1 **Identification of QTLs for grain yield and other traits in tropical maize under high and low**

2 **soil-nitrogen environments.**

3 P.F. Ribeiro[1,3], B.Badu-Apraku[2]*, V.E. Gracen[3], E.Y. Danquah[3], A.L.Garcia-Oliveira[2], M.D.

4 Asante[1], C. Afriyie-Debrah[1] and M. Gedil[2].

5 [1]CSIR – Crops Research Institute (CRI), P. O. Box 3785, Fumesua, Kumasi, Ghana.

6 [2]International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria.

7 [3]West Africa Centre for Crop Improvement, University of Ghana, Legon, Ghana.

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9 Received …… *Corresponding author (b.badu-apraku@cgiar.org).

10

11 **Abstract**

12 Low soil Nitrogen (low-N) is one of the most important abiotic stresses responsible for

13 significant yield losses in maize (Zea mays. L.). The development and commercialization of low

14 N tolerant genotypes can contribute to improved food security in developing countries. However,

15 selection for low N tolerance is difficult because it is a complex trait with strong interaction

16 between genotypes and environments. Marker assisted breeding holds great promise for

17 improving such complex traits more efficiently in less time, but requires markers associated with

18 the trait of interest. In this study, 150 BC_2F_1 families of CML 444 x CML 494 were evaluated at

19 two location for two consecutive seasons to identify SNP markers associated with quantitative

20 trait loci (QTLs) for yield and other agronomic traits under low- and high-N environments. A

21 total of 13 QTLs were identified with 158 SNP markers, of which nine and four QTLs were

22 detected under low- and high-N environments, respectively. Five QTLs one each for grain yield

23 (*qgy-1),* days to silking *(qdts-1)* and anthesis- silking interval *(qasi-6)*, and two for stay green

24 characteristic (*qsg-1* and *qsg-4*) were close to their adjacent markers, with an interval of 0.7 to

1   5.2 cM between them and explained phenotypic variance of 9 to 21%. These QTLs would be

2   invaluable for rapid introgression of genomic regions into maize populations using marker-

3   assisted selection (MAS) approaches. However, further validation of these QTLs is needed

4   before use in MAS.

5   **Key words**: Maize, low-soil nitrogen tolerance, Quantitative trait locus, Marker assisted

6   selection.

7

8   **Abbreviations**

9   **CSIR,** Council for Scientific and Industrial Research**; HN**, High Nitrogen**; h^2,** Broad sense

10   heritability; **IITA,** International Institute of Tropical Agriculture**; LN**, Low Nitrogen; **MAS,**

11   Marker assisted selection**; QTL,** Quantitative trait locus; QTLs, Quantitative trait loci.

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1   **THE INCREASE IN CROP YIELD** during the past century is attributed to the selection of

2   genotypes with higher yield potential and increased amount of nutrients, particularly nitrogen

3   (N) supplied during the growth cycle (Tuberosa, 2002). Available soil N is usually the critical

4   factor limiting plant growth. Therefore, N fertilizer is usually applied to maize fields, resulting in

5   marked increases in yield. Low N availability is a major cause of yield loss in maize in

6   developing countries (Pingali and Pandey, 2001). This is because production is usually under N-

7   deficient conditions due to limited availability of fertilizers, or low purchasing power of farmers

8   (Bänziger et al., 1997). Therefore, development of maize cultivars with tolerance to low N is the

9   most effective and sustainable approach to mitigate the problem of low N.

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11   Progress in selecting for low N tolerance is limited by large genotype x season and genotype x

12   location interactions. The efficiency of selection for yield in low N environments may be

13   improved by selecting secondary traits with high correlations to grain yield under low N

14   (Banziger and Lafitte, 1997; Badu-Apraku 2011d and 2012). Selection indices based on these

15   traits have been developed and have improved significantly the selection efficiency under stress

16   conditions (Banziger and Lafitte, 1997). The complexity of measuring the secondary traits

17   quickly and accurately, however, has limited their use in breeding programs (Monneveux and

18   Ribaut, 2006).

19
20   The introduction of molecular marker technology and the construction of saturated linkage maps

21   have facilitated the detection of the genetic loci associated with complex traits (Kang et al.,

22   1998; Li et al., 1995; Song et al., 2001). Genetic linkage maps and quantitative trait loci (QTL)

23   mapping technology have improved the efficiency of estimating the number of loci controlling

24   genetic variation in a segregating population and the characterization of the map positions in the

1   genome (Xiao et al., 1996). In maize, genetic analysis of complex traits under abiotic stresses has

2   focused mainly on drought tolerance (Agrama et al., 1996; Ribaut et al., 1996: 1997; Tuberosa et

3   al., 2002). Not as much attention has been paid to the understanding of the genetic responses of

4   segregating populations under soil nutrient deficiencies such as low phosphorus (Reiter et al.,

5   1991) or low N (Agrama et al., 1999; Hirel et al., 2001).

6   The use of marker–assisted selection (MAS) could be a very effective strategy for breeding for

7   tolerance to low N (Zhou, 2010). However, the effectiveness of MAS depends on the precise

8   locations of the QTLs and the identification of tightly linked molecular markers, which are cost

9   effective and easier to use.

10   The QTLs identified in breeding populations could be used directly for crop improvement

11   through MAS approaches (Wu¨rschum, 2012; Wang et al., 2012). The objective of this study was

12   to identify QTLs associated with yield and yield related traits under low- and high-N

13   environments.

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15   **Materials and Methods**

16   **Mapping population**

17   The two parental lines used in the present study differed for their responses to low N stress;

18   CML 494 (highly susceptible to low-N) and CML 444 (tolerant to low-N). These parental lines

19   were selected based on their performance in multi-location trials conducted under low-N in

20   Ghana. The F_1 crosses were made between the inbreds at the CSIR-Crops Research Institute,

21   Fumesua, Ghana during the major cropping season of 2013. . The F_1s were backcrossed to CML

22   494 during the minor cropping season of 2013 at Kwadaso, Ghana to obtain the BC_1F_1s. This

1   was followed by another cycle of backcrossing of BC_1F_1s to CML 494 at Fumesua to obtain 150

2   BC_2F_1 families.

3   **Field experiments**

4   The 150 BC_2F_1 families along with the parental lines and the F_1 hybrid, as well as the check

5   (ENT 70) were evaluated under low- and high-N environments during the major (April-July) and

6   minor (September- December) rainy seasons of 2014, at Fumesua (6°41' N, 1°28' W) and Ejura

7   (7°23' N, 1°21' W) in Ghana. A 11 x 14 lattice design with two replications was used for the

8   evaluations at the two locations during the two plantimg seasons. Single row plots, each 5 m

9   long, spaced 0.75 m apart with 0.5 m spacing between plants in each row were used in all the

10   environments. Three seeds were planted in each hole and thinned to two plants per hill at two

11   weeks after emergence to give a population density of 53,333plants per hectare. The low-N plots

12   received 30 kg N ha^{-1} while the high-N plots received 90 kg N ha^{-1} applied in two splits at two

13   and five weeks after planting. The low N field had been previously depleted of N by growing

14   maize crops and removing all plant material. Soil analysis was carried out at the Soil Research

15   Institute, Kumasi, Ghana. The total N in the soils was determined by Kjeldahl digestion and

16   colorimetric determination on Technicon AAII Autoanalyser (Bremner and Mulvaney 1982).

