

Quantitative trait loci for yield and correlated traits under high and low soil nitrogen conditions in tropical maize

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Abstract The first objective of this study was to map and characterize quantitative trait loci (QTL) for grain yield (GY) and for secondary traits under varying nitrogen (N) supply. To achieve this objective, a segregating F_{2:3} population previously developed for QTL mapping under water-limited conditions was used. The population was evaluated in Mexico under low N conditions in the dry winter season and under low and high N conditions in the wet summer season.

From eight QTLs identified for GY under low N conditions, two were also detected under high N conditions. Five QTLs were stable across the two low N environments and five co-localized with QTLs identified for the anthesis-silking interval (ASI) or for the number of ears per plant (ENO) under low N conditions. The percentage of the phenotypic variance expressed by all QTLs for ASI and ENO was quite different when evaluated under low N conditions during the dry winter (40% for ASI and 22% for ENO) and the wet summer seasons (22% for ASI and 46% for ENO). The results suggest optimizing different breeding strategies based on selection index depending on the growing season. Good QTL colocalization was observed for ASI (four QTLs) and ENO (three QTLs) when looking at QTL identified under low N and water-limited conditions in the same population. The results suggest that both secondary traits can be used in breeding programs for simultaneous improvement of maize against low N and drought stresses.

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Abbreviations

ASI	Anthesis-silking interval
CHLO	Chlorophyll content in ear leaf
Ch	Chromosome

cM	CentiMorgan
ENO	Number of ears per plant
FFLW	Female flowering
GY	Grain yield
HKFW	Hundred kernel fresh weight
HN	High nitrogen
KNO	Number of kernels per plant
LNw/LNd	Low nitrogen in the wet and dry season
LOD	Likelihood of odds
MFLW	Male flowering
PHT	Plant height
QTL	Quantitative trait locus
QEI	QTL by environment interaction

Introduction

Together with drought, low nitrogen conditions represent a major source of yield loss in tropical maize (Pingali and Pandey 2001). Most maize in developing countries is produced under nitrogen deficient conditions (McCown et al. 1992; Oikeh and Horst 2001) because of low nitrogen use efficiency in drought prone environments, high price ratios between fertilizer and grain, limited availability of fertilizer, and low purchasing power of farmers (Bänziger et al. 1997). There is, consequently, a growing interest in developing cultivars that perform better under low nitrogen conditions. A better understanding of the genetic basis for maize development and yield under low N conditions is required to accelerate and improve selection efficiency for these environments.

Effects of nitrogen deficiency on maize growth and development are well known. Uhart and Andrade (1995a) reported effects of N deprivation on leaf expansion, emergence rate, radiation interception and radiation use efficiency, and assimilate partitioning between vegetative and reproductive organs. N deficiency increases the anthesis-silking interval (Jacobs and Pearson 1991), enhances kernel abortion (Pearson and Jacob 1987), accelerates senescence (Moll et al. 1994), and reduces final ear and grain number (Lemcoff and Loomis 1986; Uhart and Andrade 1995b; Monneveux et al. 2005a). Progress in selecting for tolerance to low N

is slowed by large genotype \times season and genotype \times location interactions. Given the low heritability of yield under stress conditions, the use of secondary traits has often been suggested (Lafitte et al. 2003). Some traits such as ear per plant, leaf senescence, and anthesis-silking interval associated with yield under low N conditions have been proposed and used as secondary traits to select for low N tolerance in maize (Lafitte and Edmeades 1994a; Bänziger and Lafitte 1997). Selection indices based on these traits were developed and increased significantly the selection efficiency under stress conditions (Bänziger and Lafitte 1997). The difficulty of measuring some of the secondary traits quickly and precisely, however, has limited their application in breeding programs (Monneveux and Ribaut 2006).

The identification and characterization of quantitative trait loci (QTL) help to identify genomic regions associated with the expression of complex traits and their precise genetic contribution at target loci. In maize, the genetic dissection of complex traits for abiotic stress responses has focused primarily on drought tolerance (Agrama et al. 1996; Ribaut et al. 1996, 1997; Tuberosa et al. 2002), the benefit of using molecular markers in breeding programs has been evaluated (Morris et al. 2003) and marker-assisted selection experiments to improve grain yield under water-limited conditions and low temperature have been reported recently (Ribaut and Ragot 2007). Less attention has been paid to understanding the genetic response of segregating populations to field soil deficiencies like low P (Reiter et al. 1991) or low N (Agrama et al. 1999; Hirel et al. 2001). As shown by Prioul et al. (1997) and Limami and de Vienne (2001), the development of molecular marker technologies offers powerful alternative methods to examine the relationships between physiological traits controlling maize response to N, thereby contributing to a better understanding of metabolic pathways and physiological processes.

In the present study, QTLs for yield and correlated traits were mapped and characterized in a tropical maize population grown in several environments that differed for N availability. The main objectives were to (1) compare plant genetic response and QTL stability across dry winter and

wet summer cycles under low N conditions, (2) identify traits most suitable for breeding under each cycle, and (3) look for a common genetic basis between low N and drought tolerance.

Material and methods

Field experiments

A segregating $F_{2:3}$ population of 240 families derived from two tropical lines was used in this study. This population was initially created to determine the genetic basis of drought tolerance. A drought tolerant line, P_1 , Ac7643S5, derived from Population 43 (La Posta) was crossed with a drought susceptible line, P_2 , Ac7729/TZSRWS5, derived from Population 29 (Tuxpeño Caribe). Experiments were conducted under low nitrogen and high nitrogen conditions over two seasons in 1996 at the CIMMYT Poza Rica Experimental Station, Veracruz, Mexico (20°32' N, 97°26' W, elevation 60 m a.s.l.). The segregating population was evaluated under low nitrogen conditions during the dry winter season (November–April) and under low and high nitrogen conditions during the wet summer season (May–October). The three environments were thereafter referred as to LNd, LNw, and HNw, respectively. Soils at the Poza Rica station are classified as sandy loam Tropofluvent (Entisol) (Bell and van Keulen 1995). Each experiment was evaluated in an alpha (0,1) lattice design with two repetitions. In all environments plots consisted of 2.5 m rows (12 plants), with 20 cm between hills and 0.75 m between rows. Plots were overplanted with two seeds per hill, and thinned after emergence to one plant per hill. The trials were planted in 5 m rows, with two plots per row; measurements were confined to the central part of the row, leaving the first two hills on each plot as borders. In the three environments, maize was cultivated during the previous eight seasons. The low N treatment was established according to Bänziger et al. (1999). During the eight years preceding the experiment, no N was applied and residues were removed before land preparation. In LNd and LNw environments, no N fertilizer was applied during the growth cycle and the previous eight

maize cycles. At the beginning of the season only 52 kg N ha⁻¹ was present as nitrate in the top 1 m of soil, as determined by HPLC and colorimetric methods (Grunau and Swiader 1989). During the growing cycle an additional 31 kg N ha⁻¹ was mineralized in the top 0.4 m of soil. This was determined from measurements taken at 14-day intervals on soil contained in capped tubes buried within the row, according to Raison et al. (1987). In the HNw environment, the maize received 200 kg N ha⁻¹ during the growth cycle and the previous eight cycles. In the present experiment, 100 kg N ha⁻¹ were broadcasted and incorporated at sowing and 100 kg N ha⁻¹ was banded at a depth of 5 cm beside each row 40 days later. All plots received 18 kg P ha⁻¹ prior to sowing and were kept free of insects and weeds.

