KAIO OLÍMPIO DAS GRAÇAS DIAS

MODELING GENOTYPE-BY-ENVIRONMENT INTERACTION, ADDITIVE AND DOMINANCE EFFECTS INTO THE GENOMIC PREDICTION FRAMEWORK FOR DROUGHT TOLERANCE IN MAIZE

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Thesis submitted to the Federal University of Lavras, in partial fulfillment of the requirements of the Genetics and Plant Breeding Graduate Program, for the Degree of the *Doctor Scientiae*.

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To my parents, José and Maria; my sisters, Iolanda and Fernanda, my nephew, Luiz Felipe, for their unconditional love and support

I DEDICATE IT.

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GENERAL ABSTRACT

Drought is one of the major causes of severe yield losses worldwide, and it is considered as an important limiting factor for maize production in tropical areas. Maize breeding for drought tolerance is usually difficult, time consuming and expensive, since the hybrids need to be evaluated in several environments. In this context, an accurate prediction of the performance of untested hybrids in one or more environments is essential to maximize genetic gains. The main goal of this study was to evaluate the accuracy of genomic selection to predict the performance of untested maize single-cross hybrids for drought tolerance, using a statistical-genetics model that account for genotype-by-environment interaction, additive and dominance effects. Phenotypic data of five drought tolerance traits were measured in 308 single-cross hybrids in eight trials, comprising water-stressed (WS) and well-watered (WW) conditions over two years and two locations, in Brazil. The genotypes of the hybrids were inferred based on the genotypes of their parents (inbred lines), using SNP (Single Nucleotide Polymorphism) data obtained via GBS (genotyping-by-sequencing). Genomic selection analysis was done using GBLUP (Genomic Best Linear Unbiased Prediction) by fitting a factor analytic multiplicative mixed model. Our results showed differences in the predictive accuracy between additive (A) and additive+dominant (AD) models for the five traits in both water conditions. However, these differences were more evident under WS conditions. For grain yield (GY), the AD model had a predictive accuracy two times bigger than the A model. Using factor analytic mixed models, including additive and dominance effects, it was possible to investigate the stability of the additive and dominance effects across environments, as well as, the additive and dominance-by-environment interaction, with interesting applications for parental and hybrid selection. In addition, combining WW and WS trials increased the prediction accuracy of untested hybrids in WW and/or WW conditions. These results contribute to a better understanding about the genetic architecture of important traits related to drought tolerance in maize, and highlight the importance of dominance effects for grain yield genomic prediction in single-cross hybrids under both water regimes. The models applied in this study can be easily extended to other crops for which the genotypes are measured in multiple environments and the dominance effects exhibit an important role for heterosis.

RESUMO GERAL

A seca é uma das principais causas de perdas de produção em todo o mundo, e é considerada um importante fator limitante para a produção de milho em áreas tropicais. Melhoramento de milho para tolerância ao estresse hídrico é geralmente difícil, demorado e caro, uma vez que os híbridos têm de ser avaliados em vários ambientes. Neste contexto, uma predição precisa do desempenho dos híbridos simples não avaliados em um ou mais ambientes é essencial para maximizar os ganhos genéticos. Objetivou-se neste trabalho avaliar a acurácia de predição da performance de híbridos simples não avaliados para tolerância à seca, com o uso de um modelo genético estatístico que considera os efeitos da interação genótipos por ambientes e efeitos aditivos e dominantes. Dados fenotípicos para cinco caracteres foram avaliados em 308 híbridos em oito ambientes nas condições de seca e irrigado em dois anos e dois locais no Brasil. Os genótipos dos híbridos simples foram inferidos a partir do genótipo de seus genitores (linhagens) com o uso de SNP (Single Nucleotide Polymorphism) via GBS (Genotyping by Sequencing). As análises de seleção genômica foram feitas usando o GBLUP (Genomic Best Linear Unbiased Prediction) por meio do modelo misto multiplicativo fator analítico. Diferenças foram observadas na acurácia preditiva para modelos aditivos (A) e aditivos+dominantes (AD) para os cinco caracteres avaliados nas duas condições hídricas. No entanto, essas diferenças foram mais evidentes nas condições de seca. Para produção de grãos (GY) o modelo AD obteve uma acurácia duas vezes maior que o modelo A. Com o uso do modelo misto multiplicativo fator analítico com efeitos aditivos e dominantes, foi possível quantificar a estabilidade de efeitos aditivos e dominantes, bom como a interação aditiva e dominante entre ambientes. Esses resultados têm grande aplicação na seleção parental e seleção de híbridos. Além disso, combinando ambientes WW e WS houve um aumento na predição de híbridos simples não testados em ambas as condições hídricas. Esses resultados contribuem para um maior entendimento sobre a arquitetura genética dos caracteres estudados nas condições de seca e irrigado, e destaca a importância de efeitos de dominância na predição de híbridos simples não avaliados em ambas as condições híbridas. Os modelos utilizados neste estudo podem ser facilmente estendidos para outras culturas, em que os genótipos são avaliados em vários ambientes e os efeitos de dominância apresentam um papel importante para a heterose.

SUMMARY

CHAPTER I

1 INTRODUCTION

Genotyping techniques have been developed in a large scale and with a reduced cost. Associated with the development of statistical methods and computational resources for processing large data sets, it has been proposed a new approach for assisted selection for quantitative traits, known as genomic selection (GS) or genome wide selection (GWS) (MEUWISSEN; HAYES; GODDARD, 2001). Using the information of molecular markers distributed throughout the genome, GS determines the breeding values of selection candidates, based on a statistical model previously adjusted and validated in a training population, containing genotypic and phenotypic data. Thus, the selection of individuals with better performance can be made by using a model, with the estimated markers effects, to predict their breeding values without the need for phenotypic evaluations.

The benefits of GS are more evident when traits are difficult, time consuming and/or expensive to measure, or when several environments need to be evaluated. In maize, for example, the development of drought-tolerant cultivars is an effective strategy to increase yield under tropical conditions, once the water stress is a major cause of yield losses. As drought tolerance is a quantitative trait and one selection cycle is normally performed per year, the use of GS has a huge expectation to increase genetic gains. Additionally, the use of appropriate GS methods can provide accurate predictions even for untested genotypes, resulting in a considerable progress for breeding programs, due to the reduced number of genotypes tested in the field, reducing the phenotyping costs (KRCHOV; BERNARDO, 2015).

Many QTL (quantitative trait loci) studies have been increased the knowledge about the genetic architecture of drought tolerance related-traits, but none QTL with large effects has been reported (RIBAULT et al., 2009). Thus, GS could be an interesting approach to be applied to improve genetic gains, since GS uses the information of molecular markers distributed throughout the genome. In addition, GS doesn't require structured populations (F2, three way, four way, backcross, double-haploids, recombinant inbreed lines) as QTL mapping does, making this approach an interesting option to be applied in a breeding program context. Moreover, drought tolerance related traits are usually measured in well-watered (WW) and water-stressed (WS) experiments across several years and locations, which requires the inclusion of genotype-by-environment interaction (GxE) effects in multi-environment trial (MET) analysis.

Understanding GxE is one of the greatest challenges faced by plant breeders. For drought tolerance, breeders search for genotypes that are stable under WS and WW conditions. In breeding programs, genotypes are evaluated in multiple environments, over years and locations. Therefore, appropriate statistical models that account for correlations across trials and deal with unbalanced data needs to be used. Many statistical models have been proposed to understand GxE in plant breeding. Recently, some studies have shown the advantages of GS models that incorporate GxE (BURGUEÑO et al., 2012; HESLOT et al., 2014; JARQUÍN et al., 2014; LOPEZ-CRUZ et al., 2015) for quantitative traits. For this purpose, factor analytic multiplicative mixed models allow fitting flexible variance and covariance components to model genetic and residual correlations between environments (PIEPHO 1997, 1998; SMITH; CULLIS; THOMPSON, 2001). However, these studies were limited to the incorporation of additive effects.

Estimation of additive and non-additive (dominance and epistasis) effects helps to improve the knowledge about the genetic architecture of target traits and to define optimal breeding strategies. Nevertheless, orthogonal partitioning of genetic variance through molecular markers or pedigree is one important step to accurately estimate additive and non-additive effects. This partition, among other things, depends on the distribution of allele frequencies (HILL; GODDARD; VISSCHER, 2008) and the correct parametrization for genomic relationship matrices (VITEZICA; VARONA; LEGARRA, 2013; DA et al., 2014; ZHU et al., 2015). Although some studies have shown contribution of dominance effects to predictive accuracy (DA et al., 2014; BOUVET et al., 2015), most GS models have been limited to the use of additive effects.

Recently, genomic selection has been applied for drought tolerance in maize (ZYOMO; BERNARDO, 2013; BEYENE et al., 2015; ZHANG et al., 2015). However, the majority of drought-tolerance GS studies did not consider models to deal with GxE, additive and non-additive effects simultaneously. Thus, the chapters in this thesis have the goal to provide more information about the genetic basis of drought tolerance in maize growing under tropical conditions and multiple environments, using multi-environment trial analysis for phenotypic and genotypic data via complex linear mixed models.

2 LITERATURE REVIEW

2.1 Breeding for drought tolerance in maize

Maize (Zea mays *L*.) is a cereal widely used for food and feed, highlighted as a crop of highest yield production around the word. In developing countries, mainly in Africa and Asia, maize is the third most important food source, after wheat and rice (Food and Agriculture Organization - FAO, 2012). Brazil is the world's third largest maize producer, with estimated 15.9 million hectares of cultivated area, and total production estimated at 67 million tons for the 2015/2016 harvest (Companhia Nacional de Abastecimento - CONAB, 2016). Despite the increase of 10% in the second season cultivated area, the grain yield production was 24.7% lower due the water stress occurred in this season (CONAB, 2016).

One of the alternatives to improve crop production, mainly in areas that are prone to water limitation, is breeding for drought tolerance. In breeding programs, phenotypic selection in water-stressed environments is important to achieve genetic gains under drought, effectively contributing to the development of new cultivars with yield stability in areas that are prone to water limitation (LOBELL et al., 2014; COOPER et al., 2014). Grain yield is a quantitative trait, therefore moderate to low heritability under stress conditions is common. Thus, availability of space and resources is required to increase experimental precision and genetic gains under stressed conditions (EDMEADES et al., 1999). Although genetic gain for drought tolerance has been achieved through phenotypic selection (LOBELL et al., 2014), there are still many challenges about drought tolerance, such as precise phenotyping and accurate understanding about the genetic basis of drought-tolerance traits, that must be overcome to improve the response to selection (SINCLAIR, 2011; COOPER et al., 2014). In addition, in Brazil, second season maize areas are larger than the first season one, which emphasizes the importance of drought tolerance even more, since, in Brazil second season maize crops are prone to additional variation in rainfall, which can reduce grain yield due to water limitations.

Many QTL studies have been published, with an important role about the genetic architecture of target traits for drought tolerance, but none QTL with large effects has been reported (RIBAULT et al., 2009). Given these results, and due to the reduced cost of genotyping, genomic selection comes up as an interesting option for drought tolerance breeding programs. Recently, some studies have been shown the advantage of GS for drought tolerance improvement (ZYOMO; BERNARDO, 2013; BEYENE et al., 2015; ZHANG et al., 2015). Comparison of GS and selection for secondary traits were made by Ziyomo and Bernardo (2013), showing the advantage of GS to increase genetic gains. Furthermore, Zhang et al. (2015) showed the advantage of using GxE in GS models for drought tolerance. However, to our knowledge, for drought tolerance in maize, there are no GS studies that have used models that can deal with GxE and account for additive and dominance effects simultaneously.

2.2 Genotype-by-environment interaction in genomic selection

Understanding the genotype-by-environment interaction is one of the hardest challenge faced by plant breeders. Since, genotypes do not have consistent behavior in different environments, GxE could be a complicating factor for breeders. However, Chaves (2001) says that "the GxE should be seen not as a problem or an undesirable factor, which effects should be minimized in a breeding program. In contrast, should be seen as a natural biological phenomenon, and we must know it well, to better take advantage of it in the selection process". Thus, for breeding programs, the knowledge about GxE provides valuable information to evaluate genotype stability across environments, select genotypes to specific environments, evaluate breeding zones (or mega-environments), define target environments, define breeding strategies and maximize genetic gains.

Many statistical models have been proposed to understand GxE in plant breeding, including linear, bilinear and multivariate models (CROSSA, 2012). Multivariate models such as the additive main effect and multiplicative interaction (AMMI, GAUCH, 1988) and the GGE Biplot (YAN, 2000) have been widely used to evaluate genotype stability and adaptability, and to define breeding zones (MALOSETTI; RIBAULT; VAN EEUWIJK, 2013). Complex multi-trait and/or multi-environment models have been implemented in plant breeding for multi-environment trial analyses using mixed models (PIEPHO 1997, 1998; SMITH; CULLIS; THOMPSON, 2001; MEYER, 2009). These models allow fitting flexible variance and covariance components to model genetic and residual correlations between traits and/or environments.

Several modelling approaches exist to statistically explore GxE. The most interesting ones consider modelling the $s \times s$ matrix of genetic variance-covariance components between the s environments evaluated. Thus, with this matrix structure it is possible to have a good understanding of GxE and the genetic architecture for breeding traits, together with estimations of all environment-to-environment genetic correlations. One parsimonious way to model this genetic variance-covariance matrix is by using a factor analytic (FA) structure (PIEPHO, 1997, 1998; SMITH; CULLIS; THOMPSON, 2001). The FA variance-covariance structure is an approximation to the unstructured (UN) matrix but with a reduction in the number of parameters to be estimated, something that is particularly relevant when the number of environments is large (e.g. >5). Many studies have shown that FA models are good approximations and that they can be easily implemented in most breeding programs (MEYER, 2009; CULLIS et al., 2014; SMITH et al., 2015). Another advantage of the FA model is that it can be extended to estimate additive and non-additive effects simultaneously (KELLY et al., 2009).

Recently, several studies have shown the advantages of GS models that incorporate GxE (BURGUEÑO et al., 2012; HESLOT et al., 2014; JARQUÍN et al., 2014; LOPEZ-CRUZ et al., 2015) for quantitative traits. A GS study performed by Burgueño et al. (2011) showed that FA models exhibited up to 6% of advantage in the predictive accuracy over models that considered the same variance and correlation across environments. Lopez-Cruz et al. (2015) showed that GS models that account for GxE had greater prediction accuracy than models that ignores GxE. For drought tolerance, Zhang et al. (2015) showed that the prediction accuracy increased when GxE was incorporated in the GS models for grain yield. It is known that, there are high levels of GxE for most of the quantitative traits faced by breeders, which highlight the importance of the inclusion of the GxE effect in GS models when dealing with MET data.

2.3 Partition of the genetic variance in genomic selection studies

Most genetic analyses focus on the estimation of additive or total genetic effects. However, the estimation of both additive and non-additive effects helps to improve the understanding of the genetic basis of target traits and to define optimal breeding strategies. Nevertheless, estimation of these two effects, and their corresponding variance components, is often difficult requiring appropriate mating designs and large number of observations, due to the lack of orthogonality often observed between these effects. However, some studies have shown that the orthogonality and predictability of both additive and non-additive effects is greatly improved by the use of molecular-based relationship matrices (MUÑOZ et al. 2014; NAZARIAN; GEZAN, 2016). Orthogonal partitioning of genetic variance through molecular markers or pedigree is one important step to accurately estimate additive and non-additive effects. Orthogonal partitioning of genetic variance depends, among other things, on the distribution of allele frequencies (HILL; GODDARD; VISSCHER, 2008) and the correct parameterization for genomic relationship matrices (VITEZICA; VARONA; LEGARRA, 2013; DA et al., 2014; ZHU et al., 2015).

Most GS studies have been limited to the estimation of additive effects. Although few studies had applied GS using additive-dominance models (AZEVEDO et al., 2015; BOUVET et al., 2015), GxE models (BURGUEÑO et al., 2012; LOPEZ-CRUZ et al., 2015) and also models for drought tolerance in maize (ZIYOMO; BERNARDO, 2013; BEYENE et al., 2015; ZHANG et al., 2015), these studies do not fit complex linear models that incorporate GxE, additive and dominance effects simultaneously. Thus, it is not clear whether the use of additive and dominance effects can increase the accuracy to predict untested single-cross hybrids on drought tolerance breeding programs.

2.4 Application of genomic selection in maize breeding

Genomic selection can be applied at least in two contexts for drought-tolerance breeding programs in maize. First, GS can be used for parental selection and for successive cycles of intermating and selection within breeding populations (normally, biparental populations). Under this scenario, GS can be used to perform more than one breeding cycle per year, increasing the genetic gains per unit of time. A similar approach has been applied by Beyene et al. (2015), who showed the advantage of this GS strategy over phenotypic selection to increase the genetic gains in drought tolerance breeding programs. Second, GS can be used to predict untested hybrids, an important role in maize breeding programs, which allow reducing the number of tested (phenotyped) hybrids in breeding programs. In both scenarios described above, time and financial resources can be saved in genetic breeding programs. Recently, Krchov and Bernardo (2015) compared the amount of financial resources that can be saved using GS in a breeding program when compared to phenotyping costs, given that the genotyping costs are currently decreasing.

Over the recent years, many statistical models have been proposed for GS in plant and animal breeding. Although comparisons between different GS models are important, the choice of an adequate training set is one of the most important steps to apply GS in a breeding program. Thus, in maize breeding, optimizing the composition of the training set, in terms of number of lines and hybrids per line, could further increase the prediction accuracy of untested hybrids (TECHNOW et al., 2014). Moreover, biparental or multiparental training populations can be used for the breeding value prediction of selection candidates within or across breeding populations (SCHULZ-STREECK et al., 2012).

