

Full Length Research Paper

Quantitative trait loci (QTL) for cowpea resistance to flower bud thrips (*Megalurothrips sjostedti* Trybom)

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Accepted 12 July, 2017

Cowpea flower bud thrips causes about 80% yield losses in cowpea. Host plant resistance is the most easy and durable solution to the damaging effects caused by this insect. However, resistance to thrips is quantitatively inherited, thus less amenable through conventional breeding. The objective of this study was to identify QTL associated with resistance to cowpea flower bud thrips in a bid to facilitate the improvement of cowpea resistance to thrips. A mapping population consisting of 150 F₂ plants derived from cross between the highly resistant SANZI (female) and the highly susceptible VYA (male) was screened under artificial infestation in the screen house. Thrips damage scores were used as the parameter for resistance. A total of 232 polymorphic Single Nucleotide Polymorphism (SNP) markers were used for genotyping of F₂ and the parents. Three significant QTLs for thrips resistance *Fthp28*, *Fthp87* and *Fthp129* were detected on chromosomes 2, 4 and 6 accounting for 24.5, 12.2 and 6.5 % of the total phenotypic variation respectively. Transgressive segregation was observed towards the susceptible phenotype. Both additive and non-additive QTL effects were observed with additive effects being predominant. Further studies to validate these QTL for their useful exploitation in marker-assisted breeding programme are essential.

Key words: Cowpea, flower bud thrips, Quantitative Trait Loci, polymorphic SNP.

INTRODUCTION

Cowpea production worldwide is constrained by a complex of insect pests during all stages of its growth from seedling to storage. The economic importance of insect pests varied from one insect to another depending on the environment. Among these insect pests, many authors agree that cowpea flower bud thrips (*Megalurothrips sjostedti*) is the most devastating and yield loss of up to 100% has been reported in case of severe infestation (Singh and Allen, 1980; Jackai & Daout, 1986; Ngakou et al. 2008). Thrips is the major specie reported in West and Central Africa where most of the world's cowpea production is

recorded (FAOSTAT, 2015). Adults are very small size (about 1mm) black insects with high reproduction rate (Morse and Hoddle, 2006; Maharijaya et al., 2015; CABI, 2016). Thrips cause direct damage by feeding on the floral parts that lead to flowers malformation, distortion, discoloration, abortion and eventually yield reduction (Alabi et al., 2006; Maharijaye, 2013; Nyassy et al., 2016). Their biology makes them especially difficult to control because they infest a wide range of host plants and are well equipped for invasive behaviour (Morse and Hoddle, 2006; Muchero et al., 2010). Among the wide range of the existing management practices for thrips control, repeat chemical application is largely adopted (Abtey et al., 2015). However, insecticides do not completely solve thrips problem as it often leads to rapid development of

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insecticides resistance (Muchero et al., 2010; Maharijaya et al., 2015). In addition, the majority of resource-poor farmers who grow cowpea have limited resources to afford costly insecticides and well adapted application equipment (Jackai and Adalla, 1997; Muchero et al., 2010). This situation ends up rendering chemical application inefficient. An alternative and most appropriate approach that would increase the effectiveness of thrips control is the use of resistant varieties. Several cowpea accessions have been found to carry resistance to thrips, which may be exploited in breeding for thrips resistance (Abudulai et al., 2006; Alabi et al., 2006; Omo-Ikerodah et al., 2009; Asare, 2012). However, there is limited information on the molecular genetics of thrips resistance. Few studies reported the detection of Quantitative Trait Loci (QTL) for resistance to cowpea thrips *Megalurophthris sjostedti* (Omo-Ikerodah et al., 2008) and *Frankliniella* sp. (Muchero et al., 2010). QTL for resistance to thrips were detected in common bean (Frei et al., 2005) and on pepper based on F₂ populations (Maharijaya et al., 2015). Huynh et al. (2016) successfully used F₂ population derived from a cross of susceptible and resistant cowpea genotypes to map QTL for resistance to root-knot nematode with genome-wide single nucleotides polymorphism (SNP) marker. The genomic resources available in cowpea include high-throughput SNP genotyping platforms, a high-density consensus genetic map with more than 1,100 markers. With the help of molecular markers linked to QTL, the heredity of some related complex traits such as thrips resistance could be tracked. The ability of genetic manipulation through QTL analysis is greatly enhanced, thus improving the accuracy and predictability to select genotypes with superior quantitative trait loci (Tan et al., 2012). Information generated on QTL associated with resistance to cowpea flower bud thrips would facilitate the development of molecular marker to be use in breeding for thrips resistant cowpea. The objective of this study was to identify QTLs linked to the flower bud thrips resistance in cowpea.