Field measurements

To maximize the accuracy of plant phenotyping all morphological measurements at flowering time were conducted on an individual plant basis, with 10 plants per plot, in all environments. Male flowering (MFLW) was recorded as the number of days from sowing to the first anther extrusion from the tassel glumes; female flowering (FFLW) as the number of days from sowing to the first visible silk. Anthesis-silking interval (ASI) was calculated for each plant as FFLW-MFLW. Plant height (PHT) was recorded after completion of male flowering, as the distance between the ground surface and the node bearing the flag leaf. In vivo chlorophyll concentration of the ear leaf (CHLO) was assessed four weeks after male flowering, using a portable chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan) and was expressed in arbitrary absorbance (or SPAD) values (Dwyer et al. 1991). At the end of the growing cycle several yield components were measured, including grain yield (GY), ear number per plant (ENO) and hundred kernel fresh weight (HKFW). Grain was harvested about 14 days after black layer formation and dried for 3 days in a dryer room to constant weight before weighing (about 15% of remaining humidity). Within each plot, all ears with at least one grain were counted at harvest and reported on a single plant basis. A hundred grains randomly chosen were weighed to determine

HKFW. The kernel number per plant (KNO) was calculated from GY and HKFW as $KNO = (GY \times 100 / HKFW)$.

Data analysis

The means were adjusted using spatial analysis (Gleeson 1997), as described in the ASREML manual (Burgueño et al. 2000). This analysis includes all the information contained in the raw data, the repetitions, incomplete blocks, plots, rows and column position for each entry, using all the entries including the parental lines. The secondary variables obtained as linear combinations of the primary variables (e.g., KNO) were calculated using the adjusted means obtained from the spatial analysis. Simple Pearson correlation coefficients were calculated between the traits, using the adjusted means of the $F_{2:3}$ families. Broad sense heritability of the different traits, for each trial individual (over replications) and over the two levels of stress, and genetic correlations among traits were calculated using the Mixed Model Procedure (PROC MIXED) in Statistical Analysis System (SAS).

QTL analysis

Data were analyzed using the genetic map published earlier (Ribaut et al. 1996). The QTLs were identified by composite interval mapping using QTL Cartographer 1.16 model 6 (Basten et al. 2002), with a blocking window size of 30 cM. The co-factors were selected by forward and backward regressions with the in and out thresholds at a p -value of 0.01. Zmapqtl hypothesis 30 (likelihood ratio test procedure) was used to analyze single traits for the HN environment. Data from the two LN environments were analyzed jointly in a combined analysis (Jiang and Zeng 1995), allowing determining the QTL by environment interaction (QEI) for the different traits. For this type of data we can use a hypothesis similar to the hypothesis 30 for the single trait analysis, namely hypotheses 34. The presence of a QTL was considered to be significant when the LOD value was larger than 3.0 in single trait analysis and 3.5 in joint analysis. These values correspond to a type I error rate (α level)

of approximately 0.05 for single trait and joint analysis in an F_2 population (with three and five degrees of freedom, respectively), assuming that all chromosome arms segregate independently. Critical values for testing QEI was obtained based on Type I error rate for a single locus, since the position of the testing has been determined from mapping analyses. For two environments we considered a $LOD=1.3$ as the critical value with $df = 2$ as working with F_2 families. The proportion of variance (R^2) was calculated considering that for individual QTL R^2 is the proportion of the variance explained by the QTL conditioned on the background markers and any explanatory variables, and for all QTLs together R^2 is the proportion of the total variance explained by the QTL and the background markers and any explanatory variables.

Results

In all environments the target traits measured in the $F_{2:3}$ population followed approximately a normal distribution presenting a suitable phenotypic segregation for QTL mapping (Fig. 1). For several traits, the lower values of P_1 and P_2 compared to the $F_{2:3}$ population reflected the difference of inbreeding level between the parental lines and $F_{2:3}$ families. The limited amount of N in the soil due to the absence of nitrogen fertilization greatly affects the performance of the maize plants (Fig. 1). Compared with plants grown in HNw, plants grown in LNw and LNd showed 82% and 62% reduction in grain yield (GY) respectively. Low N conditions induced also a decrease in kernel (KNO) and ear number per plant (ENO), reduced the chlorophyll content in the ear leaf (CHLO), reduced plant height by about half, but increased the anthesis-silking interval (ASI) by several days. The fresh weight of 100 kernels (HKFW) was not affected much by the stress conditions as less than 20% difference was observed across the three trials. The reduction in GY appeared to be a direct consequence of a significant reduction in KNO, and to a less extent ENO. For most target traits presented here the plant performance under LN condition was also affected significantly by the growing season.

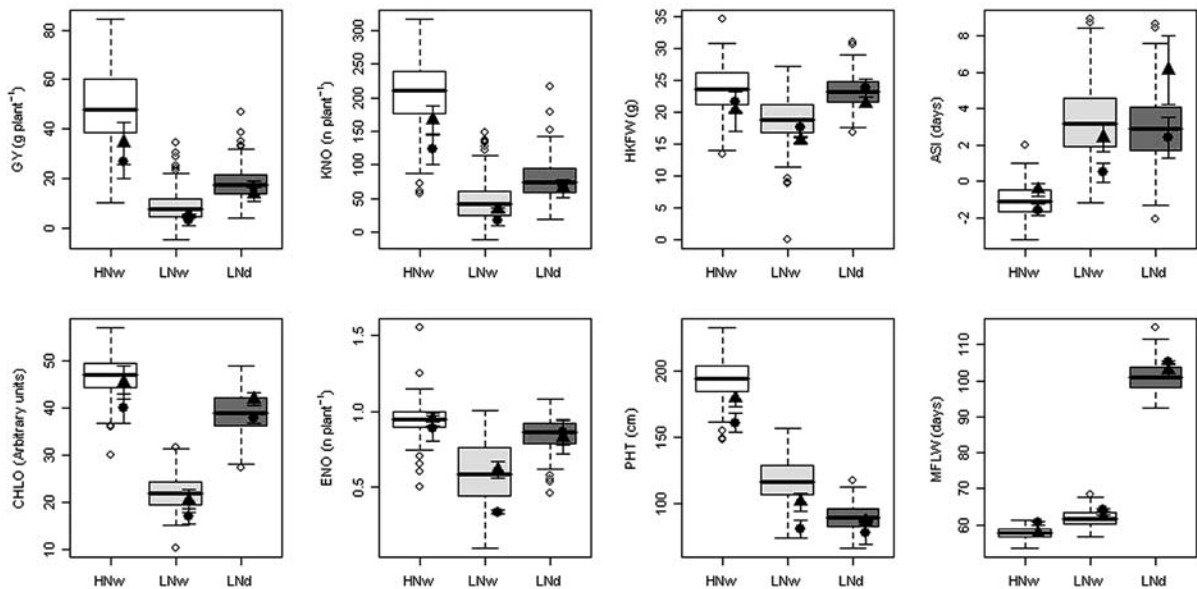


Fig. 1 Box plots of the distributions of the traits. The horizontal lines near the center of the boxes indicate the median values for the traits, and the bottom and top of the boxes represent the values for the first and third quartiles. The whiskers on the top and bottom of the boxes extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Individual data points falling outside the range defined by the whiskers are indicated by white circles (R Development

