Marker-trait associations for early season cold tolerance in sorghum

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Dedicated to my father

Abstract

Early season cold tolerance is an important breeding goal towards the establishment of sorghum as an alternative to maize for bioenergy production. Sorghum is mainly grown in the semi-arid tropics and subtropics but the adaptation to subtropical highlands gives hint for certain genetic variation in cold tolerance. To achieve high biomass yields, high and uniform emergence rates in combination with high growth rates during juvenile development are essential. The objectives were to detect marker-trait associations for parameters describing (a) the emergence process under different temperature regimes, (b) the temperature response of leaf area and dry matter growth rates and chlorophyll fluorescence and content, and (c) to verify marker-trait associations for cold tolerance during juvenile development in two F2-populations. Emergence and juvenile development of a diversity set and the F2 populations were tested in growth chamber experiments under different temperature regimes. The diversity set was fingerprinted with 171 diversity array technology (DArT), 31 simple-sequence repeat (SSR), and 2620 single nucleotide polymorphism (SNP) markers. SNP markers were available for the third part of the present study. F2-populations were only fingerprinted with SNP markers. Emergence parameters like onset or uniformity of emergence were derived from a piecewise linear regression model and final emergence rates were used to carry out stability analysis across different temperature regimes. Parameters, describing emergence as well as the temperature response of growth rates and photosynthesis related traits during juvenile development, were used as input traits for association mapping and quantitative trait loci (QTL) analysis. Already known QTL for emergence under low temperatures were verified on chromosome SBI-01, while promising new marker-traits associations for emergence were identified on chromosomes SBI-03, SBI-04, SBI-06, SBI-08, and SBI-09. Promising marker-trait associations for growth related temperature response parameters were detected on chromosomes SBI-01, SBI-03, SBI-07, and SBI-10. Many significant loci were also associated to growth rates *per se* in individual low-temperature

environments. Several marker-trait associations for mean growth rates and temperature response parameters of chlorophyll content and fluorescence were validated by QTL detected in population 1 or 2. Promising QTL regions were found on chromosomes SBI-01, SBI-02, SBI-03, and SBI-06. Interesting candidate genes involved in the abiotic stress response are located in promising QTL regions. In conclusion, several genome regions highly influencing cold tolerance during emergence and juvenile development were identified. These regions are suitable for the development of stable markers for marker-assisted selection. However, regional association studies have to be carried out and allelic diversity of candidate genes needs to be analyzed in order to reach these future goals.

Keywords: early season cold tolerance, association mapping, QTL analysis, temperature response

Kurzfassung

Kühletoleranz ist ein wichtiges Zuchtziel für die Etablierung von Sorghum als Alternative zu Energiemais für die Biogasproduktion. Sorghum wird hauptsächlich in den semi-ariden Tropen und Subtropen angebaut, die Anpassung an das subtropische Hochland gibt jedoch Hinweise auf eine gewisse genetische Variation hinsichtlich der Kühletoleranz. Wichtige Merkmale, um hohe Biomasseerträge zu erreichen, sind hohe und gleichmäßige Auflaufraten sowie rasches Wachstum während der Jugendphase. Ziele dieser Studie sind die Detektion von Marker-Merkmals Assoziationen für Parameter, die (a) das Auflaufverhalten bei verschiedenen Temperaturen und (b) Temperatureffekte auf Blattflächenentwicklung und Wachstumsraten sowie auf Chlorophyllfluoreszenz und -gehalt beschreiben, welche (c) anhand von in F2- Populationen detektierten quantitativen Merkmalsloci (QTL) verifiziert werden sollen. Ein Diversitätsset und zwei F2 Populationen wurden in Klimakammerexperimenten bei verschiedenen Temperaturstufen bezüglich ihres Auflaufverhaltens und der Jugendentwicklung getestet. Genetische Fingerabdrücke wurden im Diversitätsset mit 171 *Diversity array technology* (DArT), 31 Mikrosatelliten (SSR) Markern und 2062 Einzelnukleotid-Polymorphismen (SNP) erstellt. Für die F2 Populationen wurden nur SNP-Marker genutzt. Auflaufparameter, wie z.B. Beginn oder Gleichmäßigkeit des Auflaufs, die mithilfe eines schrittweisen, linearen Modells geschätzt wurden, sowie Auflaufraten wurden für Stabilitätsanalysen über verschiedene Temperaturstufen verwendet. Neben diesen Parametern wurden Parameter, die Temperatureffekte auf Wachstumsraten sowie Chlorophyllfluoreszenz und -gehalt beschreiben, für Assoziationskartierungen und QTL-Analysen verwendet. Bekannte QTL für Auflaufraten bei niedrigen Temperaturen konnten auf Chromosom SBI-01 verifiziert werden, vielversprechende Marker-Merkmals Assoziationen für Auflaufparameter konnten auf den Chromosomen SBI-03, SBI-04, SBI-06, SBI-08 und SBI-09 identifiziert werden. Interessante Marker-Merkmals Assoziationen für Parameter, die Temperatureffekte auf Wachstumsraten beschreiben, wurden auf

Chromosomen SBI-01, SBI-03, SBI-07 und SBI-10 detektiert. Viele dieser Genomregionen konnten auch bei der Analyse der entsprechenden Merkmale in unabhängigen, niedrigen Temperaturstufen wiedergefunden werden. Einige Marker-Merkmals Assoziationen für mittlere Wachstumsraten sowie für Chlorophyllfluoreszenz und -gehalt wurden durch identifizierte QTL in den F2 Populationen validiert. Vielversprechende Genomregionen konnten auf den Chromosomen SBI-01, SBI-02, SBI-03 und SBI-06 identifiziert werden, in denen bekannte Kandidatengene für abiotische Stresstoleranz liegen. Schlussfolgernd scheint es mehrere Genomregionen zu geben, die sich für regionale Assoziationskartierung eignen sowie interessant für die Entwicklung von stabilen Markern für die Marker-gestützte Selektion sind.

Schlagworte: Kühletoleranz, Assoziationskartierung, QTL-Analysen, temperaturabhängige Wachstumsparameter

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Abbreviations

a	exponent
ANOVA	Analysis of variance
bp	base pairs
Bt	*Bacillus thuringiensis*
°C	degree Celsius
CBF	C-repeat binding pathway
°Cd	Thermal time [°d]
CEP	Cumulative emergence percentages [%]
cM	Centimorgan
cm^2	Square centimeters
COR	Cold responsive genes
CSP	Cold shock proteins
CTAB	Cetyl trimethylammonium bromide
CV	Coefficient of variation [%]
CV_g	Coefficient of variation among and within genotypes [%]
CV_e	Coefficient of variation for averages across all environments [%]
d	Days
DArT	Diversity Array Technology markers
DAS	Days from sowing [d]
dF	Rate of change in the fluorescence
df	Degree of freedom
DM_{d6}	Dry weight 6 days after sowing [g]
DM_{dn}	Dry weight at the end of the experiment [g]
DMGR	Dry matter growth rate [g d^{-1}]
$DMGR_0$	Initial dry matter growth rate [g d^{-1}]
$DMGR_{(a)}$	exponent of dry matter growth rate
$DMGR_{(mean)}$	mean dry matter growth rate across environments [g d^{-1}]
DNA	Deoxyribonucleic acid
DREB	Dehydration responsive element

dt	Rate of change in temperature
E	Environment
env	Environmental effects
ER	Emergence rate [% d^{-1}]
$ER_{(FW)}$	Finlay-Wilkinson slope of Emergence rate [% d^{-1}]
E_{TS}	Thermal time of emergence [°Cd]
F_0'	Ground fluorescence of light adapted leaves
FEP	Final emergence percentage [%]
FEP_{cold}	Final emergence percentage under cold conditions [%]
$FEP_{(FW)}$	Finlay-Wilkinson slope of final emergence percentage [%]
$FEP_{(M)}$	Mean of final emergence percentage [%]
FEP_{normal}	Final emergence percentage under normal conditions [%]
Φ_{PSII}	Quantum yield of photosystem II
$\Phi_{PSII(sl)}$	Regression slope for quantum yield of photosystem II
$\Phi_{PSII(m)}$	Mean across environments of quantum yield of photosystem II
F_v'/F_m'	efficiency of energy harvesting by oxidized PSII reaction centers in the light
$F_v'/F_m'(sl)$	Regression slope for efficiency of energy harvesting by oxidized PSII reaction centers in the light
$F_v'/F_m'(m)$	Mean across environments of efficiency of energy harvesting by oxidized PSII reaction centers in the light
FW	Finlay-Wilkinson slopes
G	Genotype
g	Gram
GEI	Genotype x environment interaction
gen	Genotype effect
gen*env	Interaction of genotype and environment
gen*temp	Interaction of genotype and temperature
GLM	Generalized linear model
GR_0	Estimated growth rate in the lowest temperature environment [g d^{-1}]
GR_{ij}	Growth rate of the i^{th} genotype in the j^{th} environment [g d^{-1}]

h^2	heritability
ha	Hectare
LA_{d6}	Leaf area 6 days after sowing [cm²]
LA_{dn}	Leaf area at the end of the experiment [cm²]
LAR	Leaf appearance rate [d^{-1}]
$LAR_{(mean)}$	Mean leaf appearance rate [d^{-1}]
$LAR_{(sl)}$	Regression slope of leaf appearance rate [d^{-1}]
$LAR_{(Tb)}$	Base temperature of leaf appearance rate [°C]
LD	Linkage disequilibrium
LGR	Leaf area growth rate [cm²]
LGR_0	Initial leaf area growth rate [cm²]
$LGR_{(a)}$	exponent of leaf area growth rate
$LGR_{(mean)}$	Mean of leaf area growth rate [cm²]
LOD	Logarithmic odds ratio
LOESS	locally weighted scatterplot smoothing
LN_{d6}	Leaf number 6 days after sowing
LN_{dn}	Leaf number at the end of the experiment
m	mean
MLM	Mixed linear model
MSD	Mean squared difference
MW	mean across environments
n	Number of days of the temperature treatment
n	Number of environments
NES_i	Number of seeds emerged on day i
p	p-Value
PC	Principal component
PCA	Principal component analysis
PSII	Photosystem II
QTL	Quantitative trait loci
r	Number of replications

R	Pearson’s correlation coefficient
R^2	coefficient of determination
REML	Restricted maximum likelihood
RIL	Recombinant inbred lines
RNA	Ribonucleic acid
σ^2_E	Variances of the environment
σ^2_G	Variances of the genotype
σ^2_{GxE}	Variances of the genotype x environment interactions
σ^2	Error variances
SD	Standard deviations
sl	Regression slope
SNP	Single nucleotide polymorphism markers
SPAD	Leaf greenness, chlorophyll content,
$SPAD_{(sl)}$	regression slope of leaf greenness
$SPAD_{(Tb)}$	Base temperature of leaf greenness
SQR	Shan Qui Red
SSR	Simple-sequence repeat markers
T	Temperature
t	Number of days
T_1	Onset of emergence [DAS]
$T_{1(M)}$	Mean of onset of emergence [DAS]
$T_{1(FW)}$	Finlay-Wilkinson slope of onset of emergence [DAS]
T_{50}	Median time to emergence [DAS]
$T_{50(M)}$	Mean of median time to emergence [DAS]
$T_{50(FW)}$	Finlay-Wilkinson slope of median of emergence
T_{100}	End of emergence [DAS]
$T_{100(M)}$	Mean of end of emergence [DAS]
$T_{100(FW)}$	Finlay-Wilkinson slope of end of emergence [DAS]
T_{100}-T_1	Uniformity of emergence [d]
T_b	Base temperature [°C]

temp	Temperature effect
US	United states of America
μ	overall population mean
β_i	linear regression coefficient for the i^{th} genotype
e_j	effect j^{th} environment
g_i	effect of the i^{th} genotype

Chapter 1

General introduction

Background

The development of cold tolerant sorghum is a major breeding goal in order to provide farmers an alternative crop to maize for bioenergy production. The acreage cultivated to bioenergy crops increased in Germany from 700.000 ha in 2000 up to 2,526.000 ha in 2011. Currently, 21 % of the cropping area is used for energy production (FNR 2012). In Germany, nearly 1/3 of the 2,6 mio ha maize are used for energy production. Due to increasing maize production areas, missing crop rotations and the expansion of the western corn borer (*Diabrotica vigifera* LeConte) in Central Europe, a diversification is needed. Sorghum has the potential to be an alternative crop to maize due to its high biomass yields even under drought stress conditions (Farre and Faci 2006). Hence, sorghum is interesting for areas with low rainfall, a deep groundwater level, and sandy soils.

However, Sorghum is a thermophilic crop, mainly grown in the semi-arid tropics and subtropics, which has to be adapted to temperate regions. Low and non-uniform emergence rates caused by low soil temperatures in spring in combination with low growth rates during juvenile development result in a late soil coverage and canopy closure (Richards 2000), which reduces competitiveness and promotes weed growth. Early sowing dates are required to increase the vegetation period and biomass yield potential. Hence, a major issue in sorghum breeding for temperate regions is the improvement of cold tolerance.

Identification of genome regions influencing cold tolerance

The adaptation of sorghum to tropical and subtropical highlands gives hint for certain genetic variation needed for the improvement of cold tolerance. Sorghum accessions, originated from China and Russia have shown to be promising genetic resources for the improvement of cold tolerance during emergence and juvenile development (Tiryaki and Andrews 2001). Kaoling sorghum landraces from China possess higher emergence rates and seedling vigor than commercial hybrids but lack desirable agronomic trait (Cisse and Ejeta 2003). Chinese kaoling accessions exhibited higher shoot growth rates in comparison to US inbred lines under low temperature conditions (12 °C) (Franks et al. 2006). Mapping populations derived from a cross between the Chinese kaoling type 'Shan Qui Red' and an African caudatum were used to identify QTL for seedling emergence and vigor under controlled cold stress conditions (Knoll et al. 2008a). These QTL were validated in two newly developed populations under field conditions (Knoll and Ejeta 2008b). Cold tolerance during juvenile development is mainly described by shoot dry matter growth rates and photosynthesis related traits e.g. chlorophyll content and fluorescence, which were successfully used for cold tolerance selection in maize breeding programs (Fracheboud et al. 1999). A major QTL for photosynthetic performance of maize was identified only at low temperatures. The QTL co-localized to a QTL for shoot dry matter accumulation (Fracheboud et al. 2004). Thus, emergence and growth rates as well as photosynthetic related traits during juvenile development are key traits for breeding to improve cold tolerance.

In contrast to conventional QTL analyses, association studies were carried out on structured or unstructured populations, potentially carrying more than two alleles on certain loci (Flint-Garcia et al. 2003). An advantage of association mapping over conventional QTL analysis is that the time consuming and expensive development of a bi-parental crosses is not necessary (Zhu et al. 2008). Limiting factors for genome-wide association mapping is the availability of

the required number of marker loci covering the whole genome and large numbers of false positive associations. False positive marker-trait associations were reduced if analyses accounted for population structures and used kinship methods (Stich et al. 2008). The combination of association mapping and conventional QTL analyses methods enables the confirmation of major QTL and may narrow down large genome regions to those in which candidate genes are located.

The integration of genotype by environment interactions into the analysis of the genetic basis of complex traits like abiotic stress responses has always been difficult. Coupling crop models with statistical QTL models is a promising tool to overcome this problem (Collins et al. 2008). Stability analysis, as commonly used for multi-environmental trial data, is disadvantageous because of many varying factors (e.g. temperature, rainfall, radiation etc.) in field experiments controlling the traits. Using stability parameters derived from cumulative emergence model data evaluated in controlled growth chamber as input parameters for association mapping enables the detection of truly temperature response QTL due to only one varying environmental factor e.g. temperature. Likewise, response curve parameters derived for leaf elongation rate of maize in relation to temperature and soil water deficit (Reymond et al. 2003), flowering time in barley (Yin et al. 2005) and leaf senescence in potato (Malosetti et al. 2006) were successfully used as input parameters for QTL analysis. The advantage of response curve parameters is that genotype performance over a growing season or across different environments is taken into account. QTL for parameters describing the adaptability across different temperature regimes and QTL for mean genotype performance enable to distinguish between genome regions responsible for temperature dependant control of a trait and the trait itself (Via et al. 1995). QTL for the genotype specific response to temperature might be an important step in developing stable markers for marker-assisted selection for cold tolerance.

Objectives

The overall aim was to identify marker-trait associations or QTL for cold tolerance during emergence and juvenile development. Each of the following chapters focuses on a specific part of this topic, but can be read individually. Specific goals of the chapters are:

- Identification of marker-trait associations for emergence parameters derived from a cumulative emergence model and analysis of stability parameters (chapter 2).

- Identification of marker-trait associations for photosynthesis and growth related traits during juvenile development under different temperature regimes and for temperature dependant parameters describing sorghum growth (chapter 3).

- Validation of marker-trait associations for temperature dependant parameters describing sorghum growth during juvenile development using bi-parental populations (chapter 4).

Chapter 2

Genetic dissection of the temperature dependent emergence processes in sorghum using a cumulative emergence model and stability parameter

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Abstract

Among the major limitations for cultivating biomass sorghum in temperate regions is low temperature in spring that results in low and non-uniform emergence. The adaptation of sorghum to tropical and subtropical highlands gives hint of genetic variation in cold tolerance during emergence. The objective of the present study was to detect marker-trait associations for parameters describing the emergence process under different temperature regimes. A diversity set comprising 194 genotypes was tested in nine controlled environments with temperatures ranging from 9.4 to 19.9 °C. The genotypes were fingerprinted with 171 DArT markers. A piecewise linear regression model carried out on cumulative emergence was used to estimate genotype mean performance across environments and to carry out stability analysis on the parameters of the regression model. Base temperature (T_b) and thermal time required for emergence (E_{TS}) were determined based on median time to emergence data. Identified QTL positions were compared to marker-trait associations for final emergence percentages under low (FEP_{cold}) and normal (FEP_{normal}) temperatures. QTL for mean final emergence percentage (FEP), FEP_{cold} and $FEP_{normal,}$ T_b and E_{TS} were detected on SBI-01. Other QTL-rich regions were located on SBI-03, SBI-04, SBI-06, SBI-08, and SBI-09. Marker-trait associations for T_b and E_{TS} co-localized to QTL for the across environment stability of FEP and the median time to emergence or emergence rate, respectively. We conclude that genome regions on six chromosomes highly influencing cold tolerance during emergence are promising for regional association studies and for the development of stable markers for marker assisted selection.

Keywords: sorghum, cold tolerance, emergence, piecewise linear regression model, stability parameters, association mapping

Introduction

Developing cold tolerant biomass sorghum genotypes is an important breeding goal in order to have an alternative crop to maize, which presently dominates the area cultivated for methane production in Central Europe. Sorghum is a thermophilic crop, mainly grown in the semi-arid tropics and subtropics. Low soil temperature in spring may delay planting time or result in low and non-uniform emergence. Planting is recommended when stable seedbed temperatures of more than 10°C are achieved (Anda and Pinter 1994; Brar and Stewart 1994).

Agronomists and plant physiologists used different approaches to describe germination and emergence: final germination or emergence rate (Tiryaki and Buyukcingil 2009), germination indices (Afzal et al. 2008) and time to onset (T_1), end (T_{100}) and median time to emergence (T_{50}) or germination derived from functions describing the germination process regressed against time (Snapp et al. 2008). Several single-value germination indices, e.g. Kotowski's coefficient of velocity (Kotowski 1926) or Timson's cumulative germination index (Timson 1965) are widely used but final values cannot be traced back to direct measures for T_1, emergence rate (ER) and time span of emergence (T_{100}-T_1), which describe the germination or emergence process (Brown and Mayer 1988a). The emergence process is important since both final percentage of emergence and the time, when emergence occurs, are temperature dependant, and good field emergence requires high rates of uniformly germinating seeds under both optimum and low temperature conditions (Kanemasu et al. 1975). Logistic regressions carried out on cumulative germination rates are commonly used to describe germination (Hsu et al. 1984; Schimpf et al. 1977). Several functions describing the germination process were compared by Brown and Mayer (1988b) who recommended the Weibull function (Weibull 1951). However, comparability of germination curves computed from data of different temperature regimes is limited (Dumur et al. 1990). The Weibull function for instance has a parameter describing the shape of the regression, which has an

effect on the rate of increase of germination but no biological meaning. An alternative approach is the use of piecewise linear regression models (Kempenaar and Schnieders 1995). The advantages are (1) the possibility to directly compare model parameters from different datasets since parameters are not interrelated, i.e., the change of one model parameter does not necessarily lead to a change of a second parameter and (2) model parameters describe physiological processes or are simple statistical measures.

Genotypic differences in temperature response and base temperatures ranging from 5.9 to 9.8°C were reported for germination of 16 sorghum cultivars (Wade et al. 1993). Genetic variation in base temperature and emergence rates at low temperatures was assumed to be the result of adaptation processes (Tiryaki and Andrews 2001). Chinese landraces had higher germination percentages and shorter time to 50% germination at low temperatures than US breeding lines (Franks et al. 2006).

Quantitative trait loci (QTL) analysis for cold tolerance is a useful tool and a first step towards marker-assisted selection of cold tolerant genotypes (Knoll and Ejeta 2008). QTL for germination rate were found in rice (Ji et al. 2009), wild barley (Vanhala and Stam 2006) and sorghum (Burow et al. 2011; Knoll et al. 2008). Knoll et al. (2008) identified QTL for field emergence in sorghum recombinant inbred lines (RIL) developed from a cross between a caudatum of African origin and the cold tolerant Chinese kaoliang ‘Shan Qui Red’ on chromosome SBI-01. Cold tolerance QTL were detected in the same region of SBI-01 by Burow et al. (2011).

For identifying QTL for adaptation processes, multi-environment trials are needed. Lacaze et al. (2009) carried out QTL analysis in a bi-parental barley population on the slope of individual genotype trait values regressed against the population mean in different environments. Kraakman et al. (2004) used a set of modern spring barley cultivars in order to detect marker-trait associations for mean yield and yield stability across environments. El

Soda et al. (2010) used stability parameters to detect QTL for drought tolerance in wild barley introgression lines. It has been suggested that stability parameters can be used to distinguish between loci, in which constitutive genes are directly influenced by the environment and loci distinct from the constitutive genes but regulating them.

In contrast to QTL mapping in bi-parental crosses, association studies can be carried out on structured and unstructured populations, potentially carrying more than two alleles on a certain locus (Flint-Garcia et al. 2003). Advantages of association studies are that time consuming and expensive development of bi-parental crosses is not needed and a wider gene pool can be analyzed (Neumann et al. 2010). Genome-wide association studies were carried out, e.g., on traits like days to heading, culm diameter, leaf length and width in sorghum using SSR simple sequence repeat markers (Shehzad et al. 2009). Association mapping with Diversity Array Technology markers (DArT) was reported for barley (Pswarayi et al. 2008) and wheat (Crossa et al. 2007). The disadvantages are that DArT-markers are bi-allelic and dominant and are based on unknown sequences (Mace et al. 2008). However, compared to SSRs, DArT markers allow a cost efficient and fast genome-wide genotyping.

The objective of the present study was to detect marker-trait associations for emergence across different temperature regimes in sorghum. The process of emergence in the different temperature regimes was described by cumulative emergence percentages (CEP) over time in order to derive traits like FEP, T_{100}-T_1 and ER from piecewise linear regressions carried out on CEP. Since superior genotypes show high emergence percentages in a wide range of environments while emergence takes place shortly after sowing and all plants emerge nearly at the same time, the parameters FEP, ER, T_1, $T_{50,}$ T_{100} and T_{100}-T_1 are relevant. To evaluate the temperature effect on emergence, across environment means (M) and Finlay-Wilkinson slopes (FW) (Finlay and Wilkinson 1963) were estimated. FEP were computed separately for low and normal temperatures and base temperature (T_b) and thermal time (E_{TS}) were

calculated based on T_{50} data. Genome wide association studies were carried out on these parameters.

Material and Methods

Plant material

The study was carried out on a diverse set of genotypes comprising 194 biomass sorghum lines. The set includes *Sorghum bicolor* and *S. bicolor sudanense* genotypes. DNA was extracted from leaf tips using the cetyl trimethylammonium bromide (CTAB) method. The genotypes were fingerprinted with 688 polymorphic DArT markers. Marker positions were taken from Mace et al. (2008). Unmapped markers and completely linked markers were excluded from the study and further 115 markers with frequencies <5% of the rare allele were also removed. Association studies were carried out using the remaining 171 polymorphic DArTs.

Experimental design and data collection

The experiment was conducted in growth chambers set to 9 temperature regimes ranging from 9.4 to 19.9°C. Overall mean, mean night and day air and soil temperatures are shown in Table 2.1. A mean temperature of 9.4°C was used as lowest temperature treatment since pretests on a population subset revealed that the base temperature of emergence is expected to be higher than 8°C and lower than 11°C for most of the lines. Air and soil temperature was measured every 5 minutes directly above the trays and at 10 mm depth using TinyTag View 2 data loggers (Gemini Data Loggers Ltd., West Sussex, U.K.) during the entire duration of the study.

Table 2.1: Average daily mean, night and day air and mean soil temperatures in the nine temperature treatments.

Environment	Air temperature mean (night/day) °C	Soil temperature mean °C
1	9.4 (8.3/10.2)	10.0
2	10.3 (9.2/11.0)	10.3
3	10.7 (9.8/11.4)	10.7
4	10.8 (9.6/11.6)	11.0
5	11.6 (10.4/12.2)	11.6
6	12.3 (11.7/12.8)	12.5
7	16.7 (11.2/24.0)	-[a]
8	17.2 (10.0/26.0)	-
9	19.9 (15.4/25.7)	-
Cold conditions [b]	10.3	
Normal conditions [c]	17.9	

[a] Soil temperature was not measured in treatments 7-9.
[b] mean over environment 1 to 4
[c] mean over environment 7 to 9

Individual temperature regimes were arranged as randomized complete block designs with 2 replications. Light was applied for 12 h with 10 h full light and 2 h twilight. The genotypes were sown in trays filled with 50% Klasmann Potgrond P (Klasmann-Deilmann, Groß-Hesepe, Germany) and 50% loamy humic sand. A total of 18 seeds per line, treatment and replication were sown at 10 mm depth.

The number of emerged seeds was counted daily until no further seeds emerged. A plant was defined as emerged if the coleoptile was visible. Cumulative emergence percentage (CEP) for each day was calculated using the following equation:

$$CEP = \Sigma\ NES_i / 18 \times 100, \quad (2.1)$$

where NES_i is the number of seeds emerged on day *i* and 18 is the total number of seeds. Mean CEPs of the two replications were calculated and used for parameter estimation.

Data analysis

A piecewise linear regression was fitted to cumulative emergence percentages (Figure 1) in order to derive the parameters onset of emergence (T_1), median time to emergence (T_{50}), emergence rate (ER) and end of emergence (T_{100}) using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). The equation used was:

$$\begin{aligned} CEP &= 0 && t \le T_1 \\ CEP &= ER\ (t - T_1) && T_1 < t < T_{100} \\ CEP &= ER\ (T_{100} - T_1) && t \ge T_{100}, \end{aligned} \quad (2.2)$$

where t is the actual number of days from sowing (DAS). CEP equals to final emergence percentage (FEP) if $t \ge T_{100}$. The regression slope between between T_1 and T_{100} is the estimator for the daily emergence rate (ER). T_{50} was estimated as follows:

$$T_{50} = T_1 + 0.5\ FEP / ER. \quad (2.3)$$

Time span of emergence or uniformity of emergence was defined as T_{100}-T_1.

For comparing the genotypes over a series of environments, stability analysis was carried out according to Finlay and Wilkinson (1963). Genotype performance across environments was estimated by regressing individual genotypes against the population mean:

$$Y_{ij} = \mu + \beta_i e_j + g_i, \quad (2.4)$$

where μ is the overall population mean, β_i is the linear regression coefficient for the i^{th} genotype, e_j is the effect of the j^{th} environment and g_i is the effect of the i^{th} genotype.

Data was subjected to analysis of covariance using the following model for i = 1,2,3,…k genotypes and j = 1,2,3,…n environments:

$$Y_{ij} = \mu + \tau_i + \beta x_{ij} + \gamma_i x_{ij} + \varepsilon_{ij}, \quad (2.5)$$

where $\mu_i = \mu + \tau_i$ is the intercept of the i^{th} genotype, $FW_i = \beta x_{ij} + \gamma_i x_{ij}$ is the slope of the genotype performance of genotype i in nine environments regressed against the population means of the environments (Finlay and Wilkinson 1963) and ε_{ij} is the random error of the i^{th} genotype in the j^{th} environment. Analysis of covariance was carried out on the parameters T_1, T_{50}, T_{100}, T_{100}-T_1, ER and on FEP. FEP was arcsine-square root transformed prior to carrying out analysis of covariance.

Mean final emergence percentage over environments 1, 2, 3, and 4 was considered as FEP under low temperature conditions (FEP_{cold}) while FEP of environments 7, 8, and 9 was averaged to define FEP under normal conditions (FEP_{normal}). Two factorial analysis of variance (proc GLM SAS 9.1) was carried out on arcsine-square root transformed data considering FEP_{cold} and FEP_{normal} as two treatments with the individual temperature regimes as 4 or 3 replications.

Linear regression analysis was carried out on developmental rates ($1/T_{50}$) of the 9 temperature regimes. Base temperature (T_b) was estimated by linear extrapolation to define the temperature at which the development rate becomes 0:

$$T_{bi} = -\beta_{0i}/\beta_i. \quad (2.6)$$

where β_i is the regression slope and β_{0i} is the y-axis intercept of the i^{th} genotype. The temperature sum required for emergence (E_{TS}) was defined as $1 / \beta_i$.

Pearson's correlation coefficients were calculated between parameters and across environment means of traits. Variance components were assessed using restricted maximum

likelihood (REML) estimates (proc MIXED, SAS 9.1). Broad sense heritability (h^2) was calculated according to Hill et al. (1998):

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GxE}^2 \frac{1}{n} + \sigma^2 \frac{1}{n}} \quad , \qquad (2.7)$$

where σ^2_G is the genotypic variance, σ^2_{GxE} is the genotype x environment interaction variance, σ^2 is the error variance, and n is the number of environments.

The population structure of 194 individuals was determined using the software package STRUCTURE assuming an admixture model (Pritchard et al. 2000). We used a burn-in phase of 10,000 iterations followed by 10,000 Markov chain Monte Carlo iterations in order to detect the "true" number of K groups in the range of K=1-20 possible groups. δK was calculated according to Evanno et al. (2005). The cluster analysis was carried out with TASSEL 2.01 using the neighbor-joining method.

Linkage disequilibrium (LD) parameters were estimated by using the software TASSEL 2.01 (Bradbury et al. 2007). The p-values of pairwise LD were computed using 1,000 permutations. LD was calculated for all pairs of loci. The critical R^2 for unlinked loci was estimated after square root transformation of the R^2 values (Breseghello and Sorrells 2006). The 95% percentile of this distribution is the threshold beyond which LD was likely to be caused by genetic linkage. A second degree LOESS curve was plotted through the R^2 data and the point of intersection with the threshold value was used as the genome-wide estimate of LD among loci (Breseghello and Sorrells 2006).

TASSEL 2.01 (Bradbury et al. 2007) was used for identifying significant associations between the 171 markers and a total of 16 traits. The data were subjected to both a general linear model (GLM) and a mixed linear model (MLM) (Zhang et al. 2010). The Q-matrix,

which shows the probability that a genotype belongs to a subpopulation, was estimated with STRUCTURE and used in both models. A kinship matrix was computed with TASSEL 2.01 and used in MLM. An F-test with 1,000 permutations was carried out in order to adjust p-values of GLM (Churchill and Doerge 1994).

For verification of significant marker-trait associations the population was divided into two subpopulations at each relevant locus according to the allelic state of the individuals and pairwise t-tests ($p<0.05$) were performed in order to test if the marker genotypes differ significantly for the respective trait.

Results

Figure 2.1b shows that FEP increased with increasing temperature. FEP of the population mean was 95.7% at 19.9°C but less than 80% if air temperature was below 10.8°C. Lowest FEP was 35.8% at 9.4°C. Mean FEP_{cold} was 61.6% and ranged between 12.5 and 93.1% while mean FEP_{normal} was 93.3% (Table 2).

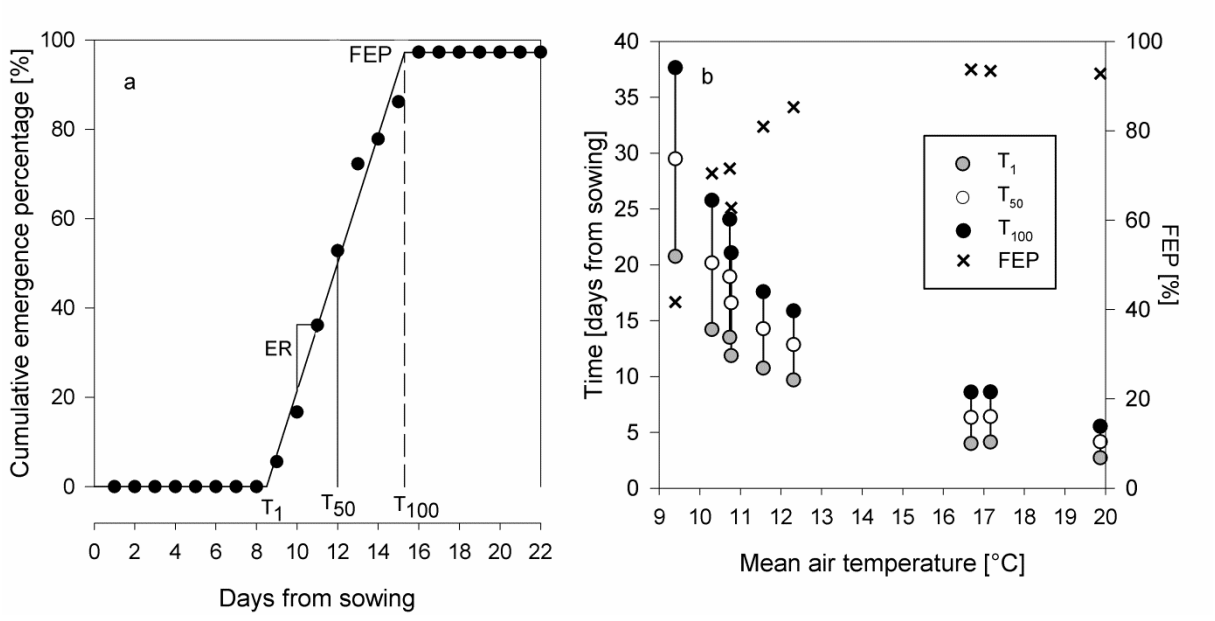

Figure 2.1 Piecewise linear regression for calculating onset (T_1) and end (T_{100}) of emergence, uniformity (T_{100}-T_1), emergence rate (ER) (regression slope) and the median of emergence time (T_{50}) (a) and T_1, T_{100}, T_{100}-T_1, T_{50} and FEP of the population mean in nine temperature treatments (b).

T_{100}-T_1 decreased with increasing temperatures and both onset and end of emergence occurred earlier at higher temperatures. Mean T_{100}-T_1 was 8.1 d across all temperature regimes (Table 2.2). Emergence started on average 10.2 DAS and ended 18.3 DAS. The population mean of ER averaged over environments was 15.4% d^{-1} and ranged between 6% d^{-1} at 9.4°C and 27% d^{-1} at 19.9°C. T_{50} of the population mean was achieved 18.5 DAS at 9.4°C and 11 DAS at 19.9°C. T_b ranged from 5.1 to 8.7°C, mean E_{TS} was 54.2°Cd and ranged between 41.6 and 93.3°Cd.

Table 2.2: Genotype mean, minimum and maximum across all nine environments and the mean, minimum, maximum and average R^2 of Finlay-Wilkinson slopes for the parameters final emergence percentage (FEP), emergence rate (ER), onset (T_1) and end (T_{100}) of emergence, the median of emergence time (T_{50}) and uniformity (T_{100}-T_1). Genotype mean, minimum and maximum for FEP under cold and normal conditions, base temperature (T_b) and thermal time (E_{TS}).

		Genotype			Average R^2
		mean	max	min	
FEP [%]	Cold	61.6	93.1	12.5	
	Normal	93.3	100	60.2	
	MW	77.0	95.7	35.8	
	FW	1	1.8	-0.06	0.80
ER [% d^{-1}]	MW	15.4	27	6.0	
	FW	1	2.4	0.3	0.78
T_1 [DAS]	MW	10.2	13.6	7.7	
	FW	1	1.5	0.6	0.95
T_{50} [DAS]	MW	14.3	18.5	11.0	
	FW	1	1.3	0.6	0.98
	T_b	7.7	8.7	5.1	
	E_{TS}	54.2	93.3	41.6	
T_{100} [DAS]	MW	18.3	22.8	13.6	
	FW	1	1.3	0.5	0.96
T_{100}-T_1 [d]	MW	8.1	11.4	4.8	
	FW	1	1.8	0.2	0.77

DAS days after sowing

Analysis of covariance revealed that both the genotype and the genotype x environment interaction (GEI) effect were significant for all analyzed traits (Table 2.3). Genotype effects were highly significant for FEP, T_1, T_{50} and ER ($p<0.001$) but also significant for T_{100} ($p<0.05$) and $T_{100}–T_1$ ($p<0.01$). Estimated h^2 was highest for FEP (0.92) (Table 2.4). For all other traits h^2 ranged between 0.73 and 0.86. Analysis of variance for FEP_{cold} and FEP_{normal} revealed that genotype and temperature effects were significant while genotype x temperature interaction effect was not statistically significant ($p=0.07$) (Table 2.5).

Results of FEP_{cold} and FEP_{normal}, stability analysis for FEP, T_1, T_{50}, T_{100}, ER, and T_{100}-T_1 as well as T_b and E_{TS} are shown in Figure 2.2. For FEP (b) and ER (g), a high genotype mean and a small FW illustrates the superiority of a genotype. A small genotype mean and FW is desirable for the traits T_1 (c), T_{50} (d), T_{100} (e) and T_{100}-T_1 (f). Ranges of FW are shown in Table 2.2. Highest variation of FW among genotypes was observed for ER. $ER_{(FW)}$ ranged from 0.3 to 2.4. Average R^2 for FW ranged between 0.77 for T_{100}-T_1 and 0.98 for T_{50} (Table 2.2). A low T_b in combination with a short E_{TS} indicates a desirable genotype (h).

Correlations between mean genotype performance and FW were significant for T_1, T_{50} and T_{100} (Table S 2.1) while correlation between $FEP_{(M)}$ and $FEP_{(FW)}$ was statistically not significant. T_b was significantly correlated to $T_{50(FW)}$ (0.54 $p<0.001$) and E_{TS} (-0.78, $p<0.001$) while correlations between $T_{50(FW)}$ and E_{TS} were statistically not significant.

Maximum value of δK occurred at K=2. Accordingly, each of the 194 lines was assigned to one of the K=2 groups, 54 lines (28%) belong to group 1 while the remaining 140 lines (72%) belong to group 2 (Figure 2.3a). Most genotypes of group 1 are members of the *S. bicolor sudanense* clusters of Figure 3b. These are the clusters from genotype 9 to 189 at the bottom and from genotype 117 to 28 on the right hand.

Table 2.3: Covariance analysis for final emergence percentage (FEP), emergence rate (ER), onset (T_1) and end (T_{100}) of emergence, the median of emergence time (T_{50}) and uniformity (T_{100}-T_1).

		Covariance analysis			
		df	Sum of Squares	Mean Squares	p
FEP	gen	193	85754	444	<.0001
	env	1	470152	470152	<.0001
	gen*env	193	52165	270	<.0001
ER	gen	193	14514	75	<.0001
	env	1	212626	212626	<.0001
	gen*env	193	47954	248	<.0001
T_1	gen	193	573	3	<.0001
	env	1	52035	52035	<.0001
	gen*env	193	2122	11	<.0001
T_{50}	gen	193	503	3	<.0001
	env	1	99248	99248	<.0001
	gen*env	193	1926	10	<.0001
T_{100}	gen	193	1063	6	0.0419
	env	1	157010	157010	<.0001
	gen*env	193	3031	16	<.0001
T_{100}-T_1	gen	193	1459.2	7.6	0.0136
	env	1	29162.1	29162.1	<.0001
	gen*env	193	2754.0	14.3	<.0001

df degree of freedom, gen genotype effect, env environment effect, gen*env interaction of genotype and environment

Table 2.4: Variance components and heritability of final emergence percentage (FEP), emergence rate (ER), onset (T_1) and end (T_{100}) of emergence, the median of emergence time (T_{50}) and uniformity (T_{100}-T_1).

	Variance components			heritability
	σ^2_G	σ^2_{GxE}	σ^2	h^2
FEP	119.7	19.2	78.4	0.92
ER	7.9	1.1	46.4	0.60
T_1	6.0	4.2	4.9	0.86
T_{50}	5.6	8.0	6.5	0.78
T_{100}	7.4	12.7	12.2	0.73
T_{100}-T_1	3.8	2.2	8.5	0.76

***,**,* significant at the 0.001, 0.01 and 0.05 probability level, σ^2_E, σ^2_G, σ^2_{GxE} and σ^2 are variances of the environment, genotype, genotype x environment interactions and error variances

Table 2.5: Analysis of variance (ANOVA) for final emergence percentage under cold (FEP_{cold}) and normal (FEP_{normal}) conditions.

	Variance analysis			
	df	Sum of Squares	Mean Squares	p
gen	193	106821	553	<.0001
temp	1	229320	229320	<.0001
gen*temp	193	20724	107	0.07
block	1	10675	10675	<.0001

df degree of freedom, gen genotype effect, temp temperature effect, gen*temp interaction of genotype and temperature

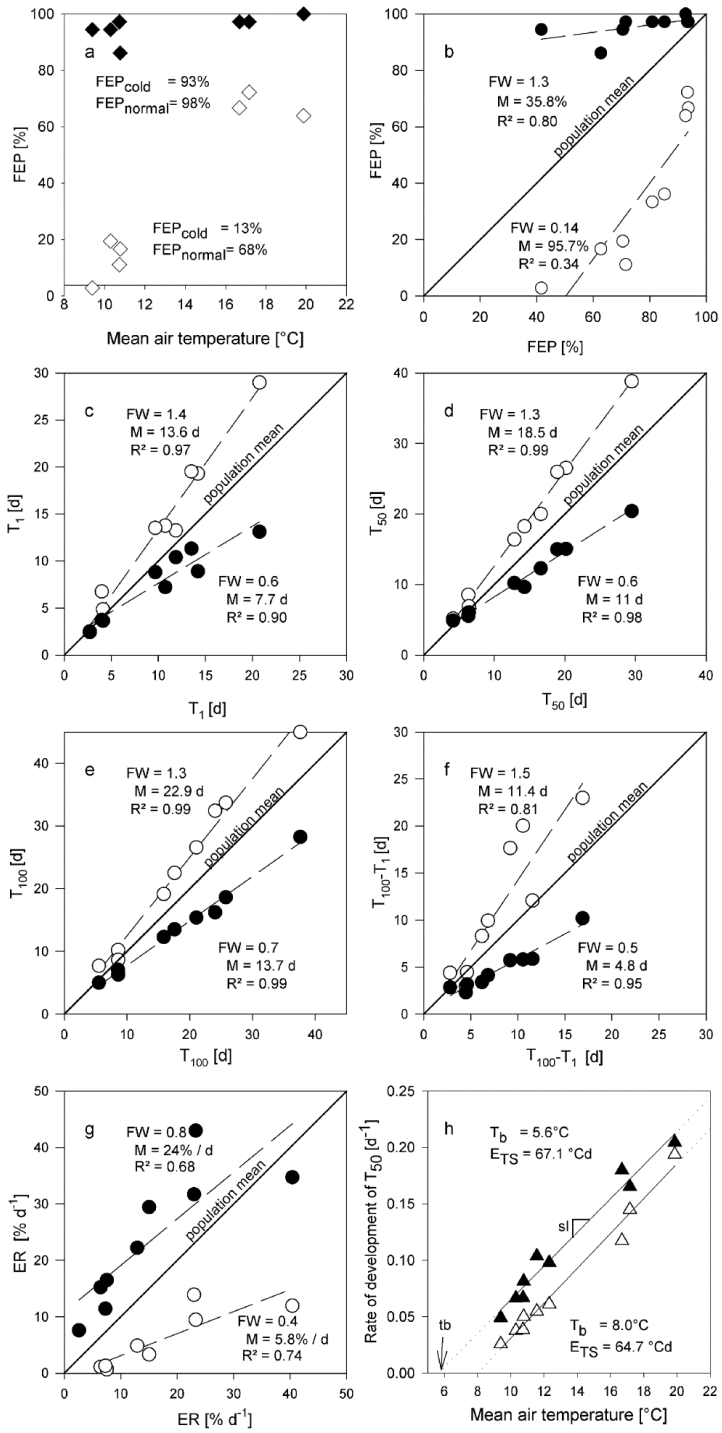

Figure 2.2 Final emergence percentage under cold (FEP_{cold}) and normal (FEP_{normal}) conditions (a), Finlay-Wilkinson regression for calculating Finlay-Wilkinson slope (FW) and across environment mean (M) for FEP (b), onset (T_1) (c), median time to emergence (T_{50}) (d), end of emergence (T_{100}) (e), uniformity (T_{100}-T_1) (f) and emergence rate (ER) (g) in nine environments and the relationship between development rates and mean air temperatures for calculating base temperature (T_b) and thermal time for emergence (E_{TS}) (h). E_{TS} is the inverse of regression slope (sl). Filled symbols indicate the genotype with highest FEP (a, b) or emergence rate (g) and development rates of T_{50} (h) or the shortest duration of T_1 (c), T_{50} (d), T_{100} (e) and uniformity (f) respectively. Unfilled symbols represent the worst performing genotype. Selection was done for each trait separately while selection criterion for T_b and E_{TS} was T_{50}.