17   Information on the soil properties of the experimental fields used in this study is presented in

18   Supplementary Table 1. Nutrient status, in accordance with Landon (1991) interpretation of

19   analyzed soils, was generally low at both locations with N levels less than 0.2%. Fertilizers were

20   applied to bring the total available N to 90 kg/ha for the high-N field and 30 kg/ha for the low-N

21   field when the soil N was less than the target level. Both low- N and high- N fields received 60

22   kg P ha^{-1} as single superphosphate (P_2O_5) and 60 kg K ha^{-1} as muriate of potash (K_2O).. The trials

23   were kept weed-free with the application of both pre- and post-emergence herbicides, primextra

1 and paraquat each at 5 l/ha. Subsequently, hand weeding was used to supplement the chemical

2 weed control.

3 **Field data collection**

4 Data were recorded on both low and high N plots for days to 50% anthesis (DA) and silking

5 (DS) as the number of days from planting to when 50% of the plants in a plot had shed pollen

6 and extruded silks, respectively. The anthesis–silking interval (ASI) was calculated as the

7 difference between DS and DA. Plant height (PHT) was measured as the distance from the base

8 of the plant to the height of the first tassel branch and ear height (EHT) as the distance to the

9 node bearing the upper ear. Plant aspect (PA) was recorded on a scale of 1–5 based on the plant

10 type, where 1 = excellent and 5 = poor. Husk cover was scored on a scale of 1–5, where 1 =

11 husks tightly arranged and extended beyond the ear tip and 5 = ear tips exposed. EASP was

12 based on a scale of 1 to 5, where 1 = clean, uniform, large and well-filled ears, and 5 = ears with

13 undesirable features. In addition, stay green characteristic (SGC) were recorded at 70 days after

14 planting on a scale of 1 to 10, where 1 = almost all leaves were still green and 10 = virtually all

15 leaves were dead (Badu-Apraku et al., 2015). Number of ears per plant (EPP) was computed by

16 dividing the total number of ears harvested per plot by the number of plants in a plot at harvest.

17 Harvested ears from each plot were shelled to determine the grain weight and the percentage

18 grain moisture for the low N experiments. Grain yield (GY) in kg ha^{-1} was adjusted to 15%

19 moisture and computed from the shelled grain weight. In the high N plots, grain yield was

20 computed based on 80% (800 g grain kg^{-1} ear weight) shelling percentage and adjusted to 15%

21 moisture content.

22

1   **Data Analysis**

2   Phenotypic data were analyzed using SAS 9.0 (SAS, 2011) with the GLM procedure. Pearson

3   correlation coefficients were calculated between the traits, using the adjusted means of the BC_2F_1

4   families. Repeatability of the traits (Falconer and Mackay, 1996) under low- and high- N

5   conditions were computed on genotypic-mean basis using the following formula:

6
$$R = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re}}$$

7   where σ_g^2 is the genotypic variance, σ_{ge}^2 is the genotype x environment and σ_e^2 is the residual

8   variance, e is the number of environments, and re is the number of replicates per environment.

9

10   **SNPs genotyping, Construction of genetic linkage map and QTL analysis**

11   A total of 153 freeze-dried leaf (two weeks old) samples consisting of 150 BC_2F_1, two parental

12   lines and the F_1 hybrid were sent to LGC Genomics for SNP genotyping. Details on the principle

13   and procedure of the DNA assays are available at http://www.lgcgroup.com/our-

14   science/genomics-solutions/#.WKgsBRrLfIU. The parental lines were genotyped with a set of

15   1250 SNP markers, for which KASP assays (Semagn et al., 2013), were designed at LGC

16   Genomics Facility in London, UK. Theoretically, the 150 BC2F1 families used in the study had

17   1/8 of the CML 444 genome in the genetic background of CML 494 with the expected genotypic

18   frequency of 0.75 and 0.25 per marker locus for the allele of CML 494 in homozygous and

19   heterozygous conditions, respectively. Segregation of marker loci was evaluated with a Chi-

20   squared test. Markers that had insufficient linkage data were excluded and the final linkage map

21   was constructed with 158 SNP markers using JoinMap4 (Van Ooijen, 2006). Markers were

22   assigned to linkage groups at independence LOD values > 6.0 and threshold values ranged from

1   2.0 to 20 with an interval of 1.0. Regression mapping algorithm was used to order the markers

2   and Haldane's mapping function was used to transform estimates of recombination frequency to

3   map distances in centimorgans (cM). The linkage groups from JoinMap were rearranged into

4   chromosomes according to their order on the reference map.

5   QTL mapping was done in R/qtl using a single-QTL model. Furthermore, composite interval

6   mapping (CIM) was used to define QTL peak position and to estimate effects of the mapped loci

7   and their constrictions to the phenotypic variances. The thresholds of the QTLs (LOD scores)

8   were obtained at p= 0.05 by 1,000 random permutations of the trait values. In addition, epistatic

9   gene interactions for grain yield and other agronomic traits were also determined under both low-

10   and high-N environments using QTL Network v2.1 (Yang et al. 2008).

11   **Results**

12   **Evaluation of BC_2F_1 population**

13   In all environments, the target traits measured in the BC_2F_1 population followed normal

14   distribution (Figs.1 and 2). The combined analysis of variance showed significant mean squares

15   of genotypes, environments and genotype by environment interaction (GEI) for GY, SG and EPP

16   across low N environments. The few exceptions included the mean squares of genotypes for ASI

17   and GEI for DTA, DTS, ASI, EHT and PHT across low N conditions which did not reach

18   significant levels (Table 1). Similarly, significant mean squares were observed for genotypes,

19   environments and GEI of all measured traits across high N environments except the genotype

20   mean squares for EHT, and the GEI mean squares for DTA, ASI, EHT, PHT, EPP and SG (Table

21   2).

22

1   The repeatability estimates of the traits ranged from 8% for ears per plant to 48 % for days to

2   silking under low N, and 32% for ear height to 72% for plant height under high N environments.

3   High repeatability estimates (i.e. ≥ 0.60) were recorded for most of the traits under high N

4   environments. A total of 23 significant correlations were detected under each environment

5   (Supplementary Table 2). The grain yield (GY) showed consistently significant and highly

6   positive correlations with ASI, PHT, EHT and EPP whereas negative correlation was found with

7   DTS under both low- and high-N environments. Similarly, the trait EHT had significant negative

8   correlation under low- and high-N environments. The associations of PHT with DTA, DTS and

9   ASI were negative under both low- and high-N environments. Similarly, EHT had significant

10   and negative associations with DTA and DTS under both environments. In contrast, significant

11   and negative correlation was observed between EHT and ASI under high N environments.

12

13   **Genetic linkage map construction**

14   Linkage analysis was performed on 150 BC_2F_1 families genotyped with 158 SNP markers.

15   Finally, a linkage map was constructed which corresponded to the ten chromosomes with length

16   of 622.7cM and an average marker interval of 3.9 cM (Supplementary Table 3).