Core Team, 2005). The means (\pm SD) of the two parental lines P1 and P2 are indicated by circles and triangles respectively. GY: grain yield (g plant^{-1}); KNO: number of kernels per plant; HKFW: fresh weight of 100 kernels (g); ASI: anthesis-silking interval (days); CHLO: chlorophyll content in the ear leaf (arbitrary units); ENO: number of ears per plant; PHT plant height (cm); MFLW: time to male flowering (days)

Environmental conditions between the dry and the wet growing cycles were pretty different as demonstrated by the difference observed on plant male flowering (MFLW), with 102 days in the LNd (winter time) and 64 days in the LNw (summer time). Because of the shorter plant cycle observed during the wet season, mainly due to higher average temperature, longer days and higher humidity level compared to the dry season, the impact of LN conditions in this season was larger for most traits compared to the values observed under the dry season.

Variance analysis was conducted using the mixed model. Difference between repetitions was not significant while entries and the interaction entry \times environment were highly significant for all traits considered here. Broad sense heritability (h) was calculated for all traits considering different data sets. With data from a single environment, using the raw data collected from each of the repetitions, heritability for most traits

was above 0.5, with the lowest values observed for ENO under HNw (0.28) and LNd (0.13). When calculated across environments, heritability for all traits increased, the lowest values remaining for ENO under both LN stress conditions (0.35) and across the three environments (0.46).

Both phenotypic and genetic correlation analysis indicated significant correlations between GY and the other traits, with most correlations above 0.5 (Table 1). Only ASI under HNw, and both HKFW and CHLO under LNd (only the genetic correlation) were not correlated significantly with GY. The highest correlation for all three environments was observed with KNO, underlying the common genetic basis between KNO and GY in this population. Except for PHT and KNO, the other secondary traits presented marked differences in their level of correlation with GY under low N when measured under the wet and the dry season. In most cases the magnitude of the correlation was higher in LNw than in LNd.

Table 1 Phenotypic (left) and genotypic (right) correlation coefficients calculated between GY and other target traits for the three trials, high N during the wet season

(HNw), low N during the wet season (LNw) and low N during the dry season (LNd)

Traits	Environment		
	HNw	LNw	LNd
KNO	0.88**/0.88**	0.98**/0.98**	0.95**/0.95**
HKFW	0.63**/0.69**	0.39**/0.13*	0.16*/ - 0.12
ASI	- 0.10/ - 0.06	- 0.41**/ - 0.33**	- 0.26**/ - 0.17**
CHLO	0.49**/0.60**	0.49**/0.40**	0.20**/ - 0.06
ENO	0.56**/0.63**	0.70**/0.80**	0.53**/0.93**
PHT	0.44**/0.53**	0.51**/0.53**	0.51**/0.47**

* and ** indicate significant Pearson's product moment correlation at $p < 0.05$ and $p < 0.01$, respectively

To assess the stability of QTLs across the two LN environments, phenotypic data obtained under LNd and LNw were analyzed jointly for each trait. The results of the joint analysis of the two low N environments are presented in Table 2. The results of the single analysis of traits in plants grown in HNw are shown in Table 3. From eight QTLs identified for GY under low N conditions, five were stable across the two growing seasons (LOD QEI < 1.3). The stable QTL on chromosome 1 (67 cM) was also identified under HNw conditions (Table 3). This QTL colocalized with a QTL for ENO, which was also stable across the two Low N environments. P₂, the drought susceptible line, contributed to the favorable allele for GY at this locus and a decrease of GY corresponded to a decrease of ENO. One other stable QTL for GY under low N environments located on chromosome 4 (128 cM), and P₁, the drought tolerant parent, carried the favorable allele at this locus. Remarkably, this region on chromosome 4 was identified by the combined analysis for all traits measured in plants grown under low N environments except HKFW, suggesting a strong pleiotropic effect at this locus, where the favorable allele for P₁ increased GY, KNO, CHLO, ENO, and PHT and decreased ASI.

Individual QTL results for chromosome 4 are presented in Fig. 2 and confirm the results obtained from the combined analysis as indicated by the number of red (significant, LOD above 3.0) and yellow (tendency, LOD above 2.5) spots identified on the QTL strips. No significant QTL were identified under HNw between 100 and 140 cM on chromosome 4, suggesting that this

genomic region clusters genes specifically involved in the regulation of plant development under low N conditions.

Two additional stable QTLs for GY in low N environments were identified on chromosomes 2 (101 cM) and 8 (136 cM). The first one overlapped with a QTL significant for KNO in LNw, and the second colocalized with significant QTL identified under low N for KNO, ASI, and ENO. As expected, the favorable allele for GY was related to shorter ASI and increased ENO and KNO.

Three QTLs with significant QEI were identified for GY in plants under low N environments. Two QTLs on chromosomes 2 (18 cM) and 3 (53 cM) were only significant for plants under LNw, and one on chromosome 3 (188 cM) was only significant under LNd. The QTL on chromosome 2 (18 cM) overlapped with a QTL for ENO and the QTL on chromosome 3 (53 cM) mapped close to QTLs significant under LNw for KNO, HKFW, and ENO. It also corresponded to a QTL for ASI significant in LNd and with a QTL for PHT significant in both low N environments. This region was also identified for GY, KNO, and PHT in plants under HNw (Table 3), suggesting a stable expression, independent of N supply.

The trait with the most stable QTLs between the two low N environments was PHT. None of the five QTLs identified for PHT showed a significant QEI. Interestingly, two of them (on chromosomes 3 and 4) overlapped with QTLs for GY in low N environments. In addition, one QTL (on chromosome 6) corresponded to QTLs identified for PHT in plants under HNw.

Table 2 Significant QTLs (LOD > 3.5) identified in joint analysis or the different target traits considered in this study when evaluated under LNw and LNd conditions

