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RESEARCH

Identification of Quantitative Trait Loci for Grain Yield and Other Traits in Tropical Maize Under High and Low Soil-Nitrogen Environments

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ABSTRACT

Low soil Nitrogen (low-N) is one of the most important abiotic stressors responsible for significant yield losses in maize (*Zea mays* L.). The development and commercialization of low-N–tolerant genotypes can contribute to improved food security in developing countries. However, selection for low-N tolerance is difficult because it is a complex trait with strong interaction between genotypes and environments. Marker-assisted breeding holds great promise for improving such complex traits more efficiently and in less time, but requires markers associated with the trait of interest. In this study, 150 BC_2F_1 families of CML 444 × CML 494 were evaluated at two locations for two consecutive seasons to identify SNP markers associated with quantitative trait loci (QTL) for yield and other agronomic traits under low- and high-N environments. A total of 13 QTL were identified with 158 SNP markers, of which nine and four QTL were detected under low- and high-N environments, respectively. Five QTL one each for grain yield (*qgy*-1), days to silking (*qdts*-1) and anthesis- silking interval (*qasi*-6), and two for stay green characteristic (*qsg*-1 and *qsg*-4) were close to their adjacent markers, with an interval of 0.7 to 5.2 cM between them and explained phenotypic variance of 9 to 21%. These QTL would be invaluable for rapid introgression of genomic regions into maize populations using marker-assisted selection (MAS) approaches. However, further validation of these QTL is needed before use in MAS.

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Abbreviations: ASI, anthesis–silking interval; CIM, composite interval mapping; CSIR, Council for Scientific and Industrial Research; DTA, days to anthesis; DTS, days to silk; EHT, ear height; EPP, ears per plant; GEI, genotype by environment interaction; GY, grain yield; HN, High Nitrogen; h^2, Broad sense heritability; IITA, International Institute of Tropical Agriculture; LOD, logarithm of the odds; MAS, Marker assisted selection; P, plant aspect; PHT, Plant height; PVE, phenotypic variance explained; QTL, Quantitative trait locus; QTLs, Quantitative trait loci; SG, stay-green; SGC, stay-green characteristic.

The increase in crop yield during the past century is attributed to the selection of genotypes with higher yield potential and an increased amount of nutrients, particularly nitrogen (N) supplied during the growth cycle (Tuberosa et al., 2002). Available soil N is usually the critical factor limiting plant growth. Therefore, N fertilizer is usually applied to maize fields, resulting in marked increases in yield. Low N availability is a major cause of yield loss in maize in developing countries (Pingali and Pandey, 2001). This is because production is usually at N-deficient conditions due to limited availability of fertilizers or low purchasing power of farmers (Bänziger et al., 1997). Therefore, development of maize cultivars with tolerance to low N is the most effective and sustainable approach to mitigate the problem of low N.

Progress in selecting for low N tolerance is limited by large genotype × season and genotype × location interactions. The

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efficiency of selection for yield in low N environments may be improved by selecting secondary traits with high correlations to grain yield under low N (Bänziger and Lafitte, 1997; Badu-Apraku 2011, 2012). Selection indices based on these traits have been developed and have improved the selection efficiency under stress conditions significantly (Bänziger and Lafitte, 1997). The complexity of measuring the secondary traits quickly and accurately, however, has limited their use in breeding programs (Monneveux and Ribaut, 2006).

The introduction of molecular marker technology and the construction of saturated linkage maps have facilitated the detection of the genetic loci associated with complex traits (Kang et al., 1998; Li et al., 1995; Song et al., 2001). Genetic linkage maps and quantitative trait loci (QTL) mapping technology have improved the efficiency of estimating the number of loci controlling genetic variation in a segregating population and the characterization of the map positions in the genome (Xiao et al., 1996). In maize, genetic analysis of complex traits under abiotic stresses has focused mainly on drought tolerance (Agrama and Moussa, 1996; Ribaut et al., 1996: 1997; Tuberosa et al., 2002). Less attention has been paid to understanding of the genetic responses of segregating populations under soil nutrient deficiencies, such as low phosphorus (Reiter et al., 1991) or low N (Agrama et al., 1999; Hirel et al., 2001).

The use of marker-assisted selection (MAS) could be a very effective strategy for breeding for tolerance to low N (Zhou, 2010). However, the effectiveness of MAS depends on the precise locations of the QTL and the identification of tightly-linked molecular markers, which are cost effective and easier to use.

The QTL identified in breeding populations could be used directly for crop improvement through MAS approaches (Würschum, 2012; Wang et al., 2012). The objective of this study was to identify QTL associated with yield and yield related traits under low- and high-N environments.

MATERIALS AND METHODS

Mapping Population

The two parental lines used in the present study differed for their responses to low N stress; CML 494 (highly susceptible to low-N) and CML 444 (tolerant to low-N). These parental lines were selected based on their performance in multilocation trials conducted under low-N in Ghana. The F_1 crosses were made between the inbreds at the CSIR-Crops Research Institute, (Fumesua, Ghana) during the major cropping season of 2013. The F_1s were backcrossed to CML 494 during the minor cropping season of 2013 in Kwadaso, Ghana to obtain the BC_1F_1s. This was followed by another cycle of backcrossing of BC_1F_1s to CML 494 in Fumesua to obtain 150 BC_2F_1 families.