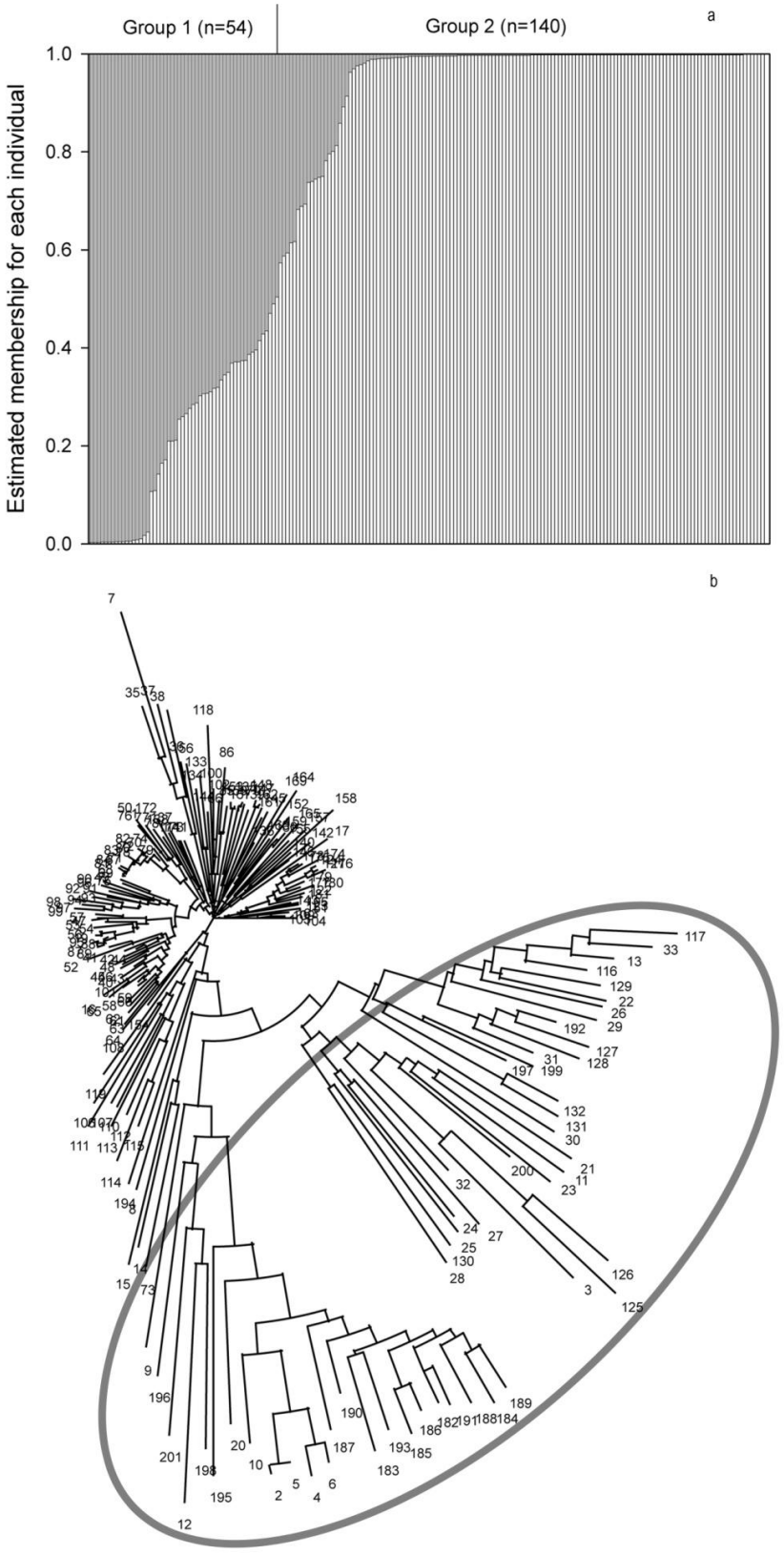

Figure 2.3 Population structure (a) and neighbor-joining dendrogram (b) of the diversity set. The population structure shows two distinct groups: Group 1 is represented by grey boxes (▭) (a) or a grey ellipse (b) and group 2 is represented by white boxes (▭).

LD in relation to the genetic distance of marker pairs on the same chromosome is shown for the whole population (Figure 2.4b) and for the two subpopulations group 1 and 2 (Figure2.4c and d). LD of marker pairs from different chromosomes is illustrated by box and whisker plots. For the whole population, significant LD ($p<0.05$) was observed for 723 marker pairs (50.6%) located on the same chromosome. Mean R^2 for all intrachromosomal marker pairs was 0.08. Group 2 showed less marker pairs (19.5%) significantly in LD compared to group 1 (28.5 %). Mean R^2 for all intrachromosomal marker pairs of group 1 was 0.08 (1427 marker pairs) and 0.05 in group 2 (1222 marker pairs). The critical R^2 value was 0.53 for the whole population and 0.54 and 0.40 for group 1 and 2, respectively. Beyond this value LD was likely to be caused by genetic linkage. Mean distance of marker pairs showing an LD beyond this threshold was 13.2 cM in the whole population while in the groups mean distance was 30 cM (group 2) and 24.4 cM (group 1). The LOESS curve did not cross the critical R^2 baseline in all cases, which gives hint that LD decayed fast. Another indicator for fast LD decay is that mean R^2 fell constantly below 0.15 if the distance was larger than 8 cM (Figure 2.4a).

A comparison of both methods, GLM without permutation test and MLM using the rank sum method according to Stich et al. (2008) shows that mean squared difference (MSD) between observed and expected p-values of GLM are for all traits higher than MSD of MLM (Table 2.6).

Table 2.6 shows the number of significant marker-trait associations identified with GLM after carrying out the permutation test and MLM. A total of 102 marker-trait associations was congruently detected by both models while 174 loci were significantly associated to one of the analyzed traits using MLM and 196 loci using GLM. The highest number of significant marker-trait associations was detected for E_{TS} and $T_{1(M)}$. Application of GLM revealed 39 marker-trait associations for E_{TS} while 14 marker-trait associations were detected using

MLM. Only 11 of the loci turned out to be significant in both models. Only 3 loci were significant applying both models on T_{100}-$T_{1(M)}$ data.

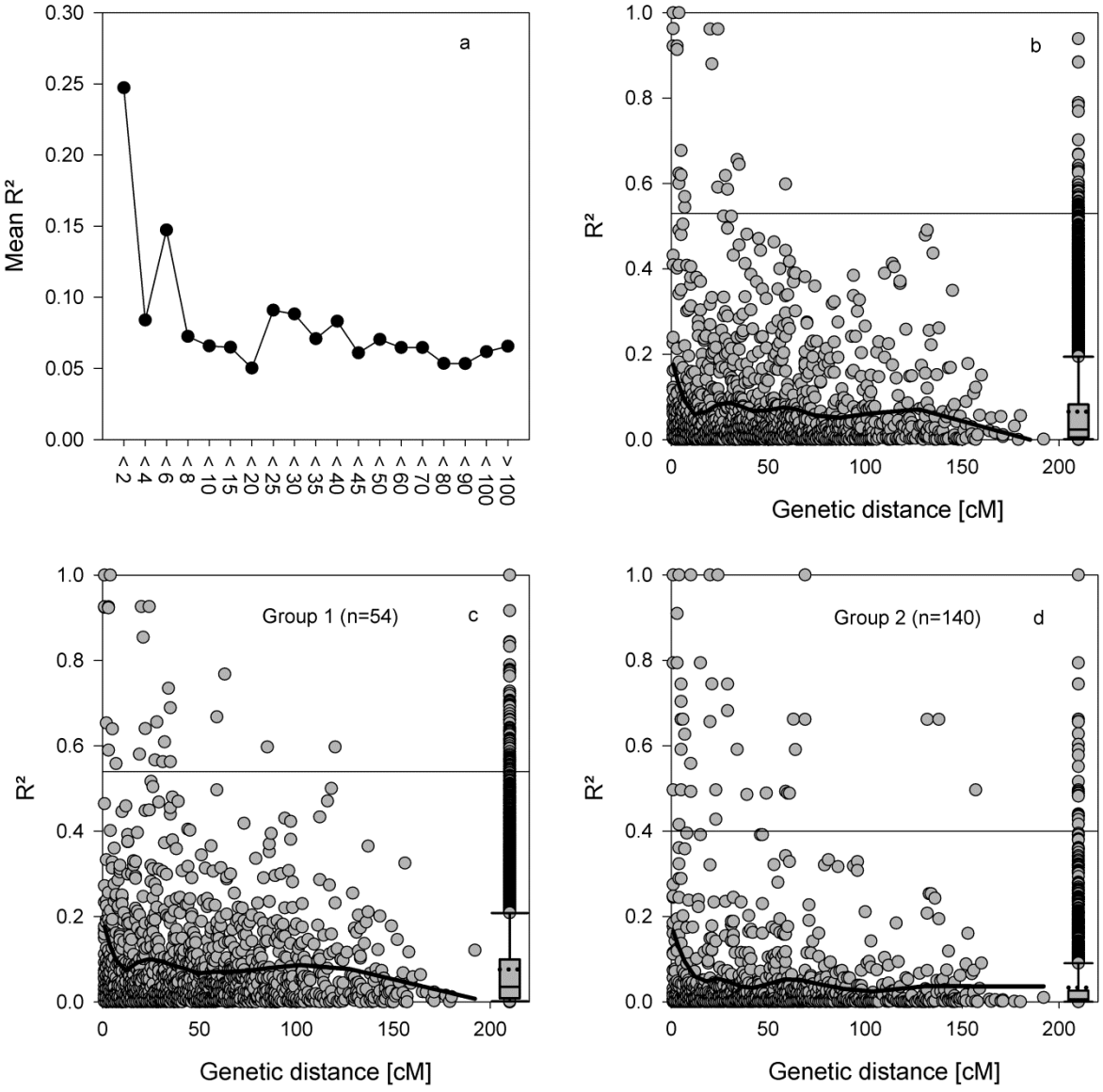

Figure 2.4 Mean R^2 values for different centimorgan (cM) classes (a). Linkage disequilibrium parameter R^2 plotted against the genetic distance in cM for the whole population (b), group 1 (54 genotypes) (c) and group 2 (140 genotypes) (d). The bottom black line shows the second degree LOESS curve. Boxplots show the distribution of R^2 derived from pairwise LD of unlinked loci, dotted lines indicate the median and straight lines represent the mean. Boxes show the 25% and 75% percentile, whiskers the 95% and 5% percentile. The critical R^2 is given by horizontal black lines.

Table 2.6: Mean squared difference (MSD) of generalized linear model (GLM) without permutation test and a mixed linear model (MLM) data, number of significant marker-trait associations using GLM with permutation test and MLM and number of significant marker-trait associations using both models.

		MSD		Number of marker-trait associations		
		GLM	MLM	GLM	MLM	GLM &MLM
FEP	Cold	0.069	0.156	7	12	5
	Normal	0.021	0.003	8	14	8
	MW	0.019	0.006	7	9	6
	FW	0.663	0.002	18	12	8
ER	MW	0.018	0.0006	7	9	6
	FW	0.312	0.001	9	9	6
T_1	MW	0.402	0.0002	12	13	9
	FW	0.139	0.031	8	11	7
T_{50}	MW	0.688	0.003	11	12	7
	FW	0.288	0.001	9	10	6
	T_b	0.744	0.001	35	10	4
	E_{TS}	0.513	0.002	39	14	11
T_{100}	MW	0.351	0.182	10	14	6
	FW	0.193	0.003	7	10	5
T_{100}-T_1	MW	0.008	0.001	3	7	3
	FW	0.009	0.001	5	8	5

FEP final emergence percentage, ER emergence rate, T_1 onset of emergence, T_{50} median time to emergence,T_{100} end of emergence, T_{100}-T_1 uniformity, FW Finlay-Wilkinson slope, MW mean across environments , cold FEP under cold conditions, normal FEP under normal conditions, T_b base temperature ,E_{TS} thermal time

Table S 2.2 shows marker-trait associations that were significant using both GLM and MLM models. Means and standard deviations for the trait values of the two groups of marker genotypes are shown. The common allele is defined as the predominant allele. Pairwise t-tests

comparing the rare and common allele revealed that 17 marker-trait associations significant in both models, GLM and MLM, were not significant. E.g., all the traits $T_{1(M)}$, $T_{1(FW)}$ and $T_{50(M)}$ were associated to marker loci sPb-6748 and sPb-3298 on chromosome SBI-09 according to GLM and MLM but the t-test showed no significance between trait values of the marker-genotype groups.

If marker-trait associations, which were not significant according to the t-test, are excluded, a total of 85 marker-trait associations are remaining for 16 traits (Figure2.5). Out of them, 24 markers were associated with only one trait and 20 markers were associated with between two and six traits. A total of 42 temperature response QTL, marker-trait associations for FW, T_b and E_{TS}, were found while 14 of them were detected on chromosome SBI-08. Some marker-traits associations were detected for genotype mean and FW at the same position, e.g. for T_{100}-T_1 on chromosome SBI-01, T_1 on chromosomes SBI-03, SBI-06 and SBI-09, T_{50} on chromosomes SBI-03, SBI-04 and SBI-09 and T_{100} on chromosomes SBI-04, SBI-08 and SBI-09. Marker-trait associations for $FEP_{(M)}$ and $FEP_{(FW)}$ did not co-localize. Four marker-trait associations for $FEP_{(M)}$ were located on chromosome SBI-01 between 25 and 66 cM. Marker-trait associations for $FEP_{(FW)}$ were found on SBI-03, SBI-04, SBI-05, SBI-08, SBI-09 and SBI-10. Co-localization of marker-trait associations for FEP_{cold} and $FEP_{(M)}$ was detected on chromosome SBI-01 and SBI-03 while marker-trait associations for FEP_{normal} did not co-localize with FEP_{cold}, $FEP_{(M)}$ or $FEP_{(FW)}$. Marker-trait associations for T_b and $T_{50(FW)}$ were co-located on chromosome SBI-01 and SBI-08, while E_{TS} and $ER_{(FW)}$ were associated with sPb-0258, sPb-1661 and sPb-1881 on chromosome SBI-08.

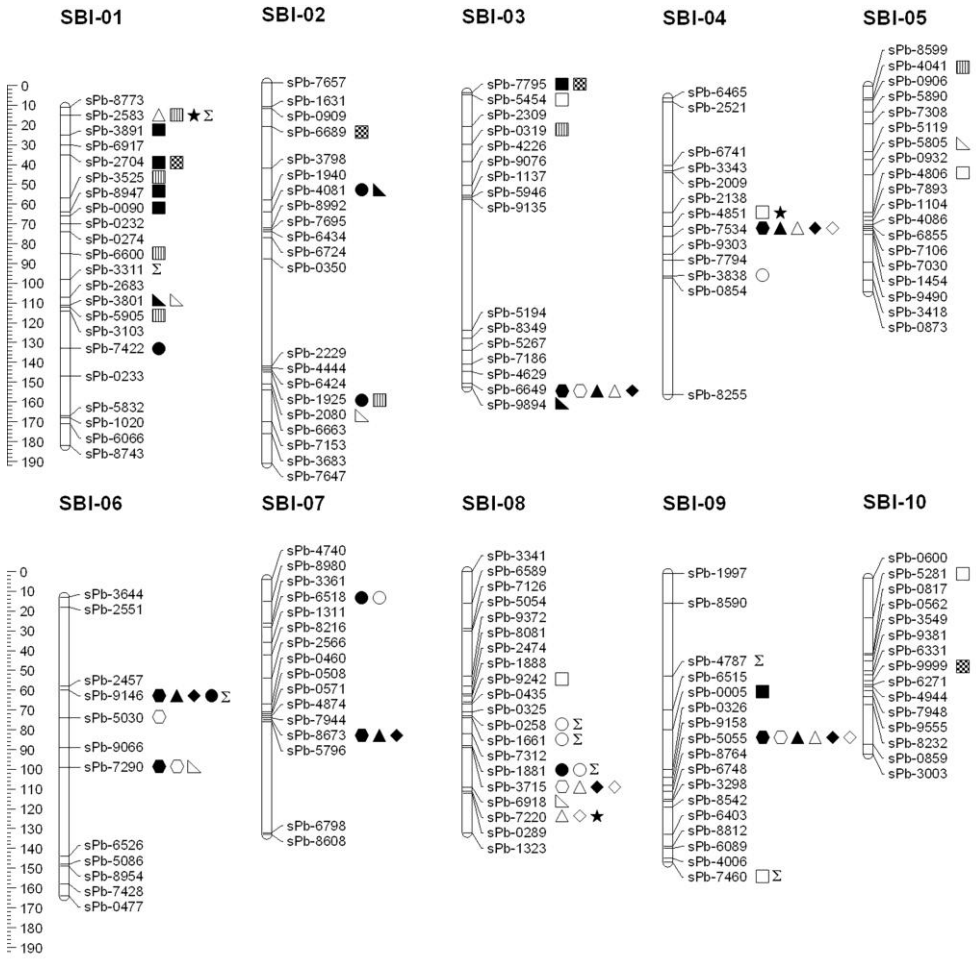

Figure 2.5 Marker-trait associations for final emergence percentage (FEP) under cold (▩) and normal (▥) conditions and for genotype mean (filled symbols) and Finlay-Wilkinson slope (unfilled symbols) of FEP (■), onset (⬣) and end (◆) of emergence, uniformity (◣), emergence rate (●) and the median of emergence time (▲) and marker-trait associations for base temperatures (★) and thermal time of T_{50} (Σ).

A positive effect of the rare allele on across environment means (reducing T_1, T_{100} and T_{100}-T_1 and increasing FEP and ER) was observed for 21 markers. The rare allele of sPb-7795 on chromosome SBI-03 increased $FEP_{(M)}$ while the rare allele of sPb-7290 on chromosome SBI-06 caused an earlier $T_{1(M)}$. Reducing T_{100}-$T_{1(M)}$ was associated with the rare alleles of sPb-3801, sPb-4081 and sPb-9894 on SBI-01, SBI-02 and SBI-03 while sPb-4081 and sPb-1925 on chromosome SBI-02 increased $ER_{(M)}$.

Discussion

Statistical models and crop models for QTL detection

The objective of the present study was to identify marker-trait associations for sorghum emergence under a broad range of temperature regimes. Sorghum cultivation in temperate climates requires the development of genotypes with high FEP under both low and optimum temperature conditions, such ideotypes should emerge uniformly and shortly after sowing. The latter makes it necessary to understand emergence as a process, which is described best by CEP, allowing a precise estimation of T_1, T_{100}, T_{50}, ER and T_{100}-T_1. Using piecewise linear regressions allowed us to describe the variability of the emergence process of 194 sorghum genotypes grown in nine temperature regimes, while it was not possible to generate model parameters for all genotypes in any environment if the Weibull function was applied (data not shown). Generally, Weibull is highly recommended for describing germination and emergence data (Brown and Mayer 1988b). However, simplicity and flexibility as well as independence and biological interpretability of all parameters (Vieth 1989) make piecewise linear regressions the model of choice, enabling the direct comparison of largely contrasting genotypes cultivated under a broad range of environmental conditions (Trudgill et al. 2000).

For marker-assisted selection it is necessary to identify QTLs that are stable across environments (Burow et al. 2011). Under situations of environmental stress, reproducibility of phenotypic data and QTL detection are low. Traits that are highly influenced by environmental factors can by definition not produce the same results in different environments. A challenge is to carry out QTL analysis directly on parameters of the response curves of a trait to its influencing factors, thus, genetic dissection of adaptation processes is done best by using mathematical functions (e.g. growth functions) for QTL detection (Reymond et al. 2003). Stability parameters are good statistical estimators for such a situation

and they have already been used to distinguish between QTL for the trait itself and for GEI effects (Kraakman et al. 2004; Lacaze et al. 2009).

In contrast to stability parameters, T_b and E_{TS} are broadly used crop modeling parameters and theoretically can be used for predicting mean emergence time of any genotype in different environments. Predicting the performance of different genotypes in different environments is a major goal for combining crop-modeling approaches with quantitative genetic analyses. However, the use of stability parameters has several advantages. Detailed environmental and climatic data is lacking in many state of the art breeding trials. Multi-environment trials with many factors, which cannot be controlled completely (e.g. temperature, soil type and structure, rainfall), are commonly used to carry out stability analyses. The linear regression model for estimating T_b and E_{TS} works since $1/T_{50}$ data of all genotypes is within the linear increase of emergence time in relation to temperature for the sampled environments. This does not hold true for all traits. Functions that fit the temperature response of FEP of individual genotypes used in the present study would include exponential, linear and monomolecular ones since not all temperatures from the minimum to the optimum for the individuals were sampled. As parameters of different functions (e.g., exponential and monomolecular) cannot be used simultaneously for QTL detection, stability parameters, which have the disadvantage not to represent real physiological responses to the environment, are an adequate compromise in many situations (El Soda et al. 2010). Stability analyses applied on data from controlled environments, e.g. varying only in temperature, are not common but have the main advantage that different reactions of genotypes can be traced back to a single influencing factor. In conclusion, QTL for FW in our study are truly temperature response QTL which did not interact with and were not affected by other environmental variables.

In our study, eleven of 32 marker-trait associations for FW co-locate with genotype mean performance QTL of the analogous trait. The only trait with no co-localization of FW and genotype mean performance was FEP. According to Kraakmann et al. (2004) the co-localization of QTL for mean trait performance and stability parameters is an indicator for genotypic differences in the allelic sensitivity, while QTL for stability parameters which are far from any QTL for the trait itself, suggest a gene regulatory network in which adaptive genes switch on or off the constitutive genes influenced by the environment. In the present study, there is always a strong positive correlation between FW and mean genotype performance of T_1, T_{50}, and T_{100}, since all genotypes emerge relatively fast under favorable conditions, while those with a low cold tolerance, emerge late at low temperatures. The same relation holds also true for T_{100}-T_1 but not for FEP. The absence of co-localization of FW and mean genotype performance QTL for FEP suggests a gene regulatory network but another reason could be that FEP is influenced by seed quality. Negative effects on seed quality may result from some extremely late flowering genotypes, i.e. seeds may not have reached maturity at harvest time and immature seeds have a reduced FEP (Shepard et al. 1996). In contrast to FEP, time of emergence includes only those seeds, which do emerge, and FEP QTL may also include QTL for flowering time. A common approach to separate seed quality QTL from QTL for cold tolerance is to use relative values (FEP at low temperatures over FEP at normal temperatures). The use of FW can be seen as a different approach to correct FEP data for differences in seed quality. A small slope indicates that FEP is not or only slightly affected by temperature regardless of FEP at higher temperatures, i.e. cold tolerant genotypes have a small $FEP_{(FW)}$ value.

Genome regions affecting the germination process

Results of the present study confirm earlier QTL studies and show that most promising regions for emergence and cold tolerance during emergence are located on SBI-01 (Knoll et al. 2008; Burow et al. 2011). Burow et al. (2011) found QTL for early emergence close to

Xtxp350 in the same region of a QTL for $FEP_{(M)}$ and FEP_{normal} at DArT markers sPb-3891 and sPb-2583, respectively (Figure 2.6). Knoll et al. (2008) described QTL for early field emergence flanked by SSR markers OPA19 and umc83. The latter was mapped close to sPb-8947 (Mace et al. 2008), which is associated with $FEP_{(M)}$ in the present study. Another interesting region is between Xtxp043 and Xtxp032, where sPb-0090 was mapped according to Mace et al. (2008). sPb-0090 is associated with $FEP_{(M)}$ and Xtxp043 is a flanking marker of a QTL detected by Burow et al. (2011). High-resolution SNP maps allowing regional association studies are needed to identify candidate genes within these important QTL regions.

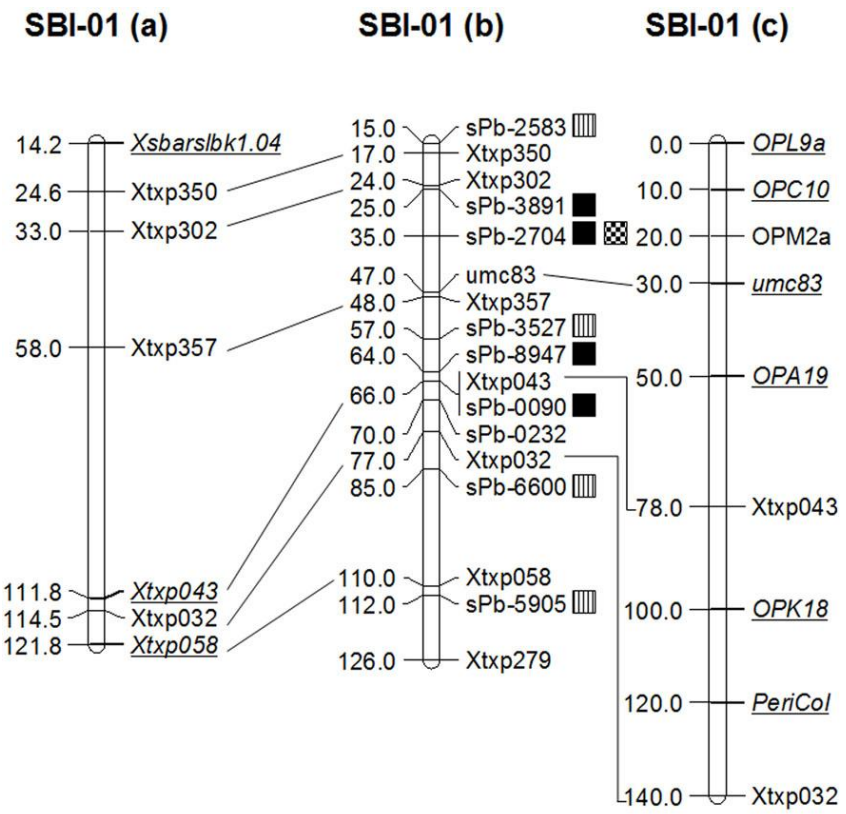

Figure 2.6 Alignment of the genetic map of chromosome SBI-01 from Burow et al. (2011) (a), the present study (b) and Knoll et al. (2008) (c). Underlined and italic markers represent flanking markers of QTL for early field emergence or germination at 30°C (a) and early vigor, early and late emergence (b). Symbols indicate final emergence percentage of over all environments (■), under cold (▩) and normal conditions (▥).

The rare allele of sPb-3801 on SBI-01 reduces T_{100}-$T_{1(M)}$ and T_{100}-$T_{1(FW)}$ and thus homogenizes the emergence process. Improving emergence percentage and uniformity leads to a better canopy establishment resulting in higher and more stable yields (Cisse and Ejeta 2003). However, low seedling vigor and a prolonged juvenile development at low temperatures may lead to a delayed canopy closure and yield reduction despite high FEPs and uniformly emerging seeds. Thus, improving cold tolerance of a crop is not simply done by improving seed emergence. QTL for early vigor and field emergence were identified between Xtxp043 and Xtxp032 by Knoll et al. (2008). QTL regions affecting emergence and seedling vigor at the same time in the same direction may be the most promising ones for improving the cold tolerance of a crop.

The QTL for T_b on SBI-01 is difficult to interpret since the marker allele, which decreases T_b, increases E_{TS}. The parameters are negatively correlated and both T_b and E_{TS} depend on the regression slope β_i (eq. 6) of the rates of development of T_{50} regressed against temperatures. If a marker allele has an effect on E_{TS} and the intersection between the regression lines of the negative and the positive allele is > 0, the increase in E_{TS} leads to a decreasing T_b and vice versa. Selection for T_b makes only sense if E_{TS} is not significantly affected (Figure 2h) or positively affected (intersection < 0) at the same time.

Knoll et al. (2008) detected a QTL for germination at high (30°C) and low (13°C) temperatures on SBI-03. The QTL region is not the same as that one we identified on SBI-03 for FEP. Flanking markers of the earlier identified QTL mapped according to Mace et al. (2008) in the large gap our map shows on SBI-03. Anyway, the region between 4 and 5cM on SBI-03 is a promising QTL region. The rare allele of sPb-7795 is associated with a positive effect on $FEP_{(M)}$ and FEP_{cold} and the rare allele of the very close marker sPb-5454 decreases $FEP_{(FW)}$, i.e., improves cold tolerance. Srinivas et al. (2009) detected QTL for maturity close

to sPb-7795 on SBI-03 (Mace and Jordan 2011), which may support the hypothesis that maturity affects seed quality.

Zhang et al. (2005) detected in a rice RIL population two QTL for germination under low temperature on chromosomes 3 and 8. Rice chromosomes 3 and 8 are widely homologous to SBI-01 and SBI-07 according to Ventelon et al. (2001) and following the nomenclature of Kim et al. (2005). A major QTL for germination at optimal temperatures was found on rice chromosome 2 in a F_2 population (Li et al. 2011), chromosome 2 is globally homologous to SBI-04 (Ventelon et al. 2001). We found marker-trait associations for $FEP_{(M)}$ on SBI-01, SBI-03 and SBI-09 and for $FEP_{(FW)}$ on SBI-04. The rare allele of sPb-4851 decreases $FEP_{(FW)}$ and T_b. Probably a reduction of T_b leads to less reductions of FEP under low temperatures.

QTL for maize germination percentage under low temperature conditions were identified on maize chromosome 4 (Hund et al. 2004). Liu et al. (2011) found QTL for maize germination percentage related to seed vigor on chromosomes 4, 7 and 10. We detected a QTL for $FEP_{(FW)}$ on SBI-04 but no QTL for $FEP_{(M)}$, FEP_{normal}, or FEP_{cold} on SBI-04 and SBI-05, which contain homologous regions of maize chromosome 4 (Whitkus et al. 1992) and for $FEP_{(FW)}$ on SBI-08 carrying homologous regions of maize chromosome 10. Another promising region on SBI-08 between 73 and 111 cM carries no FEP QTL but three marker-trait associations with a positive effect of the rare allele on $ER_{(FW)}$, E_{TS}, one QTL for T_b, and several QTL for traits related to emergence time. Limami et al. (2002) found QTL for T_{50} on maize chromosome 2, which is homologous to regions of SBI-02 and SBI-06 (Whitkus et al. 1992) and on maize chromosome 4, which is homologous to regions on SBI-04 and SBI-05 (Whitkus et al. 1992). Our results show marker-trait associations for $T_{50(M)}$ and/or $T_{50(FW)}$ on SBI-04 and SBI-06. Possibly the same genes regulate cold tolerance during emergence of maize, sorghum and rice. Also here, the identification of candidate genes is required to provide more detailed information about the genetic background.

Power and reliability of QTL detection

LD of the present sorghum population decayed within 8 cM, while average marker distance was 8.7 cM and the largest gap between markers was 66 cM. Large gaps in combination with fast LD decay make it impossible to screen the whole genome for significant marker-trait associations. However, mean LD values are useful but give no information about its local extent since high variation of LD among the genome occurs (Sorkheh et al. 2008) and LD varies also between groups of a population. We observed a higher mean R^2 and critical R^2 threshold for group 1 than for group 2. One reason could be the different population size of the groups but also differences in the number of polymorphic markers in the groups. Mean R^2 of the whole population was higher compared to values obtained by Bhosale et al. (2011) and Hamblin et al. (2004). Both studies included wild sorghum accessions. Wild sorghums have higher out-crossing rates than cultivated ones and high out-crossing rates decrease the extent of LD.

Different strategies like integrating the population structure (Pritchard et al. 2000) and familial relatedness (Yu et al. 2006) have been used to reduce false positive marker-trait associations. Kinship coefficients are used to correct association studies for familial relatedness and show the probability that homologous loci are identical by descent. The MLM approach takes both population structure and kinship matrix into account while GLM as implemented in TASSEL 2.01 uses only the population structure (Casa et al. 2008; Shehzad et al. 2009). Our results show that type I error rates of GLM are higher than those of MLM, which is in accordance with Neumann et al. (2010). Neumann et al. (2010) concluded that some associations can only be detected by GLM but, since GLM may result in many false positive marker-trait associations, both approaches GLM and MLM should be used together.

We observed that controlling GLM type I error rates with a permutation test (Churchill and Doerge 1994) reduces the number of detected marker-trait associations to a similar level as

MLM. However, approximately 50% of the identified loci were shared by applying both methods and a subsequently carried out t-test revealed that 16 of the shared marker-trait associations were not significant. In conclusion, even taking the population structure and/or familial relatedness into account both GLM and MLM may result in spurious marker-trait associations and comparing the results of different models may presently be the most useful way for detecting reliable associations (Shezad et al. 2009).

Conclusions

In accordance to previous studies we conclude from the present work that one of the most promising regions for improving FEP is located on SBI-01. However, the time-point at which emergence occurs as well as across environment stability of FEP is likely be regulated by distinct QTL regions. Piecewise linear regressions gave a good estimate of the emergence process of different genotypes. However, the emergence model in combination with stability analysis was able to precisely describe the emergence process across different temperature regimes. This combination enabled the detection of QTL for GEI effects. An interesting alternative approach is to use physiologically more meaningful parameters like T_b and E_{TS} as input traits for QTL detection. A shift in T_b without negatively affecting development processes is the most promising avenue to adapt crops to new cultivation areas with lower temperatures. However, the identification of stable markers and candidate genes for sorghum cold tolerance during emergence requires the development of high-density genetic maps.

Chapter 3

Genetic dissection of temperature dependent sorghum growth during juvenile development

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Abstract

Developing fast growing sorghum seedlings is an important breeding goal for temperate climates since low springtime temperatures are resulting in a prolonged juvenile development. The adaptation of sorghum to tropical and subtropical highlands gives hint for certain genetic variation. The goals of the present study were to detect marker-trait associations for leaf area (LGR) and dry matter growth rate (DMGR) and for chlorophyll fluorescence and content (SPAD) in relation to temperature. A diversity set comprising 194 genotypes was tested in eight controlled environments with temperatures ranging from 9.4 to 20.8 °C. Marker-trait associations were identified for each individual temperature regime and on the parameters of regression analyses describing the responses of growth or chlorophyll related traits on temperatures. The diversity set was fingerprinted with 171 diversity array technology (DArT) and 31 simple-sequence repeat (SSR) markers. SSRs were used to analyze the population structure while association studies were performed on DArT markers. Promising marker-trait associations for growth rates in relation to temperature were detected on chromosomes SBI-01, SBI-03, SBI-07, and SBI-10. Many promising loci were also significantly associated to the results obtained in individual low-temperature environments. Marker-trait associations for chlorophyll content and fluorescence did occasionally co-locate to those for growth during juvenile development but there was no evidence supporting our hypothesis that seedling growth at low temperatures is largely influenced by SPAD or fluorescence.

Keywords: sorghum, cold tolerance, juvenile development, growth rates, chlorophyll fluorescence, association mapping

Introduction

Improving sorghum cold tolerance is an important issue for breeders in order to provide farmers an alternative crop to maize for bioenergy production in temperate regions. Sorghum shows small early stage growth rates at low springtime temperatures and has high base temperatures for DMGR and LGR (Lafarge et al. 1998). However, the adaptation of sorghum to tropical and subtropical highlands gives hint for certain genetic variation in cold tolerance during juvenile development. Genotypes with high dry matter accumulation at low temperatures have early soil coverage and canopy closure (Richards 2000), which improves competitiveness with weeds, reduces water losses due to evaporation and may increase the vegetation period at the same time. Significant genotypic differences in dry matter of sorghum hybrids at early development stages were found in growth chamber experiments carried out at different temperatures (Yu and Tuinstra 2001). The authors suggested the selection of cold tolerant genotypes from growth chamber experiments since results obtained were highly correlated to field evaluation data.

High biomass accumulation is driven by high photosynthetic rates and rapid leaf growth, which may result from both high leaf appearance rates (LAR) and LGR. In general, LAR is positively linear related to temperature increases from the base (T_b) to optimum temperature. T_b is the temperature below which no growth or development takes place and temperatures above the optimum do not lead to a further increment in development rates per time unit, i.e., the temperature optimum equals to the maximum growth or development rate. Genotype specific T_b of maize varies between 2.9 and 5.0 °C and LAR, the regression slope of leaf number plotted against thermal time, ranges between 48.6 and 65.5 °Cd (Padilla and Otegui 2005). Superiority of exotic maize cultivars in LAR compared to European germplasm was observed until the third leaf stage but got lost at later development stages (Soldati et al. 1999). Genotypic differences in LAR were also observed for sorghum (Kumar et al. 2009). In

contrast to LAR, DMGR at early growth stages generally increases exponentially with increasing temperatures (Thornley and Johnson 1990). A rapid leaf area development enhances light harvesting to maximize assimilate production. Hund et al. (2008) found a high correlation between dry matter and leaf area at warm temperatures. At low temperatures dry weight was closest related to the operating efficiency of photosystem II (Φ_{PSII}). Under cold stress, photosynthetic rates may decrease due to a reduction in the membrane fluidity (Steponkus 1984), photoinhibition (Foyer et al. 2002) and changes in enzyme activities (Kocova et al. 2009). Photoinhibition affects mainly the photosystem II (PSII) while the effect on PSI is small (Krause 1988; Savitch et al. 2011). Chlorophyll fluorescence, as an indicator for the efficiency of the PSII in using photons for carbon fixation, and SPAD, which is closely related to the chlorophyll content and photosynthesis rate per unit leaf area, are useful traits to describe the photosynthetic performance of a crop under suboptimal conditions. Fluorescence was successfully used as a selection tool for cold tolerance in maize (Fracheboud et al. 1999). Trachsel et al. (2010) assumed that stage specific genetic regulation seems to play an important role since maize QTL for chlorophyll content detected during different growth stages did not co-locate. A major QTL for photosynthetic performance of maize identified only at low temperatures co-localized to a QTL for shoot dry matter accumulation, suggesting that the genetic control for photosynthesis differs depending on the temperature regime (Fracheboud et al. 2004).

Dealing with GxE interactions occurring in association studies on complex traits is important since some QTL can be found over a broad range of environments while many seem to be environment specific. Maccaferri et al. (2011) found only one stable grain yield QTL in durum wheat lines tested in environments with different soil water availability. Since the number of significant associations decreased with increasing drought stress conditions they concluded that there is limited effectiveness of association mapping under extreme conditions.

Promising tools to overcome this problem are (1) to integrate GxE interactions into the statistical framework or (2) to combine crop models with QTL analysis (Collins et al. 2008). QTL for crop model parameters were identified in bi-parental populations for maize leaf elongation rate (Reymond et al. 2003), for flowering time in barley (Yin et al. 2005), and leaf senescence in potato (Malosetti et al. 2006). QTL for parameters describing the adaptability across different temperature regimes and QTL for mean genotype performance enable to distinguish between genome regions responsible for temperature dependent control of a trait and the trait itself (Via et al. 1995; van Euwijk et al. 2010). QTL for the genotype specific response to the environment might be an important step in developing stable markers for marker-assisted selection.

The objectives of the present study were (1) to identify marker-trait associations for SPAD, chlorophyll fluorescence, and traits directly related to juvenile growth and development in eight individual environments and for simple parameters describing the adaptation to different temperature conditions, (2) to identify genetic links between chlorophyll content and fluorescence related traits and crop growth during juvenile development, (3) and to compare the results obtained by analyzing each environment separately to those of the joint analysis through regression parameters.

Material and Methods

Plant material

The study was carried out on 194 biomass sorghum lines. The diversity set includes *Sorghum bicolor* and *S. bicolor sudanense* genotypes. DNA was extracted from leaf tips using the cetyl trimethylammonium bromide (CTAB) method. The genotypes were fingerprinted with 688 polymorphic DArT markers. Marker positions were taken from Mace et al. (2008). Unmapped

markers, completely linked markers, and markers with frequencies of one allele < 5 % were removed. The final map comprised 171 informative DArT markers.

Experimental design

An experiment with eight temperature regimes was conducted in climate chambers at the Leibniz Universität Hannover. The diversity set was sown in pots with a diameter of 7 cm and filled with 50 % Klasmann Potgrond P (Klasmann-Deilmann, Groß-Hesepe, Germany) and 50 % loamy sand. Three seeds per pot and genotype were sown at 10 mm depth. All plants were grown at an optimal temperature of 25/22 °C (day/night) until most plants were in the three-leaf stage (6 d). After thinning to one plant per pot, plants were moved to different climate chambers representing the eight temperature treatments (Table S 3.1). Each single temperature treatment was designed as randomized complete block design with 3 replications of the diversity set within one climate chamber. Air temperature was measured every 5 minutes directly above the pots using TinyTag View 2 data loggers (Gemini Ltd., West Sussex, U.K.) during the entire duration of the study. Plants were grown at a photoperiod of 12 h with 10 h full light (455 µmol m^{-2} s^{-1}) and 1 h twilight in the morning and evening.

Determination of growth rates and physiological traits

The number of leaves of every plant was counted at the beginning and end of each temperature treatment. Leaf appearance rate (LAR) was calculated as follows:

$$LAR = (LN_{dn} - LN_{d6}) / n, \quad (3.1)$$

where LN_{d6} is the number of leaves 6 d after sowing, LN_{dn} is the number of leaves at the end of the experiment, and n is the number of days of the temperature treatment.

The dry matter growth rate (DMGR) was estimated as follows:

$$DMGR = (DM_{dn} - DM_{d6}) / n, \quad (3.2)$$

where DM_{dn} is the dry weight at the end of the experiment, DM_{d6} is the dry weight 6 d after sowing and n represents the number of days of temperature treatments. DM_{d6} was recorded in an additional set of plants harvested 6 d after sowing. The dry weight of leaves and stems was measured after drying at 105 °C.

Leaf area was measured at the end of temperature treatments with a leaf area meter (LICOR 3100, USA). LGR was estimated using the following equation:

$$LGR = (LA_{dn} - LA_{d6}) / n, \quad (3.3)$$

where LA_{dn} is the leaf area at the end of the experiment, LA_{d6} is the leaf area 6 d after sowing and n is the number of days of temperature treatments. An LA_{d6} of 2.2 cm² was assumed for all genotypes of the diversity set.

The greenness of the fourth leaf was recorded as mean of three measuring points using a SPAD-502plus chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan). Chlorophyll fluorescence was measured at the fourth leaf of light adapted plants with an LI-6400 instrument equipped with the LI-6400-40 pulse amplitude modulation fluorometer (LICOR, Lincoln, NE, USA) using a modified measuring protocol from Fracheboud et al. (1999). The temperature in the measurement chamber was kept at the corresponding temperature inside the growth chamber. Steady-state fluorescence (F_s') was recorded when the rate of change in fluorescence in relation to temperature (dF/dt) was < 5, indicating a stable signal. In order to

obtain the maximum fluorescence (F_m') a saturation flash of > 8000 µmol m^{-2} s^{-1} was applied for 1 s. Actinic light was turned off and leaves were illuminated with far red light to measure the ground fluorescence of light adapted leaves (F_0'). The fraction of absorbed photons used in photochemistry (Φ_{PSII}) was calculated as (F_m'- F_s')/F_m' (Genty et al. 1989). The efficiency of energy harvesting of the oxidized PSII (F_v'/F_m') was calculated as (F_m'-F_0')/F_m'.

Data analysis

Coefficients of variation (CV_g) were determined for the parameters and for each trait in every environment to describe the variation among genotypes. In addition to that, mean CVs within genotypes (CV_e) were computed based on the replications as an indicator for the error. Variance components were estimated using SAS 9.2 and broad sense heritability (h^2) was calculated according to Hill et al. (1998):

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GxE}^2 \frac{1}{n} + \sigma^2 \frac{1}{rn}} \quad , \qquad (3.4)$$

where σ^2_G is the genotypic variance, σ^2_{GxE} is the genotype x environment interaction variance, σ^2 is the error variance, r is the number of replications, and n is the number of environments. Analysis of variance (ANOVA) was carried out using the following model with i = 1,2,3,…,a genotypes, j = 1,2,3,…,n environments and k = 1,2,3,…,b genotype x environment interactions:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}, \qquad (3.5)$$

where μ is the overall mean, α_i is the effect of the i^{th} genotype, β_j is the effect of the j^{th} environment, γ_{ij} is the genotype x environment interaction, and ε_{ijk} is the random error.

Linear regression analysis was carried out on LAR, SPAD, F_v'/F_m' and Φ_{PSII} data from the nine temperature regimes using the following model:

$$y_{ij} = \beta_i + sl_i x_j + \varepsilon_{ij}, \quad (3.6)$$

where y_{ij} is trait value of the i^{th} genotype in the j^{th} temperature regime, β_i is the estimated intercept and sl_i the regression slope of the i^{th} genotype, x_j is the temperature of the j^{th} environment and ε_{ij} is a random error. Base temperature (T_b) was estimated by linear extrapolation to define the theoretical temperature below which LAR, SPAD, F_v'/F_m', and Φ_{PSII} become 0:

$$T_{bi} = -\beta_i / sl_i, \quad (3.7)$$

An exponential function was used to describe the relation between DMGR or LGR, respectively, and temperature:

$$GR_{ij} = GR_{0i}\, e^{(a\,(T - T_0))}, \quad (3.8)$$

where GR_{ij} is the growth rate of the i^{th} genotype in the j^{th} environment, GR_0 is the estimated *GR* in the lowest temperature environment T_0 (9.4 °C for DMGR and 13.5 °C for LGR), *a* is the exponent and *T* the temperature. Pearson`s correlation coefficients were calculated between parameters and the respective trait values of the individual environments and between regression parameters and across environment means of the traits using SAS 9.2.

171 DArT markers and 31 SSR markers were used to analyze the population structure of 194 individuals with the software package STRUCTURE assuming an admixture model (Pritchard et al. 2000) and using a burn-in phase of 10,000 iterations followed by 10,000 Markov chain Monte Carlo iterations in order to detect the ‘‘true’’ number of *K* groups in the range of *K* =

1–20 possible groups. *dK* was calculated according to Evanno et al. (2005). Prior to association mapping data were arcsine-square root transformed in order to achieve approximately normal distribution. For identifying significant associations between 171 DArT markers and the traits Tassel 2.1 was used (Bradbury et al. 2007). The SSR based Q-matrix and a kinship matrix were used in a mixed linear model (MLM) (Zhang et al. 2010). Association studies were carried out for all traits in each individual environment, for mean genotype performance across all environments, and for regression parameters.

Results

Figure 3.1 shows that LAR, SPAD, F_v'/F_m' and Φ_{PSII} are linearly related to temperature while DMGR and LGR increase exponentially with increasing temperatures within the range of environmental conditions used in the experiments. DMGR of the population mean across all environments was 0.009 g d^{-1} and ranged between 0.0003 at 9.4 °C and 0.037 g d^{-1} at 20.8 °C. LGR of the best performing genotype was on average over all environments 6.6 cm^2 d^{-1} while LGR of the worst performing genotype was 1.09 $cm^2 d^{-1}$ (Figure 3.1). At 20.8 °C population mean for LAR was 0.32 d^{-1}. No increase in leaf number was observed at 9.4 °C (Table 3.1).