17

18   **QTL identification**

19   A total of 13 QTLs for all the traits were detected under both low- and high-N environments with

20   the phenotypic variance explained (PVE) ranging from 531 % (Table 3; Fig.3). Of these QTLs,

21   four and nine were identified under high- and low-N environments, respectively. For GY, one

22   QTL (*qgy-10-1)* with PVE of 10% was detected under high N environment on chromosome 10

23   flanked by PZA01292_1 and PZB0049_1 markers at interval of 29.0 cM with LOD of 3.15. In

1   contrast, two QTLs were mapped for GY on chromosomes 1 (*qgy-1*) and 10 (*qgy-10-2*) under

2   low N environment. Of the QTLs, the major QTL, *qgy-1* accounted for 21% of PVE and was

3   located between markers PZA02487_1 and PZB02058_1 with marker interval of 0.7cM. The

4   QTL, *qgy-10-2* with PVE of 8% had a LOD score of 4.12. Interestingly, the QTL, *qgy-10-2* and

5   *qgy-10-1* were flanked by the same markers, but their peak positions were different on the

6   chromosome 10. QTL *qgy-10-1* had a marker interval of 29.0 cM while qgy-10-2 had a marker

7   interval of 0.7 cM.

8

9   Similarly, three QTL for DTS were identified under both environments, with QTLs *qdts*-1

10   accounting for 10.3% of PVE. This QTL was located on chromosome 1 (PHM13191_6 and

11   PZB02058_1) with LOD of 3.1 and marker interval of 0.7cM under low N environment. On the

12   other hand, two QTLs *qdts-5 and qdts-10* accounted for 8% and 31% of PVE, and were mapped

13   on chromosomes 5 and 10, respectively under high N environments,. QTL *qdts-5* was flanked

14   by markers PZA00980_1 and PZ202792_25 at a marker interval of 9.2 and had a LOD score of

15   2.8. The QTL qdts-10 with LOD 3.62 was flanked by the same markers that flanked QTLs *qgy-*

16   *10-2* and *qdts-10-1* (PZA01292_1-and PZB0049_1).

17   The QTLs, qasi-6 and qasi-10 for ASI accounted for 12% and 5% of PVE and were mapped on

18   chromosomes 6 and 10, respectively under low N environments. The markers flanked QTL, *qgy-*

19   *10-1 and qgy-10-2 for GY, and qdts-10* for DTS as well as the QTL *qasi-10* detected for ASI on

20   chromosome 10.

21

22   For three SG QTL, QTLs *qsg-8* located on chromosome 1 and two QTL, *qsg-1 and qsg-4*

23   located on chromosomes 1 and 4, were found under high- and low-N environments, respectively.

1   QTLs *qsg-8, qsg-1 and qsg-4* with PVE of 12%, 9% and 18%, were flanked by markers

2   PZA02748_3 and PZA01079_1 at 17.8cM, PZA24787_1 and PHM11000_21 at 2.8cM, and

3   PHM3587_6 and PHM3963_33 at 5.2cM, respectively. One QTL each for EPP (*qepp-1*)

4   accounted for 7% of PVE with LOD score of 2.7, and PHT (*qpht-1*) accounted for 9.6% of PVE

5   with LOD score of 3.2 were detected on chromosome 1 between the marker interval of

6   PHM174_13 and PHM1100_21 at 7.7cM and PHM16533_31 and PHM13094 at 31.9 cM,

7   respectively.

8

9   **Epistatic Interactions**

10   A total of sixteen digenic (QTL×QTL; QQ) interactions involving 22 loci were detected for the

11   studied traits in the present investigation (Table 9). Significant epistatic interactions (P≤0.05)

12   were observed for all the traits under both low- and high-N except for ASI and EPP which

13   showed epistasis only under low- and high-N conditions, respectively (Table 5). Interestingly,

14   none of these epistatic loci contained significant main effect QTLs (interaction between two

15   QTL with additive effects). All the interactions were observed either between a QTL with

16   additive effect and a locus without significant additive effect (AN or NA) or interactions between

17   two loci with only epistatic effects (NN). These epistatic QTLs explained 0.14 to 4.42 % of the

18   phenotypic variation for the studied traits. The PVE explained by the epistatic QTLs were lower

19   than the main effects QTLs for all the measured traits.

20   **Discussion**

21   Low N is one of the major constraints militating against the achievement of the full yield

22   potential of maize in sub-Saharan Africa. In depth understanding of grain yield and its related

23   traits will be beneficial for the development of low N stress resilient cultivars. Precise and

1   consistent phenotyping of such complex traits is very difficult due to highly fluctuating

2   environmental and soil conditions. Selection and release of new varieties based on inconsistent

3   phenotypic data often leads to failure in adoption by farmers. Thus, integration of genomics tools

4   with conventional breeding would facilitate the development of improved cultivars with high

5   yield under low N conditions. The target traits measured in the present study followed normal

6   distribution suggesting the suitability of the BC_2F_1 population for QTL mapping (Figures 1 and

7   2). We found significant environmental variation for GY and other measured traits indicating

8   differences in the test environments. Several researchers have previously reported variations in

9   response of maize to environmental stresses (Betran et al., 2003a and b; Badu-Apraku et al.,

10   2007; Mosisa et al., 2007; Derera et al., 2008). The highly significant GEI observed for only GY

11   indicated that the measured traits of most individual families responded similarly in the research

12   environments. This result is in agreement with the findings of Makumbi (2011), who found

13   significant GEI for GY under low N conditions. The high repeatability estimates recorded for

14   most measured traits under high N environments indicated that the expression of these traits was

15   consistent. Although the heritability estimates were lower for GY, other agronomic traits had

16   substantially higher heritability estimates indicating their potential to aid in indirect selection for

17   increased GY under these environments. This result is consistent with the findings of Ifie (2013)

18   and Maffousson (2014). Besides heritability, the strong correlation of the secondary traits with

19   GY is an important attribute that would enable their routine integration in breeding programs

20   (Banzinger et al., 2000). In the present study, significant phenotypic correlations were observed

21   between GY and other measured traits (Supplementary Table 2). This finding is in agreement

22   with the results of other researchers (Bolanos and Edmeads 1996; Ribaut et al. 1997; Zheng et al.

23   2009; Lu et al. 2011; Ifie, 2013; Maffouson, 2014).

1   We constructed a linkage map corresponding to 10 chromosomes of maize using 158 SNP

2   markers that spanned 622.7 cM in length. The results revealed that the availability of limited

3   number of polymorphic markers for the BC_2F_1 population resulted in relatively large intervals

4   between markers at some chromosomes suggesting that some QTLs may have remained

5   undetected in the corresponding regions (Li et al., 2007). However, with markers spaced about

6   10-15 cM apart, it was possible to identify markers associated with the trait of interest (Bernardo,

7   2008). Although the length of the linkage map constructed in the present study was shorter than

8   that of earlier researchers who used similar SNP markers (Almeida et al., 2014; Zaidi et al.,

9   2015), it was longer than that reported by Simic et al. (2009). The differences between the

10   results of this and other studies could be attributed to the type and size of the mapping population

11   and the number of markers used.