Field Experiments

The 150 BC_2F_1 families, along with the parental lines and the F_1 hybrids and the check (ENT 70), were evaluated under low- and high-N environments during the major (April–July) and minor (September–December) rainy seasons of 2014, at Fumesua (6°41′ N lat., 1°28′ W long.) and Ejura (7°23′ N lat., 1°21′ W long.) in Ghana. A 11 × 14 lattice design with two replications was used for the evaluations at the two locations during the two planting seasons. Single-row plots, each five meters long, spaced 0.75 m apart with 0.5-m spacing between plants in each row were used in all the environments. Three seeds were planted in each hole and thinned to two plants per hill at two weeks after emergence to give a population density of 53,333 plants per hectare. The low-N plots received 30 kg N ha^{-1} while the high-N plots received 90 kg N ha^{-1} applied in two splits at two and five weeks after planting. The low-N field had been previously depleted of N by growing maize and removing all plant material. Soil analysis was performed at the Soil Research Institute, Kumasi, Ghana. The total N in the soils was determined by Kjeldahl digestion and colorimetric determination on Technicon AAII Autoanalyser (Bremner and Mulvaney, 1982). Information on the soil properties of the experimental fields used in this study is presented in Supplementary Table 1. Nutrient status, in accordance with interpretation of analyzed soils by Landon (1991), was generally low at both locations with N levels less than 0.2%. Fertilizers were applied to bring the total available N to 90 kg ha^{-1} for the high-N field and 30 kg ha^{-1} for the low-N field when the soil N was less than the target level. Both low-N and high-N fields received 60 kg P ha^{-1} as single superphosphate (P_20_5) and 60 kg K ha^{-1} as muriate of potash (K_2O). The trials were kept weed-free with the application of both preemergence and postemergence herbicides, primextra and paraquat each at five liters per hectare. Subsequently, hand weeding was used to supplement the chemical weed control.

Field Data Collection

Data were recorded on both low- and high-N plots for days to 50% anthesis (DA) and silking (DS) as the number of days from planting to when 50% of the plants in a plot had shed pollen and extruded silks, respectively. The anthesis–silking interval (ASI) was calculated as the difference between DS and DA. Plant height (PHT) was measured as the distance from the base of the plant to the height of the first tassel branch and ear height (EHT) as the distance to the node bearing the upper ear. Plant aspect (PA) was recorded on a scale of 1 to 5 based on the plant type, where 1 = excellent and 5 = poor. Husk cover was scored on a scale of 1 to 5, where 1 = husks tightly arranged and extended beyond the ear tip, and 5 = ear tips exposed. EASP was based on a scale of 1 to 5, where 1 = clean, uniform, large and well-filled ears, and

5 = ears with undesirable features. In addition, stay-green characteristic (SG) was recorded at 70 d after planting on a scale of 1 to 10, where 1 = almost all leaves still green and 10 = virtually all leaves dead (Badu-Apraku et al., 2015). Number of ears per plant (EPP) was computed by dividing the total number of ears harvested per plot by the number of plants in a plot at harvest. Harvested ears from each plot were shelled to determine the grain weight and the percentage grain moisture for the low N experiments. Grain yield (GY) in kg ha^{-1} was adjusted to 15% moisture and computed from the shelled grain weight. In the high-N plots, grain yield was computed based on 80% (800 g grain kg^{-1} ear weight) shelling percentage and adjusted to 15% moisture content.

Data Analysis

Phenotypic data were analyzed using SAS 9.0 (SAS Institute, 2011) with the GLM procedure. Pearson correlation coefficients were calculated between the traits, using the adjusted means of the BC_2F_1 families. Repeatability of the traits (Falconer and Mackay, 1996) under low- and high-N conditions were computed on genotypic-mean basis using the following formula:

$$R = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re}}$$

where σ_g^2 is the genotypic variance, σ_{ge}^2 is the genotype × environment, and σ_e^2 is the residual variance, e is the number of environments, and re is the number of replicates per environment.

Single Nucleotide Polymorphism Genotyping and Construction of Genetic Linkage Map and Quantitative Trait Loci Analysis

A total of 153 freeze-dried leaf (two-week–old) samples consisting of 150 BC_2F_1, two parental lines and the F_1 hybrid were sent to LGC Genomics for SNP genotyping. Details on the principle and procedure of the DNA assays are available at http://www.lgcgroup.com/our-science/genomics-solutions/#.WKgsBRrLfIU. The parental lines were genotyped with a set of 1250 SNP markers, for which KASP assays (Semagn et al., 2013), were designed at LGC Genomics Facility in London, UK. Theoretically, the 150 BC2F1 families used in the study had an eighth of the CML 444 genome in the genetic background of CML 494 with the expected genotypic frequency of 0.75 and 0.25 per marker locus for the allele of CML 494 in homozygous and heterozygous conditions, respectively. Segregation of marker loci was evaluated with a Chi-squared test. Markers that had insufficient linkage data were excluded and the final linkage map was constructed with 158 SNP markers using JoinMap4 (Van Ooijen, 2006). Markers were assigned to linkage groups at independence LOD (logarithm of the odds) values greater than 6.0 and threshold values that ranged from 2.0 to 20 with an interval of 1.0. A regression mapping algorithm was used to order the markers and Haldane's mapping function was used to transform estimates of recombination frequency to map distances in centimorgans (cM). The linkage groups from JointMap were rearranged into chromosomes according to their order on the reference map.

Quantitative trait loci mapping was done in R/qtl using a single–QTL model. Furthermore, composite interval mapping (CIM) was used to define QTL peak position and to estimate effects of the mapped loci and their constributions to the phenotypic variances. The thresholds of the QTL (LOD scores) were obtained at $p = 0.05$ by 1000 random permutations of the trait values. In addition, epistatic gene interactions for grain yield and other agronomic traits were determined under both low- and high-N environments using QTL Network v2.1 (Yang et al., 2008).

