Original Research Article

The predicted genetic architecture for number of pods per plant in cowpea in phosphorus environments.

ABSTRACT

Aims: The aim of the study was to analyze quantitative trait loci for pod number per plant in cowpea under different phosphorus environments.

Study design: On the field, the experimental design was a split-plot with two replicates. The main plots were two phosphate levels: 0 P and 30 Kg P ha-1 (Triple super phosphate, TSP), while the 118 RILs and the two parents constituted the sub-plots randomized in a 12 x 10 α-lattice design. The experimental design for the pot experiment was a factorial randomized complete block design with two factors, and two replications. The factors were phosphorus levels (0 and 30 mg P per Kg soil) and genotypes.

Place and Duration of Study: The study was conducted at two sites. The first site was at the IRAD (Institut de la Recherche Agricole pour le Développement) research station, in Nkoemvone, in the HFZ of Cameroon while the second site was at Nkometou, a village in the Yaoundé neighborhoods, still within the HFZ of Cameroon.

Methodology: A RIL F11 population consisting of 118 lines derived from a cross between '58-77' and 'Yacine ' using the single seed descend method was used in the study. The line '58-77' (female parent,) is a black small-seeded local cultivar from Senegal resistant to pests and diseases with many pods per plant while 'Yacine ' (male parent) also from Senegal has large brown seeds but with very few pods per plant. Evaluation of cowpea RILs was done on low nitrogen plots both in the field and screen house and data collected on number of pods per plant. Analysis of Variance (ANOVA) was performed with the software SAS version 9.2 (SAS Institute Inc., Cary, NC, USA 2008).Marker genotype data for 118 RILs of the 58-77 x Yacine population were generated from the Illumina GoldenGate assay of 1,536 genome-wide SNP markers derived from EST sequences. The software WinQTL Cartographer 2.5. was used for composite interval mapping . QTL mapping was also performed using QTLnetwork 2.1 that uses a model that includes the effects of multiple QTL, epistasis, QTL-by-environment interactions and epistasis-by-environment interactions.

Results: Win Cartographer identified a total of eight QTL for Npod in all eight environments while QTLnetwork identified the following three main QTL (M-QTL) for Npod across the eight environments: qNpod2.1, qNpod5 and qNpod8. In total, three digenic epistatic interactions were detected for Npod across the eight environments. All three digenic pairs had epistasis main effects, and epistasis by environment interaction effect [aae] affects in one environment.

Conclusion: This study shows that, two QTL with epistasis effect were found to also have significant additive by environment effects. This means that the usual estimates of QTL effects could be confounded by epistatic interactions and result in biased estimation unless epistatic effect are isolated.

Keywords: [Epistasis, Number of Pods, Phosphorus , QTL, Vigna unguiculata }

1. INTRODUCTION

Cowpea [Vigna unguiculata (L.) Walp] is a warm weather, drought-tolerant crop well-adapted to the drier regions of the tropics, where other food legumes do not perform well. This makes cowpea an important component of traditional intercropping systems, especially in the complex, subsistence farming systems of the dry savannas in sub-Saharan Africa [1]. With its greater tolerance to heat, drought, and low soil fertility [2] and yet close evolutionary relatedness to other economically important grain legumes such as common bean (Phaseolus vulgaris) and soybean (Glycine max), cowpea can serve as a model species for crop adaptation to these stresses. Cowpea (Vigna unguiculata) is also closely related to mung bean (Vigna radiata) and shares more distant common ancestry with common bean (Phaseolus vulgaris), soybean (Glycine max), and pigeon pea (Cajanus cajans) [3]. Cowpea germplasm is notably diverse, especially when considering tolerance to several biotic and abiotic stresses; however, the genetics of these traits are not sufficiently understood in the context of modern, marker-assisted, breeding. Many agronomically important traits in cowpea display a continuous phenotypic distribution. These quantitatively inherited traits are typically influenced by several loci and the environment, and are difficult to breed using conventional methods reliant on phenotypic assessments. The progress in cowpea genomics in recent years has provided an opportunity to unravel the genetic basis of important horticultural traits in this crop as well as other subspecies like asparagus bean (Vigna unguiculata ssp sesquipedalis). The recent cowpea consensus genetic map which includes more than 1,000 loci from as many as thirteen different RIL populations [4;5] was constructed based on bead-assay SNP genotyping. Among the 13 mapping populations, one is derived from yacine-5877 cross used in this study. Associations between genotype and phenotype can expedite development of improved varieties containing favorable alleles for several traits through streamlined approaches to breeding. In cowpea, quantitative trait loci (QTL) have been detected for many traits such as seed weight and pod shattering [6], thrips resistance [7], heat tolerance [8], and aphid resistance [9] but to the best of our knowledge, no QTL have been reported for pod number per plant.