MATERIALS AND METHODS

Materials

Two contrasting parents SANZI (resistant) and VYA (susceptible) for thrips resistance were identified from previous evaluation work conducted at IRAD in 2014 (Figure 1). VITA-7 which is a very susceptible line from IITA was used to build up thrips population in the field.

Methods

Development of F₂ mapping population

A mapping population of one hundred and fifty (150) F₂ developed from bi-parental cross of two contrasting

inbred lines (SANZI and VYA) were used for the study with SANZI as female and VYA male.

Screening of F₂ population and parental lines

One hundred and fifty F₂ individuals with their parents, SANZI (resistant) and VYA (susceptible), were planted in in pots of 0.3m x 0.25 m in the screen house at the Regional Research Centre of Maroua. The pots were filled with topsoil collected from IRAD's experimental site of Guiring.

Adult thrips were introduced into the screen house from an established field of VITA-7 planted two weeks ahead. The collection of flower was done between 8:00 to 10:00 a.m. as recommended by Taylor (1969) to minimize the loss of thrips flying off after disturbance. Thirty five days after planting, infestation was carried out by dropping three flowers, containing not less than 30 thrips in each pot and continued for ten days as reported by Omo-Ikerodah et al. (2008).

Data collection

Phenotyping

Each plant was visually scored for thrip damages (THS) twice at 45 and 55 days after planting using the same scale of 1 to 9 (Jackai and Singh, 1988) as described in Table 1.

Genotyping

At three weeks after planting, DNA was collected from leaves of each F₂ individual plant and the two parents following the LGC Genomic leaf sample kit as follow:

Eight DNA discs of 6 mm in diameter were cut from leaf of each plant by placing the leaf to be sampled on the cutting mat without detaching it from the plant. The discs were cut out of the leaf by pushing the cutting tool into the leaf and twisting it at the same time to make the tool pick the disc up. After that, the discs were dispersed into a tube labelled with plant number by depressing the plunger. The procedure was repeated eight times for the same plant and the discs were dispersed into the same tube. From one plant to another, the cutting tools and the plunger of the mat were thoroughly cleaned with 70% alcohol to avoid contamination of DNA between samples.

Finally, the tubes were sealed with perforated strip caps on top of the tubes and pressed firmly to ensure caps are secured. Then, the desiccant was removed from its sealed bag and placed on top of the rack tubes. DNA leaf samples were finally sent to LGC Genomics for DNA extraction Polymerase Chain Reaction (PCR) running following K-biosciences protocol. Out of one thousand and sixty three (1063) SNPs markers used to screen the two parents, 232 polymorphic SNPs were used to screen the 150 F₂ and their parents.



P₁= SANZI (Thrips resistant having pods)

P₂= VYA (No pods on Thrips susceptible)

Figure 1. Two parental lines for mapping population development.

Table 1. Rating scale for thrips damages.

Scores	Description of the damages
1	No browning/drying of stipules, leaf of flower buds; no bud abscission
3	Initiation of browning of the stipules, leaf or flower buds; no bud abscission
5	Distinct browning/drying of stipules and leaf or flower buds; some abscission
7	Serious bud abscission accompanied by browning/drying of stipules and buds; non-Elongation of peduncles
9	Very severe bud abscission; heavy browning/drying of stipules and buds; distinct non-elongation of (most or all) peduncles