RESULTS

Evaluation of BC_2F_1 Population

In all environments, the target traits measured in the BC_2F_1 population followed normal distribution (Fig. 1 and 2). The combined analysis of variance showed significant mean squares of genotypes, environments and genotype by environment interaction (GEI) for GY, SG and EPP across low N environments. The few exceptions included the mean squares of genotypes for ASI and GEI for DTA (days to anthesis), DTS (days to silk), ASI, EHT and PHT across low-N conditions which did not reach significant levels (Table 1). Similarly, significant mean squares were observed for genotypes, environments and GEI of all measured traits across high-N environments except the genotype mean squares for EHT, and the GEI mean squares for DTA, ASI, EHT, PHT, EPP and SG (Table 2).

The repeatability estimates of the traits ranged from 8% for ears per plant to 48% for days to silking under low N, and 32% for ear height to 72% for plant height under high-N environments. High repeatability estimates (i.e., ≥ 0.60) were recorded for most of the traits under high-N environments. A total of 23 significant correlations were detected under each environment (Supplementary Table 2). The grain yield (GY) showed consistently significant and highly positive correlations with ASI, PHT, EHT and EPP, whereas negative correlation was found with DTS under both low- and high-N environments. Similarly, the trait EHT had significant negative correlations under low- and high-N environments. The associations of PHT with DTA, DTS and ASI were negative under both low- and high-N environments. Similarly, EHT had significant and negative associations with DTA and DTS under both environments. In contrast, significant and negative correlation was observed between EHT and ASI under high-N environments.

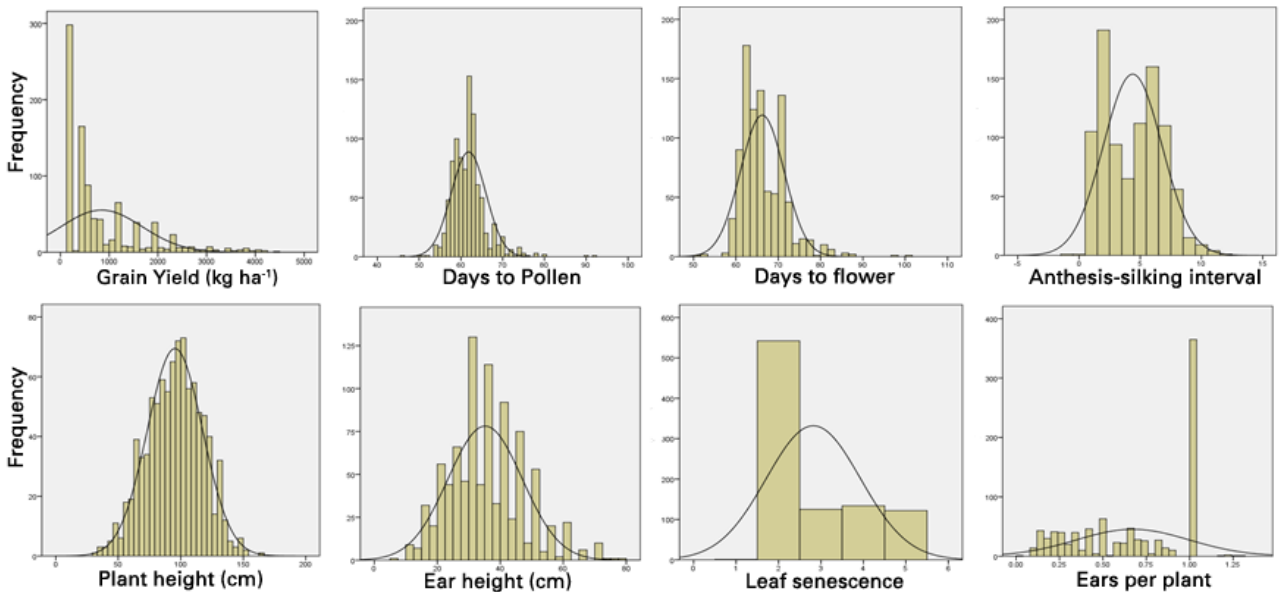

Fig. 1. Frequency distribution of eight traits in BC_2F_1 population under high-N environment.