The number of pods per plant is among the most horticulturally important traits in cowpea and is inherited quantitatively based on field behaviors, and as such, dissecting the genetic basis calls for adequate statistical methods that can integrate QTL with environment (QXE) interaction in QTL mapping. QTL by environment interaction is an important component of quantitative genetics. In the earlier studies of QTL mapping, almost all statistical methods were developed in a single environment [10;11]. These methods did not consider the correlation of data under different environments and thus may not extract maximum information from the data. QTL network software maps QTL with additive effects and their interaction with environments based on the mixed-model based composite interval mapping (MCIM) method [12]. Several studies were performed to identify the QTL by environmental effects in many crops by the QTL network in recent years, e.g., rice [13], corn [14], soybean [15], wheat [16], and groundnut [17]. These studies indicated that QTL were greatly affected by environment. Thus, it is very important to analyze QTL of pod number per plant under many phosphorus (P) environments, knowing well that P, an element usually deficient in most soils where cowpeas are grown in an essential requirement for cowpea growth. This study makes use of markers that are accessible via community genotyping platforms and are useful for modern breeding, comparative genomics, and map-based cloning.

2. METHODOLOGY

2.1 Plant material

A RIL F11 population consisting of 118 lines derived from a cross between '58-77' and 'Yacine' using the single seed descend method was used in the study. The line '58-77' (female parent,) is a black small-seeded local cultivar from Senegal resistant to pests and diseases with many pods per plant while 'Yacine ' (male parent) also from Senegal has large brown seeds but with very few pods per plant.

2.2 Field experiments

The study was conducted at two sites. The first site was at the IRAD (Institut de la Recherche Agricole pour le Développement) Research Station, in Nkoemvone, in the HFZ of Cameroon (situated between longitude 11o 6' E and 11 o 10 ' E, latitude 2o 53' N, and 2o 57' N, and altitude of 615 m). The average annual rainfall is 1820 mm with a bimodal distribution and mean daily temperature of 23.5^{o} C. The soils are Ferric Acrisols characterized by a low base saturation and a low cation exchange capacity [18]. The soils for the study area are highly acidic, with pH (1:1 H2O) 4.5 [18]. The vegetation consists of secondary humid forest. The second site, Nkometou, is a village in the Yaoundé neighborhoods, in the HFZ of Cameroon. Geographically, the study area is situated between latitude 3º51' and 3º53' N, and longitude 11º25' E and 11º27' E and has an altitude of 813 m. The climate is Equatorial with two rainy seasons corresponding to two cropping seasons: March to June and August to November. The average rainfall is 1692 mm with bimodal distribution; the mean daily temperature ranges from 19.2 to 28.6ºC. The soils are also Ferric Acrisols, characterized by low base saturation and a low cation exchange capacity. The vegetation is evergreen forest, severely degraded by human activities, especially agriculture and timber exploitation [18].

Evaluation of cowpea RILs was done on low nitrogen plots. The experimental design was a split-plot with two replicates. The main plots were two phosphate levels: 0 P and 30 Kg P ha-1 (Triple super phosphate, TSP), while the 118 RILs and the two parents constituted the sub-plots randomized in a 12 x 10 α-lattice design. Plots were fertilized uniformly with K (KCl) at 80 Kg ha-1. Lime Ca(OH)2 at the rate of 924kg of CaO per ha was incorporated into soil during land preparation. This dose followed the recommendations by KAMPRATH [19]. On the field, each of the 118 RILs was planted in a single row of 5m length at a spacing of 50cm between rows and 50 cm within rows. All plants were sprayed twice (before flowering and after pod setting) with the insecticide Thiodan® (endosulfuran organochlorine) at a concentration of 0.33 mg/L. The experimental area was bordered on either side by guard rows in order to minimize border effects. The field was hand weeded twice, two and four weeks after planting.