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CHAPTER II

Genotype-by-environment interaction and genetic correlation between drought tolerance traits in maize via factor analytic multiplicative mixed models

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ABSTRACT

Water deficit is one of the most common causes of severe crop production losses worldwide. The main goal of this study was to increase knowledge about the genetic basis of drought tolerance in maize (*Zea mays* L.) grown under tropical conditions through the estimation of genetic parameters in water-stressed (WS) and well-watered (WW) trials. For this, multi-environment trial and multi-trait multi-environment trial analyses were performed based on linear mixed models using factor analytic variance-covariance structures. A total of 308 hybrids plus four checks were evaluated in eight trials under WS and WW conditions across two years and two locations in Brazil. Combinations of water regime, location and year were designated as trials (or environments). The measured traits were grain yield (GY), ears per plot (EPP), anthesis-silking interval (ASI), female flowering time (FFT) and male flowering time (MFT). Our results indicated that estimates of the genetic parameters under WW were more accurate than those under WS conditions for most of the traits, and the genetic variances differed across WS and WW conditions. Modest genotype-by-environment interaction (GxE) was observed for FFT and MFT, with genetic correlations of 0.77 and 0.80, respectively, showing that it would be possible to make successful selections across WW and WS conditions for these traits. However, the GxE between WW and WS conditions for GY, EPP and ASI was high, with genetic correlations of 0.54, 0.48 and 0.60, respectively, which would limit the selection across conditions for these traits. Multi-trait multi-environment trial analyses can lead to an increase in the response to selection for drought-tolerance related traits in maize under water limited conditions.

INTRODUCTION

Drought is one of the major causes of severe yield losses worldwide, and it is considered an important limiting factor for maize production in tropical areas (Ribault et al., 2009; Edmeades, 2013). In breeding programs for drought tolerance, phenotypic selection under water-stressed (WS) environments is important to achieve genetic gains, effectively contributing to the development of new cultivars with yield stability in areas that are prone to water limitation (Lobell et al., 2014; Cooper et al., 2014). Grain yield is a quantitative trait, with moderate to low heritability under WS conditions, which requires an optimum number of replicates and plot size to minimize experimental error and increase experimental precision and, consequently, genetic gains through phenotypic selection (Edmeades et al., 1999). Despite the successful use of phenotypic selection in maize breeding for drought tolerance (Lobell et al., 2014), there are still many challenges that must be overcome to improve the response to selection; for example, the precise phenotyping and accurate understanding of the genetic basis of drought tolerance-related traits (Sinclair, 2011; Cooper et al., 2014).

Strategies to improve the response to phenotypic selection depend on knowledge about the genetic basis of target traits, unveiled by genotype-by-environment interactions (GxE), genetic correlations between traits, quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS). For maize, several studies have reported estimates of the genetic parameters for traits related to drought tolerance, such as grain yield, ears per plant, male and female flowering times, anthesis-silking interval (ASI), stay-green, plant height, and other physiological and root morphology traits (Ribault et al., 2009; Tuberosa, 2012; Edmeades, 2103; Cooper et al., 2014). Some of the above traits exhibited high heritability and moderate to high correlations with the grain yield (Ziyomo and Bernardo, 2013; Farfan et al., 2015; Oyekunle et al., 2015), indicating that the selection for secondary traits may lead to genetic gains for grain yield under water limited conditions.

For breeding programs, knowledge about GxE provides valuable information to evaluate the genotype stability across environments, select genotypes for specific environments, evaluate breeding zones (or mega-environments), define target environments, and develop breeding strategies to maximize genetic gains. Understanding GxE is one of the greatest challenges faced by plant breeders. For drought tolerance, breeders continually search for genotypes that are stable under water-stressed (WS) and well-watered (WW) conditions. Nevertheless, finding genotypes with good performance in both WW and WS conditions is a difficult task, since gene expression is not expected to be consistent across different water regimes. For maize, recent studies have reported high levels of GxE for grain yield under WS and WW trials (Malosetti et al., 2013; Farfan et al., 2015). These results indicate a weak concordance for the genotype rankings across different environments.

Many statistical models have been proposed to understand the GxE in plant breeding, including linear, bilinear and multivariate models (Crossa, 2012). Multivariate models such as the additive main effect and multiplicative interaction (AMMI, Gauch, 1988) and the GGE biplot (Yan, 2000) have been widely used to evaluate the genotype stability and adaptability and to define breeding zones (Malosetti et al., 2013). Complex mixed models have been used in plant breeding for multi-environment trial (MET) analyses (Piepho 1997, 1998; Smith et al., 2001; Meyer, 2009). These models allow fitting flexible variance and covariance components to model genetic and residual correlations between environments.

For MET analyses, the most flexible and informative model specifies different covariances between pairs of environments and specific variances for each environment through an unstructured (UN) variance-covariance matrix for the effect of genotypes within environments. In this model, a genetic correlation (or covariance) between any pair of environments is estimated. However, the number of parameters grows rapidly as the number of environments increases, enabling the fitting of an UN model (Piepho, 1997, 1998; Smith et al., 2001). Factor analytic (FA) variance-covariance structures approximate UN models, with a reduction in the number of parameters needed to be estimated. In multivariate statistics, FA is a variable reduction technique that fits a large number of variables into a few unobserved variables (or factors). Many studies have shown the advantages and applications of using FA models in plant breeding (Kelly et al., 2007; Meyer, 2009; Cullis et al., 2014; Smith et al., 2015). However, to our knowledge, factor analytic multiplicative mixed models have not yet been used to study the GxE and genetic correlations between drought tolerance traits in maize in the context of multi-trait and/or multi-environment trial analyses.

In maize, some studies have reported the estimation of genetic parameters under WS and WW environments for different drought tolerance-related traits (Ziyomo and Bernardo, 2013; Oyekunle et al., 2015). However, field experiments in tropical areas are limited, and in Brazil, drought tolerance is relevant mainly for second-season crops, which are often prone to strong variations in rainfall, affecting the grain yield. In addition, few studies have taken full advantage of the machinery of complex linear mixed models, such as models that can simultaneously fit different genetic and residual variance components for each environment and/or trait and also genetic and residual correlations between pairs of environments and/or traits. Thus, this study was performed to improve the knowledge of the genetic basis of drought tolerance in maize grown under tropical conditions through the estimation of genetic parameters in WS and WW trials via complex linear mixed models. The specific objectives were (i) to estimate broad-sense heritabilities and genetic variances in single and multi-environment trial analyses; (ii) to estimate genetic correlations between environments in multi-environment trial analyses to investigate the relevance of GxE; and (iii) to estimate genetic correlations through multi-trait multi-environment trial analyses to evaluate the trait-to-trait genetic correlations.

MATERIAL AND METHODS

Genetic material

A total of 308 maize hybrids were obtained from single crosses between two testers and 188 inbred lines, representing dent (85 lines) and flint (86 lines) heterotic groups, and also another group, here called group C (17 lines), which combines well with both dent and flint sources. A flint (L3) and another dent (L228-3) inbred line were used as testers. Fifty-four inbred lines were crossed only with L228-3 and 14 only with L3, whereas 120 lines were crossed with both testers. All inbred lines were derived from the maize breeding program of Embrapa Maize and Sorghum in Brazil.

Phenotypic data

Field experiments were performed under well-watered (WW) and water-stressed (WS) conditions at two locations, Janaúba (Minas Gerais state, Brazil) and Teresina (Piauí state, Brazil), in 2010 and 2011, giving a total of eight trials: WWJ10, WSJ10, WWJ11, WSJ11, WWT10, WST10, WWT11 and WST11. Combinations of water regime, location and year were designated as trials (i.e., environments). Thus, for each trial, the first two letters identify the water condition (WS or WW), followed by the first letter of the location (J for Janaúba or T for Teresina) and the last two digits of the year (10 or 11). Additional information about the geographic coordinates of the locations and the climatic data collected during the field experiments in 2010 and 2011 is provided in Figure 1. Each trial comprised the 308 maize single-cross hybrids randomly split into 6 sets of 80, 78, 60, 60, 15 and 15 hybrids. In the field, each set was augmented by four common checks (commercial maize cultivars AG7088, P30F35, BRS1010 and BRS1055) and was arranged in a randomized complete block design. Although the hybrids within each set were kept the same across trials, the hybrids and checks were randomly allocated to groups of plots within each set, and this allocation was different between replicates of the sets and between trials. The WS trials had three replicates, except for the sets of 15 hybrids and the sets evaluated in 2010 that had two replicates. All WW experiments were performed in two replicates, except in 2011, where both locations had a single replicate. Each plot consisted of a 4-meter row in Teresina and a 3.6-meter row in Janaúba, both with 0.8 meters between rows and 4 plants per meter.

The experiments were performed in a dark-red latosol and in a red-yellow argisol in Janaúba and Teresina, respectively. In Janaúba, the fertilization consisted of 40 kg/ha of N, 80 kg/ha of P_2O_5 and 60 kg/ha of K_2O at sowing and then 100 kg/ha of N applied when the plants had 6 complete leaves. In Teresina, 40 kg/ha of N, 80 kg/ha of P_2O_5 and 35 kg/ha of K_2O were applied at sowing, 40 kg/ha of N and 35 kg/ha of K_2O were applied when the plants had 6 complete leaves, and 40 kg/ha of N was applied when the plants had 8 complete leaves. For the WS experiments in Janaúba and Teresina, the water supply was interrupted before flowering, and the drought stress was imposed during flowering and grain filling. In both locations, WW conditions were ensured by applying water to completely replace water losses attributable to evapotranspiration based on local climatic data obtained from an automatic weather station. Thus, daily irrigation was maintained in the WW experiments based on the crop evapotranspiration index (ETc) of the previous day, which was calculated using the reference evapotranspiration calculated from the Penman-Monteith equation (ETo) and the crop coefficient (Kc) per phase. The water content in the soil was monitored up to a depth of 0.70 m using a DIVINER 2000® probe (Sentek Sensor Technologies, Australia). In Janaúba, both the WS and WW experiments were irrigated with a drip irrigation system, whereas in Teresina, irrigation was applied with a fixed sprinkler system (12 m x 12 m; 1.6 m^3/h). The selection of a site-specific irrigation system was based on the field characteristics, climatic and topographic conditions, and soil physical and chemical properties. All experiments were grown from May to September in Janaúba and from September to January in Teresina for the two years. Both locations have a low probability of rainfall during the specified periods. Other agronomical practices were performed as recommended for maize crops.

For all trials, five traits were evaluated: grain yield (GY, t/ha); number of ears per plot (EPP); female flowering time (FFT, days), measured as the number of days from sowing up to the time when silks had emerged on 50% of the plants in the plot; male flowering time (MFT, days), measured as the number of days from sowing up to the time when 50% of the plants in the plot had begun to shed pollen; and anthesis-silking interval (ASI, days), which is the difference in days between FFT and MFT. For the GY, all the grains of each plot were weighed, evaluated for moisture, corrected for 13% moisture, and converted to tons per hectare (t/ha), considering the differences in the plot size across trials. The EPP was corrected for the differences in the plot size across trials. The FFT, MFT and ASI were not corrected for the differences in the plot size across trials, since those traits were measured based on 50% of the plants in the plot.

Statistical analyses

For the available dataset, single and multi-environment trial (MET) analyses were performed to estimate the broad-sense heritabilities and genetic correlations between environments. In addition, multi-trait multi-environment trial analyses were performed to estimate the genetic correlation between traits. All linear mixed models were fitted using the statistical package ASReml-R v.3 (Buttler et al., 2009), which estimates variance components using the restricted maximum likelihood (REML) through the average information (AI) algorithm (Gilmour et al., 1995), followed by the estimation of fixed and random effects by solving the mixed model equations. Diagnostic plots were used to verify the presence of outliers and if the residuals of the fitted models were normally distributed. For the GY and EPP, the number of plants per plot was used as a covariate.

Single-environment trial analyses

For each trait and trial, single-environment trial analyses were performed based on the model

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{Xb} + \mathbf{Z_1r} + \mathbf{Z_2g} + \mathbf{e} \qquad [1]$$

where $\mathbf{y}$ is the vector of the phenotypes; μ is the overall mean; $\mathbf{b}$ is the vector of the fixed effects of sets; $\mathbf{r}$ is the vector of the random effects of replicates (or blocks) within sets, with $\mathbf{r} \sim \mathbf{MVN}(\mathbf{0}, \sigma_r^2\mathbf{I_r})$; $\mathbf{g}$ is the vector of the random effects of hybrids, with $\mathbf{g} \sim \mathbf{MVN}(\mathbf{0}, \sigma_g^2\mathbf{I_g})$; and **e** is the vector of the random effects of residuals, with $\mathbf{e} \sim \mathbf{MVN}(\mathbf{0}, \sigma_e^2\mathbf{I_e})$. $\mathbf{X}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ represent incidence matrices for their respective effects, **1** is a vector of ones, and $\mathbf{I_r}$, $\mathbf{I_g}$ and $\mathbf{I_e}$ are identity matrices of their corresponding orders.

Multi-environment trial analyses

Distinct MET analyses were performed by combining different trials: (i) one for the group of four trials under WW conditions; (ii) one for the group of four trials under WS conditions; and (iii) another one for all evaluated trials under WW and WS conditions. The following generic model was fitted for each trait and group of trials:

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{X_1s} + \mathbf{X_2b.s} + \mathbf{Z_1r.s} + \mathbf{Z_2g.s} + \mathbf{e} \qquad [2]$$

where $\mathbf{y}$ is the vector of the phenotypes; μ is the overall mean; $\mathbf{s}$ is the vector of the fixed effects of trials; $\mathbf{b.s}$ is the vector of the fixed effects of sets within trials; $\mathbf{r.s}$ is the vector of the random effects of replicates within sets within trials, with $\mathbf{r.s} \sim \mathrm{MVN}(\mathbf{0}, \mathbf{D_{r.s}})$; $\mathbf{g.s}$ is the vector of the random effects of hybrids within trials, with $\mathbf{g.s} \sim \mathrm{MVN}(\mathbf{0}, \mathbf{G} \otimes \mathbf{I_g})$; and **e** is the vector of residuals, with $\mathbf{e} \sim \mathrm{MVN}(\mathbf{0}, \mathbf{D_e})$. **G** is a variance-covariance (VCOV) matrix for the effect of hybrids across trials**,** with dimensions of 8 x 8 or 4 x 4, when considering all evaluated WW and WS trials or solely the group of trials under WW or WS conditions, respectively. A FA structure of order *k*, where *k* is the number of multiplicative components (FA_k, see Appendix), was considered to model **G**. $\mathbf{D_{r.s}}$ and $\mathbf{D_e}$ are diagonal VCOV matrices, in which each trial has a specific and independent variance component for the effects of replicates within sets and for the residuals, respectively. $\otimes$ denotes the Kronecker product, and all other vectors and matrices were as previously defined.

Multi-trait multi-environment trial analyses

Genetic correlations between pairs of traits were estimated via multi-trait multi-environment trial analyses by fitting two traits at a time across groups of trials. Thus, these analyses were performed for (i) a group of four trials under WW conditions and (ii) a group of four trials under WS. For this, the following model was used:

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{X_1 t} + \mathbf{X_2 b.t} + \mathbf{Z_1 r.t} + \mathbf{Z_2 g.t} + \mathbf{e} \qquad [3]$$

where $\mathbf{y}$ is the vector of the phenotypes; μ is the overall mean; $\mathbf{t}$ is the vector of the fixed effects of trait-trial combinations, with a total of 8 combinations (2 traits x 4 trials) per group; $\mathbf{b.t}$ is the vector of the fixed effects of sets within trait-trial combinations; $\mathbf{r.t}$ is the vector of the random effects of replicates within sets and within trait-trial combinations, with $\mathbf{r.t} \sim \text{MVN}(\mathbf{0}, \mathbf{D_{r.t}})$; $\mathbf{g.t}$ is the vector of the random effects of hybrids within trait-trial combinations, with $\mathbf{g.t} \sim \text{MVN}(\mathbf{0}, \mathbf{G} \otimes \mathbf{I_g})$; and $\mathbf{e}$ is the vector of residuals, with $\mathbf{e} \sim \text{MVN}(\mathbf{0}, \mathbf{R} \otimes \mathbf{D_e})$. Here, **G** is an 8 x 8 matrix of variances and covariances between trait-trial combinations modeled by a FA_k covariance structure. $\mathbf{D_{r.t}}$ is a diagonal matrix, in which each trait-trial combination has a specific and independent variance component for the effects of replicates within sets. **R** is an unstructured (UN) 2 x 2 VCOV matrix for the residuals of the two traits, and $\mathbf{D_e}$ is a diagonal matrix, in which each trial has a specific and independent component of variance for the residuals. The Kronecker product $\mathbf{R} \otimes \mathbf{D_e}$ results in an 8 x 8 residual matrix, considering the specific variances for each trait-trial combination and the covariances between the pairs of trait-trial combinations. All other vectors and matrices were as previously defined.

The broad-sense heritability (H^2) was estimated for each trait using $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_r^2 + \sigma_e^2)$ for model [1] and $H^2 = \bar{\sigma}_{g.s}^2/(\bar{\sigma}_{g.s}^2 + \bar{\sigma}_{r.s}^2 + \bar{\sigma}_e^2)$ for model [2], where the bars over the components represent the simple average values across all trials. All the estimates were obtained considering an FA_2 variance-covariance structure (see Appendix). Approximate standard errors were obtained through the delta method (Kendall and Stuart, 1963), using the package *nadiv* (Wolak, 2012) available in the software R (R Core Team, 2015).

RESULTS

Single-environment trial analyses

The broad-sense heritabilities varied considerably between traits and trials (Table 1). For all measured traits, except in a few cases, the heritabilities were lower in the water-stressed trials than in the well-watered ones. High to moderate heritabilities were found for MFT, with averages of 0.43 and 0.52 under the WS and WW trials, respectively, and similar results were found for FFT. The smallest values of heritabilities under WS conditions were found for EPP, with an average of 0.25, followed by GY, with an average of 0.29. However, under WW conditions, the EPP and GY also had the lowest values of heritability, with averages of 0.22 and 0.34, respectively. For all traits in the single-environment trial analyses, the estimates of the genetic variances differed significantly from zero, based on the likelihood ratio test (LRT, $\alpha = 0.05$). However, an exception was found for the genetic variance of the EPP in trial WWT11, where the estimate was zero (data not shown). These results indicate the presence of important and significant levels of genetic variability among the evaluated hybrids.