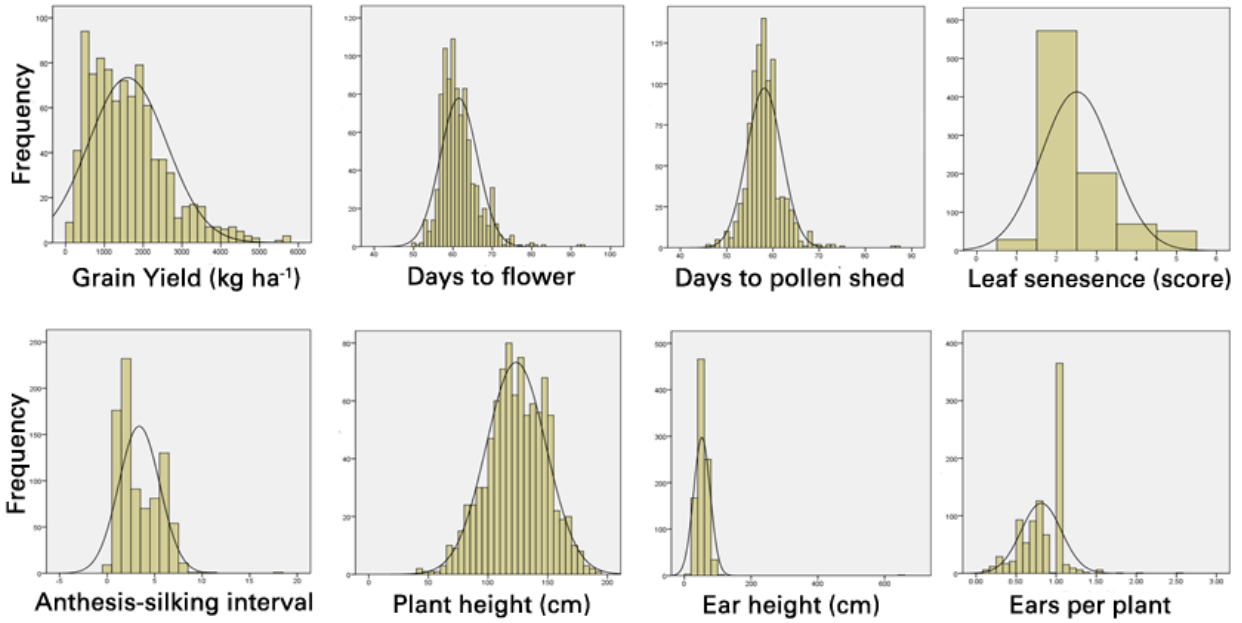

Fig. 2. Frequency distribution of eight traits in BC_2F_1 population under low-N environment.

Genetic Linkage Map Construction

Linkage analysis was performed on 150 BC_2F_1 families genotyped with 158 SNP markers. A linkage map was then constructed that corresponded to the ten chromosomes with length of 622.7cM and an average marker interval of 3.9 cM (Supplementary Table 3).

Quantitative Trait Loci Identification

A total of 13 QTL for all the traits were detected under both low- and high-N environments with the phenotypic variance explained (PVE) ranging from 5 to 31% (Table 3; Fig. 3). Of these QTL, four and nine were identified under high- and low-N environments, respectively. For GY, one QTL (*qgy-10–1)* with PVE of 10% was detected under high-N environment on chromosome 10 flanked by PZA01292_1 and PZB0049_1 markers at interval of 29.0 cM with LOD of 3.15. In contrast, two QTL were mapped for GY on Chromosomes 1 (*qgy-1*) and 10 (*qgy-10–2*) under low-N environment. Of the QTL, the major QTL, *qgy-1* accounted for 21% of PVE and was located between markers PZA02487_1 and PZB02058_1 with marker interval of 0.7cM. The QTL, *qgy-10–2* with PVE of 8% had a LOD score of 4.12. Interestingly, the QTL, *qgy-10–2* and *qgy-10–1* were flanked by the same markers, but their peak positions were different on the Chromosome 10. QTL *qgy-10–1* had a marker interval of 29.0 cM while *qgy*-10–2 had a marker interval of 0.7 cM.

Table 1. Mean squares of BC_2F_1 population evaluated across low-N environments§.

Source	DF	GY	DTA	DTS	ASI	EHT	PHT	SG	EPP
Envt	2	176548648.9**	55.54**	1805.59**	1314.29**	6653.92**	2933.90**	430.73**	30.93**
Blk (Rep×Envt)	78	552478.4**	30.94**	44.28**	4.50**	297.79**	1309.32**	0.67**	0.04**
Rep (Envt)	3	3745158.4**	675.63**	762.35**	13.30**	178.09ns	1968.28**	0.19**	0.25**
Entry	153	272850.2**	17.07**	22.62**	2.65NS†	117.4936**	415.11**	0.26**	0.04**
Envt (Entry)	306	255538.1**	8.45NS	12.27NS	2.52NS	76.89NS	277.826NS	0.22*	0.04*
Error	381	192721.80	9.94	12.50	2.23	72.84	255.93	0.18	0.02
h^2‡		16	48	47	37	35	34	14	8

* Significant at p = 0.05

** Significant at p = 0.01

† NS, not significant

‡ h^2, Broad sense heritability

§ ASI, anthesis silking interval; DF, Degree of freedom; DTA, days to anthesis; DTS, days to silk; EHT, ear height; EPP, number of ears per plant; GY, Grain yield; PHT, plant height; SG, Stay green characteristic.

Table 2. Mean squares of BC_2F_1 population evaluated across high N environments§.

Source	DF	GY	DTS	DTA	ASI	EHT	PHT	EPP	SG
Envt	2	189200830.2**	2230.58**	144.66**	1406.34**	25285.19**	59359.99**	7.64**	199.25**
Blk(Rep×Envt)	78	1024333.8**	34.79**	26.71**	2.19**	789.05**	1379.33**	0.06**	0.76**
Rep(Envt)	3	9677253.8**	321.13**	358.14**	15.47**	2943.70**	6490.33**	1.17**	3.48**
Entry	153	756050.8**	18.37**	16.66**	1.52*	558.86NS†	434.72**	0.06**	0.29*
Envt(Entry)	306	448258.3*	11.18NS	8.79NS	1.39NS	499.14NS	212.62NS	0.04NS	0.22NS
Error	380	397162.8	10.22	7.75	1.22	462.62	238.98	0.04	0.23
h^2‡		46	62	69	52	32	72	61	53

* Significant at p = 0.05

** Significant at p = 0.01

† NS, not significant

‡ h^2, Broad sense heritability

§ ASI, anthesis silking interval; DF, Degree of freedom; DTA, days to anthesis; DTS, days to silk; EHT, ear height; EPP, number of ears per plant; GY, Grain yield; PHT, plant height; SG, Stay green characteristic.

Table 3. QTL identified based on BC_2F_1 population from CML 444 × CML 494 across two nitrogen (N) environments.