QTL analysis

Spider Map version 1.5.7b software was used to construct the genetic linkage map based on 232 polymorphic SNPs detected between the two parents. The mapping distances were estimated based on Kosambi mapping function, which assumes that recombination events influence the occurrence of adjacent recombination events (Collard et al., 2005). The map was set to display only markers with significant level less than 0.1%. QTL for score of thrips damage were identified using Breeding View Standalone QTL package (Malosetti et al., 2013). First, Simple Interval Mapping (SIM) was performed to estimate the genetic predictors that cover the genome, the software set the corresponding significant $-\log_{10}(p)$ threshold Li and Ji (2005). The Maximum stepwise along the genome was set to 5 cM and the genome-wide significant level alpha was equal to 5%. Secondly, Composite Interval Mapping (CIM) (Zen, 1994; Jansen

and Stam, 1994) was performed to improve accuracy of detection and the marker close to the QTL was used as co-factor. The maximum rounds of QTL scan which was set to 2 and the maximum cofactor proximity and minimum separator of QTL was 30 (Malosetti et al., 2013). A QTL was considered significant when $-\log_{10}(p)$ value was above the threshold determined by p-value of Wald statistic test (Malosetti et al., 2013).

RESULTS

Distribution of thrips resistance in F₂ population

The frequency distribution of score of thrips damage in F₂ deviated from the normality showing a continuous variation and skewed towards the resistant parent (Figure 2). There was also transgressive segregation for susceptibility of phenotype.

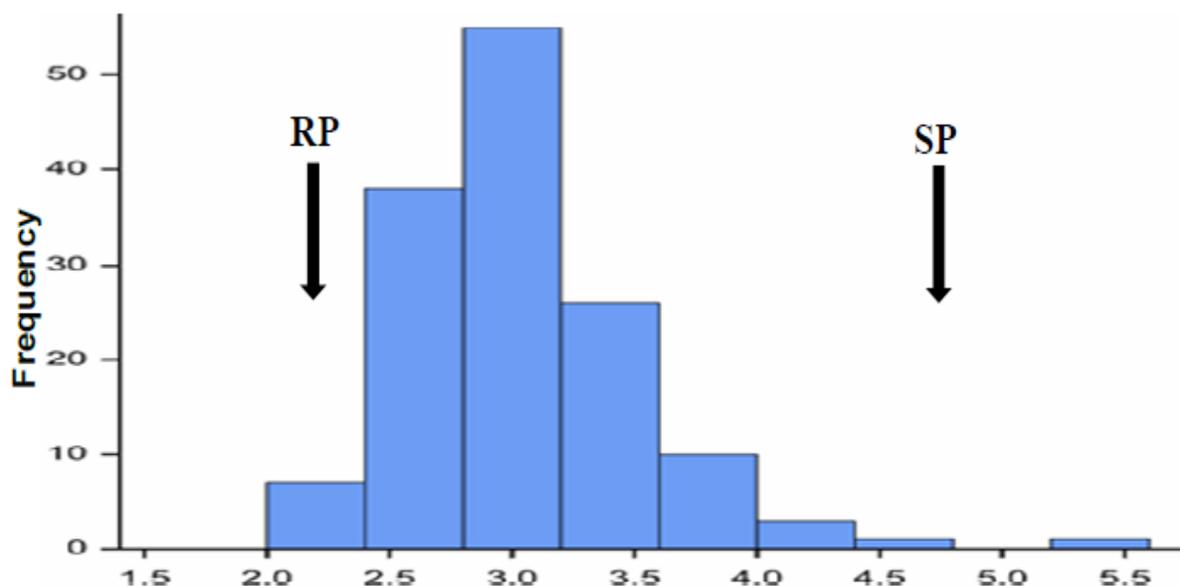


Figure 2. Frequency distribution of thrips damage score. RP = Resistant parent; SP = Susceptible parent.

Genetic linkage map

Among the SNP markers tested, 232 revealed polymorphism between SANZI (resistant) and VYA (susceptible) parents. The genetic map based on cross SANZI x VYA and the polymorphic SNPs showed that the markers were distributed over 11 Linkage Groups (LG) covering a total distance of 620.1 cM (Table 2). The length of linkage groups varied between 33.7 and 79.1 cM. The number of markers per linkage group ranged from 12 (LG7) to 39 (LG3) while median distance between markers varied from 0.4cM (LG11) to 2.1cM (LG10 and LG1).