A summary of the statistics for all traits and the percentages of yield losses attributable to drought stress in each location-year combination are presented in Table S1. The yield reductions under WS conditions differed among location-year combinations, with values ranging from 15.57% (WST11) to 57.24% (WST10). In Teresina, for the year 2011, the yield reduction under drought was lower than expected, probably because of unusual rain events during the field experiments (Figure 1). On average, the grain yield reduction in drought-stressed trials reached 43% compared to that in well-watered trials. Thus, with this level of grain yield reduction, it is expected that the drought stress was effectively imposed.

Multi-environment trial analyses

In the MET analyses, the broad-sense heritabilities ranged from 0.27 (EPP) to 0.43 (MFT) for WS and from 0.18 (EPP) to 0.43 (FFT) for WW (Table 1). In both water conditions, the highest estimates for the heritability were observed for MFT and FFT, as also observed in the single-environment trial analyses. Genetic variances of lower magnitudes were found for the grain yield in drought-stressed trials (Table S2) when compared with those in WW conditions (Table S3). On the other hand, for EPP, ASI and FFT in WS environments, the genetic variances were greater than those observed in WW environments, except for FFT in trial WST11.

For all traits, the genetic correlations between trials were fitted through an FA structure of order 2, which varied from 0.19 (WSJ10 *vs.* WST10, GY) to 0.92 (WSJ10 *vs.* WSJ11, MFT) under drought conditions (Table S2) and from 0.12 (WWT10 *vs.* WWT11, GY) to 0.94 (WWJ10 *vs.* WWJ11, ASI) under WW conditions (Table S3). The lowest average correlations were 0.43 and 0.47 for ASI and EPP under WS and 0.474 and 0.66 for GY and ASI under WW conditions.

The level of GxE under WW conditions, inferred by the genetic correlation between pairs of trials, was higher than that under WS conditions for GY and MFT (Table 2). The ASI, EPP and FFT showed higher GxE interactions under WS conditions. However, higher levels of GxE were found under both conditions for GY and EPP, with the genetic correlations between pairs of trials being smaller than 0.60, when compared with the ASI, FFT and MFT. The ASI and FFT showed large decreases in the genetic correlations in the WS trials when compared with those in the WW trials. The average genetic correlation between the WS and WW trials ranged from 0.47 (EPP) to 0.79 (MFT). The GY, EPP and ASI exhibited lower genetic correlations between the WS and WW trials.

The percentage of GxE variance explained by two factors of the FA2 VCOV structure in model [2] (see Appendix eq. A7), considering all trials in a joint analysis, ranged from 56.74% (EPP) to 83.92% (FFT) (data not shown). For this, models fitted with a FA2 VCOV structure estimated 23 variance-covariance parameters (24 less one constraint) against 36 of an unstructured VCOV model (UN). When the number of trials increases, the difference in the parameter estimates between the FA and UN models also increases, showing that FA models are parsimonious methods to address moderate to large MET studies. For this study, the differences between FA2 and FA3 were not significant by the LRT in the majority of cases (data not shown). Therefore, an FA of order 2 was used in all fitted models.

Based on the genetic correlations between all pairs of trials estimated via model [2], it was possible to identify mega-environments, in which different trials grouped together because of the consistent levels of GxE interaction. For this purpose, biplots are an interesting tool to identify mega-environments or clusters of trials. Thus, the estimated loadings (after rotation) of each trial are presented for the first *vs.* second common factors, generating different mega-environments for the evaluated traits (Figure 2). For the ASI, FFT and MFT, one mega-environment with trials evaluated in Teresina and another with Janaúba trials were identified. However, for the GY and EPP, it was not possible to observe a tendency in the clustering of trials. For example, for GY, trials WWJ10 and WSJ10 formed one group, whereas the other trials clustered in the opposite group.

The experimental precision, inferred through the accuracy (i.e., the square root of the heritability, Table 1) to predict the genetic effects of hybrids via model [2], were lower in the WS trials. Only the EPP and MFT exhibited higher accuracy values in the WS than in the WW trials. The standard errors (SE) associated with the genetic effects of hybrids, obtained via BLUP (Best Linear Unbiased Predictor), are shown in Figure S1 for all measured traits under WW and WS conditions. In general, higher SE were observed for the WS conditions, even for the WS trials with an additional replicate (Figure S1). The four checks, corresponding to the last four hybrids in the Figure S1 curves, exhibited the smallest standard errors, since they were present in all replicates and all sets. Based on these results, it is possible to observe that both the accuracy and SE reflect the existence of lower experimental precision under the WS trials.

Multi-trait multi-environment trial analyses

The genetic correlations between pairs of traits under the WS and WW environments showed different magnitudes (Table 3), with higher absolute values found between traits under the WS conditions. Correlations ranged from -0.18 (GY vs. ASI) to 0.90 (FFT vs. MFT) under WW conditions and from -0.44 (GY vs. FFT) to 0.78 (FFT vs. MFT) under WS conditions. A high positive correlation was found between the FFT and MFT under both WS and WW conditions, and another was found between the GY and EPP in both water conditions. The GY had a moderate negative correlation with the ASI, FFT and MFT in the WS trials but only a small correlation with the FFT and MFT under the WW trials. The EPP had different patterns of correlation with the FFT and MFT across the water conditions.

Figure S2 illustrates the genetic effects of hybrids under WW plotted against WS conditions. It is possible to observe a high correlation between the genetic effects predicted in contrasting water regimes for the FFT and MFT. On the other hand, the GY, EPP and ASI exhibited smaller correlations for the genetic effects predicted between WS and WW conditions. These results are in accordance with the levels of GxE interaction presented in Table 2 for the different traits, where the GY, EPP and ASI showed high to moderate levels of GxE interaction across water conditions.

DISCUSSION

For all traits, using single and multi-environment trial analyses, it was possible to observe that genetic parameters, such as the heritability, genetic variances and GxE interaction, significantly differed across water conditions. Higher values for the genetic variances of the EPP, ASI and FFT were expected under drought because those traits frequently exhibit enhanced expression of stress-associated genes under water-stressed conditions (Milkelbart et al., 2015). Differences in the heritability between single and multi-environment trial analyses showed that selection based on a single trial was influenced by the GxE, which affects the response to selection. Thus, breeders should always perform phenotypic selection based on MET results. Moreover, these results suggested that the selection of the best hybrids could achieve small genetic gains for the GY in WS trials because of the low heritability observed for this trait under drought stress. Similar results were reported by Zhang et al. (2015) using 19 biparental populations in maize, in which smaller heritabilities were found for the grain yield, anthesis day, and plant height under WS compared with WW conditions. It is important to emphasize that the accuracies were lower in the WS trials, even in those trials with more replicates. Experimental precision is indispensable for the accurate estimation of the genetic parameters, therefore, for planning future trials under water-stressed conditions, additional replicates are required to achieve the same level of accuracy found in well-watered trials.

Low levels of GxE for the FFT and MFT showed that it was possible to make a successful selection of hybrids across WW and WS conditions. However, the high levels of GxE found between WW and WS for the GY, EPP and ASI limit the selection across different water conditions. These findings suggested that, at least for some evaluated traits, it is important to select genotypes exclusively under WS conditions when the goal is to identify superior drought-tolerant hybrids. For example, based on maize and soybean field trials performed in the USA from 1995 to 2012, Lobell et al. (2014) showed that selection under water-stressed environments was efficient to increase drought tolerance. In Brazil, the second-season maize areas are larger than the first-season ones, which emphasizes the importance of drought tolerance, since second season maize crops are prone to additional variations in rainfall, which can reduce the grain yield because of water limitations.

Modeling genetic variances-covariances (VCOV) across environments using factor analytic (FA) mixed models represents an efficient way to perform multi-environment trial (MET) analyses because FA models approximate the most complex unstructured VCOV model. For data sets with complex GxE, Burgueño et al. (2011) showed that using FA mixed models improved the predictability by up to 6% compared with models considering no genetic correlations between environments and assuming the homogeneity of variances across environments. However, under low levels of GxE, the majority of their models had a high predictability. Data from breeding programs are often unbalanced because different sets of genotypes are generally evaluated in different years and trials. In this context, the statistical methods appropriated to analyze unbalanced data should be used to model different variances and covariances between environments, such as the ones used in this study.

According to Smith et al. (2001), the graphical display of loadings based on two factors informs the genetic variance explained by these factors, which corresponds to the squared length of the vector for an environment, and the cosine of the angle between the vectors for two environments corresponds to the genetic correlation between them. Hence, trials close to each other in a biplot have similar biological responses for all genotypes (Figure 2). This tool helps breeders to better define target breeding zones (i.e., mega-environments) and to decide when genotypes should be selected for specific environments. In this study, the trials located in Janaúba and the trials located in Teresina tended to group separately for the ASI, FFT and MFT. This result was expected because these locations are in different geographic regions and are exposed to different climatic conditions (see Figure 1). A similar study performed by Figueiredo et al. (2015) showed the efficiency of using a FA covariance structure in a linear mixed model framework to study GxE and to identify mega-environments in a maize data set based on unbalanced data from a breeding program.

Multi-trait multi-environment trial analyses showed that the EPP, ASI, FFT and MFT could be used as secondary traits in the selection for maize drought tolerance in tropical environments. Our results were similar to a recent study on maize under WS conditions (Oyekunle et al., 2015), in which correlations of the EPP, ASI, FFT and MFT with the GY were 0.78, -0.22, -0.50 and -0.45, respectively. However, under WW conditions, the results reported by Oyekunle et al. (2015) differed from those found in the present study. Thus, it is important to highlight that the genetic correlations reported here were estimated via multi-trait and multi-environment trial analyses, which considered the genetic correlations between traits, genetic correlations between environments and the existence of heterogeneity in the error variances across environments, being more realistic for MET data. In addition, selecting for secondary traits is advantageous because their heritabilities remain high even under WS conditions, as observed for the FFT and MFT in this study.

An easy way to evaluate the importance of a secondary trait is by calculating the level of genetic gain through indirect selection, defined as $\Delta G_{xy} = i \times h_y \times h_x \times r_{G_{xy}} \times \sigma_{p_y}$, where ΔG_{xy} is the indirect gain in trait y by selection over trait x; i is the selection intensity; h_y and h_x are the square roots of the respective heritabilities; $r_{G_{xy}}$ is the genetic correlation between traits; and σ_{p_y} is the phenotypic standard deviation of trait y. In this study, for example, the genetic gain for GY by selecting over the FFT in WS conditions resulted in a value of -0.287, which means that a decrease of 1 day in the FFT will increase the GY by 0.287 ton/ha. Thus, through selection based on secondary traits, it is possible to achieve genetic gains in the GY under drought-stressed conditions. However, the measurement of secondary traits, such as ASI, FFT and MFT, can be more expensive than evaluating the GY directly. Rebetzke et al. (2013) commented that several secondary traits had been proposed to improve the drought tolerance performance, but few had been used in breeding programs as a routine practice. According to these authors, one reason for this is the fact that there is limited knowledge about the economic benefits of selecting one trait over another for a breeding program.

The simultaneous analysis of the GY and other secondary traits can lead to an improvement in the response to the selection for drought tolerance. However, the advantages of a multi-trait over a single-trait analysis are more evident when the genetic correlations between traits are high and when the secondary trait has a higher heritability than the target trait (Mrode, 2014). Thus, based on our results from multi-trait multi-environment trial analyses, bivariate analyses such as GY-EPP and GY-FFT would be expected to achieve an increased response to the selection for drought tolerance. Then, even if these secondary traits are difficult and expensive to measure, information about them is relevant to improving the response to the selection of low-heritability target traits, such as the grain yield under drought. Furthermore, Malosetti et al. (2008) showed the advantages of a multi-trait multi-environment analysis in the context of quantitative trait locus mapping for drought tolerance-related traits in maize.

Breeders select superior hybrids with stable responses across WS and WW conditions. This can be carried out by using the factor scores from the FA model in a biplot analysis, where genotypes close to the origin have stable performance across water regimes (Cullis et al., 2014; Smith et al., 2015). For this study, the moderate levels of GxE for the GY, EPP and ASI make it difficult to find stable hybrids. Moreover, for the GY, EPP and ASI, the results showed that there were low correlations for the genetic effects of hybrids between WS and WW conditions (Figure S2), which reflects the low consistency in the ranks of genotypes between water regimes. Modern molecular tools, such as genome-wide selection (GWS) or genomic selection (GS - Meuwissen, 2001), have provided excellent results for breeding programs with a focus on drought tolerance (Beyene et al., 2015; Zhang et al., 2015). However, it is important to highlight that the use of GS does not overcome the need for good knowledge of the genetic basis of drought tolerance-related traits, and there is still a need for appropriate direct or indirect selection decisions to integrate the breeding program strategy. Moreover, accurate phenotypic data are required for the successful use of any breeding tool. The models presented in this study can be easily extended to application in future GWS studies.

CONCLUSION

This study demonstrated that (i) estimates of genetic parameters under WW conditions were more accurate than those under WS conditions for the majority of evaluated traits; (ii) genetic variances for the GY, EPP, ASI and FFT differed across WS and WW conditions; (iii) low levels of GxE were observed for the FFT and MFT, showing that it was possible to make successful selection across WW and WS conditions; however, the high levels of GxE found between WW and WS conditions for the GY, EPP and ASI would limit the indirect selection across different water conditions; and (iv) multi-trait multi-environment analyses, such as GY-EPP and GY-FFT, could achieve an increased response to selection for drought tolerance in maize under water limited conditions.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflict of interests regarding the publication of this paper.

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APPENDIX

Modeling factor analytic covariance structures

In this appendix, a compilation about FA models (Smith et al., 2001; Resende and Thompson, 2004; De los Campos and Gianola, 2007) is presented in order to provide more information about the statistical method used in this study. In the context of MET analyses, the genetic effect of individual i within trial s in an FA model can be expressed as

$$\mu_{is} = \lambda_{1s}\, x f_{1i} + \ldots + \lambda_{ks}\, x f_{ki} + \delta_{is} \qquad [A1]$$

where μ_{is} is the genetic effect of individual i within trial s; λ_{ks} is the k^{th} factor loadings for trial s; f_{ki} is the k^{th} factor score for the i^{th} individual; and δ_{is} is the i^{th} specific factor for trial s. Equation [A1], in matrix notation is

$$\boldsymbol{\mu} = (\mathbf{I_s} \otimes \boldsymbol{\Delta})\mathbf{f} + \boldsymbol{\delta} \qquad [A2]$$

where $\mathbf{I_s}$ is an identity matrix of dimension *sxs*; $\boldsymbol{\Delta} = \{\lambda_{ks}\}$ is the matrix of factor loadings; **f** is a vector of factor scores; $\boldsymbol{\delta}$ is a diagonal matrix of specific factors; and $\otimes$ is the Kronecker product.

An important assumption is that the factor scores and specific factors are assumed to be independent and normally distributed as

$$\begin{pmatrix} \mathbf{f} \\ \boldsymbol{\delta} \end{pmatrix} \sim \text{MVN}\left[\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \end{pmatrix}, \begin{pmatrix} \mathbf{I_s} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{\psi} \end{pmatrix}\right] \qquad [A3]$$

where $\boldsymbol{\psi} = \text{cov}(\delta_{is})$, which is a specific variance.

From equation [A2], $\text{cov}(\boldsymbol{\mu}) = \boldsymbol{\Delta}\boldsymbol{\Delta}' + \boldsymbol{\psi}$ is the variance-covariance matrix for the genetic effects of individuals in environment s, defined by an FA_k model. Hence, under the above definition, equation [A2] in mixed model presented in model [2] can be written as

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{X_1 s} + \mathbf{X_2 b.s} + \mathbf{Z_1 r.s} + \mathbf{Z_2}(\mathbf{I_s} \otimes \boldsymbol{\Delta})\mathbf{f} + \mathbf{Z_3}\boldsymbol{\delta} + \mathbf{e} \qquad [A4]$$

where the model terms were previously defined in the section Material and Methods.

After estimating the variance components, and solving the mixed model equations from the above model, the factor scores and specific factors can be calculated as described in Resende and Thompson (2004)

$$\tilde{\mathbf{f}} = \left[\widehat{\mathbf{\Delta}}' \left(\widehat{\mathbf{\Delta}}\widehat{\mathbf{\Delta}}' + \widehat{\boldsymbol{\psi}} \right)^{-1} \otimes \mathbf{I_s} \right] \tilde{\boldsymbol{\mu}} \qquad [A5]$$

$$\tilde{\boldsymbol{\delta}} = \left[\widehat{\boldsymbol{\psi}}' \left(\widehat{\mathbf{\Delta}}\widehat{\mathbf{\Delta}}' + \widehat{\boldsymbol{\psi}} \right)^{-1} \otimes \mathbf{I_s} \right] \tilde{\boldsymbol{\mu}} \qquad [A6]$$

Here, when the number of factors k is greater than 1, constraints must be imposed on the factor analytic parameters to obtain a unique model (Smith et al., 2001). In an FA model with k factors, the number of restrictions is given by $k(k-1)/2$ (Smith et al., 2001). Even after assuming some constraints, the loadings need to have an orthonormal rotation. The factor rotation was performed using the Varimax rotation.

The proportion of the GxE variance accounted for by k multiplicative terms (v_s) can be calculated as

$$v_s = 100 \times [\mathrm{tr}(\mathbf{\Delta\Delta}') / \mathrm{tr}(\mathbf{\Delta\Delta}' + \boldsymbol{\psi})] \qquad [A7]$$

where all of the terms were previously defined.

