(RESEARCH ARTICLE)

Trait-QTL-heritability of grain yield and other agronomic traits under low nitrogen conditions in bi-parental maize populations

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Abstract

Limited or low Nitrogen is a wanting abiotic stress in maize mainly in Sub-Sahara Africa, affecting yields and quality development of maize crop. As an approach to getting a breeding solution; mapping of QTLs and understanding the heritability factor can provide useful information and guide for breeders in developing low nitrogen resilient maize. QTL mapping which is a molecular breeding component forms an actual basis in estimation of genomic regions associated to the expression of quantitative traits, and how heritable are such traits. Conducting a selection for Low N-tolerance is challenging due to its complex nature with strong interaction between genotypes and environments; therefore, marker assisted breeding is key to improving such complex traits, but at the same time requires markers associated with the trait of interest. In this study, three bi-parental populations were subjected to either or both low and optimum N conditions to detect and determine the QTLs heritability for grain yield and other agronomic traits. Essential to the study; genotype by environmental interaction, significance and heritability was examined for each population with most traits expressing low (<0.2) and moderate to high heritabilities (0.3>). These QTLs with high heritabilities across environments will be of great value for rapid introgression into maize populations using marker assisted selection approach. The study was a preliminary and therefore require further validation on heritability and fine mapping for them to be useful in MAS.

Keywords: Heritability; QTL Mapping; Grain yield; Low nitrogen conditions

1. Introduction

Food security is a great challenge in the twenty first century in the developing world, particularly in sub-Saharan Africa. Maize is one of the most important food crops in SSA and is the staple food with expected demand to increase in a sharp gradient (Jayne et al., 2012).

The 2011-13 FAOSTAT showed that more than 2.1 million ha of Kenya's 5.3 million ha of all crops' harvested area was occupied by maize. In other words, maize accounts for 40% of all crop area in Kenya. Maize is grown throughout the world, although there are large differences in yields.

To fulfill this projected demand, maize production in the future has to be realized predominantly on the existing cultivated land, since an expansion of cultivated land is severely limited because of population increase, environmental concerns, urbanization and diminishing water resources (Smale et al., 2013).

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However, other aspects that limit maize production in Kenya and in Africa in general include major abiotic stresses listed as; diseases and devastating pests (Kari, 2012; Wokabi, 2013). However, other factors include limited production resources and inconsistent marketing system of maize crop output.

Nowadays, limited nitrogen available in the soil has forced some of the farming communities to do their farming under nitrogen deficient conditions; this happens due to expensive acquisition of Nitrogen fertilizers and its limited availability(Rochiman & Purnobasuki, 2013), the situation has seen many farmers record very low yields down to approximately 1-1.5 t/ha (Gondwe, 2014).

On the other hand application of above optimum or excessive synthetic-N has now become a concern due to accumulation of a large amount of nitrogen in ecosystem. This leads to significant direct and indirect negative effects on environmental quality, biodiversity decrease and negative impact to human health (Hao et al., 2008). This is because only about 33% N fertilizer removed by plant and the rest lost by leaching, denitrification, and volatilization. Under tropical conditions, maize crop only absorb about 40-50% N fertilizer.

Incomplete uptake and poor conversion of N fertilizer causes global warming through emissions of nitrous oxide (N_2O). On the other hand, low nitrogen conditions lead to poor crop growth and yield, reduced shoot biomass production, chlorosis, leaf discoloration, and impaired reproduction (Hirel et al., 2001). Optimizing N fertilization and planting genotypes with better Nitrogen use Efficiency (NUE) are the ways to overcome the N application problems (Han et al., 2016).

Another major challenge in understanding the adaptive nature of maize genotype for it to perform well under low nitrogen condition will require sequential approaches which include phenotypic approach which involve morphological analysis of the maize key components (traits) expressing maximum utilization and mobilization of organic and inorganic N. However, This is not enough because the above approach is subject to influence by both the genotype, environment and the interaction, and therefore it may not reflect exactly the genotypic influence in utilizing N (Carvalho et al., 2012).

Generally, Mineral N applied does not always correspond to an increase in yield; this indicates that not all applied N is utilized by the crop. Some of N applied to maize in most cases has not been accounted for meaning a substantial amount is lost through the system (Hirel, 2001). Nitrogen use efficiency technology is worth exploring to address the challenges of limited accessibility to N fertilizers among the resource poor farmers. Nitrogen use efficiency (NUE) technology produces crops with yields comparable to conventional varieties which on the contrary require significantly less nitrogen. Even though breeders have sought to breed for N efficient genotypes to improve yields among small-scale farmers, specific information on particular N efficient genotypes is still lacking.

Contrary to farmers' practice, most breeding programs in the region develop new varieties under optimally managed on-station experimental plots (Collins et al., 2008). The genetic mechanism for grain yield under optimum and low N stress conditions are different, and varieties developed for optimal environments often responds differently under N limiting environments (Das & Harikrishnan, 2015).

Ideally breeding for low nitrogen tolerance is a polygenic trait and typically has low heritability and characterized by high genotype x Environment interaction (GEI); therefore its complexity in nature gives breeders a hard-time to achieve.(Bertrand Hirel, Bertin, et al., 2017)

Breeders are highly involved in improvement of maize based on its uses; therefore, quality and quantity are enhanced through development of new technologies in the breeding industry. The cutting age technologies utilize precision and accuracy with maximum optimization of time, based on the market demand for the product. The complexity nature of breeding maize under low nitrogen conditions has necessitated the application of Quantitative trait loci (QTL)-based approaches which can contribute significantly to the understanding of the genetic basis of crop performance and stability under low nitrogen conditions. (Semagn et al., 2014)

Most traits of interest in plant breeding show quantitative inheritance, which complicate the breeding process since phenotypic performances only partially reflects the genetic values of individuals. The genetic variation of a quantitative trait is assumed to be controlled by the collective effects of a quantitative trait loci (QTLs), the environment, and interaction between QTL and Environment (Semagn et al., 2010).

Exploiting molecular markers in breeding has proved to enhance the finding of a subset of markers associated with one or more QTLs that regulate the expression of complex traits. The molecular markers therefore, form the concrete basis for the genomic selection after sequential mapping of the desired population (Würschum, 2012)

studies conducted for the last twenty years, identified QTLs that generally explained a significant portion of the phenotypic variance, and therefore gave rise to an optimistic assessment of the prospects of markers assisted selection (Semagn et al., 2010). The advancement to the technology has been enhanced with the availability of whole genome sequence information in maize, most Single Nucleotide Polymorphic Markers (SNPs) have been physically anchored and are very useful for linkage mapping and QTLs identification in maize(Sabadin et al., 2008).

Set against the backdrop of nitrogen deficiency; physiological and genetic backgrounds are necessary in development of low nitrogen tolerant maize lines with excellent NUE under same conditions(R. R. Pathak et al., 2011)

Efforts are currently underway to improve NUE through a variety of strategies. These include profiling the transcriptional response of N metabolism-related genes to diverse stimuli, and mapping of quantitative crop phenotypes to loci associated with metabolic genes also linked to grain yield (Amiour et al., 2012).

The success of modern molecular breeding has stimulated interest in adopting other state-of-the-art scientific strategies to this traditional agricultural problem. therefore it will become a major importance to select for maize cultivars that absorb and metabolize nitrogen in the most efficient way for grain production(Ravi Ramesh Pathak et al., 2008).

According to (Coque & Andreallais, 2006), the detected marker QTL associations in maize plant revealed the consistency of the involvement of some traits, such as root architecture and glutamine synthase activity, which would be of major importance for grain yield setting whatever the nitrogen condition.