2.3 Pot experiments

Two screen house experiments were carried out at IITA Cameroon with soils collected from low N plots at Nkometou and Nkoemvone at 0– 20 cm depth. The soil was air dried, sieved through a 2mm screen and homogenized. The 118 RILs and their parents were grown. Plants were grown in 5L capacity pots containing 4.3 kg of non-sterile soil with one plant growing per pot after thinning. Amount of soil per pot was calculated based on soil bulk density. The experimental design was a factorial randomized complete block design with two factors, and two replications (two pots per RIL per replication for nodulation and yield traits, respectively). The factors were phosphorus level (0 and 30 mg P per Kg soil) and genotypes. Phosphorus and potassium were supplied as KH2PO4 and muriate of potash, respectively. Prior to sowing, seeds were surface sterilized with 95% ethanol for 1 min, and 3% H2O2 for

5 min, then rinsed with sterile water [20]. Three seeds of each genotype were sown in each pot and thinned to one plant per pot one week after emergence. Before sowing, P and K nutrients were applied as mentioned above. One milliliter of a combination of micronutrients per kg soil was also applied [20]. Pots were watered and maintained at field capacity. Soil rhizobial population was estimated using the Most Probable Number (MPN) method [21; 20]. The soil rhizobia population was found to be high (>103 rhizobium bacteria per g soil) which made artificial inoculation unnecessary for the soils [22; 23].

2.4 Linkage Analysis and QTL mapping

Analysis of Variance (ANOVA) was performed with the software SAS version 9.2 (SAS Institute Inc., Cary, NC, USA 2008). Factors in the ANOVA model were cowpea lines and blocks. Normality was tested per environment. The means of parents were compared using a student t test. A 5% false-positive value was chosen as a significant criterion. Marker genotype data for 118 RILs of the 58-77 x Yacine population were obtained from LUCAS

et al. [5] and generated from the Illumina GoldenGate assay of 1,536 genome-wide SNP markers derived from EST sequences [4]. The Illumina GoldenGate Assay with the BeadStation 500G (http://www.illumina.com) was used to genotype 1,536 SNPs using the USLP 1.0 array. The Illumina GenomeStudio software (Illumina, Inc., San Diego, CA, USA) was used to call SNP alleles. Additional SNPs that were excluded in USLP 1.0 markers were genotyped with a KASP (K-Bioscience, Hoddesdon Herts, UK), and these SNPs were analyzed by a LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA) based on endpoint genotyping. Linkage maps were constructed with the software QTL IciMapping 3.1 (http://www.isbreeding.net) using the Kosambi function, and alignment with the cowpea consensus genetic map [5] available at HarvEST:Cowpea (http://harvest-web.org/).

The software WinQTL Cartographer 2.5. was used for composite interval mapping [CIM, 24]. For CIM, the stepwise selection was used for background marker selection as co-factors in the model. An alpha value of 0.05 was used to avoid model over-fitting. A 1,000-repetition permutation [25] was performed to find the genomewide critical likelihood ratio test (LRT) value according to trait and year at an overall a value of 0.05. A window size of 1 cM was applied to control background marker effects and produce a precise LOD profile.

QTL mapping was also performed using QTLnetwork 2.1 [26] that uses a model that includes the effects of multiple QTL, epistasis, QTL-by-environment interactions and epistasis-by-environment interactions. The map distances were estimated based on the Kosambi function. This mapping strategy is based on marker interval selection, detection of marker interval interactions and genome scans, to evaluate putative locations of multiple QTL and their interactions. An F-statistics was used for hypothesis tests. In each of the mapping procedures, permutation testing was exploited to control for genome-wide false positive rate, and model selection was used to reduce ghost peaks in F-statistic profile. The thresholds of the QTL (LOD scores) were obtained at $p = 0.05$ by 1,000 random permutations of the trait values. Parameters of the full-QTL model were estimated using a Bayesian method via Gibbs sampling. The different stages in QTL mapping using the the QTLnetwork software involved mapping main QTL by one dimensional (1D) genome scan and epistasis by two-dimensional (2D) genome scan.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Trait performance of the parents and population

The phenotypic behavior for number of pods per plant (Npod) for the RIL population and its parents under the eight environments are described in Table 1 for the pot experiments and Table 2 for the field experiments. The parent 58-77 had higher means than Yacine in both experiments. The means were different under different environments and transgressive segregants were observed across all eight environments with some RILs higher than the better parent, 58-77, or lower than the poor parent, Yacine. The Npod of the RIL population under study segregated continuously as indicated by the absolute skew and kurt values (Tables 1 and 2).