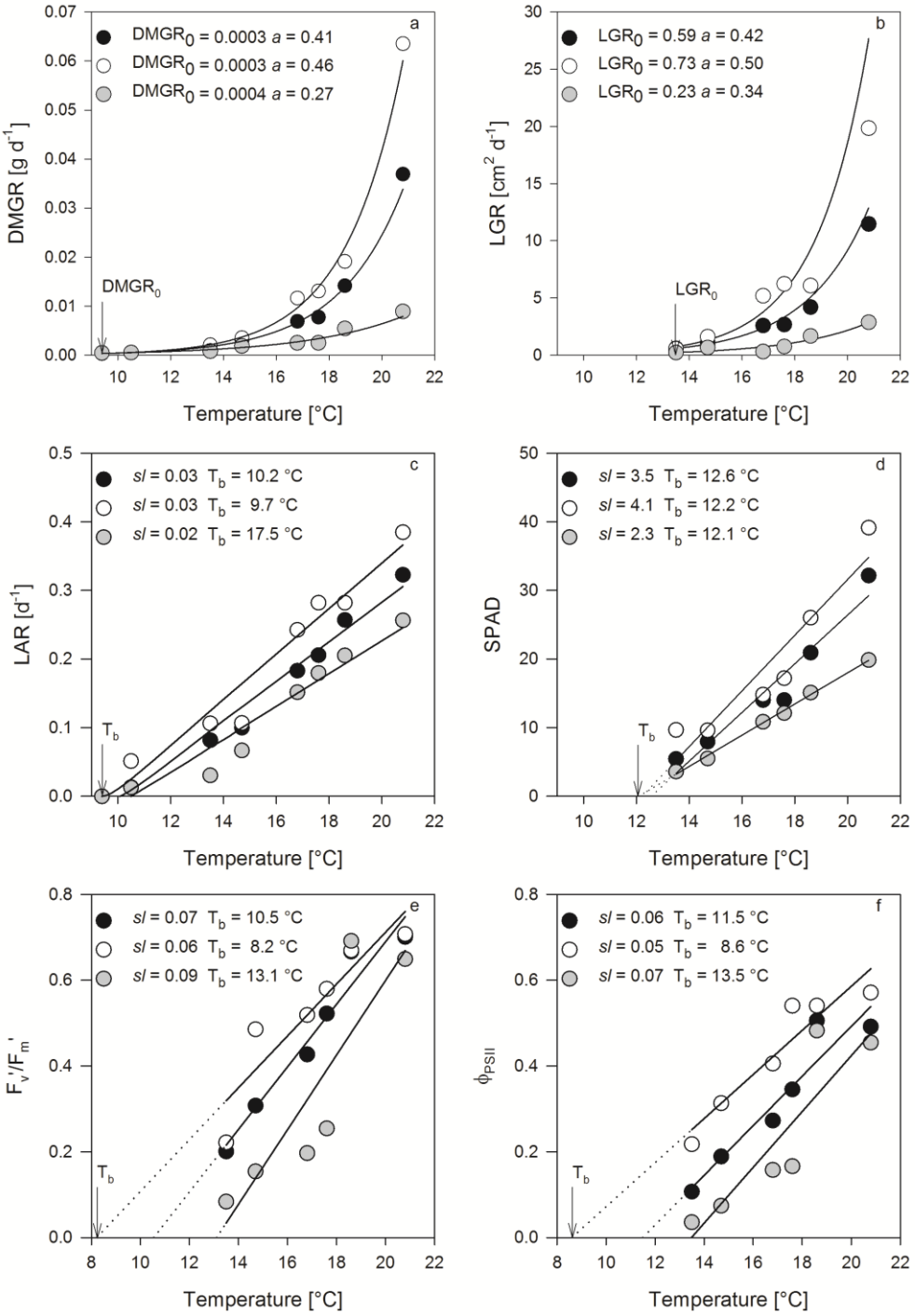

Figure 3.1: Relationship between dry matter growth rates (DMGR) (a), leaf area growth rates (LGR) (b), leaf appearance rates (LAR) (c), chlorophyll contents (SPAD) (d), fluorescence (Φ_{PSII} (e) and F_v'/F_m' (f)] and temperature for calculating the exponent (*a*), initial growth rates ($DMGR_0$, LGR_0), temperature effects (*sl*) and base temperatures (T_b). Black circles (●) indicate population means, unfilled circles (○) represent the best and grey circles (◯) the worst performing genotype. Selection criterion was mean across environments.

Table 3.1: Genotype means, ranges and coefficients of variation among (CV_g) and within genotypes (CV_e) for averages across all environments, the parameters regression slope (*sl*) and base temperature (T_b) of leaf appearance rate (LAR), chlorophyll content (SPAD), and chlorophyll fluorescence (F_v'/F_m' and Φ_{PSII}), and for exponents (*a*) and initial growth rates ($DMGR_0$ or LGR_0) of dry matter growth rate (DMGR) and leaf area growth rate (LGR). Mean R^2 values of regression analyses are shown.

	Genotype mean, min and max across all environments						*sl* or *a*						T_b or LGR_0 or $DMGR_0$					R^2
	mean	min	max	CV_g [%]	CV_e [%]		mean	min	max	CV_g [%]	CV_e [%]		mean	min	max	CV_g [%]	CV_e [%]	
DMGR	0.009	0.003	0.014	25.3	16.5	$DMGR_{(a)}$	0.41	0.27	0.56	11.8	11.2	$DMGR_0$	0.0003	0.0001	0.0007	36.1	35.8	0.97
LGR	3.78	1.09	6.59	23.8	13.5	$LGR_{(a)}$	0.44	0.26	0.77	15.4	19.5	LGR_0	0.57	0.09	1.24	36.7	38.5	0.94
LAR	0.15	0.11	0.18	9.0	6.4	$LAR_{(sl)}$	0.03	0.02	0.04	8.6	10.4	$LAR_{(Tb)}$	10.2	9.2	10.9	2.8	4.8	0.95
SPAD	15.76	11.17	19.41	9.9	9.0	$SPAD_{(sl)}$	3.55	2.05	4.94	16.7	17.2	$SPAD_{(Tb)}$	12.5	9.4	13.7	5.7	7.9	0.90
F_v'/F_m'	0.47	0.34	0.58	9.3	6.2	$F_v'/F_m'_{(sl)}$	0.07	0.04	0.09	11.4	12.2	$F_v'/F_m'_{(Tb)}$	10.5	8.0	13.1	9.6	12.3	0.89
Φ_{PSII}	0.32	0.23	0.45	11.3	21.5	$\Phi_{PSII(sl)}$	0.06	0.03	0.09	17.4	13.0	$\Phi_{PSII(Tb)}$	11.3	8.0	14.0	9.7	10.2	0.79

Estimations for T_b of LAR ($LAR_{(Tb)}$) varied between 9.2 and 10.9 °C. SPAD of the population mean averaged over the environments was 15.8 and ranged between 11.2 and 19.4. Mean F_v'/F_m' and Φ_{PSII} across treatments were 0.47 and 0.32, respectively. CV_g of the estimated DMGR at the lowest temperature ($DMGR_0$) was 36 %, and CV_g of *a* of DMGR ($DMGR_{(a)}$) was 11.8 % (Table 3.1). CV_e of $DMGR_{(a)}$ and *a* of LGR ($LGR_{(a)}$) were also relatively low, while the comparatively high CV_g for $DMGR_0$ and for the estimated LGR at the lowest temperature (LGR_0) corresponded to a high CV_e.

Analysis of variance (ANOVA) revealed that both environment and genotype effects were significant for all analyzed traits (Table 3.2). Genotype x environment interaction effects were highly significant for DMGR, LGR, LAR, SPAD and F_v'/F_m' ($p < 0.001$) but not significant for Φ_{PSII} ($p = 0.06$). Estimated h^2 was lowest for Φ_{PSII} (0.34). For all other traits h^2 ranged between 0.46 and 0.67.

Table 3.2: Variance components and heritability for dry matter growth rate (DMGR), leaf area growth rate (LGR), leaf appearance rate (LAR), chlorophyll content (SPAD), and chlorophyll fluorescence (Φ_{PSII} and F_v'/F_m').

	Variance components [a]				heritability
	σ^2_E	σ^2_G	σ^2_{GxE}	σ^2	h^2
DMGR [g d^{-1}]	0.00017***	0.000003***	0.000017***	0.000003	0.60
LGR [cm^2 d^{-1}]	16.09***	0.56***	1.03***	1.81	0.67
LAR [d^{-1}]	0.01166***	0.00014***	0.00013***	0.00117	0.62
SPAD	94.42***	1.37***	1.75***	12.57	0.58
F_v'/F_m'	0.0453***	0.0005***	0.0027***	0.0003	0.53
Φ_{PSII}	0.0293***	0.0004***	0.0013n.s.	0.0056	0.46

***, **, * significant at the 0.001, 0.01, or 0.05 probability level

σ^2_E, σ^2_G, σ^2_{GxE}, and σ^2 are environment, genotype, genotype x environment interaction and error variances

Pearson`s correlation coefficients between response curve parameters and trait values of single temperature regimes are presented in Table 3.3. Highest correlation coefficients were found between mean DMGR ($DMGR_{(mean)}$) and mean LGR ($LGR_{(mean)}$) and DMGR and LGR at 20.8 °C. $DMGR_0$ and LGR_0 were highly correlated with DMGR and LGR at 9.4 or 13.5 °C, respectively. $DMGR_{(a)}$ and $LGR_{(a)}$ were negatively correlated to DMGR and LGR at low temperature regimes. In case of LAR and SPAD, highest correlations were found between the slopes of LAR ($LAR_{(sl)}$) and SPAD ($SPAD_{(sl)}$) and the respective trait values at 20.8 °C. Highly negative correlations were observed between T_b and the low temperature environment trait values of LAR, SPAD, F_v'/F_m', and Φ_{PSII}. Pearson`s correlation coefficients between across environment means of the traits revealed that DMGR and LGR were highly correlated while both traits were not significantly correlated to LAR (Table S 3.2). Mean SPAD was significantly correlated to all other traits except $LAR_{(mean)}$ and $LAR_{(Tb)}$. The genotype with the highest DMGR across all environments had a much higher LGR, SPAD and Φ_{PSII} in comparison to the population mean, while LAR and F_v'/F_m' were only slightly increased (Figure 3.2).

There was for all traits a strong negative correlation between temperature and CV_g (Table 3.4). The correlation between temperature and CV_e was always negative as well and there was a strong correlation between CV_g and CV_e (R = 0.96).

Table 3.3: Pearson's correlation coefficients between the traits dry matter growth rate (DMGR), leaf area growth rate (LGR), leaf appearance rate (LAR), chlorophyll content (SPAD) and chlorophyll fluorescence (F_v'/F_m' and Φ_{PSII}) measured in each temperature regime and means across environments, exponents (*a*), initial growth rates ($DMGR_0$ and LGR_0), temperature effects (*sl*) and base temperatures (T_b).

	Temperature	DMGR			LGR			LAR		
	[°C]	mean	*a*	$DMGR_0$	mean	*a*	LGR_0	mean	*sl*	T_b
DMGR/LGR/LAR in single environments	20.8	0.95***	0.38***	0.22**	0.94***	0.15*	0.43***	0.72***	0.82***	0.21*
	18.6	0.82***	0.29***	0.27***	0.79***	0.09n.s.	0.42***	0.62***	0.60***	-0.02n.s.
	17.6	0.77***	0.24***	0.37***	0.78***	-0.05n.s.	0.56***	0.52***	0.48***	-0.04n.s.
	16.8	0.75***	0.14*	0.46***	0.77***	-0.04n.s.	0.55***	0.63***	0.47***	-0.21**
	14.7	0.57***	0.01n.s.	0.46***	0.65***	-0.41***	0.74***	0.47***	0.25**	-0.32***
	13.5	0.47***	0.02n.s.	0.45***	0.47***	-0.74***	0.92***	0.60***	0.23**	-0.56***
	10.5	0.26***	-0.47***	0.70***				0.24***	-0.20**	-0.68***
	9.4	0.34***	-0.61***	0.86***						

***, **, * significant at the 0.001, 0.01, and 0.05 probability level

Table 3.3: continued

	Temperature	SPAD			F_v'/F_m'			Φ_{PSII}		
	[°C]	mean	*sl*	T_b	mean	*sl*	T_b	mean	*sl*	T_b
SPAD, F_v'/F_m', Φ_{PSII} in single environments	20.8	0.74***	0.85***	0.49***	0.25***	0.16*	0.02n.s.	0.43***	0.77***	0.47***
	18.6	0.73***	0.65***	0.27***	0.25***	0.13n.s.	-0.01n.s.	0.37***	0.18*	-0.07n.s.
	17.6	0.65***	0.31***	-0.08n.s.	0.71***	-0.09n.s.	-0.35***	0.68***	0.11n.s.	-0.29***
	16.8	0.55***	0.23***	-0.09n.s.	0.76***	-0.21**	-0.45***	0.54***	-0.08n.s.	-0.36***
	14.7	0.29***	-0.32***	-0.55***	0.58***	-0.71***	-0.76***	0.52***	-0.40***	-0.67***
	13.5	0.33***	-0.45***	-0.74***	0.48***	-0.69***	-0.67***	0.38***	-0.32***	-0.48***

***, **, * significant at the 0.001, 0.01, and 0.05 probability level

Table 3.4: Coefficients of variation among genotypes (CV_g) and within genotypes (CV_e) for dry matter growth rate (DMGR), leaf area growth rate (LGR), leaf appearance rate (LAR), chlorophyll content (SPAD), and chlorophyll fluorescence (Φ_{PSII} and F_v'/F_m') in different temperature regimes.

Temperature	DMGR		LGR		LAR		SPAD		F_v'/F_m'		Φ_{PSII}	
	CV_g	CV_e	CV_g	CV_e	CV_g	CV_e	CV_g	CV_e	CV_g	CV_e	CV_g	CV_e
20.8	29.5	23.5	28.0	22.6	10.5	8.5	11.5	10.2	11.5	1.7	14.8	18.7
18.6	28.9	28.1	28.9	30.7	14.2	13.4	14.9	17.9	14.9	2.4	12.0	12.0
17.6	27.7	27.2	29.6	28.4	13.0	15.6	19.4	23.7	19.4	13.5	26.5	25.4
16.8	32.7	25.8	31.0	28.5	12.6	11.9	16.6	21.1	16.6	16.0	30.3	31.0
14.7	33.8	40.4	33.5	39.6	16.6	21.4	22.8	30.7	22.8	27.4	34.6	34.8
13.5	37.5	47.2	45.4	53.3	27.6	30.2	43.3	63.2	43.3	33.9	32.8	33.7
10.5	31.9	44.0			106.1	117.7						
9.4	51.7	37.2										
R	-0.74	-0.79	-0.84	-0.90	-0.80	-0.82	-0.85	-0.87	-0.94	-0.97	-0.86	-0.82

R: Pearson`s correlation coefficient between temperature and CV

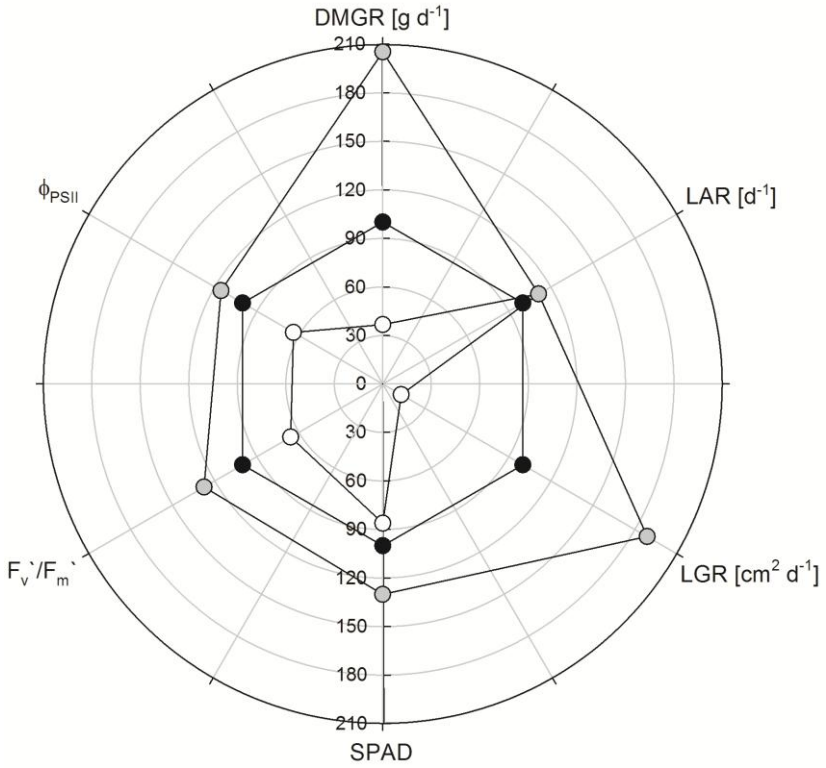

Figure 3.2: Percentage deviations for the best (◌) and worst (○) performing genotype compared to the population mean (●) for the traits dry matter growth rate (DMGR), leaf appearance rate (LAR), leaf area growth rate (LGR), chlorophyll content (SPAD), and fluorescence (Φ_{PSII} and F_v'/F_m'). Selection criterions were highest or lowest DMGR at 13.5°C.

Figure 3.3 shows the estimated population structure for 194 individuals based on 31 SSR or 171 DArT markers, respectively. The population consists of two distinct groups. According to DArT marker data, 140 lines (72 %) belong to group 1 while 54 lines (28 %) belong to group 2. Using SSRs, 131 genotypes (68 %) were considered to belong to group 1 while 61 genotypes (32 %) were part of group 2.

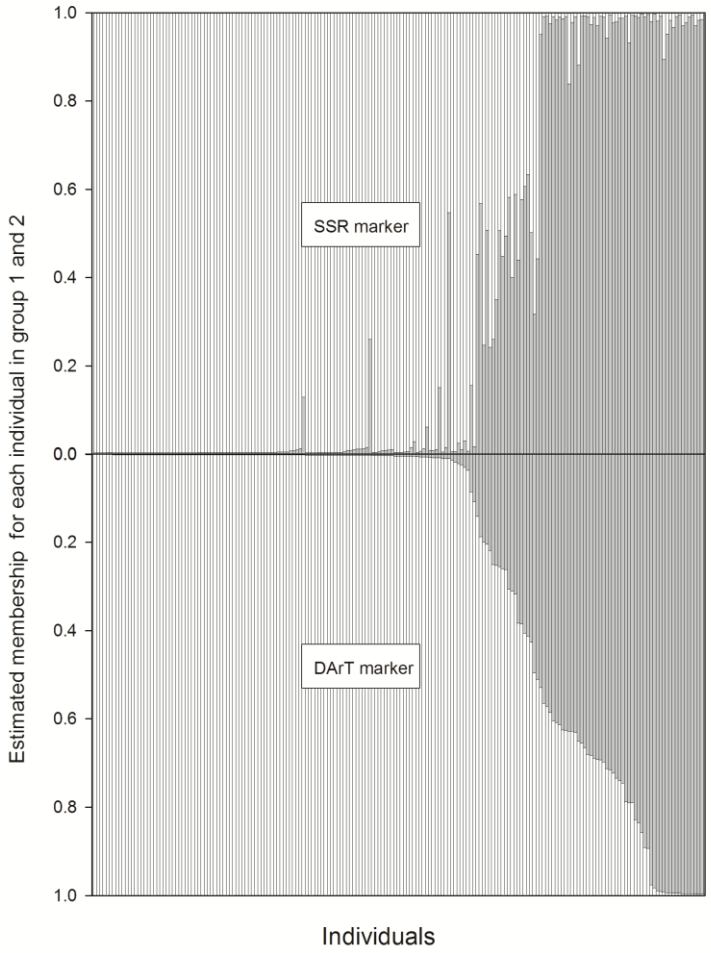

Figure 3.3: Estimated population structure for 194 individuals of a diversity set using 31 SSR markers and 171 DArT markers. Both marker systems distinguish between group 1 (▭) and group 2 (▭).

A total of 138 marker-trait associations were identified for the response curve parameters and 449 QTL were detected in the individual environments (Table 3.5). The highest number of significant marker-trait associations was found for DMGR; 22 marker-trait associations for DMGR were found at 13.5 °C. The number of significant marker-trait associations for response curve parameters ranged between four ($\Phi_{PSII(sl)}$) and 18 ($DMGR_0$).

Table 3.5: Number of marker-trait associations for dry matter growth rate (DMGR), leaf are growth rate (LGR), leaf appearance rate (LAR), chlorophyll content (SPAD), and chlorophyll fluorescence (F_v'/F_m' and Φ_{PSII}) in each environment and number of marker-trait associations for means across environments, exponents (*a*), initial growth rates ($DMGR_0$ and LGR_0), temperature effects (*sl*), and base temperatures (T_b).

Temperature	Number of marker-trait associations					
[°C]	DMGR	LGR	LAR	SPAD	F_v'/F_m'	Φ_{PSII}
20.8	5	8	8	14	15	3
18.6	10	10	10	12	10	13
17.6	9	7	7	13	15	12
16.8	11	11	8	17	12	11
14.7	15	14	8	11	14	15
13.5	22	15	11	13	8	12
10.5	18		6			
9.4	16					
Mean	11	9	9	14	12	12
a or *sl*	11	16	6	13	15	4
T_b/$DMGR_0$/ LGR_0	18	15	9	12	10	9

The marker with the highest number of associations to the traits was sPb-4874 on chromosome SBI-07 (Figure 3.4, Table S 3.3). The marker was significantly associated with DMGR in seven of the eight environments while 32 marker-trait associations for DMGR were found in only one environment. Marker-trait associations found in only one environment were rarely co-located with QTL for response curve parameters. 29 marker-trait associations for DMGR in individual environments were co-located with QTL for $DMGR_{(mean)}$ while only 12 QTL for LAR in the different temperature regimes coincided with QTL for $LAR_{(mean)}$.

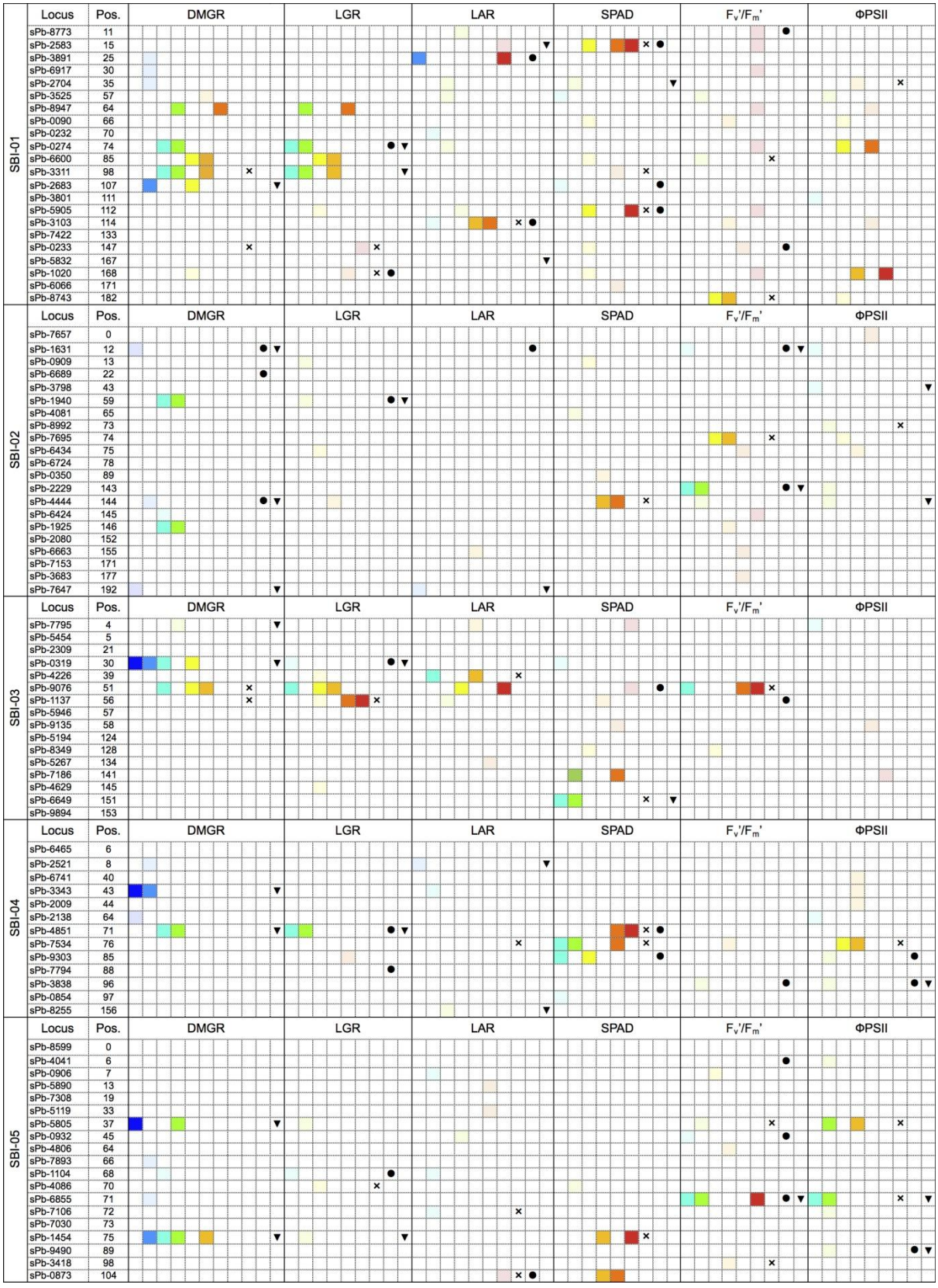
SBI-01
Locus
Pos.
DMGR
LGR
LAR
SPAD
Fv'/Fm'
ΦPSII
sPb-8773 11
sPb-2583 15
sPb-3891 25
sPb-6917 30
sPb-2704 35
sPb-3525 57
sPb-8947 64
sPb-0090 66
sPb-0232 70
sPb-0274 74
sPb-6600 85
sPb-3311 98
sPb-2683 107
sPb-3801 111
sPb-5905 112
sPb-3103 114
sPb-7422 133
sPb-0233 147
sPb-5832 167
sPb-1020 168
sPb-6066 171
sPb-8743 182
SBI-02
Locus
Pos.
DMGR
LGR
LAR
SPAD
Fv'/Fm'
ΦPSII
sPb-7657 0
sPb-1631 12
sPb-0909 13
sPb-6689 22
sPb-3798 43
sPb-1940 59
sPb-4081 65
sPb-8992 73
sPb-7695 74
sPb-6434 75
sPb-6724 78
sPb-0350 89
sPb-2229 143
sPb-4444 144
sPb-6424 145
sPb-1925 146
sPb-2080 152
sPb-6663 155
sPb-7153 171
sPb-3683 177
sPb-7647 192
SBI-03
Locus
Pos.
DMGR
LGR
LAR
SPAD
Fv'/Fm'
ΦPSII
sPb-7795 4
sPb-5454 5
sPb-2309 21
sPb-0319 30
sPb-4226 39
sPb-9076 51
sPb-1137 56
sPb-5946 57
sPb-9135 58
sPb-5194 124
sPb-8349 128
sPb-5267 134
sPb-7186 141
sPb-4629 145
sPb-6649 151
sPb-9894 153
SBI-04
Locus
Pos.
DMGR
LGR
LAR
SPAD
Fv'/Fm'
ΦPSII
sPb-6465 6
sPb-2521 8
sPb-6741 40
sPb-3343 43
sPb-2009 44
sPb-2138 64
sPb-4851 71
sPb-7534 76
sPb-9303 85
sPb-7794 88
sPb-3838 96
sPb-0854 97
sPb-8255 156
SBI-05
Locus
Pos.
DMGR
LGR
LAR
SPAD
Fv'/Fm'
ΦPSII
sPb-8599 0
sPb-4041 6
sPb-0906 7
sPb-5890 13
sPb-7308 19
sPb-5119 33
sPb-5805 37
sPb-0932 45
sPb-4806 64
sPb-7893 66
sPb-1104 68
sPb-4086 70
sPb-6855 71
sPb-7106 72
sPb-7030 73
sPb-1454 75
sPb-9490 89
sPb-3418 98
sPb-0873 104

Figure 3.4: Marker-trait associations for dry matter growth rate (DMGR), leaf area growth rate (LGR), leaf appearance rate (LAR), chlorophyll content (SPAD), and fluorescence (F_v'/F_m' and Φ_{PSII}) in eight different temperature regimes (9.4 | 10.5 | 13.5 | 14.7 | 16.8 | 17.6 | 18.6 | 20.8 °C) and for means across environments (×), exponents or temperature effects (●) and initial growth rates or base temperatures (▼). Pale colors were used for marker-trait associations significant in only one environment.

QTL for $DMGR_{(a)}$ were found at the same positions of markers associated with DMGR at low temperature regimes. Nine marker-trait associations for $DMGR_0$ co-located with QTL for DMGR at 9.4 °C. The highest number of co-localizations of *sl* and trait QTL detected in only one environment was observed for SPAD. Eight $SPAD_{(sl)}$ marker-trait associations co-located with QTL for SPAD at 20.8 °C. Likewise, QTL for $LAR_{(sl)}$ often co-located with QTL for LAR obtained in the high temperature treatments. Most of the significant marker-trait associations for T_b of LAR, SPAD, F_v'/F_m' and Φ_{PSII} coincided with QTL for the same traits at low temperatures regimes.

Marker-trait associations for DMGR and LGR were co-localized on all chromosomes. Promising regions were identified on chromosome SBI-01 between 74 and 107 cM and on chromosome SBI-03 between 30 and 56 cM. The region on SBI-03 carries also QTL for LAR and F_v'/F_m'. Another interesting region was identified on chromosome SBI-07. sPb-4874 was associated with DMGR in many temperatures and with $SPAD_{(sl)}$ and $SPAD_{(Tb)}$. Further co-localizations between QTL for DMGR and SPAD temperature response parameters were detected on chromosome SBI-10 between 42 and 46 cM.

Discussion

Multi-environment data in association mapping

For analyzing juvenile development, a certain leaf number is often the harvest time criterion, which makes results comparable. Testing many different lines in different environments makes the use of a fixed leaf number as harvest time criterion nearly impossible. To work with growth rates, as done in the present study, has the advantage of being widely independent of exactly identical harvest times, if the goal is to compare results or analyze them together. Plant growth rates change during different development stages (El-Lithy et al. 2004), however, within short periods during certain development stages as the juvenile phase

or later pre-flowering development stages, growth rates can assumed to be constant. Relating growth rates to temperature enabled us to dissect the genetic basis of processes regulated by temperature, and parameterizing simple functions allowed us to characterize the genotype specific temperature response of sorghum during juvenile development.

For marker-assisted selection, the identification of QTL, which are stable across environments, is required (Burow et al. 2011) but the detection of stable QTL across experimental conditions is difficult even in controlled experiments, varying only in one environmental factor. In the present study, one QTL on SBI-07 for DMGR was found in seven of eight environments while many marker-trait associations were environment specific. Maccaferri et al. (2011) suggested that the lack of stable marker-trait associations is due to similar phenotypes of genotypes, which have different physiological mechanisms to cope with stress, if complex traits like grain yield are analyzed in diverse populations. They detected less QTL under more stressful situations. Since the plant material of the present study shows strong variation in the adaptation to low temperatures and more similar phenotypes under optimum growing conditions, we detected a decreased number of marker-trait associations with increasing temperatures. However, if the chance for the detection of a QTL was highest due high phenotypic variation, the increasing error or variation within genotypes in the more stressful environment made the circumstances for the identification of a marker-trait association sub-optimal. Consequently, it might be useful to find the optimum compromise between the variation within and among genotypes for each trait or to increase the number of observations if stress increases.

It was suggested that QTL mapping approaches using repeated measurements on growth curves and functions describing the adaptation to environmental factors, provide maximum

information about QTL effects and positions and reduce random errors (Ma et al. 2002; Reymond et al. 2003; Uptmoor et al. 2009). Our results show a very similar trend for the model parameters *sl*, *a*, T_b, $DMGR_0$, and LGR_0. A high CV_g, which is advantageous for the detection of significant marker-trait associations, came always along with an increasing CV_e. Using more observations from extreme environments as carried out by Fiedler et al. (2012) may increase the accuracy of parameter estimations if linearity can be assumed. We often found non-significant correlations between *a* or *sl* and the trait values at intermediate temperature regimes, suggesting that mainly the high and low temperature environments contributed to the parameter estimation.

Sadok et al. (2007) found no co-localization of QTL for response parameters and QTL detected in stress environments and concluded that trait QTL, which were detected in stress environments, might have another genetic network than QTL for response parameters. We found co-localizations between low-temperature and response curve QTL especially for LGR but also for other traits. Most co-localizations were found between QTL for treatments at low temperatures and T_b or $DMGR_0$ and LGR_0, respectively. These parameters are closely correlated to the response parameters *sl* and *a*, i.e, small *sl* or *a* parameters lead to a low T_b, $DMGR_0$, or LGR_0 and co-localizations between QTL for the parameters and/or low temperature QTL are likely to occur.

Identification of physiological mechanisms, which promote growth at low temperatures

We analyzed plant growth and several chlorophyll content and fluorescence related traits and LAR in order to see if these traits may have positive impacts on crop performance under low temperatures. We assumed high correlations between these traits and plant growth and that the co-localization of marker-trait associations are strong indicators for significant influences

on cold tolerance. Across environment means of SPAD values were significantly correlated with DMGR and LGR. QTL for mean SPAD and DMGR co-localized on SBI-01 and SBI-07. In both cases the reduced mean SPAD value of one marker allele was associated with a smaller mean DMGR, i.e. higher chlorophyll contents may have improved photosynthesis and growth. However, there was no strong evidence that the preservation of high chlorophyll contents under unfavorable conditions promoted growth at low temperatures since both $SPAD_{(sl)}$ and $SPAD_{(Tb)}$ were positively correlated to mean DMGR, i.e., a strong increase in SPAD with increasing temperatures was correlated with high $DMGR_{(mean)}$. As mentioned before, the marker-trait association for DMGR on SBI-07 was found to be significant in seven environments, while a marker-trait association for SPAD was detected at the same locus only in the two environments with highest temperatures. Fracheboud et al. (2004) identified overlapping positions of SPAD and carbon exchange rate QTL but no co-localizations of QTL for SPAD and shoot dry-matter in maize.

T_b and *sl* of F_v'/F_m' were negatively correlated with $DMGR_0$, i.e., higher energy harvesting efficiencies at low temperatures may promote growth in low temperature environments. However, at sPb-1631 on SBI-02, the only locus at which QTL for T_b and *sl* of F_v'/F_m' and $DMGR_0$ were co-located, the same allele was associated with increasing $DMGR_0$ and T_b as well as *sl* of F_v'/F_m'. $SPAD_{(Tb)}$ was significantly correlated with $F_v'/F_m'_{(Tb)}$. Accordingly, high chlorophyll contents at low temperatures may improve the efficiency of PSII. The marker alleles on SBI-10, which were associated with an decreasing $SPAD_{(Tb)}$ were also associated with decreasing $F_v'/F_m'_{(Tb)}$ or $\Phi_{PSII(Tb)}$, respectively. However, one of the alleles was also associated with an increasing $DMGR_0$ (Table S3). Trachsel et al. (2010) found a QTL allele with positive effects on $\Phi_{PSII(Tb)}$ close to a QTL allele with negative effects on shoot dry weight in maize. Pleiotropic effects of a single gene seem less likely than the

occurrence of different genes, which affect growth, SPAD and fluorescence, and are associated to the same marker loci on SBI-10.

Comparison of results to earlier QTL studies

Rami et al. (1998) and Ritter et al. (2008) found QTL for plant height and Haussmann et al. (2002) detected QTL for stay green on SBI-01. Mace and Jordan (2011) integrated the flanking markers of QTL from different studies into a consensus map. txp-37, a flanking marker of the mentioned QTL was mapped within the genomic region spanning from 71 to 107 cM, where nine marker-trait associations for DMGR were identified. Another co-localization of marker trait associations for DMGR and a stay green QTL identified by Haussmann et al. (2002) was described on chromosome SBI-10 between 64 and 68 cM (Mace and Jordan 2011). Shiringani et al. (2010) detected a QTL for plant height on SBI-08 in the region where sPb-0325 was mapped. sPb-0325 showed significant marker-trait associations with DMGR in four environments of the present study. Since plant height is closely correlated to biomass, the same genetic mechanisms may regulate growth during early and later development stages.

Stay green is closely related to traits like SPAD and fluorescence, which are relevant for photosynthesis (Thomas and Howarth 2000). Marker-trait associations for SPAD and fluorescence of the present study were detected in genomic regions, where stay green QTL were found in earlier studies. We detected marker-trait associations for SPAD in five environments, for $SPAD_{(mean)}$ and $SPAD_{(sl)}$ on SBI-04 between 71 and 85 cM. A stay green QTL was found by Kebede et al. (2001) in the same region. The authors found another QTL for stay green on chromosome SBI-05. The flanking markers were mapped close to sPb-6855 (Mace and Jordan 2011), a significant locus for F_v'/F_m' and Φ_{PSII}. A QTL for stay green

found by Subudhi et al. (2000) was mapped in the region of sPb-6518 on SBI-07 (Mace and Jordan 2011), which was associated to Φ_{PSII} in two and with LGR in four temperature regimes. The same genetic mechanisms may have effects on leaf growth at early development stages and on a delayed senescence. Between 43 and 46 cM on SBI-10, four QTL for Φ_{PSII}, five for SPAD and six for F_v'/F_m' were identified. Tao et al. (2000) found a QTL for stay green and Ritter et al. (2008) detected a QTL for time to maturity in the same region. The QTL for Φ_{PSII}, SPAD, and F_v'/F_m' co-localized with marker-trait associations for DMGR and LGR in the present study. However, as mentioned before, situations are less clear at this locus. The alleles associated to increased photon harvesting efficiencies were also associated to decreased growth rates (Table S3).

Sugars play an important role in the cold acclimation of plants (Stitt and Hurry 2002). sPb-0319 was associated with DMGR, LGR, and SPAD at low temperatures. In the region on SBI-03, where sPb-0319 was mapped (Mace and Jordan 2011), QTL for glucose content were detected by Shiringani et al. (2010). However, the allele, which was associated with higher SPAD values in our study, was associated with smaller growth rates (Table S 3.3). Shiringani et al. (2010) identified another QTL for sugar content on chromosome SBI-09 between 100 and 108 cM. We detected QTL for SPAD, $SPAD_{(sl)}$, and $SPAD_{(Tb)}$ in the same region.

Conclusions

Several loci with effects on sorghum growth at low temperatures were identified. Most marker-trait associations for $DMGR_0$ did co-locate with those for DMGR at low temperatures, so that association studies carried out on a regression parameter like $DMGR_0$ might be advantageous only if the response to an environmental factor is more important than the development in an extreme environment itself. An important application for marker-trait

association based modeling approaches may arise if the behavior of progenies in response to environmental factors can be predicted by parameter estimates of their parental lines. While DMGR and LGR were highly correlated and marker-trait associations for the traits often co-localized, marker-trait associations for chlorophyll content and fluorescence co-localized only occasionally with those for plant growth during juvenile development and gave no hint for a major direct contribution to dry matter and leaf area accumulation. Since earlier studies on maize described the influence of these traits on carbon exchange rates, it has to be verified if high leaf greenness and the efficiency of PSII positively influence seedling survival in the field.

Chapter 4

Combining linkage analysis and association mapping to validate QTL for cold tolerance during juvenile development

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Abstract

Cold tolerance of sorghum during the juvenile phase is a major breeding goal for the development of new sorghum varieties to have an alternative for maize as energy crop in temperate regions. Existing genetic variation can be used to develop breeding populations, which segregate for cold tolerance. The objective of the present study was to identify marker-trait associations for cold tolerance in a sorghum diversity panel fingerprinted with 2620 single nucleotide polymorphism (SNP) markers and to verify the quantitative trait loci (QTL) in two F2- populations. Traits of interest were dry matter growth rates (DMGR), leaf appearance rates (LAR), chlorophyll contents (SPAD) and chlorophyll fluorescence (F_v'/F_m' and Φ_{PSII}) in relation to temperature. The association panel comprised 194 genotypes, while the F2 populations comprised 80 and 92 genotypes, respectively. All populations were tested in a minimum of four temperature regimes ranging from 9.8 to 20.8 °C. QTL were identified for mean across environments and regression parameters describing temperature effects. Several marker-trait associations for mean (*m*) DMGR, base temperature (T_b) of SPAD and Φ_{PSII}, and temperature effect on LAR were validated by QTL detected in population 1 or 2. Promising QTL regions were found on chromosome SBI-01, SBI-02, SBI-03 and SBI-06 where candidate genes involved in the C-repeat binding (CBF) pathway or encode cold shock proteins are located.

Keywords: sorghum, cold tolerance, juvenile development, growth rates, association mapping, QTL analysis

Introduction

The maize acreage in Central Europe increased dramatically during the last decade. The bioenergy boom boosted maize cultivation and the range expansion of pests like the western corn rootworm (*Diabrotica vigifera* LeConte) accompanied by missing crop rotations makes its cultivation more difficult and endangers crop yields. Recent studies have shown that the western corn rootworm is evolving a Cry3Bb1 resistance in *Bt*-maize (Gassmann et al. 2011). Hydrocyanic acid has lethal effects on western corn rootworm larvae feeding on sorghum roots (Branson et al. 1969) and sorghum can be cultivated and harvested with the same machinery and technique as maize. Consequently, sorghum has the potential to replace a part of the maize acreage and diversify cropping systems in temperate climates. However, the sorghum growing area is limited due to the low cold tolerance of the crop.

Promising genetic resources for improving cold tolerance in sorghum are known. Chinese landraces showed higher emergence rates and improved early seedling vigor under low temperature conditions; however, they also have undesirable agronomic characteristics (Knoll et al. 2008a). Quantitative trait loci (QTL) for sorghum cold tolerance during emergence were detected in several studies (Knoll et al. 2008a; Burow et al. 2011; Fiedler et al. 2012). Early season crop vigor is characterized by high DMGR and leaf expansion rates. Nondestructive indirect selection criteria, suitable for high throughput phenotyping like chlorophyll fluorescence were successfully used for QTL detection in a bi-parental maize population segregating for cold tolerance (Fracheboud et al. 2004). Chlorophyll content (SPAD) and the operating efficiency of photosystem II (Φ_{PSII}) are nowadays routinely used for selecting maize genotypes at low temperatures since the traits are involved in the maintenance of photosynthetic performance under chilling stress (Trachsel et al. 2010). It was shown that QTL can be detected on the parameters of mathematical functions describing the response of agronomic or physiological traits to environmental factors (Reymond et al. 2003). Marker-

trait associations for growth as well as chlorophyll fluorescence and content related traits in response to temperature at early growth stages were identified in a recent study carried out on a sorghum diversity panel (chapter 3).

False positive marker-trait associations hamper the power of association studies. Association mapping approaches routinely take population structures into account in order to reduce false positives. Kinship methods were shown to reduce false positive marker-trait associations more effectively than others but had also deficiencies in the effectiveness (MacKenzie and Hackett 2012). Linkage mapping based QTL analyses have the disadvantage of large confidence intervals even for major QTL (Collard and Mackill 2008), which makes the identification of candidate genes difficult. Brown et al. (2003) highlighted the importance of using multiple genetic backgrounds to verify QTL identified in single populations.

The goal of the present study was to verify significant marker-trait associations for cold tolerance during juvenile development identified in a sorghum diversity panel (chapter 3) by QTL analyses for cold tolerance in two F2 populations. The parental lines of one of the populations were taken from the diversity panel, while the other population was developed from completely independent parental lines. The diversity panel and the two bi-parental crosses were genotyped using a recently developed 3k SNP array (Bekele et al. 2013). The phenotype data from the diversity panel (chapter 3) was reanalyzed based on SNP markers and the two F2 mapping populations were phenotyped in four temperature regimes.

Material & Methods

Plant material

The study was carried out on two F2 mapping populations, comprising 80 (pop. 1) and 92 (pop. 2) genotypes, respectively, and a diversity set of 194 breeding lines including *Sorghum bicolor* and *S. bicolor sudanense* genotypes. DNA was extracted from leaf tips using the cetyl

trimethylammonium bromide (CTAB) method. All genotypes were fingerprinted with 2620 SNP markers. Monomorphic markers and markers where the parental genotype has no allele or is heterozygote were removed. 307 and 714 remaining SNP marker for population 1 and 2, respectively, were used to create a genetic map with JoinMap 4.0 (van Ooijen 2006) Additional markers were excluded if more than one marker was mapped at the same locus. Grouping was based on recombination frequency and the Haldane function was used as regression mapping algorithm. The final maps comprised 255 or 544 markers covering 1053 cM or 1211 cM for population 1 and 2, respectively (Table 4.1). The average interval length was 6.3 cM for population 1 and 5.3 cM for population 2. Markers with frequencies of one allele < 5% were removed from the diversity panel, 803 polymorphic markers were used for association mapping. Only 72 markers were present in all three populations.

Table 4.1: Number of filtered SNP markers per chromosome for QTL analysis (population 1 and 2) and association panel.

	Number of SNP marker			
Chromosome	Association panel	Population 1	Population 2	Shared marker
SBI-01	89	32	77	15
SBI-02	91	37	85	12
SBI-03	100	39	55	6
SBI-04	67	20	65	8
SBI-05	99	32	56	7
SBI-06	54	14	58	7
SBI-07	42	10	27	2
SBI-08	71	18	23	2
SBI-09	94	32	44	5
SBI-10	96	21	54	8
Total	803	255	544	72

Experimental design

Experiments with four temperature regimes for populations 1 and 2 and eight temperature regimes for the diversity set were conducted in climate chambers at the Leibniz Universität Hannover (Germany). The genotypes were sown in pots with a diameter of 7 cm and filled with 50% Klasmann Potgrond P (Klasmann-Deilmann, Groß-Hesepe, Germany) and 50% loamy sand. Three seeds per pot and genotype were sown at 10 mm depth. All plants were grown at an optimal temperature of 25/22 °C (day/night) until most plants were in the three-leaf stage (6 d after sowing). After thinning to one plant per pot, plants were moved to different climate chambers representing four (Table 4.2) or eight temperature regimes ranging from 9.8 and 20.8 °C (chapter 3). Each single temperature treatment was designed as randomized complete block design with 3 replications within one climate chamber. Air temperature was measured every 5 minutes directly above the pots using TinyTag View 2 data loggers (Gemini Ltd., West Sussex, U.K.) during the entire duration of the experiment. Plants were grown at a photoperiod of 12 h with 10 h full light (385 µmol m^{-2} s^{-1} on canopy level) and 1 h twilight in the morning and evening.

Table 4.2: Average daily mean, night and day air temperatures in four temperature treatments and the duration of temperature treatment for population 1 and 2. Temperature treatments for the association panel are shown in Table S 3.1.

Environment	Air temperature			Duration of temperature treatment
	Mean	Night	Day	
	[°C]			[d]
1	13.5	12.2	15.5	15
2	14.4	13.5	16.6	14
3	17.0	15.7	18.7	14
4	20.8	18.8	23.6	13

Determination of growth rates and physiological traits

The number of leaves of every plant was counted at the beginning and end of each temperature treatment. Leaf appearance rate (LAR) was calculated as follows:

$$LAR = (LN_{dn} - LN_{d6}) / n, \quad (4.1)$$

where LN_{dn} is the number of leaves at the end of the experiment, LN_{d6} is the number of leaves 6 d after sowing, and n is the number of days of the temperature treatment.