Specifically, this study has grounded its data on a double objective of identifying the genomic regions associated with grain yield and other agronomic traits, estimation of their effect phenotypically and their heritability status under optimum and low nitrogen management in multiple biParental populations.

2. Material and methods

2.1. Plant materials

Four F3 tropical maize populations were phenotypically evaluated and mapped under two Kenyan locations. The populations were developed by the Global Maize Program of CIMMYT. Population 1(CML494/CLWN201) constituted of 330 $F_{2:3}$ developed by selfing the F_2 plants; Population 2 (CKL5017/CML536) constituted of 271 $F_{2:3}$ developed by selfing F_2 plants; Population 3 (VL081452/VL058589) consisted of 158 lines. These lines are adapted to mid-altitude regions (1000-1500 M Above sea level) of sub-Saharan Africa. The lines are bred on good-by-good basis, adapted to stress conditions.

Generally, the CML lines have been test crossed to CML 312 and CML 395 as testers for phenotypic evaluations.

The CIMMYT lines utilized for the project were derived from breeding programs targeting tolerance to low nitrogen; hence best choice for QTL mapping with most of the lines included in two association Mapping panel (AM) constituted under IMAS (improved maize for Africa soils) and DTMA (Drought Tolerant Maize for Africa) (Semagn et al., 2012). The four populations formed set of multiple Bi-parental populations used for the project.

2.2. Field trial

CIMMYT lines were identified as suitable candidate for this project and were evaluated for response to Low N conditions at two different locations; Kiboko station (Longitude 37^0 E, Latitude 2^0 S, 975M A.S.L), Embu station (Longitude 37^0 E, Latitude 3^0 S, 1560M A.S.L) with an average annual rainfall of 1200mm per. The lines were evaluated on an alpha lattice incomplete block design under two N levels. The two N treatments are low N (N-depleted field/plot) and at normal farmer practice conditions (optimum N 200 kg/Ha).

Single row plots measuring 5m long at 0.75m row spacing was sown at a density of 50 seeds for each Bi-parental line. All entries were planted on the same day in conventionally tilled plots and maintained under rain fed and irrigated conditions.

2.3. Data collection

2.3.1. Phenotyping of important agronomic traits

Four plants were randomly selected for each genotype for phenotypic evaluation of major agronomic traits associated with Nitrogen use. Phenotypic components measured and analyzed included: plant height (from base of the plant to tip of the head, in centimeters), days of anthesis silking (number of days from planting to 50% anthesis), Silking date (recorded as the number of days from sowing until silks had emerged on at least 50% of the plants, Anthesis silking interval (ASI) Was calculated as the difference between SD and AD (J. Bolanos and G.O. Edmeades, 1996)Mature ears were; harvested dry, manually bagged, air dried and shelled on an electric shelling device. Total grain yield of each plot was weighed on an electronic balance and converted to (GY in t/ha) by dividing the total grain weight per plot by the plot area, plant aspect and ear Aspect were calculated and recorded from Normal-N and low-N fields.

Best linear unbiased predictors (BLUPs) were estimated, considering genotype entries as fixed terms and the rest of the terms as random. Estimating broad sense heritability, all the terms were considered random. Broad sense heritability was estimated by the formula:

$$h^2 = \sigma^2_g / (\sigma^2_G + \sigma^2_{GE}/l + \sigma^2/lr)$$

Where σ^2_g is the genotypic variance, σ^2_{GE} is the Genotypic by environment interaction (GEI), σ^2 is the error variance, l is the number of environments and **r** is the number of replications in each trials. The phenotypic and genotypic correlations among traits were evaluated as described by (Bertrand Hirel, et al., 2017)

2.4. Genotype analysis

Total DNA was extracted from bulked young leaves of the lines according to the CTAB Method (CIMMYT, 2005), and the DNA quality for each sample was checked using gel-electrophoresis and spectrophotometer (NanoDrop ND8000 Thermo Scientific) before genotyping. Genotyping was performed using the Illumina MaizeSNP1500 Bead Chip evenly spaced SNP to cover the whole maize genome (Ganal et al., 2011). The above task was performed at LGC genomic labs in UK (https://www.lgcgroup.com/genotyping/) Linkage maps in all the four populations were constructed using QTL IciMapping version 3.2 software (http://www.Isbreeding.net) using the twin criterion of more than 2.5 logarithm of odds(LOD), and the recombination frequency between linked loci were transformed into Cm(CentiMorgan's units) using Kosambi"s mapping function(KOSAMBI, 1943)

Two procedures were carried out to identify QTL in each population: (1) mapping QTL for each individual environment, (2) Mapping stable QTL across all environments. In all procedures, the progressive step in QTL scanning was 1 cM and LOD threshold of 3.0 was checked for declaring putative QTL(Ribaut et al., 1997).

3. Results

3.1. Evaluation of the populations

Population 1 (CML494XCLWN201) generally exhibited a good heritability on grain yield (GY), Anthesis date (AD), in optimum environment and uniquely consistent heritability in Low N conditions. However, a good heritability was recorded on grain yield (GY), Anthesis date (AD), plant height (PH), ear position (EPO) with low heritability recorded on plant aspect (PA) and ear aspect (EA). Table 1a

Under low N condition in population 2 (CKL05017X CML 536); recorded a lower heritability on grain yield (GY), but a moderate heritability on anthesis date (AD), plant height (PH), ear height (EA) and on ear position (EPO) as shown on table x. Still on population two there is a moderate heritability on grain yield and low heritability >3.0 on other measured traits.

Across population two, measured traits exhibited low heritability n grain yield (GY) but good heritabilities on plant height, ear height and ear position and moderate heritability on plant Aspect.

In population three, under low nitrogen conditions showed a moderate heritability on grain yield, Anthesis date (AD) and plant height.

Table 1a A summary statistic table on trait heritability, variance, grand mean and genotype significance under optimum and low nitrogen condition for population

CML494XCLWN201 (POP 1)									
Environment (EMBU)	**Statistic**	**GY**	**AD**	**ASI**	**PH**	**EH**	**Epos**	**EA**	**PA**
Opt N	Heritability (h²)	0.43	0.44	0.13	0.28	0.26	0.32	0.24	0.22
	Genotype Variance	0.38	1.08	0.09	56.64	14.13	0.00	0.02	0.02
	Residual Variance	0.98	2.78	1.16	285.76	80.72	0.00	0.15	0.11
	Grand Mean	5.21	75.40	0.84	248.13	112.06	0.45	2.42	2.49
	LSD	0.94	1.60	0.54	12.93	6.55	0.02	0.27	0.22
	CV	19.06	2.21	127.62	6.81	8.02	6.86	16.29	13.49
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.00	0.00	0.20	0.01	0.02	0.00	0.01	0.04
Environment (KIBOKO)									
LN	Heritability (h²)	0.48	0.43	0.02	0.54	0.58	0.49	0.10	0.14
	Genotype Variance	0.22	0.60	0.01	16.56	10.12	0.00	0.01	0.01
	Residual Variance	0.49	1.62	0.61	28.04	14.60	0.00	0.21	0.16
	Grand Mean	3.58	64.03	0.67	130.46	39.56	0.30	2.49	2.49
	LSD	0.68	1.21	0.16	5.80	4.34	0.03	0.20	0.21
	CV	19.47	1.99	116.18	4.06	9.66	8.34	18.48	16.13
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.00	0.00	0.85	0.00	0.00	0.00	0.36	0.20