Trait	Ch	cM	LOD score				Flanking markers	Interval ^a	A ^b	D ^b	R ²		
			Joint	LNw	LNd	QEI					LNw	LNd	
GY	1	67	3.5	1.8	2.3	0.13	csu111	umc11	49–82	-0.10	5.21	2.3	4.9
	2	18	4.4	4.0	0.1	2.91*	umc53a	umc6	0–55	-0.82	1.96	5.7	0.1
	2	101	4.5	2.4	2.5	0.90	umc8b	umc55a	85–115	0.23	-4.75	4.2	4.6
	3	53	6.4	6.4	0.2	2.48*	csu29b	npi114	27–83	-1.64	-2.39	8.0	0.5
	3	188	5.2	0.4	5.1	2.10*	umc39a	umc96	150–217	1.60	-0.14	0.5	6.8
	4	128	6.3	4.6	2.5	0.50	umc133a	umc15a	107–135	2.09	-1.09	6.0	4.2
	8	136	3.5	2.2	1.5	1.08	umc30a	umc150a	107–184	1.52	1.00	2.7	2.7
	9	64	5.1	1.5	4.1	1.09	bnl3.06	umc114	29–83	-1.70	2.18	1.8	8.1
												31.7	26.9
KNO	2	103	3.8	2.4	0.9	3.59*	umc8b	umc55a	81–118	-0.55	-5.64	4.2	2.2
	3	54	4.5	4.2	0.1	3.11*	npi114	umc10	27–99	-4.65	-4.98	5.7	0.1
	4	128	4.8	4.8	0.1	1.87*	umc133a	umc15a	109–137	7.15	-9.12	5.2	0.7
	8	153	5.0	4.5	0.4	2.82*	umc65c	csu38b	108–176	6.75	-8.45	7.8	0.6
											22.4	4.4	
HKFW ASI	3	54	4.2	4.1	0.2	2.63*	npi114	umc10	25–99	-0.51	-0.66	7.5	0.3
	1	209	5.6	1.6	5.2	0.71	umc174b	umc65b	196–237	0.71	-0.33	2.5	7.5
	3	65	5.3	0.2	4.4	3.26*	umc10	csu30	25–97	-0.43	0.01	1.1	7.4
	4	117	4.1	2.1	0.7	3.85*	umc104a	umc133a	94–133	-0.14	-0.24	4.1	1.3
	6	67	3.6	1.8	2.6	0.19	csu111b	csu116a	33–85	-0.60	-0.47	4.6	5.3
	6	116	6.1	1.1	3.2	5.31*	bnl8.05b	umc132a	91–140	-0.23	-0.49	3.4	8.6
	7	88	5.3	0.0	5.1	2.68*	bnl8.39	csu16c	73–111	0.44	-0.49	0.2	7.8
	8	70	5.7	0.1	5.5	2.55*	umc91b	umc152b	52–92	-0.51	-0.86	0.2	10.6
	8	134	6.7	4.1	0.6	6.41*	umc30a	umc150a	107–157	-0.19	-0.15	9.6	1.0
	10	46	6.8	0.8	6.1	2.81*	npi223b	umc64	34–87	-0.55	-0.39	1.1	7.7
											22.0	40.1	
CHLO	1	138	5.9	5.4	0.6	2.45*	umc167	csu61	117–175	-1.11	-0.27	10.6	1.1
	4	81	6.0	4.9	2.8	0.37	umc156a	csu26b	44–100	-1.37	-1.27	10.2	4.7
	4	133	4.9	4.3	0.5	2.25*	umc15a	csu20b	117–154	0.88	-0.63	6.2	0.7
	7	0	3.9	1.6	2.7	1.56*	csu13	csu34a	0–11	0.80	1.58	2.8	5.3
											23.8	10.5	
ENO	1	67	3.8	1.6	2.8	0.11	csu111	umc11	49–84	0.00	0.13	1.9	8.0
	2	12	3.8	2.8	1.4	1.26	npi239	umc53a	0–42	-0.03	0.01	2.9	3.4
	3	53	6.9	6.5	1.0	3.57*	csu29b	npi114	41–91	-0.03	-0.06	6.5	1.2
	4	125	5.7	5.4	0.4	3.66*	umc104a	umc133a	93–139	0.03	-0.01	7.2	1.3
	5	148	3.7	2.0	2.2	0.76	umc68	umc104b	123–174	-0.03	0.03	2.9	4.1
	6	97	6.3	6.2	0.0	4.68*	csu60	umc38a	71–136	-0.03	0.00	7.9	1.1
	7	115	3.8	3.7	0.2	2.41*	umc91a	umc35	86–115	0.02	0.01	3.5	0.7
	8	149	10.7	10.6	0.3	7.75*	umc150a	umc65c	128–170	0.04	0.02	12.4	1.0
	10	46	6.3	1.2	5.1	2.21*	npi223b	umc64	14–53	-0.01	0.11	1.4	9.1
												46.1	22.0
PHT	3	70	9.2	5.3	6.7	0.98	csu30	csu134d	22–93	-3.86	8.22	10.6	10.4
	4	137	3.6	0.6	3.5	0.16	csu20b	csu39	102–168	2.82	-0.49	0.6	4.4
	6	118	3.5	0.6	3.2	0.49	bnl8.05b	umc132a	71–148	2.80	0.84	1.7	5.5
	9	56	8.1	2.5	7.4	0.15	umc105a	bnl3.06	25–69	-4.11	4.31	4.5	9.5
	10	79	4.3	1.6	3.2	0.99	csu86	umc182	65–123	-3.25	-0.80	4.6	5.6
											15.8	28.7	

Table 2 continued

Trait	Ch	cM	LOD score				Flanking markers	Interval ^a	A ^b	D ^b	R ²	
			Joint	LNw	LNd	QEI					LNw	LNd
MFLW	1	177	4.7	3.5	3.8	0.81	umc33a umc83a	158–195	0.88	– 0.40	5.0	6.4
	2	170	4.5	0.3	4.1	0.68	umc150b csu64a	143–194	0.97	– 0.30	0.6	12.0
	4	83	7.0	6.7	3.7	3.32*	umc156a csu26b	53–100	0.85	– 0.09	10.2	7.1
	6	71	3.7	2.3	0.4	3.29*	csu111b csu116a	17–97	0.19	0.37	3.6	1.2
	8	101	10.6	8.2	0.2	10.28*	csu68c umc89	60–128	– 0.25	– 0.18	14.6	0.8
	9	89	6.1	4.5	0.0	5.98*	umc95 csu59	56–109	– 0.05	– 0.17	6.0	0.4
											25.8	28.8

The QTL characteristics include the chromosome (Ch), the position of the QTL peak in cM, the LOD score for the joint analysis, the individual and the QEI (* indicates a significant QEI at LOD > 1.30), the flanking markers and the QTL interval as well as the additive (A) and dominance (D) genetic effects at the QTL peak, and finally the percentage of phenotypic variance (R²) explained by the individual QTLs and considering all significant QTLs together

^a Positions around peak where LOD at peak dropped by 1/2

^b Additive (A) and dominance (D) effects are defined as the contribution of the allele of P₁

Table 3 Significant QTLs (LOD > 3.0) identified for the different target traits considered in this study when evaluated under HNw conditions

The QTL characteristics include the chromosome (Ch), the position of the QTL peak in cM, the LOD score for the single analysis, the flanking markers and the QTL interval as well as the additive (A) and dominance (D) genetic effects at the QTL peak, and finally the percentage of phenotypic variance (R²) explained by the individual QTLs and considering all significant QTLs together

^a Positions around peak where LOD at peak dropped by 1/2

^b Additive (A) and dominance (D) effects are defined as the contribution of the allele of P₁