Trait†	N level	QTL	Chromosome	Markers	Marker Interval	Position‡	Add§	LOD¶	R^2#
GY	HN	*qgy*-10–1	10	PZA01292_1- PZB0049_1	29	18.2	310.13	3.15	10
	LN	*qgy*-1	1	PZA02487_1- PZB02058_1	0.7	58.5	-10.4	3.6	21
		qgy-10–2	10	PZA01292_1- PZB0049_1	29	10.3	–52.8	4.12	8
DTS	LN	*qdts*-1	1	PHM13191_6- PZB02058_1	0.7	59.3	2.34	3.1	10.3
	HN	*qdts*-5	5	PZA00980_1- PZ202792_25	9.2	51.2	-1.53	2.8	8
		qdts-10	10	PZA01292_1-PZB0049_1	29	1.3	-2.24	3.62	31
SG	HN	*qsg*-8	8	PZA02748_3-PZA01079_1	17.8	25.3	3.27	3.3	12
	LN	*qsg*-1	1	PZA24787_1-PHM1100_21	2.8	58.5	1.55	3.8	9
		qsg-4	4	PHM3587_6-PHM3963_33	5.2	3.8	0.56	4.13	18
ASI	LN	*qasi*-6	6	PZB00414_2-PHM15251_3	4.3	15.2	0.26	4.1	12
		qasi-10	10	PZA01292_1-PZB0049_1	29	5.8	1.2	2.8	5
EPP	LN	*qepp*-1	1	PHM174_13-PHM1100_21	7.7	58.5	0.2	2.7	7
PHT	LN	*qpht*-1	1	PHM16533_31-PHM13094_8	31.9	128.9	9.06	3.2	9.6

† ASI, anthesis silking interval; DTS, days to silk; EPP, number of ears per plant; GY, Grain yield; HN, high N; LN, low N; PHT, plant height; SG, Stay green characteristic.

‡ Position of peak marker in centiMorgans.

§ Add = Additive effect;- and + sign indicate favorable alleles came from CML494 and CML444, respectively.

¶ LOD = log10 of odds ratio.

R^2 = Percentage of phenotypic variation explained by QTL.

Similarly, three QTL for DTS were identified under both environments, with QTL *qdts*-1 accounting for 10.3% of PVE. This QTL was located on Chromosome 1 (PHM13191_6 and PZB02058_1) with LOD of 3.1 and marker interval of 0.7cM under low-N environment. On the other hand, two QTL *qdts*-5 *and qdts*-10 accounted for 8% and 31% of PVE, and were mapped on Chromosomes 5 and 10, respectively under high N environments.

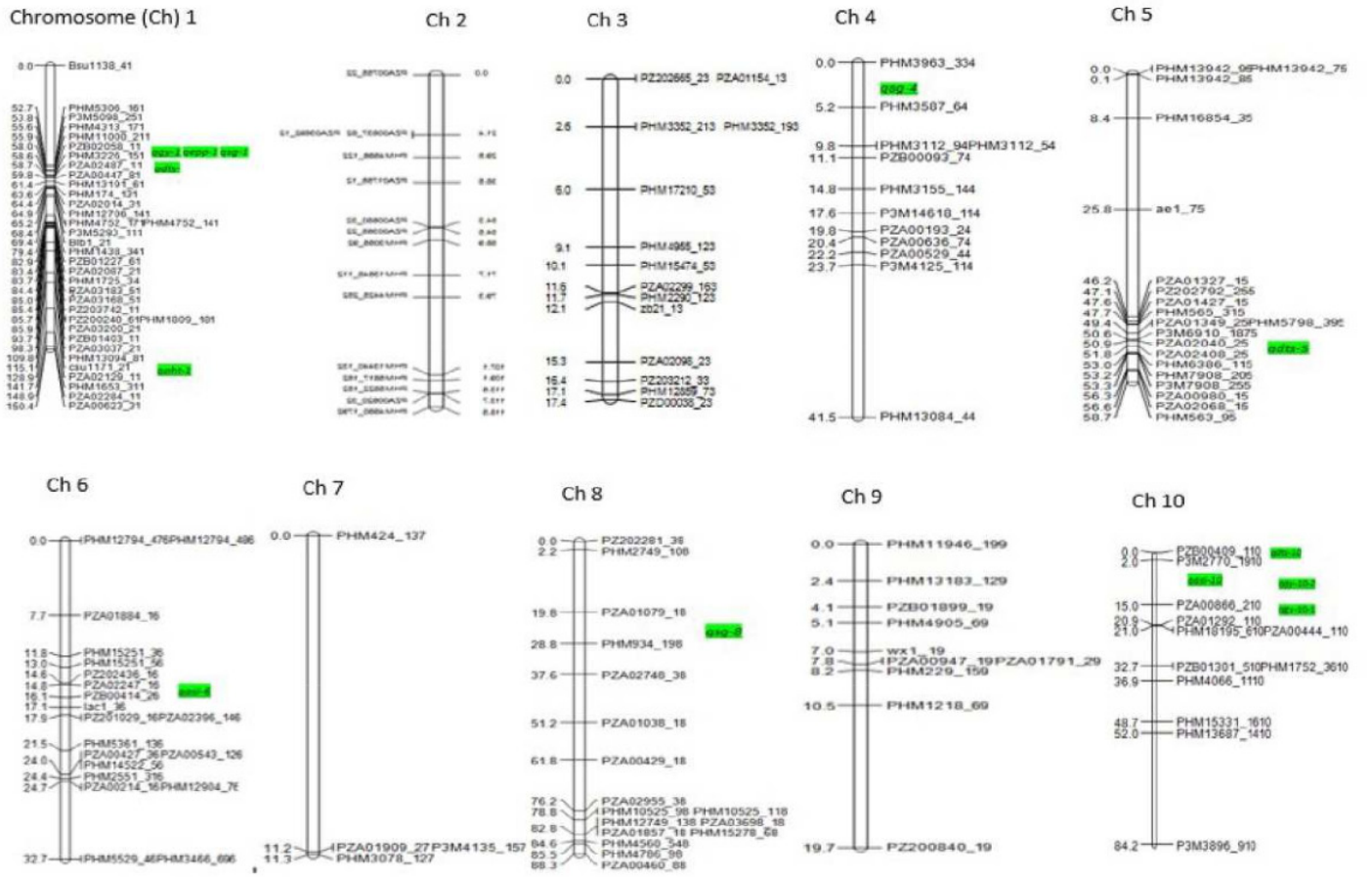

Fig. 3. Linkage map showing QTL on Chromosomes 1, 4, 5, 6, 8, and 10 for six traits (GY, ASI, DTS, SG, EPP and PHT).