12

13   QTL analysis resulted in the identification of 13 QTLs for six different traits under low- and

14   high-N (4 QTL) environments. Some QTLs for different traits overlapped in some specific

15   genomic regions. For instance, interval PZA01292_1 – PZB0049 at chromosome 10 harbored

16   overlapping QTL for GY, DTS and ASI. These QTLs may have pleiotropic effects explaining

17   the correlation observed among these traits. Similar overlapping genomic regions for GY and

18   ASI on chromosome 10 were reported by Ribaut et al. (1997) and Malosetti et al. (2008). This

19   explains the strong correlation of ASI with GY across a broad range of germplasm suggesting

20   the possibility of a cluster of tightly linked loci controlling low N tolerance through coordinated

21   expression of these traits. Higher heritability was recorded for ASI and DTS than for GY for

22   both low- and high-N environments. Thus, the understanding of the genetic basis of ASI and

23   DTS would aid in designing efficient marker-based breeding strategies for enhanced selection for

1   GY under low N environments. Some earlier studies have reported QTL for grain yield and its

2   related traits on chromosome 10 under optimal and water stress conditions (Li et al., 2010;

3   Zheng et al., 2009).

4

5   Similarly, the co-location of QTLs for GY, SG and EPP on chromosome 1 confirmed the

6   physiological relationship and strong correlation among these traits. Close linkage between GY

7   and EPP has been reported in numerous classical studies (Agrama and Mousaa, 1996; Ifie, 2013;

8   Mafouasson, 2014). The mapping of the traits in the same region could indicate that this region is

9   a hotspot for yield related traits and introgression of this region into maize genotypes will lead

10 to varieties with improved yield. In maize, QTLs for GY has been reported previously on

11 chromosome 1 under low N (Table 4). Correspondingly, a QTL for EPP has also been reported

12 on chromosome 1 under low N and drought stress conditions (Ribaut et al., 1997). The

13 identification of common QTL under drought and low N conditions has important implications

14 for maize breeding, because maize yield would be expected to suffer due to the insufficient N

15 supply in drought prone areas located particularly in developing countries. In maize, it has been

16 observed that selection for tolerance to mid-season drought stress is crucial for yield

17 enhancement under N deficiency (Bazinger et al., 2002; Badu-Apraku et al., 2013).

18 The quest for stress tolerance, high yield and good quality is unending for crop breeders, so the

19 desirable crop production characteristics of functional stay-green genotypes make them very

20 attractive. Beavis et al. (1994), identified three and five QTL for SG in an F_4 and a top- cross

21 maize populations generated from B73_Mo17 while Zheng et al. (2009), detected 14 QTLs in an

22 F_2 population. In the present study, only three QTLs for SG including one QTLs on chromosome

23 8 and two QTL on chromosomes 1 and 4, were identified inder high- and low-N, respectively.

1 Wang et al (2012) also identified QTL for SG on chromosomes 1 and 4 indicating the important

2 role of these loci for improving SG trait in maize. A QTL for PHT (*qpht-1*) with PVE of 9.6%

3 was detected on chromosome 1 in the present study. No QTL for PHT has ever been reported

4 on chromosome 1 (Table 4), indicating that this is a new QTL associated with PHT in maize.

5 Plant height was also shown to be correlated with yield, hence, it is an important trait for

6 selection for improved yield. Overall, the favourable alleles at QTL   *qgy-10-1* for GY, *qdts-1* for

7 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

8 PHT, were contributed by the inbred CML 444, while the favourable alleles at QTL qgy-1 and

9 qgy-10-2 for GY and qdts-5 and qdts-10 for DTS were contributed by the inbred CML 494.

10

11 It is noteworthy that QTLs for GY, ASI, EPP and PHT detected in the present study have also

12 been previously reported by other researchers (Table 4). However, our results differ substantially

13 from earlier reports in many respects in terms of QTL positions and their contributions in trait

14 expression. Another notable aspect of our study is the detection of epistatic QTL under low- and

15 high-N environments, although their contributions were limited. The maximum epistatic

16 interactions were detected for GY under both high- and low-N conditions, contributing from

17 0.72% to 1.49% of the variance indicating the complex nature of GY and its contributing traits.

18 In the present study, all the observed interactions were either between a QTL with main effect

19 and a locus without significant effect or interactions between two loci with only epistatic effects.

20 These results are consistent with the findings of Yan et al. (2006), who also detected epistatic

21 QTL for GY and its contributing traits in maize, suggesting that many QTLs are affecting trait

22 expressions, not directly but indirectly through interactions with other loci.

23

1   **Conclusions**

2   A total of 13 QTLs were identified on a linkage map spanning a total length of 622.7 cM with

3   marker density of 3.9 cM. The co-localization of QTL for GY and other agronomic traits is a

4   good indication of their strong associations. The identification of QTL for yield related traits

5   that improve crop growth and performance, especially under low N environments, will certainly

6   assist breeders in rapid introgression of these genomic regions into desired elite germplasm. Five

7   QTL, one each for GY (*qgy-1), DTS (qdts-1) and ASI (qasi-6*), and two for SG (*qsg-1* and *qsg-4*)

8   were close to their adjacent markers with an interval of 0.7 to 5.2cM between them. These QTL

9   with PVE of 9-21% suggested that the markers were linked with the genes controlling the traits

10 and could be used for MAS. However, other QTLs identified for these traits were far ($\geq$10 cM)

11 from their linked markers, indicating that there will be the need for further fine mapping of these

12 chromosomal regions to narrow down the marker interval. The detection of several epistatic

13 interactions for the measured traits, especially GY in both high- and low-N conditions, indicated

14 the complex nature of yield and its contributing traits. Finally, the validation of these QTL in

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

16 Therefore, in a follow up study, fine mapping of the identified QTL will be performed with a

17 larger population size and a saturated map (GBS or DArT-seq).

18

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4

References

6 Agrama, H.A.S., and M.E. Moussa. 1996. Mapping QTLs in breeding for drought tolerance in

7   maize (Zea mays L.). Euphytica. 91,89–97

8 Agrama, H.A.S., A.G. Zakaria, F.B. Said, and M.Tuinstra. 1999. Identification of quantitative

9   traits loci for nitrogen use efficiency in maize. Molecular Breeding. 5,187–195.

10 Almeida, G., S. Nair, A. Borém, J. Cairns, S. Trachsel, and J.M. Ribaut. 2014. Molecular

11   mapping across three populations reveals a QTL hotspot region on chromosome 3 for

12   secondary traits associated with drought tolerance in tropical maize. Molecular Breeding.

13   34,701–715.

14 Badu-Apraku, B., R. O. Akinwale, J. Franco, and M. Oyekunle. 2012. Assessment of reliability

15   of secondary traits in selecting for improved grain yield in drought and low-nitrogen

16   environments. Crop Science. 11.12.0629.

17 Badu-Apraku, B., M. A. B. Fakorede, and A. F. Lum. 2007. Evaluation of experimental

18   varieties from recurrent selection for *Striga* resistance in two extra-early maize

19   populations in the savannas of West and Central Africa. Experimental Agriculture. 43,

20   183-200.