Trait	Ch	cM	LOD score	Flanking markers	Interval (cM) ^a	A ^b	D ^b	R ²
GY	1	95	4.7	umc185 umc26b	65–128	6.33	5.72	10.2
	3	39	4.8	csu16 umc50	20–73	– 6.25	2.93	8.5
	10	63	10.1	umc64 csu86	44–93	– 8.85	3.02	17.8
KNO	3	75	3.5	csu134d bnl8.01	41–99	– 17.4	34.5	9.3
	10	61	8.0	umc64 csu86	34–91	– 27.8	31.0	17.3
HKFW	1	97	5.4	umc185 umc26b	76–125	1.72	2.80	14.7
	1	166	3.7	umc119 umc33a	141–191	1.45	– 0.88	7.5
	8	18	3.0	npi114a umc91b	0–40	– 1.22	5.40	18.9
	8	138	3.1	umc30a umc150a	114–155	– 0.82	2.66	5.7
	10	67	5.7	csu86 umc182	50–97	– 1.61	– 0.12	9.4
ASI	1	211	5.5	umc174b umc65b	196–231	0.40	– 0.21	8.8
	6	71	7.6	csu111b csu116a	45–95	– 0.49	– 0.21	15.6
	7	92	4.1	csu16c umc91a	44–111	0.31	– 0.13	6.4
	10	44	3.7	csu30b npi223b	19–63	– 0.31	– 0.03	5.7
CHLO	1	185	3.5	umc33a umc83a	166–215	– 1.29	2.13	5.7
	4	77	13.9	umc156a csu26b	51–98	– 2.69	– 0.39	21.3
	6	63	5.3	csu111b csu116a	17–85	1.71	1.02	9.3
	9	89	4.2	umc95 csu59	60–101	1.41	0.97	6.2
	10	75	6.9	csu86 umc182	34–95	– 2.04	1.81	12.6
ENO	10	65	3.9	umc64 csu86	48–91	– 0.04	0.06	7.8
PHT	4	59	3.8	bnl5.46 bnl5.71b	36–73	2.77	17.65	10.3
	6	120	3.7	bnl8.05b umc132a	89–152	5.24	– 0.73	6.1
	7	69	7.6	umc149 bnl15.07b	39–79	– 7.16	1.74	12.4
	8	90	3.6	bnl10.39 csu68c	30–108	– 5.25	11.56	10.3
MFLW	9	60	5.9	bnl3.06 umc114	31–83	– 6.21	3.72	9.3
								34.0
MFLW	4	73	5.7	bnl5.71b umc156a	49–96	0.76	– 0.30	12.4

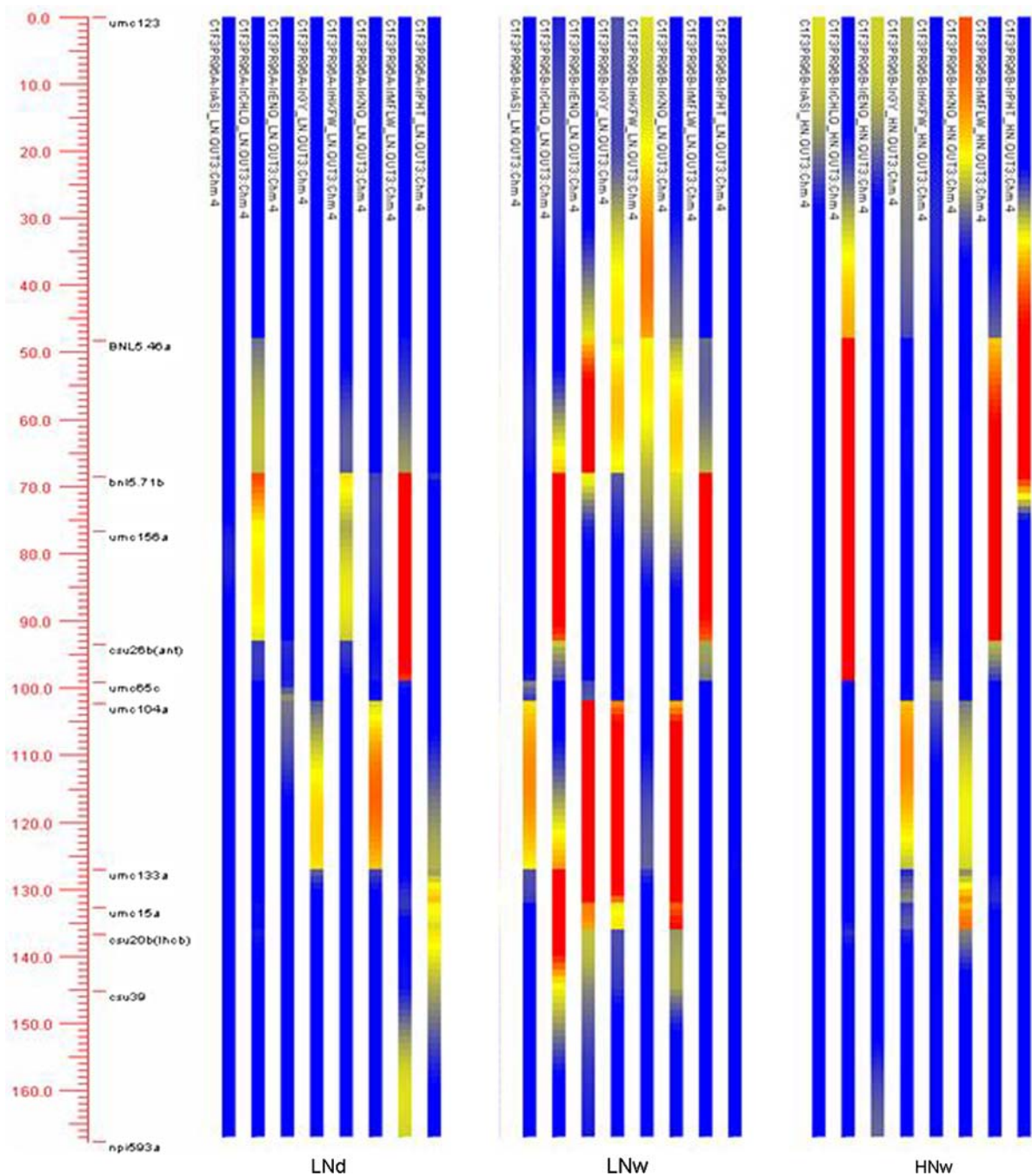


Fig. 2 Chromosome 4 with complete QTL raw data for the eight morphological traits (ASI, CHLO, ENO, GY, HKFW, KNO, MFLW AND PHT) reported in this paper under LNd, LNw and HNw experimental conditions. The

input file for each trait is associated to a LR score per cM, and the colors range from blue (low LR score) to red (high and significant LR score)

In contrast, QTLs for KNO, ASI, and ENO were mostly not stable across the two low N environments. QTLs for ASI were mainly significant for

plants grown in LNd, while QTLs for KNO and ENO were mainly significant for plants grown in LNw. However, QTLs for ASI and ENO showed

remarkable overlaps on chromosomes 3, 4, 6, 8, and 10. Those results suggest a similar genetic control of both traits. However, the direction of additivity of ASI and ENO was not consistent across all significant loci. On chromosome 3 (25–97 cM), P_1 carried a favorable allele for ASI (shorter ASI), but contributed to a decrease in ENO, and finally had a negative effect on GY. This might be due to opposite effects of the same genes on ASI and ENO or to the presence of linked loci controlling ASI and ENO independently. All QTLs for ASI together accounted for a total of 40.1%, 22.0%, and 29.7% of the phenotypic variance under LNd, LNw, and HNw, respectively, and all QTLs for ENO expressed 22.0%, 46.1%, and 7.8% of the total phenotypic variance.