Table 1: Phenotypic values of number of pods per plant among parents and RIL population per evnironment in pot experiments.

		Parents		RIL population						
ENV	**Trait**	**58-77**	**Yacine**	**Mean**	**Max**	**Min**	**Stdev**	**C V(%)**	**Skew**	**Kurt**
PHP1	Npod	14±4.24	4.5±0.71	7.89	20.00	2.50	2.64	33.41	1.22	4.09
PLP1	Npod	7.5±2.12	2±0.00	2.81	6.50	1.00	1.34	47.71	0.90	0.72
PHP2	Npod	7.5±3.54	3±1.41	9.05	24.00	3.00	3.70	40.89	1.25	2.74
PLP2	Npod	4.5±2.12	1.5±0.71	3.97	11.00	1.00	1.84	46.42	1.41	2.69

ENV = Environment; PHP1 = Nkometou high p, PLP1 = Nkometou low P , PHP2 = Nkoemvone high P and PLP2 = Nkoemvone low p in pot experiments. Stdev = standard deviation and CV is coefficient of variation. The means of the parents are given ± stdev.

Table 2: Phenotypic values of number of pods per plant among parents and RIL population per environment in field experiments.

		Parents		RIL population						
ENV	**Trait**	**58-77**	**yacine**	**mean**	**max**	**min**	**Stdev**	**cv (%)**	**skew**	**Kurt**
FHP1	**Npod**	61.5±4.95	28.5±2.12	37.11	71.00	22.50	10.63	28.66	1.57	2.58
FLP1	**Npod**	5.1±0.42	1.2±0.85	3.47	10.50	1.00	1.66	47.96	1.30	3.28
FHP2	**Npod**	74.55±7.67	39.7±4.38	62.15	177.50	29.00	32.07	51.61	1.58	2.53
FLP2	**Npod**	6.15±1.06	1.8±0.85	8.30	29.50	1.00	5.57	67.10	1.28	2.07

ENV = Environment; FHP1 = Nkometou high p, FLP1 = Nkometou low P , FHP2 = Nkoemvone high P and FLP2 = Nkoemvone low p in field experiments. Stdev = standard deviation and CV is coefficient of variation. The means of the parents are given ± stdev.

3.1.2 Analysis of QTL and QTL by Environment (QE) interactions of number of pods per plant

Win Cartographer identified a total of eight QTL for Npod (Table 3) in all eight environments. A “Constitutive” QTL, qNpod 6.2 (Table 3) was identified in low phosphorus conditions both in the screen house and on the field by Win Cartographer but this QTL was not detected by QTLnet work software after isolating the effect of epistasis. QTLnetwork identified the following three main QTL (M-QTL) for Npod across the eight environments (Figure 1) qNpod2.1, qNpod5 and qNpod8. The positions of these QTL (Table 4) are indicated by the distance between the QTL and the first marker of the relevant linkage group. The interval refers to the flanking markers of the QTL while the range is the support interval of QTL position.

Table 3: Number of pods (Npod) per plant QTL with main effects identified by Win Cartographer in eight environments.

Trait	**EVN**	**QTL**	**LG**	**Marker**	**Lod score**	**additive**	**R^2**
Npod	FHP1	qNpod3.1	3	1_0139	5.05	12.64	0.4
(08QTL, 8	PLP1	qNpod1.1	1	1_0972	2.67	1.31	0.4
EVNs)	PHP2	qNpod1.1	1	1_0972	4.23	2.18	0.4
	FLP2	qNpod10.1	10	1_1098	3.21	4.61	0.4
	FLP2	qNpod10.2	10	1_0416	3.97	4.75	0.4

PLP2	qNpod5.1	5	1_0032	4.33	2.18	0.4
FLP2	qNpod6.2*	6	1_0326	2.86	4.54	0.3
PLP2	qNpod6.2*	6	1_0326	2.63	2.02	0.4

*Represent a constitutive QTL detected in more than one Environment. LG = linkage group. ENV = Environment; FHP1 = Nkometou high p, FLP2 = Nkoemvone low p in field experiments. PLP1 = Nkometou low P, PHP2 = Nkoemvone high P and PLP2 = Nkoemvone low p in pot experiments. The QTL are named beginning with "q" standing for QTL, followed by trait name and the linkage group number. In cases where there are more than one QTL on a linkage group for the same trait, the serial number is added after the linkage group number separated by a dot.