QTL *qdts*-5 was flanked by markers PZA00980_1 and PZ202792_25 at a marker interval of 9.2 and had a LOD score of 2.8. The QTL *qdts*-10 with LOD 3.62 was flanked by the same markers that flanked QTL *qgy*-10–2 and *qdts*-10–1 (PZA01292_1-and PZB0049_1).

The QTL *qasi*-6 and *qasi*-10 for ASI accounted for 12% and 5% of PVE and were mapped on Chromosomes 6 and 10, respectively under low-N environments. The markers flanked QTL, *qgy*-10–1 and *qgy*-10–2 for GY, *qdts*-10 for DTS, and the QTL *qasi*-10 detected for ASI on Chromosome 10.

Three SG QTL, QTL *qsg*-8 located on Chromosome 1 and two QTL, *qsg*-1 *and qsg*-4 located on Chromosomes 1 and 4, were found under high- and low-N environments, respectively.

QTL *qsg*-8, *qsg*-1 *and qsg*-4 with PVE of 12%, 9% and 18%, were flanked by markers PZA02748_3 and PZA01079_1 at 17.8 cM, PZA24787_1 and PHM11000_21 at 2.8 cM, and PHM3587_6 and PHM3963_33 at 5.2 cM, respectively. One QTL each for EPP (*qepp*-1) accounted for 7% of PVE with a LOD score of 2.7, and PHT (*qpht*-1) accounted for 9.6% of PVE with an LOD score of 3.2 were detected on Chromosome 1 between the marker interval of PHM174_13 and PHM1100_21 at 7.7cM and PHM16533_31 and PHM13094 at 31.9 cM, respectively.

Epistatic Interactions

A total of sixteen digenic (QTL×QTL; QQ) interactions involving 22 loci were detected for the studied traits in the present investigation (Table 4). Significant epistatic interactions ($P \leq 0.05$) were observed for all the traits under both low- and high-N except for ASI and EPP which showed epistasis only under low- and high-N conditions, respectively (Table 4). Interestingly, none of these epistatic loci contained significant main effect QTL (interaction between two QTL with additive effects). All the interactions were observed either between a QTL with additive effect and a locus without significant additive effect (AN or NA) or interactions between two loci with only epistatic effects (NN). These epistatic QTL explained 0.14 to 4.42% of the phenotypic variation for the studied traits. The PVE explained by the epistatic QTL were lower than the main effects QTL for all the measured traits.

DISCUSSION

Low N is one of the major constraints militating against the achievement of the full yield potential of maize in sub-Saharan Africa. In depth understanding of grain yield and its related traits will be beneficial for the development of low N stress resilient cultivars. Precise and consistent phenotyping of such complex traits is very

Table 4. Epistatic (QTL×QTL) interactions for grain yield and its contributing traits under high and low nitrogen in BC_2F_1 maize population by QTL Network v2.1.

Traits§¶	N Level	Chr_i	$Marker\ interval_i$	P_i	Chr_j	$Marker\ interval_j$	P_j	AA	$h^2(aa)(\%)$‡	Interaction‡	*P*-value
GY	High	1	PHM4752_14- P3M5293_11	68.2	6	PHM15251_5- PZ202436_1	14.0	176.6	1.17	NN†	0.000
		1	PZA03200_2- PZB01403_1	92.9	9	PHM11946_19- PHM13183_12	1.0	-251.0	1.49	NN	0.001
		5	PHM16854_3- ae1_7	9.4	5	PZA02068_1- PHM563_9	57.6	209.2	1.17	NN	0.001
		8	PHM934_19- PZA02748_3	27.6	8	PHM15278_6- PHM4560_54	81.6	-355.8	1.06	N*N	0.000
	Low	2	PHM13648_11- PHM4425_25	72.7	5	ae1_7- PZA01327_1	45.8	-220.4	0.73	NN	0.000
		4	P3M3963_33- PHM3587_6	5.0	6	PHM15251_5- PZ202436_1	14.0	87.5	0.84	N*N*	0.009
		8	PHM2749_10- PZA01079_1	1.0	10	PZA00444_1- PZB01301_5	32.0	-165.1	0.72	N*N	0.047
DTS	High	1	PHM1438_34- PZB01227_6	80.4	6	PHM12904_7- PHM5529_4	25.7	-1.304	2.11	NN	0.000
	Low	1	PHM13191_6- PHM174_13	62.4	1	PHM13094–8- csu1171_2	114.8	2.140	2.61	AN*	0.000
SG	High	4	PHM3155_14- P3M14618_14	16.8	5	ae1_7- PZA01327_1	45.8	0.073	0.19	NN	0.012
		5	ae1_7- PZA01327_1	45.8	8	PHM2749_10- PZA01079_1	17.0	-0.109	0.14	NA	0.044
	Low	6	PZA02247_1- PZB00414_2	15.8	10	PHM4066_11- PHM15331_16	47.9	0.103	0.29	N*N	0.022
ASI	Low	1	Blb1_2- PHM1438_34	73.4	2	PHM13648_11- PHM4425_25	72.7	0.576	1.27	NN	0.003
EPP	High	1	PZA03200_2- PZB01403_1	92.9	10	PHM1752_36- PHM4066_11	36.7	-0.139	4.42	NN	0.000
PHT	High	6	PHM12904_7- PHM5529_4	26.7	10	P3M2770_19- PZA00866_2	14.0	12.917	3.41	NN	0.000
	Low	1	csu1138_4- PHM5306_16	1.0	10	PZA00866_2- PZA01292_1	18.0	-17.485	3.58	NN*	0.000

†Without significant additive QTL for this trait but with significant additive QTL for other traits in the present study

‡ $h^2(aa)(\%)$ represents percentage of phenotypic variance explained by individual epistatic effects of the mapped QTL.