Table 1. Broad-sense heritabilities (H^2) from single-environment trial analyses (model [1]) and from multi-environment trial analyses (model [2]) performed separately for well-watered (WW) and water-stressed (WS) groups of trials in Janaúba (J) and Teresina (T) in 2010 (10) and 2011(11).

Model	Trial	GY†	EPP	ASI	FFT	MFT
[1]	WSJ10	0.30 (0.03)	0.29 (0.03)	0.37 (0.03)	0.51 (0.03)	0.55 (0.03)
	WSJ11	0.32 (0.03)	0.28 (0.03)	0.39 (0.03)	0.46 (0.03)	0.55 (0.03)
	WST10	0.28 (0.04)	0.25 (0.05)	0.23 (0.04)	0.22 (0.04)	0.29 (0.04)
	WST11	0.24 (0.03)	0.17 (0.03)	0.22 (0.03)	0.38 (0.04)	0.33 (0.03)
	WWJ10	0.43 (0.04)	0.23 (0.05)	0.49 (0.04)	0.58 (0.03)	0.57 (0.03)
	WWJ11	0.12 (0.11)	0.41 (0.19)	0.41 (0.12)	0.60 (0.12)	0.76 (0.07)
	WWT10	0.48 (0.04)	0.27 (0.05)	0.12 (0.04)	0.12 (0.04)	0.12 (0.04)
	WWT11	0.32 (0.11)	0.00 (-)‡	0.42 (0.15)	0.67 (0.10)	0.64 (0.11)
[2]	WS	0.29 (0.01)	0.27 (0.01)	0.28 (0.03)	0.34 (0.05)	0.43 (0.02)
	WW	0.37 (0.01)	0.18 (0.02)	0.37 (0.02)	0.43 (0.03)	0.41 (0.02)

† GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days). Values within parentheses correspond to approximate standard errors. ‡ Standard errors are not presented when the parameters were bounded to zero.

Table 2. Average genetic correlations between well-watered (WW) and water-stressed (WS) trials from multi-environment trial analyses (model [2]) performed jointly for all evaluated trials. Values within parentheses are approximate standard errors.

Traits	Trials	WW	WS
GY [†] (60.85%)[‡]	WW	0.46 (0.06)	0.53 (0.06)
	WS	-	0.52 (0.07)
EPP (56.74%)	WW	0.588 (0.060)	0.47 (0.08)
	WS	-	0.46 (0.16)
ASI (58.23%)	WW	0.70 (0.07)	0.60 (0.06)
	WS	-	0.44 (0.07)
FFT (79.09%)	WW	0.80 (0.04)	0.76 (0.03)
	WS	-	0.69 (0.03)
MFT (84.61%)	WW	0.74 (0.03)	0.79 (0.03)
	WS	-	0.82 (0.04)

[†] GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days). [‡] Percentage of the variance explained by two factors of the FA2 model.

Table 3. Average genetic correlations between pairs of traits obtained via multi-trait multi-environment trial analyses (model [3]) performed separately for the well-watered (WW) (above the diagonal) and water-stressed (WS) (below the diagonal) groups of trials. Values within parentheses are approximate standard errors.

Traits	GY	EPP	ASI	FFT	MFT
GY [†]	-	0.31 (0.15)	-0.18 (0.09)	-0.09 (0.10)	-0.02 (0.10)
EPP	0.70 (0.06)	-	-0.11 (0.18)	0.18 (0.13)	0.26 (0.12)
ASI	-0.31 (0.07)	-0.30 (0.08)	-	0.41 (0.09)	0.14 (0.12)
FFT	-0.44 (0.07)	-0.37 (0.07)	0.49 (0.08)	-	0.90 (0.02)
MFT	-0.31 (0.07)	-0.24 (0.08)	0.10 (0.07)	0.78 (0.03)	-

[†] GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days).

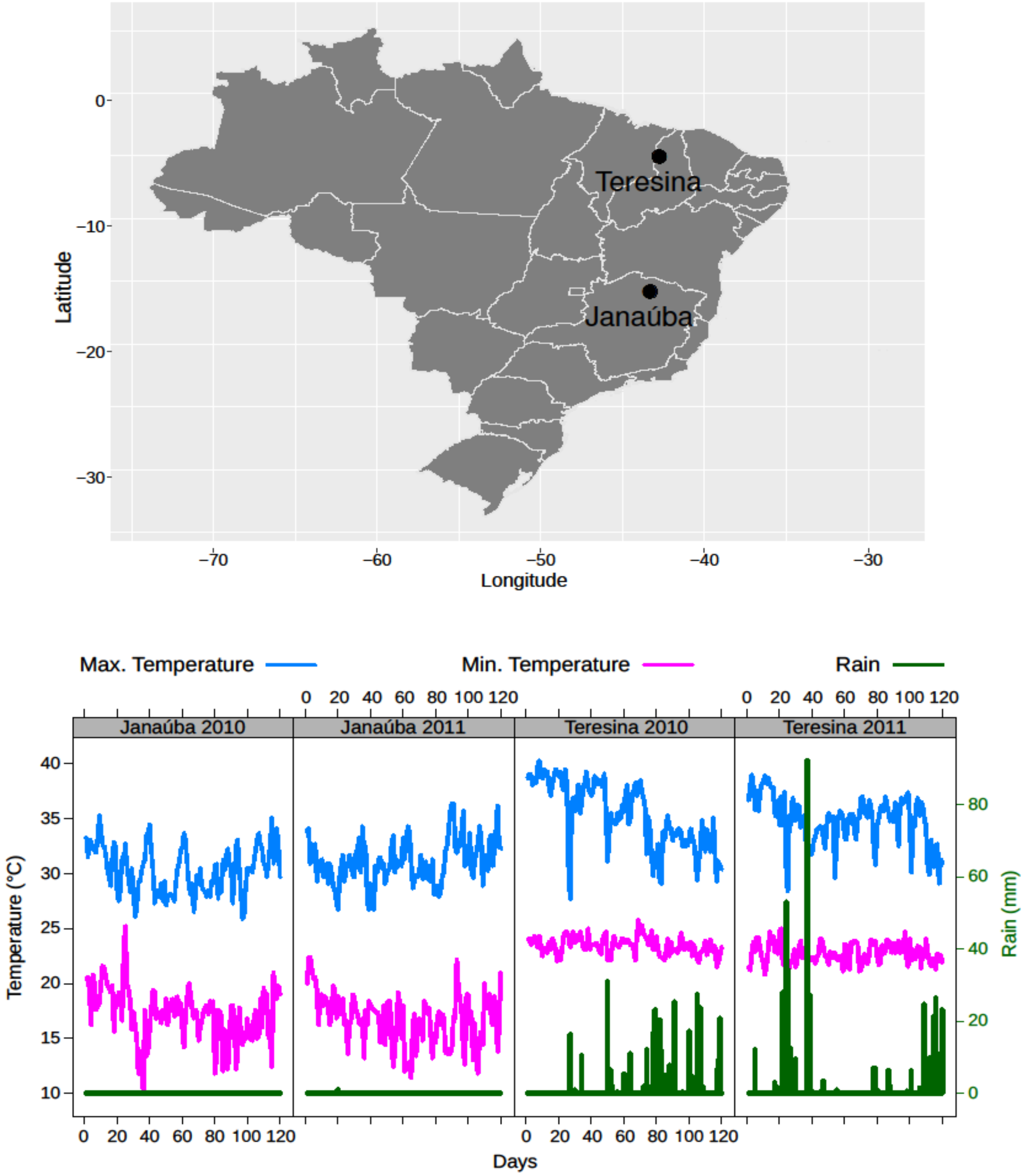

Figure 1. Geographic coordinates of Janaúba (Minas Gerais state, Brazil) and Teresina (Piauí state, Brazil) and rainfall (mm), and minimum and maximum temperatures (°C) collected during the field experiments in 2010 and 2011.

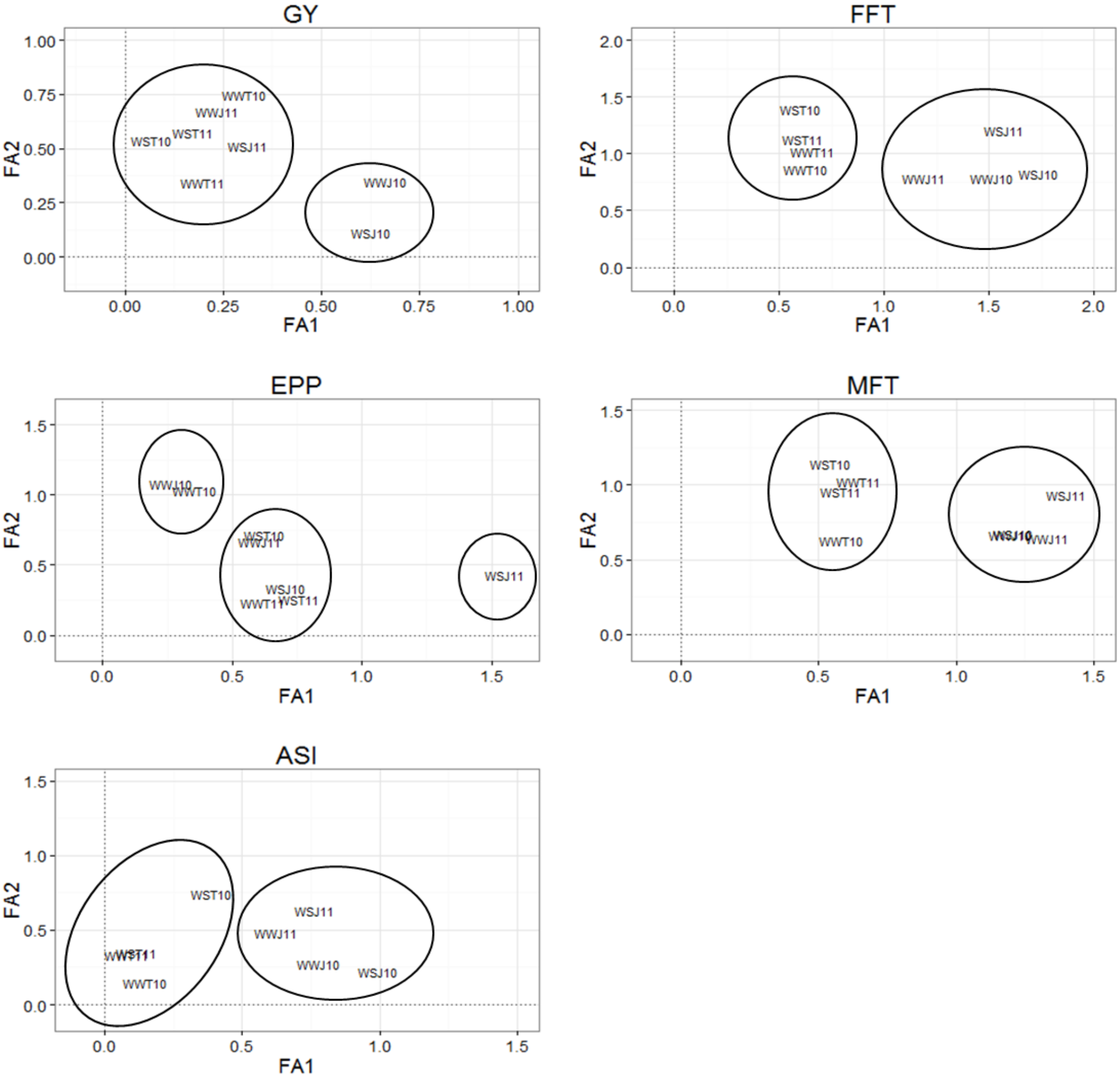

Figure 2. Estimated loadings (after Varimax rotation) of each trial for the first (FA1) and second (FA2) common factors obtained through the multi-environment trial analyses (model [2]) performed jointly for well-watered (WW) and water-stressed (WS) trials conducted in Janaúba (J) and Teresina (T) in 2010 (10) and 2011(11). GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days).

SUPPLEMENTARY INFORMATION

Table S1. Average phenotypic values observed for well-watered (WS) and water-stressed (WS) trials for all measured traits in Janaúba (J) and Teresina (T) for 2010 (10) and 2011(11). Values within parentheses are the standard deviations.

Trait	WSJ10	WSJ11	WST10	WST11	WWJ10	WWJ11	WWT10	WWT11
GY[†]	3.47	3.34	2.86	4.88	6.59	7.11	6.69	5.78
	(1.35)	(1.24)	(1.76)	(1.68)	(1.68)	(1.83)	(1.77)	(2.27)
EPP	12.34	15.04	10.34	17.05	17.86	17.09	16.39	15.15
	(3.86)	(4.07)	(4.57)	(4.18)	(3.72)	(3.67)	(3.54)	(5.13)
ASI	1.07	2.86	4.31	2.56	0.47	0.63	2.36	2.77
	(1.83)	(2.24)	(3.98)	(1.03)	(1.26)	(1.38)	(0.79)	(0.68)
FFT	64.34	70.69	55.12	53.66	64.78	68.36	54.28	52.52
	(2.60)	(3.25)	(5.48)	(2.54)	(3.11)	(2.20)	(2.81)	(2.07)
MFT	63.28	67.84	50.66	51.10	64.30	67.72	51.91	49.75
	(1.91)	(2.35)	(2.68)	(2.27)	(3.09)	(1.96)	(2.68)	(1.87)
Yield reduction (%)	47.34	53.02	57.24	15.57	-	-	-	-

[†] GY: grain yield (t/ha); EPP: number de ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days).

Table S2. Genetic correlations between water-stressed (WS) trials in Janaúba (J) and Teresina (T) for 2010 (10) and 2011(11), estimated through multi-environment trial analyses (model [2]). Values on diagonal correspond to the genetic variances of each trial, and values in parentheses are approximate standard errors.

Trait	Trial	WSJ10	WSJ11	WST10	WST11
GY[†] (73.57%)[‡]	WSJ10	0.38 (0.04)	0.62 (0.05)	0.19 (0.08)	0.41 (0.08)
	WSJ11		0.44 (0.05)	0.44 (0.09)	0.79 (0.06)
	WST10			0.64 (0.11)	0.72 (0.04)
	WST11				0.43 (0.06)
EPP (58.93%)	WSJ10	1.83 (0.18)	0.51 (0.05)	0.17 (0.07)	0.43 (0.08)
	WSJ11		2.52 (0.33)	0.34 (0.18)	0.77 (0.03)
	WST10			3.94 (0.67)	0.52 (0.08)
	WST11				1.09 (0.17)
ASI (49.24%)	WSJ10	1.02 (0.42)	0.67 (0.08)	0.26 (0.10)	0.40 (0.11)
	WSJ11		1.81 (0.52)	0.35 (0.12)	0.57 (0.10)
	WST10			2.83 (0.09)	0.37 (0.08)
	WST11				0.19 (0.07)
FFT (85.88%)	WSJ10	3.61 (0.68)	0.84 (0.04)	0.42 (0.18)	0.81 (0.09)
	WSJ11		5.12 (0.60)	0.54 (0.24)	0.80 (0.02)
	WST10			4.92 (4.09)	0.66 (0.24)
	WST11				1.64 (0.24)
MFT (91.84%)	WSJ10	1.99 (0.23)	0.92 (0.009)	0.68 (0.07)	0.85 (0.03)
	WSJ11		3.05 (0.37)	0.72 (0.07)	0.90 (0.04)
	WST10			2.09 (0.35)	0.88 (0.03)
	WST11				1.20 (0.14)

[†] GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days). [‡] Percentage of the variance explained by two factors.

Table S3. Genetic correlations between well-watered (WW) trials in Janaúba (J) and Teresina (T) for 2010 (10) and 2011(11), estimated through multi-environment trial analyses (model [2]). Values on diagonal correspond to the genetic variances of each trial, and values in parentheses are approximate standard errors.

Trait	Trial	WWJ10	WWJ11	WWT10	WWT11
GY[†] (78.99%)[‡]	WWJ10	0.76 (0.10)	0.77 (0.09)	0.43 (0.07)	0.34 (0.10)
	WWJ11		0.50 (0.11)	0.91 (0.06)	0.25 (0.11)
	WWT10			1.38 (0.16)	0.12 (0.11)
	WWT11				0.81 (0.07)
EPP (87.19%)	WWJ10	1.27 (0.28)	0.54 (0.136)	0.76 (0.072)	0.97 (0.15)
	WWJ11		1.85 (0.59)	0.58 (0.12)	0.70 (0.53)
	WWT10			1.72 (0.33)	0.79 (0.05)
	WWT11				0.16 (0.19)
ASI (97.09%)	WWJ10	0.64 (0.08)	0.94 (0.04)	0.66 (0.08)	0.45 (0.12)
	WWJ11		0.60 (0.11)	0.70 (0.06)	0.68 (0.14)
	WWT10			0.06 (0.01)	0.53 (0.16)
	WWT11				0.15 (0.06)
FFT (86.34%)	WWJ10	3.00 (0.36)	0.78 (0.05)	0.76 (0.02)	0.68 (0.07)
	WWJ11		2.76 (0.63)	0.79 (0.07)	0.59 (0.09)
	WWT10			1.32 (0.22)	0.80 (0.07)
	WWT11				2.08 (0.38)
MFT (84.85%)	WWJ10	2.08 (0.37)	0.76 (0.05)	0.69 (0.11)	0.92 (0.06)
	WWJ11		3.04 (0.30)	0.72 (0.08)	0.81 (0.02)
	WWT10			1.04 (0.26)	0.90 (0.10)
	WWT11				1.17 (0.19)

[†] GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days). [‡] Percentage of the variance explained by two factors.

