

## QTL MAPPING OF BACTERIAL BLIGHT RESISTANCE IN CLUSTER BEAN BY MOLECULAR MARKERS

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### SUMMARY

Guar is a legume crop resistant to drought and susceptible to diseases like bacterial blight and alternaria leaf spot. Its a major crop in various developed and developing countries. It has been producing guar gum for industrial uses. In the present investigation bacterial blight resistant QTL were identified using RAPD markers. Statistical analysis was done using Mapmanager QTX software. DNA estimation was done on uv spectrophotometer, further analysed by gel electrophoresis for quality. Resistant and susceptible genotypes were used to generate F2-3 population used further for linkage map construction for the disease bacterial blight. F2-3 population was undertaken for disease percent survival in contrast to molecular marker analysis for the disease. Polymorphism studies were undertaken among molecular markers, molecular mapping was done and distances were calculated at  $p=0.05$  markers linked to each other for disease were selected Molecular mapping was done with three markers covering a map distance of 43.9 cM. Two markers (D1 & AB7) were found linked to each other and were grouped together in group 2 whereas L19 was found unlinked to other markers and contributing to disease resistance. These QTLs can be used for creation of superior genotypes of clusterbean using molecular breeding techniques for resistance to bacterial blight.

**Key words:** Bacterial blight, QTLs, Molecular mapping, Clusterbean, Polymorphism

Guar or cluster bean (*Cyamopsis tetragonoloba* (L.) Taub) is a summer legume traditionally grown in the semi-arid regions of India and Pakistan as a vegetable, forage and grain crop. It is tolerant of hot, dry conditions and has a deep taproot. This crop thrives well in rainfall range of 300-450 mm with 3-4 spells, at temperature 25-40°C, relative humidity 50-65%, and longer warmer days with 8-9 h sunshine (Satpal *et al.*, 2018). The crop was introduced to Australia from the United States in the early 20th Century and evaluated in New South Wales, Queensland and the Northern Territory (Jackson and Doughton 1982). Subsequent research conducted in Queensland and the Northern Territory in the 1960s, 1970s and 1980s demonstrated guar was well adapted to the dryland cropping environments of northern Australia. Recently world demand for guar gum has increased and continues to grow at 2% per annum, current production levels are estimated at 190 000 tonnes processed gum (500 000 tonnes grain), 90% of which is produce by India (Bryceson and Cover 2004). India is the major producer of Guar Seed followed by Pakistan and US. India's guar seed production fluctuates between years and has been

around 200000 - 600000 tons in the recent years. India's guar production in 2003 is estimated at around 600000 tons. (<http://www.agrocrops.com/guar-gum.php>) Now, it has emerged as a new industrial crop because of its gum content mainly present in endosperm which constitutes an important raw material for a wide range of industries like, textile, cosmetic, mining and oil (Dhugga *et al.* 2004; Joshi and Kumar 2004). Guar seed or guar meal can be detoxified by various processing technologies. Guar meal contains 40-45 % protein and used as animal feed (Panchta *et al.*, 2016). Guar gum is a good foreign exchange earner for the country and India earned Rs.814 crores during 1999-2000 by exporting over one lac tonnes of galactomannan (Singh and Dahiya 2004).

India and Pakistan export much of their guar crop to the United States and other countries in the form of partially processed endosperm material. World demand for guar has increased in recent years, leading to crop introductions in several countries. In Asia, guar beans are used as a vegetable for human consumption, and the crop is also grown for cattle feed and as a green manure crop. In the United States,

highly refined guar gum is used as a stiffener in soft ice cream, a stabilizer for cheeses, instant puddings and whipped cream substitutes, and as a meat binder. Most of the crop in the United States, however, is grown for a lower grade of guar gum, which is used in cloth and paper manufacture, oil well drilling muds, explosives, ore flotation, and a host of other industrial applications. Guar is an excellent soil-improving crop and fits well in a crop-rotation program with grain sorghum, small grains or vegetables. Increased yields can be expected from crops following guar because of increased soil nitrogen reserves. (<http://corn.agronomy.wisc.edu/Crops/Guar.aspx>).