The dry matter growth rate (DMGR) was estimated as follows:

$$DMGR = (DM_{dn} - DM_{d6}) / n, \quad (4.2)$$

where DM_{dn} is the dry weight at the end of the experiment, DM_{d6} is the dry weight 6 d after sowing and n represents the number of days of temperature treatments. DM_{d6} was recorded in an additional set of plants harvested 6 d after sowing. The dry weight of leaves and stems was measured after drying at 105 °C.

The greenness of the fourth leaf was recorded as mean of three measuring points using a SPAD-502plus chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan). Chlorophyll fluorescence was measured at the fourth leaf of light adapted plants with an LI-6400 instrument equipped with the LI-6400-40 pulse amplitude modulation fluorometer (LICOR, Lincoln, NE, USA) using a modified measuring protocol from Fracheboud et al. (1999). The temperature in the measurement chamber was kept at the corresponding temperature inside the growth chamber. Steady-state fluorescence (F_s') was recorded when the rate of change in fluorescence in relation to temperature (dF/dt) was < 5, indicating a stable signal. In order to obtain the maximum fluorescence (F_m') a saturation flash of > 8000 µmol m^{-2} s^{-1} was applied for 1 s. Actinic light was turned off and leaves were illuminated with far red light to measure the ground fluorescence of light adapted leaves (F_0'). The fraction of absorbed PSII photons

used in photochemistry (Φ_{PSII}) was calculated as (F_m'- F_s')/F_m' (Genty et al. 1989). The efficiency of energy harvesting of the oxidized PSII (F_v'/F_m') was calculated as (F_m'-F_0')/F_m'.

Data analysis

Variance components were estimated using SAS 9.2 and broad sense heritability (h^2) was calculated according to Hill et al. (1998):

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GxE}^2 \frac{1}{n} + \sigma^2 \frac{1}{rn}} \quad , \qquad (4.3)$$

where σ^2_G is the genotypic variance, σ^2_{GxE} is the genotype x environment interaction variance, σ^2 is the error variance, r is the number of replications, and n is the number of environments. Analysis of variance (ANOVA) was carried out using the following model with $i = 1,2,3,\ldots,a$ genotypes, $j = 1,2,3,\ldots,n$ environments and $k = 1,2,3,\ldots,b$ genotype x environment interactions:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}, \qquad (4.4)$$

where μ is the overall mean, α_i is the effect of the i^{th} genotype, β_j is the effect of the j^{th} environment, γ_{ij} is the genotype x environment interaction, and ε_{ijk} is a random error.

Linear regression analysis was carried out on LAR, SPAD, F_v'/F_m' and Φ_{PSII} data from the nine temperature regimes using the following model:

$$y_{ij} = \beta_i + sl_i x_j + \varepsilon_{ij}, \qquad (4.5)$$

where y_{ij} is the trait value of the i^{th} genotype in the j^{th} temperature regime. β_i is the estimated intercept of the i^{th} genotype and sl_i is the estimated regression slope of the i^{th} genotype, x_j is the temperature of the j^{th} environment and ε_{ij} is a random error. Base temperature (T_b) of the

i^{th} genotype was estimated by linear extrapolation to define the theoretical temperature below which LAR, SPAD, F_v'/F_m', and Φ_{PSII} become 0:

$$T_{bi} = -\beta_i / sl_i, \quad (4.6)$$

An exponential function was used to describe the relation between DMGR and temperature:

$$GR_{ij} = GR_{0i}\, e^{(a\,(T - T_0))}, \quad (4.7)$$

where GR_{ij} is the growth rate of the i^{th} genotype in the j^{th} environment, GR_0 is the estimated *GR* in the lowest temperature environment T_0, a is the exponent and T the temperature. Pearson`s correlation coefficients were calculated between across environment means of the traits using SAS 9.2 for each population separately. Coefficients of variation (CV) were determined for all traits. For the determination of genetic relatedness between the populations, principal component analysis (PCA) was performed using R (R Development Core Team 2008) assuming 5 components.

QTL analyses and association studies were carried out on mean genotype performance across all environments and on regression parameters. QTL were detected with PLABQTL 1.2 (Utz and Melchinger 2006) by composite interval mapping with cofactors. The logarithmic odds ratio (LOD) threshold was set 2.5. Tassel 3.0 (Bradbury et al. 2007) was used for identifying significant associations between 803 SNP markers and the traits described above. The SSR based Q-matrix and a kinship matrix were used to account for the population structure as described in chapter 3. A mixed linear model (MLM) was assumed to detect significant marker-trait associations (Zhang et al. 2010). Locus positions were subjected to BLAST analysis using Phytozome v 9.1 (Goodstein et al. 2011).

Results

Figure 4.1 shows box plots for all analyzed traits of each population. The mean daily increase in dry weight across all environments of population 2 was approximately two-fold less compared to population 1 and the association panel (Figure 4.1a), however, SPAD(*m*) of population 2 was highest (Figure 4.1g) and mean SPAD(T_b) was lowest compared to the other populations (Figure 4.1i). The highest $DMGR_0$ was identified in population 1 (Figure 4.1c). Minimum T_b for LAR of the association panel was 4 °C higher compared to the other populations (Figure 4.1f). The highest CV of 16 % and 23 % for F_v'/F_m' (*sl*) and F_v'/F_m' (T_b), respectively, was found for population 2 (Figure 4.1k, l), while the highest CV for Φ_{PSII} (*sl*) and $\Phi_{PSII}(T_b)$ was detected in the association panel (Figure 4.1n, o). Average R^2 for fitting linear and exponential functions ranged between 0.80 for F_v'/F_m' and 0.95 for DMGR in population 1 (data not shown).

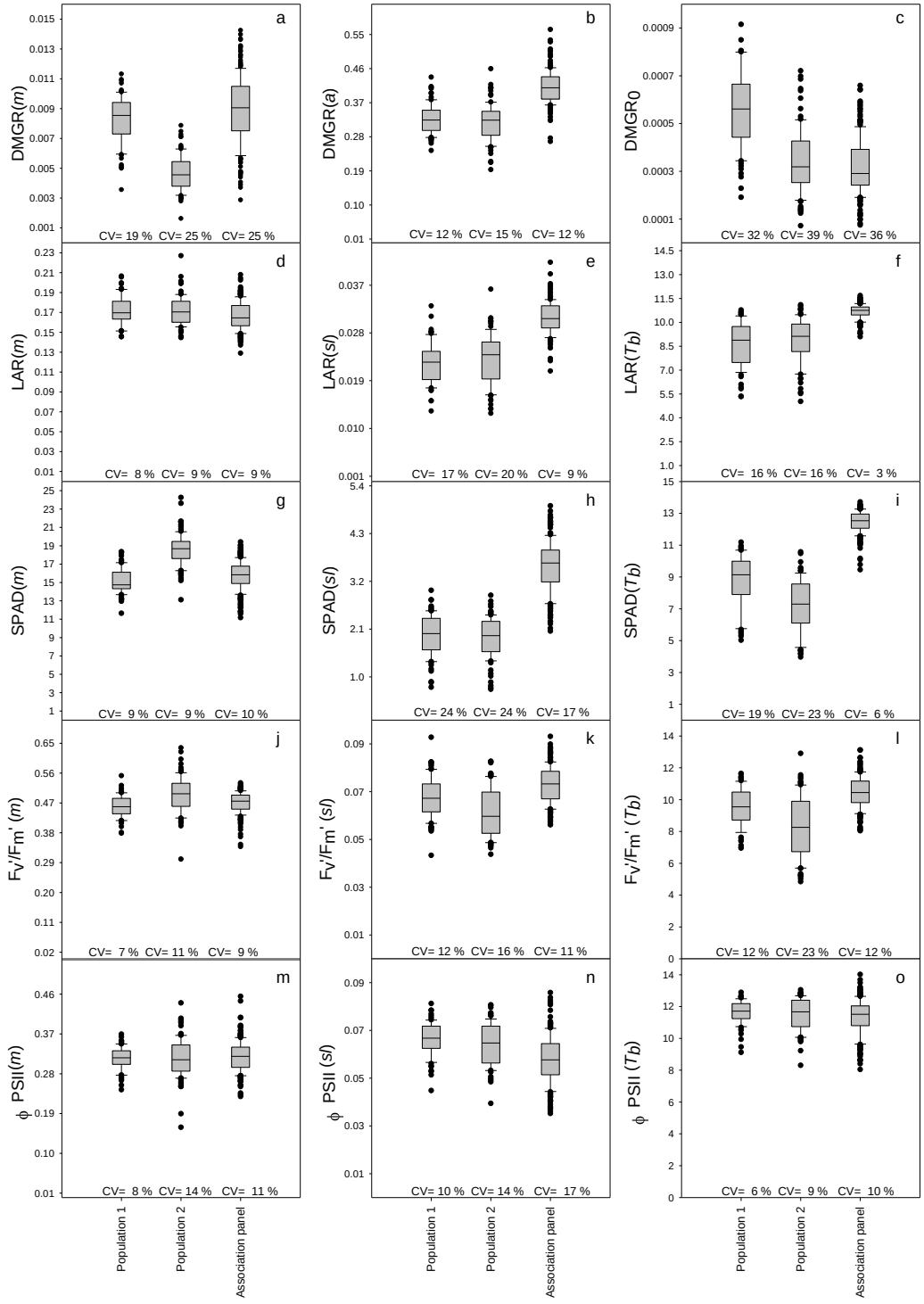


Figure 4.1: Boxplots for mean across environments (*m*), exponents (*a*) and initial growth rate ($DMGR_0$) for dry matter growth rate (DMGR) (a,b,c) and *m*, regression slopes (*sl)*, and base temperatures (T_b) for leaf appearance rate (LAR) (d,e,f), chlorophyll content (SPAD) (g,h,i), and fluorescence (F_v'/F_m' and Φ_{PSII}) (j,k,l,m,n,o) of population 1, 2 and the association panel.

Analysis of variance (ANOVA) revealed that the environmental and genotypic effects were significant for all analyzed traits (Table 4.3). Genotype x environment interaction effects were highly significant for DMGR, LAR, F_v'/F_m' and Φ_{PSII} but not significant for SPAD. Estimated h^2 ranged between 0.10 for Φ_{PSII} and 0.73 for SPAD.

Table 4.3: Variance components and heritability for dry matter growth rate (DMGR), leaf appearance rate (LAR), chlorophyll content (SPAD) and chlorophyll fluorescence (Φ_{PSII} and F_v'/F_m') of population 1 and 2.

	Variance components [a]				heritability
	σ^2_E	σ^2_G	σ^2_{GxE}	σ^2	h^2
DMGR [g d^{-1}]	0.000046***	0.000003***	0.000006***	0.000007	0.56
LAR [d^{-1}]	0.0059***	0.00008***	0.00016***	0.0016	0.32
SPAD	43.5***	3.74***	0.42 n.s.	15.59	0.73
F_v'/F_m'	0.05***	0.00025***	0.0017***	0.0064	0.21
Φ_{PSII}	0.04***	0.0001***	0.002***	0.004	0.10

a***,**,* significant at the 0.001, 0.01 and 0.05 probability level
σ^2_E, σ^2_G, σ^2_{GxE} and σ^2 are variances of the environment, genotype, genotype x environment interactions and error variances

The highest correlation coefficients were found between mean across environments of F_v'/F_m' and Φ_{PSII} in all populations (Figure 4.2). DMGR(*m*) of the association panel was highly significantly correlated to SPAD (0.34, $p < 0.0001$), the traits were moderately correlated in population 2 (0.23, $p < 0.05$) but not significantly correlated in population 1. DMGR(*m*) was significantly negatively correlated with F_v'/F_m' and Φ_{PSII} in population 2.

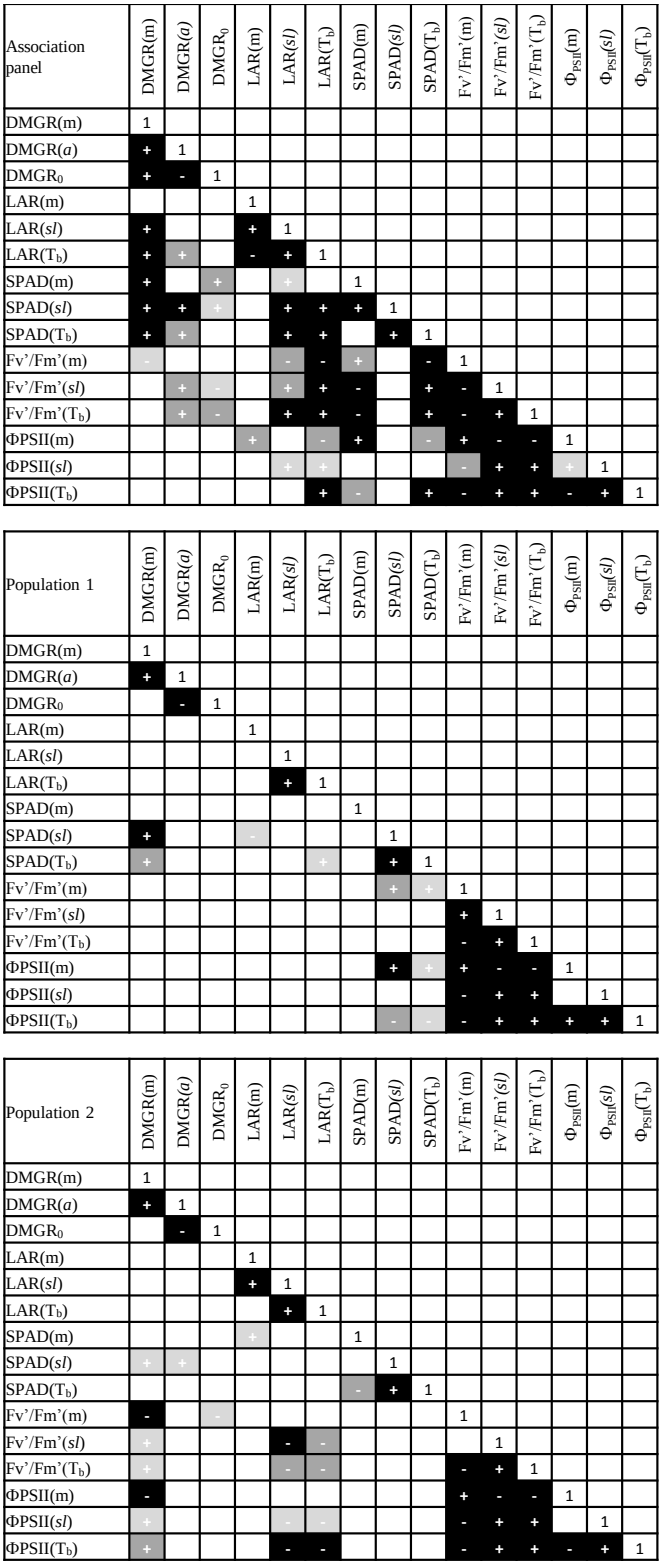


Figure 4.2: Pearson's correlation coefficients for mean across environments (*m*), exponents (*a*) and initial growth rate ($DMGR_0$) for dry matter growth rate (DMGR) and *m*, regression slopes (*sl)* and base temperatures (T_b) for leaf appearance rate (LAR), chlorophyll content (SPAD), and fluorescence (F_v'/F_m' and Φ_{PSII}) of the association panel, population 1 and 2. Shadings show significant correlations at the 0.001 (■), 0.01 (■), and 0.05 (■) probability level.

The principal component analysis revealed that population 2 had no close relation to the association panel (Figure 4.3) while population 1 and the association panel were closely related. 52.8 % of the relatedness between the genotypes was explained by component 1 (PC 1) and 8% by component 2 (PC 2).

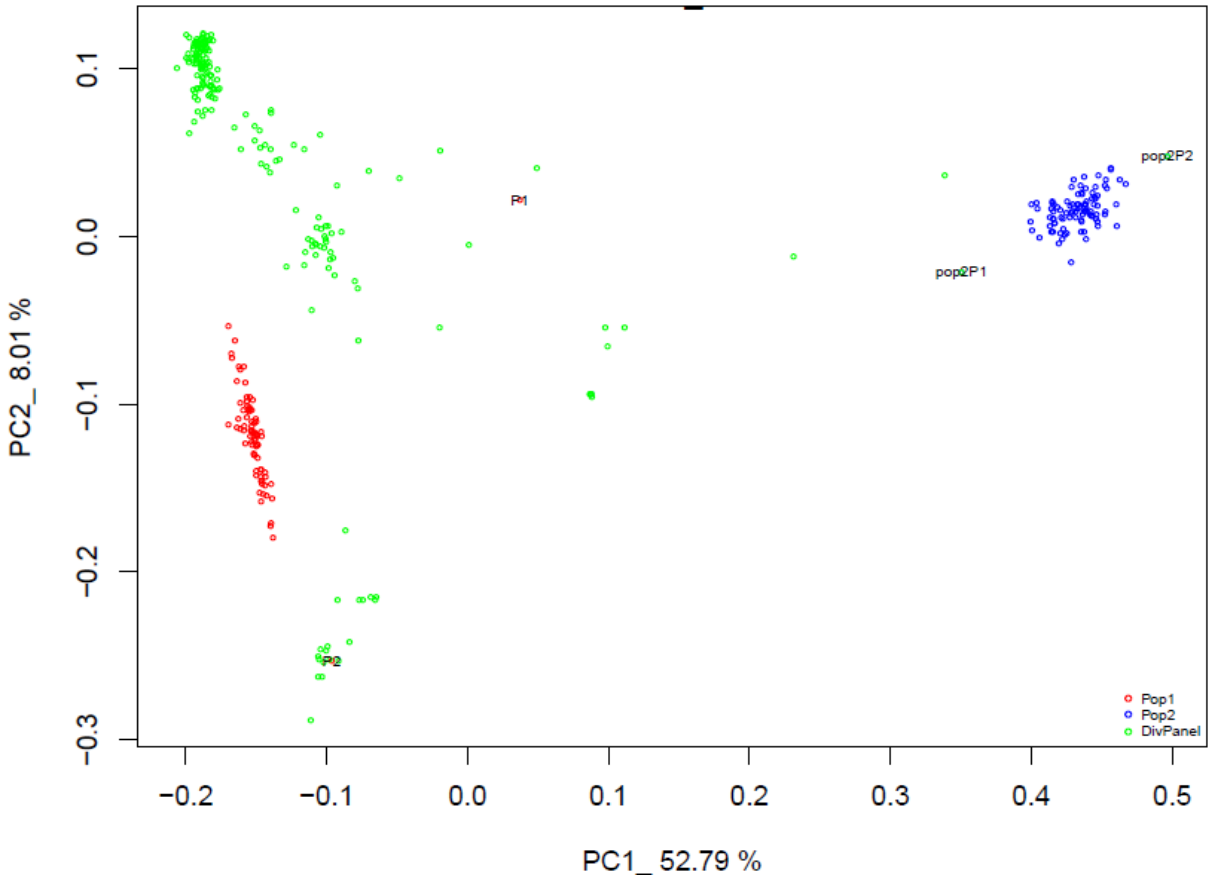


Figure 4.3: Principal component analysis for genetic relatedness of the association panel (green circles), population 1 (red circles) and population 2 (blue circles). P1 and P2 indicate the parents of population 1, pop2P1 and pop2P2 indicate the parents of population 2.

Results of QTL analysis for population 1 and 2 are presented in Table 4.5 and marker-trait associations are shown in Table S 4.1. A total of 32 and 37 QTL were identified for population 1 and 2, respectively. Most of them were detected on SBI-03 (Figure 4.4), 7 in population 1 and 8 in population 2. On chromosome SBI-06 no QTL were identified in population 1, but two QTL were detected in population 2. A total of 434 marker-trait

associations were identified, 66 of them were found on chromosome SBI-03. 38 markers were associated with $\Phi_{PSII}(T_b)$, only 22 marker-trait associations were detected for LAR(*m*).

Co-localizations between QTL identified in population 1 and marker-trait associations were observed 31 times, only 16 QTL co-localizations were found between the association panel and population 2. Several QTL were identified in population 1 on SBI-03 in a QTL-hotspot region of population 2, however, only one trait SPAD(T_b) was significant in both populations.

QTL for DMGR(*m*) were often co-located with temperature response (*sl* and T_b) QTL for SPAD. On chromosome SBI-01, UGSDI_03647 was associated with SPAD(T_b) and was located in the support interval of a QTL for DMGR(*m*) detected in population 1. UGSDI_07470 on chromosome SBI-02 was associated with SPAD(*m*), QTL for F_v'/F_m'(*m, sl,* T_b) were identified in population 1 at UGSDI_07470. On chromosome SBI-03, marker-trait associations for DMGR(*a*) and SPAD(T_b) were found in a region of a QTL for Φ_{PSII} (*sl*) identified in population 1. Another interesting region was identified on chromosome SBI-06. Marker- trait associations for $DMGR_0$ and F_v'/F_m'(*m*) are co-located with a QTL for Φ_{PSII} (*m*) identified in population 2.

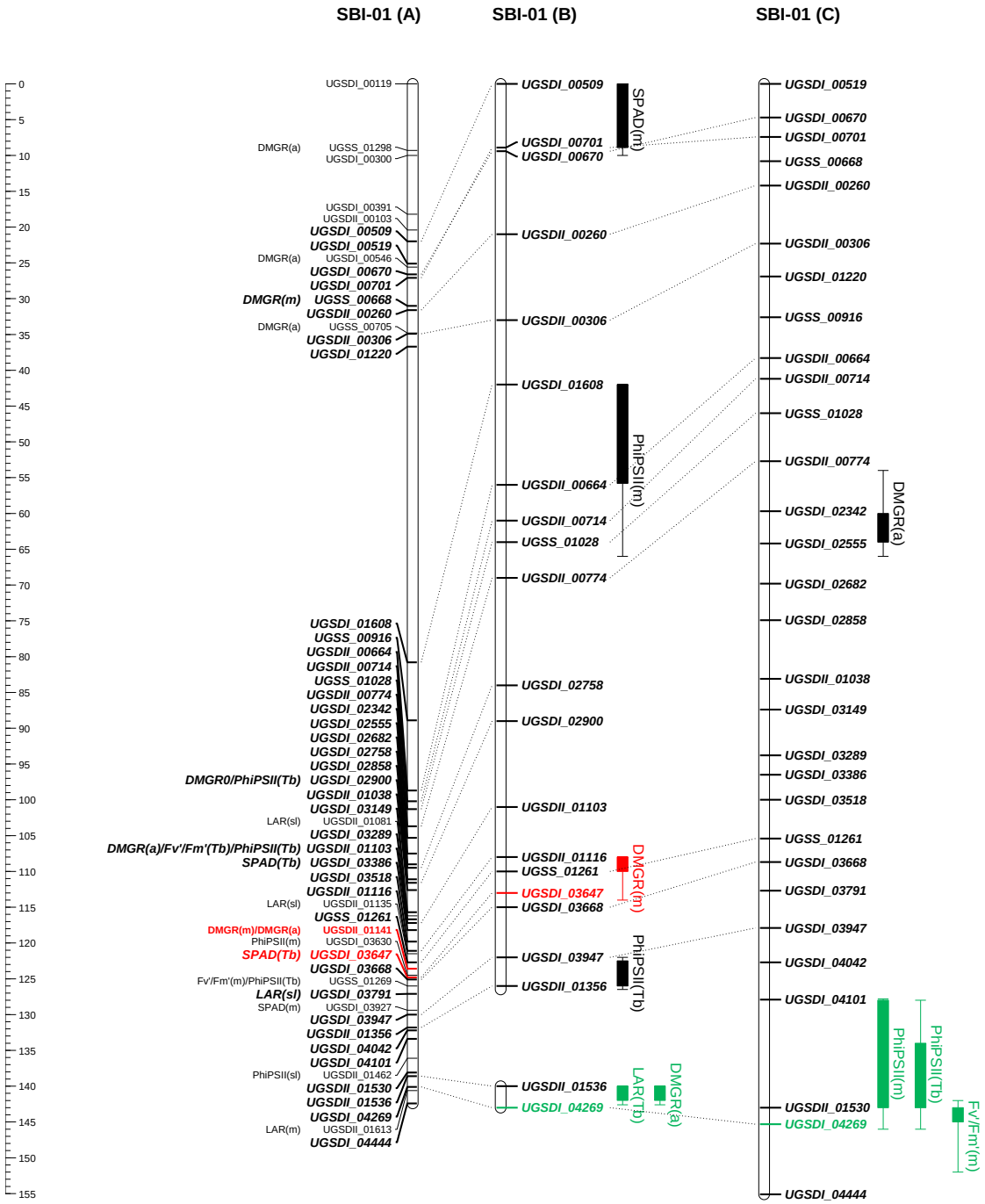
Table 4.4: QTL for mean across environments (*m*), exponents (*a*) and initial growth rate ($DMGR_0$) for dry matter growth rate (DMGR) and for *m*, regression slopes (*sl*) and base temperatures (T_b) for leaf appearance rate (LAR), chlorophyll content (SPAD), and fluorescence (F_v'/F_m' and Φ_{PSII}) of population 1 and 2.

Population 1

Trait	Chr.	Pos. [cM]	Nearest marker	LOD intervall	LOD	R^2	Additiv effects
DMGR(*m*)	SBI-01a	110	UGSS_01261	108-114	3.81	19.9	-0.007
	SBI-09b	8	UGSS_05885	2-14	3.08	16.4	0.01
	SBI-09b	24	UGSDII_14662	18-28	3.61	19	-0.01
	SBI-010	16	UGSDI_51874	10-18	4.16	21.5	-0.008
DMGR(*a*)	SBI-01b	0	UGSDI_00509	0-2	2.59	14.3	-0.017
	SBI-02b	24	UGSS_02293	18-28	2.95	16	0.018
	SBI-04a	26	UGSDII_05715	17-38	2.6	14.2	-0.018
$DMGR_0$	SBI-10	42	UGSS_00364	38-44	5.06	25.3	-0.123
LAR(*m*)	SBI-05a	94	UGSDI_21008	85-100	10.23	44.9	0.014
	SBI-07a	32	UGSS_05156	25-33	5.74	28.8	0.009
	SBI-09a	50	UGSDII_14970	48-52	3.34	17.7	0.006
	SBI-09b	12	UGSDI_33946	6-18	3.03	16.2	-0.005
LAR(*sl*)	SBI-02c	18	UGSS_02113	12-31	5.69	28.2	2.085
	SBI-03a	2	UGSDI_11887	0-4	5.26	28.9	-3.479
	SBI-03a	62	UGSDI_09649	54-66	2.5	13.6	1.771
	SBI-04c	24	UGSDI_18656	20-43	3.48	18.4	-3.173
	SBI-08a	24	UGSDII_14222	16-36	3.04	16.2	-1.7
LAR(T_b)	SBI-01b	0	UGSDII_01536	0-2	2.67	14.6	-0.494
	SBI-02c	18	UGSS_02113	14-31	6.78	32.7	0.801
	SBI-03a	0	UGSDI_12252	0-4	3.16	18.6	-0.937
	SBI-03a	14	UGSDI_12395	6-18	2.51	13.6	0.959
	SBI-04c	42	UGSDI_17190	20-48	2.99	16	-0.667
	SBI-05a	94	UGSDI_21008	85-102	3.5	18.5	-0.686
SPAD(*m*)	SBI-01a	4	UGSDI_00509	0-10	2.81	15	-0.799
SPAD(*sl*)	SBI-03a	66	UGSS_02698	60-66	4.03	21	-0.281
SPAD(T_b)	SBI-03a	66	UGSS_02698	60-66	4.26	24.4	-1.091
F_v'/F_m'(*m*)	SBI-02b	46	UGSDI_07470	44-47	3.9	20.1	0.019
F_v'/F_m'(*sl*)	SBI-02b	44	UGSDI_07470	38-46	2.33	12.9	-0.395
F_v'/F_m'(T_b)	SBI-02b	46	UGSDI_07470	44-47	2.98	16.1	-0.644
Φ_{PSII} (*m*)	SBI-01a	54	UGSDII_00664	42-60	3.77	19.8	0.015
Φ_{PSII} (*sl*)	SBI-03b	14	UGSDI_12780	6-16	2.77	15.1	-0.04
Φ_{PSII} (T_b)	SBI-01a	126	UGSDII_01356	122-126	3.44	18.4	-0.36

Table 4.4: continued

			Population 2				
Trait	Chr.	Pos.	Nearest marker	LOD intervall	LOD	R^2	Additiv effects
DMGR(*m*)	SBI-03a	48	UGSDI_09749	46-58	2.72	13.1	0.617
DMGR(*a*)	SBI-01a	60	UGSDI_02342	54-66	4.19	19.1	0.022
	SBI-05	16	UGSS_03995	12-22	5.52	24.4	0.031
$DMGR_0$	SBI-03a	22	UGSDI_10780	16-28	2.84	13.2	-0.063
	SBI-05	44	UGSDI_25362	40-47	2.72	12.7	0.128
	SBI-05	66	UGSDI_22978	62-68	4.37	19.6	-0.107
LAR(*m*)	SBI-02	102	UGSDII_03978	98-109	2.52	12.2	-0.081
	SBI-02	116	UGSDI_06880	112-122	4.48	20.7	0.129
	SBI-02	186	UGST_00195	178-189	4.34	20.1	-0.083
LAR(*sl*)	SBI-09	80	UGSDI_41002	76-80	2.57	12.3	0.214
$LAR(T_b)$	SBI-07	26	UGSDI_30920	20-32	2.94	14.3	-1.039
	SBI-10	60	UGSDI_44150	54-64	2.78	13.5	-0.742
SPAD(*m*)	SBI-07	24	UGSDI_30920	16-32	3.47	16	1.054
SPAD(*sl*)	SBI-08b	10	UGSDII_11548	8-16	2.82	13.3	-0.213
$SPAD(T_b)$	SBI-02	30	UGSDI_04854	26-36	2.62	13.5	-0.815
	SBI-02	170	UGSDI_08529	162-178	3.27	16.6	1.631
	SBI-03a	60	UGSDII_04396	50-66	3.67	18.4	1.143
	SBI-06	104	UGSDI_26836	102-119	3.66	18.4	1.777
	SBI-07	16	UGSDI_31029	2-18	2.75	14.2	-0.893
$F_v'/F_m'(m)$	SBI-01a	144	UGSDII_01530	142-152	3.16	14.8	0.024
	SBI-03a	12	UGSS_02500	6-18	2.65	12.6	0.026
	SBI-03a	34	UGSDI_10436	28-38	6.09	26.5	-0.04
	SBI-04a	88	UGSDI_17961	80-97	3.49	16.2	0.023
$F_v'/F_m'(sl)$	SBI-03a	34	UGSDI_10436	28-38	3.4	17.8	0.007
	SBI-08a	24	UGSDII_14277	16-30	3.03	16	0.01
	SBI-10	86	UGSS_00181	74-96	2.77	14.7	-0.006
$F_v'/F_m'(T_b)$	SBI-08a	26	UGSDII_14277	16-34	3	15.9	0.926
	SBI-10	78	UGSDI_43636	74-94	3.59	18.7	-1.053
Φ_{PSII} (*m*)	SBI-01a	140	UGSDII_01530	128-146	2.81	13.3	0.021
	SBI-03a	40	UGSDI_10250	36-46	2.84	13.4	-0.018
	SBI-04a	94	UGSDI_18462	82-98	2.55	12.1	0.018
	SBI-06	22	UGSS_04975	14-26	3.68	17	0.021
Φ_{PSII} (*sl*)	SBI-10	56	UGSS_00319	54-61	6.71	32	-0.023
Φ_{PSII} (T_b)	SBI-01a	142	UGSDI_01530	128-146	2.92	15.7	-0.474
	SBI-03a	30	UGSDI_10674	20-34	3.03	16.2	0.576
	SBI-04b	22	UGSDII_05715	20-26	3.87	20.2	-0.493
	SBI-07	56	UGSDI_30425	52-62	4.33	22.3	-0.657


SBI-01 (A)
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SBI-01 (C)
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UGSDII_00103
UGSDI_00509
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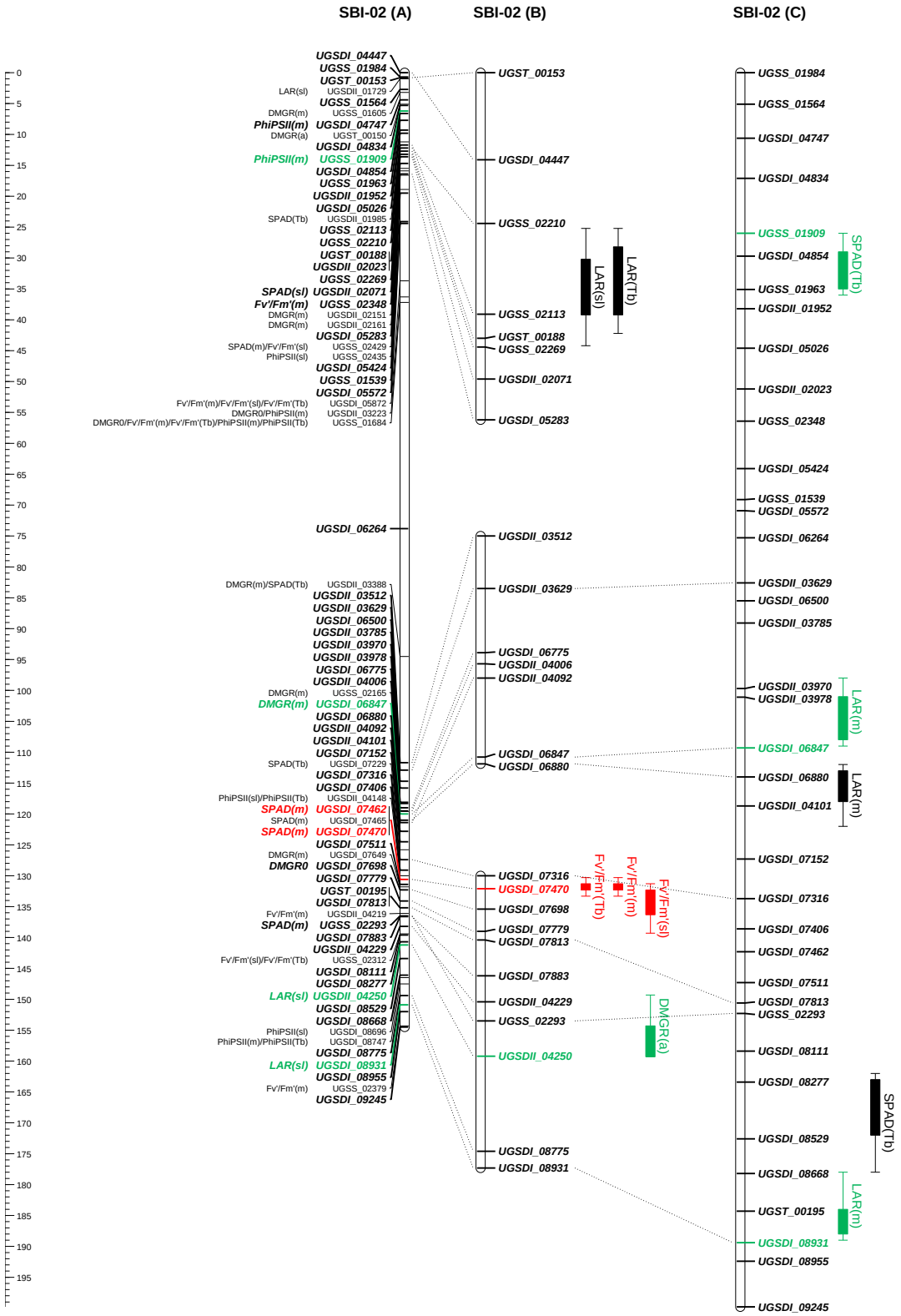
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DMGR(m)/SPAD(Tb) UGSDII_03388
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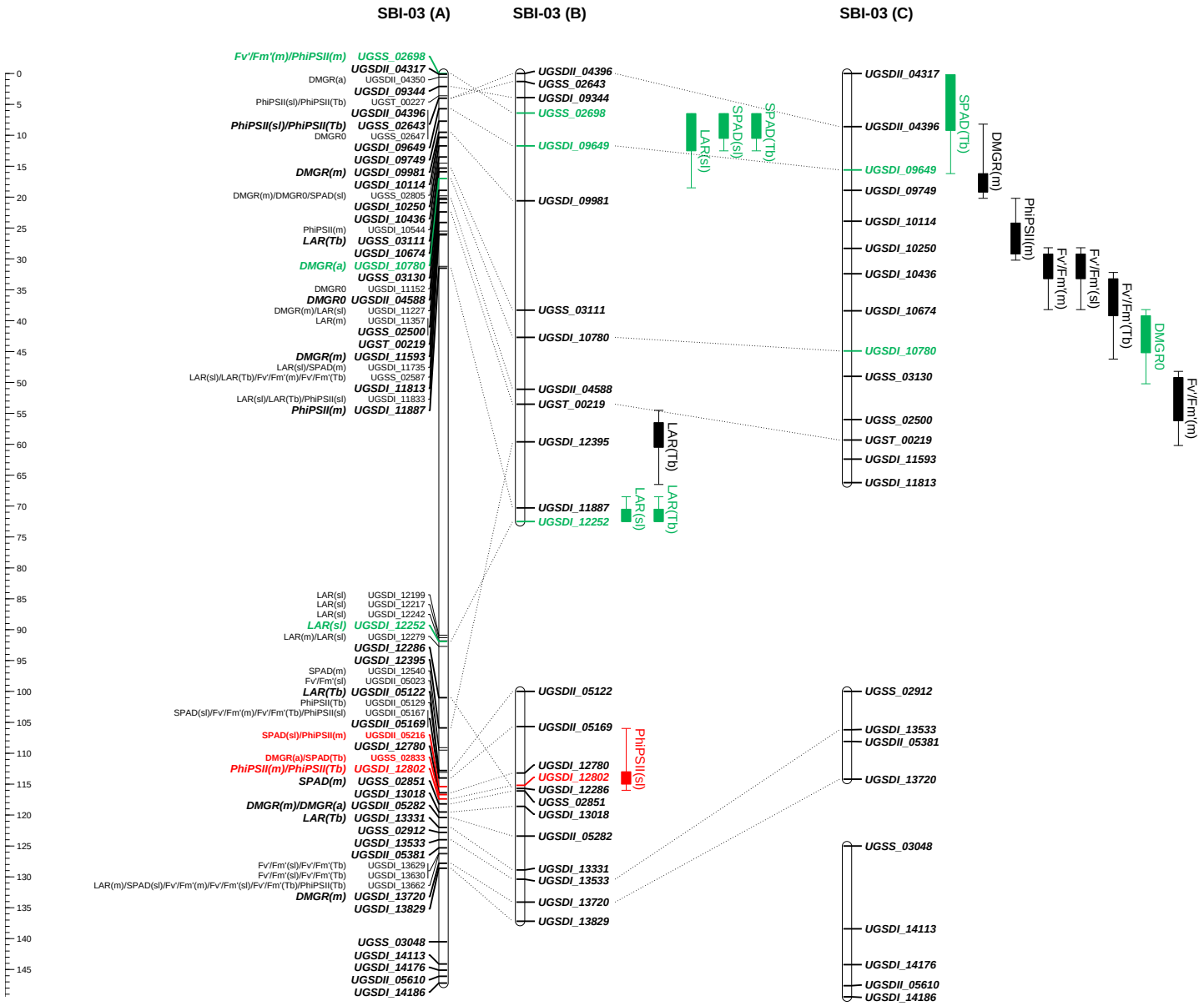
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145
Fv'/Fm'(m)/PhiPSII(m)
DMGR(a)
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LAR(m)/LAR(sl)
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Fv'/Fm'(sl)
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SPAD(m)
DMGR(m)/DMGR(a)
LAR(Tb)
Fv'/Fm'(sl)/Fv'/Fm'(Tb)
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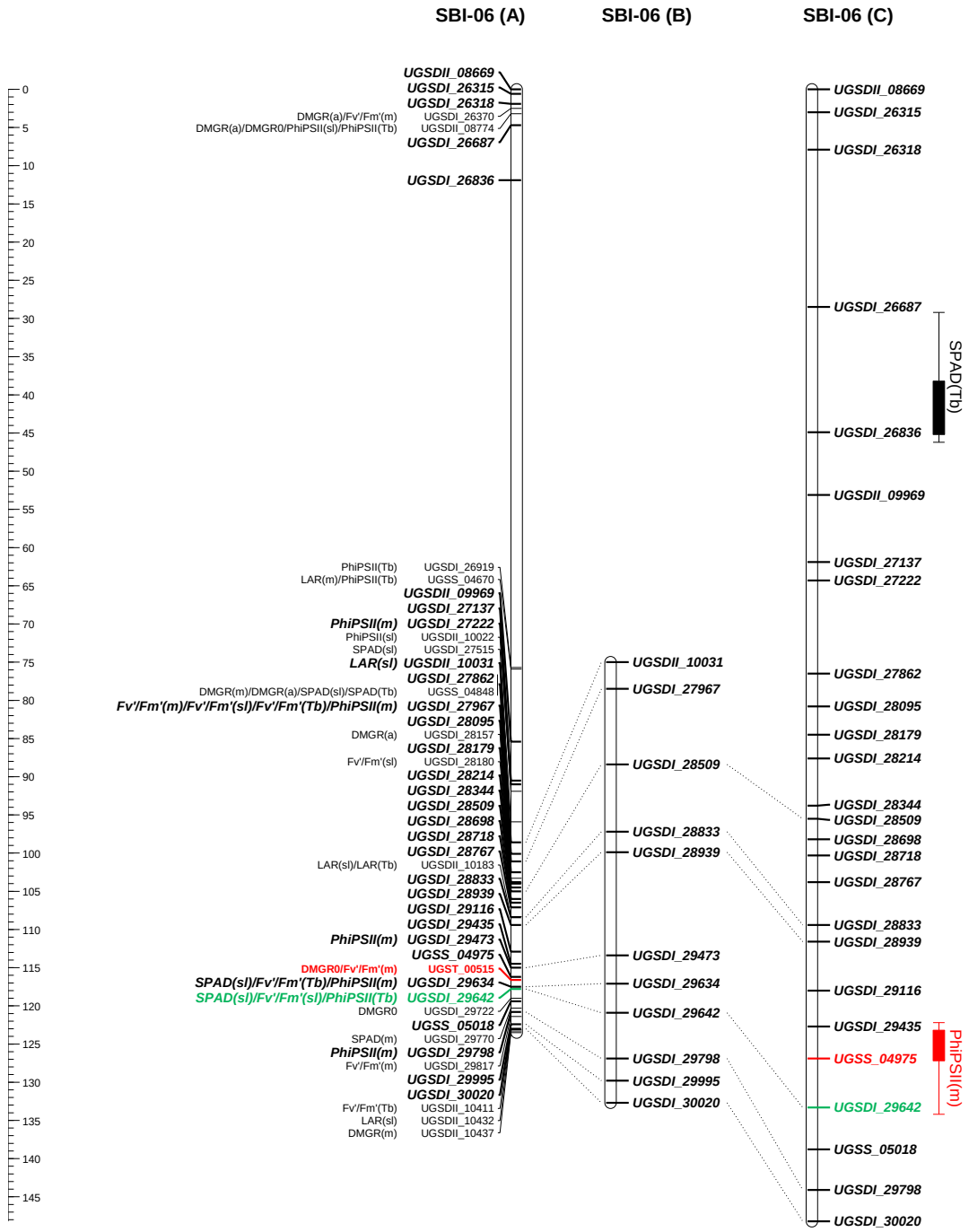

Figure 4.4: Marker-trait associations (A) and QTL for population 1 (B) and population 2 (C) for mean across environments (*m*), exponents (*a*) and initial growth rate ($DMGR_0$) for dry matter growth rate (DMGR) and *m*, regression slopes (*sl)*, and base temperatures (T_b) for leaf appearance rate (LAR), chlorophyll content (SPAD) and fluorescence (F_v'/F_m' and Φ_{PSII}). Only markers significantly associated with a trait or present in at least two populations are presented. Bold and italic markers are present in all three populations. Positions of markers of the association panel are given in $2*10^{-6}$ bp. Distances between markers in the cross populations are given in cM. Green markers are associated with a trait and located in a QTL region of population 1 or 2 or in both populations. Red markers are located in a genome region of a candidate gene.

Discussion

One of the goals of the present study was to validate marker-trait association detected in a diversity panel with QTL found in bi-parental crosses. Using parameters derived from regression analyses enabled us to compare the temperature effect on growth and photosynthesis of three populations tested in several temperature conditions.

The combination of conventional QTL analysis, a well established method in plant breeding, and association mapping is an option to verify QTL positions (Breseghello and Sorrel, 2006). However, several problems are obvious in the present study: (1) The small number of markers present in all populations makes it difficult to verify many significant marker-trait associations in one of the two F2 populations. (2) The low genetic relatedness of population 2 to population 1 and to the diversity panel makes it even more difficult to verify QTL (Figure 4.3). Knoll et al. (2008b) validated QTL for cold tolerance in two F3 sorghum populations; however, both populations shared one parent, a cold tolerant "Shan Qui Red" (SQR) line. Nevertheless, the genetic background of the progeny is different but the favorable alleles for cold tolerance will be contributed from the SQR line. In the present study, the two populations shared no parental line and the progenies differ in their genetic variability depending how diverse the parents are (Figure 4.3). Reduced allele variability between the parents of population 2 could be one reason of the lack for QTL verification. Another reason might be population specific epistatic effects. Reyna and Sneller (2001) argued that epistasis and recombination might be a reason for the lack of confirmation of yield QTL in near isogenic populations developed from crosses with a high yield adapted soybean parent. The chance of validating marker trait associations by classical QTL analysis would have been higher if both bi-parental crosses were developed from parental lines taken from the association panel.

Promising genome regions involved in cold response

Exposure of plants to cold stress cause rapid inhibition of growth due to the reduction of membrane fluidity (Orvar et al. 2000) and photoinhibition, the imbalance between light harvesting and consumption of the energy results in a damage of the photosystem II (PSII) (Oquist and Huner 2003). Low temperature stress responses are regulated by many interacting genes involved in different pathways. Best described are C-repeat binding factor/dehydration responsive element (CBF/DREB) based pathways, which play a key role in the regulation of cold responsive (*COR*) genes (Zhou et al. 2011). UGSDI_07462 and UGSDI_07470 are located on chromosome SBI-02 and were associated with SPAD(*m*) in the present study (Table 4.5, Figure 4.4). UGSDI_07470 is also a flanking marker of a QTL for Φ_{PSII} (*sl* and T_b). Both markers are located next to loci described as "similar to SbCBF 6*"*. Jurczyk et al. (2012) showed that the expression of *CBF6* in *Festuca pratensis* Huds. cv. Skra is strongly affected by an interaction between light and low temperature. Besides of the light quality, day length seems to play a role in the expression of *CBF6* suggesting that the cold acclimation process might be linked to the circadian clock. UGSS_02833 on chromosome SBI-03 is associated with DMGR(*a*) and SPAD(T_b). A QTL identified in population 2 was found for Φ_{PSII} (*sl)* next to the marker-trait associations. In the same genome region a locus annotated as "similar to the cold acclimation protein COR413-TM1" is located. Breton et al. (2003) speculated that COR413 proteins found in wheat are targeted to plasma and thylakoid membranes and may, thus, play important roles in the improvement of cold tolerance.