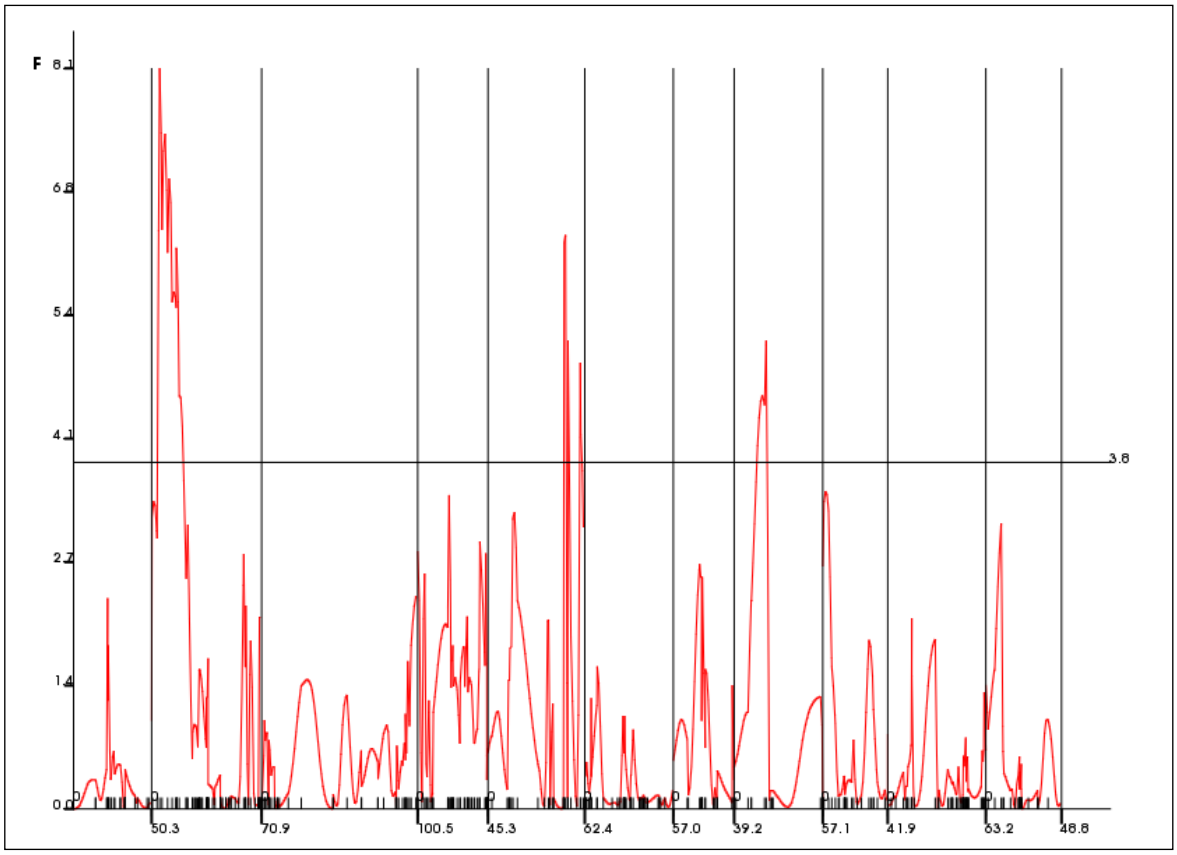

Figure 1: F-statistic plots from one dimensional genome scan for QTL with individual effects for number of pods per plant . Three peaks exceed the threshold F-value (3.7) calculated by permutation tests on LG 2, 5 and 8, respectively.