Stresses like diseases reduces the yield of the crop drastically. In addition to its direct effects on yield, diseases can also reduce the potential beneficial effects of improved crop management practices such as fertilizer application or pest and disease management. Looking at its importance in the local market and its foreign exchange earning capacity, there is a need for detailed molecular analysis of guar. Bacterial blight of clusterbean incited by *Xanthomonas campestris* pv. *Cyamopsidis*, is a devastating disease (Mali *et al.*, 1989). Yield loss up to 68% has been reported in India (Gandhi and Chand 1985). The monsoon season enhances the intensity of the disease (Karwasra *et al.*, 1985). The disease is systemic and the pathogen is seed borne and located in the seed coat (Srivastava and Rao 1963; Karwasra *et al.*, 1983; Parashar and Sharma, 1984). This seed-borne disease causes loss of plants from the seedling stage until maturity. Symptoms include large angular necrotic lesions at the tips of leaves, which cause defoliation and black streaking of the stems. This is potentially the greatest disease hazard to clusterbean. Quantitative trait loci (QTLs), the class of genetic loci, which account for the inheritance of many complex measures of plant productivity, resistance to biotic and abiotic stresses and quality traits. Quantitative trait loci (QTLs) provide the basic information required for the mapping of a genome and cloning of economically important genes. Progress has been made in mapping and tagging of many agriculturally important genes with molecular markers which forms the foundation for MAS in crop plants. Mapping and sequencing of plant genome would help to elucidate the gene function, gene regulation and their expression. Identification of the markers linked to useful traits has been based on complete linkage maps. However, alternative methods, such as the construction of partial maps and combination of pedigree and marker information, have also proved useful in identifying marker/trait

associations. Map based cloning facilitates the isolation of genes of economic importance that have been located on a linkage map. Based on the linkage between a molecular marker and the gene of interest, we can predict that whether the gene is present or absent, before the actual identification of gene. DNA markers linked closely to the simply inherited traits can be isolated efficiently using RAPD and SSRs on DNA pools.

## MATERIALS AND METHODS

### Plant Material

Cluster bean varieties HG 75 (resistant) and PNB (susceptible) to bacterial blight were grown at dryland area fields and crosses were made between them early morning (5:30 a.m.) so that successful crosses can be obtained. The crosses were covered with paper bag to avoid chances of self pollination and environmental contamination. Individual pods were harvested from each plant, threshed and seeds were collected. Seeds of F<sub>1</sub> generation were grown at net house and field and studied. The F<sub>1</sub> seeds were grown to harvest F<sub>2</sub> seeds. A total of 50 F<sub>2</sub> seeds were harvested. All the 50 F<sub>2</sub> seeds were grown and individual F<sub>2</sub> plants seeds were harvested. Individual F<sub>2-3</sub> population was used for further studies.

### Isolation of Genomic DNA

Parental and F<sub>2-3</sub> seeds of clusterbean were obtained and were grown in the dryland experimental area of Forage Section, CCSHAU Hisar. Genomic DNA was isolated from young leaves of 3-4 week old seedlings using standardized CTAB (Cetyl trimethyl ammonium bromide) extraction method as used in experiment No.1 of both parental and 50 F<sub>2-3</sub> genotypes. Visualization of highly intense discrete band of high molecular weight DNA free from RNA was observed through electrophoresis of isolated DNA samples was done on 0.8 per cent (w/v) horizontal agarose gel. The genomic DNA was free from mechanical or enzymatic degradation.

### Qualitative and Quantitative Estimation of DNA

Quantification of DNA was done by UV spectrophotometer analysis, taking UV absorbance at 260 (Table 4.5). Amount of DNA was found to range between 214 to 1230µg/ml. It was observed that modified CTAB extraction protocol yielded good

genomic DNA from cluster bean genotype plants. To check the purity of genomic DNA, UV absorbance was also taken at 280 nm. The ratio of UV absorbance at 260 and 280 nm equals to 1.8 which showed that the DNAs was free from all cellular contaminants like RNA, polyphenols, polysaccharides and proteins. O. D. ratio of  $A_{260}/A_{280}$  of the DNA ranged from 1.34 to 1.92 (Table 4.5). The quality and quantity of genomic DNA of all the genotypes was also checked on agarose gel for its size and RNA contamination. RNase treatment was given wherever required to make DNA free from RNA. From all observations, it was found that the DNA of all the genotypes was of high molecular weight, free from RNA, intact and free from any mechanical or enzymatic degradation and cellular contaminants.