Besides of photoinhibition, membrane changes to maintain fluidity play a role in cold responsiveness. In particular, the ratio of unsaturated to saturated fatty acids in plasma membranes affects the fluidity (Steponkus 1984). On chromosome SBI-01 and SBI-03 several marker-trait associations and QTL for DMGR(*m*), DMGR(*a*) and SPAD(*sl*) were located next to loci annotated as "similar to Lipase, similar to OSIGBa0111L12.4"

(belonging to cytochrome P450 enzymes), and "similar to an acid phosphatase-like gene" belonging to the fatty acid hydroxylase superfamily (Figure 4.4). These findings underline that lipids and fatty acids are involved in plant response under low temperatures.

UGST_00515 on chromosome SBI-06 is associated with $DMGR_0$ and F_v'/F_m'(*m*). In the same genome region a flanking marker (UGSS_04975) of a QTL for Φ_{PSII} (*m*) identified in population 2 and a locus annotated as "similar to putative cold shock protein-1" are located. The function of plants cold shock proteins (CSP) is poorly understood, however CSP in *E. coli* are well known as up-regulated proteins when a fast drop of temperature occurs (Jones and Inouye 1994). CSP were identified in *Arabidopsis thaliana* (Sasaki et al. 2007), *Brassica rapa* (Ryzhova et al. 2013) and wheat (Nakaminami et al. 2006) as well. CSP may act as RNA chaperone and play a role in translation under low temperatures (Nakaminami et al. 2006). The identified marker-trait association for dry matter growth under low temperatures, which are closely located to putative *CSP* genes, may support the hypothesis of the involvement of CSP in the improvement of cold tolerance in addition to CBF/DREB pathway.

Table 4.5: Summary of the most promising marker-trait associations, QTL regions, and candidate genes.

Gene				Marker-Trait Association			QTL		
Chr.	Locus name	Description	Position [bp]	Trait	Locus	Position [bp]	Trait	Locus	Position [bp]
SBI-01	Sb01g039970	similar to Lipase, putative, expressed	63,402,298 – 63,405,523	SPAD (T_b)	UGSDI_034647	64,449,310	DMGR(*m*) (Pop. 1)	UGSS_01261	63,405,482
SBI-01	Sb01g040500	similar to OSIGBa0111L12.4 protein,Cytochrome P450	63,835,078 – 63,839,162	DMGR(*m*) DMGR(*a*)	UGSDII_01141	63,852,726			
SBI-02	Sb02g030330	similar to SbCBF6	65,390,448 – 65,391,233	SPAD(*m*)	UGSDI_07462	65,391,657			
SBI-02	Sb02g03034	similar to SbCBF6	65,399,042 – 65,399,776	SPAD(*m*)	UGSDI_07470	65,397,174	F_v'/F_m'(*m*, *sl*, T_b) (Pop.1)	UGSDI_07470	65,397,174
SBI-03	Sb03g030030	similar to Acid phosphatase-like, Fatty acid hydroxylase superfamily	58,323,038 – 58,325,931	SPAD(*sl*)	UGSDII_05216	58,207,290			
SBI-03	Sb03g030420	similar to Cold acclimation protein COR413-TM1	58,665,766 – 58,666,964	DMGR(*a*) SPAD(T_b) Φ_{PSII} (*m*) Φ_{PSII} (T_b)	UGSS_02833 UGSDI_12802	58,838,889 59,222,133	Φ_{PSII} (*sl*) (Pop.1)	UGSDI_12780 UGSDI_12802	58,691,855 59,222,133
SBI-06	Sb06g029650	similar to Putative cold shock protein-1	58,229,032 – 58,230,131	$DMGR_0$ F_v'/F_m'(*m*)	UGST_00515	58,367,647	Φ_{PSII} (*m*) (Pop. 2)	UGSS_04975	58,149,192

Conclusion

The verification of marker-trait associations using classical QTL analysis was partly successful. QTL, which were identified in different mapping populations, may serve as a powerful tool for the identification of candidate genes. A main benefit of the concurrent application of classical QTL studies with association mapping approaches is that the advantages of QTL mapping in cross populations (e.g. low rate of false positives) is combined with the advantages of association studies, which, e.g., make QTL fine mapping in relatively small populations much easier. The regression approach for phenotype data analysis turned out to be useful if several populations are analyzed at the same time. Since a comparison of parameters describing the temperature effect over a range of environments is more reliable than the comparison of data from single environments if different populations have to be phenotyped separately. However, a large number of polymorphic markers should be present in all populations, parental lines of cross populations should be carefully selected from the association panel, the diversity panel should be genetically diverse and unstructured, and include as much as variation for the traits of interest as possible. The results of the present study are a first step towards marker-assisted selection for cold tolerance in sorghum. Allele diversity of the most promising candidate genes should be determined and regional association studies have to be done to develop stable markers.

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Chapter 5

General discussion

The ideotype of an energy plant exhibits a fast and uniform emergence with high emergence rates under low temperature conditions. Chapter 2 presents the application of parameter derived from a cumulative emergence model and stability parameters to identify markers associated with cold tolerance during emergence. Piecewise linear regression models are suitable to describe the emergence process in a more detailed way and to determine parameters related to uniformity and speed of emergence. The combination of emergence models and stability analysis allows characterizing the temperature response of the emergence model parameters. These parameters are used in association mapping for the identification of genome regions involved in the temperature regulation of the emergence process.

Improving cold tolerance means both improving emergence and growth during juvenile development. Therefore chapter 3 deals with the application of temperature response curve parameter in association mapping studies to detect stable marker across different temperature regimes. Finally chapter 4 examines the validation of marker-trait associations using conventional QTL analysis in order to identify stable marker across different genetic backgrounds. Relating developmental and growth rates as well as chlorophyll fluorescence and content to temperatures enable the estimation of base temperatures and temperature response parameters, i.e., regression slope describing temperature effects (chapter 3 and 4). Response curve parameters allow the identification of QTL for genotype x environment interactions (GEI), which may describe crop development in a wide range of environments. However, these QTL did not often coincide with environment specific QTL as shown in chapter 3. Besides describing GEI effects, temperature response parameters are suitable to

describe genotype specific reactions even if not all populations evaluated in exactly the same environments (chapter 4).

To estimate temperature response parameters, experiments under controlled conditions are necessary in order to ensure that only one environmental factor, e.g. temperature, influences growth and development of plants. QTL detected from field experiments data have the disadvantage of many varying environmental factors which make interpretation quite difficult. Field experiments with different temperatures regimes can only be realized by different sowing dates in one location. Whereas e.g. early sown plants may have experienced lower temperatures but also different radiation intensities and soil water contents. A major problem of cold-tolerance trials in the field are unpredictable weather conditions especially during the phase of juvenile development. Further disadvantages of field emergence trials are the insufficient plant densities of cold sensitive genotypes resulting from low emergence rates and making the trial unusable for biomass evaluation. However, irregular plant densities can be neglected for the evaluation of cold tolerance at juvenile development because during early growth stages row closure and competition between plants for resources did not affect plant growth.

QTL detected on the data basis of growth chamber experiments with different temperatures can be considered as true temperature response QTL, which did not interact and were not affected by other environmental factors. Growth chamber experiments guarantee stable conditions during cultivation time but are expensive and have limited growing areas. However, cold tolerance means also coping with fluctuating environmental conditions in field experiments which are difficult to imitate in growth chamber experiments due to daily and annually changing temperature ranges. However, Yu et al. (2004) demonstrated that growth chamber experiments with only one cold and one warm treatment are suitable to pre-select sorghum genotypes with improved cold tolerance during emergence and juvenile

development. As presented in chapter 3 marker-trait associations for temperature response parameter of growth rates and chlorophyll related traits coincided often with QTL for the traits detected under stable low temperature conditions. Hence, QTL for temperature response parameter increase the knowledge about the temperature regulation of a trait across a broad range of stable temperature regimes, but cold tolerance under fluctuating temperature conditions have to be verified in field experiments (Burow et al. 2010). If temperature response parameters evaluated in growth chamber experiments will be integrated into the breeding process a minimum of four temperature regimes in growth chamber experiments is required to derive adequate temperature response parameters from regression analysis, if responses are not linear or cannot be linearized, more than four temperature regimes will be needed. In conclusion, evaluating the genotypes in field trials is necessary but pre-selection can be done in growth chamber assays.

Stability parameters, as used in chapter 2 as well as physiologically meaningful parameters like base temperature and regression slopes describing temperature effects on a trait (chapter 3 and 4) characterize GEI effects. In combination with mean genotype performance stability parameters can successfully distinguish between QTL for the trait itself and for GEI effects (Kraakman et al. 2004; Lacaze et al. 2009). The cited authors concluded that co-locations of constitutive QTL and stability QTL mean that the regulation of the underlying genes depend on the environment. Whereas, if the constitutive QTL and stability QTL are located far from each other this may indicate that regulatory genes are affected by the environment and cause the occurrence/absence of constitutive genes (Via et al. 1995). Correspondingly, Sadok et al. (2007) stated that response parameter QTL might have another genetic network than QTL found in stress environments if no co-localization of these QTL is found. In the present study, for all traits related to emergence (except final emergence percentage) stability and mean genotype performance QTL were co-located at least once. These co-localizations suggest that

there are genotypic differences in the allelic sensitivity (chapter 2). Likewise, markers associated with mean genotype performance for growth and photosynthesis related traits did coincide with temperature response QTL for these traits. QTL identified in low temperature environments were often located in the same regions of QTL for base temperature. While QTL detected in single environments and QTL for temperature effects (*sl*) were rarely co-located (chapter 3). This suggests that abiotic stress responses are regulated by many genes, which are interacting differently in varying temperature environments. These findings are in line with the result of Fracheboud et al. (2004) who concluded that the genetic control for photosynthesis of maize differs depending on the temperature regime. For marker assisted selection, stable QTL across environments are needed (Burow et al. 2011). However, most marker-trait associations were environment specific, despite of treatments with small temperature differences between test environments e.g. only one maker on chromosomes SBI-07 was associated with DMGR across seven temperature regimes (chapter 3). In conclusion, environment specific and temperature response QTL have to be interpreted differently. Co-localizations between QTL for traits detected under low temperature conditions and QTL for base temperatures prove the reliability of temperature response parameters.

The main disadvantage of temperature response parameters is that intercept and slope of the regression are interacting and both parameters have to be taken into account for the selection of superior genotypes. Stability parameter and mean genotype performance as well as base temperature and temperature effect (*sl*) of traits have to be considered together. A parallel shift of base temperatures without affecting negatively the development under warmer temperatures regimes indicates superiority of a genotype in all environments. Very interesting regions for marker-assisted selection are those, where base temperature QTL co-segregate with temperature sum QTL (chapter 2) e.g. marker alleles, which are linked to a lower base

temperature and also linked to a lower temperature sum required for emergence. However, developing selection indices might be an alternative step to integrate several parameters into breeding programs.

The combination of association mapping and conventional QTL-analysis aids in narrow down major QTL regions (Breseghello and Sorrells 2006) because large confidence intervals detected from linkage mapping based QTL analyses hamper the identification of candidate genes. The results presented in chapter 4 show that the number of shared QTL loci between an association panel and bi-parental populations is smaller if the bi-parental crosses are genetically only loosely related to the association panel. Optimal is an association panel covering the whole genetic variation and the number of genotypes belonging to the subgroups should be nearly equal to be able to detect association with rare alleles (Vinod et al. 2011). Besides of the familial relatedness, the number of shared polymorphic markers is essential for the validation of marker-trait associations. Nevertheless, interesting genome regions with known candidate genes involved in cold response were identified (chapter 4).

Analyzing plant growth and photosynthesis related traits like chlorophyll content and fluorescence revealed that chlorophyll content is significantly correlated with growth rates and several marker-trait associations for both traits were co-localized (chapter 3). Coincided positive marker alleles of both traits indicate that photosynthesis and growth might be positively affected by higher chlorophyll contents. However, positive correlations between base temperatures of chlorophyll contents and growth rates are contrary to these findings. Likewise, Fracheboud et al. (2004) found no co-localizations of QTL for SPAD and shoot dry-matter in maize. Base temperature of chlorophyll contents and fluorescence were significantly correlated suggesting an improvement of the efficiency of PSII due to high chlorophyll contents under low temperatures. Due to the contrary effects of marker-trait associations there are no strong indications that growth under low temperature conditions is

to a large extent influenced by chlorophyll contents or fluorescence. However, more research is needed to understand the underlying physiological mechanism for growth under low temperature regimes.

QTL for emergence found in earlier studies (Knoll et al. 2008; Burow et al. 2011) could be verified and underlined the most important genome regions are located on chromosome SBI-01 (chapter 2). Recently, physical positions of several DArT- markers are available (Bouchet et al. 2012). On chromosome SBI-01, sPb-2583 is associated with FEP_{normal}, T_b, and E_{TS} (chapter 2) as well as LAR(T_b), SPAD(*m*) and SPAD(*sl*) (chapter 3). In the same region a loci annotated as "Sb01g007395, similar to putative uncharacterized protein" belonging to cytochrome P450 enzymes is located (Table 5.1). Likewise on chromosome SBI-01, marker-trait associations for DMGR(*m*) and DMGR(*a*) were located next to a loci annotated as "similar to Lipase, similar to OSIGBa0111L12.4" also belonging to cytochrome P450 enzymes (chapter 4) which are involved in the biosynthesis of plant hormones, lipids and secondary metabolites as well as in plant defense against chemicals e.g. herbicides (Werck-Reichert et al. 2000). Next to this locus on chromosome SBI-01, Bekele et al. (2013) found a QTL hotspot containing 206 genes involved in abiotic stress stimuli. Additionally, on chromosome SBI-01 sPb-3311 associated with E_{TS} and DMGR(*m*), LGR_0 and SPAD(*m*) was mapped in a region where Sb01g033060 annotated as "similar to Sucrose synthase 2" is located. In this sorghum genome region a QTL for sucrose content was found by Ritter et al. (2008). Further marker- trait associations for juvenile development on chromosome SBI-03 coincided with QTL for glucose content (Shiringani et al. 2010) (chapter 3) indicating the importance of sugars in the role of cold acclimation of plants (Stitt and Hurry 2002).

Table 5.1: Summary of the most promising DArT-marker associations, and candidate genes for emergence and juvenile development.

Gene				Marker-Trait Association for			
Chr.	Locus name	Description	Position [bp]	Emergence	Juvenile development	Locus	Position [bp]
SBI-01	Sb01g007395	similar to putative uncharacterized protein, Cytochrome P450	6,333,799-6,334,231	FEP_{normal} T_b E_{TS}	$LAR(T_b)$ SPAD(*m*) SPAD(*sl*)	sPb-2583	6,333,392-6,333,828
SBI-01	Sb01g033060	similar to Sucrose synthase 2	56,177,529-56,184,240	E_{TS}	DMGR(*m*) LGR_0 SPAD(*m*)	sPb-3311	56,163,666-56,163,982

Additionally, marker-trait association for emergence and QTL for maturity (Srinivas et al. 2009) were co-located on chromosome SBI-03, but physical position of this DaRT markers were not available. Further promising genome regions associated with traits describing the juvenile development were identified on chromosome SBI-03 which encode cold acclimation proteins and on chromosome SBI-06 annotated as “similar to putative cold shock proteins” (chapter 4). First promising candidate genes for temperature response of emergence and juvenile development in sorghum are identified, however high-resolution SNP maps allowing regional association studies are needed to identify more candidate genes within important QTL regions to close the gaps in some genome regions.

Future research needs

Breeding for cold tolerance, a trait with quite complex genetic pathways, is challenging due to the involvement of many candidate genes. The development of high density genetic maps to identify stable marker and candidate genes will be no more a limiting factor due to lower costs per marker and rapidly growing techniques for marker development. Statistical methods like association mapping are routinely integrated in the breeding process to identify candidate genes. For the development of new breeding material, bi-parental crosses carrying positive alleles for emergence and growth rates can be developed for marker assisted pyramiding of alleles (Collard and Mackill 2008). The QTL and candidate genes for cold tolerance identified in the present study can be a first steps towards marker-assisted selection but these QTL have to be verified in field experiments.

The prediction of the progenies phenotype performance in different environments is an important issue to fasten the breeding process. Beside of genomic selection (Jannink et al. 2010) crop models might help in the prediction of phenotype performance. QTL information can be used in crop models to improve genotype specific model parameters (Tardieu and Tuberosa 2010) and to predict ideotypes having the best allele combination using parental parameters (Chenu et al. 2009), but these ideotypes have to be verified in field experiments.

However, the development of more precise high throughput phenotyping methods for breeding programs will be the challenge for the future (Xu and Crouch 2008). An alternative for scoring and dry weight measurements which are too laborious and time consuming is demanded. New, fast and automated phenotyping methods like field laser scanner or simple RGB pictures taken from a tractor or unmanned aerial vehicles might be new options.

Concluding remarks

Using response curve parameters for association mapping and QTL analysis enables the identification and validation of genome regions responsible for the temperature dependant emergence and growth during juvenile development. The results presented in this thesis highlighted the possibility to implement genotype x environment interaction by using stability analysis and growth function parameters. Piecewise linear regressions in combination with stability analysis were able to precisely describe the emergence process across different temperature regimes. Temperature response parameters for juvenile growth e. g. T_b for growth related traits were proven to be reliable due to a high correlation with these traits measured in single low temperature environments. A shift in T_b without negatively affecting development processes is the most promising way to adapt crops to new cultivation areas with lower temperatures. Temperature response parameters were suitable for the verification of QTL across environments and genetic backgrounds. Promising candidate genes for emergence and juvenile development were identified on chromosomes SBI-01, SBI-02, SBI-03 and SBI-06 which are known to be involved in cold response of plants. The development of high density SNP marker is needed to close the gaps in some genome regions and to identify more candidate genes. Nevertheless, the identified genome regions might be an aid for the development of stable markers which can be used in marker-assisted selection for cold tolerance in sorghum.

Supplementary Material

Table S 2.1 : Pearson's correlation coefficients among mean across environments (MW) and Finlay-Wilkinson slopes (FW) for the parameters final emergence percentage (FEP), emergence rate (ER), onset (T_1), end (T_{100}) of emergence, median time of emergence (T_{50}) and uniformity (T_{100}-T_1) as well as for base temperature (T_b) and thermal time (E_{TS}) and final emergence percentage under cold (FEP_{cold}) and normal (FEP_{normal}) conditions.

		FEP			ER		T_1		T_{50}				T_{100}		T_{100}-T_1	
		FW	cold	normal	MW	FW	MW	FW	MW	FW	T_b	E_{TS}	MW	FW	MW	FW
FEP	MW	-0.12 n.s.	0.96 ***	0.83 ***	0.58 ***	0.13 n.s.	-0.005 n.s.	-0.08 n.s.	-0.13 n.s.	-0.09 n.s.	-0.08 n.s.	-0.05 n.s.	-0.19 **	-0.09 n.s.	-0.26 ***	-0.02 n.s.
	FW		-0.26 ***	0.20 **	0.06 n.s.	0.19 **	0.13 n.s.	0.22 **	0.14 *	0.29 ***	0.40 ***	-0.26 ***	0.14 n.s.	0.29 ***	0.07 n.s.	0.11 n.s.
	cold			0.68 ***	0.50 ***	0.04 n.s.	-0.02 n.s.	-0.11 n.s.	-0.13 n.s.	-0.15 *	-0.18 *	0.06 n.s.	-0.18 **	-0.14 *	-0.25 ***	-0.04 n.s.
	normal				0.57 ***	0.26 ***	0.07 n.s.	0.05 n.s.	-0.04 n.s.	0.05 n.s.	0.15 *	-0.22 **	-0.11 n.s.	0.03 n.s.	-0.24 **	-0.008 n.s.
ER	MW					0.77 ***	-0.18 *	-0.18 *	-0.44 ***	-0.18 *	0.25 ***	-0.57 ***	-0.56 ***	-0.14 n.s.	-0.60 ***	0.01 n.s.
	FW						-0.14 n.s.	-0.09 n.s.	-0.27 ***	-0.03 n.s.	0.55 ***	0.73 ***	-0.34 ***	0.03 n.s.	-0.31 ***	0.09 n.s.
T_1	MW							0.87 ***	0.88 ***	0.76 ***	0.32 ***	0.20 **	0.67 ***	0.49 ***	0.05 n.s.	-0.27 ***
	FW								0.75 ***	0.85 ***	0.39 ***	0.03 n.s.	0.59 ***	0.53 ***	0.02 n.s.	-0.36 ***
T_{50}	MW									0.82 ***	0.33 ***	0.29 ***	0.95 ***	0.68 ***	0.51 ***	0.03 n.s.
	FW										0.54 ***	-0.06 n.s.	0.76 ***	0.89 ***	0.35 ***	0.17 *
	T_b											-0.78 ***	0.29 ***	0.52 ***	0.11 n.s.	0.19 **
	E_{TS}												0.30 ***	-0.12 n.s.	0.24 ***	-0.14 *
T_{100}	MW													0.72 ***	0.75 ***	0.23 **
	FW														0.56 ***	0.59 ***
T_{100}-T_1	MW															0.58 ***

***,**,* significant at the 0.001, 0.01 and 0.05 probability level, n.s. not significant

Table S 2.2: Marker-trait associations for final emergence percentage und cold (FEP_{cold}) and under normal (FEP_{normal}) conditions, mean and Finlay-Wilkinson slope (FW) of the traits final emergence rate (FEP), emergence rate (ER), onset (T_1) and end (T_{100}) of emergence, the median of emergence time (T_{50}) and uniformity **(**T_{100}-T_1) and marker- trait associations for base temperature (T_b) and thermal time (E_{TS}). Means and standard deviations (SD) comparing two allelic groups for each significant locus. The common allele is defined as the most often occurring allele.

	Trait	Locus	Chr.	Position [cM]	MLM p	GLM p	Rare allel			Common allel			t-test p	QTL effect of rare allel [%]
							Mean	SD	n	Mean	SD	n		
FEP	Cold	sPb-8773	1	11	0.0078	0.00900	46.2	13.1	13	52.27	9.79	181	0.1265	-13.1
		sPb-2704	1	35	0.0147	0.00400	44.0	8.4	10	52.46	9.85	175	0.0117	-19.2
		sPb-6689	2	22	0.0171	0.00700	56.0	9.7	64	50.01	9.85	118	0.0001	10.7
		sPb-7795	3	4	0.0122	0.00100	58.4	9.7	33	50.61	9.69	160	0.0001	13.3
		sPb-9999	10	56	0.0200	0.03300	54.3	8.7	85	50.00	10.90	105	0.0034	8.0
	Normal	sPb-2583	1	15	0.0003	0.00100	72.2	8.6	25	77.74	7.92	164	0.0050	-7.70
		sPb-3525	1	57	0.0118	0.01100	82.8	8.2	16	76.57	7.91	176	0.0096	7.50
		sPb-6600	1	85	0.0069	0.00300	81.5	7.7	24	76.25	8.12	159	0.0039	6.49
		sPb-5905	1	112	0.0099	0.00500	81.5	7.2	23	76.34	8.11	171	0.0037	6.29
		sPb-1925	2	146	0.0426	0.04000	79.9	7.5	31	76.23	8.21	159	0.0176	4.63
		sPb-0319	3	30	0.0099	0.00700	81.7	7.1	22	76.41	8.08	171	0.0032	6.47
		sPb-4041	5	6	0.0031	0.03100	79.9	6.5	46	75.98	8.43	137	0.0015	4.92
		sPb-1997	9	1	0.0166	0.01300	74.6	8.3	38	77.65	8.12	150	0.0497	-4.06
	Mean	sPb-3891	1	25	0.0220	0.0030	69	14	14	77	11	180	0.01	-10
		sPb-2704	1	35	0.0012	0.0010	66	11	10	78	11	175	0.003	-15
		sPb-8947	1	64	0.0197	0.0050	68	11	7	77	12	186	0.048	-12
		sPb-0090	1	66	0.0394	0.0250	72	12	33	78	12	157	0.02	-8
		sPb-7795	3	4	0.0346	0.0110	82	12	33	76	11	160	0.001	8
		sPb-0005	9	80	0.0043	0.0040	70	16	15	77	11	178	0.04	-9
	FW	sPb-6424	2	145	0.0010	0.0345	1.01	0.31	33	1.01	0.34	160	0.9	0
		sPb-5454	3	5	0.0010	0.0132	0.73	0.34	23	1.05	0.31	166	<0.0001	30
		sPb-4851	4	71	0.0010	0.0094	0.61	0.33	17	1.06	0.31	170	<0.0001	-42
		sPb-4806	5	64	0.0110	0.0278	1.11	0.30	87	0.94	0.35	106	0.004	18
		sPb-9242	8	66	0.0010	0.0094	1.20	0.22	12	0.99	0.34	177	0.039	21
		sPb-1323	8	132	0.0010	0.0244	1.06	0.29	53	0.99	0.35	140	0.17	7
		sPb-7460	9	147	0.0010	0.0069	1.15	0.33	22	0.99	0.34	170	0.039	16
		sPb-5281	10	24	0.0010	0.0394	0.65	0.34	19	1.05	0.31	174	<0.0001	-38

Trait		Locus	Chr.	Position [cM]	MLM p	GLM p	Rare allel			Common allel			t-test p	QTL effect of rare allel [%]
							Mean	SD	n	Mean	SD	n		
ER	Mean	sPb-7422	1	133	0.0191	0.0090	13.2	2.8	23	15.6	4.0	170	0.005	-15
		sPb-4081	2	65	0.0026	0.0020	16.4	4.2	82	14.6	3.6	97	0.001	12
		sPb-1925	2	146	0.0253	0.0360	16.6	3.5	31	15.0	4.0	159	0.04	11
		sPb-9146	6	60	0.0239	0.0130	13.2	3.0	22	15.7	4.0	166	0.007	-16
		sPb-6518	7	28	0.0338	0.0160	13.5	3.5	32	15.7	4.0	162	0.004	-14
		sPb-1881	8	88	0.0136	0.0021	14.2	3.9	69	16.1	4.0	118	0.002	-12
	FW	sPb-1925	2	146	0.0420	0.0060	1.1	0.4	31	1.0	0.5	159	0.249	10
		sPb-3838	4	96	0.0313	0.0040	0.82	0.43	72	1.10	0.47	114	0.001	-25
		sPb-6518	7	28	0.0214	0.0010	0.69	0.31	32	1.05	0.48	162	0.0001	-34
		sPb-0258	8	73	0.0249	0.0070	1.14	0.42	38	0.94	0.47	151	0.01	21
		sPb-1661	8	74	0.0013	0.0010	1.23	0.43	22	0.96	0.47	170	0.009	28
		sPb-1881	8	88	0.0048	0.0010	0.82	0.42	69	1.11	0.48	118	0.0001	-26
T_1	Mean	sPb-6649	3	151	0.0419	0.0010	11.8	1.5	10	10.1	1.0	184	<0.0001	17
		sPb-7534	4	76	0.0464	0.0030	11.0	1.4	12	10.1	1.0	182	0.005	9
		sPb-9146	6	60	0.0257	0.0010	10.9	1.6	22.0	10.1	0.9	166	0.002	8
		sPb-7290	6	99	0.0381	0.0060	9.8	1.0	54.0	10.3	1.1	140	0.02	-5
		sPb-7428	6	158	0.0286	0.0020	10.4	1.6	39.0	10.1	0.9	149	0.062	3
		sPb-8673	7	75	0.0024	0.0040	10.7	1.3	27.0	10.1	1.0	156	0.01	6
		sPb-5055	9	108	0.0368	0.0010	11.2	1.7	9.0	10.1	1.0	185	0.007	11
		sPb-6748	9	115	0.0212	0.0030	10.3	1.5	52.0	10.1	0.9	136	0.15	2
		sPb-3298	9	116	0.0242	0.0020	10.3	1.5	56.0	10.1	0.9	136	0.12	2
	FW	sPb-6649	3	151	<0.0001	0.0010	1.23	0.25	10	0.99	0.19	184	0.0001	24
		sPb-5030	6	74	0.0278	0.0070	0.86	0.23	20	1.02	0.19	174	0.0008	-16
		sPb-7290	6	99	0.0209	0.0030	0.92	0.18	54	1.03	0.20	140	0.0003	-11
		sPb-3715	8	89	0.0381	0.0030	1.14	0.27	10	0.99	0.19	182	0.02	15
		sPb-5055	9	108	0.0101	0.0020	1.16	0.27	9	0.99	0.19	185	0.01	17
		sPb-6748	9	115	0.0403	0.0120	0.99	0.24	52	1.00	0.18	136	0.9	-1
		sPb-3298	9	116	0.0434	0.0040	1.0	0.2	56	1.0	0.2	136	0.9375	0

MLM mixed linear model, GLM general linear model

Trait		Locus	Chr.	Position [cM]	MLM p	GLM p	Rare allel			Common allel			t-test p	QTL effect of rare allel [%]
							Mean	SD	n	Mean	SD	n		
T_{50}	Mean	sPb-6649	3	151	0.0001	0.0010	19.9	1.9	10	18.2	1.6	184	0.001	9
		sPb-7534	4	76	0.0191	0.0010	19.6	1.7	12	18.2	1.6	182	0.003	8
		sPb-9146	6	60	0.0173	0.0010	19.3	2.1	22	18.1	1.5	166	0.001	7
		sPb-8673	7	75	0.0004	0.0010	19.1	1.8	27	18.2	1.5	156	0.003	5
		sPb-5055	9	108	0.0043	0.0010	20.1	1.7	9	18.2	1.6	185	0.0004	10
		sPb-6748	9	115	0.0223	0.0150	18.3	2.3	52	18.2	1.3	136	0.56	1
		sPb-3298	9	116	0.0205	0.0110	18.4	2.2	56	18.2	1.3	136	0.42	1
	FW	sPb-2583	1	15	0.0003	0.0350	0.91	0.20	25	1.02	0.12	164	0.0002	-11
		sPb-6649	3	151	0.0001	0.0010	1.13	0.15	10	1.01	0.14	184	0.002	12
		sPb-7534	4	76	0.0132	0.0010	1.08	0.17	12	1.00	0.14	182	0.04	8
		sPb-3715	8	89	0.0025	0.0010	1.12	0.16	10	1.00	0.14	182	0.008	12
		sPb-7220	8	111	0.0231	0.0010	0.91	0.19	30	1.02	0.12	147	<0.0001	-11
		sPb-5055	9	108	0.0003	0.0010	1.14	0.15	9	0.99	0.14	185	0.0017	15
	T_b	sPb-2583	1	15	0.00021	0.00100	7.05	0.89	25	7.84	0.34	164	<.0001	-11.2
		sPb-4851	4	71	0.01840	0.00100	6.78	0.93	17	7.83	0.34	170	<.0001	-15.6
		sPb-8673	7	75	0.00700	0.00100	7.83	0.37	27	7.76	0.46	156	0.382	0.9
		sPb-7220	8	111	0.00420	0.00100	7.19	0.88	30	7.84	0.34	147	<.0001	-9.0
	E_{TS}	sPb-2583	1	15	0.035	0.0010	63.19	10.94	25	53.06	5.89	164	<.0001	16.0
		sPb-3311	1	98	0.0335	0.0010	66.79	10.48	18	53.20	5.86	173	<.0001	20.3
		sPb-1925	2	146	0.0247	0.0010	52.41	5.90	31	54.90	7.80	159	0.05	-4.8
		sPb-9146	6	60	0.0241	0.0010	62.09	11.03	22	53.28	6.28	166	<.0001	14.2
		sPb-2566	7	54	0.0462	0.0010	52.36	5.48	21	54.71	7.66	170	0.0869	-4.5
		sPb-0258	8	73	0.04	0.0010	51.38	5.79	38	55.14	7.80	151	0.006	-7.3
		sPb-1661	8	74	0.0318	0.0010	51.55	5.65	22	54.82	7.71	170	0.0206	-6.3
		sPb-1881	8	88	0.0372	0.0010	57.56	9.45	69	52.29	5.28	118	<.0001	9.2
		sPb-4787	9	53	0.0055	0.0010	51.16	4.71	28	54.78	7.80	154	0.0183	-7.1
		sPb-7460	9	147	0.0297	0.0010	51.70	5.93	22	54.73	7.68	171	0.0375	-5.9
		sPb-9555	10	64	0.0268	0.0010	51.13	4.67	14	54.66	7.67	180	0.091	-6.9

Trait		Locus	Chr.	Position (cM)	MLM p	GLM p	Rare allele			Common allele			t-test p	QTL effect of rare allele [%]
							Mean	SD	n	Mean	SD	n		
T_{100}	Mean	sPb-6649	3	151	0.0013	0.0010	19.9	1.9	10	18.2	1.6	184	0.001	9
		sPb-7534	4	76	0.0097	0.0010	19.6	1.7	12	18.2	1.6	182	0.003	8
		sPb-9146	6	60	0.0118	0.0010	19.3	2.1	22.0	18.1	1.5	166	0.001	7
		sPb-8673	7	75	0.0006	0.0070	19.1	1.8	27	18.2	1.5	161	0.003	5
		sPb-3715	8	89	0.0292	0.0010	19.6	2.1	10.0	18.2	1.6	182	0.004	8
		sPb-5055	9	108	0.0026	0.0010	20.1	1.7	9.0	18.2	1.6	185	0.0004	10
	FW	sPb-6649	3	151	0.0029	0.0220	1.08	0.12	10	1.00	0.14	184	0.07	8
		sPb-7534	4	76	0.0023	0.0010	1.08	0.17	12	1.00	0.14	182	0.03	8
		sPb-3715	8	89	0.0010	0.0010	1.10	0.13	10	1.00	0.14	182	0.01	10
		sPb-7220	8	111	0.0013	0.0010	0.91	0.18	30	1.02	0.12	147	<0.0001	-11
		sPb-5055	9	108	0.0001	0.0010	1.14	0.15	9	0.99	0.14	185	0.002	15
$T_{100}-T_1$	Mean	sPb-3801	1	111	0.0031	0.0050	7.7	1.2	38	8.2	1.1	153	0.009	-6
		sPb-4081	2	65	0.0029	0.0020	7.8	1.2	82	8.4	1.1	97	0.0007	-7
		sPb-9894	3	153	0.0178	0.0160	7.8	1.2	74	8.3	1.2	119	0.0035	-6
	FW	sPb-3801	1	111	0.0026	0.0050	0.88	0.31	38	1.04	0.29	153	0.002	-15
		sPb-2080	2	152	0.0083	0.0050	0.88	0.31	49	1.04	0.30	122	0.002	-15
		sPb-5805	5	37	0.0352	0.0430	1.15	0.31	21	0.98	0.30	168	0.01	17
		sPb-7290	6	99	0.0044	0.0070	1.09	0.29	54	0.96	0.29	140	0.008	14
		sPb-6918	8	109	0.0072	0.0050	0.92	0.31	82	1.05	0.29	105	0.005	-12

Table S 3.1: Average daily mean, night and day air temperatures in eight temperature treatments and the duration of temperature treatment.

Environment	Air temperature			Duration of temperature treatment
	mean	night	day	
	[°C]			[d]
1	9.4	8.7	10.1	23
2	10.5	10.4	10.7	26
3	13.5	13.0	14.1	22
4	14.7	14.0	15.6	25
5	16.8	16.1	17.6	11
6	17.6	17.1	17.8	13
7	18.6	18.2	19.2	13
8	20.8	20.0	21.8	13

Table S 3.2: Pearson's correlation coefficients between mean across environments (*m*), exponent (*a*) and initial growth rate ($DMGR_0$ and LGR_0) for dry matter growth rate (DMGR), leaf area growth rate (LGR) and mean across environments, regression slope (*sl*) and base temperatures (T_b) for leaf appearance rate (LAR), chlorophyll content (SPAD) and chlorophyll fluorescence (F_v'/F_m' and Φ_{PSII})

		DMGR		LGR			LAR			SPAD			F_v'/F_m'			Φ_{PSII}		
		a	$DMGR_0$	*m*	*a*	LGR_0	*m*	*sl*	T_b	*m*	*sl*	T_b	*m*	*sl*	T_b	*m*	*sl*	T_b
DMGR	*m*	0.33** *	0.35 ***	0.94 ***	0.03 n.s.	0.54 ***	-0.04 n.s.	0.19 **	0.36 ***	0.34 ***	0.58 ***	0.46 ***	-0.14 n.s.	0.10 n.s.	0.13 n.s.	-0.06 n.s.	0.04 n.s.	0.07 n.s.
	a		-0.70 ***	0.33 ***	0.22 **	0.02 n.s.	-0.02 n.s.	0.09 n.s.	0.19 **	0.07 n.s.	0.20 **	0.19 **	-0.13 n.s.	0.20 **	0.20 **	-0.05 n.s.	0.05 n.s.	0.08 n.s.
	$DMGR_0$			0.34 ***	-0.33 ***	0.50 ***	0.05 n.s.	0.04 n.s.	-0.03 n.s.	0.19 **	0.17 *	0.08 n.s.	0.11 n.s.	-0.19 **	-0.19 **	0.12 n.s.	-0.02 n.s.	-0.09 n.s.
LGR	*m*				0.02 n.s.	0.60 ***	0.02 n.s.	0.21 **	0.30 ***	0.20 **	0.46 ***	0.41 ***	-0.13 n.s.	0.14 n.s.	0.16 *	-0.03 n.s.	0.04 n.s.	0.04 n.s.
	a					-0.73 ***	-0.18 *	0.03 n.s.	0.33 ***	-0.21 **	0.11 n.s.	0.26 ***	-0.18 *	0.37 ***	0.35 ***	-0.25 ***	0.05 n.s.	0.20 **
	LGR_0						0.17 *	0.12 n.s.	-0.08 n.s.	0.30 ***	0.19 **	0.04 n.s.	0.12 n.s.	-0.19 **	-0.19 *	0.22 **	0.05 n.s.	-0.09 n.s.
LAR	*m*							0.80 ***	-0.27 ***	0.11 n.s.	0.05 n.s.	-0.06 n.s.	0.04 n.s.	-0.03 n.s.	-0.02 n.s.	0.24 ***	0.05 n.s.	-0.1n .s.
	sl								0.36 ***	0.15 *	0.30 ***	0.20 **	-0.17 *	0.17 *	0.20 **	0.09 n.s.	0.15 *	0.05 n.s.
	T_b									0.06 n.s.	0.39 ***	0.42 ***	-0.32 ***	0.29 ***	0.34 ***	-0.23 **	0.15 *	0.26 ***

		DMGR		LGR			LAR			SPAD			F_v'/F_m'			Φ_{PSII}		
		a	*DMGR$_0$*	*m*	*a*	*LGR$_0$*	*m*	*sl*	T_b	*m*	*sl*	T_b	*m*	*sl*	T_b	*m*	*sl*	T_b
SPAD	*m*										0.52 ***	-0.06 n.s.	0.22 **	-0.25 ***	-0.28 ***	0.24 ***	-0.08 n.s.	-0.20 **
	sl											0.80 ***	-0.12 n.s.	0.13 n.s.	0.15*	-0.05 n.s.	0.05 n.s.	0.07 n.s.
	T_b												-0.31 ***	0.33 ***	0.37 ***	-0.23 **	0.14*	0.25 ***
F_v'/F_m'	*m*													-0.53 ***	-0.78 ***	0.63 ***	-0.21 **	-0.53 ***
	sl														0.93 ***	-0.35 ***	0.38 ***	0.52 ***
	T_b															-0.52 ***	0.34 ***	0.58 ***
Φ_{PSII}	*m*																0.16*	-0.41 ***
	sl																	0.81 ***

Table S 3.3: Marker-trait associations for dry matter growth rate (DMGR), leaf area growth rate (LGR), leaf appearance rate (LAR), chlorophyll content (SPAD), and fluorescence (F_v'/F_m' and Φ_{PSII}) in eight different temperature regimes and for means across environments (*m*), exponents (*a*) or regression slopes (*sl*) and initial growth rates ($DMGR_0$ and LGR_0)or base temperatures (T_b). Mean, standard deviation (SD) comparing two allelic groups for each significant locus. The common allele is defined as the most often occurring allele.

						rare allel			common allele		
Trait	**Locus**	**Chr.**	**Position [cM]**	**p_Marker**	**Effect**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
DMGR_9.4	sPb-1631	2	12	0.011400	0.0015	0.00044	0.00018	26	0.00029	0.00015	166
	sPb-7647	2	192	0.043200	0.0017	0.00048	0.00020	11	0.00029	0.00015	181
	sPb-0319	3	30	0.010300	0.002	0.00048	0.00018	22	0.00029	0.00015	171
	sPb-3343	4	43	0.029800	0.0018	0.00025	0.00015	28	0.00032	0.00016	163
	sPb-2138	4	64	0.019200	0.0019	0.00026	0.00017	31	0.00032	0.00016	160
	sPb-5805	5	37	0.036300	0.0013	0.00047	0.00025	21	0.00029	0.00014	168
	sPb-5086	6	148	0.036800	0.0016	0.00027	0.00013	32	0.00032	0.00017	156
	sPb-0477	6	164	0.044200	0.00087	0.00035	0.00017	58	0.00029	0.00016	133
	sPb-8608	7	133	0.041500	0.0014	0.00028	0.00016	29	0.00032	0.00016	161
	sPb-2474	8	62	0.035300	0.002	0.00018	0.00017	7	0.00031	0.00016	187
	sPb-0005	9	80	0.044800	0.0015	0.00041	0.00021	15	0.00030	0.00015	178
	sPb-8542	9	119	0.018000	0.0016	0.00026	0.00014	35	0.00032	0.00016	157
	sPb-0562	10	43	0.007600	0.0013	0.00024	0.00012	75	0.00036	0.00017	113
	sPb-3549	10	46	0.040600	0.001	0.00025	0.00013	70	0.00035	0.00017	121
	sPb-9555	10	64	0.028100	0.0017	0.00046	0.00020	14	0.00030	0.00015	180
	sPb-8232	10	68	0.040500	0.0014	0.00023	0.00012	23	0.00032	0.00017	164
DMGR_10.5	sPb-3891	1	25	0.031800	0.0015	0.00047	0.00014	14	0.00060	0.00019	180
	sPb-6917	1	30	0.033700	0.0017	0.00045	0.00017	9	0.00060	0.00019	182
	sPb-2704	1	35	0.030300	0.0017	0.00048	0.00020	10	0.00060	0.00019	175
	sPb-2683	1	107	0.032000	0.0013	0.00075	0.00024	19	0.00057	0.00018	171
	sPb-4444	2	144	0.002900	0.0015	0.00051	0.00020	25	0.00061	0.00018	158
	sPb-0319	3	30	0.011900	0.0016	0.00075	0.00022	22	0.00057	0.00018	171
	sPb-2521	4	8	0.005700	0.0013	0.00048	0.00014	37	0.00062	0.00020	152
	sPb-3343	4	43	0.035800	0.0014	0.00050	0.00019	28	0.00060	0.00018	163
	sPb-7893	5	66	0.016000	0.0014	0.00055	0.00022	47	0.00061	0.00018	145
	sPb-6855	5	71	0.041600	0.0013	0.00070	0.00024	14	0.00059	0.00018	173
	sPb-1454	5	75	0.012200	0.0014	0.00073	0.00022	25	0.00057	0.00018	166
	sPb-2457	6	58	0.005500	0.002	0.00084	0.00013	9	0.00058	0.00019	182
	sPb-4874	7	73	0.010300	0.0021	0.00046	0.00016	20	0.00061	0.00019	172
	sPb-0325	8	71	0.000099	0.0023	0.00047	0.00014	27	0.00062	0.00019	157
	sPb-9999	10	56	0.027400	0.00080	0.00054	0.00017	85	0.00064	0.00020	105
	sPb-9555	10	64	0.002800	0.002	0.00081	0.00026	14	0.00058	0.00018	180
	sPb-8232	10	68	0.016200	0.0014	0.00046	0.00015	23	0.00061	0.00019	164
	sPb-0859	10	88	0.023800	0.0014	0.00055	0.00023	44	0.00061	0.00018	145