Table 4: Positions of Main QTL (M-QTL) identified by QTLnetwork in eight environments for Npod in cowpea

Trait	QTL	LG	interval	Position cM	R^2 (%) range of QTL
	qNpod2.1	2	1_1067-1_0113	5.2	4.5-6.2
	qNpod5	5	1_0032-1_0945	49.9	48.5-50.9
Npod	***qNpod8***	8	1_0762-1_1123	23.8	19.3-24.9

QTL with both detectable additive and epistasis effects are presented in bold italic form. .LG= linkage group.

3.1.3 Epistatic QTL and QE interactions of number of pods per plant

Digenic epistatic interactions with epistatic main effect [aa] and /or epistasis by environment interaction effect [aae] were detected for the number of pods per plant in cowpea. Two M-QTL (qNpod2.1 and qNpod8) with both [ae] effects, but without [a] effects (Table 5) were involved in digenic interactions (Table 6), same as qNpod2.2, qNpod2.3 and qNpod2.4 which had no detectable [ae] and/or [a] effects.In total, three digenic epistatic interactions were detected for Npod across the eight environments (Table 6). All three digenic pairs had [aa] main effects (Table 7), and epistasis by environment interaction effect [aae] affects in one environment. The QTL qNpod2.1 was involved in two digenic interactions on different LGs, LG 2 and LG 8 (Figure 2).

Table 5: Additive and /or additive x environment interaction effects of M-QTL across eight environments

Gene effect and environment	**qNpod2.1**	qNpod5	**qNpod8**
[a]	-0.734	0.617	-0.596
ae FHP1	0.122	-0.845	0.673
ae FLP1	0.654	-0.448	0.245
ae PHP1	0.576	-0.424	0.184
aePLP1	0.478	-0.454	0.242
aeFHP2	-2.992**	4.704**	-1.721*
aeFLP2	0.091	-0.691	-0.114
aePHP2	0.538	-1.017	0.243
aePLP2	0.561	-0.776	0.242

[a], ae represent additive main effect and additive x environment interaction effect, respectively. Environments are defined as follows: FHP1 = Nkometou high P, FLP1 = Nkometou low P, FHP2 = Nkoemvone high P and FLP2 = Nkoemvone low P in

field experiments. PHP1 = Nkometou high P, PLP1 = Nkometou low P, PHP2 = Nkoemvone high P and PLP2 = Nkoemvone low P in pot experiments. * and **represent the significance level of p=.05 and .01 respectively. Npod = number of pods per plant.

Table 6: Positions of epistatic QTL (E-QTL) identifed by QTLNETWORK for Npod across eight environments.

Trait	QTL_i	interval_i	position_i	QTL_j	interval_j	position_j
Npod	***qNpod2.1***	1_1067-1_0113	5.2	***Npod8***	1_0762-1_1123	23.8
	qNpod2.1	1_1067-1_0113	5.2	Npod2.4	1_0709-1_0513	63.8
	qNpod2.2	1_0062-1_0687	35.2	Npod2.3	1_0115-1_0885	59.4

QTL with both detectable additive and epistasis effects are presented in bold italic form. QTL_i and QTL_j are the two QTL involved in epistatic interaction. Interval_i = the flanking markers of QTL_i, LG= linkage group, interval_j = the flanking markers of QTL_j,

Table 7: Additive x additive and /or additive x additive x environment interaction effects of E-QTL for number of pods across eight environments.

Gene effect and environment	**qNpod2.1 (QTL_i) qNpod8 (QTL_j)**	**qNpod2.1 (QTL_i) qNpod2.4 (QTL_j)**	**qNpod2.2 (QTL_i) qNpod2.3 (QTL_j)**
[aa]	1.224**	1.570**	1.061**
aae FHP1	0.439	-0.48	-0.396
aae FLP1	-0.841	-1.728	-0.603
aae PHP1	-0.66	-1.473	-0.759
aaePLP1	-0.887	-1.618	-0.901
aaeFHP2	4.887**	9.676**	4.187**
aaeFLP2	-0.648	-1.022	0.195
aaePHP2	-1.322	-1.659	-0.828
aaePLP2	-1.071	-1.673	-0.876

[aa], ***[aae]*** represent epistatic main effect and epistasis x environment interaction effect, respectively. Environments are defined as follows: FHP1 = Nkometou high P, FLP1 = Nkometou low P, FHP2 = Nkoemvone high P and FLP2 = Nkoemvone low P in field experiments. PHP1 = Nkometou high P, PLP1 = Nkometou low P, PHP2 = Nkoemvone high P and PLP2 = Nkoemvone low P in pot experiments. * and **represent the significance level of p=.05 and .01 respectively.