## RESULTS AND DISCUSSION

### Screening for Bacterial Blight disease reaction Preparation of inoculum for disease screening

Parental genotypes HG 75 and PNB and their

F<sub>2-3</sub> crossed plant material were screened for disease resistance at net house under artificial conditions as well as at dryland experimental area. Pure culture strain of *Xanthomonas* was isolated from diseased samples of clusterbean. Diseased samples of cluster bean were surface sterilized in 0.1% mercuric chloride for one minute followed by two washings with sterilized distilled water. Culture was inoculated from diseased leaves on YEGCA medium and streak plate method was used to get a single colony. The purified culture was mass multiplied further in broth medium in 500 ml flasks at 24°C in the incubator. Disease inoculum ( $10^6$  cells / ml) was prepared in sterile distilled water and sprayed in field and net house in the evening in the month of October when the temperature was around 20-25°C. High level of humidity was maintained by covering the net house with polypropylene sheet after giving sprinkling water treatment every 24 hrs to the net house area so as to provide maximum possible conditions for spreading of disease. Screening of the samples was done after disease is clearly visible on leaves. Disease scoring was done at an interval of

TABLE 4.5  
Quantity of Genomic DNA isolated from parents and F2-3 plants of *Cyamopsis tetragonoloba* using modified CTAB extraction method.

S. No.	Plant no	O.D. Ratio of DNA at A260/280	Concentration of DNA (µg/ml)	S. No.	Plant no.	O.D. Ratio of DNA at A260/280	Concentration of DNA (µg/ml)
1.	P1	1.67	247	26.	P26	1.62	248
2.	P2	1.56	214	27.	P27	1.55	458
3.	P3	1.74	650	28.	P28	1.76	1036
4.	P4	1.55	292	29.	P29	1.77	1234
5.	P5	1.74	382	30.	P30	1.83	296
6.	P6	1.45	370	31.	P31	1.83	898
7.	P7	1.78	260	32.	P32	1.84	1230
8.	P8	1.86	455	33.	P33	1.82	660
9.	P9	1.92	580	34.	P34	1.76	820
10.	P10	1.57	460	35.	P35	1.75	1044
11.	P11	1.54	380	36.	P36	1.59	360
12.	P12	1.82	454	37.	P37	1.84	436
13.	P13	1.85	780	38.	P38	1.32	624
14.	P14	1.83	898	39.	P39	1.34	456
15.	P15	1.84	1070	40.	P40	1.76	1036
16.	P16	1.83	460	41.	P41	1.56	260
17.	P17	1.76	820	42.	P42	1.77	416
18.	P18	1.75	1283	43.	P43	1.76	556
19.	P19	1.83	360	44.	P44	1.62	578
20.	P20	1.84	436	45.	P45	1.74	240
21.	P21	1.83	624	46.	P46	1.84	726
22.	P22	1.78	640	47.	P47	1.66	608
23.	P23	1.76	894	48.	P48	1.74	854
24.	P24	1.77	980	49.	P49	1.72	650
25.	P25	1.34	870	50.	P50	1.22	765
Parent	HG75	1.23	865	parent	PNB	1.54	664