Detection and mapping of QTL for thrip resistance

Three QTL were consistently detected on chromosome 2, 4 and 6 at a threshold level of $-\log_{10}(p)$ equal to 3.3 using both Simple Interval Mapping (SIM) and Composite interval Mapping (CIM) approach. The three QTL *Fthp129*, *Fthp28* and *Fthp87* mapped at the peak position of 40.3, 20.2 and 19.2 cM respectively on linkage LG2, LG4 and LG6 (Figure 3). QTL *Fthp129* and *Fthp87* in LG2 and LG6 were directed toward the resistant parent whereas *Fthp28* on LG4 was in direction of the susceptible parent (Figure 4).

Estimate of QTL effects for thrips resistance

The effects of the QTL *Fthp129*, *Fthp28* and *Fthp87* were significant ($p < 0.001$) using the Wald statistic test.

They accounted together for 43.2% of the phenotypic variation observed for score of thrips damage. The highest value of thrips score damage in F_2 population tested was observed for *Fthp129* which contributed 24.5% followed by *Fthp28* and *Fthp87* accounting for 12.2 and 6.5%, respectively (Table 3). *Fthp129* and *Fthp87* accounted each for more than 10% to the total phenotypic variation observed. The resistant parent (SANZI) contributed for high value allele in two QTL (*Fthp129* and *Fthp28*). For QTL *Fthp87* on LG4 the susceptible parent (VYA) contributed the favourite allele. Estimate of QTL effect showed significant additive and epistasis effects. The negative sign of the estimate indicated contribution of the resistant parent while positive sign estimate was associated with the contribution of the susceptible parent.

DISCUSSION

The frequency distribution of phenotypic data of score of thrips damage on F_2 population showed continuous distribution between the two parents, and was skewed toward the resistant parent indicating dominance over susceptible parent. Transgressive segregation phenotypes were observed in the F_2 . These results were in agreement with works of Muchero et al. (2010) and Maharijaya et al. (2015). The 232 SNP markers generated a linkage map with eleven linkage groups which was constructed corresponding to a genome length of 620.1 cM which was not far from the total

Table 2. Linkage group length and number of markers per chromosome.

Linkage group	Length (cM)	Number of Markers	Median distance between markers	95% percentile of distances
1	58.4	18	2.1	10.7
2	68.1	24	1.9	10
3	76.8	39	1.3	6.5
4	45.6	19	1.2	12.5
5	51.8	23	0.7	10.5
6	79.1	28	0.6	12
7	46.5	12	1.4	16.9
8	61.5	19	1.9	12.4
9	37.2	17	1.4	6.6
10	61.4	20	2.1	9.2
11	33.7	13	0.4	11.7
Genome	620.1	232	1.5	10.7