Table 1b A summary statistic table on trait heritability, variance, grand mean and genotype significance under optimum and low nitrogen condition across environment in population 1

CML494XCLWN201(POP 1)								
Environment KIBOKO vs EMBU	**Statistic**	**GY**	**AD**	**PH**	**EH**	**Epos**	**EA**	**PA**
	Heritability (h^2)	0.44	0.40	0.31	0.38	0.50	0.29	0.25
	Genotype Variance	0.19	0.49	21.49	8.52	0.00	0.02	0.01
	GenxLoc Variance	0.11	0.36	15.23	4.14	0.00	0.00	0.00
	Residual Variance	0.74	2.19	156.78	47.22	0.00	0.18	0.14
Across environment	Grand Mean	4.39	69.71	189.29	75.81	0.38	2.45	2.49
	LSD	0.65	1.10	7.76	4.69	0.02	0.23	0.19
	CV	19.52	2.12	6.61	9.06	7.45	17.42	14.83
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	n Environments	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	GenxEnv significance	0.01	0.01	0.11	0.16	0.30	1.00	0.63

Table 2a A summary statistic table on trait heritability, variance, grand mean and genotype significance under optimum and low nitrogen condition for population 2 on individual environments

CKL5017XCML536 (POP 2)									
Environment KIBOKO	**Statistic**	**GY**	**AD**	**ASI**	**PH**	**EH**	**EPO**	**EA**	**PA**
LOW N	Heritability (h^2)	0.22	0.42	0.25	0.47	0.51	0.43	0.10	0.10
	Genotype Variance	0.08	1.00	0.31	19.37	14.38	0.00	0.01	0.02
	Residual Variance	0.55	2.75	1.80	43.83	27.92	0.00	0.22	0.26
	Grand Mean	2.77	68.36	3.08	166.04	70.68	0.43	2.66	2.62
	LSD	0.49	1.57	0.96	6.63	5.49	0.02	0.21	0.23
	CV	26.75	2.43	43.59	3.99	7.48	6.10	17.70	19.59
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.07	0.00	0.02	0.00	0.00	0.00	0.42	0.41
Environment KIBOKO									
OPT N	Heritability (h^2)	0.40	0.28	0.04	0.51	0.28	0.06	0.01	0.11
	Genotype Variance	0.33	0.63	0.03	48.78	15.33	0.00	0.01	0.01
	Residual Variance	0.98	3.30	1.30	92.51	79.91	0.00	1.09	0.17
	Grand Mean	8.95	60.82	0.69	261.76	143.78	0.55	2.44	2.67
	LSD	0.91	1.37	0.33	10.26	6.71	0.01	0.16	0.20
	CV	11.06	2.99	164.63	3.67	6.22	4.93	42.67	15.66
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.00	0.03	0.74	0.00	0.02	0.61	0.93	0.41

Table 2b A summary statistic table on trait heritability, variance, grand mean and genotype significance under optimum and low nitrogen condition for population 2 across environments

CKL5017XCML536 (POP 2)							
Environment (KIBOKO) LN vs OPT N	Statistic	GY	AD	PH	EH	EPO	PA
Across environment	Heritability (h^2)	0.13	0.29	0.56	0.60	0.45	0.27
	Genotype Variance	0.04	0.39	26.95	19.00	0.00	0.02
	GenxLoc Variance	0.17	0.44	8.31	0.00	0.00	0.00
	Residual Variance	0.75	3.03	67.90	50.21	0.00	0.21
	Grand Mean	5.86	64.59	213.90	107.23	0.49	2.64
	LSD	0.37	1.06	7.14	5.67	0.02	0.24
	CV	14.80	2.69	3.85	6.61	5.43	17.44
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00
	n Environments	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.32	0.02	0.00	0.00	0.00	0.01
	GenxEnv significance	0.00	0.04	0.08	1.00	1.00	1.00

Table 3 A summary statistic table on trait heritability, variance, grand mean and genotype significance low nitrogen condition for population 3

VL081452Xvl058589 (POP 3)								
Environment (KIBOKO)	Statistic	GY	AD	ASI	PH	EH	EPO	EA
LN	Heritability (h^2)	0.30	0.57	0.17	0.46	0.17	0.04	0.00
	Genotype Variance	0.13	0.71	0.18	33.91	5.57	0.00	0.00
	Residual Variance	0.59	1.09	1.85	78.87	52.60	0.00	2.88
	Grand Mean	3.36	60.85	2.30	180.63	86.04	0.48	2.65
	LSD	0.61	1.14	0.78	8.86	4.30	0.01	0.00
	CV	22.86	1.71	59.18	4.92	8.43	6.02	64.09
	n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00
	Genotype significance	0.05	0.00	0.30	0.00	0.33	0.83	1.00

The combined analysis of variation on genotype by environment interaction (GEI) in all the populations showed significance on grain yield, anthesis date, anthesis silking interval (ASI), plant height, ear height, ear aspect and plant Aspect; the variation was far much greater under low nitrogen condition.

The percentage trait CV's exhibited was cumulative of individual plant; hence exhibiting inconsistent low to large figures. Clear observation on Anthesis silking interval (large figure) and plant height (low figure).