For plants grown under HNw conditions (Table 3), a QTL detected for all traits except PHT and MFLW was present on chromosome 10 around 60 cM. This QTL was a major one for GY and KNO in this environment, as it was identified at a LOD score of 10.1 and 8.0 for GY and KNO, respectively. This locus expressed 17% of the phenotypic variance for both traits. The favorable allele for GY at this locus, carried by P_2 , contributed to an increase in GY, KNO, HKFW, CHLO, ENO, and also ASI. This region was also identified under low N environments for ASI, ENO, and PHT. In contrast, the two other loci involved in the control of GY in HNw on chromosomes 1 and 3 (Table 3) were also detected for GY under low N environments (Table 2), suggesting that the genetic control of GY was partially independent of N supply. Similarly, the QTLs identified for KNO in plants grown under HNw corresponded to QTLs identified for GY in this environment. In general reduced colocalization was observed among significant QTLs identified for the different traits, except for the locus on chromosome 10 mentioned above. Also, there were only few correspondences with the QTLs identified for these traits under low N environments (Table 2), suggesting distinct genetic controls in low and high N environments.

Discussion

The reductions of GY under low N conditions were mainly related to reductions in the number

of kernels per plant (KNO), while hundred kernels fresh weight (HKFW), reflecting the grain size, was hardly affected (Fig. 1), confirming the results of Hirel et al. (2001). According to Gallais and Hirel (2004), the reduction of KNO under low N conditions, attributed to ovule abortion, is the result of a limitation in the source of photosynthetic products. The observation that all QTLs identified for KNO matched with QTLs identified for GY in low N environments (Table 2) confirmed, at the genetic level, the tight relation between GY and KNO under those conditions. While a single QTL was identified for HKFW under low N environments, GY was correlated to HKFW under high N conditions, and the two traits shared two QTLs in common, including a major one on chromosome 10. Those results indicate that assimilates and N supply, contributing to grain development and therefore GY, are more important factors under high N than under low N conditions. The relatively large difference in GY between LNw and LNd conditions is mainly due to differences in other environmental factors affecting plant development. The lower mean temperature and shorter days in the dry season compared to the wet season (Lafitte and Edmeades 1994b) explained most of the difference in plant precocity observed between the two growing seasons (about 40 days). Additionally, in our experiments, the fact that plants grown in the dry season had to be irrigated while plants grown in the wet season were mostly grown under rainfed conditions should also have influenced their performance.

ASI and ENO, which were significantly correlated with GY, showed remarkable QTL correspondences with GY under low N conditions, confirming the common genetic basis between GY, ASI and ENO reported in previous studies (Lafitte and Edmeades 1994a; Bänziger and Lafitte 1997). According to Gallais and Hirel (2004), the genetic control of the performance under low N supply is related to genes encoding for enzymes of N metabolism (in particular, glutamine synthetase). However, we found little correspondences with the QTLs identified by these authors. This might be related to the fact that yield reduction under low N was much more pronounced in our experiments. In contrast, we

obtained good QTL correspondences with those previously identified by Agrama et al. (1999). The QTLs for GY under low N (Table 2) on chromosomes 1 (67 cM), 2 (18 cM), and 9 (64cM) mapped in the same bins as three QTLs identified for GY under low N by Agrama et al. (1999), near the markers *bnl9.13*, *bnl12.06*, and *umc20*, respectively. This may indicate that, while N metabolism is important for the genetic control of maize performance under moderate N stress, other factors may become more important under severe N stress. Although ASI and ENO had several QTLs in common, most QTLs for ASI were significant only in LNd, while most of those identified for ENO were significant only in LNw, suggesting that common loci can control both traits independently and that this control depends on factors other than N supply. Since shorter ASI was mainly related to earlier silk emergence, it can be hypothesized that the common loci for ASI and ENO contain genes involved in the initiation and development of female flowers, their action being modulated by other factors such as hormonal status and carbohydrate and N supply.

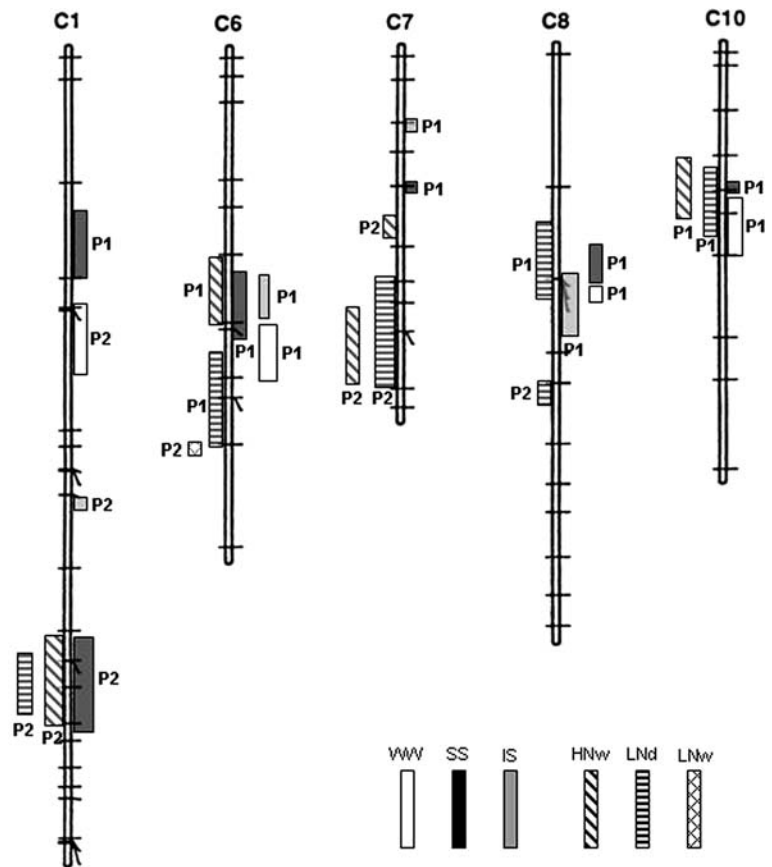
The different genetic control of the expression of the traits presented in this study suggest that selection for low N tolerance will be more efficient under low N conditions, as already postulated by Bänziger and Lafitte (1997). The sign of the QTL additive effects identified for the different traits across low and high N supply was identical, suggesting that selecting for these traits under low N conditions should not have negative effects under high N conditions, as reported by Laffite and Edmeades (1994a). The good QTL correspondences observed between GY and ASI or ENO under low N indicate that ASI and ENO are pertinent secondary traits for the improvement of low N tolerance in maize. The fact that five of eight loci involved in the control of GY were identified for ASI or ENO (or both), and that the two loci with favorable alleles for both ASI and ENO under low N (on chromosomes 4 and 8) corresponded to two QTLs for GY presenting the largest genetic effects, fully supports this view. However, genetic regulation of both ASI and ENO differed between the two types of growing seasons and opposite allelic

effects for those two traits were also observed at some loci. Therefore, different selection indices need to be used depending on the experimental conditions.