21 Badu-Apraku, B., Fakorede, M. A. B., Oyekunle, M. and Akinwale, R. O. (2015). Genetic gains

22 in grain yield under nitrogen stress following three decades of breeding for drought tolerance and

23 *Striga*   resistance   in   early   maturing   maize.   Jour.   Agric.   Sci.

1   doi:10.1017/S0021859615000593.Badu-Apraku, B., Oyekunle, M., Akinwale, R. O., and Lum,

2   A. F. 2011. Combining ability of   early-maturing white maize inbreds under stress and non-

3   stress environments. *Agronomy*   *Journal,* 103**,** 544-557.

4   Badu-Apraku, B., M. Oyekunle, A. Menkir, K. Obeng-Antwi, and C.G. Yallou. 2013.

5   Comparative performance of early maturing maize cultivars developed in three eras

6   under drought stress and well-watered environments in West Africa. Crop Science. doi:

7   10.2135/cropsci2012.11.0640.

8   Banziger, M., and H.R. Lafitte. 1997. Efficiency of secondary traits for improving maize for low

9   nitrogen target environments. Crop Science. 37, 1110-1117.

10   Bänziger, M., F. J. Betrán, and H. R. Lafitte. 1997. Efficiency of high-nitrogen selection

11   environments for improving maize for low-nitrogen target environments. Crop Science.

12   37, 1103-1109.

13   Bänziger, M., G. O. Edmeades, and H.R. Lafitte. 2002. Physiological mechanisms contributing

14   to the increased N stress tolerance of tropical maize for drought tolerance. Field Crops

15   Research. 75, 223-233.

16   Beavis, W.D., O.S. Smith, D. Grant, R. and Fincher. 1994. Identification of quantitative trait

17   loci using a small sample of topcrossed and F4 progeny from maize. Crop Science. 34,

18   882_896.

19   Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: Learning from

20   the last 20 years. Crop science. 48**,** 1649-1664.

21   Betran, F.J., J.M. Ribaut, D.L. Beck, and D. Gonzalez de Leon. 2003a. Genetic diversity,

22   specific combining ability, and heterosis in tropical maize under stress and non stress

23   environments. Crop Science. 43, 797-806.

1   Betran, F.J., Ribaut, J.M., D.L. Beck, and D. Gonzalez de Leon. 2003b. Genetic analysis of

2   inbred and hybrid grain yield under stress and non stress environments. Crop Science. 43,

3   807-817.

4   Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. Pages 595–624 in. A.L. Page et al.,

5   eds. Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA,

6   Madison.

7   Derera, J., P. Tongoona, B.S. Vivek, and M.D. Laing. 2008. Gene action controlling grain yield

8   and secondary traits in southern African maize hybrids under drought and nondrought

9   environments. Euphytica. 162, 411-422.

10   Hirel, B., P. Bertin, I. Quillere, W. Bourdoncle, C. Attagnant, C. Dellay, Y. Gouy, S. Cadiou,

11   C. Retailliau, M. Falque, and A. Gallais. 2001. Towards a better understanding of the

12   genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiology.

13   125,1258–1270.

14   Ifie, B.E. 2013. Genetic analysis of *Striga* resistance and low soil nitrogen tolerance in early

15   maturing maize (*Zea mays* L.) inbred lines. PhD. *University of Ghana Legon.* West

16   Africa Centre for Crop Improvement/ School of Agriculture/ College of Agriculture and

17   Consumer Sciences. pp 110-123.

18   Kang, H.J., Y.G. Cho, Y.T. Lee, M.Y. Eun, and J.U. Shim. 1998. QTL mapping of genes

19   conferring days to heading, culm length and panicle length based on molecular map of

20   rice (Oryza sativa L.). RDA. J. Crop Science. 40, 55−61.

21   Li W.J., Z.Z. Liu, Y.S. Shi, Y.C. Song, T.Y. Wang, C.W. Xu, and Y. Li. 2010. Detection of

22   consensus genomic region of QTL relevant to drought-tolerance in maize by QTL meta-

23   analysis and bioinformatics approach. Acta Agronomica Sinica. 36,1457–1467.

Li, H., G. Ye, and J. Wang. 2007. A modified algorithm for the improvement of composite interval mapping. Genetics. 175,361–374.

Li, Z., S.R.M. Pinson,.J.W. Stansel, and W.D. Park. 1995. Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (Oryza sativa L.). Theoretical and Applied Genetics. 91, 374-381.

Liu R, H .Jia, X .Cao, J. Huang and F. Li. 2012. Fine Mapping and Candidate Gene Prediction of a Pleiotropic Quantitative Trait Locus for Yield-Related Trait in Zea mays. PLoS ONE 7(11): e49836. doi:10.1371/journal.pone.0049836

Mafouasson, A.H.N. 2014. Genetic analysis of tolerance to low soil nitrogen in intermediate maturing maize (*Zea mays* L.) inbred lines. PhD Thesis, University of Ghana Legon, West Africa Centre for Crop Improvement/ School of Agriculture/ College of Agriculture and Consumer Sciences.pp 64-82.

Makumbi, D., F.J. Betràn, M. Bänziger, and J. M. Ribaut. 2011. Combining ability, heterosis and genetic diversity in tropical maize (Zea mays L.) under stress and non-stress conditions. Euphytica. 180, 143-162.

Malosetti, M., J.M. Ribaut, M. Vargas, J. Crossa, and F.A. Van Eeuwijk. 2008. A multi-trait multi-environment QTL mixed model with an application to drought and nitrogen stress trials in maize (*Zea mays* L.) Euphytica. 161, 241–257.

Monneveux, P., and J.M. Ribaut. 2006. Secondary traits for drought tolerance improvmement in cereals. In: Ribaut JM (ed) Drought tolerance in cereals. The Haworth Press Inc., Binghamtown, NY, USA, pp 97–144.

Mosisa, W., M. Banziger, G.S. Erley, D. Friesen, A.O. Diallo, and W.J. Horst. 2007. Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. Crop Science. 47, 519-528.

Pingali, P.L., and S. Pandey. 2001. Meeting world maize needs: technological opportunities and priorities for the public sector. In: Pingali, P.L. (ed) 1999/2000 World Maize Facts and Trends. CIMMYT, Mexico. pp 1-24.

Reiter, R.S., J.G. Coors, M.R. Sussman, and W.H. Gabelman. 1991. Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphism. Theoretical and Applied Genetics. 82, 561–568.

Ribaut, J.M, D.H. Hoisington, J.A, Deutsch, C. Jiang, and D. Gonzalez-de-Leon. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theoretical and Applied Genetics. 94, 887–896.

Ribaut, J.M., D.H. Hoisington, J.A, Deutsch, C. Jiang, and D. Gonzalez-de-Leon. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. 1.Flowering parameters and the anthesis-silking interval. Theoretical and Applied Genetics. 92:905-914.

Ribaut, J,M., and M. Ragot. 2007. Marker-assisted selection to improve drought adaptation in maize. The backcross approach. Perspective, limitations and alternatives. Journal of Experimental Botany. 58:351-360 doi:10.1093/jxb/erl214.