TABLE 4.6  
Disease scoring of clusterbean plants for bacterial blight disease

Plant number	Disease score	Percentage disease	Plant number	Disease score	Percentage of disease
1	1	1-10%	26	5	20-40%
2	3	11-20%	27	5	20-40%
3	5	20-40%	28	3	11-20%
4	3	11-20%	29	5	20-40%
5	3	11-20%	30	5	20-40%
6	9	>60%	31	5	20-40%
7	5	20-40%	32	3	11-20%
8	9	>60%	33	5	20-40%
9	3	11-20%	34	5	20-40%
10	9	>60%	35	5	20-40%
11	7	40-60%	36	1	1-10%
12	7	40-60%	37	1	1-10%
13	5	20-40%	38	9	>60%
14	3	11-20%	39	3	11-20%
15	3	11-20%	40	3	11-20%
16	5	20-40%	41	5	20-40%
17	3	11-20%	42	1	1-10%
18	5	20-40%	43	7	40-60%
19	5	20-40%	44	5	20-40%
20	9	>60%	45	3	11-20%
21	5	20-40%	46	5	20-40%
22	5	20-40%	47	9	>60%
23	5	20-40%	48	1	1-10%
24	7	40-60%	49	3	11-20%
25	1	1-10%	50	5	20-40%
HG 75	1	1-10%	PNB	9	>60%

24 hrs according to 0-9 scale. Disease appeared on leaves 3-4 days after inoculation. The scores 1 and 3 is for resistant and 5, 7, 9 are for susceptible genotypes. All the progenies were categorized into two different categories *i.e.* resistant and susceptible.

A total of fifteen plants of each of parental genotypes (HG 75 and PNB) and 50  $F_{2-3}$  genotypes were grown and screened for disease reaction. There are a total of 6 genotypes with 1-10% disease, 13 were having 11-20% disease, 21 were having 20-40% disease, 4 were having 40-60% disease and 6 were having > 60% disease.

The genotyping data screened by using the nine markers and the phenotyping data of disease score of all the genotypes was used for linkage analysis. The 50 genotypes along with two parental lines were also used for identification of QTL/gene linked to disease bacterial blight. The resistant and susceptible parents were screened with random (10-mer) primers. Eighty three RAPD primers were used to differentiate between the two parental genotypes HG 75 and PNB as shown in Plate 4.5, lane 3, lane 9, lane 12 and lane 21, markers D1, QLTY 30, L5 and E7 and in plate 4.6, lane 15 marker AB2 were found linked to bacterial blight.



Plate 4.3: Xanthomonas isolated from diseased sample of *Cyamopsis tetragonoloba*.

Seventy four RAPD primers produced good amplification. Nine markers, L19, D1, AB2, E7, D6, AB7, QLTY 30, QLTY 12 and L5 were found polymorphic in these two parents. The markers polymorphic to resistant and susceptible parent were used to screen all the individual  $F_{2-3}$  genotypes.

#### Linkage mapping for bacterial blight

The genotyping data was obtained by the nine markers, L19, D1, AB2, E7, D6, AB7, QLTY30, QLTY12 and L5. Linkage analysis was accomplished



Plate 4.4: Bacterial blight infected plants of Clusterbean at Dryland area.

using the software Mapmaker/Exp version 3.0b and Map manager QTX version 0.30. Distances were calculated in centimorgans (cM) between marker loci on clusterbean genome. The “sequence” and “group” and “map” command were performed for linkage mapping and “build” command to place new markers from genotypic dataset in the most appropriate position within the identified linkage group.

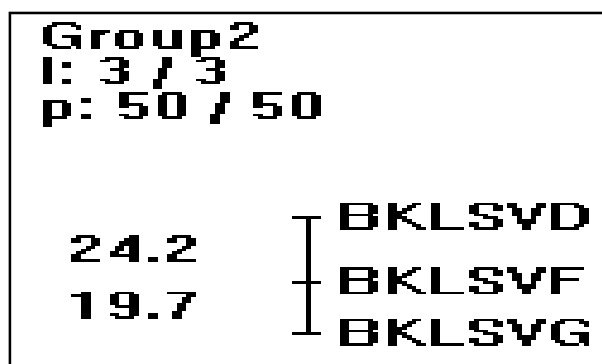


Figure 4.5: Linkage Mapping of RAPD markers for bacterial blight.

TABLE 4.7  
Details of primers used for disease bacterial blight.