In contrast to ASI and ENO, the QTLs for CHLO under low N conditions showed little correspondence with those identified for GY. The genetic regulation for CHLO seems to be pretty different between LNd and LNw, as demonstrated by the diverse set of QTLs identified for both environments, suggesting different adaptive mechanisms for low N conditions in wet and dry seasons. This result disagrees with the general opinion that chlorophyll content is a good indicator of low N tolerance, but confirms the absence of genetic correlation between chlorophyll content and GY observed in maize by Laffite and Edmeades (1994a) under low N conditions. PHT, which reflects plant vigor and perhaps indirectly root system development, showed a significant and stable correlation with GY across environments (about 0.5). QTL for PHT colocalized with GY, and all QTLs for PHT presented a reduced QEI. Therefore, PHT should be included in the SI as an important trait for plant performance per se, including under low N.

The QTL results obtained in this study were compared to those obtained under water-limited conditions by Ribaut et al. (1996, 1997), using the same segregating population. Although the two experiments were conducted under different types of stress (drought vs. low N), at different locations (Tlaltizapan vs. Poza Rica), and during different years (1994 vs. 1996), the correlations obtained between ASI measured under LNd and under intermediate and severe drought stress conditions (dry winter season) were relatively high (0.50 and 0.58, respectively). Those correlations were much higher than those calculated for ASI between LNd and LNw seasons (0.13). As presented in Fig. 3, the significant correlation between ASI measured under drought and LNd conditions was validated by the identification of four common QTLs located on chromosomes 1, 6, 8, and 10. Additionally, at all four loci, the genetic contribution was consistent across the two parental lines as the allele contributing to a decrease of ASI under drought also shortened ASI under low N. As observed under drought conditions, the

Fig. 3 Positions of QTLs identified in single environment analysis for ASI under well watered conditions (WW), intermediate (IS) and severe (SS) drought stress, high nitrogen (HN) and low nitrogen (LN) fertilization in the dry (d) and in the wet (w) seasons



increase of ASI in LNd was induced by a delay in silking rather than a change in anthesis.

Three of the QTLs identified for ENO under low N, located on chromosomes 1 (67 cM), 3 (53 cM), and 6 (97 cM) (Table 2), also corresponded to QTLs identified for ENO under drought stress (Ribaut et al. 1997). The identification of common genomic regions for the regulation of ASI and ENO under drought and LNd conditions has important implications for breeding maize for drought prone environments (Eeuwijk et al. 2002). Many drought areas are located in developing countries, where, for economical reasons, N supply is insufficient. Based on phenotypic data, Bänziger et al. (2002) and Monneveux et al. (2005b) suggested that selection for tolerance to mid-season drought stress lead to morphological and physiological changes that increase yield under N deficiency. Our QTL data confirm this hypothesis as demonstrated by the QTL colocalization observed across water and N

deficiency stresses. The common genetic basis in the genetic control of ASI and ENO under drought and under low N stresses brings the interesting question of the nature of genes at those genomic regions. Different hypothesis can be proposed to explain some common response to low N and drought. First, under both stresses, the performance of photosynthesis is likely to be strongly reduced. Therefore, the common genetic control of ASI might be related to genes involved in the capacity of the plants to maintain sufficient rates of photosynthesis under stress conditions (Bänziger et al., 2002). A major locus has been identified for the tolerance of photosynthesis to low temperature on chromosome 3 in a RIL population derived from the F_{2:3} population used in the present study (Fracheboud et al. 2002), suggesting the presence of a large genetic variability in the tolerance of the photosynthetic apparatus to stress conditions in this population. However, the response of the photosynthetic

apparatus has not been investigated yet under low N and drought stress in this material.

Another explanation is that the genes at the common loci are involved in the supply of photosynthetic products or in the translocation of assimilates to the high energy-demanding growing flower organs. Bänziger et al. (2002) suggested that constitutive changes enabled drought tolerant selections to also partition more assimilates to the young ear under N stress. Monneveux et al. (2005b) postulated that increase in yield and ears per plant under low N associated with selection for drought was driven more by changes in dry matter partitioning than by changes in biomass production or senescence.

A third hypothesis for the existence of QTLs common to drought and low N situations is the fact that tolerance to both stresses could involve common key enzymes. It has been observed, for example, that low water potential during pollination disrupts carbohydrate metabolism by inhibiting the activity of acidic invertase in maize ovaries, leading to the accumulation of sucrose and to kernel abortion (Westgate and Boyer 1986; Zinselmeier et al. 1995). In this context it is interesting to note that QTLs identified on chromosome 2 around 12–18 cM for GY and ENO under low N conditions (Table 2) mapped very close to the *Ivr1* gene on the map IBM2 2004 neighbors 2 (<http://www.maizegdb.org>), coding for invertase 1, known to be induced by sugar depletion (Xu et al. 1995).

Finally, a fourth possibility is a genetic variation of the structure and efficiency of the root system. In theory, a more developed root system may enable the plant to access water and N at a greater depth. Selection for GY in low N environments was accompanied by a larger increase in root biomass than shoot biomass (Lafitte and Edmeades 1994b). It is still not clear, however, if deeper roots really confer higher drought tolerance in maize. Studies of water extraction patterns by maize show that little water is removed below 70 cm soil depth (Mugo et al. 1998) and suggest that deep or extensive root systems do not represent a clear advantage under drought in maize. Moreover, increased root growth comes at a carbon cost to the plant, perhaps at a time when C flux to reproductive

organs is already limiting sink size (Lemcoff and Loomis 1994).

In conclusion, this paper demonstrates that the genetic basis for low N tolerance differed depending on the target environment and that to apply similar selection across growing season at the same location might be counterproductive. To be most efficient, the selection index must be consequently adjusted depending on climatic conditions, with more emphasis on ENO in the wet season and ASI in the dry season. The common genetic basis observed between ASI, and to a lesser extent ENO, measured under drought and LNd conditions in the same segregating population contributed to explain the increased N stress tolerance of tropical maize selected for drought tolerance. Alternate selection under low N and water-limited selection across plant cycles represents an attractive option to make genetic gains for both types of stress under marginal environments.

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References

- Agrama HAS, Moussa ME (1996) Mapping QTLs in breeding for drought tolerance in maize (*Zea mays* L.). *Euphytica* 91:89–97
- Agrama HAS, Zakaria AG, Said FB, Tuinstra M (1999) Identification of quantitative traits loci for nitrogen use efficiency in maize. *Mol Breed* 5:187–195
- Bänziger M, Betran FJ, Lafitte HR (1997) Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. *Crop Sci* 37:556–561
- Bänziger M, Lafitte HR (1997) Efficiency of secondary traits for improving maize for low-nitrogen target environments. *Crop Sci* 37:1110–1117
- Bänziger M, Edmeades GO, Lafitte HR (1999) Selection for drought tolerance increases maize yields across a range of nitrogen levels. *Crop Sci* 39:1035–1040
- Bänziger M, Edmeades GO, Lafitte HR (2002) Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. *Field Crop Res* 75:223–233
- Basten CJ, Weir BS, Zeng ZB (2002) QTL cartographer, a reference manual and tutorial for QTL mapping. Department of Statistics North Carolina State University, Raleigh, 177 pp.
- Bell MA, van Keulen H (1995) Soil pedotransfer functions for four Mexican soils. *Soil Sci Soc Am J* 59:865–871