Semagn, K., Y. Beyene, M. Warburton, A. Tarekegne, S. Mugo, B. Meisel, P. Sehabiague, and B. Prasanna, 2013. Meta-analyses of QTL for grain yield and anthesis silking interval

1   in 18 maize populations evaluated under water-stressed and well-watered environments.

2   BMC Genomics. 14,313. doi:10.1186/1471-2164-14-313

3   Šimić, D., T. Ledenčan, A. Jambrović, Z. Zdunić, J. Brkić, A. Brkić, Mladenović, S. Drinić,

4   and I. Brkić. 2009. SNP and SSR marker analysis and mapping of a maize population.

5   Genetika. 41(3), 237 -246.

6   Song, M. T., J.H. Lee, Y.S. Cho, O.Y. Jeong, and H.G. Hwang. 2001. Construction of linkage

7   map and heading date related QTL analysis using the F2 population between Japonica

8   rices. Korean J. Genetics. 23, 401−406.

9   Tuberosa, R., S. Salvi, M.C. Sanguineti, P. Landi, M. Maccaferri, and S. Conti. 2002.

10   Mapping QTLs regulating morpho-physiological traits and yield: Case studies,

11   shortcomings and perspectives in drought-stressed maize. Annal of Botany. (Lond.)

12   89:941−963. doi:10.1093/ aob/mcf134.

13   Van Ooijen, J.W. 2006. Joinmap 4. Software for the calculation of genetic linkage maps in

14   experimental populations. Kyazma B.V. Wageningen, Netherlands

15   Wang, A.-y., Y. Li, and C.-q. Zhang. 2012. QTL mapping for stay-green in maize (Zea mays).

16   Canadian Journal of Plant Science. 92, 249-256.

17   Wang, H., K.P. Smith, E. Combs, T. Blake, R.D. Horsley, and G.J. Muehlbauer. 2012. Effect

18   of population size and unbalanced data sets on QTL detection using genome-wide

19   association mapping in barley breeding germplasm. Theoretical and Applied Genetics.

20   124(1), 111−124.

21   Wüʳschum, T. 2012. Mapping QTL for agronomic traits in breeding populations. Theoretical

22   and Applied Genetics. 125,201−210.

1   Xiao, J., J. Li, and D.D. Tanksley. 1996. Identification of QTLs affecting traits of agronomic

2   importance in a recombinant inbred population derived from a subspecific rice cross.

3   Theoretical and Applied Genetics. 92, 230−244.

4   Yan, J., H. Tang, Y. Zheng, S. Chander and J. Li. 2006. A genome scan for quantitative trait

5   loci affecting grain yield and its components of maize both in single-and two-locus

6   levels. Chinese Science Bulletin, 51(12), 1452-1461.

7   Yang J, C.C. Hu, H. Hu, R.D. Yu, Z. Xia, X.Z. Ye and J. Zhu. 2008. QTLNetwork: mapping and

8   visualizing genetic architecture of complex traits in experimental populations.

9   Bioinformatics. 24, 721-723.

10 Zaidi, P.H., Z. Rashid, M.T. Vinayan, G.D. Almeida, R.K. Phagna, and R. Babu. 2015. QTL

11   Mapping of Agronomic Waterlogging Tolerance Using Recombinant Inbred Lines

12   Derived from Tropical Maize (*Zea mays L*) Germplasm. PLoS ONE 10(4): e0124350.

13   doi:10.1371/journal.pone.0124350.

14 Zheng, H. J., A. Z. Wu, C.C. Zheng, Y.F. Wang, R. Cai, X.F. Shen, R.R. Xu, P. Liu, L.J. Kong,

15   and S.T. Dong. 2009. QTL mapping of maize (Zea mays) stay-green traits and their

16   relationship to yield. Plant Breeding. 128, 54–62.

17 Zhou, M. Z. 2010. Improvement of plant water logging tolerance In: Mancuso S, Shabala S,

18   eds. Water logging signalling and tolerance in plants. Heidelberg, Germany: Springer-

19   Verlag. pp267–285.

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1   **Figure Captions**

2   Figure 1. Figure 1 Frequency distribution of eight traits in BC_2F_1 population under high N

3   environment

4   Figure 2 Frequency distribution of eight traits in BC_2F_1 population under low N environment

5   Figure 3. Linkage map showing QTL on chromosomes 1, 4, 5, 6, 8 and 10 for six traits (GY,

6   ASI, DTS, SG, EPP and PHT).

Table 1. Mean squares of BC$_2$F$_1$ 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

DF: Degree of freedom; GY: Grain yield;   **DTS**: days to silk;   **DTA**: days to anthesis;   **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic;   *, **, Significant at 0.05 and 0.01probability levels, respectively, and ns, not significant **h$^{2:}$** Broad sense heritability

Table 2. Mean squares of BC$_2$F$_1$ 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

DF: Degree of freedom; GY: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height; **EPP**: number of ears per plant; **SG**: Stay green characteristic; *, **, Significant at 0.05 and 0.01 probability levels, respectively, and ns, not significant; **h^2;** Broad sense heritability

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

Trait	N level	QTL	Chromosome	Markers	Marker Interval	[a]Position	[b]Add	[c]LOD	[d]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

GY: Grain yield; **DTS**: days to silk; **ASI**: anthesis silking interval; **PHT**: plant height; **EPP**: number of ears per plant; **SG**: Stay green characteristic; **HN** = high N; **LN** = low N . [a]Position of peak marker in centiMorgans . [b]**Add**=Additive effect; - and + sign indicate favorable alleles came from CML494 and CML444, respectively; [c]**LOD** = log10 of odds ratio; [d]R^2 Percentage of phenotypic variation explained by QTL.

1 **Table 4. Comparison of QTLS 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 et al., 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., 2010
		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 et al., 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

2 §**GY**, Grain yield.

3 †**ASI**, anthesis silking interval.

4 ‡**PHT**: plant height.

5 ††**EPP**: number of ears per plant.

6

1   **Table 5: Epistatic (QTL×QTL) interactions for grain yield and its contributing traits under high and low nitrogen in BC$_2$F$_1$**
2   **maize population by QTL Network v2.1.**
3

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

4   *Without significant additive QTL for this trait but with significant additive QTL for other traits in the present study
5   [†]h^2(aa)(%) represents percentage of phenotypic variance explained by individual epistatic effects of the mapped QTL.
6   [‡]Types of epistatic (QQ) interaction: NN interaction between two loci with epistatic effects only whereas NA/AN represents interactions between a QTL
7   with additive effects and a locus without significant additive effects or vice versa, respectively.
[§]**GY**: Grain yield; **DTS**: days to silk; **SG**: Stay green characteristic; **ASI**: anthesis silking interval; **EPP**: number of ears per plant; **PHT**: plant height.
9

1

Supplementary Table 1. Soil chemical properties of experimental sites

Soil Properties	Ejura		Fumesua		Landon (1991) interpretation	
	0-15cm	15-30cm	0-15cm	15-30cm	High	Low
pH (1:1)	4.78	4.472	4.67	4.66	>6.5	<5.8
Organic C (%)	0.41	0.26	1.31	1.1	>10.0	<4.0
Total N (%)	0.03	0.02	0.12	0.11	>0.5	<0.2
Ex Ca (Cmolc/kg)	1.9	1.73	2.73	2.81	>10.0	<4.0
Ex Mg (Cmolc/kg)	1.24	1.4	0.53	0.6	>4.0	<0.5
Ex K (Cmolc/kg)	0.04	0.02	0.28	0.29	>0.6	<0.2
Ex Na (Cmolc/kg)	0.13	0.12	0.52	0.41	>1.0	<1.0
Av P (Mg/kg)	17.41	13.52	27.89	32.12	>50.0	<15.0
Second season N levels	0.04	0.04	0.13	0.12		