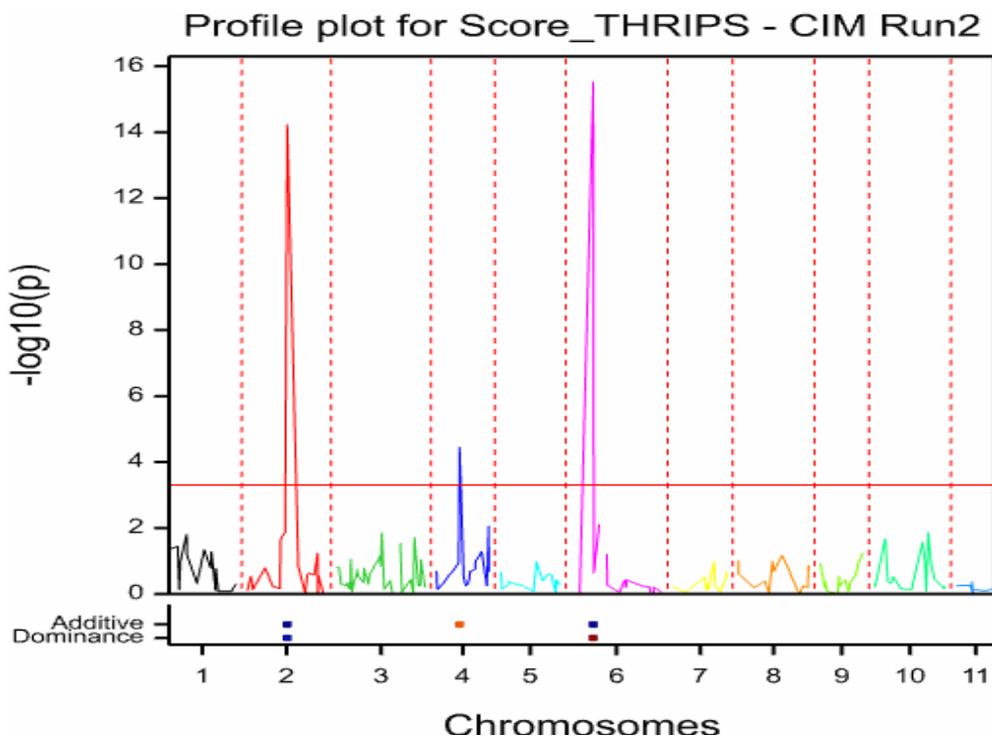


Figure 3. Magnitude profile of three significant QTLs detected. $-\log_{10}(p)$ = p value of Wald test for QTL effect; Red horizontal line = threshold value for significance equal to 3.3; Blue dot below = allele contribution of resistant parent; Red dot below = allele contribution of susceptible parent.

genetic distance of 643 that cM Muchero et al. (2010) mapped using 306 Amplified Fragment Length

Polymorphism (AFLP) markers. The study detected three significant QTL *Fthp129*, *Fthp28* and *Fthp87* located

Table 3. Estimate of additive and epistasis QTL effects for thrips resistance in cowpea.

QTL name	Linkage group	Marker at QTL peak	QTL PP (cM)	$-\log_{10}(p)$	PVE (%)	Additive effect	Dominant effect	H.Val. allele
<i>Fthp28</i>	LG2	9895_292	40.3	11.8	24.5	-0.323±0.043*	0.321±0.067*	RP
<i>Fthp87</i>	LG4	1202_1215	20.2	4.7	12.2	0.228±0.051*	-	SP
<i>Fthp129</i>	LG6	14610_202	19.2	12.6	6.5	-0.166±0.033*	-1.514±0.023*	RP

*:QTL effect significant if zero is outside the Confidential Interval (CI). CI = estimate ± SE (standard error). LG = Linkage Group. QTL PP = QTL peak position, PVE = proportion of phenotypic variation explained, H. Val= high value, RP = resistant parent, SP = susceptible parent.