Legend for figure 2
QTL with both additive *[a]* and additive by environment *[ae]effects*
Blue dashed lines linking QTL means the epistatis interaction has both main *[aa]* and epistasis × environment interaction effect *[aae]*
QTL with no additive *[a]* effect

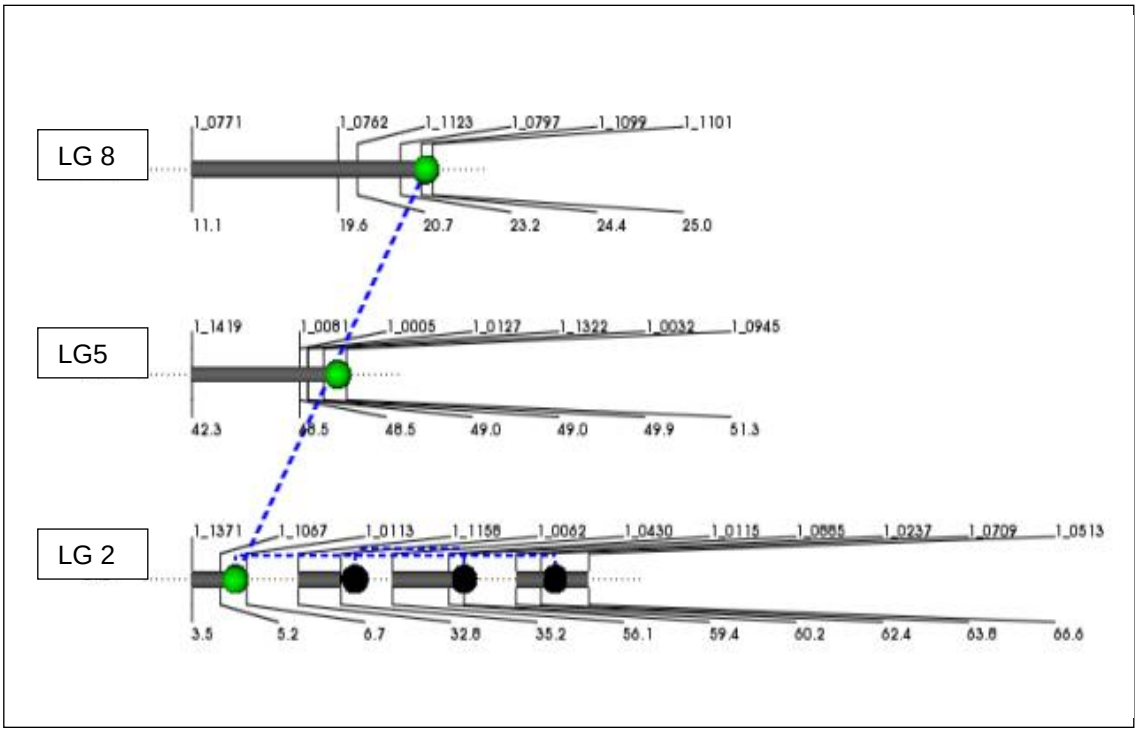

Figure 2: The predicted genetic architecture for number of pods per plant in cowpea. The figure was identified by QTLNetwork v2.0. It shows additive and epistatic QTL for numbe r of pods per plant in cowpea. The interaction loci between epistatic QTL are shown by dashed lines.

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3.2 DISCUSSION

The genetic architecture of number of pods per plant in cowpea can be determined through QTL identified under different environments. The use of different P environments not only greatly facilitated the detection of QTL, but also allowed the identifcation of QTL by environment interactions. Win Cartographer identified a total of eight QTL for Npod in all eight environments while QTLnetwork identified only three main QTL (M-QTL) for Npod across the eight environments: qNpod2.1, qNpod5 and qNpod8.