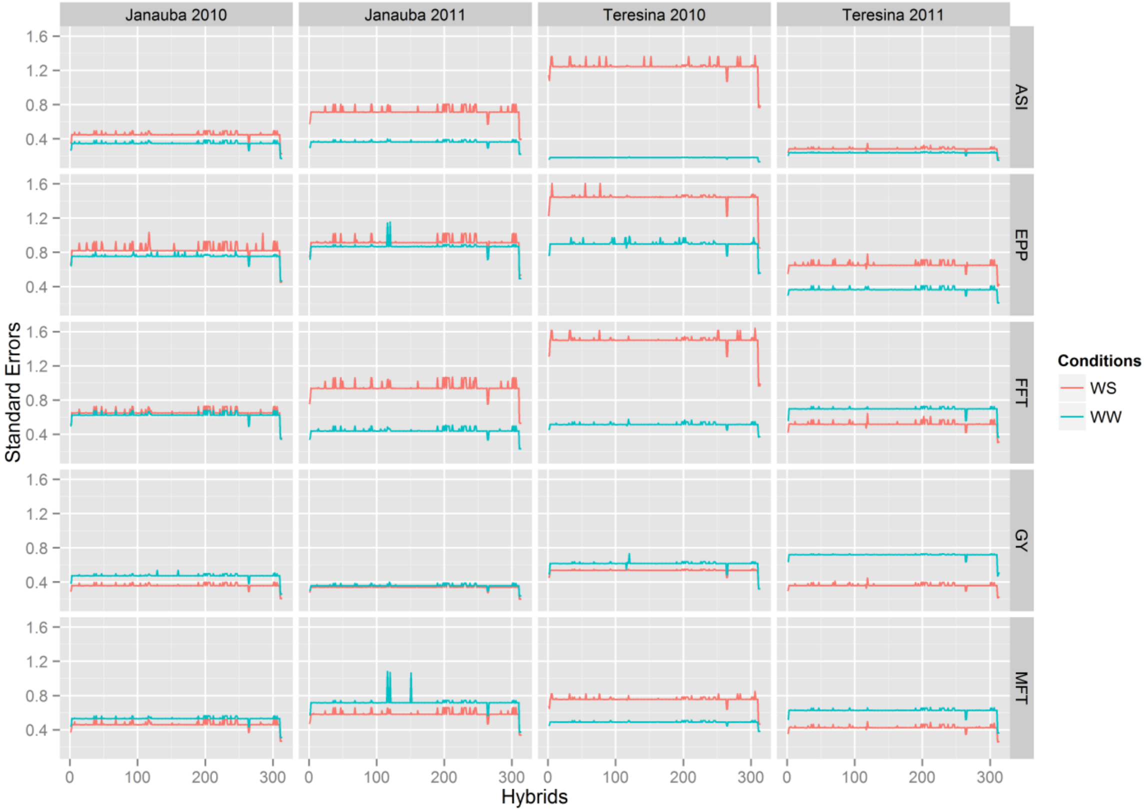

Figure S1. Standard errors associated with the genetic effects of 308 single-cross hybrids and 4 common checks, obtained via best linear unbiased predictor (BLUP) through the multi-environment trials analysis (model [2]) performed separately for well-watered (WW) and water-stressed (WS) trials. GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days).

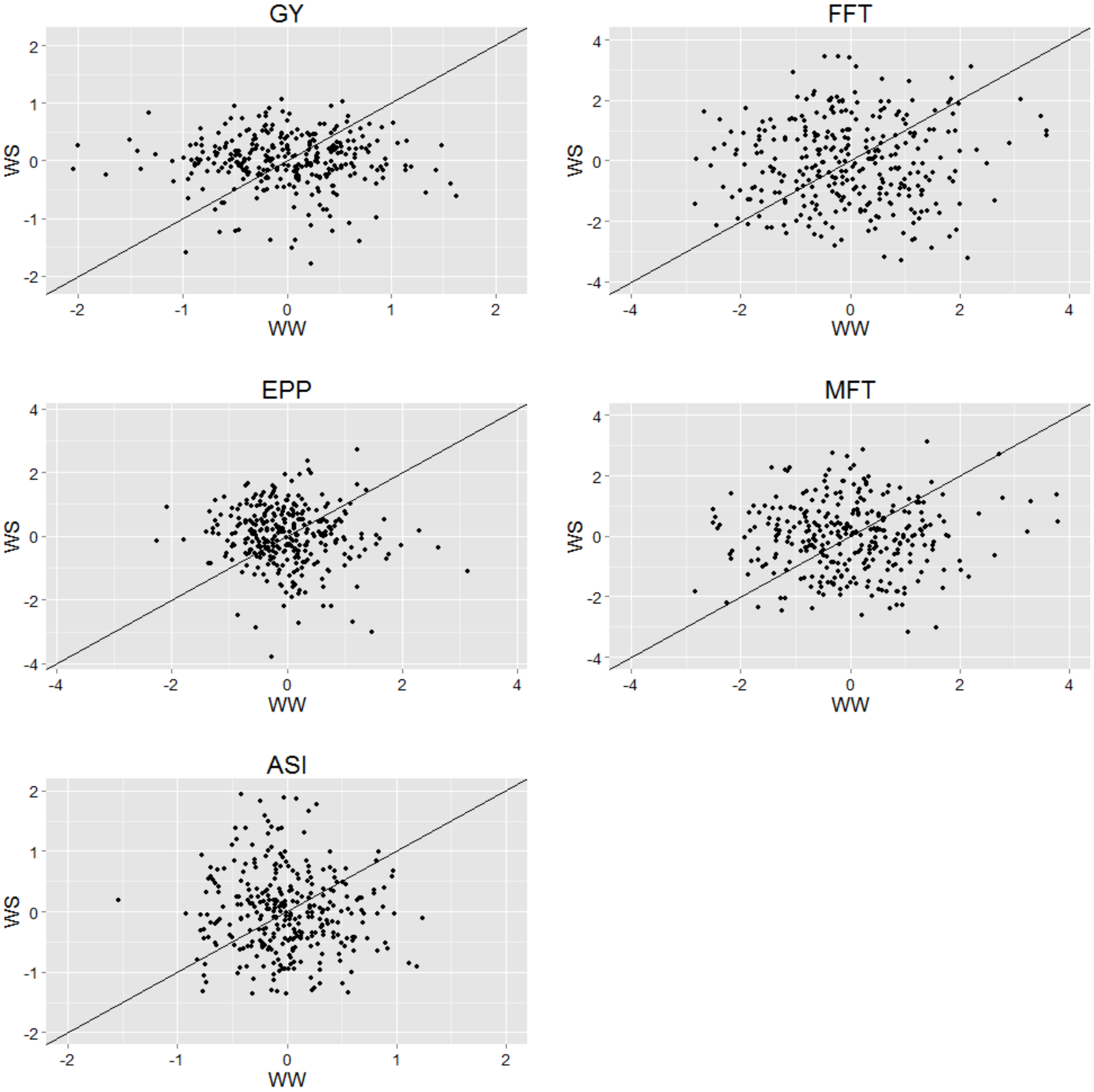

Figure S2. Genetic effects of 308 single-cross hybrids and 4 common checks, obtained via best linear unbiased predictor (BLUP) through the multi-environment trials analysis (model [2]) performed jointly for well-watered (WW) and water-stressed (WS) trials. GY: grain yield (t/ha); EPP: number of ears per plot; ASI: anthesis-silking interval (days); FFT: female flowering time (days); and MFT: male flowering time (days).

CHAPTER III

Improving accuracies of genomic predictions for drought tolerance in maize by joint modeling of additive and dominance effects in multi-environment trials

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ABSTRACT

Due to the difficulties of conventional breeding for drought tolerance, the use of genomic selection could increase the selection efficiency and the genetic gain. The main goal of this study was to evaluate the accuracy of genomic selection to predict the performance of untested maize single-cross hybrids for drought tolerance, using a statistical-genetics model that account for genotype-by-environment interaction, additive and dominance effects. Phenotypic data of five drought tolerance traits were measured in 308 hybrids in eight trials under water-stressed (WS) and well-watered (WW) conditions over two years and two locations in Brazil. Hybrids genotypes were inferred based on their parents genotypes (inbred lines) using SNP (single nucleotide polymorphism) data obtained via GBS (genotyping-by-sequencing). Genomic selection analysis was done using GBLUP (Genomic Best Linear Unbiased Prediction) by fitting a factor analytic multiplicative mixed model. Differences were observed in predictive accuracy between additive models and additive and dominance models for the five traits in both water conditions. However, these differences were more evident under WS conditions. These results contribute to a better understanding about the genetic architecture of important traits evaluated under WW and WS conditions, and highlight the importance of dominance effects for grain yield genomic predictions in maize single-cross hybrids under both water regimes.

INTRODUCTION

An accurate prediction of the performance of untested genotypes in one or more environments is essential to maximize genetic gains in breeding programs (Bernardo, 1994). Traditionally, pedigree-based analyses have been used to analyze field experiments and to estimate genetic parameters and breeding values (Nunes et al., 2008; Piepho et al., 2008). However, due to the decreasing costs of genotyping with thousands or millions of markers and the increasing costs of phenotyping (Krchov and Bernardo 2015), genomic selection (GS, Meuwissen et al., 2001) has been emerged as an alternative molecular-based method to predict yet-to-be seen genetic responses. The use of appropriate GS methods can provide accurate predictions even for untested genotypes, resulting in a considerable progress for breeding programs, due to the reduction in the number of field-tested genotypes, with a consequent reduced phenotyping costs (Krchov and Bernardo 2015). The benefits of GS are more evident when traits are difficult, time consuming and/or expensive to measure, or when several environments need to be evaluated.

In breeding programs for drought tolerance, genotypes are evaluated under water-managed environments (such as well-watered and water-stressed conditions), in which an effective phenotypic screening for several traits is often laborious and time-consuming. Thus, the release of new cultivars with yield stability over areas that are prone to water limitations is often considered a critical and challenge task (Cooper et al., 2014). In general, most of the drought tolerance related-traits are controlled by many genes of small effects and, as expected, are strongly influenced by the environment (Ribault et al., 2009; Zhang et al., 2015). Thus, an increased genetic gain is expected with GS, once it is possible to perform early predictions of the genetic values (untested genotypes) in a breeding program and, consequently, more than one selection cycle can be carried out per year for drought related traits, accelerating genetic gains. Comparisons between

GS and selection based on secondary traits were made by Ziyomo and Bernardo (2013), showing the advantage of GS to increase genetic gains. Furthermore, recently, Beyene et al. (2015) and Zhang et al. (2015) showed promising results for the application of GS for drought tolerance in maize.

Among many GS approaches, genomic best linear unbiased prediction (GBLUP) has shown high levels of predictive accuracy, ranking often among the best predictive models (for further details see Heslot al., 2012 and Resende et al., 2012). GBLUP considers an observed genomic relationship matrix obtained from marker information (VanRanden, 2008), instead of a pedigree-based relationship matrix (Henderson, 1984). The flexibility of GBLUP, in comparison to other GS methods, allows fitting complex linear mixed models, such as the ones applied to multi-environment trials (MET) and multi-trait analysis (Meyer, 2009; Smith et al., 2015). Moreover, its use often results in reduced computing time and a better exploration of all information available.

Most genetic analyses focus on the estimation of additive or total genetic effects. However, the estimation of both additive and non-additive effects (dominance and epistasis) helps to improve the understanding about the genetic architecture of target traits and to define optimal breeding strategies. Nevertheless, the estimation of these effects, and their corresponding variance components, are often difficult, requiring appropriate mating designs and large number of observations, due to the lack of orthogonality that occurs in the estimation process. However, some studies have shown that the orthogonality and predictability of both additive and non-additive effects are greatly improved by the use of molecular-based relationship matrices (Muñoz et al., 2014; Nazarian and Gezan 2016b). Orthogonal partitioning of genetic variance through molecular markers or pedigree is one important step to accurately estimate additive and non-additive effects, and depends on, among other things, on the distribution of allele frequencies (Hill et al., 2008) and the correct parameterization for genomic relationship matrices (Vitezica et al., 2013; Da et al., 2014; Zhu et al., 2015). Although some studies have shown contribution of dominance effects to predictive accuracy (Da et al., 2014; Bouvet et al., 2015), most GS models have been limited to the use of additive effects.

Models that evaluate genotype-by-environment interaction (GxE) are critical to any plant breeding program regardless the method used for genomic prediction. Understanding GxE provides valuable information for breeders including, among others: i) evaluation of stability of genotype's response across environments; ii) selection of genotypes to specific environments; iii) evaluation/definition of breeding zones, iv) definition of target environments; and v) definition of strategies to maximize genetic gain. Recent studies have shown advantages of GS models that incorporate GxE (Burgueño et al., 2012; Heslot et al., 2014; Jarquín et al., 2014; Lopez-Cruz et al., 2015) for quantitative traits; however, these studies were limited to the incorporation of only additive effects.

Several modelling approaches exist to explore GxE. The most interesting ones consider modelling the genetic variance-covariance matrix across environments. Thus, with this matrix structure it is possible to better understand GxE and the genetic architecture of breeding traits, together with the estimation of all environment-to-environment genetic correlations. One parsimonious way to model this genetic variance-covariance matrix is by using a factor analytic (FA) structure (Piepho, 1997, 1998; Smith et al., 2001). The FA variance-covariance structure is an approximation to the unstructured (UN) matrix but with a reduction in the number of parameters to be estimated, something that is particularly relevant when the number of environments is large (e.g. greater than 5). Many studies have shown that FA models are good approximations of the unstructured models and that they can be easily implemented in most breeding programs (Kelly et al., 2007; Burgueño et al., 2008; Cullis et al., 2014; Smith et al., 2015). Another advantage of the FA model is that it can be extended to estimate additive and non-additive effects simultaneously (Kelly et al., 2009).

Applications of GS have been limited to the estimation of additive effects, although few research had applied GS for additive and dominance models (Azevedo et al., 2015; Bouvet et al., 2015), for GxE (Burgueño et al., 2012; Lopez-Cruz et al., 2015) and also for drought tolerance in maize (Ziyomo and Bernardo, 2013; Zhang et al., 2015; Beyene et al., 2015). However, these implementations do not fit complex linear models that incorporate GxE, additive and dominance effects simultaneously. So far, it is not clear whether the use of additive and dominance effects can increase the accuracy of genomic predictions in drought tolerance breeding programs.

Therefore, the goals of this study were: i) to evaluate the predictive accuracy of single-cross maize hybrids for drought-tolerance related traits under two different water conditions, using a high-density SNP marker panel and multi-environmental trials analyses; ii) to compare the predictive accuracy achieved by models that account for additive (A) *vs.* additive and dominance (AD) effects; iii) to investigate the partition of the genetic variance based on a high-density marker panel for drought tolerance related traits; iv) to explore the stability of hybrids using latent regression plots in AD models; and v) to investigate the impact in the predictive accuracy of separating the training set for each water conditions (well-watered and water-stressed) *vs.* combing both water conditions in a single training set.

MATERIAL AND METHODS

Phenotypic data

Field data comprise 308 single-cross maize hybrids evaluated in drought tolerance experiments for two water conditions, well-watered and water-stressed, at two locations in Brazil (Janaúba, Minas Gerais State, and Teresina, Piauí State) over two years (2010 and 2011), giving a total of 8 trials. Each trial comprised the 308 maize single-cross hybrids randomly split into 6 sets with 80, 78, 60, 60, 15 and 15 hybrids. In the field, each set was augmented by four common checks (commercial maize cultivars), and was arranged as a randomized complete block design. Although the hybrids within each set were kept the same across trials, hybrids and checks were randomly allocated to groups of plots within each set, and this allocation was different between replicates of sets and between trials.

Five drought tolerance traits were evaluated: grain yield (GY, ton/ha); number of ears per plot (EPP); female and male flowering time (FFT and MFT, both in days), measured as the number of days from sowing up to the time when silks have emerged on 50% of the plants, and 50% of the plants have begun to shed pollen, respectively; and anthesis-silking interval (ASI, days), which corresponds to the time between FFT and MFT. Further details about the experimental design and field trials can be found in Chapter II.

Genotypic data

Genomic DNA was extracted from young leaves based on the CTAB method (Saghai et al., 1984). DNA samples were quantified using the Fluorometer Qubit® 2.0 with compatible quantification reagents, following the manufacturer´s instructions (Life Technologies^{TM}). DNA samples were also evaluated on 1% agarose gel in TAE (Tris-acetate-EDTA) buffer stained with GelRed^{TM} (Biotium) and recorded under white light in the Imager Gel Doc L-PIX (Loccus Biotecnologia). Genotyping-by-sequencing (GBS) was carried out by the Genomic Diversity Facility at Cornell University (Ithaca, NY, USA), using the standard GBS protocol (Elshire et al. 2011), with restriction enzyme *Ape*KI and 96 samples per sequencing lane. Burrows-Wheeler alignment (BWA) tool (Li and Durbin 2009) was used to align tags to the B73 reference genome (RefGen_v3). Then, single nucleotide polymorphisms (SNP) were called using the GBS pipeline

(Glaubitz et al. 2014), available in the software TASSEL v.4 (Bradbury et al. 2007). SNP were obtained for the 188 inbred lines and the two testers (two inbred lines of the total 190) used as parents of the 308 maize hybrids. A particular SNP marker was discarded if: (i) its minor allele frequency was smaller than 5%; (ii) it had more than 20% of missing genotypes; and/or (iii) it had more than 5% of heterozygous genotypes. After filtering, missing genotypes were imputed using NPUTE (Roberts et al. 2007). Then, for each SNP, the genotype of each hybrid was inferred based on the genotype of its parents (inbred line and tester). The number of SPN per chromosome ranged from 7.638 (chromosome 1) to 3.086 (chromosome 10), with a total of 46,603 markers.