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
DMGR_13.5	sPb-0274	1	74	0.010900	0.0034	0.0012	0.0005	24	0.0017	0.0006	169
	sPb-3311	1	98	0.008700	0.004	0.0010	0.0003	18	0.0017	0.0006	173
	sPb-1940	2	59	0.010300	0.0035	0.0010	0.0004	17	0.0017	0.0006	175
	sPb-6424	2	145	0.015700	0.0026	0.0018	0.0006	34	0.0016	0.0006	160
	sPb-1925	2	146	0.017700	0.0026	0.0018	0.0007	31	0.0016	0.0006	159
	sPb-0319	3	30	0.022400	0.0027	0.0019	0.0006	22	0.0016	0.0006	171
	sPb-9076	3	51	0.025400	0.0033	0.0013	0.0005	9	0.0016	0.0006	180
	sPb-4851	4	71	0.007400	0.0043	0.0010	0.0003	17	0.0017	0.0006	170
	sPb-1104	5	68	0.025800	0.0016	0.0014	0.0006	52	0.0017	0.0006	128
	sPb-1454	5	75	0.011100	0.0025	0.0019	0.0006	25	0.0016	0.0006	166
	sPb-6518	7	28	0.012700	0.0021	0.0012	0.0005	32	0.0017	0.0006	162
	sPb-0571	7	72	0.036400	0.003	0.0011	0.0003	11	0.0017	0.0006	177
	sPb-4874	7	73	0.001500	0.0044	0.0011	0.0003	20	0.0017	0.0006	172
	sPb-0325	8	71	0.000053	0.0045	0.0011	0.0004	27	0.0017	0.0006	157
	sPb-4787	9	53	0.027400	0.0022	0.0019	0.0007	28	0.0016	0.0006	154
	sPb-0326	9	100	0.042200	0.0016	0.0014	0.0006	51	0.0017	0.0006	141
	sPb-9158	9	104	0.027600	0.0017	0.0014	0.0006	52	0.0017	0.0006	142
	sPb-8542	9	119	0.005500	0.0028	0.0012	0.0004	35	0.0017	0.0006	157
	sPb-7460	9	147	0.040600	0.0023	0.0019	0.0007	22	0.0016	0.0006	171
	sPb-5281	10	24	0.000394	0.0048	0.0010	0.0003	20	0.0017	0.0006	174
	sPb-4944	10	59	0.004500	0.0034	0.0011	0.0005	21	0.0017	0.0006	168
	sPb-0859	10	88	0.038000	0.0024	0.0014	0.0006	44	0.0017	0.0006	145
DMGR_14.7	sPb-8947	1	64	0.032100	0.0042	0.0018	0.0006	7	0.0029	0.0010	186
	sPb-0274	1	74	0.000191	0.006	0.0018	0.0008	24	0.0031	0.0009	169
	sPb-3311	1	98	0.010000	0.0051	0.0018	0.0008	18	0.0030	0.0009	173
	sPb-1940	2	59	0.000096	0.0061	0.0016	0.0007	17	0.0030	0.0009	175
	sPb-1925	2	146	0.043900	0.0027	0.0032	0.0008	31	0.0029	0.0010	159
	sPb-7795	3	4	0.023500	0.0025	0.0022	0.0009	33	0.0031	0.0009	160
	sPb-4851	4	71	0.001400	0.0068	0.0017	0.0006	17	0.0030	0.0009	170
	sPb-5805	5	37	0.009400	0.0031	0.0033	0.0008	21	0.0029	0.0010	168
	sPb-1454	5	75	0.018400	0.0028	0.0034	0.0008	25	0.0028	0.0010	166
	sPb-6518	7	28	0.003400	0.0029	0.0021	0.0008	32	0.0031	0.0009	162
	sPb-4874	7	73	0.014300	0.0046	0.0018	0.0008	20	0.0030	0.0009	172
	sPb-0325	8	71	0.000869	0.0046	0.0020	0.0008	27	0.0031	0.0009	157
	sPb-4787	9	53	0.003400	0.0034	0.0033	0.0007	28	0.0029	0.0010	154
	sPb-5281	10	24	0.000003	0.008	0.0016	0.0006	20	0.0031	0.0009	174
	sPb-4944	10	59	0.002400	0.0042	0.0019	0.0008	21	0.0031	0.0009	168

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
DMGR_16.8	sPb-6600	1	85	0.007300	0.0051	0.0086	0.0023	24	0.0066	0.0021	159
	sPb-2683	1	107	0.004300	0.006	0.0092	0.0024	19	0.0066	0.0021	171
	sPb-1020	1	168	0.007800	0.0056	0.0071	0.0031	29	0.0069	0.0021	164
	sPb-0319	3	30	0.007000	0.0061	0.0089	0.0021	22	0.0066	0.0022	171
	sPb-9076	3	51	0.042500	0.0054	0.0059	0.0023	9	0.0070	0.0023	180
	sPb-4874	7	73	0.000173	0.0112	0.0045	0.0016	20	0.0072	0.0022	172
	sPb-0325	8	71	0.040800	0.005	0.0053	0.0022	27	0.0072	0.0022	157
	sPb-7312	8	82	0.031300	0.004	0.0054	0.0023	17	0.0070	0.0022	177
	sPb-0562	10	43	0.009900	0.0036	0.0059	0.0020	75	0.0077	0.0021	113
	sPb-3549	10	46	0.022500	0.0033	0.0058	0.0020	70	0.0075	0.0022	121
	sPb-6331	10	53	0.020300	0.007	0.0089	0.0023	11	0.0069	0.0022	171
DMGR_17.6	sPb-3525	1	57	0.032500	0.0042	0.0089	0.0030	16	0.0076	0.0020	176
	sPb-6600	1	85	0.023300	0.0039	0.0089	0.0023	24	0.0075	0.0021	159
	sPb-3311	1	98	0.040000	0.0068	0.0050	0.0014	18	0.0080	0.0020	173
	sPb-9076	3	51	0.005800	0.0063	0.0061	0.0020	9	0.0078	0.0021	180
	sPb-1454	5	75	0.021900	0.0039	0.0091	0.0018	25	0.0075	0.0021	166
	sPb-4874	7	73	0.007800	0.0079	0.0050	0.0013	20	0.0081	0.0020	172
	sPb-0435	8	67	0.044300	0.0039	0.0065	0.0024	40	0.0080	0.0020	152
	sPb-6748	9	115	0.018900	0.0044	0.0066	0.0021	52	0.0081	0.0019	136
	sPb-3549	10	46	0.026500	0.0033	0.0067	0.0020	70	0.0083	0.0021	121
DMGR_18.6	sPb-8947	1	64	0.035100	0.0078	0.009	0.001	7	0.014	0.004	186
	sPb-2457	6	58	0.037500	0.0059	0.013	0.003	9	0.014	0.004	182
	sPb-4874	7	73	0.014600	0.0102	0.009	0.002	20	0.015	0.004	172
	sPb-1881	8	88	0.034600	0.0034	0.012	0.003	69	0.015	0.004	118
	sPb-1323	8	132	0.042700	0.0044	0.014	0.004	53	0.014	0.004	141
	sPb-1997	9	1	0.004200	0.0084	0.011	0.004	38	0.015	0.004	150
	sPb-6748	9	115	0.014800	0.0061	0.011	0.004	52	0.015	0.004	136
	sPb-3298	9	116	0.021900	0.0057	0.012	0.004	56	0.015	0.004	136
	sPb-0562	10	43	0.012300	0.0046	0.012	0.003	75	0.016	0.004	113
	sPb-3549	10	46	0.014400	0.0047	0.012	0.003	70	0.016	0.004	121
DMGR_20.8	sPb-7428	6	158	0.041600	0.0095	0.029	0.011	39	0.039	0.010	149
	sPb-6518	7	28	0.010600	0.0088	0.026	0.008	32	0.039	0.010	162
	sPb-4874	7	73	0.044700	0.0148	0.023	0.007	20	0.039	0.010	172
	sPb-0562	10	43	0.003400	0.0098	0.032	0.011	75	0.040	0.010	113
	sPb-3549	10	46	0.031100	0.0076	0.032	0.011	70	0.040	0.010	121

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
DMGR_mean	sPb-3311	1	98	0.030700	0.0069	0.0056	0.0013	18	0.0092	0.0021	173
	sPb-0233	1	147	0.037800	0.004	0.0084	0.0026	15	0.0089	0.0022	178
	sPb-9076	3	51	0.024200	0.0049	0.0073	0.0017	9	0.0090	0.0023	180
	sPb-1137	3	56	0.032300	0.0034	0.0096	0.0023	31	0.0088	0.0023	155
	sPb-6518	7	28	0.043300	0.0028	0.0065	0.0017	32	0.0094	0.0021	162
	sPb-4874	7	73	0.007800	0.0078	0.0056	0.0012	20	0.0093	0.0020	172
	sPb-8608	7	133	0.040900	0.0038	0.0071	0.0028	29	0.0092	0.0020	161
	sPb-1997	9	1	0.022200	0.0048	0.0071	0.0024	38	0.0094	0.0020	150
	sPb-6748	9	115	0.035500	0.0039	0.0075	0.0023	52	0.0094	0.0020	136
	sPb-0562	10	43	0.003400	0.0039	0.0077	0.0021	75	0.0097	0.0020	113
	sPb-3549	10	46	0.016700	0.0034	0.0077	0.0022	70	0.0096	0.0020	121
DMGR_*a*	sPb-1631	2	12	0.005300	0.0159	0.38	0.03	26	0.42	0.04	166
	sPb-6689	2	22	0.041500	0.0094	0.40	0.04	64	0.42	0.04	118
	sPb-4444	2	144	0.002200	0.0176	0.42	0.06	25	0.41	0.04	158
	sPb-9146	6	60	0.004200	0.0169	0.38	0.05	22	0.42	0.04	166
	sPb-5030	6	74	0.043700	0.0158	0.39	0.04	20	0.42	0.04	174
	sPb-8081	8	58	0.032700	0.0149	0.42	0.06	14	0.41	0.04	177
	sPb-2474	8	62	0.003700	0.026	0.44	0.09	7	0.41	0.04	187
	sPb-7220	8	111	0.020800	0.013	0.42	0.05	30	0.41	0.04	147
	sPb-0005	9	80	0.019300	0.016	0.38	0.06	15	0.42	0.04	178
	sPb-5055	9	108	0.024500	0.0188	0.37	0.05	9	0.42	0.04	185
	sPb-9999	10	56	0.013900	0.0079	0.42	0.04	85	0.41	0.04	105
$DMGR_0$	sPb-8773	1	11	0.500400	0.00036	0.00038	0.00020	13	0.00031	0.00011	181
	sPb-2683	1	107	0.025800	0.00110	0.00044	0.00014	19	0.00030	0.00010	171
	sPb-1631	2	12	0.009400	0.00110	0.00040	0.00012	26	0.00030	0.00011	166
	sPb-4444	2	144	0.029900	0.00091	0.00029	0.00013	25	0.00032	0.00011	158
	sPb-7647	2	192	0.035300	0.00120	0.00042	0.00013	11	0.00031	0.00011	181
	sPb-7795	3	4	0.042800	0.00083	0.00025	0.00010	33	0.00033	0.00011	160
	sPb-0319	3	30	0.001200	0.00160	0.00044	0.00011	22	0.00030	0.00010	171
	sPb-3343	4	43	0.043400	0.00110	0.00026	0.00012	28	0.00032	0.00011	163
	sPb-4851	4	71	0.036700	0.00170	0.00023	0.00008	17	0.00033	0.00011	170
	sPb-5805	5	37	0.005700	0.00120	0.00043	0.00016	21	0.00030	0.00010	168
	sPb-1454	5	75	0.025900	0.00097	0.00041	0.00012	25	0.00030	0.00011	166
	sPb-5086	6	148	0.019000	0.00120	0.00027	0.00011	32	0.00032	0.00011	156
	sPb-4874	7	73	0.003400	0.00200	0.00023	0.00008	20	0.00033	0.00011	172
	sPb-0325	8	71	0.000555	0.00170	0.00025	0.00009	27	0.00032	0.00011	157
	sPb-8542	9	119	0.016500	0.00110	0.00027	0.00011	35	0.00033	0.00011	157
	sPb-5281	10	24	0.000621	0.00230	0.00022	0.00008	20	0.00033	0.00011	174
	sPb-0562	10	43	0.028100	0.00074	0.00027	0.00010	75	0.00035	0.00012	113
	sPb-6331	10	53	0.025800	0.00140	0.00046	0.00017	11	0.00031	0.00010	171
	sPb-9555	10	64	0.009400	0.00140	0.00044	0.00013	14	0.00031	0.00011	180

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
LGR_13.5	sPb-0274	1	74	0.004500	0.01	0.407	0.207	24	0.610	0.267	169
	sPb-3311	1	98	0.044500	0.0085	0.378	0.161	18	0.601	0.270	173
	sPb-0319	3	30	0.002900	0.0088	0.824	0.284	22	0.551	0.251	171
	sPb-9076	3	51	0.044600	0.0069	0.473	0.255	9	0.593	0.266	180
	sPb-4851	4	71	0.030200	0.0101	0.366	0.157	17	0.603	0.264	170
	sPb-1104	5	68	0.037400	0.0036	0.493	0.288	52	0.609	0.258	128
	sPb-6518	7	28	0.028400	0.0046	0.431	0.218	32	0.613	0.268	162
	sPb-4874	7	73	0.002500	0.0115	0.373	0.148	20	0.611	0.268	172
	sPb-0325	8	71	0.001400	0.0094	0.405	0.207	27	0.607	0.267	157
	sPb-4787	9	53	0.036200	0.0053	0.746	0.281	28	0.563	0.260	154
	sPb-0005	9	80	0.003000	0.0085	0.398	0.314	15	0.599	0.260	178
	sPb-8542	9	119	0.024300	0.0056	0.454	0.230	35	0.605	0.261	157
	sPb-5281	10	24	0.007800	0.0105	0.370	0.146	20	0.608	0.268	174
	sPb-4944	10	59	0.005700	0.008	0.382	0.207	21	0.614	0.265	168
	sPb-0859	10	88	0.000699	0.0092	0.485	0.259	44	0.616	0.265	145
LGR_14.7	sPb-8947	1	64	0.027600	0.0087	0.651	0.184	7	1.082	0.354	186
	sPb-0274	1	74	0.000790	0.012	0.706	0.370	24	1.121	0.327	169
	sPb-3311	1	98	0.008900	0.0111	0.671	0.337	18	1.104	0.334	173
	sPb-0909	2	13	0.016100	0.0048	0.888	0.343	44	1.124	0.348	139
	sPb-1940	2	59	0.000412	0.0114	0.622	0.344	17	1.110	0.331	175
	sPb-4851	4	71	0.001200	0.0154	0.633	0.305	17	1.110	0.333	170
	sPb-5805	5	37	0.021800	0.0055	1.278	0.349	21	1.042	0.350	168
	sPb-1454	5	75	0.022800	0.0055	1.336	0.329	25	1.025	0.344	166
	sPb-6518	7	28	0.003100	0.006	0.766	0.343	32	1.128	0.332	162
	sPb-4874	7	73	0.004200	0.0116	0.682	0.325	20	1.116	0.335	172
	sPb-0325	8	71	0.012000	0.0075	0.763	0.384	27	1.119	0.328	157
	sPb-4787	9	53	0.000957	0.0078	1.331	0.262	28	1.032	0.351	154
	sPb-5281	10	24	0.000004	0.0172	0.621	0.286	20	1.119	0.329	174
	sPb-4944	10	59	0.004200	0.0081	0.713	0.339	21	1.121	0.331	168
LGR_16.8	sPb-6600	1	85	0.024500	0.0081	3.286	0.849	24	2.460	0.735	159
	sPb-5905	1	112	0.039100	0.0081	3.242	0.795	23	2.472	0.748	171
	sPb-6434	2	75	0.044100	0.008	2.277	0.889	13	2.594	0.795	173
	sPb-4226	3	39	0.022800	0.0059	2.238	0.723	54	2.703	0.790	135
	sPb-9076	3	51	0.008200	0.013	2.257	0.919	9	2.592	0.777	180
	sPb-1137	3	56	0.014100	0.0083	3.109	0.767	31	2.476	0.747	155
	sPb-4629	3	145	0.037900	0.0052	2.653	0.674	95	2.438	0.891	89
	sPb-4086	5	70	0.040200	0.0073	2.471	1.032	29	2.539	0.719	150
	sPb-6518	7	28	0.032300	0.0064	2.044	0.690	32	2.668	0.762	162
	sPb-4874	7	73	0.000130	0.0191	1.922	0.538	20	2.655	0.767	172
	sPb-6331	10	53	0.020000	0.013	3.360	0.929	11	2.537	0.743	171

						rare allel			common allele		
Trait	**Locus**	**Chr.**	**Position [cM]**	**p_Marker**	**Effect**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
LGR_17.6	sPb-6600	1	85	0.022600	0.0082	3.234	1.049	24	2.558	0.711	159
	sPb-3311	1	98	0.021400	0.0159	1.772	0.500	18	2.755	0.768	173
	sPb-4444	2	144	0.024500	0.0078	2.811	1.154	25	2.645	0.710	158
	sPb-9076	3	51	0.011000	0.0121	2.202	1.033	9	2.693	0.776	180
	sPb-4874	7	73	0.014900	0.0153	1.801	0.508	20	2.783	0.754	172
	sPb-6918	8	109	0.035100	0.0058	2.506	0.847	82	2.799	0.739	105
	sPb-6748	9	115	0.023500	0.0089	2.326	0.806	52	2.784	0.738	136
LGR_18.6	sPb-8947	1	64	0.044400	0.0135	2.709	0.575	7	4.246	1.213	186
	sPb-1020	1	168	0.039600	0.0091	3.916	1.759	29	4.249	1.096	164
	sPb-1137	3	56	0.016100	0.0095	4.698	1.571	31	4.125	1.121	155
	sPb-9303	4	85	0.021500	0.0111	3.473	0.706	13	4.217	1.211	178
	sPb-2457	6	58	0.044900	0.0105	4.027	1.099	9	4.219	1.230	182
	sPb-1997	9	1	0.000911	0.0176	3.330	1.249	38	4.396	1.132	150
	sPb-6748	9	115	0.013500	0.0112	3.486	1.136	52	4.455	1.157	136
	sPb-3298	9	116	0.018300	0.0106	3.554	1.161	56	4.455	1.157	136
	sPb-0562	10	43	0.029000	0.0074	3.638	0.982	75	4.557	1.235	113
	sPb-3549	10	46	0.033500	0.0075	3.604	0.980	70	4.534	1.239	121
LGR_20.8	sPb-0233	1	147	0.042100	0.0171	12.279	3.748	15	11.313	3.173	178
	sPb-1137	3	56	0.033500	0.0149	12.825	2.626	31	11.118	3.304	155
	sPb-7428	6	158	0.040400	0.0165	10.180	3.415	39	11.654	3.084	149
	sPb-6518	7	28	0.008400	0.0157	9.070	2.567	32	11.866	3.109	162
	sPb-2566	7	54	0.025000	0.0164	13.482	3.096	21	11.127	3.070	170
	sPb-8608	7	133	0.019900	0.0186	9.951	3.398	29	11.670	3.069	161
	sPb-8081	8	58	0.039000	0.0167	12.735	3.917	14	11.305	3.143	177
	sPb-0562	10	43	0.012000	0.0147	10.537	3.009	75	12.106	3.214	113
LGR_mean	sPb-0233	1	147	0.032300	0.0086	3.891	1.245	15	3.727	0.915	178
	sPb-1020	1	168	0.043100	0.0075	3.657	1.265	29	3.770	0.872	164
	sPb-1137	3	56	0.015800	0.0081	4.251	0.920	31	3.658	0.928	155
	sPb-4086	5	70	0.021200	0.0077	3.482	1.208	29	3.737	0.859	150
	sPb-6518	7	28	0.024000	0.0065	2.937	0.752	32	3.906	0.884	162
	sPb-4874	7	73	0.011000	0.0153	2.745	0.601	20	3.881	0.886	172
	sPb-8608	7	133	0.015200	0.0093	3.230	1.126	29	3.841	0.867	161
	sPb-1997	9	1	0.011200	0.0113	3.258	1.097	38	3.870	0.873	150
	sPb-0562	10	43	0.020400	0.0068	3.412	0.856	75	3.998	0.932	113

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
LGR_*a*	sPb-0274	1	74	0.004400	0.0329	0.466	0.086	24	0.431	0.064	169
	sPb-1020	1	168	0.008400	0.0245	0.456	0.086	29	0.431	0.063	164
	sPb-1940	2	59	0.034100	0.0251	0.470	0.098	17	0.433	0.063	175
	sPb-0319	3	30	0.020700	0.0245	0.396	0.054	22	0.441	0.068	171
	sPb-4851	4	71	0.027500	0.0317	0.468	0.097	17	0.432	0.064	170
	sPb-7794	4	88	0.031500	0.0123	0.451	0.061	75	0.425	0.071	114
	sPb-1104	5	68	0.021000	0.0139	0.460	0.067	52	0.430	0.066	128
	sPb-6589	8	16	0.011300	0.0254	0.455	0.082	30	0.432	0.064	164
	sPb-1888	8	63	0.043500	0.0236	0.388	0.052	10	0.439	0.068	179
	sPb-0325	8	71	0.036400	0.0225	0.457	0.088	27	0.434	0.064	157
	sPb-4787	9	53	0.029300	0.0203	0.404	0.053	28	0.442	0.070	154
	sPb-0005	9	80	0.009400	0.0271	0.480	0.107	15	0.432	0.062	178
	sPb-6403	9	133	0.042600	0.0193	0.457	0.088	28	0.432	0.063	166
	sPb-5281	10	24	0.005000	0.0369	0.470	0.090	20	0.431	0.064	174
	sPb-4944	10	59	0.000390	0.0349	0.477	0.084	21	0.429	0.064	168
	sPb-0859	10	88	0.009800	0.0253	0.448	0.080	44	0.431	0.063	145
LGR_0	sPb-0274	1	74	0.000873	0.0309	0.391	0.196	24	0.596	0.201	169
	sPb-3311	1	98	0.018500	0.0264	0.367	0.165	18	0.587	0.204	173
	sPb-1940	2	59	0.009500	0.0223	0.350	0.188	17	0.588	0.200	175
	sPb-0319	3	30	0.004900	0.0213	0.776	0.192	22	0.541	0.199	171
	sPb-4851	4	71	0.005300	0.035	0.348	0.149	17	0.590	0.200	170
	sPb-1454	5	75	0.031700	0.0135	0.741	0.225	25	0.543	0.196	166
	sPb-6518	7	28	0.011100	0.0135	0.416	0.181	32	0.600	0.203	162
	sPb-4874	7	73	0.000364	0.0355	0.362	0.143	20	0.596	0.203	172
	sPb-0325	8	71	0.002700	0.023	0.407	0.195	27	0.593	0.201	157
	sPb-8590	9	16	0.041000	0.0097	0.487	0.202	50	0.597	0.203	138
	sPb-4787	9	53	0.004300	0.0181	0.735	0.205	28	0.547	0.199	154
	sPb-0005	9	80	0.012700	0.0182	0.418	0.251	15	0.582	0.203	178
	sPb-5281	10	24	0.000351	0.0364	0.350	0.139	20	0.595	0.203	174
	sPb-4944	10	59	0.001400	0.0236	0.373	0.177	21	0.599	0.201	168
	sPb-0859	10	88	0.022400	0.0167	0.496	0.247	44	0.594	0.194	145

						rare allel			common allele		
Trait	**Locus**	**Chr.**	**Position [cM]**	**p_Marker**	**Effect**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
LAR_10.5	sPb-3891	1	25	0.0209	0.0311	0.027	0.013	14	0.013	0.014	180
	sPb-7647	2	192	0.0274	0.0294	0.030	0.016	11	0.012	0.013	181
	sPb-2521	4	8	0.0066	0.0252	0.024	0.014	37	0.011	0.013	152
	sPb-0326	9	100	0.0014	0.0235	0.022	0.013	51	0.010	0.013	141
	sPb-9158	9	104	0.0041	0.0215	0.021	0.013	52	0.011	0.014	142
	sPb-6271	10	58	0.0195	0.0156	0.010	0.013	68	0.016	0.015	122
LAR_13.5	sPb-0232	1	70	0.0412	0.0086	0.075	0.018	38	0.083	0.025	155
	sPb-3103	1	114	0.0026	0.0151	0.094	0.020	54	0.076	0.024	140
	sPb-4226	3	39	0.0102	0.0102	0.086	0.028	54	0.079	0.022	135
	sPb-3343	4	43	0.0396	0.0131	0.098	0.021	28	0.078	0.024	163
	sPb-0906	5	7	0.0175	0.0102	0.080	0.022	54	0.081	0.025	114
	sPb-1104	5	68	0.0208	0.0086	0.072	0.023	52	0.084	0.024	128
	sPb-7106	5	72	0.0130	0.0101	0.074	0.023	39	0.083	0.024	150
	sPb-9066	6	89	0.0294	0.0124	0.097	0.021	24	0.079	0.024	169
	sPb-7126	8	29	0.0145	0.014	0.098	0.016	32	0.078	0.024	161
	sPb-9242	8	66	0.0004	0.0228	0.069	0.014	12	0.083	0.024	177
	sPb-0817	10	42	0.0220	0.011	0.075	0.021	26	0.082	0.024	166
LAR_14.7	sPb-2704	1	35	0.0023	0.0184	0.085	0.025	10	0.099	0.019	175
	sPb-3525	1	57	0.0195	0.0118	0.105	0.013	16	0.098	0.020	176
	sPb-0274	1	74	0.0006	0.0245	0.099	0.022	24	0.099	0.019	169
	sPb-1137	3	56	0.0067	0.0113	0.094	0.022	31	0.100	0.019	155
	sPb-8255	4	156	0.0104	0.011	0.090	0.026	16	0.100	0.018	177
	sPb-8954	6	149	0.0209	0.0114	0.110	0.016	13	0.098	0.020	176
	sPb-2566	7	54	0.0056	0.0116	0.091	0.024	21	0.100	0.019	170
	sPb-5054	8	30	0.0132	0.0107	0.108	0.015	31	0.097	0.020	163
LAR_16.8	sPb-8773	1	11	0.0016	0.0162	0.172	0.031	13	0.182	0.030	181
	sPb-5905	1	112	0.0069	0.0131	0.198	0.018	23	0.179	0.030	171
	sPb-9076	3	51	0.0317	0.0128	0.175	0.028	9	0.182	0.030	180
	sPb-0932	5	45	0.0283	0.0099	0.169	0.020	14	0.183	0.031	169
	sPb-3361	7	26	0.0361	0.0089	0.197	0.030	43	0.177	0.028	150
	sPb-2474	8	62	0.0019	0.0202	0.216	0.037	7	0.180	0.029	187
	sPb-6515	9	70	0.0035	0.0145	0.202	0.030	32	0.177	0.028	161
	sPb-9555	10	64	0.0100	0.0133	0.206	0.018	14	0.179	0.030	180

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
LAR_17.6	sPb-3103	1	114	0.0042	0.0117	0.218	0.025	54	0.198	0.035	140
	sPb-6663	2	155	0.0221	0.0062	0.206	0.039	93	0.200	0.028	94
	sPb-7795	3	4	0.0176	0.0097	0.221	0.031	33	0.200	0.034	160
	sPb-4226	3	39	0.0003	0.0098	0.209	0.049	54	0.201	0.026	135
	sPb-7312	8	82	0.0014	0.0139	0.184	0.021	17	0.205	0.034	177
	sPb-0600	10	4	0.0024	0.0092	0.195	0.035	81	0.211	0.032	106
	sPb-9999	10	56	0.0002	0.0091	0.213	0.025	85	0.195	0.038	105
LAR_18.6	sPb-3103	1	114	0.0008	0.0172	0.275	0.042	54	0.248	0.043	140
	sPb-5267	3	134	0.0409	0.0114	0.274	0.040	23	0.254	0.041	164
	sPb-5890	5	13	0.0388	0.0089	0.253	0.041	66	0.255	0.049	102
	sPb-5119	5	33	0.0432	0.0074	0.246	0.050	91	0.265	0.037	99
	sPb-3361	7	26	0.0142	0.0135	0.278	0.039	43	0.249	0.044	150
	sPb-3341	8	0	0.0156	0.0173	0.288	0.058	9	0.253	0.044	179
	sPb-6589	8	16	0.0047	0.0196	0.286	0.038	30	0.250	0.044	164
	sPb-1661	8	74	0.0196	0.0136	0.250	0.048	22	0.256	0.045	170
	sPb-8542	9	119	0.0214	0.0136	0.281	0.036	35	0.250	0.045	157
	sPb-0859	10	88	0.0415	0.013	0.278	0.038	44	0.249	0.045	145
LAR_20.8	sPb-2583	1	15	0.0246	0.0128	0.320	0.041	25	0.318	0.049	164
	sPb-3891	1	25	0.0308	0.0144	0.315	0.045	14	0.320	0.048	180
	sPb-9076	3	51	0.0325	0.0149	0.328	0.032	9	0.319	0.049	180
	sPb-0873	5	104	0.0038	0.0085	0.323	0.059	90	0.316	0.034	96
	sPb-6526	6	144	0.0094	0.0148	0.347	0.038	33	0.313	0.047	157
	sPb-1323	8	132	0.0191	0.0109	0.330	0.043	53	0.316	0.048	141
	sPb-0600	10	4	0.0425	0.0072	0.307	0.052	81	0.330	0.043	106
	sPb-0859	10	88	0.0044	0.016	0.332	0.038	44	0.316	0.050	145
LAR_mean	sPb-3103	1	114	0.0001	0.0083	0.178	0.014	54	0.162	0.012	140
	sPb-4226	3	39	0.0045	0.0047	0.173	0.014	54	0.164	0.014	135
	sPb-7534	4	76	0.0241	0.0065	0.184	0.010	12	0.165	0.014	182
	sPb-7106	5	72	0.0098	0.0046	0.161	0.012	39	0.168	0.014	150
	sPb-0873	5	104	0.0449	0.0033	0.169	0.014	90	0.164	0.014	96
	sPb-3361	7	26	0.0395	0.0049	0.178	0.015	43	0.163	0.012	150
	sPb-1311	7	36	0.0188	0.006	0.172	0.015	57	0.164	0.012	117
	sPb-2474	8	62	0.0170	0.0084	0.189	0.013	7	0.166	0.014	187
	sPb-0600	10	4	0.0193	0.0039	0.160	0.013	81	0.172	0.013	106
LAR_*sl*	sPb-3891	1	25	0.0209	0.0034	0.030	0.004	14	0.031	0.003	180
	sPb-3103	1	114	0.0041	0.0027	0.032	0.003	54	0.031	0.003	140
	sPb-1631	2	12	0.0287	0.0022	0.031	0.003	26	0.031	0.003	166
	sPb-0873	5	104	0.0042	0.0019	0.032	0.003	90	0.030	0.003	96
	sPb-1323	8	132	0.0424	0.002	0.031	0.004	53	0.031	0.003	141
	sPb-0600	10	4	0.0062	0.002	0.030	0.003	81	0.032	0.003	106

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
LAR_T_b	sPb-2583	1	15	0.0372	0.0014	9.954	0.348	25	10.213	0.259	164
	sPb-5832	1	167	0.0357	0.0018	10.117	0.336	56	10.206	0.257	136
	sPb-7647	2	192	0.0114	0.0021	9.997	0.350	11	10.195	0.272	181
	sPb-2521	4	8	0.0011	0.0018	9.977	0.291	37	10.229	0.263	152
	sPb-8255	4	156	0.0316	0.0014	10.283	0.216	16	10.167	0.289	177
	sPb-9242	8	66	0.0431	0.0017	10.269	0.305	12	10.165	0.283	177
	sPb-8764	9	111	0.0300	0.0016	10.102	0.309	56	10.236	0.246	131
	sPb-8542	9	119	0.0332	0.0014	9.979	0.296	35	10.225	0.259	157
	sPb-6271	10	58	0.0202	0.001	10.267	0.219	68	10.124	0.307	122

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
SPAD_13.5	sPb-3525	1	57	0.02870	0.0187	4.2	2.4	16	5.5	2.4	176
	sPb-2683	1	107	0.02420	0.0181	4.2	2.6	19	5.5	2.4	171
	sPb-0319	3	30	0.04110	0.0171	4.4	2.3	22	5.5	2.4	171
	sPb-6649	3	151	0.00053	0.0337	8.4	2.4	10	5.2	2.3	184
	sPb-7534	4	76	0.00150	0.028	7.8	2.5	12	5.2	2.3	182
	sPb-9303	4	85	0.00730	0.0237	3.7	1.5	13	5.6	2.4	178
	sPb-0854	4	97	0.00530	0.0123	5.9	2.6	95	4.9	2.1	98
	sPb-2457	6	58	0.01920	0.0234	3.6	1.8	9	5.5	2.4	182
	sPb-9146	6	60	0.00023	0.0266	7.4	2.6	22	5.2	2.3	166
	sPb-9066	6	89	0.00300	0.0205	6.9	2.5	24	5.2	2.3	169
	sPb-3715	8	89	0.00740	0.0263	7.8	2.7	10	5.3	2.3	182
	sPb-5055	9	108	0.00770	0.028	8.0	3.2	9	5.3	2.3	185
	sPb-3549	10	46	0.03760	0.0107	6.2	2.2	70	5.0	2.4	121
SPAD_14.7	sPb-2704	1	35	0.01380	0.0165	9.2	1.9	10	7.8	1.9	175
	sPb-4081	2	65	0.01240	0.0083	7.6	1.7	82	8.2	2.2	97
	sPb-7186	3	141	0.00460	0.009	8.5	1.7	68	7.6	2.0	124
	sPb-6649	3	151	0.00750	0.0175	9.5	1.8	10	7.8	1.9	184
	sPb-7534	4	76	0.02680	0.0131	9.1	1.9	12	7.8	1.9	182
	sPb-4086	5	70	0.00800	0.0126	7.5	1.9	29	8.0	1.9	150
	sPb-5054	8	30	0.01970	0.0114	8.5	2.0	31	7.8	1.9	163
	sPb-1888	8	63	0.00370	0.0167	9.6	2.6	10	7.8	1.9	179
	sPb-3715	8	89	0.02840	0.0149	9.2	1.6	10	7.8	2.0	182
	sPb-7220	8	111	0.01640	0.012	7.2	1.8	30	8.0	2.0	147
	sPb-8764	9	111	0.01870	0.0135	8.3	2.1	56	7.8	1.9	131

						rare allel			common allele		
Trait	**Locus**	**Chr.**	**Position [cM]**	**p_Marker**	**Effect**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
	sPb-2583	1	15	0.02620	0.0129	12.8	2.6	25	14.0	2.8	164
	sPb-0090	1	66	0.01830	0.0088	15.0	3.5	33	13.6	2.5	157
	sPb-6600	1	85	0.02340	0.0112	14.5	2.8	24	13.8	2.7	159
	sPb-5905	1	112	0.00280	0.0161	14.5	2.7	23	13.8	2.7	171
	sPb-0233	1	147	0.03380	0.0119	14.5	2.6	15	13.8	2.8	178
	sPb-1020	1	168	0.03370	0.0109	13.8	2.5	29	13.8	2.8	164
	sPb-0909	2	13	0.00770	0.0098	15.1	2.5	44	13.4	2.7	139
	sPb-8349	3	128	0.03370	0.0135	15.0	3.0	11	13.8	2.7	183
SPAD_16.8	sPb-9303	4	85	0.02060	0.0135	14.7	3.1	13	13.8	2.7	178
	sPb-7428	6	158	0.03500	0.0117	13.8	2.7	39	13.9	2.8	149
	sPb-8980	7	15	0.01510	0.0094	12.6	2.4	38	14.2	2.7	155
	sPb-8673	7	75	0.03860	0.0092	14.2	2.1	27	13.8	2.9	156
	sPb-7126	8	29	0.01130	0.0137	13.8	2.6	32	13.9	2.8	161
	sPb-0258	8	73	0.04380	0.0081	13.1	2.0	38	14.1	2.9	151
	sPb-8590	9	16	0.02440	0.0073	14.8	2.3	50	13.4	2.7	138
	sPb-0326	9	100	0.01660	0.0091	13.0	2.3	51	14.2	2.8	141
	sPb-9158	9	104	0.01810	0.0089	13.0	2.2	52	14.2	2.8	142
	sPb-0350	2	89	0.03120	0.0108	12.7	3.0	34	14.1	3.0	159
	sPb-4444	2	144	0.00150	0.0153	12.3	2.9	25	14.2	2.9	158
	sPb-1137	3	56	0.04300	0.0091	14.1	2.2	31	13.8	3.1	155
	sPb-1454	5	75	0.02030	0.0117	14.6	2.6	25	13.7	3.1	166
	sPb-0873	5	104	0.02220	0.0076	14.1	3.4	90	13.4	2.7	96
	sPb-6526	6	144	0.01320	0.0126	12.5	3.4	33	14.1	2.9	157
SPAD_17.6	sPb-5086	6	148	0.01030	0.013	12.3	3.3	32	14.1	2.9	156
	sPb-0325	8	71	0.01840	0.0145	12.3	3.9	27	14.0	2.8	157
	sPb-7312	8	82	0.02190	0.0117	12.6	2.5	17	14.0	3.0	177
	sPb-0326	9	100	0.02310	0.0075	13.1	2.9	51	14.1	3.1	141
	sPb-9158	9	104	0.02210	0.0075	13.1	2.9	52	14.1	3.1	142
	sPb-5055	9	108	0.03080	0.0168	14.8	2.6	9	13.8	3.0	185
	sPb-8232	10	68	0.00280	0.0142	12.0	2.8	23	14.0	3.0	164
	sPb-2583	1	15	0.04420	0.0109	17.7	3.4	25	21.1	3.6	164
	sPb-3311	1	98	0.03570	0.0192	16.7	3.2	18	21.1	3.6	173
	sPb-6066	1	171	0.01250	0.0138	21.4	3.3	31	20.6	3.9	160
	sPb-4444	2	144	0.02230	0.0112	18.6	3.7	25	21.2	3.7	158
	sPb-9135	3	58	0.00650	0.0093	21.5	4.6	77	20.1	3.0	109
	sPb-7186	3	141	0.03350	0.0071	19.5	3.3	68	21.3	3.9	124
SPAD_18.6	sPb-4851	4	71	0.01410	0.0261	16.5	3.2	17	21.1	3.6	170
	sPb-7534	4	76	0.02190	0.0144	20.4	3.1	12	20.7	3.8	182
	sPb-0873	5	104	0.02560	0.0076	21.2	4.5	90	20.1	3.1	96
	sPb-4874	7	73	0.00074	0.0292	16.5	3.1	20	21.2	3.6	172
	sPb-5055	9	108	0.02030	0.0175	20.4	2.5	9	20.7	3.8	185
	sPb-5281	10	24	0.01040	0.0222	16.6	3.0	20	21.2	3.6	174
	sPb-9555	10	64	0.01840	0.0146	22.3	3.0	14	20.6	3.8	180

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
SPAD_20.8	sPb-2583	1	15	0.00017	0.0214	27.1	3.5	25	32.5	4.8	164
	sPb-5905	1	112	0.00040	0.0197	34.2	2.9	23	31.5	5.1	171
	sPb-7795	3	4	0.03330	0.0103	28.4	3.9	33	32.5	4.8	160
	sPb-9076	3	51	0.04000	0.0143	30.0	3.0	9	31.9	5.0	180
	sPb-4851	4	71	0.01000	0.0295	25.9	3.4	17	32.4	4.8	170
	sPb-1454	5	75	0.03540	0.0108	33.3	3.3	25	31.6	5.1	166
	sPb-1311	7	36	0.03280	0.0122	30.3	4.5	57	32.4	5.2	117
	sPb-4874	7	73	0.00720	0.0236	26.4	3.4	20	32.5	4.7	172
	sPb-3715	8	89	0.00440	0.0203	31.3	3.8	10	31.9	5.0	182
	sPb-0326	9	100	0.02680	0.0094	29.6	3.4	51	32.7	5.1	141
	sPb-9158	9	104	0.01350	0.0105	29.5	3.5	52	32.6	5.1	142
	sPb-0562	10	43	0.03030	0.0089	30.4	3.8	75	32.8	5.5	113
	sPb-9555	10	64	0.04000	0.0128	34.0	4.0	14	31.6	5.0	180
	sPb-8232	10	68	0.00660	0.0149	27.5	4.1	23	32.3	4.9	164
SPAD_mean	sPb-2583	1	15	0.00130	0.0106	14.0	1.6	25	16.0	1.4	164
	sPb-3311	1	98	0.04100	0.0106	13.7	2.0	18	16.0	1.3	173
	sPb-5905	1	112	0.00250	0.0099	16.1	1.2	23	15.7	1.6	171
	sPb-4444	2	144	0.00530	0.0075	14.5	1.8	25	16.0	1.4	158
	sPb-6649	3	151	0.00350	0.0116	15.9	1.4	10	15.7	1.6	184
	sPb-4851	4	71	0.01760	0.0153	13.6	2.1	17	15.9	1.4	170
	sPb-7534	4	76	0.00380	0.0102	15.9	1.5	12	15.7	1.6	182
	sPb-1454	5	75	0.02270	0.0067	16.0	1.6	25	15.7	1.6	166
	sPb-1311	7	36	0.04270	0.0066	14.9	1.8	57	16.1	1.3	117
	sPb-4874	7	73	0.03990	0.0106	13.8	2.1	20	16.0	1.3	172
	sPb-7126	8	29	0.03970	0.0068	15.1	2.1	32	15.9	1.4	161
	sPb-3715	8	89	0.00019	0.015	16.2	1.6	10	15.7	1.6	182
	sPb-5055	9	108	0.00120	0.0138	16.0	1.5	9	15.7	1.6	185
	sPb-8232	10	68	0.00370	0.009	14.0	1.8	23	16.0	1.4	164
SPAD_*sl*	sPb-2583	1	15	0.00870	0.006	2.8	0.5	25	3.6	0.5	164
	sPb-2683	1	107	0.04280	0.0045	3.9	0.5	19	3.5	0.6	171
	sPb-5905	1	112	0.00300	0.0066	3.9	0.5	23	3.5	0.6	171
	sPb-9076	3	51	0.04210	0.0055	3.1	0.5	9	3.6	0.6	180
	sPb-4851	4	71	0.03840	0.0101	2.6	0.3	17	3.6	0.5	170
	sPb-9303	4	85	0.02400	0.0055	3.8	0.8	13	3.5	0.6	178
	sPb-9146	6	60	0.01840	0.005	2.9	0.6	22	3.6	0.5	166
	sPb-4874	7	73	0.00049	0.0125	2.6	0.3	20	3.7	0.5	172
	sPb-0325	8	71	0.03140	0.0053	2.8	0.5	27	3.7	0.5	157
	sPb-0326	9	100	0.01560	0.0042	3.1	0.6	51	3.7	0.5	141
	sPb-9158	9	104	0.01140	0.0044	3.1	0.6	52	3.7	0.5	142
	sPb-0562	10	43	0.02310	0.0037	3.2	0.5	75	3.8	0.5	113
	sPb-0859	10	88	0.04000	0.0049	3.1	0.7	44	3.7	0.5	145

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
$SPAD\_T_b$	sPb-2704	1	35	0.00800	0.0056	11.8	1.0	10	12.5	0.7	175
	sPb-6649	3	151	0.00001	0.0085	11.1	1.1	10	12.5	0.6	184
	sPb-9146	6	60	0.00032	0.0055	11.6	1.0	22	12.6	0.6	166
	sPb-8980	7	15	0.00760	0.0032	12.6	0.6	38	12.4	0.7	155
	sPb-4874	7	73	0.01350	0.0068	11.6	0.9	20	12.6	0.6	172
	sPb-6798	7	132	0.03010	0.0037	11.9	1.1	27	12.5	0.6	159
	sPb-8608	7	133	0.02850	0.0037	11.9	1.0	29	12.5	0.6	161
	sPb-5054	8	30	0.04450	0.0031	12.0	1.0	31	12.6	0.6	163
	sPb-1888	8	63	0.01690	0.0041	12.0	1.3	10	12.5	0.7	179
	sPb-5055	9	108	0.00380	0.0062	11.4	1.4	9	12.5	0.6	185
	sPb-0562	10	43	0.00950	0.0031	12.1	0.8	75	12.7	0.6	113
	sPb-3549	10	46	0.02310	0.0029	12.1	0.8	70	12.7	0.6	121

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
Fv'/Fm'_13.5	sPb-1631	2	12	0.0235	0.0205	0.15	0.05	26	0.19	0.07	166
	sPb-2229	2	143	0.0043	0.0207	0.15	0.07	55	0.20	0.06	128
	sPb-9076	3	51	0.0178	0.0318	0.11	0.09	9	0.19	0.07	180
	sPb-0932	5	45	0.0317	0.0208	0.16	0.07	14	0.19	0.07	169
	sPb-6855	5	71	0.0392	0.024	0.17	0.08	14	0.19	0.07	173
	sPb-5796	7	76	0.0352	0.0113	0.18	0.07	83	0.20	0.07	110
	sPb-1661	8	74	0.0252	0.0207	0.19	0.06	22	0.19	0.07	170
	sPb-7220	8	111	0.0008	0.0301	0.18	0.08	30	0.19	0.07	147
Fv'/Fm'_14.7	sPb-3525	1	57	0.0069	0.0354	0.32	0.11	16	0.28	0.10	176
	sPb-6600	1	85	0.0209	0.0259	0.31	0.12	24	0.29	0.10	159
	sPb-2229	2	143	0.0316	0.019	0.26	0.12	55	0.30	0.09	128
	sPb-4444	2	144	0.0259	0.0247	0.24	0.11	25	0.29	0.10	158
	sPb-3838	4	96	0.0404	0.015	0.27	0.11	72	0.30	0.10	114
	sPb-5805	5	37	0.0285	0.0251	0.29	0.14	21	0.29	0.10	168
	sPb-6855	5	71	0.0099	0.0364	0.30	0.16	14	0.29	0.09	173
	sPb-3644	6	13	0.0217	0.0302	0.28	0.14	34	0.29	0.09	159
	sPb-7428	6	158	0.0418	0.0268	0.24	0.14	39	0.30	0.09	149
	sPb-2566	7	54	0.0100	0.0299	0.26	0.11	21	0.29	0.10	170
	sPb-7126	8	29	0.0176	0.0286	0.27	0.15	32	0.29	0.09	161
	sPb-5054	8	30	0.0314	0.0254	0.27	0.14	31	0.29	0.09	163
	sPb-8764	9	111	0.0093	0.0363	0.28	0.13	56	0.29	0.08	131
	sPb-8542	9	119	0.0449	0.0261	0.26	0.15	35	0.30	0.09	157

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
Fv'/Fm'_16.8	sPb-8743	1	182	0.0033	0.0259	0.43	0.13	74	0.38	0.12	96
	sPb-7695	2	74	0.0172	0.0325	0.41	0.18	30	0.40	0.12	160
	sPb-8349	3	128	0.0077	0.0458	0.44	0.18	11	0.40	0.13	183
	sPb-0906	5	7	0.0381	0.0245	0.42	0.13	54	0.40	0.12	114
	sPb-3644	6	13	0.0015	0.0503	0.40	0.18	34	0.40	0.12	159
	sPb-0460	7	67	0.0098	0.0472	0.37	0.24	11	0.41	0.12	183
	sPb-7944	7	74	0.0173	0.0372	0.35	0.14	28	0.41	0.13	162
	sPb-0289	8	112	0.0413	0.0278	0.39	0.20	17	0.40	0.12	177
	sPb-0562	10	43	0.0024	0.0281	0.42	0.16	75	0.39	0.11	113
	sPb-3549	10	46	0.0064	0.0259	0.42	0.15	70	0.39	0.11	121
	sPb-6331	10	53	0.0086	0.0548	0.40	0.20	11	0.40	0.13	171
	sPb-6271	10	58	0.0405	0.0176	0.39	0.11	68	0.41	0.14	122
Fv'/Fm'_17.6	sPb-0090	1	66	0.0169	0.0203	0.48	0.11	33	0.49	0.15	157
	sPb-3103	1	114	0.0297	0.0201	0.44	0.18	54	0.51	0.12	140
	sPb-8743	1	182	0.0134	0.0186	0.51	0.14	74	0.47	0.13	96
	sPb-7695	2	74	0.0295	0.0232	0.47	0.19	30	0.50	0.12	160
	sPb-1925	2	146	0.0217	0.0272	0.44	0.15	31	0.50	0.14	159
	sPb-7534	4	76	0.0306	0.0298	0.37	0.28	12	0.50	0.12	182
	sPb-4806	5	64	0.0253	0.015	0.48	0.14	87	0.50	0.14	106
	sPb-3418	5	98	0.0121	0.0306	0.44	0.22	32	0.50	0.12	157
	sPb-2457	6	58	0.0291	0.0278	0.44	0.10	9	0.49	0.14	182
	sPb-5054	8	30	0.0259	0.0231	0.45	0.21	31	0.50	0.12	163
	sPb-7220	8	111	0.0334	0.0215	0.48	0.17	30	0.49	0.14	147
	sPb-6515	9	70	0.0295	0.0264	0.45	0.20	32	0.50	0.12	161
	sPb-4006	9	145	0.0122	0.02	0.44	0.18	61	0.52	0.11	128
	sPb-0562	10	43	0.0233	0.0174	0.50	0.18	75	0.49	0.11	113
	sPb-3549	10	46	0.0021	0.0244	0.50	0.18	70	0.48	0.11	121
Fv'/Fm'_18.6	sPb-0233	1	147	0.0386	0.0077	0.54	0.28	15	0.64	0.14	178
	sPb-6434	2	75	0.0444	0.0069	0.61	0.19	13	0.63	0.16	173
	sPb-6663	2	155	0.0038	0.0039	0.61	0.18	93	0.64	0.14	94
	sPb-3683	2	177	0.0352	0.0078	0.59	0.23	16	0.63	0.15	178
	sPb-9076	3	51	0.0013	0.0146	0.50	0.30	9	0.63	0.15	180
	sPb-9146	6	60	0.0372	0.0066	0.48	0.30	22	0.65	0.10	166
	sPb-0571	7	72	0.0246	0.0106	0.59	0.20	11	0.63	0.15	177
	sPb-4874	7	73	0.0042	0.018	0.49	0.29	20	0.64	0.12	172
	sPb-7220	8	111	0.0123	0.0078	0.60	0.20	30	0.63	0.15	147
	sPb-6271	10	58	0.0281	0.0045	0.64	0.12	68	0.62	0.18	122