Across the correlation tables under low nitrogen condition, negative correlation was recorded on Grain yield against Anthesis date and Anthesis silking interval, generally Low N reduced grain yield significantly and increased anthesis silking interval (ASI) and Anthesis date (AD).
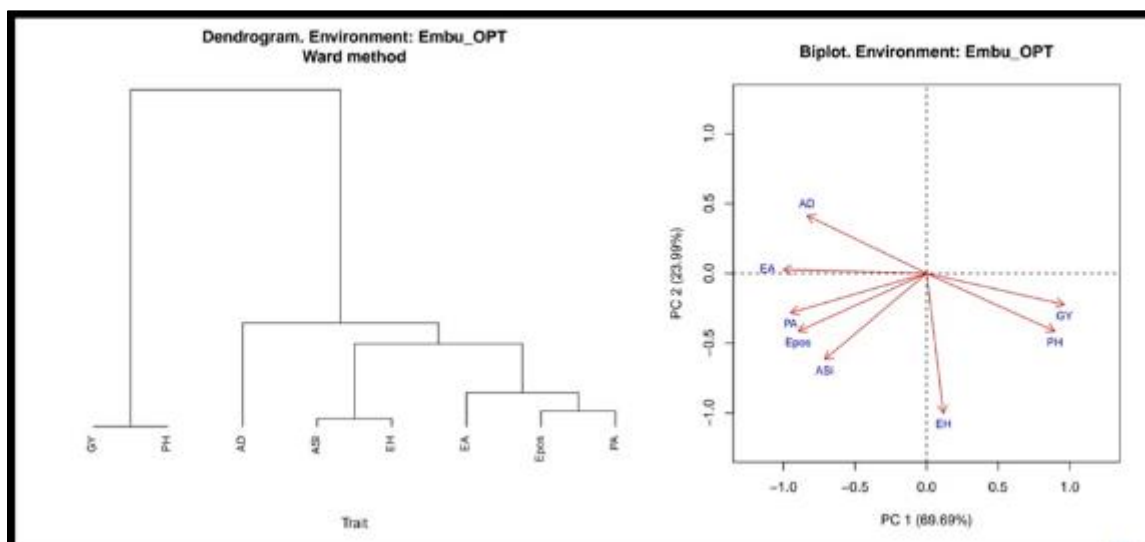
Figure 1 Cluster and Principal Component analysis for grain yield and other agronomic traits in population 1
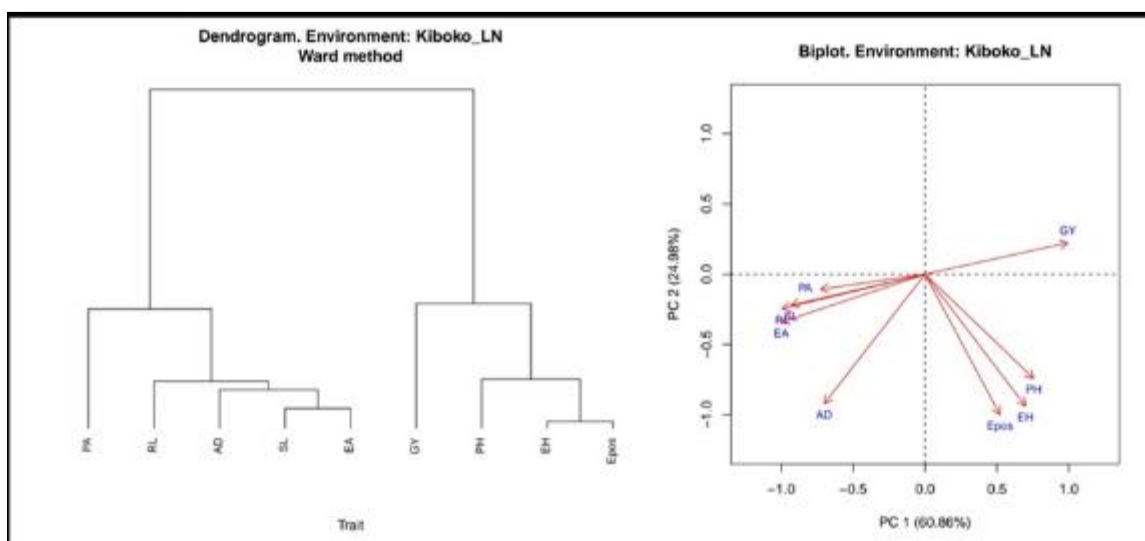
Figure 2 Cluster and Principal component analysis for grain yield and other agronomic traits in population 2
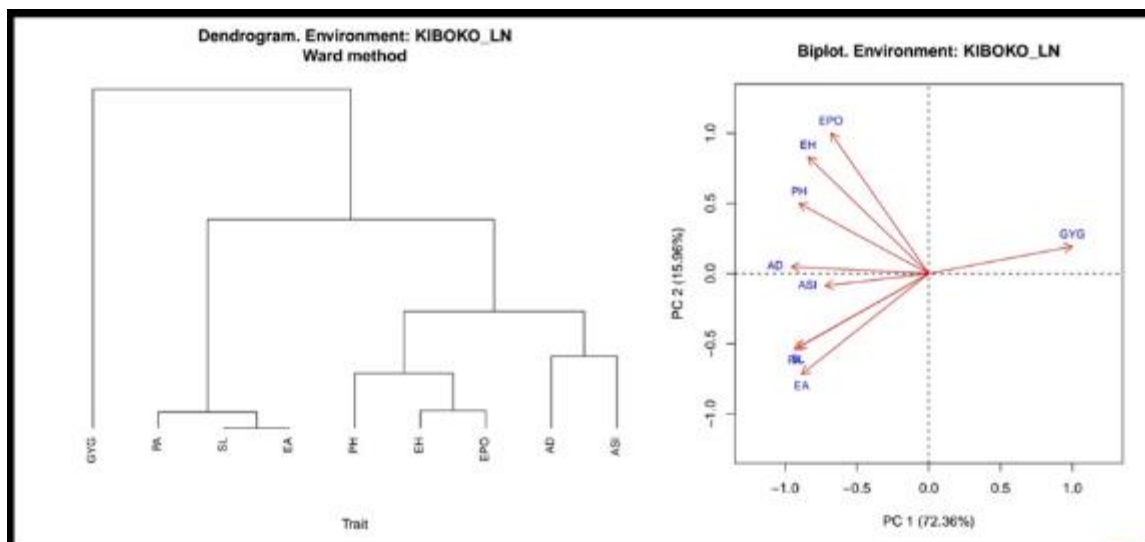
Figure 3 Cluster and principal component analysis for grain yield and other agronomic traits in population 3

4. Discussion

Nitrogen stress and optimal N conditions expressed diverse heritability estimates and correlations between grain yield and other agronomic traits, Considering Low N; is one of the major constraints hindering the optimization of high yields per unit area (Wu et al., 2011).

In-depth understanding of grain yield and other related agronomic traits will be a plus in coming up and development of low N resilient cultivars (Derera et al., 2008). Phenotypic characterization of such complex traits is very challenging due to unpredictable environmental and edaphic conditions (XiaoHong Liu et al., 2010).

Selection in Nitrogen stress conditions is very inconsistent and will not give correct phenotypic data thus a failed breeding that will not give substantive results for farmer adoption(Almeida et al., 2013).

With the integration of precise genomic tools coupled with conventional breeding, would accelerate the development of N-stress adaptive cultivars with high yields under Low N conditions.

In this research, two typical environments which include low N and optimal N served as basis for identification of QTLs related to grain yield and other agronomic traits (PH, EH, EPo, Epp, ASI, AD) and their cross-cutting heritability's of the three maize populations under study.

The target traits measured followed a normal distribution suggesting that the F3 population is stable and suitable for QTL mapping (X. Li et al., 2003)

In nearly all the studied traits, there was a significant environmental variation in their output moreso on grain yield, plant height and ASI indicating adaptive differences in the test environments. Several scientists have highlighted major variations in response of maize to environmental stresses (Derera et al., 2008).

In population 1, grain yield exhibited low heritability under low N conditions, this result is backed up with significant genotypic by environment interaction (GEI), results are in agreement with the findings of (Abate T, Menkir A, MacRobert JF, Tesfahun G, Abdoulaye T, Setimela P, Badu-Apraku B, Makumbi D, Magorokosho C, 2013).

Although the heritabilities of all the traits were moderate to low, it is significant enough to aid in indirect selection for increased grain yield under stress N conditions; results consistent with the findings of (Monneveux et al., 2005).

Generally, across the two environments, the most responsive trait was ASI; N-stress condition always increased genetic variance for ASI. This clearly shows the adaptive nature of this trait (Ba, 2014).

Across the three populations there was almost a consistent moderate heritability on all the studied traits in both Low N and optimum N; but population 1 showed higher heritabilities in almost all traits. This imply stability of an N tolerant genotype across diverse environment.

The pairwise genetic correlation showed a strong negative correlation between ASI and grain across population 3 under low These relationships are tandem to the study of (Ngugi, 2013)(Westgate et al., 2013). The negative correlation between Grain yield, ASI and AD is influenced by the co-location of their QTLs on chromosome as detected in Chr 4 for grain yield and Chr 1 for Anthesis Silking Interval and Chr 5 for Anthesis date in population 1 under low N.

In population three under Low N (Grain yield QTL detected on Chr 3, Anthesis date on Chr. 5 and ASI on Chr.4. The above QTLs are expressing pleiotropic effects explaining the negative correlation observed among these three traits (R. Liu et al., 2012). Overlapping of genomic regions for grain yield and ASI were reported by (Almeida et al., 2014a). These correlations suggest the high chances of tightly linked loci controlling Low-N tolerance through coordinated expression of these traits.