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3

1   **Suplementary Table 2. Correlation among traits under low- (above diagonal) and high-N (below diagonal)**
2   **environments**

Traits[§]	DTA	DTS	ASI	PHT	EHT	EPP	GY	SG
DTA	-	0.89**	0.18**	-0.49**	-0.43**	-0.00ns	-0.14**	0.19**
DTS	0.89**	-	0.61**	-0.46**	-0.33**	0.05ns	0.02**	0.40**
ASI	0.21**	0.62**	-	-0.15**	0.03	0.13**	0.28**	0.51**
PHT	-0.49**	-0.59**	-0.45**	-	0.79**	0.18**	0.37**	-0.09ns
EHT	-0.29**	-0.34**	-0.24**	0.53**	-	0.30**	0.53**	0.13**
EPP	-0.04ns	0.12**	0.35**	0.03ns	0.02ns	-	0.62**	0.59**
GY	-0.19**	0.12**	0.39**	0.16**	0.13**	0.51**	-	0.53**
SG	0.24**	0.49**	0.65**	-0.46ns	-0.24ns	0.36**	0.36**	-

3   [§]**GY**: Grain yield; **DTS**: days to silk; **DTA**: days to anthesis; **ASI**: anthesis silking interval; **PHT**: plant height; **EHT**: ear height;
4   **EPP**: number of ears per plant; **SG**: Stay green characteristic

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1 **Supplementary Table 3. Genetic map from 158 SNP markers for 150 BC$_2$F$_1$**
2 **population for CML 444 x CML 494**

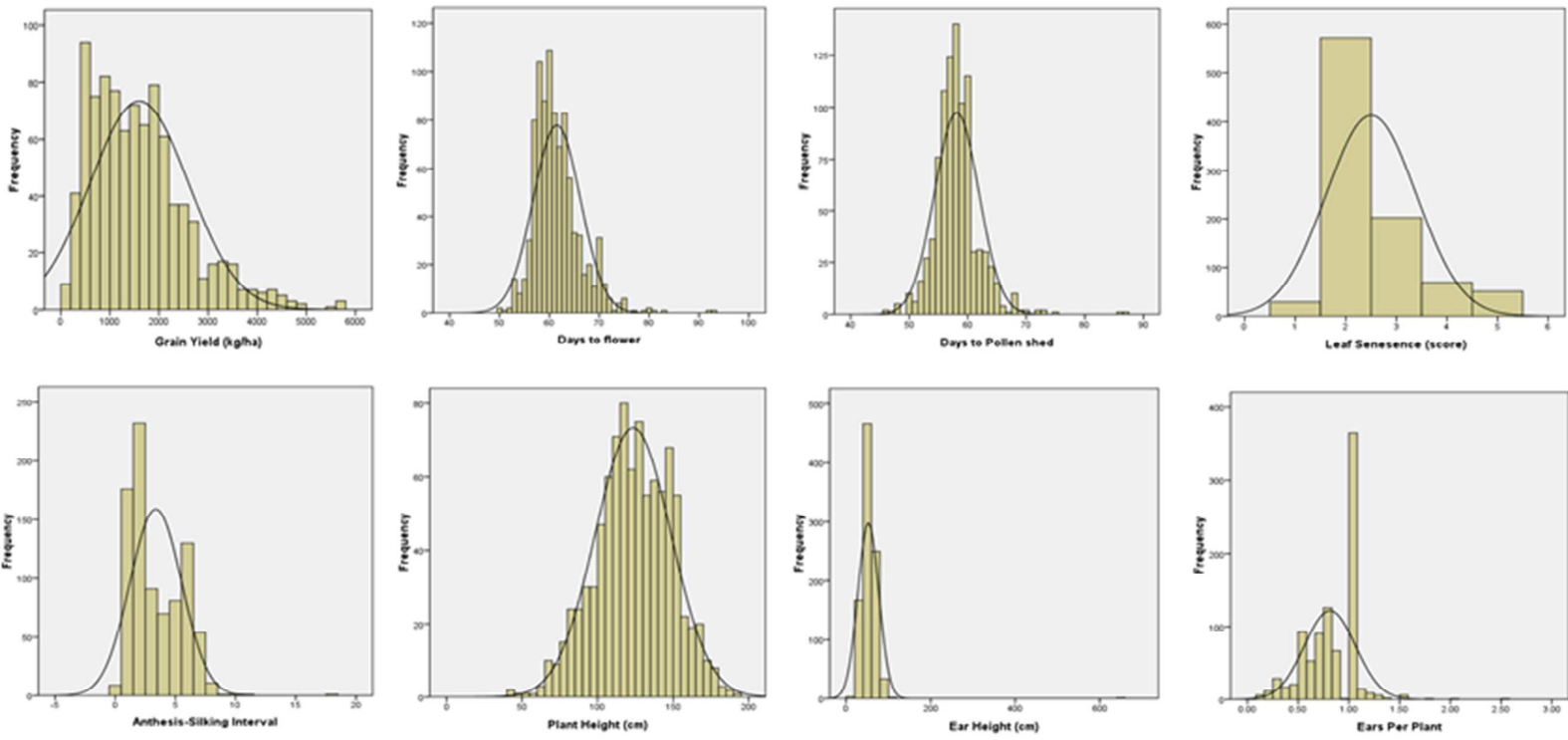| Linkage group	Length (cM)	Number of mapped markers	Marker spacing (cM)
LG 1 (Chr 1)	150.4	35	4.3
LG 2 (Chr 2)	118.5	15	7.9
LG 3 (Chr 3)	17.4	14	1.2
LG 4 (Chr 4)	41.5	12	3.4
LG 5 (Chr 5)	58.7	20	2.9
LG 6 (Chr 6)	32.7	20	1.6
LG 7 (Chr 7)	11.3	4	2.8
LG 8 (Chr 8)	88.3	16	5.5
LG 9 (Chr 9)	19.7	10	2.0
LG 10 (Chr 10)	84.2	12	7.0
Total/Avearge	622.7	158	3.9