Genomic relationship matrices

Genetic relationships between hybrids were constructed based on the information from SNP markers. Additive ($\mathbf{A_g}$) and dominance ($\mathbf{D_g}$) genomic relationship matrices were calculated following the methods described by Yang et al. (2010) and Vitezica et al. (2013), respectively. Both methods consider two alleles (A and a) for a given kth marker locus. Then, $\mathbf{A_g}$ was estimated through the following equation:

$$\mathbf{A_g} = \mathbf{W_A W_A^T} \Big/ \left[\tfrac{1}{m} \sum_k 2p_k(1\text{-}p_k) \right]$$

where

$$\mathbf{W_A W_A^T} = \begin{cases} \tfrac{1}{m} \sum_k (g_{ki} - 2p_k)(g_{kj} - 2p_k) & \text{if } i \neq j \\ \tfrac{1}{m} \sum_k 2p_k(1\text{-}p_k) + \tfrac{1}{m} \sum_k \left[g_{ki}^2 - (1 + 2p_k) + 2p_k^2 \right] & \text{if } i = j \end{cases}$$

in which m is the total number of markers, p_k is the observed allele frequency of the kth SNP, g_{ki} and g_{kj} represent the number of copies of a given allele A for individuals i and j at SNP k, assuming values 2, 1 and 0 for the SNP genotypes AA, Aa and aa, respectively. The denominator is the sum of the SNP variances. **D_g** was calculated as following:

$$\mathbf{D_g} = \mathbf{W_D}\mathbf{W_D^T} \Big/ \left\{ \tfrac{1}{m} \sum_k 4[p_k(1\text{-}p_k)]^2 \right\}$$

$$\text{where } \mathbf{W_D} = \begin{cases} -2p_k^2 - 2p_k^2 & \text{if SNP} = \text{AA} \\ 2p_k(1\text{-}p_k) - 2p_k^2 & \text{if SNP} = \text{Aa} \\ (1\text{-}p_k)^2 - 2p_k^2 & \text{if SNP} = \text{aa} \end{cases}$$

in which all terms were previously defined. Additive and dominance genomic relationship matrices were estimated using the software GenoMatrix (Nazarian and Gezan, 2016a), which also produces the inverse of the genomic relationship matrices as output. Thus, if $\mathbf{A_g}$ and $\mathbf{D_g}$ were not positive definite, their inverse was obtained by iterative bending methods (Schaeffer et al., 1989).

Dependence between additive and dominance variances were evaluated as described by Muñoz et al. (2014). For this, the portions of the asymptotic variance-covariance matrices due to the additive and dominance components were used to evaluate the non-independence among variance components by calculating and plotting the eigenvalues of the corresponding correlation matrix.

Genomic selection analyses

Genomic best linear prediction (GBLUP) for additive+dominance effects in multi-environment trials were performed as describe below.

Multi-environment trial analyses

Several multi-environment trial analyses, combining different groups of trials, were carried out. Three different groups were defined: (i) all four trials under WW conditions; (ii) all four trials under WS conditions; and (iii) all eight trials under both WW and WS conditions. The following generic linear mixed model was fitted for each trait and group:

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{X_1 s} + \mathbf{X_2 b.s} + \mathbf{Z_1 r.s} + \mathbf{Z_2 a.s} + \mathbf{Z_3 d.s} + \mathbf{e} \qquad [1]$$

where $\mathbf{y}$ is the vector of phenotypes; μ is the overall mean; $\mathbf{s}$ is the vector of fixed effects of trials; $\mathbf{b.s}$ is the vector of fixed effects of sets within trials; $\mathbf{r.s}$ is the vector of random effects of replicates within sets within trials, with $\mathbf{r.s} \sim \text{MVN}(\mathbf{0}, \mathbf{D_{r.s}})$; $\mathbf{a.s}$ is the vector of random additive effects of hybrids within trials, with $\mathbf{a.s} \sim \text{MVN}(\mathbf{0}, \mathbf{A_g} \otimes \mathbf{G_{SA}})$; $\mathbf{d.s}$ is the vector of random dominance effects of hybrids within trials, with $\mathbf{d.s} \sim \text{MVN}(\mathbf{0}, \mathbf{D_g} \otimes \mathbf{G_{SD}})$; and $\mathbf{e}$ is the vector of residuals, with $\mathbf{e} \sim \text{MVN}(\mathbf{0}, \mathbf{R_e})$. $\mathbf{G_{SA}}$ and $\mathbf{G_{SD}}$ are variance-covariance (VCOV) matrices for the additive and dominance genetic effects of hybrids, across trials, with dimensions 4x4 for the group of trials under WW or WS conditions, and 8x8 considering all trials. An FA model of order k (FA$_k$) was considered for the $\mathbf{G_{SA}}$ and $\mathbf{G_{SD}}$ structure, in which k is the number of multiplicative components. $\mathbf{D_{r.s}}$ and $\mathbf{R_e}$ are diagonal VCOV matrices, where each trial has a different and independent variance component for the replicates within sets and for the residuals, respectively. The Kronecker product is denoted by $\otimes$. The additive model is a particular case of the model [1], in which there is no inclusion of dominance effects.

Under the above model, narrow-sense heritability (h^2) and the proportion of the variance explained by the dominance effects (δ^2) were estimated for each trait using the following expressions: $h^2 = \bar{\sigma}^2_{a.s} \Big/ \left(\bar{\sigma}^2_{a.s} + \bar{\sigma}^2_{d.s} + \bar{\sigma}^2_{r.s} + \bar{\sigma}^2_{e}\right)$ and $\delta^2 = \bar{\sigma}^2_{d.s} \Big/ \left(\bar{\sigma}^2_{a.s} + \bar{\sigma}^2_{d.s} + \bar{\sigma}^2_{r.s} + \bar{\sigma}^2_{e}\right)$, where the bars over the variance components represent the average variance (i.e.

diagonal terms) across all trials. Broad-sense heritability was estimated as $H^2 = h^2 + \delta^2$. Standard errors for h^2, δ^2 and H^2 were estimated through the Delta method (Kendall and Stuart, 1963), implemented in the package *nadiv* (Wolak, 2012) available in R v3.2.5 (R Core Team 2016), which estimates approximated standard errors of the variance components.

For MET analysis, considering additive and dominance effects, a FA structure was fitted as described in Oakey et al. (2007) and Kelly et al. (2009), in which the vector of genetic effects ($\mathbf{u_g}$), including both additive and dominance effects, is defined as:

$$\mathbf{u_g} = \mathbf{u_A} + \mathbf{u_D} \qquad [2]$$

where $\mathbf{u_A}$ and $\mathbf{u_D}$ are random vectors of additive and dominance effects within *s* trials, respectively. These vectors are assumed to be independent with a multivariate normal distribution with zero mean and VCOV matrices $\mathbf{G_A}$ and $\mathbf{G_D}$, respectively, where $\mathbf{G_A} = \mathbf{A_g} \otimes \mathbf{G_{SA}}$ and $\mathbf{G_D} = \mathbf{D_g} \otimes \mathbf{G_{SD}}$. Here, $\mathbf{G_{SA}}$ and $\mathbf{G_{SD}}$ are $s\,x\,s$ VCOV matrices for the additive and dominance genetic effects. The structure of $\mathbf{G_{SA}}$ and $\mathbf{G_{SD}}$ matrices were defined based on an FA$_k$ (i.e. of order *k*) model as:

$$\mathbf{G_{SA}} = (\mathbf{\Delta_A \Delta_A^T} + \boldsymbol{\psi}_\mathbf{A}) \qquad [3]$$

$$\mathbf{G_{SD}} = (\mathbf{\Delta_D \Delta_D^T} + \boldsymbol{\psi}_\mathbf{D}) \qquad [4]$$

where, $\mathbf{\Delta_A} = \{\lambda_A\}$ and $\mathbf{\Delta_D} = \{\lambda_D\}$ are $s\,x\,k$ matrices of factor loadings (common factors) for the additive and dominance effects, respectively, for s trials; and $\boldsymbol{\psi}_\mathbf{A}$ and $\boldsymbol{\psi}_\mathbf{D}$ (diagonal matrices of dimension $s\,x\,s$) are specific factors for the additive and dominance effects, respectively. Common and specific factors are assumed to be independent and normally distributed. Hence, under the above definitions $\mathrm{cov}(\mathbf{u_g})$ can be obtained as:

$$\begin{aligned}\mathrm{cov}(\mathbf{u_g}) &= \mathbf{A_g} \otimes \mathbf{G_{SA}} + \mathbf{D_g} \otimes \mathbf{G_{SD}} \\ &= \mathbf{A_g} \otimes (\mathbf{\Delta_A \Delta_A^T} + \boldsymbol{\psi}_\mathbf{A}) + \mathbf{D_g} \otimes (\mathbf{\Delta_D \Delta_D^T} + \boldsymbol{\psi}_\mathbf{D})\end{aligned} \qquad [5]$$

where all terms were previously defined.

Under a FA$_k$ structure, adaptability and stability of genetic effects can be easily assessed using latent regression plots (Cullis et al., 2014; Smith et al., 2015). These plots show the genetic responses to each environment considering the genetic effects, i.e. predicted breeding values from marginal prediction as shown in Cullis et al. (2014), as dependent variables against the independent variables, the rotated estimated factor loadings ($\lambda_{rs,r=1,2...k}$). Here we extended the regression plots proposed by Cullis et al. (2014) for additive effects, to dominance effects as well. Plots for the first and second factor were obtained using the following equations:

plot FA$_1$: $\mathbf{y_j} = \mathbf{u_{is}}$ against $\mathbf{x_j} = \boldsymbol{\lambda}^*_{\mathbf{1s}}$

plot FA$_2$: $\mathbf{y_j} = \mathbf{u_{is}} - \boldsymbol{\lambda}^*_{\mathbf{1s}}\, x\, \mathbf{f}^*_{\mathbf{1s}}$ against $\mathbf{x_j} = \boldsymbol{\lambda}^*_{\mathbf{2s}}$

where $\mathbf{u_{is}}$ is the vector of breeding values or dominance deviations of hybrid *i* within trial s, **f** is a vector of factors scores, and * denotes the vectors after rotation. The important difference here is that plot FA$_1$ and FA$_2$ were constructed for additive and dominance effects separately. Therefore, patterns of additive and dominance effects in terms of adaptability and stability can be easily identified. The rotation for factor loading ($\boldsymbol{\lambda}$) and factor scores was used in order to facilitate and simplify the results of FA models using Varimax rotation.

All five traits and all linear mixed models were fitted using the statistical package ASReml-R v. 3 (Buttler et al., 2009) that estimates variance components using restricted maximum likelihood (REML) through the average information (AI) algorithm (Gilmour et al., 1995), followed by estimation of fixed and random effects by solving the mixed model equations. Diagnostic plots were used to verify the outliers and normality of the residuals. For GY and EPP, the number of plants per plot was used as a covariate. Model comparisons were done using the Akaike information criterion (AIC, Akaike, 1974).

Cross-validation scheme

Predictive accuracy was calculated as the correlation between the phenotype (obtained from single-environment trial analysis without molecular markers and considering hybrids as fixed effects) and the predicted genotypic effects (additive or additive+dominance). A 10-fold cross-validation procedure was implemented to calculate the predictive accuracies in which the total set of hybrids was randomly split into 10 groups. The cross-validation procedure was performed separately for each trait using the MET model [1], where hybrids in the validation set were considered as not evaluated in any other environments (trials). Two different scenarios for the cross-validation scheme were implemented to evaluate the predictive accuracy of the measured traits: i) the training and validation procedures were performed separately in well-watered or water-stressed trials (CV1), and ii) all trials, combing both watering regimes, were used to predict the performance of single-cross hybrids under well-watered and water-stressed conditions (CV2).

RESULTS

Estimates of genetic parameters using high-density SNP markers

Broad and narrow sense heritabilities varied considerably between WW and WS conditions (Table 1) for MET analysis (model [1]). For GY, ASI and FFT h^2 were lower in WS conditions. Higher values of heritabilities were found for FFT and MFT in both conditions. ASI showed h^2 under WW conditions almost 2 times greater than under WS conditions. Similar trends were found to broad sense heritabilities (H^2) for ASI, with values of 0.339 and 0.521 under WS and WW conditions, respectively. Models including dominance effects exhibited a decrease in the additive variance component, and consequently in the narrow sense heritability (h^2) (Table 1). For example, FFT under WS conditions showed a decrease of almost 45% in the h^2. Only for EPP under the WW condition, the h^2 were almost the same for A and AD models. Similar results were found for single-environment trial analysis (Table S1-S5).

For almost all traits, except GY, the proportion of the genetic variance explained by the dominance effects was smaller under WW than in WS conditions (Table 1). ASI and EPP had dominance variances under WS almost 44% and 32%, respectively, higher than in WW conditions. The ratio VA/VD ranged from 1.36 (GY) to 4.69 (MFT) under WS conditions, and from 1.36 (GY) to 7.98 (ASI) under WW conditions. MFT showed smaller proportions of dominance effects in the genetic variance for both conditions, compared to other traits.

The dependency between additive and dominance genomic relationship matrices for GY are presented in Figure 1. A small dependency between $\mathbf{A_g}$ and $\mathbf{D_g}$ can be observed when compared with a hypothetical situation of orthogonality (diagonal line, Figure 1). Similar trends were observed for the other traits (Figure S1). However, in WWJ11 and WWT11 trial, where unreplicated trials were used to evaluate hybrids under WW conditions, the dependency between $\mathbf{A_g}$ and $\mathbf{D_g}$ was higher for the secondary traits (ASI, EPP, FFT and MFT). Probably, it is due to the smaller experimental precision, which can be inferred by the square root of the broad sense heritability, observed for these trials. Moreover, the AIC criterion showed that the inclusion of dominance effects in general improved the model fitted compared to model A, being the AD the best model in all the cases (Table 1).

Accuracy of MET-GS models for drought tolerance related traits

Predictive accuracy was different across WW and WS conditions (CV1, Table 2). In general, under WW conditions, the predictive accuracy was higher, ranging from 0.391 (GY) to 0.688 (FFT) and from 0.474 (GY) to 0.723 (FFT) for models A and AD, respectively (CV1, Table 2). For GY, an increase of 17.5% and 51.8% in the predictive accuracy were observed when the dominance effects were included in the GS model under WW and WS conditions, respectively. FFT and EPP under WS showed an increase of 19.67% and 9.56%, respectively, when the dominance effects were accounted. However, ASI and MFT under WS conditions, and ASI, MFT, FFT and EPP under WW conditions, do not exhibited much increase (lower than 5%) with the inclusion of dominance effects in the MET-GS model.

Differences in the predictive accuracies were observed between GY and the secondary traits (CV1, Table 2). For all secondary traits, models AD under WW conditions resulted in a predictive accuracy higher than 0.64, which was at least 25% higher than the predictive accuracy observed for GY. On the other hand, under WS conditions, the differences between the predictive accuracies obtained for secondary traits and GY were smaller than 10% for models AD, whereas for models including solely additive effects those differences were more evident, almost two times greater than the predictive accuracy observed for GY (CV1, Table 2).

An increase in the predictive accuracy was observed when the hybrids performance was predicted in both conditions simultaneously (CV2, Table 2). In general, higher predictive accuracies were observed under WW compared to WS conditions, considering models A or AD, except for GY that exhibited a higher predictive accuracy under WS conditions when using the model AD. The benefits of considering both conditions simultaneously to predict hybrids performance were more evident under WS conditions, with predictive accuracies ranging from 0.49 (EPP) to 0.63 (MFT). For GY, under WS conditions, the predictive accuracy increased up to 15% when using model AD. However, for EPP, the use of model AD resulted in a little decrease in the predictive accuracies in both WW and WS conditions. FFT and MFT showed little increments when using model AD under WW conditions.

GxE interaction model for additive and additive+dominance effects

Additive and dominance genetic correlations estimated through FA models are shown in Figure 2. The additive genetic correlations ranged from 0.41 (WS_GY) to 0.83 (WS_MFT) and the dominance genetic correlations ranged from 0.08 (WS_ASI) to 0.69 (WS_MFT). Based on the additive and dominance genetic correlations across environments, it is possible to observe that the dominance effects exhibited a more evident interaction with environments, except for WS_EPP and WS_GY. The additive effects showed low levels of interaction with environments for MFT in both water conditions, with correlations greater than 0.75. However, the dominance genetic correlations, under WW for EPP, ASI, FFT and MFT, and under WS for ASI showed values of low magnitude or equal to zero (Table 1).

Stability responses of some superior hybrids are shown in (Table 3). Values close to the origin (FA1 and FA2 close to zero) indicate a stable performance of a given hybrid across WW and WS conditions. The stability for best hybrids varied between additive and dominant effects. For example, the hybrid 11 showed only stable additive effects, whereas the hybrid 211 exhibited only stable dominance effects. Figures 3 show the latent regression plots, for the first and second factors, for the additive and dominance effects. For hybrid 211, as suggested by the latent regression, the additive effects increased for trials with high estimated loadings (Figure 3A). Another example, for hybrid 11, the dominance effects increased for trials with high estimated loadings (Figure 3B).

DISCUSSION

To increase genomic predictions for untested hybrids in maize is a recurrent challenge for the successful application of genomic selection in breeding programs. This task requires the use of models that account for multi-environment data, as well as non-additive effects. Our results, based on tropical maize germplasm cultivated in Brazil, showed that it is possible to achiev high levels of predictive accuracy for untested hybrids for drought tolerance related traits through including GxE, additive and dominance effects into the genomic selection models.

Partition of the genetic variance through SNP markers

Orthogonal partitions of the genetic variance were found by genomic relationship matrices (Figure 1). Partitions of genetic variance into additive and dominance effects contribute to a better understanding about the genetic architecture of target traits. In maize, for instance, the knowledge about the genetic architecture help breeders to decide if target traits should be evaluated into inbred lines or hybrids. Genomic relationship matrices used in this study were based on the theory of quantitative genetics, where the covariance between additive and dominance effects is zero. This parameterization has been used in plants (Bouvet et al., 2015), humans (Zhu et al., 2015) and also in simulations studies (Da et al., 2014; Nazarian and Gezan, 2016b) and showed reasonable orthogonal partition of additive and non-additive effects. These studies also highlighted the importance of this correct partition of genetic variance to better understand the genetic architecture of traits though GBLUP.