Other Agronomic traits showed positive genomic correlations; for instance in the case of plant height, ear height and Ears per plant (Epp) had a strong correlation to grain yield suggesting that mapping of the positively correlated traits is a hot-spot for yield related traits and even introgressing of this localized regions into other maize genotypes will lead to improved maize varieties with high yield potentials (reported in numerous studies; recent studies being done by (Abate T, Menkir A, MacRobert JF, Tesfahun G, Abdoulaye T, Setimela P, Badu-Apraku B, Makumbi D, Magorokosho C, 2013; Ba, 2014; Z. Q. Li et al., n.d.; Sehgal et al., 2017)

The variation on genotype by environment interaction (GEI), all the populations showed significant variations Table 4, 5 and 6; however the variation was far much greater under Low N condition. This is a clear indication of susceptibility of grain yield related traits to be influenced by stress (Badu-Apraku et al., 2016; Wu et al., 2011). The inconsistency and high genetic and phenotypic variation has been a major bottle-neck for Low N breeding program in Maize (Bänziger et al., 1997; Geiger, 2009).

5. Conclusion

Cross-cutting observation in all the populations showed that heritability and genetic variance for grain yield usually decreases under abiotic stress as yield levels fall

Also, peak genotypic by environment interaction involved in stressed environments produce rankings that differ significantly from one trial to another making it hard to identify the best germplasm.

Heritability and correlation are basic indicators of germplasm performance. However, in the two environments (Low and optimum N) the results on the indicators were very inconsistent; this is clear that as severity of N stress increases, the correlation between genotype performance under low and optimum N conditions diminishes.

Other populations (2 and 3) also had favorable alleles and even the colocalization of QTLs for grain yield and other agronomic traits is key indicator of their strong association.

Observation

Based on the results obtained from the study, breeding for N-stress tolerance require a stable population for mapping and heritability study; therefore, it is recommended that breeding under N-stress conditions require controlled environment to minimize environmental co-variants that compromise the mapping and heritability.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

No conflict of interest.

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Appendices

Appendix I Summary of all chromosomes hosting the QTLs identified for each trait across the three populations in Low and optimum N condition.

Traits	Mapped Chromosomes				
	Population 1		Population 2		Population 3
	Optimum N	Low N	Optimum N	Low N	Low N
Grain yield	1, 10	4	2, 11	7	3
Anthesis date	NA	5	1, 2, 3, 5, 6, 8, 9, 10	2, 11	5, 7, 10
Anthesis silking interval	NA	1	NA	NA	4
Plant Height	11	1 , 3, 7, 11	1, 5, 8, 9, 11	6, 8, 9, 11	1, 2, 3, 8, 9
Ear Height	1, 6	7, 8, 11	1, 3, 5, 8, 9, 11	2, 3, 4, 5, 7, 8, 9	2, 9
Ear Position	6, 8	6, 8, 11,	NA	1, 4, 11	NA
Ear Aspect	6	NA	NA	NA	NA