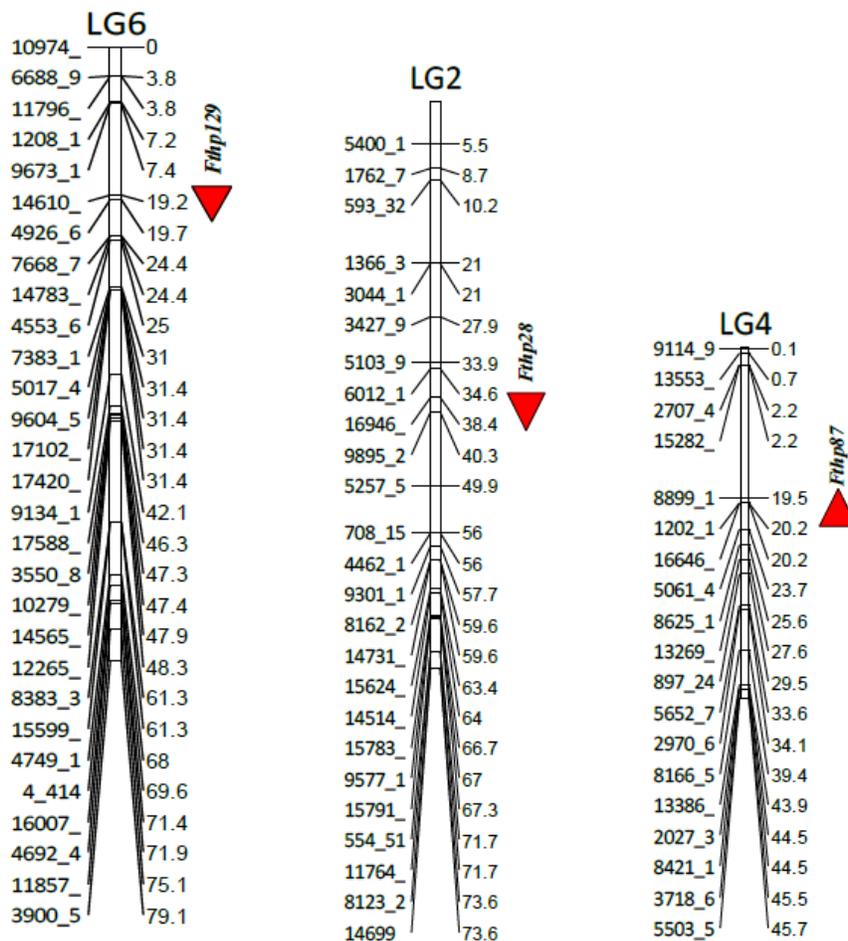


Figure 4: Three QTLs mapped on LG2, LG4 and LG6. ▼ = QTL peak position and direction toward resistant parent; ▲ = QTL peak position and direction toward susceptible parent

on LG2, LG4 and LG6 respectively. This indicated that resistance to cowpea flower bud thrips (*Megalurothrips sjostedti*) may be different from resistance to foliar

thrips (*Thrips tabaci* and *Frankliniella schultzei*). QTL conferring resistance to these thrips were identified rather on LG5 and LG7 (Muchero et al., 2010). These results

results could be explained by differences that exist in the biology and the feeding habit of the two species of cowpea thrips. However, the results were partially in agreement with findings of Omo-Ikerodah et al. (2008) who identified similar QTL on LG2 and LG6 but failed to detect the QTL at LG4. These results also corroborated the findings of Lucas et al. (2012) who reported two major QTLs and one minor QTL for cowpea foliar thrips. The QTL at LG4 in this study was not reported so far for cowpea flower bud thrips, therefore it may be considered as novel. The identification of this novel QTL compared to the earlier study (Omo-Ikerodah et al., 2008) may be explained by the differences of the experimental conditions used in our study and the earlier study reported. This may suggest the need to validate the three QTLs detected in different environment as the same resistance parent was used in the two experiments. In addition, the QTLs identified need to be tested on different genetic background to confirm their robustness. QTL *Fthp129* and *Fthp87* which accounted each for more than 10% of the total phenotypic variation observed may be considered as QTLs of major effects (Singh and Singh, 2015). The additive effects of the QTL *Fthp28* and *Fthp129* at LG2 and LG6 were negative in favour of the resistant parent indicating that the alleles at these loci contributed to increase the resistance genes. Whereas, for QTL *Fthp87* at LG4 the additive effects were positive in direction of the susceptible parent. Allele at this QTL contribute to increase the susceptibility, suggesting to select against the QTL *Fthp87* at LG4 when breeding cowpea for resistance to flower bud thrips. Furthermore, the transgressive segregation observed towards the susceptibility on F_2 phenotypes illustrated the complex nature of thrips resistance trait. Moreover, resistance may be recessive in the susceptible parent, thus the F_2 individuals that lacked the three QTL expressed more symptoms of thrips damage than the susceptible parent did as indicated by transgressive susceptible phenotypes. These results corroborate the earlier works conducted by Lucas et al., (2012) and Maharijaya et al. (2015). The combination of QTL *Fthp129* and *Fthp87* in the resistant parent SANZI, may have been effective to confer sufficient resistance to cowpea flower bud thrips. Further studies need to be undertaken to validate these QTL for their useful exploitation in marker-assisted breeding programme. The development of a RIL population from cross SANZI x VYA would help to repeat the experiment across different environments in order to assess the consistency of these QTL.

CONCLUSIONS

Three significant QTL *Fthp129*, *Fthp28* and *Fthp87*, were detected and mapped on LG2, LG4 and LG6, respectively accounting for 24.5, 12.2 and 6.5% of the phenotypic variation for the score of thrips damage. The

QTL, *Fthp28* at LG4 is the major finding of the current study while those on LG2 and LG6 were confirmed. The resistant parent contributed to high value alleles in two QTL *Fthp129* and *Fthp87* while the susceptible parent contributed to high value allele in QTL *Fthp28*. Both additive and epistatic QTL effects were significant. There was transgressive segregation of susceptibility of F_2 population for score of thrips damage. The QTL identified give the opportunity for improvement of cowpea resistance to thrips through the application of marker assisted selection.

ACKNOWLEDGEMENT

The authors of the present study are very thankful to the KirkHouse Trust project and the West Africa Centre for Crop Improvement (WACCI) for the financial support used to conduct this work.

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