Both epistatic interaction effects and QTL by environment interactions effects were found to be very important genetic factors in this study. To infer epistasis between QTL, interaction effects between molecular markers were widely assayed by two-way analysis of variance [27]. But his method usually cannot give unbiased estimation for QTL parameters. The possibility of QTL by environment interactions was also indicated simply by comparing results from different environments [28] as was identified by Win Cartograper in this study. A "Constitutive" QTL, qNpod 6.2 was identified in low phosphorus conditions both in the screen house and on the field by Win Cartographer but this QTL was not detected by QTLnet work software after isolating the effect of epistasis. The results of this study indicated comparing QTL from different environments to identify QTL by environment interactions leads to biased results. Quantitative trait loci (QTL) mapping has often been used to test for epistasis. But, numerous problems hinder estimation of QTL main effects, and these problems are exacerbated for QTL-by-QTL epistasis.

In this study, QTLnetwork program allowed the detection of QTL with epistasis and QE interactions and estimated their effects in multi-environments. QTLnetwork has also been used in other studies for similar purposes [29; 30]. The dissection of epistasis from other genetic components of variation is in no doubt helpful in obtaining reliable estimates of QTL effects. This can be seen in the difference in main QTL identified by Win Cartograper compared with QTLnetwork that estimates epistatic effects. In addition, considering epistasis in QTL analysis enhances the understanding of the inheritance of the traits under consideration. This study shows that, two QTL with epistasis effect were found to also have significant additive by environment effects. This means that the usual estimates of QTL effects could be confounded by epistatic interactions and result in biased estimation unless epistatic effect are isolated.

Three other loci qNpod2.2, qNpod2.3 and qNpod2.4 involved in epistasis did not have any significant single effects of their own. These epistatic QTL have not been reported before as focus has always been on QTL with main effects. Epistatic interactions can occur between loci that have no significant main effects. WADE [31] suggested caution when considering the importance of significant main effects, stating, "the existence of a statistical main effect is not an indication that a gene has any effect independent of its genetic background". HOLLAND et al. [32] detected several QTL for heading date and plant height in oat that were involved in epistatic interactions. They also found epistasis among loci that were not individually significant for trait effects and concluded that all pairs of loci should be tested for epistatic interactions, not merely the significant ones. LECOMTE et al. [33] also reported variability among fruit traits in tomato that were attributed to epistatic interactions between QTL and the genetic backgrounds.

Disimilarity coefficient

The successful detection of significant epistasis effects resulting from QTL without additive and additive by environment main effects indicates that, many loci even without significantly affecting the trait on their own could still affect the trait in combination with other loci. Such loci may play the part of modifying agents which tend to activate other loci or modify the action of other loci [34]. At a specific environment, the total effect of a QTL includes all the genetic main effects and QE interaction effects.

Two M-QTL, qNpod2.1 and qNpod8 were also found to have both epistasis and QE effects, implying that major gene or QTL could also interact with other genes under different environments. PRIOUL et al. [35] reported that environmental or stress-specific gene regulation affects the detection rates and approximate genomic locations of QTL.

From the signs of the additive effects, it shows that two QTL (with positive additive effects) are from the less performant parent, Yacine. This suggests that alleles for improving these traits may be dispersed within the two parents. So pyramiding of all alleles increasing these traits from the two parents will produce segregants higher than the better parent.

Pyramiding of all these minor QTL for the improvement of Npod in cowpea may not be possible through marker-assisted backcrossing (MABC), since MABC involves the transfer of limited number of QTL from one genetic background to another [36]. Therefore, to improve this trait, alternative and more efficient approaches (genome wide marker approaches) like MARS (marker-assisted recurrent selection) and GWS (genome wide selection), which allows selection for several QTL with small effects [37] will have to be used in cowpea.

4. CONCLUSION

The QTLnetwork program analyzed QTL with epistasis and QTL by environment interactions and estimated their effects in multi-environments. These interactions could not be detected by win Cartographer. This study shows that, two QTL with epistasis effect were found to also have significant additive by environment effects. This means that the usual estimates of QTL effects could be confounded by epistatic interactions and result in biased estimation unless epistatic effect are isolated. Since two main QTL were also found to have both epistasis and QTL by environment effects it may be concluded that major gene or QTL could also interact with other genes under different environments.

COMPETING INTERESTS

No competing interest.

All authors read and approved the final manuscript

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable

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