Appendix II QTL maps showing individual trait qtls in population 1 under optimum N condition.
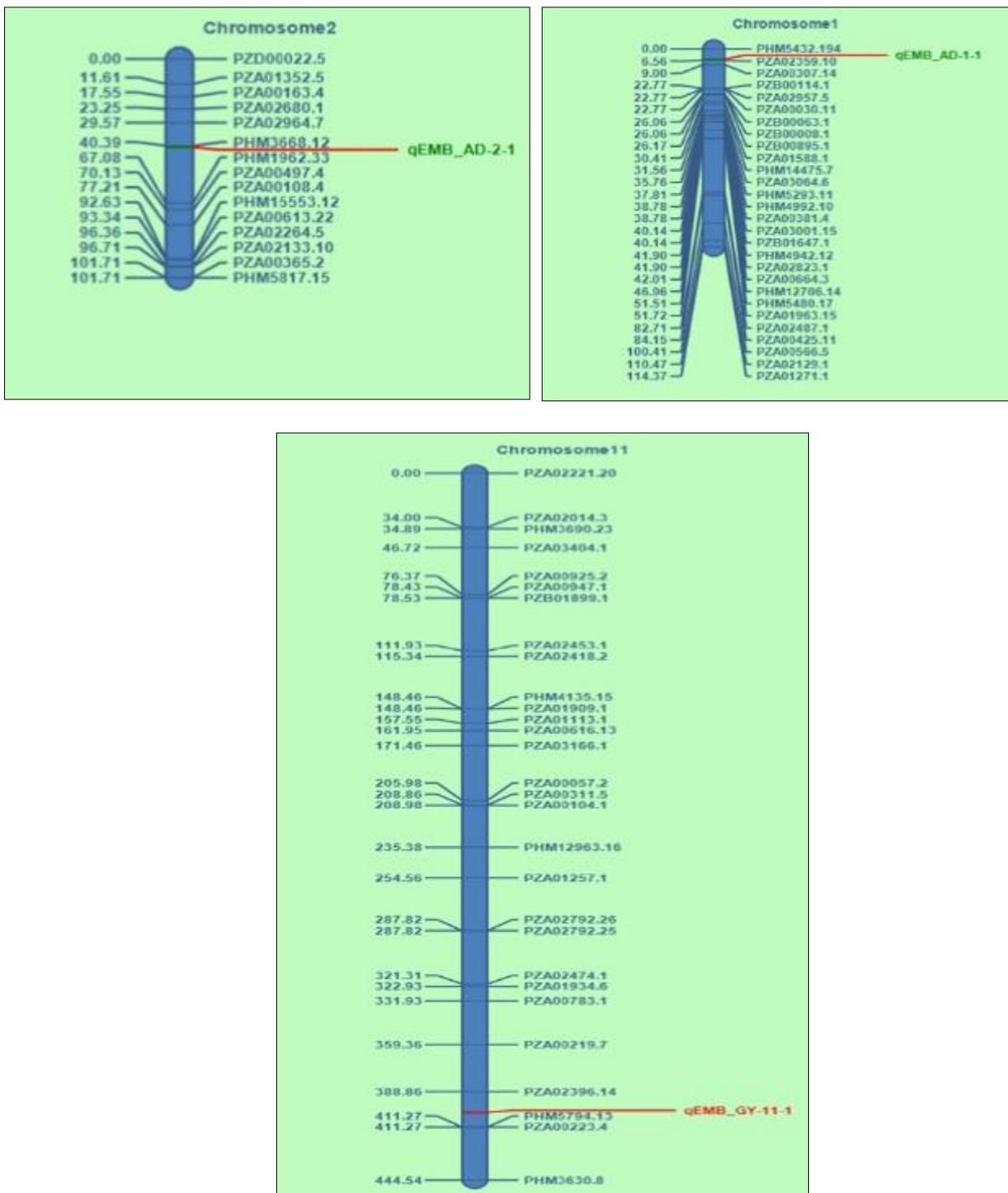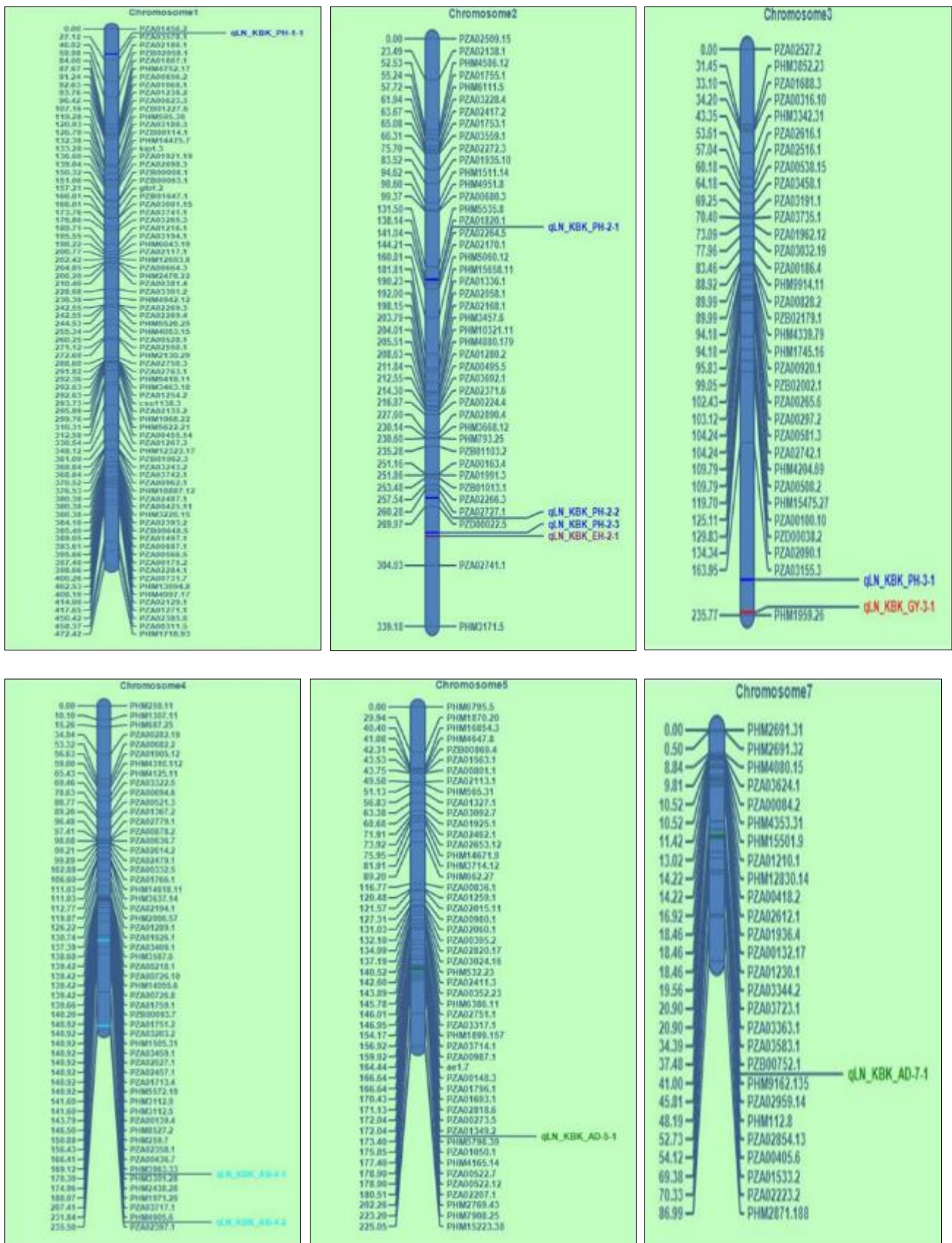
Appendix III QTL maps in population 2
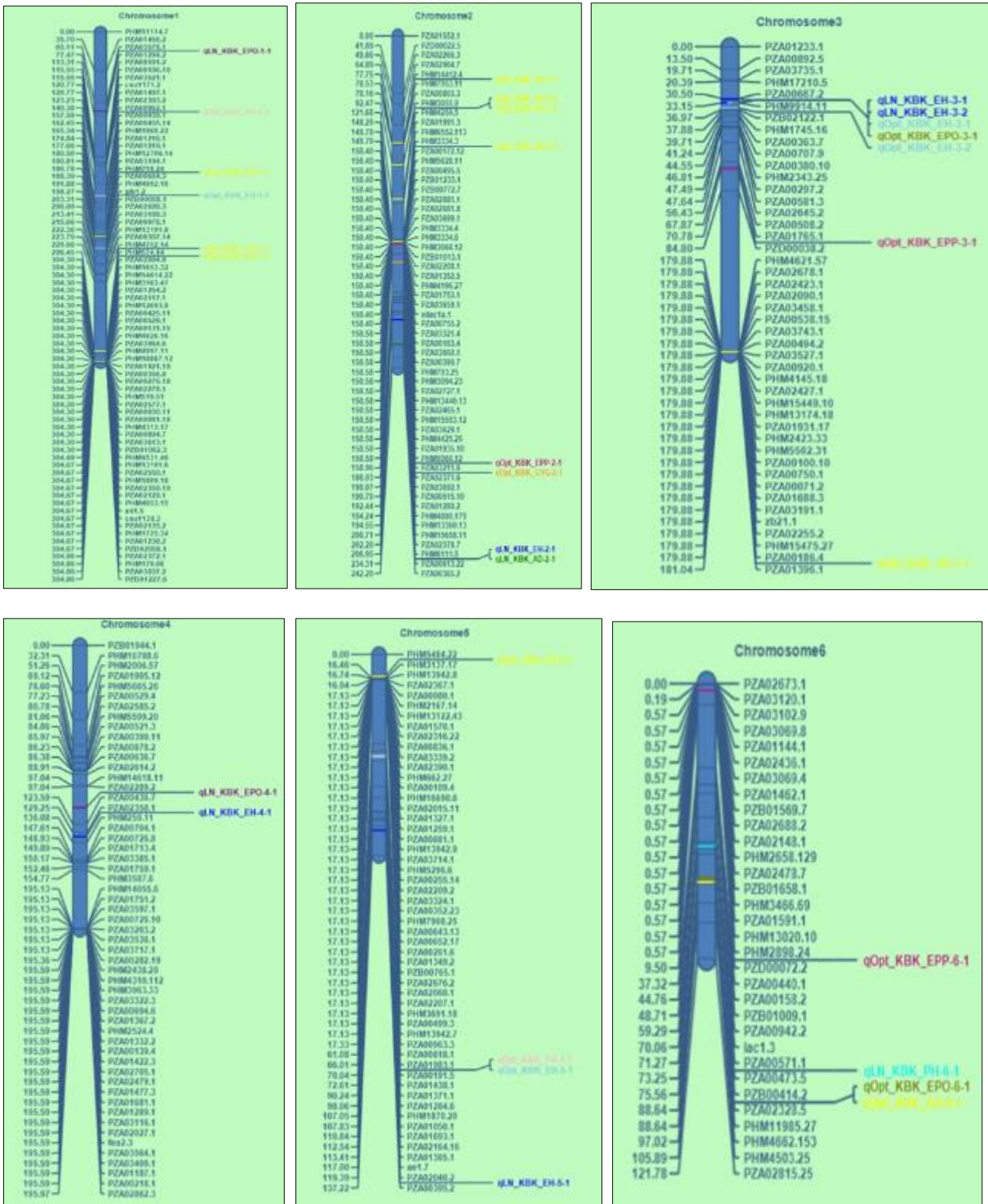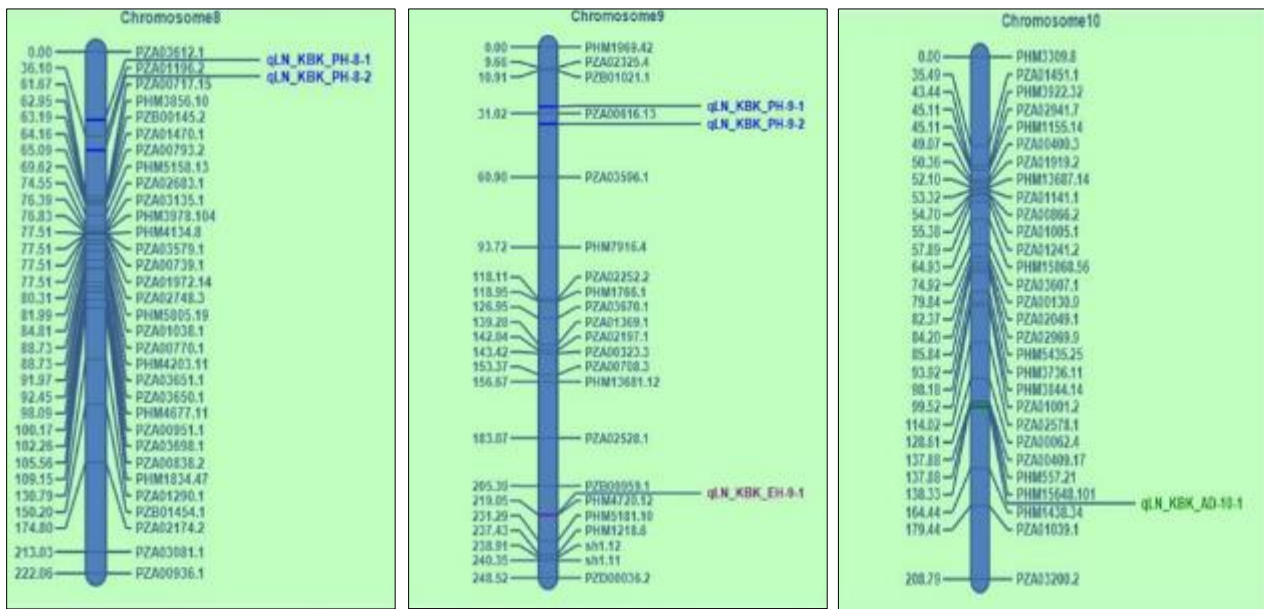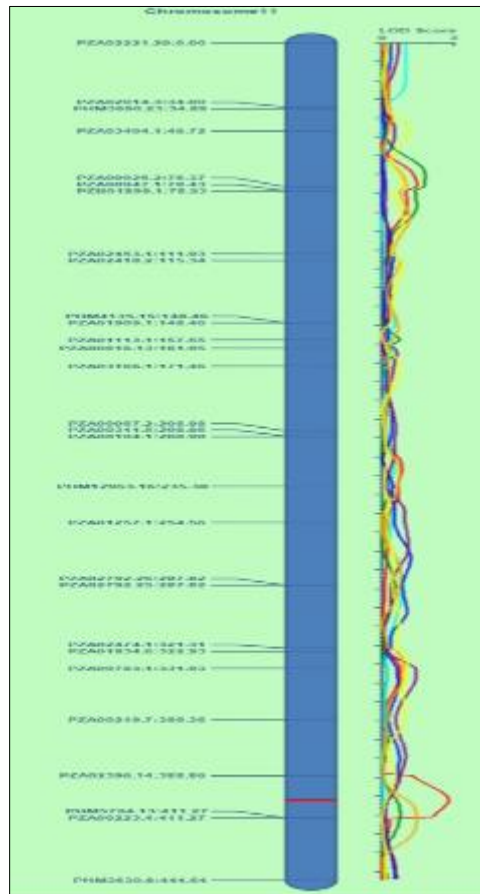