Total no. of primers used	83
Primers which showed amplification	74
Primers which produced polymorphism	9

TABLE 4.8  
Primers which produced polymorphism for disease Bacterial Blight

L19	D1	AB2	E7	D6
AB7	QLTY30	QLTY 12	L5	

**QTL Mapping and analysis**

Interval QTL mapping was carried out using software programme map manager QTX version 0.30. Polymorphic scoring obtained from amplification by RAPD markers were used for analysis using Map

manager QTX version 0.30 for Linkage Group Analysis and Molecular mapping of genes. Search & find linkage option was used to make the linkage markers in ordered loci and to calculate the map distances. Simple linear regression analysis and interval QTL mapping analysis were done using polymorphic markers scores along with the trait scores of resistant and susceptible groups. The linear regression function calculated the likelihood ratio statistic (LRS) for the association of the trait with the loci, the probability of an association and estimate of the 95% confidence interval size for a QTL (CI). Marker Regression report was generated to get the detailed information. A clear demarcation was found for resistance at ten loci in progeny genotypes.

The markers polymorphic to parents were further screened with all the  $F_{2-3}$  genotypes. Markers L19, D1, AB7, QLTY30, AB2, QLTY12, E7, L5, D6 were found polymorphic among parents and these 9 markers (Table 4.8) were selected out of 83 markers for further analysis in  $F_{2-3}$  population. Presence or absence of allele was observed and data was scored for all markers in all genotypes for further analysis. Molecular markers linked to disease bacterial blight were identified. Linkage among these markers was analyzed and map distances calculated at  $p=0.05$  with three loci covering a map distance of 43.9 cM. Two markers were found linked to each other in group 1, three markers were found linked to each other and were grouped together in group 2 and four markers were found unlinked with each other. Three QTLs were identified which contributed to the variation for disease reaction. The QTL BKSLVI was the major QTL which contributed 11% to the total variation for the disease reaction whereas QTL BKLSVD and BKLSVF were found linked to each other and contributed 4 % (1%+3%) of total variation for disease reaction. The LOD scores for the loci detected proves close linkage with bacterial blight trait were 9.2 between BKLSVF and BKLSVG and 6.8 between BKLSVD and BKLSVF. Molecular markers linked to disease bacterial blight were found to be L19, AB7 and D1 and QLTY30.

TABLE 4.9  
Molecular markers linked to disease Bacterial Blight

Locus	Marker	Locus	Marker
1	BKLSVF	L19	3 BKLSVI
2	BKLSVD	AB7	4 BKLSVG
			D1
			QLTY30

Disease resistant trait in  $F_{2-3}$  progeny genotypes were found to be linked with three markers, L19, D1 and AB 7. Molecular mapping was done and

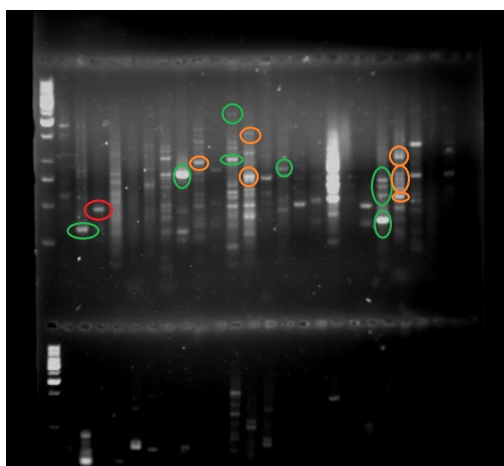


Plate 4.5: Molecular markers showing polymorphism among parents for resistance to disease bacterial blight.

distances were calculated at  $p=0.05$  with three markers covering a map distance of 43.9 cM. Two markers (D1 & AB7) were found linked to each other and were grouped together in group 2 whereas L19 was found unlinked to other markers and contributing to disease resistance. These QTLs can be used for creation of superior genotypes of clusterbean using molecular breeding techniques for resistance to bacterial blight.