- Burgueño J, Cadena A, Crossa J, Banziger M, Gilmour AR, Cullis BR (2000) User's guide for spatial analysis of field variety trials using ASRML. CIMMYT, México
- Dwyer LM, Tollenaar M, Houwing L (1991) A non-destructive method to monitor leaf greenness in corn. *Can J Plant Sci* 71:505–509
- Fracheboud Y, Ribaut J-M, Vargas M, Messmer R, Stamp P (2002) Identification of quantitative trait loci for cold-tolerance of photosynthesis in maize (*Zea mays* L.). *J Exp Bot* 53:1967–1977
- Gallais A, Hirel B (2004) An approach to the genetics of nitrogen use efficiency in maize. *J Exp Bot* 55:295–306
- Gleeson AC (1997) Spatial analysis. In: Kempton RA, Fox PN (eds) *Statistical methods for plant variety evaluation*. Chapman & Hall, London, pp 68–85
- Grunau JA, Swiader J-M (1989) Ion chromatography in nutrient depletion studies: measurements of very low nitrate concentrations. *Commun Soil SciPlant Anal* 20:383–402
- Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy Y, Cadiou S, Retailiau C, Falque M, Gallais A (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol* 125:1258–1270
- Jacobs BC, Pearson CJ (1991) Potential yield of maize determined by rates of growth and development of ears. *Field Crop Res* 27:281–298
- Jiang C, Zeng Z (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* 140:1111–1127
- Lafitte R, Blum A, Atlin G (2003) Using secondary traits to help identify drought-tolerant genotypes. In: Fischer KS, Lafitte R, Fukai S, Atlin G, Hardy B (eds) *Breeding rice for drought-prone environments*. IRRI, Los Baños, Philippines, pp 37–48
- Lafitte HR, Edmeades GO (1994a) Improvement for tolerance to low soil nitrogen in tropical maize. I. Selection criteria. *Field Crop Res* 39:1–14
- Lafitte HR, Edmeades GO (1994b) Improvement for tolerance to low soil nitrogen in tropical maize. III. Variation in yield across environments. *Field Crop Res* 39:27–38
- Lemcoff JH, Loomis RS (1986) Nitrogen influence on yield determination in maize. *Crop Sci* 26:1017–1022
- Lemcoff JH, Loomis RS (1994) Nitrogen and density influences on silk emergence, endosperm development, and grain yield in maize (*Zea mays* L.). *Field Crop Res* 38:63–72
- Limami AM, de Vienne D (2001) Natural genetic variability in nitrogen metabolism. In: Lea PJ, Morot-Gaudry JF (eds) *Plant nitrogen*. Springer-INRA Editions, 407 pp
- McCown RL, Keating BA, Probert ME, Jones RK (1992) Strategies for sustainable crop production in semi-arid Africa. *Outlook Agric* 21:21–31
- Moll RH, Jackson WA, Mikkelsen RL (1994) Recurrent selection for maize grain yield: dry matter and nitrogen accumulation and partitioning changes. *Crop Sci* 34:874–881
- Monneveux P, Ribaut J-M (2006) Secondary traits for drought tolerance improvement in cereals. In: Ribaut JM (ed) *Drought tolerance in cereals*. The Haworth Press Inc., Binghamtown, NY, USA, pp 97–144
- Monneveux P, Zaidi PH, Sanchez C (2005a) High density and low nitrogen affects yield and associated traits in tropical maize. *Crop Sci* 45:535–545
- Monneveux P, Sanchez C, Beck D, Edmeades GO (2005b) Drought tolerance improvement in tropical maize source populations: evidence of progress. *Crop Sci* 45:180–191
- Morris M, Dreher K, Ribaut J-M, Khairallah M (2003) Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. *Mol Breed* 11:235–247
- Mugo SN, Smith ME, Bänziger M, Setter TL, Edmeades GO, Elings A (1998) Performance of early maturing Katumani and Kito maize composites under drought at the seedling and flowering stages. *Afr Crop Sci J* 6:329–344
- Oikeh SO, Horst WJ (2001) Agro-physiological responses of tropical maize cultivars to nitrogen fertilization in the moist savanna of West Africa. In: Horst WJ (ed) *Plant nutrition—food security and sustainability of agro-ecosystems*. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 804–805
- Pearson CJ, Jacob BC (1987) Yield components and nitrogen partitioning of maize in response to nitrogen before and after anthesis. *Aust J Agric Res* 38:1001–1009
- Pingali PL, Pandey S (2001) Meeting world maize needs: technological opportunities and priorities for the public sector. In: Pingali PL (eds) *1999/2000 world maize facts and trends*. CIMMYT, Mexico, pp 1–24
- Prioul JL, Quarrie S, Causse M, de Vienne D (1997) Dissecting complex physiological functions into elementary components through the use of molecular quantitative genetics. *J Exp Bot* 48:1151–1163
- R Development Core Team (2005) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>
- Raison RJ, Connell MJ, Khanna PK (1987) Methodology for studying fluxes of soil mineral-N in situ. *Soil Biol Biochem* 19:521–530
- Reiter RS, Coors JG, Sussman MR, Gabelman WH (1991) Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphism. *Theor Appl Genet* 82:561–568
- Ribaut J-M, Hoisington DH, Deutsch JA, Jiang C, Gonzalez-de-Leon D (1996) Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theor Appl Genet* 92:905–914
- Ribaut J-M, Hoisington DH, Deutsch JA, Jiang C, Gonzalez-de-Leon D (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theor Appl Genet* 94:887–896
- Ribaut J-M, Ragot M (2007) Marker-assisted selection to improve drought adaptation in maize: the backcross

- approach, perspectives, limitations, and alternatives. *J Exp Bot* 58:351–360
- Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S (2002) Mapping QTLs regulating morpho-physiological traits and yield in drought-stressed maize: case studies, shortcomings and perspectives. *Ann Bot* 89:941–963
- Uhart SA, Andrade FH (1995a) Nitrogen deficiency in maize. I. Effects on crop growth, development, dry matter partitioning, and kernel set. *Crop Sci* 35:1376–1383
- Uhart SA, Andrade FH (1995b) Nitrogen deficiency in maize. II. Carbon-nitrogen interaction effects on kernel number and grain yield. *Crop Sci* 35:1384–1389
- Van Eeuwijk FA, Crossa J, Vargas M, Ribaut J-M (2002) Analysing QTL by environment interaction by factorial regression, with an application to the CIMMYT drought and low nitrogen stress programme in maize. In: Kang MS (ed) *Quantitative genetics, genomics, and plant breeding*. CABI Publishing, Wallingford, UK, pp 245–256
- Westgate ME, Boyer JS (1986) Reproduction at low silk and pollen water potentials in maize. *Crop Sci* 26:951–956
- Xu J, Pemberton GH, Almira EC, McCarty DR, Koch KE (1995) The *Ivr 1* gene for invertase in maize. *Plant Physiol* 108:1293–1294
- Zinselmeier C, Westgate ME, Schussler JR, Jones RJ (1995) Low water potential disrupts carbohydrate metabolism in maize (*Zea mays* L.) ovaries. *Plant Physiol* 107:385–391