§ Types of epistatic (QQ) interaction: NN interaction between two loci with epistatic effects only whereas NA/AN represents interactions between a QTL with additive effects and a locus without significant additive effects or vice versa, respectively.

¶ ASI, anthesis silking interval; DTS, days to silk; EPP, number of ears per plant;GY, Grain yield; PHT, plant height; SG, Stay green characteristic.

difficult due to highly fluctuating environmental and soil conditions. Selection and release of new varieties based on inconsistent phenotypic data often leads to failure in adoption by farmers. Thus, integration of genomics tools with conventional breeding would facilitate the development of improved cultivars with high yield under low-N conditions. The target traits measured in the present study followed normal distribution suggesting the suitability of the BC_2F_1 population for QTL mapping (Fig. 1 and 2). We found significant environmental variation for GY and other measured traits indicating differences in the test environments. Several researchers have previously reported variations in response of maize to environmental stresses (Betrán et al. 2003; Badu-Apraku et al., 2007; Worku et al., 2007; Derera et al., 2008). The highly significant GEI observed for only GY indicated that the measured traits of most individual families responded similarly in the research environments. This result is in agreement with the findings of Makumbi et al., (2011), who found significant GEI for GY under low N conditions. The high repeatability estimates recorded for most measured traits under high N environments indicated that the expression of these traits was consistent. Although the heritability estimates were lower for GY, other agronomic traits had substantially higher heritability estimates indicating their potential to aid in indirect selection for increased GY under these environments. This result is consistent with the findings of Ifie (2013) and Mafouasson (2014). Besides heritability, the strong correlation of the secondary traits with GY is an important attribute that would enable their routine integration in breeding programs (Bänziger et al., 2002). In the present study, significant phenotypic correlations were observed between GY and other measured traits (Supplementary Table 2). This finding is in agreement with the results of other researchers (Bolaños and Edmeades, 1996; Ribaut et al., 1997; Zheng et al., 2009; Lu et al., 2011; Ifie, 2013; Mafouasson, 2014).

We constructed a linkage map corresponding to 10 chromosomes of maize using 158 SNP markers that spanned 622.7 cM in length. The results revealed that the availability of limited number of polymorphic markers for the BC_2F_1 population resulted in relatively large intervals between markers at some chromosomes, suggesting that some QTL may have remained undetected in the corresponding regions (Li et al., 2007). However, with markers spaced about 10 to 15 cM apart, it was possible to identify markers associated with the trait of interest (Bernardo, 2008). Although the length of the linkage map constructed in the present study was shorter than that of earlier researchers who used similar SNP markers (Almeida et al., 2014; Zaidi et al., 2015), it was longer than that reported by Šimić et al. (2009). The differences between the results of this study and other studies could be attributed to the type and size of the mapping population and the number of markers used.

Quantitative trait loci analysis resulted in the identification of 13 QTL for six different traits under low- and high-N (4 QTL) environments. Some QTL for different traits overlapped in some specific genomic regions. For instance, interval PZA01292_1 through PZB0049 at Chromosome 10 harbored overlapping QTL for GY, DTS and ASI. These QTL may have pleiotropic effects

explaining the correlation observed among these traits. Similar overlapping genomic regions for GY and ASI on Chromosome 10 were reported by Ribaut et al. (1997) and Malosetti et al. (2008). This explains the strong correlation of ASI with GY across a broad range of germplasm, suggesting the possibility of a cluster of tightly-linked loci controlling low-N tolerance through coordinated expression of these traits. Higher heritability was recorded for ASI and DTS than for GY for both low- and high-N environments. Thus, the understanding of the genetic basis of ASI and DTS would aid in designing efficient marker-based breeding strategies for enhanced selection for GY under low-N environments. Some earlier studies have reported QTL for grain yield and its related traits on Chromosome 10 under optimal and water stress conditions (Li et al., 2010; Zheng et al., 2009).

Similarly, the co-location of QTL for GY, SG and EPP on Chromosome 1 confirmed the physiological relationship and strong correlation among these traits. Close linkage between GY and EPP has been reported in numerous classical studies (Agrama and Moussa, 1996; Ifie, 2013; Mafouasson, 2014). The mapping of the traits in the same region could indicate that this region is a hotspot for yield-related traits and introgression of this region into maize genotypes will lead to varieties with improved yield. In maize, QTL for GY have been reported previously on Chromosome 1 under low N (Table 5). Correspondingly, a QTL for EPP has also been reported on Chromosome 1 under low-N and drought-stress conditions (Ribaut et al., 1997). The identification of common QTL under drought and low-N conditions has important implications for maize breeding, because maize yield would be expected to suffer due to the insufficient N supply in drought-prone areas, located particularly in developing countries. In maize, it has been observed that selection for tolerance to midseason drought stress is crucial for yield enhancement under N deficiency (Bänziger et al., 2002; Badu-Apraku et al., 2013).