Appendix III Contd'
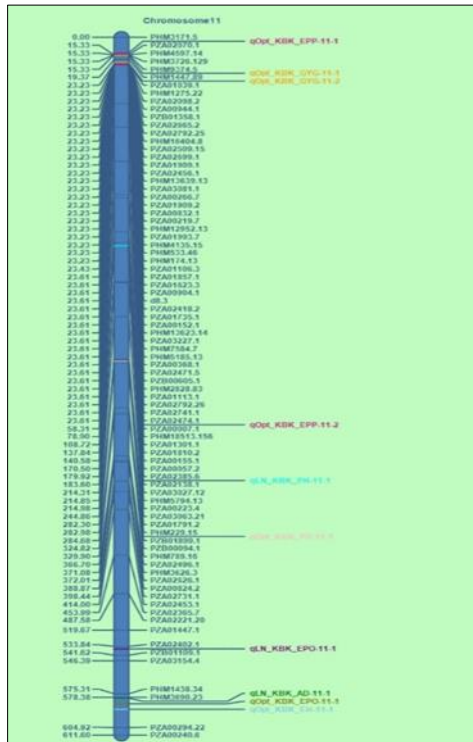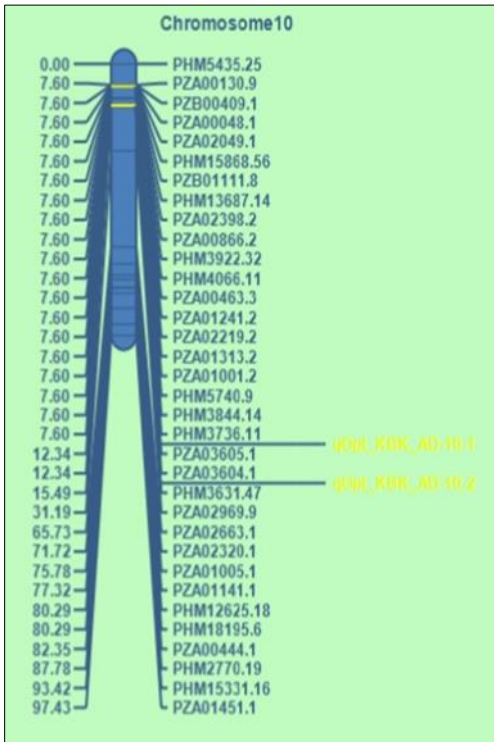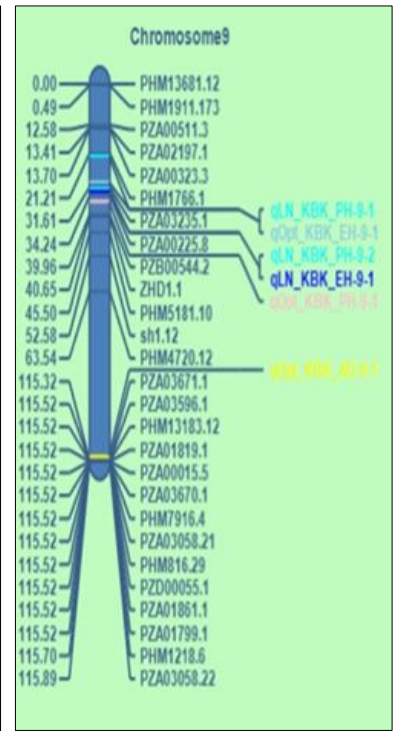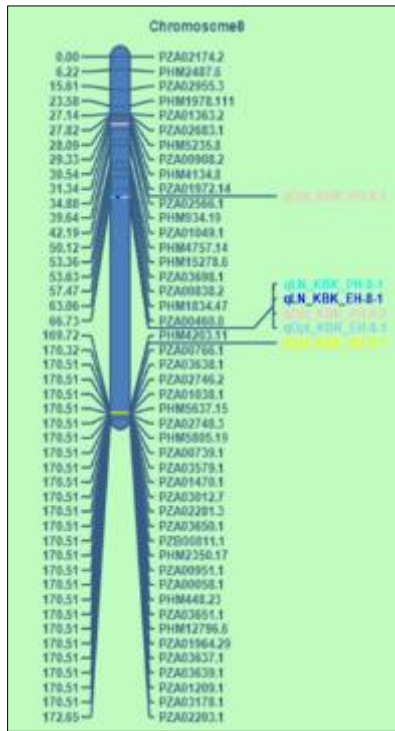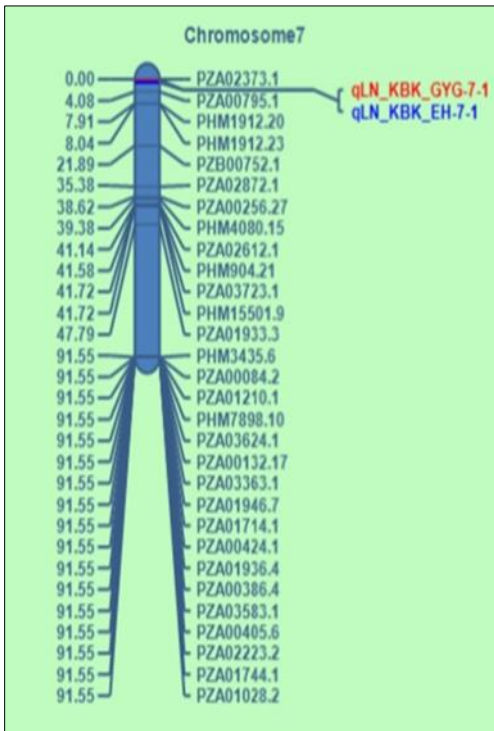
Appendix IV QTL maps in population 3 under low nitrogen condition
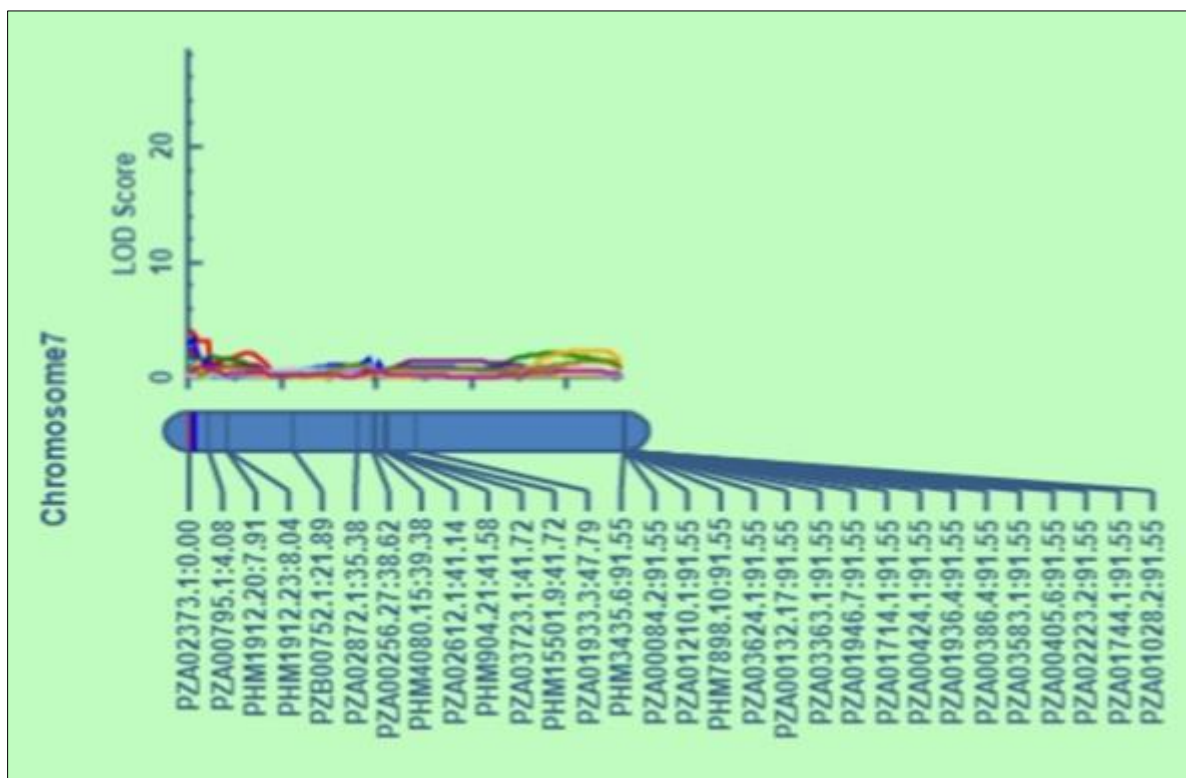
Appendix IV Contd'