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
Fv'/Fm'_20.8	sPb-8773	1	11	0.0008	0.0163	0.52	0.30	13	0.67	0.15	181
	sPb-2583	1	15	0.0266	0.0113	0.60	0.23	25	0.67	0.15	164
	sPb-6917	1	30	0.0203	0.0141	0.63	0.23	9	0.66	0.16	182
	sPb-8947	1	64	0.0302	0.0142	0.70	0.01	7	0.66	0.17	186
	sPb-0274	1	74	0.0056	0.0192	0.57	0.26	24	0.68	0.13	169
	sPb-5905	1	112	0.0180	0.0104	0.64	0.20	23	0.66	0.16	171
	sPb-1020	1	168	0.0401	0.0084	0.53	0.30	29	0.68	0.11	164
	sPb-6424	2	145	0.0171	0.0107	0.65	0.17	34	0.66	0.16	160
	sPb-9076	3	51	0.0016	0.0167	0.51	0.32	9	0.67	0.15	180
	sPb-6855	5	71	0.0254	0.0098	0.60	0.25	14	0.68	0.13	173
	sPb-3644	6	13	0.0364	0.0099	0.59	0.25	34	0.68	0.14	159
	sPb-5086	6	148	0.0151	0.0111	0.57	0.28	32	0.68	0.13	156
	sPb-4874	7	73	0.0011	0.0081	0.52	0.31	20	0.68	0.13	172
	sPb-9242	8	66	0.0291	0.0108	0.68	0.01	12	0.66	0.17	177
	sPb-5055	9	108	0.0049	0.0177	0.36	0.37	9	0.68	0.13	185
Fv'/Fm'_mean	sPb-6600	1	85	0.0343	0.0108	0.47	0.03	24	0.47	0.03	159
	sPb-8743	1	182	0.0351	0.0083	0.48	0.03	74	0.46	0.03	96
	sPb-7695	2	74	0.0372	0.0111	0.48	0.03	30	0.47	0.03	160
	sPb-9076	3	51	0.0069	0.0187	0.43	0.06	9	0.47	0.03	180
	sPb-5805	5	37	0.0363	0.0107	0.48	0.02	21	0.47	0.03	168
	sPb-3418	5	98	0.0440	0.0125	0.48	0.03	32	0.47	0.03	157
	sPb-3644	6	13	0.0012	0.0203	0.48	0.03	34	0.47	0.03	159
	sPb-7126	8	29	0.0363	0.0115	0.48	0.03	32	0.47	0.03	161
	sPb-7220	8	111	0.0156	0.0127	0.48	0.03	30	0.47	0.03	147
	sPb-8764	9	111	0.0187	0.0146	0.47	0.04	56	0.47	0.03	131
	sPb-4006	9	145	0.0303	0.0089	0.46	0.04	61	0.48	0.03	128
	sPb-3549	10	46	0.0325	0.0095	0.49	0.02	70	0.46	0.03	121
Fv'/Fm'_*sl*	sPb-8773	1	11	0.0330	0.0058	0.07	0.01	13	0.07	0.01	181
	sPb-0233	1	147	0.0337	0.0055	0.08	0.01	15	0.07	0.01	178
	sPb-1631	2	12	0.0447	0.004	0.08	0.01	26	0.07	0.01	166
	sPb-2229	2	143	0.0005	0.0056	0.08	0.01	55	0.07	0.01	128
	sPb-1137	3	56	0.0293	0.0042	0.08	0.01	31	0.07	0.01	155
	sPb-3838	4	96	0.0346	0.0029	0.07	0.01	72	0.07	0.01	114
	sPb-4041	5	6	0.0403	0.0029	0.08	0.01	46	0.07	0.01	137
	sPb-0932	5	45	0.0158	0.0056	0.08	0.01	14	0.07	0.01	169
	sPb-6855	5	71	0.0200	0.006	0.07	0.01	14	0.07	0.01	173
	sPb-3644	6	13	0.0423	0.0051	0.07	0.01	34	0.07	0.01	159
	sPb-2566	7	54	0.0191	0.005	0.08	0.01	21	0.07	0.01	170
	sPb-5796	7	76	0.0337	0.0027	0.07	0.01	83	0.07	0.01	110
	sPb-5054	8	30	0.0267	0.0049	0.07	0.01	31	0.07	0.01	163
	sPb-0258	8	73	0.0321	0.0036	0.07	0.01	38	0.07	0.01	151
	sPb-8764	9	111	0.0412	0.0052	0.07	0.01	56	0.07	0.01	131

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
Fv'/Fm'_T_b	sPb-1631	2	12	0.0287	0.005	11.03	0.99	26	10.37	0.97	166
	sPb-2229	2	143	0.0035	0.0057	10.87	1.00	55	10.34	0.97	128
	sPb-6855	5	71	0.0093	0.0076	9.95	1.02	14	10.51	1.00	173
	sPb-3644	6	13	0.0114	0.0074	10.29	1.01	34	10.48	1.00	159
	sPb-2566	7	54	0.0145	0.0059	11.14	1.12	21	10.36	0.96	170
	sPb-5796	7	76	0.0228	0.0035	10.67	0.93	83	10.31	1.04	110
	sPb-7126	8	29	0.0340	0.0056	10.30	1.04	32	10.47	0.98	161
	sPb-7220	8	111	0.0306	0.0053	10.35	1.09	30	10.53	1.01	147
	sPb-8764	9	111	0.0199	0.0069	10.58	1.14	56	10.45	0.94	131
	sPb-3549	10	46	0.0424	0.0038	10.19	0.84	70	10.60	1.07	121

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
$\Phi_{PSII}\_13.5$	sPb-3801	1	111	0.030	0.0152	0.107	0.049	38	0.103	0.037	153
	sPb-1631	2	12	0.031	0.016	0.087	0.036	26	0.106	0.039	166
	sPb-3798	2	43	0.020	0.0158	0.091	0.037	54	0.109	0.039	139
	sPb-7795	3	4	0.033	0.0158	0.091	0.031	33	0.106	0.041	160
	sPb-2138	4	64	0.037	0.0192	0.107	0.052	31	0.103	0.036	160
	sPb-6855	5	71	0.041	0.0191	0.112	0.046	14	0.103	0.039	173
	sPb-0508	7	71	0.043	0.0218	0.078	0.023	11	0.105	0.040	183
	sPb-0571	7	72	0.034	0.0226	0.078	0.023	11	0.106	0.039	177
	sPb-7126	8	29	0.036	0.0181	0.105	0.048	32	0.104	0.037	161
	sPb-7220	8	111	0.001	0.0241	0.117	0.049	30	0.101	0.038	147
	sPb-0817	10	42	0.031	0.015	0.092	0.033	26	0.106	0.040	166
	sPb-3549	10	46	0.037	0.0134	0.110	0.044	70	0.100	0.036	121
$\Phi_{PSII}\_14.7$	sPb-3525	1	57	0.014	0.0335	0.214	0.072	16	0.184	0.061	176
	sPb-8992	2	73	0.045	0.0237	0.169	0.059	29	0.189	0.062	157
	sPb-2229	2	143	0.043	0.0193	0.176	0.067	55	0.190	0.063	128
	sPb-4444	2	144	0.044	0.0234	0.173	0.075	25	0.188	0.062	158
	sPb-9303	4	85	0.030	0.0297	0.152	0.056	13	0.190	0.063	178
	sPb-3838	4	96	0.041	0.016	0.181	0.070	72	0.193	0.058	114
	sPb-4041	5	6	0.026	0.0191	0.172	0.064	46	0.192	0.061	137
	sPb-5805	5	37	0.018	0.0291	0.211	0.078	21	0.184	0.061	168
	sPb-6855	5	71	0.001	0.0453	0.239	0.082	14	0.180	0.059	173
	sPb-7106	5	72	0.027	0.0163	0.169	0.060	39	0.193	0.063	150
	sPb-9490	5	89	0.034	0.0184	0.166	0.061	29	0.192	0.063	154
	sPb-6518	7	28	0.007	0.0279	0.170	0.066	32	0.190	0.062	162
	sPb-2566	7	54	0.013	0.0287	0.159	0.065	21	0.191	0.062	170
	sPb-7126	8	29	0.024	0.0287	0.208	0.062	32	0.183	0.062	161
	sPb-8542	9	119	0.033	0.0274	0.207	0.070	35	0.183	0.061	157

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
	sPb-0090	1	66	0.035	0.0227	0.239	0.058	33	0.281	0.087	157
	sPb-0274	1	74	0.040	0.0333	0.319	0.064	24	0.266	0.083	169
	sPb-8743	1	182	0.007	0.0222	0.294	0.078	74	0.251	0.082	96
	sPb-7695	2	74	0.005	0.0354	0.319	0.084	30	0.263	0.080	160
	sPb-7534	4	76	0.012	0.0391	0.348	0.074	12	0.268	0.081	182
Φ_{PSII}_16.8	sPb-7312	8	82	0.036	0.0265	0.319	0.061	17	0.269	0.084	177
	sPb-0289	8	112	0.003	0.0389	0.337	0.080	17	0.267	0.081	177
	sPb-0562	10	43	0.026	0.018	0.293	0.073	75	0.259	0.086	113
	sPb-3549	10	46	0.043	0.0168	0.294	0.074	70	0.261	0.085	121
	sPb-6271	10	58	0.011	0.0198	0.249	0.082	68	0.285	0.081	122
	sPb-7948	10	61	0.044	0.0303	0.326	0.101	12	0.268	0.081	175
	sPb-2704	1	35	0.044	0.0388	0.401	0.111	10	0.338	0.086	175
	sPb-1020	1	168	0.013	0.0373	0.371	0.118	29	0.340	0.087	164
	sPb-6434	2	75	0.043	0.0305	0.395	0.117	13	0.339	0.084	173
	sPb-6741	4	40	0.020	0.0402	0.300	0.074	32	0.354	0.093	161
	sPb-3343	4	43	0.036	0.0391	0.299	0.067	28	0.353	0.094	163
Φ_{PSII}_17.6	sPb-2009	4	44	0.015	0.041	0.300	0.074	32	0.354	0.093	162
	sPb-7534	4	76	0.044	0.0348	0.395	0.126	12	0.341	0.089	182
	sPb-5805	5	37	0.027	0.0316	0.400	0.134	21	0.338	0.084	168
	sPb-9066	6	89	0.016	0.0339	0.388	0.126	24	0.339	0.086	169
	sPb-8954	6	149	0.007	0.0434	0.419	0.142	13	0.340	0.086	176
	sPb-7460	9	147	0.033	0.0329	0.312	0.093	22	0.349	0.092	171
	sPb-6331	10	53	0.007	0.0606	0.429	0.127	11	0.340	0.089	171
	sPb-8947	1	64	0.018	0.0337	0.445	0.090	7	0.508	0.059	186
	sPb-0274	1	74	0.010	0.0366	0.482	0.079	24	0.509	0.058	169
	sPb-3103	1	114	0.034	0.0165	0.517	0.056	54	0.501	0.063	140
	sPb-7657	2	0	0.016	0.0151	0.518	0.052	83	0.494	0.066	101
	sPb-9135	3	58	0.009	0.0141	0.522	0.051	77	0.494	0.064	109
	sPb-9066	6	89	0.012	0.0219	0.532	0.052	24	0.503	0.062	169
Φ_{PSII}_18.6	sPb-7126	8	29	0.033	0.02	0.523	0.054	32	0.502	0.062	161
	sPb-9242	8	66	0.005	0.0305	0.456	0.099	12	0.509	0.058	177
	sPb-0435	8	67	0.020	0.0222	0.521	0.053	40	0.502	0.063	152
	sPb-7312	8	82	0.034	0.0182	0.469	0.085	17	0.509	0.058	177
	sPb-6748	9	115	0.030	0.0215	0.513	0.061	52	0.503	0.061	136
	sPb-3298	9	116	0.027	0.0217	0.513	0.062	56	0.503	0.061	136
	sPb-0817	10	42	0.008	0.0194	0.472	0.078	26	0.511	0.056	166
	sPb-1020	1	168	0.033	0.0241	0.520	0.070	29	0.485	0.073	164
Φ_{PSII}_20.8	sPb-7186	3	141	0.019	0.0157	0.472	0.069	68	0.501	0.073	124
	sPb-6518	7	28	0.006	0.0252	0.457	0.076	32	0.497	0.071	162

Trait	Locus	Chr.	Position [cM]	p_Marker	Effect	rare allel			common allele		
						Mean	SD	n	Mean	SD	n
Φ_{PSII} _mean	sPb-2704	1	35	0.044	0.0167	0.345	0.065	10	0.316	0.032	175
	sPb-8992	2	73	0.020	0.0139	0.309	0.040	29	0.320	0.034	157
	sPb-7534	4	76	0.002	0.0213	0.356	0.053	12	0.316	0.033	182
	sPb-5805	5	37	0.003	0.0174	0.343	0.042	21	0.315	0.034	168
	sPb-6855	5	71	0.012	0.016	0.344	0.045	14	0.315	0.033	173
	sPb-9146	6	60	0.030	0.0131	0.341	0.049	22	0.316	0.033	166
	sPb-6518	7	28	0.025	0.0116	0.312	0.032	32	0.320	0.036	162
	sPb-7126	8	29	0.003	0.0188	0.334	0.047	32	0.316	0.032	161
	sPb-5054	8	30	0.013	0.015	0.328	0.038	31	0.316	0.035	163
	sPb-0289	8	112	0.006	0.0166	0.349	0.050	17	0.316	0.033	177
	sPb-6331	10	53	0.020	0.0218	0.351	0.051	11	0.316	0.034	171
	sPb-7948	10	61	0.011	0.0168	0.349	0.045	12	0.316	0.034	175
Φ_{PSII} _*sl*	sPb-9303	4	85	0.008	0.009	0.066	0.011	13	0.057	0.010	178
	sPb-3838	4	96	0.018	0.0048	0.060	0.011	72	0.057	0.009	114
	sPb-9490	5	89	0.011	0.0056	0.062	0.011	29	0.057	0.009	154
	sPb-2551	6	18	0.027	0.0069	0.064	0.009	23	0.057	0.010	168
Φ_{PSII} _T_b	sPb-3798	2	43	0.027	0.0051	11.620	1.311	54	11.251	1.011	139
	sPb-4444	2	144	0.022	0.0059	11.710	1.329	25	11.290	1.069	158
	sPb-3838	4	96	0.012	0.0047	11.500	1.170	72	11.233	1.033	114
	sPb-6855	5	71	0.003	0.0089	10.688	1.276	14	11.439	1.060	173
	sPb-9490	5	89	0.026	0.0044	11.821	0.834	29	11.213	1.133	154
	sPb-2551	6	18	0.042	0.0058	11.892	1.031	23	11.273	1.111	168
	sPb-3715	8	89	0.002	0.011	10.228	1.625	10	11.414	1.057	182
	sPb-0817	10	42	0.028	0.0055	11.804	0.981	26	11.272	1.118	166
	sPb-0562	10	43	0.022	0.0047	11.061	1.162	75	11.552	1.033	113

Table S 4.1: Marker-trait associations for mean across environments (*m*), exponents (*a*) and initial growth rate ($DMGR_0$) for dry matter growth rate (DMGR) and *m*, regression slopes (*sl)*, and base temperatures (T_b) for leaf appearance rate (LAR), chlorophyll content (SPAD) and fluorescence (F_v'/F_m' and Φ_{PSII}).

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
DMGR_mean	UGSS_00668	1	17541120	0.0393	G:G	0.0001	141	A:G	0.002	8
	UGSDII_01141	1	63852726	0.0040	C:C	0.0020	159	A:A	0.002	12
	UGSS_01605	2	1689223	0.0364	C:C	-0.0009	59	A:A	0.001	39
	UGSDII_02151	2	7833117	0.0335	C:C	0.0008	139	A:A	-0.001	33
	UGSDII_02161	2	8039258	0.0070	A:A	0.0022	162	C:C	-0.002	20
	UGSDII_03388	2	47350373	0.0298	C:C	0.0031	168	A:G	0	20
	UGSS_02165	2	59908438	0.0150	C:C	0.0006	101	A:A	0.001	60
	UGSDI_06847	2	60113519	0.0438	C:C	0.0005	98	A:A	0.001	69
	UGSDI_07649	2	66009499	0.0197	C:C	-0.0001	170	A:A	0.003	10
	UGSDI_09981	3	5239039	0.0179	G:G	0.0000	149	C:C	-0.002	20
	UGSS_02805	3	5722599	0.0005	C:C	-0.0006	95	G:G	-0.002	78
	UGSDI_11227	3	10689401	0.0392	A:A	0.0009	138	A:C	0	48
	UGSDI_11593	3	12534912	0.0401	A:A	0.0024	164	A:C	0.002	17
	UGSDII_05282	3	60724435	0.0357	A:A	0.0013	100	C:C	0.001	65
	UGSDI_13720	3	64401625	0.0310	G:G	-0.0012	112	C:C	-0.0002	57
	UGSDI_17961	4	61121730	0.0233	G:G	0.0009	166	C:C	0.003	10
	UGSS_04848	6	50089590	0.0084	G:G	0.0011	162	C:C	-0.002	21
	UGSDII_10437	6	61778333	0.0209	G:G	-0.0019	158	A:G	-0.0001	11
	UGSDII_11636	8	2260138	0.0239	C:C	0.0002	141	G:G	-0.001	31
	UGSDII_14005	8	47609884	0.0274	G:G	-0.0021	135	A:G	-0.001	12
	UGSDI_33330	8	53100069	0.0260	G:G	-0.0025	145	C:C	-0.001	31
	UGSDI_41411	9	55957743	0.0011	G:G	0.0015	103	C:C	-0.00007	62
	UGSDI_51828	10	49460379	0.0335	G:G	0.0001	109	C:C	0.001	67
	UGSDI_52729	10	56100805	0.0272	C:C	0.0017	159	G:G	-0.001	21
DMGR_*a*	UGSS_01298	1	6688268	0.0088	C:C	-0.1750	27	A:A	-0.165	14
	UGSDI_00546	1	14867608	0.0184	G:G	0.0586	164	A:G	0	24
	UGSS_00705	1	19430430	0.0160	G:G	0.1414	169	A:A	0.114	18
	UGSDII_01103	1	60627976	0.0135	A:A	0.0215	132	A:G	-0.0004	21
	UGSDII_01141	1	63852726	0.0394	C:C	0.0345	159	A:A	0.034	12
	UGST_00150	2	2674509	0.0044	C:C	0.0118	54	A:A	-0.022	48
	UGSDII_04350	3	792285	0.0165	C:C	0.0119	159	A:G	0.049	13
	UGSDI_10780	3	8994716	0.0114	C:C	-0.0640	149	A:A	-0.075	29
	UGSS_02833	3	58838889	0.0199	C:C	0.0166	150	G:G	-0.017	22
	UGSDII_05282	3	60724435	0.0346	A:A	0.0300	100	C:C	0.027	65
	UGSDI_14390	4	1458984	0.0339	G:G	-0.0178	161	C:C	0.028	10
	UGSDII_05877	4	5059995	0.0042	A:A	-0.0657	166	G:G	-0.075	10
	UGST_00314	4	55810400	0.0041	A:A	0.0966	95	G:G	0	7
	UGSDI_20942	5	4011154	0.0068	G:G	0.0244	102	C:C	0.039	67
	UGSDII_07691	5	10363515	0.0178	A:C	-0.0178	85	A:A	0.004	45
	UGSDI_23501	5	19066824	0.0015	A:A	-0.1419	164	C:C	-0.118	20
	UGSDI_26370	6	1295049	0.0223	C:C	-0.0260	155	G:G	-0.058	27
	UGSDII_08774	6	1644212	0.0317	A:G	-0.0416	161	C:C	0	25
	UGSS_04848	6	50089590	0.0193	G:G	-0.0318	162	C:C	-0.088	21
	UGSDI_28157	6	51722531	0.0422	G:G	-0.0180	135	C:C	0.001	40
	UGSS_05184	7	54293787	0.0231	A:A	0.0428	136	G:G	0.023	38

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
	UGSDII_11310	7	55410183	0.0104	C:C	0.0383	167	A:G	-0.038	7
	UGSDII_13488	8	41596866	0.0253	G:G	-0.0260	134	A:G	0.018	21
	UGSDII_14100	8	48649540	0.0072	G:G	0.0874	174	A:A	0.035	12
	UGSS_05725	8	54325450	0.0041	A:A	-0.0976	147	G:G	-0.069	33
	UGSDI_33699	9	3699	0.0248	G:G	-0.0278	87	A:A	-0.034	82
	UGSDI_35770	9	9255371	0.0046	C:C	-0.0333	149	A:G	0	36
	UGSDI_36313	9	13698114	0.0317	C:C	0.0362	166	A:G	0.060	9
DMGR_*a*	UGSDI_36510	9	14195682	0.0063	G:G	0.0239	165	A:G	0.074	9
	UGSDI_36583	9	14429762	0.0109	C:C	0.0479	169	A:G	0.068	9
	UGSDI_39851	9	48745718	0.0094	G:G	-0.0779	170	C:C	-0.090	15
	UGSS_06272	9	54095775	0.0078	A:G	-0.1461	145	G:G	-0.111	27
	UGSDI_41984	9	58328284	0.0145	C:C	0.1196	105	G:G	0.171	7
	UGSDII_15599	10	4586779	0.0027	C:C	0.0310	154	A:A	-0.002	15
	UGSDI_44580	10	13833465	0.0032	C:C	-0.0015	151	A:A	-0.070	7
	UGSS_00404	10	55123291	0.0255	A:G	0.0470	131	G:G	0.006	43
	UGSDII_00917	1	55283082	0.0315	A:A	-0.0002	141	G:G	-0.00017	26
	UGSDI_02900	1	57831374	0.0087	G:G	-0.0001	134	A:A	-0.00008	35
	UGSDII_03223	2	18252899	0.0368	G:G	-0.0001	172	A:C	0	9
	UGSS_01684	2	18705142	0.0448	A:A	-0.0001	152	A:G	-0.00010	7
	UGSDI_07698	2	66254819	0.0379	A:A	0.0000	79	G:G	0.00006	91
	UGSS_02647	3	2514380	0.0435	C:C	-0.0002	136	G:G	-0.00025	13
	UGSS_02805	3	5722599	0.0242	C:C	-0.0001	95	G:G	-0.00008	78
	UGSDI_11152	3	10414688	0.0034	G:G	0.0000	164	A:G	0.00012	11
	UGSDII_04588	3	10605987	0.0261	A:A	-0.0001	145	C:C	-0.00015	28
	UGSDI_14390	4	1458984	0.0144	G:G	0.0000	161	C:C	-0.00010	10
	UGST_00314	4	55810400	0.0088	A:A	-0.0002	95	G:G	0	7
	UGSDI_20070	4	67130172	0.0318	G:G	-0.0001	156	A:G	-0.00013	20
	UGSDI_25802	5	59296539	0.0437	C:C	0.0000	147	A:G	0.00010	12
	UGSDII_08774	6	1644212	0.0224	A:G	0.0001	161	C:C	0	25
$DMGR_0$	UGST_00515	6	58367647	0.0373	C:C	0.0001	176	A:G	0	10
	UGSDI_29722	6	59527825	0.0366	G:G	0.0000	151	A:C	0.00008	15
	UGSDI_31596	7	54256039	0.0357	C:C	-0.0001	159	A:G	0	22
	UGSS_05337	8	2431200	0.0171	G:G	-0.0001	145	A:G	-0.00014	1
	UGSDI_32247	8	3014692	0.0248	G:G	0.0001	34	C:C	0.00008	132
	UGSS_05405	8	3611531	0.0190	G:G	0.0001	172	A:G	0	10
	UGSS_05462	8	4635734	0.0143	G:G	0.0000	115	A:G	0	73
	UGSDI_33048	8	51493476	0.0213	C:C	-0.0001	144	A:G	-0.00005	12
	UGSS_06434	9	8532527	0.0084	G:G	-0.0001	157	C:C	-0.00006	14
	UGSDI_35770	9	9255371	0.00007	C:C	0.0001	149	A:G	0	36
	UGSDI_36848	9	15163484	0.0381	A:A	0.0000	168	G:G	0.00010	10
	UGSDII_15599	10	4586779	0.0005	C:C	-0.0001	154	A:A	0.00003	15
	UGSDI_44580	10	13833465	0.0154	C:C	0.0000	151	A:A	0.00015	7
	UGSDI_51856	10	49712399	0.0430	C:C	0.0000	118	A:A	-0.00001	12
	UGSS_00389	10	54612219	0.0379	A:G	-0.0001	141	G:G	0.00002	9
	UGSDII_00629	1	48693864	0.0135	G:G	0.0130	110	A:A	0.007	57
	UGSDII_00714	1	52162190	0.0362	G:G	0.0039	117	A:A	-0.008	44
	UGSDI_02152	1	52429487	0.0035	G:G	-0.0040	131	A:G	0.008	15
	UGSDII_00774	1	53923432	0.0222	G:G	0.0219	155	A:A	0.008	27
LAR_mean	UGSDII_01613	1	72338217	0.0144	G:G	-0.0191	160	A:G	-0.024	7
	UGSDI_11357	3	10939353	0.0122	G:G	-0.0041	45	C:C	-0.009	129
	UGSDI_12279	3	46865461	0.0314	C:C	0.0167	112	G:G	0.016	64
	UGSDI_13662	3	63671432	0.0154	A:A	0.0297	171	A:C	0.028	11
	UGSDII_05677	4	2017303	0.0064	A:A	-0.0201	162	G:G	-0.013	16

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
LAR_mean	UGSDII_05835	4	4849962	0.0326	A:G	-0.0204	159	G:G	-0.013	23
	UGSS_03531	4	6170533	0.0422	A:A	-0.0049	94	C:C	0	74
	UGSDI_15208	4	6670841	0.0114	G:G	-0.0135	122	A:A	-0.016	48
	UGSDII_06959	4	52026584	0.0085	A:C	0.0045	46	A:G	-0.006	41
	UGSDI_23501	5	19066824	0.0290	A:A	-0.0233	164	C:C	-0.009	20
	UGSS_04670	6	37986408	0.0296	G:G	0.0020	169	A:C	0.022	7
	UGSS_05093	7	1303996	0.0337	G:G	0.0022	76	A:A	-0.007	25
	UGSDII_10492	7	2513027	0.0070	A:A	0.0078	164	G:G	0.019	17
	UGSDI_31170	7	7143645	0.0201	G:G	0.0148	145	C:C	0.002	31
	UGSDI_33048	8	51493476	0.0334	C:C	0.0063	144	A:G	0.009	12
	UGSDI_33807	9	770096	0.0443	G:G	-0.0036	162	A:A	-0.014	18
	UGSDI_40415	9	52529034	0.0001	G:G	-0.0287	161	A:A	-0.035	19
	UGSDI_41411	9	55957743	0.0045	G:G	-0.0001	103	C:C	-0.009	62
	UGSDI_43410	10	6936670	0.0157	C:C	0.0004	77	G:G	-0.007	70
	UGSDI_51856	10	49712399	0.0056	C:C	0.0068	118	A:A	-0.002	12
	UGSDI_51874	10	49934866	0.0252	G:G	0.0059	112	A:C	-0.002	16
	UGSDII_16082	10	52955823	0.0114	C:C	0.0061	78	G:G	-0.001	88
LAR_*sl*	UGSS_00873	1	43739095	0.0268	A:A	0.0004	157	A:G	-0.003	8
	UGSDII_00629	1	48693864	0.0210	G:G	0.0022	110	A:A	0.001	57
	UGSDI_02152	1	52429487	0.0100	G:G	-0.0008	131	A:G	0.002	15
	UGSDII_01135	1	62817379	0.0338	C:C	0.0018	35	A:A	-0.00009	135
	UGSDI_03947	1	67072069	0.0401	A:A	0.0009	71	G:G	0.002	94
	UGSDII_01333	1	67686926	0.0182	A:A	-0.0016	105	G:G	-0.002	64
	UGSDII_04250	2	70719219	0.0354	G:G	0.0035	140	A:C	0.001	10
	UGSDI_08931	2	75537855	0.0195	C:C	0.0005	109	A:G	0.003	12
	UGSS_02643	3	2503519	0.0212	G:G	0.0029	127	C:C	0.003	49
	UGSS_02587	3	13465929	0.0435	G:G	-0.0034	173	A:G	0	14
	UGSDI_12279	3	46865461	0.0394	C:C	0.0030	112	G:G	0.004	64
	UGSDII_05677	4	2017303	0.0426	A:A	-0.0021	162	G:G	-0.002	16
	UGSDII_05835	4	4849962	0.0243	A:G	-0.0040	159	G:G	-0.003	23
	UGSDII_07067	4	53501586	0.0032	A:A	0.0008	161	C:C	-0.003	9
	UGSDI_17206	4	58518488	0.0289	A:G	0.0032	151	G:G	0.001	25
	UGSDI_19962	4	66849200	0.0251	A:G	0.0009	145	A:C	0.005	5
	UGSDII_10183	6	54207715	0.0355	C:C	0.0031	151	G:G	0.004	21
	UGSS_05156	7	3752805	0.0423	G:G	-0.0001	27	C:C	0.003	105
	UGSDI_31649	7	56738319	0.0440	G:G	0.0031	51	C:C	0.004	125
	UGSDI_32430	8	5044579	0.0120	C:C	0.0058	158	G:G	0.006	26
	UGSDII_13488	8	41596866	0.0373	G:G	0.0006	134	A:G	-0.002	21
	UGSS_05654	8	53899866	0.0054	C:C	0.0002	146	A:G	-0.004	20
	UGSDI_33699	9	3699	0.0109	G:G	-0.0018	87	A:A	-0.001	82
	UGSDI_40415	9	52529034	0.0088	G:G	-0.0041	161	A:A	-0.004	19
	UGSDI_41411	9	55957743	0.0129	G:G	0.0011	103	C:C	-0.00028	62
	UGSDI_41523	9	56515391	0.0225	C:C	0.0017	161	A:G	0.005	9
	UGSS_06358	9	56812162	0.0375	A:C	-0.0014	61	G:G	0	122
	UGSDI_41631	9	57002334	0.0142	A:A	0.0007	98	G:G	-0.001	77
	UGSDI_48260	10	34936799	0.0120	A:C	-0.0038	165	A:G	0	21
	UGSDI_51856	10	49712399	0.0088	C:C	0.0010	118	A:A	-0.001	12
LAR_T_b	UGSS_02587	3	13465929	0.0202	G:G	-0.4153	173	A:G	0	14
	UGSDII_07067	4	53501586	0.0077	A:A	0.2645	161	C:C	-0.291	9
	UGSDI_17206	4	58518488	0.0046	A:G	0.5932	151	G:G	0.473	25
	UGSDII_10183	6	54207715	0.0246	C:C	0.3961	151	G:G	0.553	21
	UGSDI_31649	7	56738319	0.0205	G:G	0.4819	51	C:C	0.506	125
	UGSDII_13488	8	41596866	0.0271	G:G	0.2554	134	A:G	-0.164	21
	UGSDI_33699	9	3699	0.0219	G:G	-0.3474	87	A:A	-0.149	82

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
SPAD_mean	UGSDII_00605	1	48452824	0.0440	A:A	-1.4459	154	G:G	-0.784	15
	UGSDI_03927	1	66764796	0.0038	A:A	0.7814	162	C:C	-1.185	15
	UGSS_02429	2	8412341	0.0067	G:G	0.3747	173	A:A	-2.054	12
	UGSDI_07462	2	65391657	0.0350	C:C	0.5194	111	A:A	0.929	57
	UGSDI_07465	2	65391758	0.0298	A:A	0.3349	92	C:C	0.864	59
	UGSDI_07470	2	65397174	0.0447	C:C	0.3722	94	A:A	0.863	74
	UGSS_02293	2	68325941	0.0423	G:G	1.4426	166	A:A	0	16
	UGSDI_11735	3	13240931	0.0243	A:A	0.8426	158	C:C	1.328	19
	UGSDI_12540	3	55056783	0.0294	C:C	0.6360	119	A:G	1.131	21
	UGSS_02851	3	59616226	0.0029	C:C	-0.2461	170	A:A	-3.528	12
	UGSDI_14722	4	2640061	0.0385	G:G	-0.9954	157	C:C	0	30
	UGST_00343	4	7393846	0.0406	A:A	1.1595	151	C:C	0	36
	UGSDI_16824	4	55043311	0.0252	A:A	-0.0792	52	C:C	0.863	22
	UGSDI_17277	4	59185316	0.0042	A:A	1.3809	167	C:C	2.739	11
	UGSDI_21785	5	6174255	0.0222	C:C	-0.7032	157	G:G	-2.383	21
	UGSDII_07856	5	11295914	0.0340	G:G	0.7331	109	C:C	0.028	47
	UGSDI_25887	5	59979232	0.0388	A:A	0.2871	153	C:C	-0.985	22
	UGSDI_29770	6	60209320	0.0308	C:C	1.0139	141	G:G	-0.214	23
	UGSDI_30175	7	499539	0.0402	C:C	-0.7891	109	G:G	0	27
	UGSS_05159	7	37743870	0.0020	C:C	-0.5421	131	G:G	-3.651	12
	UGSDII_11303	7	54965057	0.0116	G:G	-0.8435	161	C:C	-2.498	14
	UGSDI_32430	8	5044579	0.0379	C:C	-2.4268	158	G:G	-1.275	26
	UGSS_05898	9	11633552	0.0278	C:C	-0.7556	171	A:G	0	15
	UGSDI_40153	9	50756495	0.0031	G:G	0.2861	121	A:A	-1.207	46
	UGSDII_15573	10	3199826	0.0066	C:C	1.7394	160	G:G	-0.049	21
	UGSDI_42951	10	4383980	0.0286	C:C	-1.3619	165	A:A	0	9
	UGSDI_43070	10	5424999	0.0040	A:G	0.5504	144	G:G	1.587	28
	UGSDII_15626	10	5931119	0.0128	G:G	1.2511	144	A:G	-0.329	29
	UGSDI_43410	10	6936670	0.0181	C:C	0.8038	77	G:G	0.152	70
	UGSDI_43971	10	9655431	0.0388	G:G	-1.1025	95	C:C	-1.204	72
SPAD_*sl*	UGSDI_01005	1	18392063	0.0341	A:G	0.4935	157	G:G	0	27
	UGSDII_00306	1	19503946	0.0390	A:A	-0.2479	152	G:G	-0.657	27
	UGSDI_02152	1	52429487	0.0019	G:G	-0.2558	131	A:G	0.125	15
	UGSDII_00774	1	53923432	0.0151	G:G	0.8320	155	A:A	0.247	27
	UGSDI_02758	1	56816253	0.0155	C:C	0.1426	41	A:A	0.352	124
	UGSDII_02071	2	6897226	0.0100	A:A	0.2216	154	G:G	-0.631	24
	UGSS_02805	3	5722599	0.0278	C:C	-0.2759	95	G:G	-0.430	78
	UGSDII_05167	3	57484015	0.0122	G:G	0.5076	170	C:C	-0.100	11
	UGSDII_05216	3	58207290	0.0029	G:G	-0.3923	109	C:C	-0.166	64
	UGSDI_13662	3	63671432	0.0400	A:A	0.9773	171	A:C	0.978	11
	UGSS_03587	4	7362300	0.0037	G:G	-0.5679	147	A:G	-1.055	31
	UGSDI_18656	4	62505274	0.0134	G:G	-0.3893	83	A:G	-0.219	80
	UGSDI_20942	5	4011154	0.0044	G:G	-0.1413	102	C:C	0.166	67
	UGSS_04552	5	9137603	0.0168	A:A	1.3245	147	A:C	0.940	14
	UGST_00480	5	9295511	0.0396	C:C	-0.2902	83	G:G	-0.237	75
	UGSDI_22377	5	10113990	0.0305	C:C	-0.3070	118	G:G	-0.081	52
	UGSDI_22523	5	10612724	0.0184	G:G	-0.1946	118	A:G	0	66
	UGSDI_27515	6	47991367	0.0156	G:G	-0.5335	144	C:C	-0.676	35
	UGSS_04848	6	50089590	0.0352	G:G	0.0348	162	C:C	-0.716	21
	UGSDI_29634	6	58810203	0.0235	C:C	0.3550	111	G:G	0.447	64
	UGSDI_29642	6	58960437	0.0287	G:G	0.3971	152	A:A	-0.228	26
	UGSDI_30425	7	2104261	0.0157	A:A	-0.1498	154	G:G	0.163	27
	UGSDII_11117	7	51671137	0.0322	A:A	-0.9916	159	G:G	-1.070	24

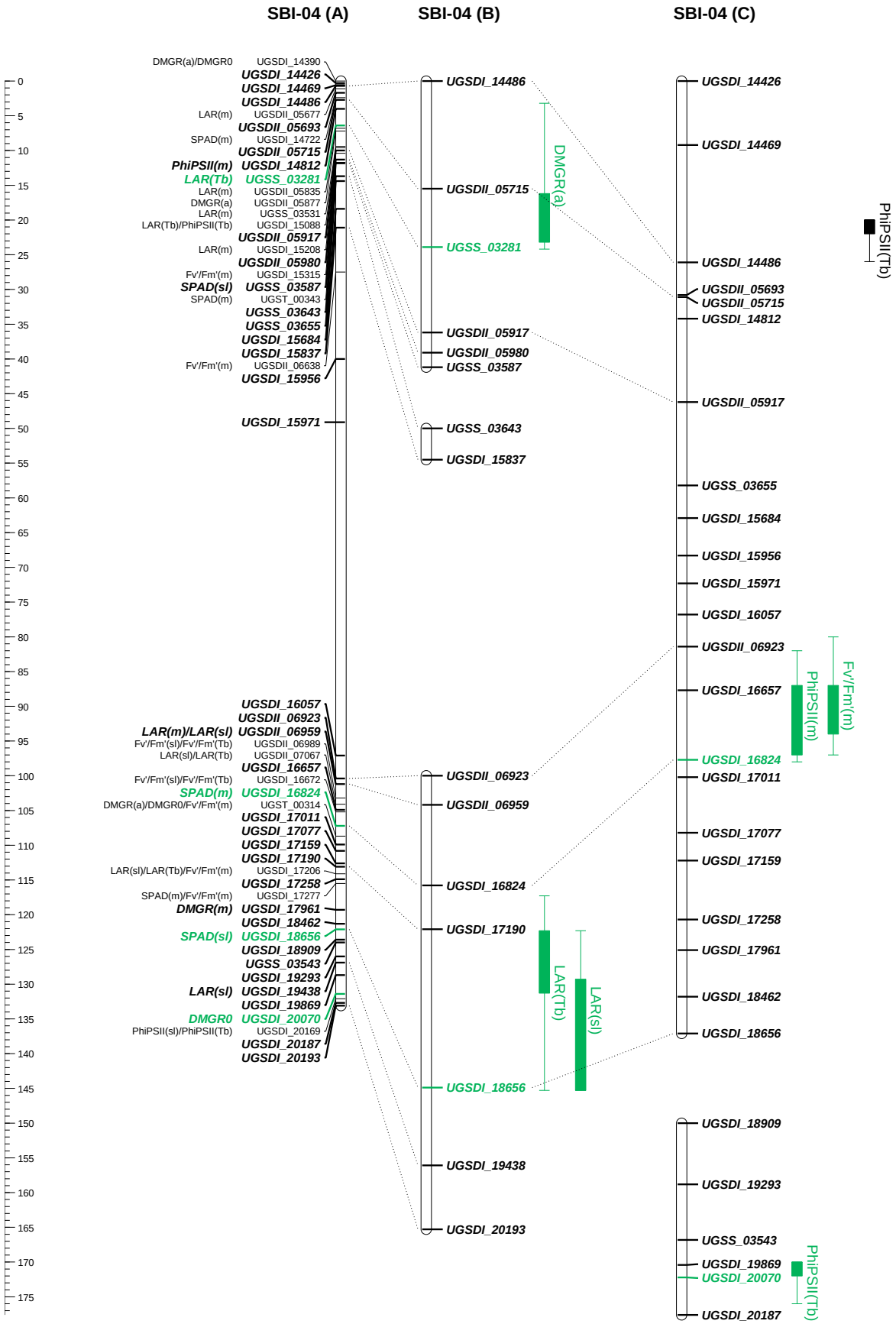
Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
	UGSDII_11324	7	55772900	0.0306	G:G	-0.4591	141	C:C	-0.277	33
	UGSS_05405	8	3611531	0.0243	G:G	0.5125	172	A:G	0	10
	UGSS_05610	8	52661661	0.0063	C:C	-0.7413	159	G:G	-0.696	19
	UGSDI_33699	9	3699	0.0081	G:G	-0.4153	87	A:A	-0.350	82
	UGSS_05885	9	1130820	0.0015	G:G	0.7630	150	A:G	0.446	12
SPAD_*sl*	UGSDII_14706	9	3201246	0.0328	C:C	0.2070	156	G:G	-0.688	23
	UGSDI_35992	9	9909969	0.0111	C:C	-0.5129	119	G:G	-0.778	39
	UGSDI_36313	9	13698114	0.0170	C:C	0.5876	166	A:G	0.578	9
	UGSDI_36510	9	14195682	0.0185	G:G	0.6105	165	A:G	0.470	9
	UGSDI_36583	9	14429762	0.0350	C:C	0.5405	169	A:G	0.574	9
	UGSDI_41411	9	55957743	0.0363	G:G	0.0678	103	C:C	-0.193	62
	UGSDI_03386	1	61170263	0.0074	A:A	0.3458	147	G:G	-0.160	27
	UGSDI_03647	1	64449310	0.0019	A:A	-0.2290	133	A:G	0.048	13
	UGSDII_01985	2	5704182	0.0356	A:A	-0.2682	160	G:G	-0.110	12
	UGSDII_03388	2	47350373	0.0167	C:C	0.7584	168	A:G	0	20
	UGSDI_07229	2	63000610	0.0314	A:A	-0.1360	117	G:G	-0.272	59
	UGSS_02833	3	58838889	0.0272	C:C	0.3325	150	G:G	0.199	22
	UGSDI_20728	5	2697685	0.0128	C:C	0.5036	147	G:G	0.128	29
	UGSDI_21684	5	5585914	0.0115	A:C	0.8769	96	C:C	0.738	84
	UGSDI_22254	5	9348710	0.0256	C:C	-0.4511	103	G:G	-0.423	68
	UGSDII_08007	5	13749806	0.0100	C:C	0.2304	169	A:C	0.649	7
	UGSDI_23454	5	18307595	0.0420	A:G	-0.0673	115	G:G	-0.779	19
	UGSDI_24739	5	46500037	0.0106	G:G	0.1179	171	C:C	0.316	12
	UGSS_04848	6	50089590	0.0039	G:G	0.0348	162	C:C	-0.716	21
	UGSS_05405	8	3611531	0.0346	G:G	0.5125	172	A:G	0	10
SPAD_T_b	UGSDII_12241	8	9018898	0.0143	A:G	0.3039	154	C:C	0.478	32
	UGSDII_13149	8	35611060	0.0334	A:G	-0.3114	135	C:C	0	48
	UGSDII_13771	8	43751430	0.0279	G:G	0.3394	159	C:C	0.217	23
	UGSS_05729	8	54372482	0.0253	G:G	-0.1251	126	A:A	-0.359	44
	UGSDI_33699	9	3699	0.0305	G:G	-0.4153	87	A:A	-0.350	82
	UGSS_05885	9	1130820	0.0001	G:G	0.7630	150	A:G	0.446	12
	UGSDI_36313	9	13698114	0.0020	C:C	0.5876	166	A:G	0.578	9
	UGSDI_36510	9	14195682	0.0024	G:G	0.6105	165	A:G	0.470	9
	UGSDI_36583	9	14429762	0.0051	C:C	0.5405	169	A:G	0.574	9
	UGSDI_41002	9	54609996	0.0118	G:G	0.2589	169	C:C	-0.052	12
	UGSDI_41631	9	57002334	0.0261	A:A	0.2423	98	G:G	0.008	77
	UGSDI_43070	10	5424999	0.0104	A:G	-0.4636	144	G:G	-0.354	28
	UGSDI_43410	10	6936670	0.0343	C:C	-0.2245	77	G:G	-0.216	70
	UGSS_00174	10	34142244	0.0369	C:C	0.2289	172	A:A	0	14
	UGSDII_16082	10	52955823	0.0250	C:C	-0.3185	78	G:G	-0.237	88
	UGSDI_00990	1	17772279	0.0173	G:G	-0.0083	142	A:A	-0.044	25
	UGSDI_01005	1	18392063	0.0348	A:G	0.0312	155	G:G	0	22
	UGSS_01028	1	52678585	0.0040	A:A	0.0023	139	C:C	0.039	27
	UGSDII_00774	1	53923432	0.0076	G:G	0.0760	152	A:A	0.064	23
	UGSDII_00801	1	54074608	0.0413	A:A	0.0409	152	G:G	0.050	17
	UGSS_01269	1	65048795	0.0412	C:C	-0.0851	150	A:A	-0.088	27
Fv'/Fm'_mean	UGSS_02348	2	7464637	0.0391	A:A	-0.0445	43	C:C	-0.001	6
	UGSDI_05872	2	16967911	0.0057	A:A	-0.0012	148	G:G	0.052	19
	UGSS_01684	2	18705142	0.0001	A:A	0.0043	145	A:G	-0.062	7
	UGSDII_04219	2	68165681	0.0260	G:G	-0.0383	152	A:A	-0.008	17
	UGSS_02379	2	77273043	0.0322	A:A	-0.0098	152	A:C	-0.042	21
	UGSS_02698	3	497579	0.0322	G:G	0.0315	136	C:C	0.001	35
	UGSS_02587	3	13465929	0.0059	G:G	0.0411	167	A:G	0	11

