.

Overall, the results presented through this dissertation provide a better understanding of the response of maize inbred lines to the variation of N input. Variation among to the environments should be controlled, and more environments should be tested to provide more solid data for better statistical analyses. This study can represent the basis for more in depth investigation into the genetics underlying the response to N variation. As for any QTL mapping study, a validation process should be followed to determine candidate genomic regions to be analyzed in fine mapping projects. For the present case, studies of testcross versions of the IBM2Sn10-DH population are in process to determine QTL that are stable for response to N variation at the hybrid level. Those results could contribute with more ideas for further genetic studies to help assess the difficulty of breeding for improved N-use efficiency maize varieties.

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