The quest for stress tolerance, high yield and good quality is unending for crop breeders, so the desirable crop production characteristics of functional stay-green genotypes make them very attractive. Beavis et al. (1994), identified three and five QTL for SG in an F_4 and a top-cross maize population generated from B73_Mo17, while Zheng et al. (2009) detected 14 QTL in an F_2 population. In the present study, only three QTL for SG, including one QTL on Chromosome 8 and two QTL on Chromosomes 1 and 4, were identified under high and low N, respectively. Wang et al. (2012) also identified QTL for SG on Chromosomes 1 and 4, indicating the important role of these loci for improving SG trait in maize. A QTL for PHT (*qpht*-1) with PVE of 9.6% was detected on Chromosome 1 in the present study. No QTL for PHT has ever been reported on Chromosome 1 (Table 5), indicating that this is a new QTL associated with PHT in maize. Plant height was also shown to be correlated with yield; hence, it is an important trait for selection for improved yield. Overall, the favourable alleles at QTL *qgy*-10–1 for GY, *qdts*-1 for DTS, *qsg*-1, *qsg*-4 and *qsg*-8 for SG, *qasi*-6 and *qasi*-10 for ASI, *qepp*-1 for EPP, and *qpht*-1 for PHT, were contributed by the inbred CML 444, while the favourable alleles at QTL *qgy*-1 and *qgy*-10–2 for GY and *qdts*-5 and *qdts*-10 for DTS were contributed by the inbred CML 494.

It is noteworthy that QTL for GY, ASI, EPP and PHT detected in the present study have also been previously reported by other researchers (Table 5). However, our results differ substantially from earlier reports in many respects in terms of QTL positions and their contributions in trait expression. Another notable aspect of our study is the detection of epistatic QTL under low- and high-N environments, although their contributions were limited. The maximum epistatic interactions were detected for GY under both high- and low-N conditions, contributing from 0.72% to 1.49% of the variance, indicating the complex nature of GY and its contributing traits. In the present study, all the observed interactions were either between a QTL with main effect and a locus without significant effect or interactions between two loci with only epistatic effects. These results are consistent with the findings of Yan et al. (2006), who also detected epistatic QTL for GY and its contributing traits in maize, suggesting that many QTL are affecting trait expressions indirectly through interactions with other loci.

CONCLUSIONS

A total of 13 QTL were identified on a linkage map spanning a total length of 622.7 cM with marker density of 3.9 cM. The colocalization of QTL for GY and other agronomic traits is a good indication of their strong associations. The identification of QTL for yield-related traits that improve crop growth and performance, especially under low-N environments, will certainly assist breeders in rapid introgression of these genomic regions into desired elite germplasm. Five QTL, one each for GY (*qgy*-1), DTS (*qdts*-1) and ASI (*qasi*-6), and two for SG (*qsg*-1 and *qsg*-4) were close to their adjacent markers with an interval of 0.7 to 5.2cM between them. These QTL with PVE of 9 to 21% suggested that the markers were linked with the genes controlling the traits and could be used for MAS. However, other QTL identified for these traits were far (≥ 10 cM) from their linked markers, indicating that there will be the need for further fine mapping of these chromosomal regions to narrow down the marker interval. The detection of several epistatic interactions for the measured traits, especially GY in both high- and low-N conditions, indicated the complex nature of yield and its contributing traits. Finally, the validation of these

Table 5. Comparison of QTL for ASI, PHT, GY and EPP for two N levels with those of other studies.

Trait	Mapping population	Chromosome	Marker type	N level	QTL position	Authors
ASI†	$F_{2:3}$	1,3,10	RFLP	High	75, 39, 63	Ribaut and Ragot, 2007
		1,3,4,6,7,8,10		Low	1.08, 3.05, 4.08, 6.05, 7.04, 8.02, 8.06, 10.03	
	RIL	3, 6, 7, 8		high	3.06, 3.07, 6.01, 7.02, 8.02, 8.06	Liu et al., 2012
		6, 7, 8	SSR	Low	6.01,7.02,8.03	
	BC_2F_1	6	SNP	High	4.4	Present study
		10		Low	29	
PHT‡	$F_{2:3}$	3,5,9	RFLP	High	48.6, 85.7, 21.1	Agrama et al., 1999
		2,3,5,9		Low	51.4, 57.1,58.9,137.7,32.6	
	$F_{2:3}$	4,6,7,8,9	RFLP	High	59,120,69,90,60	Ribaut and Ragot, 2007
	BC_2F_1	1	SNP	Low	31.9	Present study
GY§	$F_{2:3}$	1,4,5,9,10,	RFLP	High	131.4,33.6, 8.5, 122.7, 74.8	Agrama et al. 1999
		1,2,7,9,10		Low	46.9,90.6, 110.8, 59.6, 120.7, 69.4	
	$F_{2:3}$	1,3,10	RFLP	High	95,39,63	Ribaut et al.,2007
		1,2,3.4,8,9		Low	67,18,101,53,188,128,136,64	
	BC_2F_1	10	SNP	High	18.1	Present study
EPP¶	$F_{2:3}$	1,4,6,9	RFLP	High	196.4,55.3,30,122.7	Agrama et al., 1999
		1,3.6,9		Low	94.5,144.3,35.6,102.1	
	BC_2F_1	1	SNP	Low	7.7	Present study

† ASI, anthesis silking interval.

‡ PHT, plant height.

§ GY, Grain yield.

¶ EPP, number of ears per plant.

QTL in another mapping population would be necessary before their use in MAS.

Therefore, in a follow up study, fine mapping of the identified QTL will be performed with a larger population size and a saturated map (GBS or DArT-seq).

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