Figure 1 - Frequency distribution of eight traits in BC_2F_1 population under high N environment
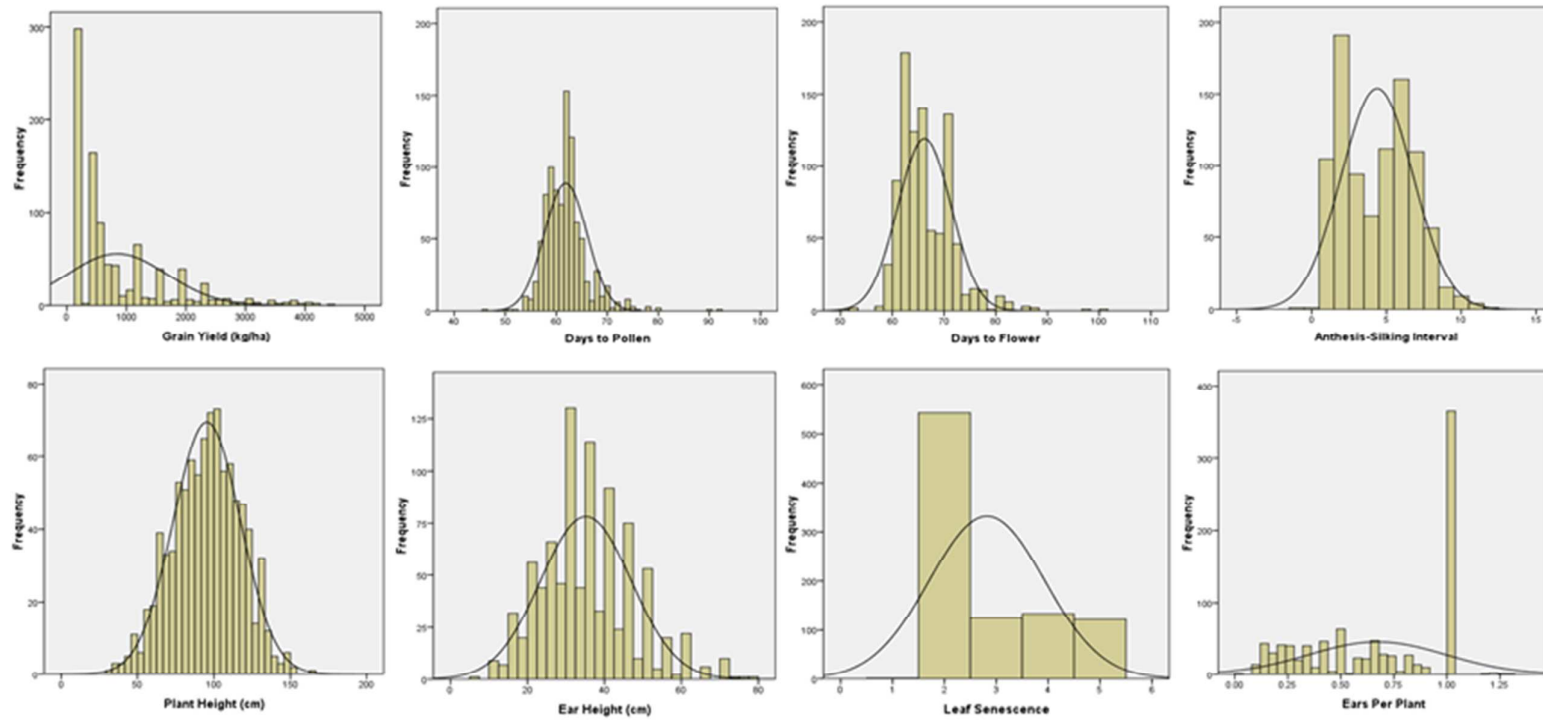
Figure 2 - Frequency distribution of eight traits in BC$_2$F$_1$ population under low N environment
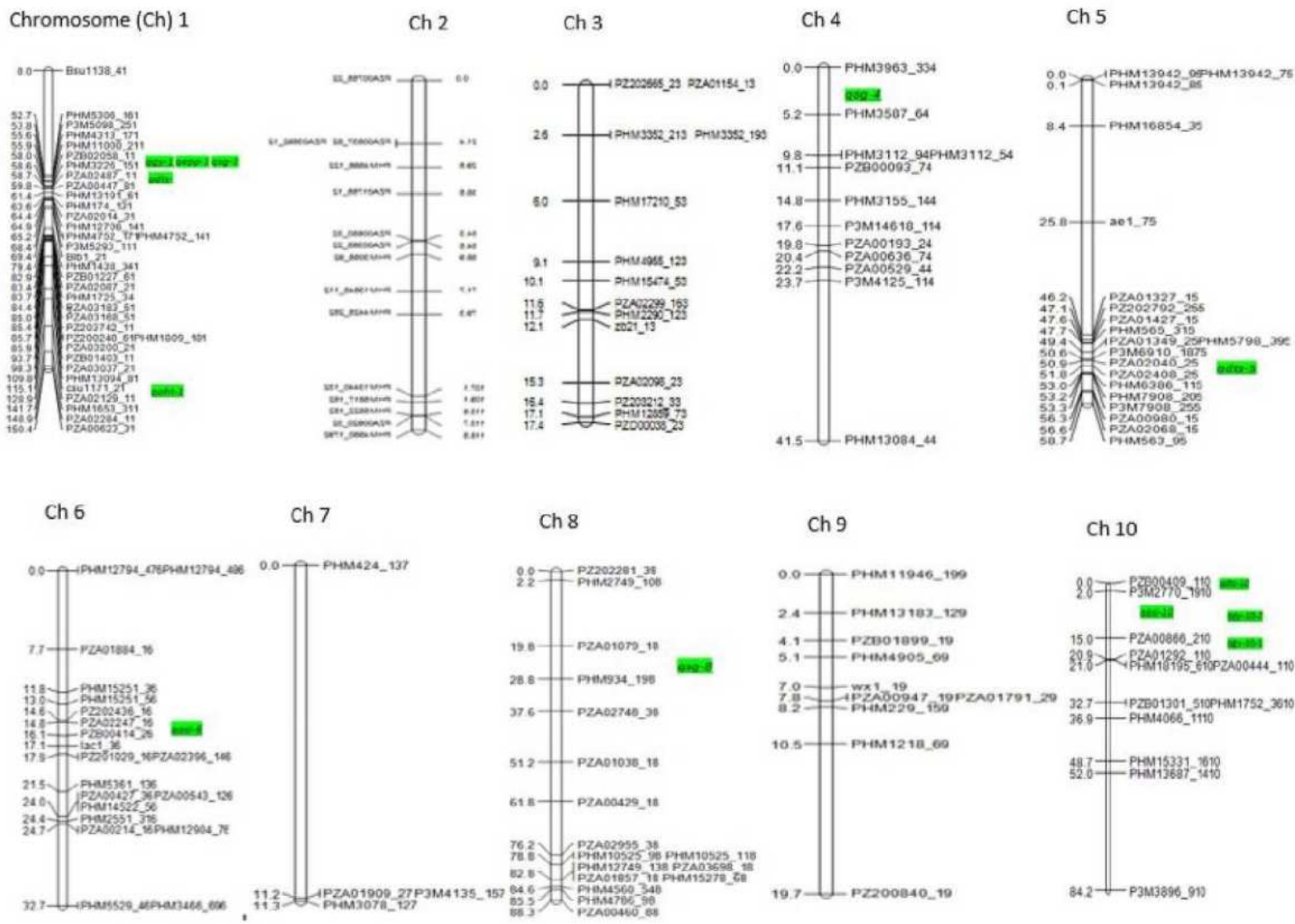
Fig. 3. Linkage map showing QTLs on chromosomes 1, 4, 5, 6, 8 and 10 for six traits (GY, ASI, DTS, SG, EPP and PHT)