Narrow sense heritabilities decreased when dominance effects were included into the GS models (Table 1). These decreases on narrow sense heritabilities were probably due to the alleles frequencies distributions (Hill et al., 2008). In this case, when allele frequencies are distributed towards extreme values, even in the presence of non-additive effects, part of these effects can be estimate as additive variance (Hill et al., 2008). Our findings that narrow sense heritabilities decreased when dominance effects were included in the models, corroborates the findings of Muñoz et al. (2014) and Bouvet et al. (2015) in pine and eucalyptus, respectively. However, to our knowledge, our study was the first one to incorporate GxE, additive and dominance effects into the genomic prediction models for drought tolerance traits in maize.

Even with a small dependency between additive and dominance effects as showed here, the additive variance can have part of dominance effects, which highlight the importance of the alleles frequencies distribution. Therefore, even when there is no difference between A and AD models for the predictive accuracy for some traits, such as MFT, it is important to use AD models to estimate genetic parameters because these models can split better the composition of the genetic variance. Mainly, when working with a species that exhibits high heterosis, and hybrids are the main type of cultivar, like maize. The presence of dominance into the models is important to have a better and more realistic partitioning of the genetic variance. Thus, the narrow sense heritability can be overestimated if just an additive model is considered and the genetic gain may be overestimated as well.

Accuracy of GS models for drought tolerance traits

Under WS conditions the differences in predictive accuracy between A and AD models were more evident (Table 2). As an example, under WS the AD model for GY had the predictive accuracy two times bigger than A model. However, under WW, for ASI, EPP, FFT and MFT, A and AD models had similar results. There are two possible explanations for these results. First, these traits had a higher heritability under WW conditions than under WS conditions. Da et al. (2014) showed that AD models tend to be less efficient when the heritabilities of the traits are high. Second, these traits with small differences in the predictive accuracy between A and AD models exhibited a smaller contribution of the dominance effects in the genetic variance compared to the additive effects. Recently Almeida filho et al. (2016) showed via simulation that the AD models were significantly better than A models when the ratio between the dominance variance and the total phenotypic variance reached 0.2. Thus, differences between A and AD models under WW were small for these traits probably due to the high heritability and the small contribution of the dominance effects to the genetic variance.

Predictive accuracy increased when all trials were used in a training set (CV2, Table 2). This result is probably due to the use of a one-stage model analysis, which accounted for the genetic correlations across trials. For GY, the AD model resulted in an increment of 18% in the prediction accuracy under WS conditions. This result showed that the prediction accuracy of untested single-cross hybrids can be increased when WW and WS trials are used together for the training data set. Aeron et al. (2012) showed that to increase the size of training sets using unrelated individuals does not always result in a higher predictive accuracy. In our study, although the number of individuals in the training set was kept the same, combining the information of WW and WS trials improved the prediction accuracy under WW and WS conditions.

The genetic architecture of drought tolerance traits affects the prediction accuracy (Table 2). Secondary traits, such as ASI and FFT had higher prediction accuracies than GY in both water conditions. Selection for secondary traits for drought tolerance has been reported as an interesting way to increase genetic gains for GY (Oyekunle et al., 2015). However, Zyomo and Bernardo (2013) showed that GS is more efficient to increase genetic gains for GY than indirect selection for secondary traits on temperate maize. Comparisons between GS and phenotypic selection for drought tolerance in biparental maize populations showed that after three cycles of selection GS achieved greater genetic gains (Beyene et al., 2015). The results found in our study, suggest that it is possible to enhance the predictive accuracy of untested hybrids for drought tolerance traits, such as GY, FFT and EPP, by using AD models.

GxE interaction model for additive and additive+dominance effects

The incorporation of GxE in multi-environment trial analysis, provide valuable information about stability of hybrids under WW and WS conditions. Therefore, appropriate statistical model that account for correlations across trials and deal with unbalanced data needs to be used. Explicit models that have a main genetic effect and a genotype-by-environment interaction effect, have the same results that implicit models (the effect of genotypes within environments) with compound symmetry structure for the variance and covariance matrices (VCOV), which means that explicit models consider the same variance and covariance across trials (Smith et al., 2011; Smith et al., 2015). Implicit models have some advantages: allow to consider different structures for the VCOV between trials (i.e unstructured or FA models). Burgueño et al. (2011) showed that FA models exhibited an advantage of up to 6% in the prediction accuracy over the models that considered the same variance and correlation across environments. Recently, Lopez-Cruz et al. (2015) showed that GS models that account for GxE resulted in greater prediction accuracy than models without GxE. For drought tolerance, Zhang et al. (2015) showed that the prediction accuracy was increased when using a model that incorporates GxE for GY. It is known that there are high levels of GxE for most of the traits faced by breeders, which increases the importance of models that can deal with MET data.

Although previous studies have applied genomic selection for drought tolerance in maize (Zyomo and Bernardo, 2013; Beyene et al., 2015; Zhang et al., 2015), they did not take the advantage of using models that can deal with GxE and account for additive and dominance effects simultaneously. Our results emphasize the importance of dominance effects for traits such as FFT, EPP and GY under drought conditions. Also, our study contributes to a better understanding about the genetic architecture of these traits based on models that represent the reality of breeding programs, where genotypes are unbalanced and measured in multi-environment trials.

Over recent years, many statistical models have been proposed for the application of GS in plant and animal breeding. Azevedo et al. (2015) compared different statistical methods for GS, incorporating additive and dominance effects, and concluded that GBLUP was between the best methods and provided an accurate prediction for the breeding values, as well as, for the additive and dominance effects. Factor analytic multiplicative mixed models showed as an efficient way to perform MET analysis including additive and dominance effects for GS through GBLUP.

Based on FA models with additive and dominance effects, latent regression plots were done to understand GxE (Figure 3). Using these plots, inferences about the stability of the breeding values and the dominance deviation for a given hybrid can be made. Based on these findings, breeders can analyze the partition of the genetic variance and how GxE acts on the additive and dominance variance components. Also, based on these plots it is possible to select the hybrids with stability for the additive effects and to intermate their parents (inbred lines) for the next breeding cycles. This approach can be easily extended to other crops, such as eucalyptus and pine, in which the evaluated individual is used to generate the next breeding cycle. Then, optimized crosses can be performed through the selection of the best parents with the goal of improving the expected genetic gain (Toro and Varona, 2010). Since in these species the dominance effects cannot pass from parents to the offspring over generations, increases in the genetic gains can be reached through the selection of the best parents based on the additive effects.

Implementation of GS for drought tolerance breeding programs in maize

Genomic selection can be applied at least in two contexts for drought-tolerance breeding programs in maize. First, GS can be used for parental selection and for successive cycles of intermating and selection within breeding populations (normally, biparental populations). Under this scenario, GS can be used to perform more than one breeding cycle per year, increasing the genetic gains per unit time. A similar approach has been applied by Beyene et al. (2015), who showed the advantage of this GS strategy over phenotypic selection to increase the genetic gains in drought tolerance breeding programs. Second, GS can be used to predict untested hybrids, an important role in maize breeding programs, which allow reducing the number of tested (phenotyped) hybrids in breeding programs. In both scenarios described above, time and financial resources can be saved in genetic breeding programs. Recently, Krchov and Bernardo (2015) compared the amount of financial resources that can be saved using GS in a breeding program when compared to phenotyping costs, given that the genotyping costs are currently decreasing.

Over the recent years, many statistical models have been proposed for GS in plant and animal breeding. Although comparisons between different GS models are important, the choice of an adequate training set is one of the most important steps to apply GS in a breeding program. Thus, in maize breeding, optimizing the composition of the training set, in terms of number of lines and hybrids per line, could further increase the prediction accuracy of untested hybrids (TECHNOW et al., 2014). Moreover, biparental or multiparental training populations can be used for the breeding value prediction of selection candidates within or across breeding populations (SCHULZ-STREECK et al., 2012).

Our findings suggest that GxE, additive and dominance effects should be simultaneously incorporated into the GS models in maize breeding programs; and that GBLUP models that account for GxE, additive and dominance effects can be easily used for high density SNP panels. In addition, these GBLUP models can be easily extended to other crops, such as outcrossing species, in which non-additive effects are very important. Through these models it is possible to select the best genotypes based on the performance and stability across different environments. Moreover, it is also possible to select the hybrids with stability for the additive effects and intermate their parents (inbred lines) for the next breeding cycles. Thus, using FA models, breeders can select superior hybrids with stable responses across WS and WW conditions. For a breeding program, one-stage model analysis may be challenge to fit, since hybrids are usually evaluated across many locations and years. Then, one option to solve this problem is to perform a two-stage model analysis (for some details about the two-stage model analysis see Mohring and Piepho, 2009).

As demonstrated so far, greater prediction accuracies for the hybrids performance can be achieved when dominance effects are incorporated in the GS models for drought tolerance. Our results showed a higher contribution of dominance effects for GY in both water conditions, while for ASI, EPP and FFT, the dominance effects were more evident under WS condition. Furthermore, the orthogonal partition of the genetic variance was performed the drought tolerance traits based on the genomic relationship matrices. Differences were observed in the predictive accuracy between models A and AD for the five evaluated traits in both water conditions. However, these differences were more evident under WS conditions. Using factor analytic mixed models, including additive and dominance effects, it was possible to investigate the stability of the additive and dominance effects across environments, as well as, the additive and dominance-by-environment interaction, with interesting applications for parental and hybrid selection. In addition, combining WW and WS trials increased the prediction accuracy of untested hybrids. These results contribute to a better understanding about the genetic architecture of important traits related to drought tolerance under WW and WS conditions, and highlight the importance of dominance effects for the grain yield genomic prediction of maize single-cross hybrids under both water regimes.

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CONFLICTS OF INTERESTS

The authors declare that there are no conflict of interests regarding the publication of this paper.

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Table 1. Estimates of the genetic parameters and goodness-of-fit-measures of the multi-environment trials models (model [1]) for well-watered (WW) and water-stressed (WS) conditions for additive (A) and additive-dominance (AD) models.

		WS		WW	
		A	AD	A	AD
GY	h^2	0.345 (0.026)	0.191 (0.025)	0.397 (0.029)	0.264 (0.031)
	δ^2	-	0.140 (0.017)	-	0.193 (0.022)
	H^2	-	0.331 (0.023)	-	0.457 (0.024)
	LogL	-2374.205	-2251.968	-1706.948	-1641.089
	AIC	4788.411	4567.935	3448.895	3342.178
EPP	h^2	0.311 (0.024)	0.236 (0.024)	0.218 (0.028)	0.214 (0.028)
	δ^2	-	0.114 (0.013)	-	0.078 (0.024)
	H^2	-	0.349 (0.021)	-	0.293 (0.031)
	LogL	-5187.843	-5137.087	-2768.141	-2754.757
	AIC	10415.690	10388.170	5572.281	5569.515
ASI	h^2	0.276 (0.042)	0.235 (0.057)	0.483 (0.031)	0.463 (0.034)
	δ^2	-	0.103 (0.014)	-	0.058 (0.017)
	H^2	-	0.339 (0.049)	-	0.521 (0.029)
	LogL	-3512.529	-3478.561	-681.806	-669.321
	AIC	7065.057	7021.122	1399.613	1398.642
FFT	h^2	0.410 (0.029)	0.276 (0.028)	0.440 (0.033)	0.387 (0.033)
	δ^2	-	0.092 (0.022)	-	0.078 (0.015)
	H^2	-	0.368 (0.027)	-	0.466 (0.032)
	LogL	-4962.642	-4881.973	-2344.532	-2316.392
	AIC	9965.284	9827.946	4725.065	4692.784
MFT	h^2	0.544 (0.027)	0.399 (0.030)	0.441 (0.034)	0.375 (0.034)
	δ^2	-	0.085 (0.016)	-	0.075 (0.016)
	H^2	-	0.485 (0.027)	-	0.449 (0.033)
	LogL	-3671.602	-3608.921	-2157.249	-2126.197
	AIC	7383.204	7281.842	4350.497	4312.395

h^2 narrow-sense heritability, δ^2 proportion of the total genetic variance explained by the dominance effects, H^2 broad-sense heritability, LogL is the likelihood of the fitted model; ρ^2_A and ρ^2_D are the average additive and dominance correlations between pairs of trials, respectively. Traits correspond to grain yield (GY, t/ha), number of ears per plot (EPP), anthesis-silking interval (ASI, days), female flowering time (FFT, days) and male flowering time (MFT, days). Values within parentheses are approximated standard errors.

Table 2. Predictive accuracy based on a 10-fold cross-validation (CV1 and CV2) procedure for five traits evaluated in WS and WW conditions, using additive (A) and additive+dominance (AD) models [1].

Predictive accuracy		GY	EPP	ASI	FFT	MFT
		----------------------------------	----------------------------------	CV1	----------------------------------	----------------------------------
WS	A	0.245	0.501	0.549	0.494	0.613
	A+D	0.508	0.554	0.577	0.615	0.614
WW	A	0.391	0.625	0.639	0.688	0.633
	A+D	0.474	0.663	0.636	0.723	0.662
		----------------------------------	----------------------------------	CV2	----------------------------------	----------------------------------
WS	A	0.268	0.518	0.557	0.539	0.621
	A+D	0.604	0.490	0.613	0.631	0.635
WW	A	0.311	0.584	0.681	0.683	0.657
	A+D	0.552	0.551	0.674	0.729	0.686

Traits considered correspond to grain yield (GY, t/ha), number de ear per plot (EPP), anthesis silking interval (ASI, days), female flowering time (FFT, days) and male flowering time (MFT, days).

Table 3. Predicted factor scores for the 15 best hybrids based on grain yield (GY, t/ha). Factor scores are rotated with the Varimax rotation from model [1] for all trials.

Hybrids	Additive		Dominance	
	Factor 1	Factor 2	Factor 1	Factor 2
11	**-0.14**	**0.42**	1.82	1.12
42	1.44	0.71	0.92	1.58
44	1.30	0.83	1.47	0.35
98	1.40	1.11	1.33	0.85
109	1.03	-0.09	0.80	2.23
121	0.69	1.01	0.52	1.22
133	1.16	0.73	1.38	1.25
139	0.93	0.51	0.81	1.54
141	1.30	0.29	0.89	1.03
143	1.29	0.31	0.82	1.91
182	1.27	0.28	1.22	1.35
186	0.40	0.65	1.66	1.26
188	1.30	0.70	0.54	1.22
211	1.70	1.52	**0.68**	**0.43**
285	0.71	1.25	1.62	0.62

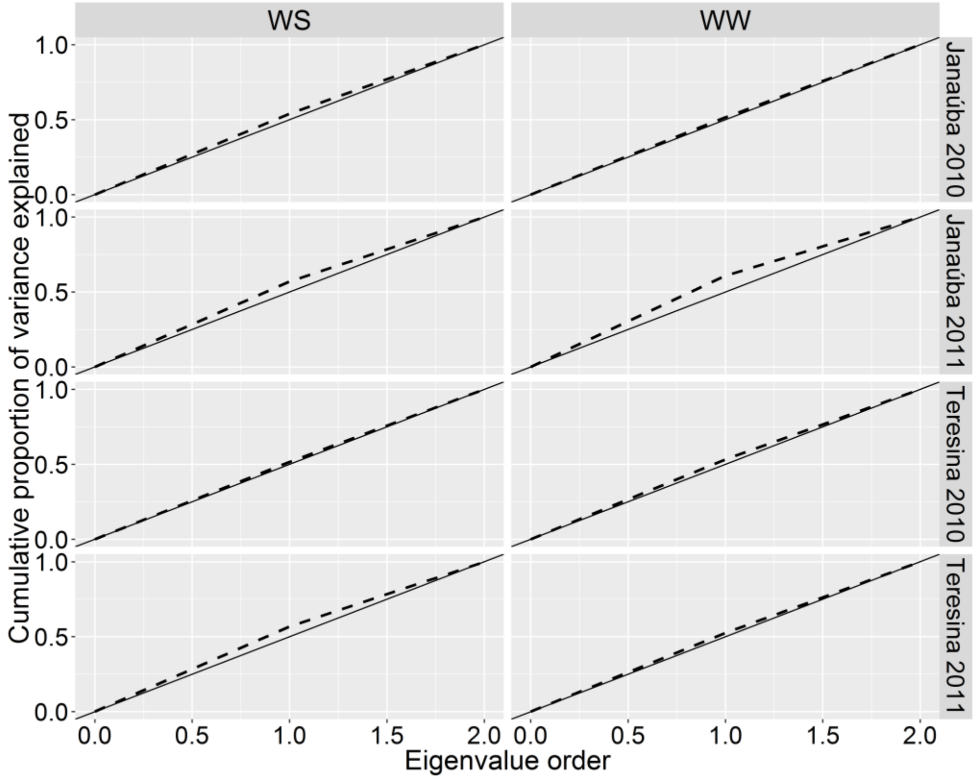

Figure 1. Proportion of the genetic variance explained by the additive and dominance effects for grain yield (GY, t/ha), based on the eigenvalues and using model [1]. The diagonal represents a hypothetical situation of orthogonality between two distinct matrices.