Appendix V LoD score for grain yield qtl under optimum N condition in population 1
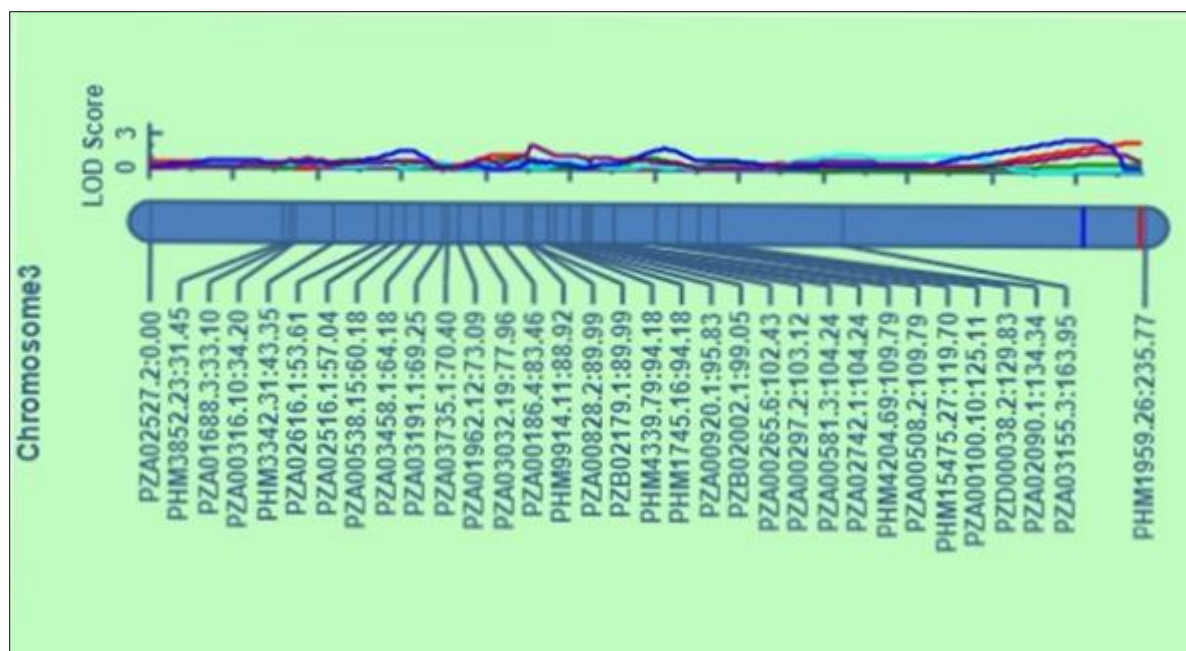

LoD score =2.75

Appendix VI LoD score for grain yield in population 2 (Across low and optimum N)

LoD score (=3.83)

Appendix VII LoD score for grain yield in population 3 under low N condition

LoD score =2.67