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
	UGSDII_05167	3	57484015	0.0015	G:G	0.0065	164	C:C	-0.045	9
	UGSDI_13662	3	63671432	0.0065	A:A	0.0655	164	A:C	0.083	9
	UGSDI_15315	4	7285512	0.0150	A:A	-0.0263	153	C:C	0	20
	UGSDII_06638	4	15198012	0.0297	A:A	0.0180	161	G:G	0.046	10
	UGST_00314	4	55810400	0.0447	A:A	0.0588	94	G:G	0	6
	UGSDI_17206	4	58518488	0.0231	A:G	-0.0249	147	G:G	0.006	23
	UGSDI_17277	4	59185316	0.0129	A:A	0.0437	161	C:C	0.031	8
	UGSDI_20501	5	1190661	0.0392	G:G	0.0170	146	C:C	-0.014	19
	UGSS_03995	5	3372746	0.0026	C:C	-0.0381	136	G:G	0.013	26
	UGSDII_07441	5	7096990	0.0014	G:G	-0.0207	145	C:C	-0.051	12
	UGSS_04552	5	9137603	0.0049	A:A	0.1084	146	A:C	0.104	13
Fv'/Fm'_mean	UGSDI_23923	5	25498005	0.0128	G:G	-0.0509	95	A:G	-0.052	77
	UGSDI_24232	5	41421154	0.0152	C:C	0.0574	162	A:A	0.023	9
	UGSDI_25517	5	56504980	0.0101	C:C	0.0056	154	G:G	-0.039	16
	UGSDI_26370	6	1295049	0.0363	C:C	0.0308	151	G:G	0.00038	22
	UGSDI_27967	6	50580527	0.0169	A:A	-0.0414	140	G:G	-0.006	27
	UGST_00515	6	58367647	0.0155	C:C	0.0439	168	A:G	0	9
	UGSDI_29817	6	60768705	0.0426	G:G	0.0093	168	A:A	0.039	9
	UGSDII_14100	8	48649540	0.0249	G:G	0.0591	168	A:A	0.065	9
	UGSS_05515	8	48798667	0.0133	G:G	0.0311	146	A:C	0	24
	UGSDI_40000	9	49133114	0.0360	C:C	0.0015	147	A:G	0.044	5
	UGSDI_40067	9	49380411	0.0145	G:G	0.0103	161	A:G	-0.036	9
	UGSS_00104	10	17631024	0.0285	C:C	-0.0376	164	G:G	0	13
	UGSDI_01005	1	18392063	0.0426	A:G	-0.0067	155	G:G	0	22
	UGSS_00882	1	45813048	0.0060	G:G	0.0095	146	A:A	0.005	14
	UGSDI_02342	1	54720245	0.0187	A:A	-0.0048	116	A:G	-0.003	16
	UGSDI_02758	1	56816253	0.0022	C:C	-0.0008	39	A:A	0.005	122
	UGSS_02429	2	8412341	0.0432	G:G	0.0160	166	A:A	0.023	11
	UGSDI_05872	2	16967911	0.0207	A:A	-0.0082	148	G:G	-0.016	19
	UGSS_02312	2	69795464	0.0088	A:C	0.0031	55	A:A	0.007	104
	UGSDII_05023	3	55267162	0.0409	C:C	0.0046	74	G:G	0.003	74
	UGSDI_13629	3	63576281	0.0191	C:C	-0.0020	109	A:G	-0.007	17
	UGSDI_13630	3	63599252	0.0244	C:C	-0.0017	102	A:C	-0.009	8
	UGSDI_13662	3	63671432	0.0295	A:A	-0.0091	164	A:C	-0.016	9
	UGSDII_06989	4	53058228	0.0062	A:G	0.0029	33	G:G	0.006	122
	UGSDI_16672	4	54037709	0.0012	A:A	0.0088	75	G:G	0.008	89
	UGSDII_07441	5	7096990	0.0312	G:G	0.0065	145	C:C	0.009	12
Fv'/Fm'_*sl*	UGSDI_23923	5	25498005	0.0161	G:G	0.0112	95	A:G	0.012	77
	UGSDI_25464	5	56063892	0.0094	C:C	0.0063	99	G:G	0.009	51
	UGSDI_27967	6	50580527	0.0074	A:A	0.0115	140	G:G	0.010	27
	UGSDI_28180	6	51982555	0.0319	A:G	-0.0072	161	C:C	0	14
	UGSDI_29642	6	58960437	0.0299	G:G	0.0118	150	A:A	0.011	21
	UGSDI_30175	7	499539	0.0363	C:C	0.0047	106	G:G	0	24
	UGSDII_11324	7	55772900	0.0309	G:G	-0.0058	133	C:C	-0.008	33
	UGSDII_11795	8	3757426	0.0297	G:G	0.0093	123	C:C	0.006	40
	UGSDII_14270	8	50648581	0.0441	G:G	0.0025	76	C:C	0.005	75
	UGSDI_32962	8	50784624	0.0194	G:G	-0.0069	141	A:G	-0.003	5
	UGSDI_34812	9	5493017	0.0192	C:C	-0.0073	155	A:G	-0.012	11
	UGSDI_35770	9	9255371	0.0389	C:C	-0.0045	146	A:G	0	30
	UGSDI_36872	9	15491737	0.0287	G:G	-0.0025	153	C:C	-0.010	14
	UGSS_06107	9	47130339	0.0230	G:G	-0.0059	163	A:A	0.004	10
	UGSDII_14939	9	49639894	0.0200	C:C	-0.0082	165	G:G	-0.001	1

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
	UGSDI_01005	1	18392063	0.0331	A:G	-0.9588	155	G:G	0	22
	UGSDI_02342	1	54720245	0.0085	A:A	-0.6848	116	A:G	-0.418	16
	UGSDI_02758	1	56816253	0.0087	C:C	-0.0854	39	A:A	0.621	122
	UGSDII_01103	1	60627976	0.0261	A:A	0.0517	127	A:G	-0.659	19
	UGSDI_05872	2	16967911	0.0185	A:A	-0.5743	148	G:G	-1.882	19
	UGSS_01684	2	18705142	0.0229	A:A	0.3065	145	A:G	1.401	7
	UGSS_02312	2	69795464	0.0045	A:C	0.3765	55	A:A	0.948	104
	UGSS_02587	3	13465929	0.0339	G:G	-0.9690	167	A:G	0	11
	UGSDII_05167	3	57484015	0.0213	G:G	0.3110	164	C:C	1.400	9
	UGSDI_13629	3	63576281	0.0137	C:C	-0.1795	109	A:G	-1.018	17
	UGSDI_13630	3	63599252	0.0287	C:C	-0.1115	102	A:C	-1.126	8
	UGSDI_13662	3	63671432	0.0192	A:A	-1.3454	164	A:C	-2.288	9
Fv'/Fm'_T_b	UGSDII_06989	4	53058228	0.0028	A:G	0.2467	33	G:G	0.795	122
	UGSDI_16672	4	54037709	0.0063	A:A	0.9799	75	G:G	0.952	89
	UGSDII_07441	5	7096990	0.0068	G:G	0.8872	145	C:C	1.449	12
	UGSDI_23923	5	25498005	0.0077	G:G	1.6760	95	A:G	1.781	77
	UGSDI_25464	5	56063892	0.0430	C:C	0.3845	99	G:G	0.796	51
	UGSDI_25517	5	56504980	0.0175	C:C	-0.2189	154	G:G	1.058	16
	UGSDI_27967	6	50580527	0.0072	A:A	1.6049	140	G:G	1.034	27
	UGSDI_29634	6	58810203	0.0442	C:C	-0.0319	108	G:G	0.472	59
	UGSDII_10411	6	61585843	0.0331	G:G	-1.1320	146	A:G	-0.236	8
	UGSDI_30175	7	499539	0.0279	C:C	0.6569	106	G:G	0	24
	UGSDII_11795	8	3757426	0.0339	G:G	1.1937	123	C:C	0.758	40
	UGSDI_32962	8	50784624	0.0148	G:G	-0.9524	141	A:G	-0.591	5
	UGSDI_34812	9	5493017	0.0229	C:C	-0.8161	155	A:G	-1.600	11
	UGSDI_00412	1	12780924	0.0132	A:A	0.0429	153	G:G	0.051	13
	UGSDI_00670	1	15363035	0.0040	G:G	-0.0226	141	A:G	0.006	13
	UGSS_00916	1	46522086	0.0154	A:G	-0.0138	36	G:G	-0.033	67
	UGSS_01028	1	52678585	0.0157	C:C	0.0313	31	A:A	-0.005	138
	UGSDI_02242	1	54468634	0.0093	C:C	-0.0152	92	A:C	0	73
	UGSDI_03630	1	64293897	0.0242	A:A	-0.0272	140	A:G	-0.002	17
	UGSDI_04747	2	2277079	0.0074	C:C	-0.0004	123	A:G	0.037	12
	UGSS_01909	2	3221343	0.0189	C:C	-0.0368	134	A:A	-0.054	38
	UGSDII_03223	2	18252899	0.0132	G:G	0.0358	167	A:C	0	9
	UGSS_01684	2	18705142	0.0133	A:A	0.0097	147	A:G	-0.040	7
	UGSDI_08747	2	73826945	0.0337	C:C	-0.0032	96	A:G	0.020	19
	UGSS_02698	3	497579	0.0274	G:G	0.0391	137	C:C	0.019	38
	UGSDI_10544	3	7736014	0.0235	C:C	-0.0097	163	G:G	0.039	11
Φ_{PSII}_mean	UGSDI_11887	3	16264864	0.0420	G:G	-0.0014	31	C:C	0.025	135
	UGSDII_05216	3	58207290	0.0293	G:G	0.0227	106	C:C	0.010	63
	UGSDI_12802	3	59222133	0.0150	G:G	-0.0316	57	C:C	-0.021	106
	UGSDI_14812	4	3443239	0.0178	A:A	-0.0287	150	G:G	-0.014	18
	UGSDI_21684	5	5585914	0.0416	C:C	-0.1241	81	A:C	-0.125	94
	UGSS_04354	5	5697301	0.0369	C:C	0.0069	70	A:G	0.023	20
	UGSS_04552	5	9137603	0.0420	A:A	0.0899	144	A:C	0.099	13
	UGSDII_07808	5	11048639	0.0310	C:C	-0.0027	87	A:G	0.016	30
	UGSDI_23923	5	25498005	0.0217	G:G	-0.0499	97	A:G	-0.051	79
	UGSDI_25517	5	56504980	0.0167	G:G	-0.0517	17	C:C	-0.028	156
	UGSDI_27222	6	45529043	0.0325	C:C	-0.0273	41	G:G	-0.028	121
	UGSDI_27967	6	50580527	0.0226	G:G	-0.0151	32	A:A	-0.044	139
	UGSDI_29473	6	57559466	0.0440	C:C	-0.0232	25	G:G	-0.053	147
	UGSDI_29634	6	58810203	0.0157	G:G	-0.0246	62	C:C	-0.009	108
	UGSDI_29798	6	60459760	0.0373	C:C	0.0087	70	A:G	0.032	10

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
	UGSDII_11202	7	53130716	0.0391	G:G	-0.0388	40	C:C	-0.041	130
	UGSDI_31651	7	56757202	0.0146	G:G	0.0641	79	C:C	0.054	26
	UGSDI_32247	8	3014692	0.0392	C:C	-0.0062	129	A:G	0.018	18
	UGSDII_12241	8	9018898	0.0153	A:G	-0.0690	149	C:C	-0.097	32
	UGSS_05515	8	48798667	0.0060	G:G	0.0338	147	A:C	0	27
	UGSDI_32858	8	49411280	0.0356	G:G	-0.0333	22	C:C	-0.011	150
Φ_{PSII} _mean	UGSDII_14270	8	50648581	0.0439	C:C	-0.0198	76	G:G	-0.021	78
	UGSDI_32962	8	50784624	0.0257	G:G	0.0263	142	A:G	0.009	5
	UGSDI_35992	9	9909969	0.0421	C:C	0.0236	115	G:G	0.037	38
	UGSDI_40000	9	49133114	0.0365	C:C	-0.0103	147	A:G	0.033	6
	UGSDI_40415	9	52529034	0.0237	A:A	-0.0514	18	G:G	-0.054	157
	UGSDII_15599	10	4586779	0.0312	C:C	0.0038	149	A:A	0.030	15
	UGSDI_43971	10	9655431	0.0098	C:C	0.0116	70	A:A	0.033	12
	UGSDI_00990	1	17772279	0.0386	A:A	0.0156	29	G:G	0.011	140
	UGSS_00882	1	45813048	0.0412	G:G	0.0069	145	A:A	-0.00013	18
	UGSDII_01462	1	70099164	0.0400	C:C	0.0045	154	A:A	-0.004	17
	UGSS_02435	2	9526405	0.0306	A:A	0.0058	60	C:C	0.018	6
	UGSDII_04148	2	65113602	0.0137	C:C	-0.0073	21	A:A	-0.015	144
	UGSDI_08696	2	73353359	0.0407	A:A	-0.0098	156	A:G	-0.010	13
	UGST_00227	3	2304449	0.0111	G:G	0.0102	52	C:C	0.005	119
	UGSS_02643	3	2503519	0.0268	C:C	0.0096	48	G:G	0.006	124
	UGSDI_11833	3	16099516	0.0096	A:A	0.0020	163	A:C	-0.010	14
	UGSDII_05167	3	57484015	0.0171	G:G	0.0141	165	C:C	0.009	11
	UGSDI_20169	4	67503756	0.0221	G:G	0.0098	158	C:C	0.009	10
	UGSDII_07441	5	7096990	0.0299	G:G	0.0079	148	C:C	0.011	12
	UGSS_04052	5	49987259	0.0370	C:C	0.0040	30	G:G	0.011	140
	UGSDI_25517	5	56504980	0.0338	G:G	0.0026	17	C:C	-0.007	156
Φ_{PSII} _*sl*	UGSDII_08774	6	1644212	0.0242	C:C	-0.0090	24	A:G	0	157
	UGSDII_10022	6	45995024	0.0353	C:C	0.0070	153	A:G	0	29
	UGSDI_30352	7	1624829	0.0020	C:C	0.0157	40	G:G	0.013	126
	UGSDI_32052	8	234578	0.0168	A:C	0.0082	16	G:G	0	167
	UGSDII_14222	8	50227065	0.0327	C:C	-0.0085	54	G:G	0.001	120
	UGSS_05729	8	54372482	0.0340	A:A	0.0083	42	G:G	0.010	123
	UGSS_05745	8	54424095	0.0332	A:A	0.0118	127	G:G	0.004	47
	UGSS_05937	9	2429648	0.0312	A:A	0.0154	4	G:G	0	16
	UGSDI_39240	9	46511577	0.0065	A:A	0.0102	167	A:C	0	12
	UGSS_06157	9	51319279	0.0373	G:G	0.0099	28	C:C	0.003	142
	UGSDI_42010	9	58369291	0.0348	A:A	0.0059	116	G:G	0.003	42
	UGSDII_15532	10	2243753	0.0142	C:C	-0.0018	68	A:A	0.006	18
	UGSDI_44580	10	13833465	0.0379	C:C	-0.0051	146	A:A	-0.001	7
	UGSDI_44715	10	14912907	0.0267	C:C	-0.0049	156	A:A	0	22
	UGSDI_46065	10	20416232	0.0184	A:A	0.0048	34	C:C	0	146
	UGSDI_00670	1	15363035	0.0299	G:G	0.2542	141	A:G	-0.706	13
	UGSDI_00990	1	17772279	0.0426	A:A	1.9635	29	G:G	1.386	140
	UGSS_00989	1	52324950	0.0161	A:C	1.7263	112	A:A	1.351	39
	UGSDI_02342	1	54720245	0.0305	A:A	-0.6048	117	A:G	-0.358	16
	UGSDI_02900	1	57831374	0.0328	A:A	0.7961	34	G:G	-0.544	130
Φ_{PSII} _T_b	UGSDII_01103	1	60627976	0.0272	A:A	0.3012	128	A:G	-0.420	20
	UGSS_01269	1	65048795	0.0398	C:C	3.3638	151	A:A	3.109	29
	UGSS_01684	2	18705142	0.0103	A:A	-0.5391	147	A:G	1.340	7
	UGSDII_04148	2	65113602	0.0144	C:C	-0.7581	21	A:A	-1.828	144
	UGSDI_08747	2	73826945	0.0333	C:C	0.0707	96	A:G	-0.655	19
	UGST_00227	3	2304449	0.0048	G:G	0.6429	52	C:C	-0.046	119

Trait	Locus	Chr.	Position [cM]	p_Marker	Allel	Effect	n	Allel	Effect	n
	UGSS_02643	3	2503519	0.0160	C:C	0.9832	48	G:G	0.413	124
	UGSDII_05129	3	57060236	0.0359	A:A	-0.1130	122	A:C	-0.772	18
	UGSDI_12802	3	59222133	0.0237	G:G	0.5214	57	C:C	-0.009	106
	UGSDI_13662	3	63671432	0.0436	A:A	-1.6725	166	A:C	-2.440	11
	UGSDI_15088	4	6261583	0.0184	C:C	0.4125	59	A:A	0	120
	UGSDI_20169	4	67503756	0.0028	G:G	1.4002	158	C:C	1.662	10
	UGSDII_07441	5	7096990	0.0039	G:G	0.9787	148	C:C	1.709	12
	UGSDII_07808	5	11048639	0.0007	C:C	0.1594	87	A:G	-0.742	30
	UGSDI_24739	5	46500037	0.0152	C:C	-0.1662	12	G:G	-1.554	166
	UGSDI_25517	5	56504980	0.0006	G:G	1.3337	17	C:C	-0.672	156
	UGSDII_08774	6	1644212	0.0363	C:C	-1.1061	24	A:G	0	157
	UGSDI_26919	6	37887169	0.0177	G:G	0.9017	149	A:G	-0.079	12
	UGSS_04670	6	37986408	0.0350	G:G	1.1589	165	A:C	0.260	7
	UGSDI_29642	6	58960437	0.0402	A:A	1.5096	25	G:G	1.594	148
	UGSDI_30352	7	1624829	0.0016	C:C	1.9090	40	G:G	1.305	126
Φ_{PSII} _ T_b	UGSDII_11202	7	53130716	0.0225	G:G	1.2238	40	C:C	0.744	130
	UGSDI_32052	8	234578	0.0043	A:C	1.2255	16	G:G	0	167
	UGSDII_11636	8	2260138	0.0417	G:G	0.1836	31	C:C	-0.618	137
	UGSDII_11940	8	5700178	0.0327	A:G	-1.1006	32	C:C	-0.157	128
	UGSDI_33170	8	52033501	0.0390	G:G	1.0432	127	A:A	1.136	42
	UGSS_05729	8	54372482	0.0067	A:A	1.0652	42	G:G	1.402	123
	UGSS_05885	9	1130820	0.0041	A:G	1.7128	12	G:G	1.170	146
	UGSDI_34812	9	5493017	0.0205	C:C	-1.0094	155	A:G	-1.887	14
	UGSDI_35770	9	9255371	0.0170	A:G	0.6316	34	C:C	0	146
	UGSDI_39240	9	46511577	0.0044	A:A	1.2684	167	A:C	0	12
	UGSS_06138	9	51058868	0.0254	A:G	1.1468	37	C:C	0	142
	UGSS_06272	9	54095775	0.0286	G:G	3.6194	26	A:G	2.812	142
	UGSDI_43091	10	5773366	0.0215	G:G	-0.3847	127	A:A	0.646	12
	UGSDI_44580	10	13833465	0.0338	C:C	-0.5757	146	A:A	-0.171	7
	UGSDI_44715	10	14912907	0.0410	C:C	-0.4985	156	A:A	0	22
	UGSDI_46065	10	20416232	0.0176	A:A	0.5260	34	C:C	0	146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SPAD(m) UGSDI_14722
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PhiPSII(m) UGSDI_14812
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LAR(m) UGSDII_05835
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LAR(m) UGSS_03531
LAR(Tb)/PhiPSII(Tb) UGSDI_15088
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UGSDII_05980
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SPAD(sl) UGSS_03587
SPAD(m) UGST_00343
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DMGR(a)/DMGR0/Fv'/Fm'(m) UGST_00314
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UGSDI_17077
UGSDI_17159
UGSDI_17190
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SPAD(m)/Fv'/Fm'(m) UGSDI_17277
DMGR(m) UGSDI_17961
UGSDI_18462
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PhiPSII(m)
Fv'/Fm'(m)
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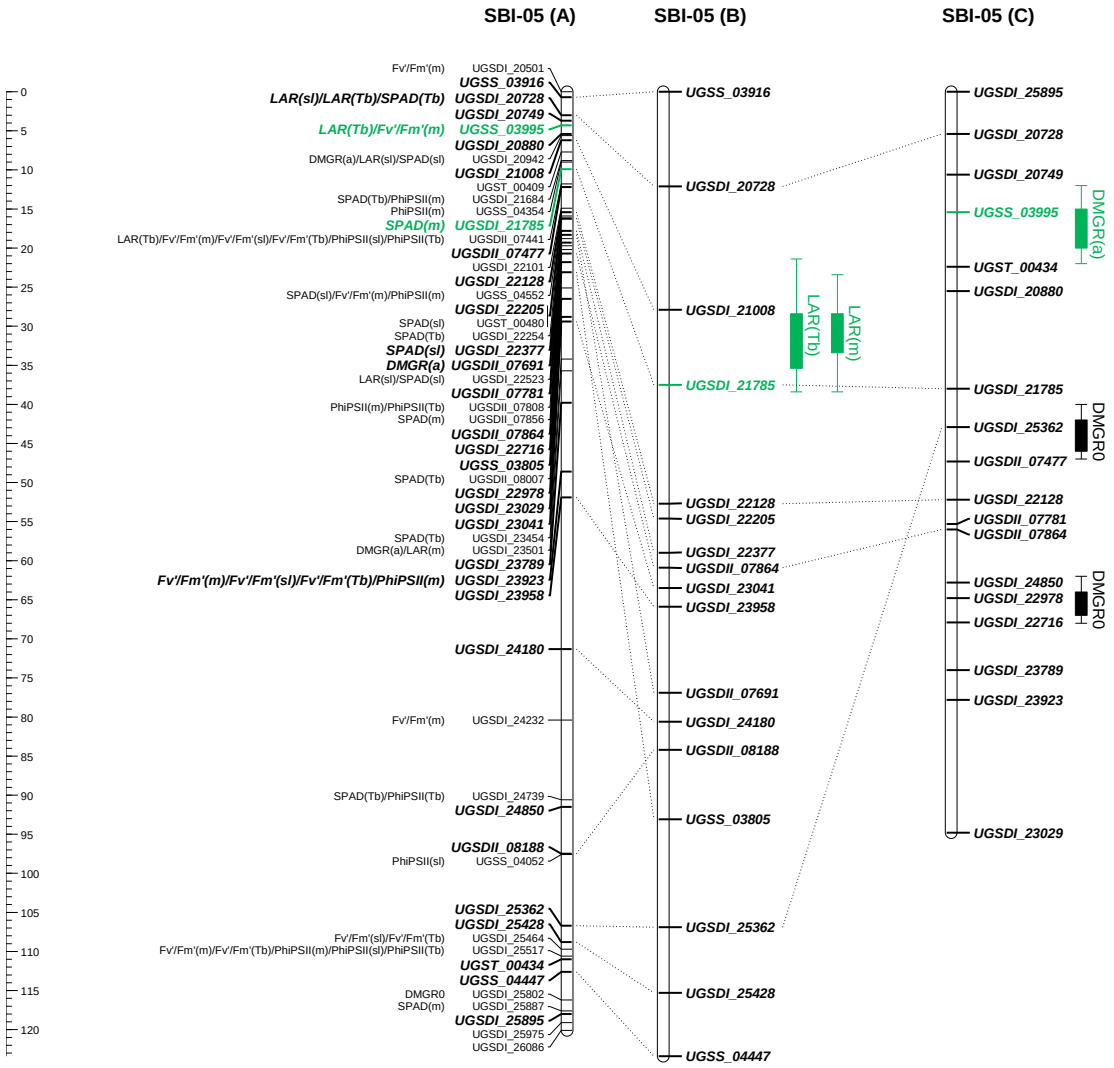
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120
Fv'/Fm'(m)
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LAR(sl)/LAR(Tb)/SPAD(Tb)
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UGSDI_20749
LAR(Tb)/Fv'/Fm'(m)
UGSS_03995
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DMGR(a)/LAR(sl)/SPAD(sl)
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UGSDI_21008
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SPAD(Tb)/PhiPSII(m)
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PhiPSII(m)
UGSS_04354
SPAD(m)
UGSDI_21785
LAR(Tb)/Fv'/Fm'(m)/Fv'/Fm'(sl)/Fv'/Fm'(Tb)/PhiPSII(sl)/PhiPSII(Tb)
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UGSDII_07477
UGSDI_22101
UGSDI_22128
SPAD(sl)/Fv'/Fm'(m)/PhiPSII(m)
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SPAD(sl)
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SPAD(Tb)
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SPAD(sl)
UGSDI_22377
DMGR(a)
UGSDII_07691
LAR(sl)/SPAD(sl)
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UGSDII_07781
PhiPSII(m)/PhiPSII(Tb)
UGSDII_07808
SPAD(m)
UGSDII_07856
UGSDII_07864
UGSDI_22716
UGSS_03805
SPAD(Tb)
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UGSDI_22978
UGSDI_23029
UGSDI_23041
SPAD(Tb)
UGSDI_23454
DMGR(a)/LAR(m)
UGSDI_23501
UGSDI_23789
Fv'/Fm'(m)/Fv'/Fm'(sl)/Fv'/Fm'(Tb)/PhiPSII(m)
UGSDI_23923
UGSDI_23958
UGSDI_24180
Fv'/Fm'(m)
UGSDI_24232
SPAD(Tb)/PhiPSII(Tb)
UGSDI_24739
UGSDI_24850
UGSDII_08188
PhiPSII(sl)
UGSS_04052
UGSDI_25362
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Fv'/Fm'(sl)/Fv'/Fm'(Tb)
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DMGR0
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SPAD(m)
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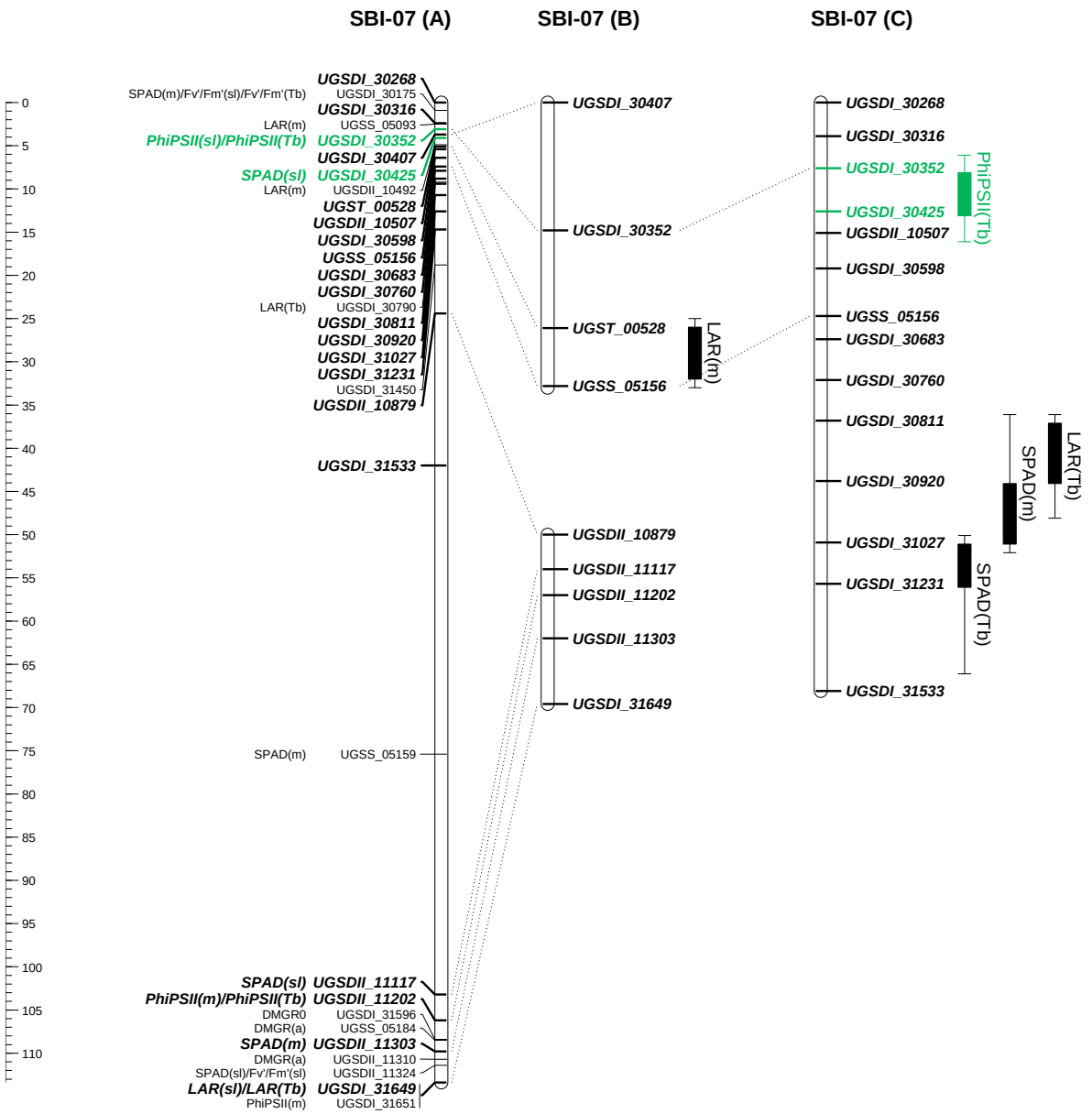
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SPAD(m)/Fv'/Fm'(sl)/Fv'/Fm'(Tb)
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UGSDI_30316
LAR(m)
UGSS_05093
PhiPSII(sl)/PhiPSII(Tb)
UGSDI_30352
UGSDI_30407
SPAD(sl)
UGSDI_30425
LAR(m)
UGSDII_10492
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UGSDI_30598
UGSS_05156
UGSDI_30683
UGSDI_30760
LAR(Tb)
UGSDI_30790
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UGSDI_31450
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SPAD(m)
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SPAD(sl)
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PhiPSII(m)/PhiPSII(Tb)
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DMGR(a)
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SPAD(m)
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DMGR(a)
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SPAD(sl)/Fv'/Fm'(sl)
UGSDII_11324
LAR(sl)/LAR(Tb)
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PhiPSII(m)
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SPAD(m)
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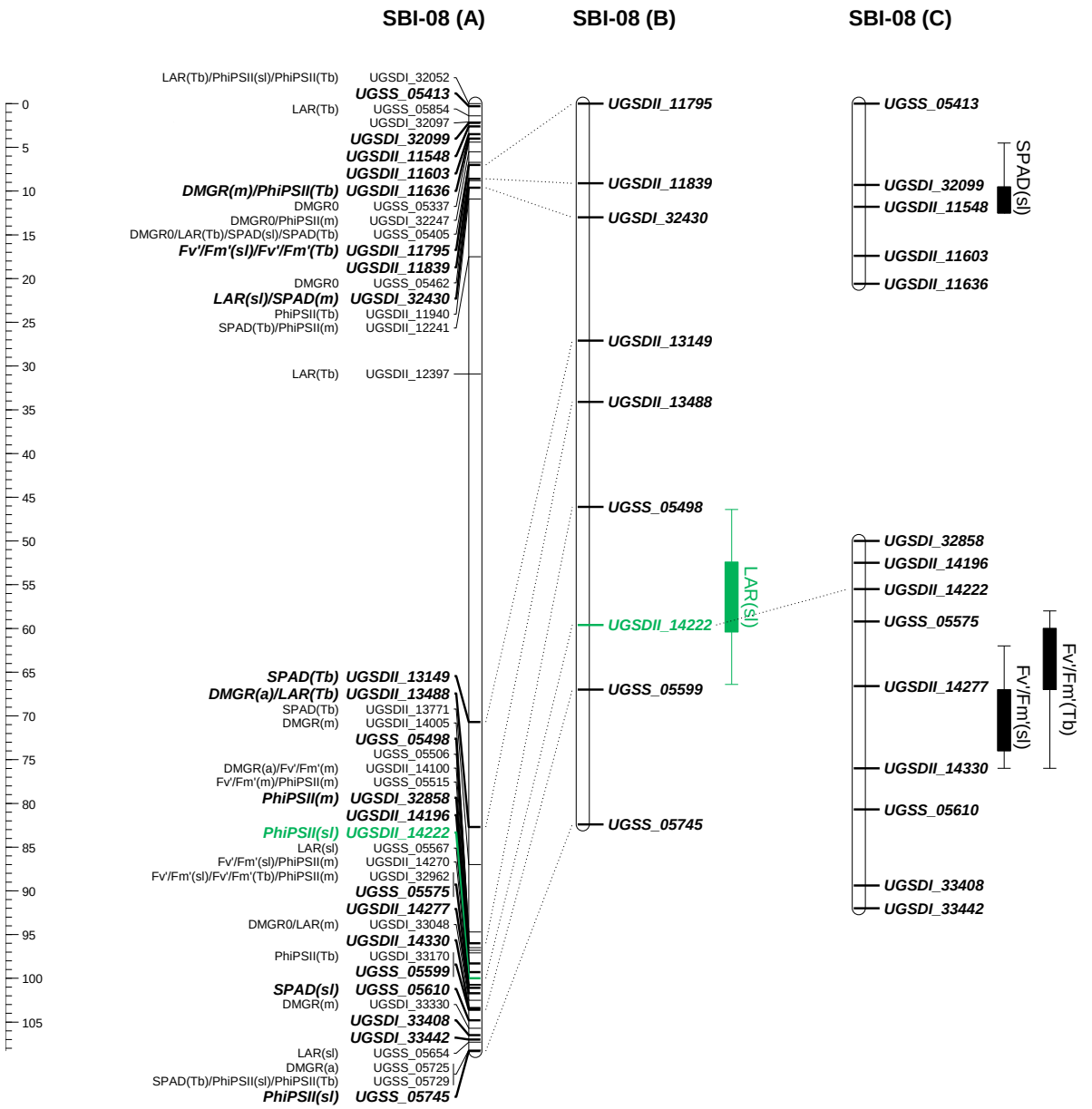
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105
LAR(Tb)/PhiPSII(sl)/PhiPSII(Tb) UGSDI_32052
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LAR(Tb) UGSS_05854
UGSDI_32097
UGSDI_32099
UGSDII_11548
UGSDII_11603
DMGR(m)/PhiPSII(Tb) UGSDII_11636
DMGR0 UGSS_05337
DMGR0/PhiPSII(m) UGSDI_32247
DMGR0/LAR(Tb)/SPAD(sl)/SPAD(Tb) UGSS_05405
Fv'/Fm'(sl)/Fv'/Fm'(Tb) UGSDII_11795
UGSDII_11839
DMGR0 UGSS_05462
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SPAD(Tb) UGSDII_13771
DMGR(m) UGSDII_14005
UGSS_05498
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DMGR(a)/Fv'/Fm'(m) UGSDII_14100
Fv'/Fm'(m)/PhiPSII(m) UGSS_05515
PhiPSII(m) UGSDI_32858
UGSDII_14196
PhiPSII(sl) UGSDII_14222
LAR(sl) UGSS_05567
Fv'/Fm'(sl)/PhiPSII(m) UGSDII_14270
Fv'/Fm'(sl)/Fv'/Fm'(Tb)/PhiPSII(m) UGSDI_32962
UGSS_05575
UGSDII_14277
DMGR0/LAR(m) UGSDI_33048
UGSDII_14330
PhiPSII(Tb) UGSDI_33170
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DMGR(m) UGSDI_33330
UGSDI_33408
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SPAD(Tb)/PhiPSII(sl)/PhiPSII(Tb) UGSS_05729
PhiPSII(sl) UGSS_05745
UGSDII_11795
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UGSS_05745
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SPAD(sl)
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Fv'/Fm'(sl)
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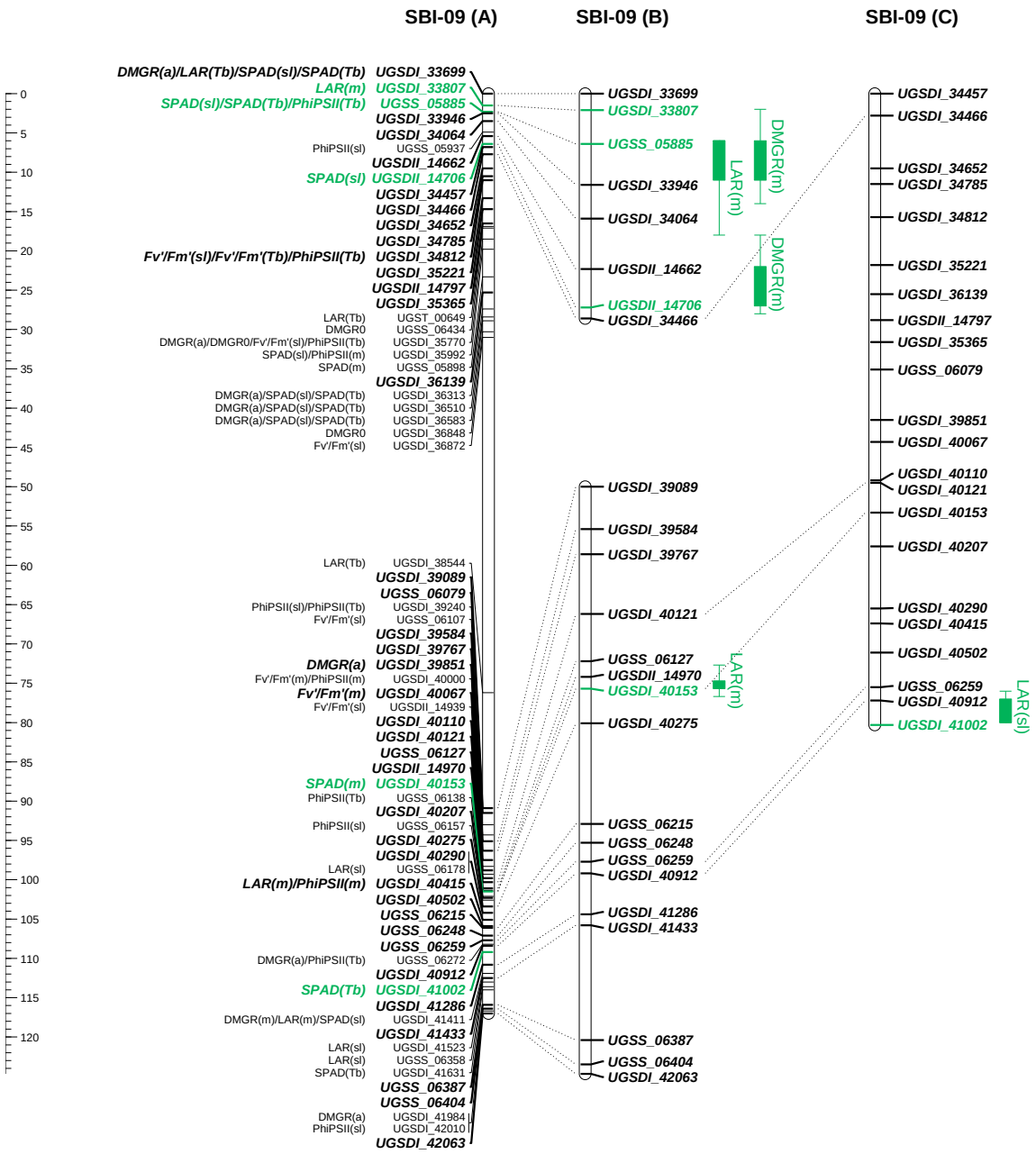
SBI-09 (A)
SBI-09 (B)
SBI-09 (C)
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LAR(m)
SPAD(sl)/SPAD(Tb)/PhiPSII(Tb)
SPAD(sl)
Fv'/Fm'(sl)/Fv'/Fm'(Tb)/PhiPSII(Tb)
SPAD(m)
DMGR(a)
Fv'/Fm'(m)
SPAD(Tb)
LAR(m)
DMGR(m)
LAR(sl)

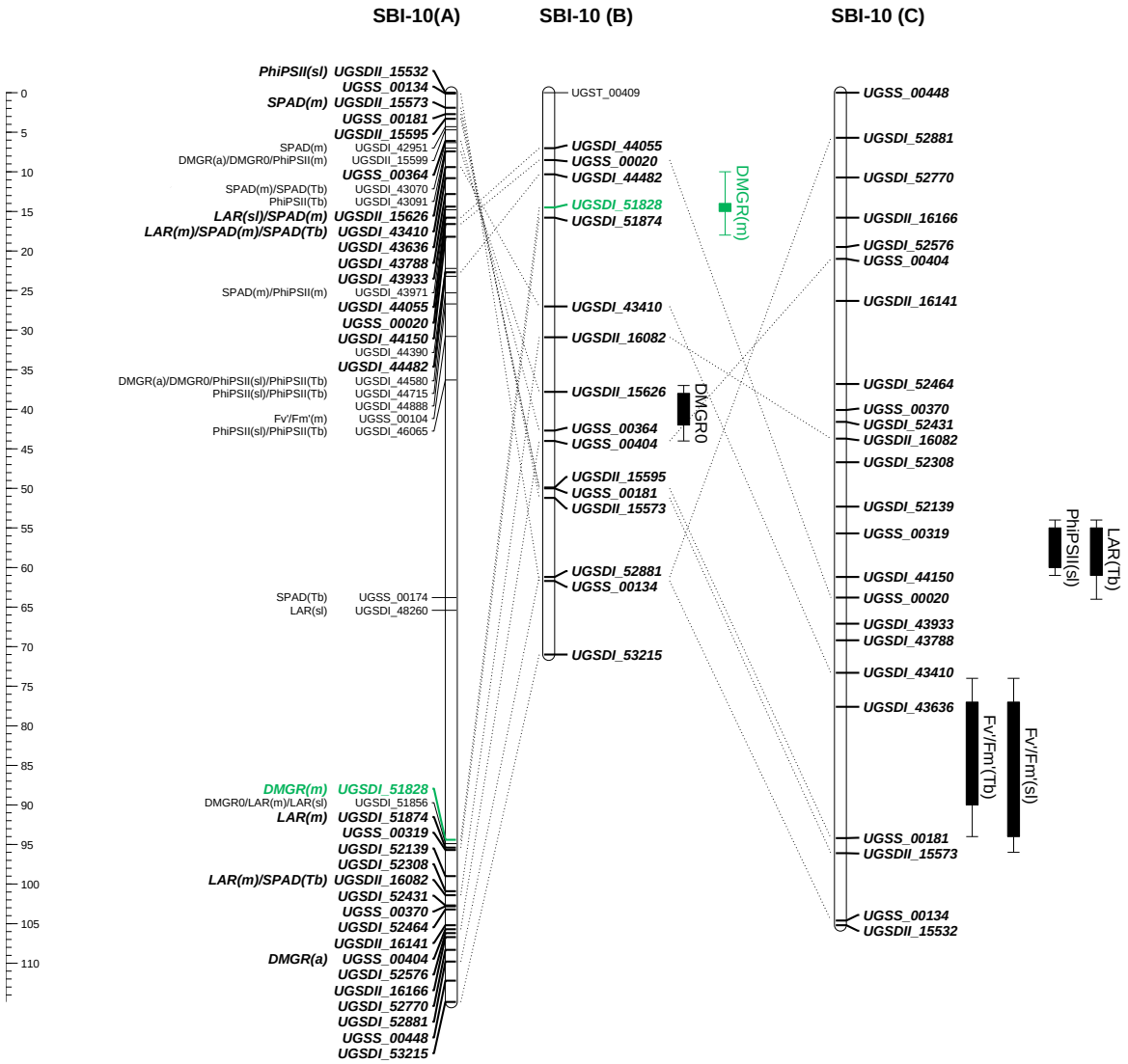

Figure S 4.1: Marker-trait associations (A) and QTL for population 1 (B) and population 2 (C) for mean across environments (*m*), exponents (*a*) and initial growth rate ($DMGR_0$) for dry matter growth rate (DMGR) and *m*, regression slopes (*sl)*, and base temperatures (T_b) for leaf appearance rate (LAR), chlorophyll content (SPAD) and fluorescence (F_v'/F_m' and Φ_{PSII}). Only markers significantly associated with a trait or present in at least two populations are presented. Bold and italic markers are present in all three populations. Positions of markers of the association panel are given in $2*10^{-6}$ bp. Distances between markers in the cross populations are given in cM. Green markers are associated with a trait and located in a QTL region of population 1 or 2 or both populations.

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Selbstständigkeitserklärung

gemäß § 6 Abs. 1 PromO für die Promotion zum Dr. rer. hort.

Ich erkläre, dass ich meine Dissertation mit dem Titel: „Marker-trait associations for early season cold tolerance in sorghum" entsprechend der Vorgaben des § 6 Abs.1 S. 2 lit. d PromO verfasst habe.

Im Einzelnen erkläre ich:

1.die Regeln der geltenden Promotionsordnung zu kennen und eingehalten zu haben und mit einer Prüfung nach den Bestimmungen der Promotionsordnung einverstanden zu sein,

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4. die Dissertation noch nicht als Prüfungsarbeit für eine andere Prüfung eingereicht zu haben,

5. ferner, die gleiche oder eine in wesentlichen Teilen ähnliche Arbeit nicht bei einer anderen Fakultät oder bei einer anderen Hochschule als Dissertation eingereicht zu haben, keine andere Abhandlung als Dissertation anderswo eingereicht zu haben.

6. damit einverstanden zu sein, dass die Dissertation einer Überprüfung der Einhaltung allgemein geltender wissenschaftlicher Standards unterzogen wird, insbesondere auch unter Verwendung elektronischer Datenverarbeitungsprogramme.

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