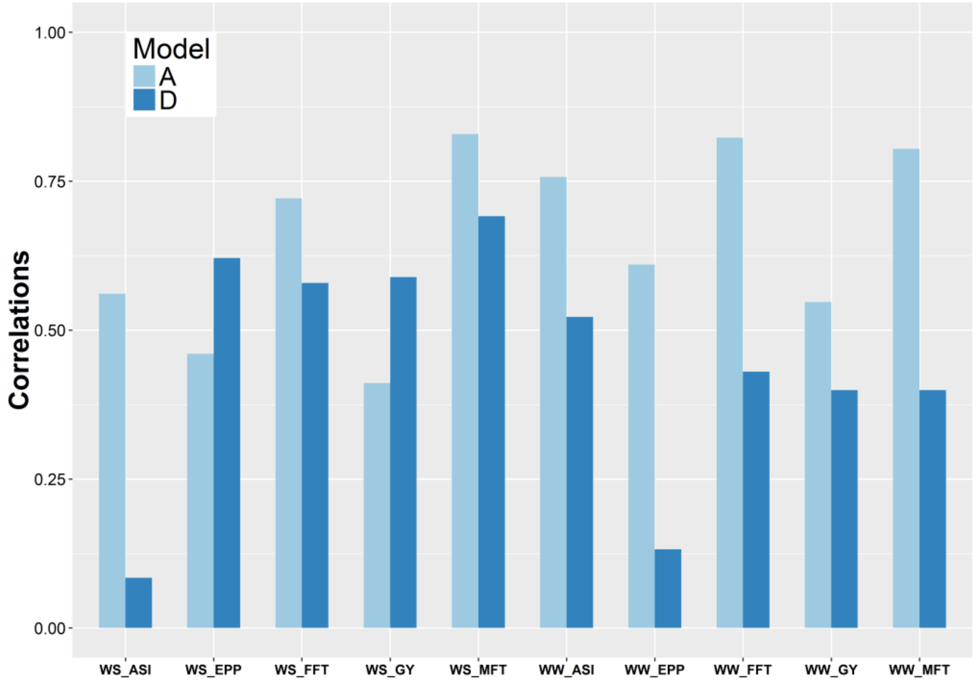

Figure 2. Additive (A) and dominance (AD) correlations between pairs of trials for the five evaluated traits under WS and WW conditions, using A (additive) and AD (additive and dominance) models (model [2]). Traits correspond to grain yield (GY, t/ha), number of ears per plot (EPP), anthesis-silking interval (ASI, days), female flowering time (FFT, days) and male flowering time (MFT, days).

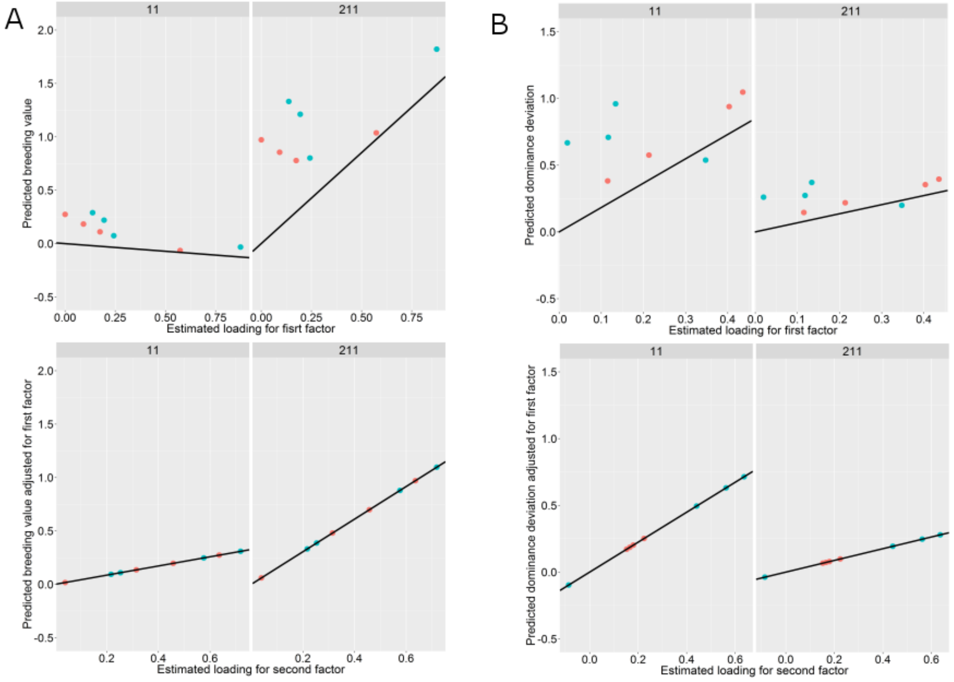

Figure 3. Latent regression plots for the first and second factors of additive effects (A). Latent regression plots for the first and second factors of dominance effects (B). Dots in blue and red represent WW and WS trials, respectively.

SUPPLEMENTARY INFORMATION

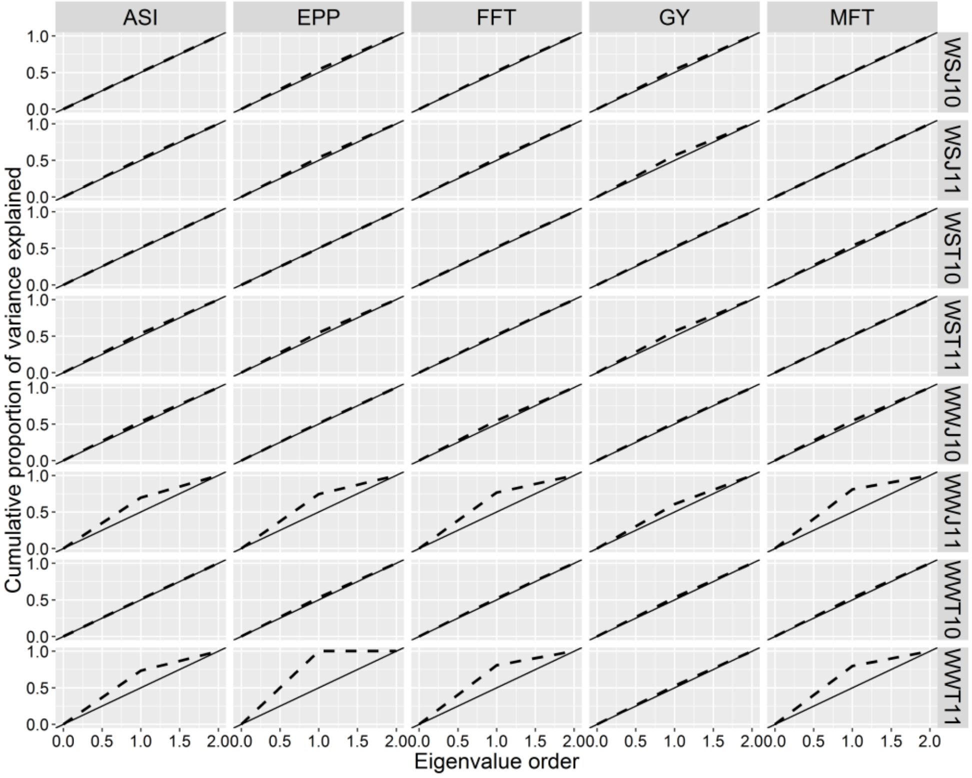

Figure S1. Proportion of the genetic variance explained by the eigenvalues of the models considering additive and dominance effects for all measured traits. The diagonal represents a hypothetical situation of orthogonality between two distinct matrices. The traits considered correspond to grain yield (GY, t/ha), number de ears per plot (EPP), anthesis silking interval (ASI, days), female flowering time (FFT, days) and male flowering time (MFT, days). For each trial, the two first letters identify the water condition (WS or WW), followed by the first letter of location (J for Janaúba or T for Teresina) and the last two digits corresponds to the year (10 or 11).

Table S1. Estimates of the genetic parameters and goodness-of-fit-measures from single trial analyses. The trait considered correspond to grain yield (GY, t/ha). Values in parentheses are approximate standard errors.

	A	AD	A	AD
	WSJ10		WWJ10	
h^2	0.274 (0.044)	0.235 (0.045)	0.490 (0.050)	0.415 (0.053)
δ^2	-	0.111 (0.037)	-	0.112 (0.036)
H^2	-	0.346 (0.043)	-	0.527 (0.046)
LogL	-549.429	-537.187	-490.156	-469.821
AIC	1104.858	**1082.376**	986.312	**947.642**
	WSJ11		WWJ11	
h^2	0.294 (0.046)	0.176 (0.042)	0.185 (0.086)	0.188 (0.087)
δ^2	-	0.193 (0.042)	-	0.131 (0.073)
H^2	-	0.346 (0.043)	-	0.319 (0.104)
LogL	-549.429	-537.187	-295.725	-290.786
AIC	1104.858	**1082.376**	595.450	**587.573**
	WST10		WWT10	
h^2	0.234 (0.053)	0.196 (0.053)	0.460 (0.053)	0.363 (0.055)
δ^2	-	0.128 (0.053)	-	0.221 (0.050)
H^2	-	0.324 (0.059)	-	0.584 (0.043)
LogL	-604.768	-599.931	-623.869	-594.239
AIC	1215.538	**1207.863**	1253.738	**1196.478**
	WST11		WWT11	
h^2	0.250 (0.043)	0.159 (0.040)	0.171 (0.067)	0.066 (0.059)
δ^2	-	0.144 (0.038)	-	0.323 (0.094)
H^2	-	0.303 (0.043)	-	0.389 (0.094)
LogL	-674.647	-635.490	-350.572	-341.526
AIC	1355.295	**1278.982**	705.144	**689.053**

h^2 narrow-sense heritability, δ^2 proportion of the total genetic variance explained by dominance, H^2 broad-sense heritability, LogL is the likelihood of the fitted model. For each trial, the two first letters identify the water condition (WS or WW), followed by the first letter of location (J for Janaúba or T for Teresina) and the last two digits corresponds to the year (10 or 11).

Table S2. Estimates of the genetic parameters and goodness-of-fit-measures from single trial analyses. The trait considered correspond to ears per plot (EPP, t/ha). Values in parentheses are approximate standard errors.

	A	AD	A	AD
	WSJ10		WWJ10	
h^2	0.384 (0.049)	0.331 (0.051)	0.317 (0.054)	0.300 (0.055)
δ^2	-	0.064 (0.031)	-	0.047 (0.036)
H^2	-	0.396 (0.047)	-	0.347 (0.057)
LogL	-1239.732	-1235.195	-891.311	-889.722
AIC	2485.504	**2478.390**	1788.623	**1787.445**
	WSJ11		WWJ11	
h^2	0.318 (0.046)	0.243 (0.045)	0.253 (0.089)	0.294 (0.087)
δ^2	-	0.115 (0.035)	-	0.126 (0.090)
H^2	-	0.358 (0.042)	-	0.420 (0.125)
LogL	-1519.157	-1494.454	-386.686	-385.061
AIC	3044.314	**2996.908**	777.373	**776.129**
	WST10		WWT10	
h^2	0.225 (0.054)	0.195 (0.055)	0.236 (0.054)	0.231 (0.055)
δ^2	-	0.140 (0.054)	-	0.137 (0.054)
H^2	-	0.335 (0.060)	-	0.367 (0.061)
LogL	-1230.183	-1225.576	-973.507	-968.894
AIC	2466.366	**2459.153**	1953.015	**1945.79**
	WST11		WWT11	
h^2	0.194 (0.042)	0.170 (0.042)	0.114 (0.068)	0.111 (0.059)
δ^2	-	0.082 (0.035)	-	0.000 (-)
H^2	-	0.252 (0.044)	-	0.111 (0.059)
LogL	-1253.852	-1247.434	-571.272	-565.517
AIC	2513.703	**2502.869**	**1146.545**	1139.035

h^2 narrow-sense heritability, δ^2 proportion of the total genetic variance explained by dominance, H^2 broad-sense heritability, LogL is the likelihood of the fitted model. For each trial, the two first letters identify the water condition (WS or WW), followed by the first letter of location (J for Janaúba or T for Teresina) and the last two digits corresponds to the year (10 or 11).

Table S3. Estimates of the genetic parameters and goodness-of-fit-measures from single trial analyses. The trait considered correspond to anthesis silking interval (ASI, days). Values in parentheses are approximate standard errors.

	A	AD	A	AD
	WSJ10		WWJ10	
h^2	0.444 (0.042)	0.431 (0.044)	0.535 (0.045)	0.511 (0.044)
δ^2	-	0.035 (0.025)	-	0.059 (0.032)
H^2	-	0.466 (0.042)	-	0.567 (0.044)
LogL	-865.201	-863.928	-371.382	-369.194
AIC	1736.403	**1735.856**	748.765	**746.388**
	WSJ11		WWJ11	
h^2	0.466 (0.044)	0.371 (0.040)	0.527 (0.073)	0.527 (0.073)
δ^2	-	0.116 (0.0337)	-	0.007 (0.052)
H^2	-	0.487 (0.040)	-	0.535 (0.090)
LogL	-1148.622	-1127.608	-238.010	-238.000
AIC	2303.245	**2263.216**	**480.020**	482.001
	WST10		WWT10	
h^2	0.176 (0.051)	0.155 (0.053)	0.155 (0.049)	0.145 (0.049)
δ^2	-	0.126 (0.054)	-	0.066 (0.045)
H^2	-	0.281 (0.062)	-	0.211 (0.059)
LogL	-1143.980	-1140.427	-132.667	-131.134
AIC	2293.960	**2288.854**	271.334	**270.026**
	WST11		WWT11	
h^2	0.277 (0.044)	0.251 (0.045)	0.248 (0.083)	0.228 (0.083)
δ^2	-	0.043 (0.028)	-	0.287 (0.095)
H^2	-	0.295 (0.045)	-	0.515 (0.112)
LogL	-402.327	-399.530	-5.920	-0.628
AIC	810.654	**807.061**	15.840	**7.250**

h^2 narrow-sense heritability, δ^2 proportion of the total genetic variance explained by dominance, H^2 broad-sense heritability, LogL is the likelihood of the fitted model. For each trial, the two first letters identify the water condition (WS or WW), followed by the first letter of location (J for Janaúba or T for Teresina) and the last two digits corresponds to the year (10 or 11).

Table S4. Estimates of the genetic parameters and goodness-of-fit-measures from single trial analyses. The trait considered correspond to female flowering time (FFT, days). Values in parentheses are approximate standard errors.

	A	AD	A	AD
	WSJ10		WWJ10	
h^2	0.544 (0.038)	0.512 (0.041)	0.599 (0.041)	0.537 (0.046)
δ^2	-	0.055 (0.022)	-	0.106 (0.033)
H^2	-	0.567 (0.036)	-	0.644 (0.037)
LogL	-1227.789	-1219.396	-758.423	-741.650
AIC	2461.579	**2446.793**	1522.847	**1491.302**
	WSJ11		WWJ11	
h^2	0.531 (0.044)	0.342 (0.046)	0.465 (0.080)	0.482 (0.076)
δ^2	-	0.170 (0.037)	-	0.086 (0.066)
H^2	-	0.512 (0.039)	-	0.568 (0.097)
LogL	-1495.668	-1444.274	-408.579	-406.954
AIC	2997.336	**2896.548**	821.158	**819.908**
	WST10		WWT10	
h^2	0.197 (0.054)	0.169 (0.054)	0.210 (0.049)	0.188 (0.048)
δ^2	-	0.104 (0.049)	-	0.043 (0.027)
H^2	-	0.274 (0.059)	-	0.231 (0.053)
LogL	-1336.340	-1332.434	-952.937	-949.650
AIC	2678.680	**2672.868**	1911.874	**1907.301**
	WST11		WWT11	
h^2	0.457 (0.047)	0.353 (0.042)	0.497 (0.078)	0.509 (0.068)
δ^2	-	0.103 (0.029)	-	0.210 (0.063)
H^2	-	0.457 (0.045)	-	0.720 (0.070)
LogL	-1041.652	-1016.160	-342.169	-324.353
AIC	2089.305	**2040.320**	688.339	**654.707**

h^2 narrow-sense heritability, δ^2 proportion of the total genetic variance explained by dominance, H^2 broad-sense heritability, LogL is the likelihood of the fitted model. For each trial, the two first letters identify the water condition (WS or WW), followed by the first letter of location (J for Janaúba or T for Teresina) and the last two digits corresponds to the year (10 or 11).

Table S5. Estimates of the genetic parameters and goodness-of-fit-measures from single trial analyses. The trait considered correspond to male flowering time (MFT, days). Values in parentheses are approximate standard errors.

	A	AD	A	AD
	WSJ10		WWJ10	
h^2	0.596 (0.037)	0.518 (0.042)	0.0564 (0.043)	0.488 (0.048)
δ^2	-	0.095 (0.026)	-	0.129 (0.036)
H^2	-	0.614 (0.034)	-	0.617 (0.037)
LogL	-906.270	-881.837	-658.286	-636.513
AIC	1818.542	**1771.676**	1322.573	**1281.025**
	WSJ11		WWJ11	
h^2	0.603 (0.037)	0.482 (0.044)	0.529 (0.073)	0.566 (0.066)
δ^2	-	0.119 (0.031)	-	0.105 (0.057)
H^2	-	0.602 (0.035)	-	0.672 (0.081)
LogL	-1120.695	-1090.680	-369.899	-365.964
AIC	2247.391	**2189.360**	743.798	**737.928**
	WST10		WWT10	
h^2	0.032 (0.053)	0.281 (0.054)	0.207 (0.049)	0.187 (0.049)
δ^2	-	0.063 (0.035)	-	0.035 (0.027)
H^2	-	0.344 (0.053)	-	0.222 (0.051)
LogL	-876.604	-872.102	-925.355	-923.192
AIC	1759.209	**1752.206**	1856.711	**1854.385**
	WST11		WWT11	
h^2	0.398 (0.047)	0.327 (0.047)	0.515 (0.076)	0.531 (0.066)
δ^2	-	0.077 (0.026)	-	0.170 (0.058)
H^2	-	0.405 (0.046)	-	0.701 (0.070)
LogL	-974.274	-958.445	-326.322	-312.657
AIC	1954.549	**1924.890**	656.644	**631.315**

h^2 narrow-sense heritability, δ^2 proportion of the total genetic variance explained by dominance, H^2 broad-sense heritability, LogL is the likelihood of the fitted model. For each trial, the two first letters identify the water condition (WS or WW), followed by the first letter of location (J for Janaúba or T for Teresina) and the last two digits corresponds to the year (10 or 11).