## Discussion

### Mapping QTL for bacterial blight resistance

### Screening of bacterial blight disease reaction in clusterbean

In this study QTLs were identified for bacterial blight resistance in guar. Under disease screening experiments, parental genotypes HG75 and PNB and their 50  $F_{2-3}$  crossed plant material were screened under artificial conditions in net house and field. It was observed that, out of fifty  $F_{2-3}$  progenies, six progenies were with 1-10% disease, eleven progenies with 11-20%, twenty three progenies with 20-40% and four progenies were having >60% disease. The disease was first recorded in India as bacterial leaf spot in 1953 by Patel *et al.* and was identified as bacterial blight (Patel and Patel, 1958; Srivastava and Rao, 1963a). Bacterial blight has also been reported from various parts of U.S.A. (Orellana *et al.*, 1965; Mihail and Alcorn, 1985) and Brazil (Almeida *et al.*, 1992). Disease surveys undertaken during 1977-87 in arid zones of Rajasthan in India revealed yield losses of around 50-67 per cent due to bacterial blight (Lodha *et al.*, 1986; Gupta *et*

*al.*, 1998). All the yield components i.e. number of clusters/plant, pods/plant, 1000-seed weight as well as yield per plant are affected adversely. Gandhi and Chand (1985a) recorded losses to the tune of 68 per cent and 32 per cent in highly susceptible cv. Pusa Navbahar and moderately resistant cv. HG 75, respectively, when both were tested under artificial inoculation conditions.

Tyagi *et al.* (2006) screened 54 varieties of cotton for resistance to bacterial blight caused by *Xanthomonas malvacearum* under natural field conditions and artificial conditions using hypodermic needle inoculation method. They found that there was no plant with complete immunity, 18.5% were highly resistant, 29.6% were resistant and 20.4% were susceptible to the disease. Yang *et al.* (2003), inoculated 500 rice plants with 12 *xoo* strains at booting stage of plants using leaf clipping method, on ten uppermost fully expanded leaves of each plant. They scored disease intensity after 2-3 weeks and found that the results were in support with the mendelian ratio (3:1), as out of 500 plants, 388 plants were resistant and 112 plants were susceptible to the disease.

### Genotyping of parents with RAPD primers

The parents HG75 (resistant) and PNB (susceptible) to disease bacterial blight were screened with eighty-three RAPD markers for polymorphic alleles. Out of eighty-three RAPD markers used, seventy-four produced amplification. The markers polymorphic to resistant and susceptible parent were selected. Nine markers L19, D1, AB7, QLTY30, AB2, QLTY12, E7, L5 and D6 were found to be polymorphic and associated to bacterial blight resistance. In a similar pattern, Identification of two markers *Xtxp 309* and *Xtxp 274* for resistant trait had been done by Mittal and Boora (2005) in sorghum. They reported that marker *Xtxp 309* produced an approximately 700 bp band unique in resistant parent and resistant bulk only and other unique band of 450 bp in susceptible parent and susceptible bulk only. The other marker, *Xtxp 274*, produced a unique band of an approximately 100 bp unique in resistant parent and resistant bulk only. Korinsak *et al.* (2009) used sixty-two polymorphic markers covering all rice chromosomes to identify the location and linked markers of the resistance gene. Four SSR markers, viz. *RM30*, *RM7243*, *RM5509* and *RM400* were located on the long arm of rice chromosome 6, could clearly discriminate between resistant and susceptible phenotypes.

Encircled orange: linked to bacterial blight resistant gene.

Encircled green: linked to drought tolerant gene.

Unlinked (marker regression report/single marker analysis)											
Chr	Locus	Stat	%	P	CI	Dom					
Unlinked	: BKLSVI	5.9	11	0.01493	95	-0.75					
Interval mapping report											
Group1	P = 0.05		1:2/2	p:50/50		2 X	2.0 cM				
Locus	Mat	Het	Pat	X	N	Map	SE	low	hi	LOD	
n BKLSVE	16	0	33	2	49	2.0	1.4	0.6	7.2	25.3	
Group 2 (Marker regression report/single marker analysis)											
	Chr	Locus	Stat	%	P	CI	Dom				
•	Group2	:: BKLSVD	0.5	1	0.49751	1157	0.24				
•	Group2	:: BKLSVF	1.3	3	0.25973	423	0.39				
•	Group2	:: BKLSVG	0.1	0	0.71564	4000	-0.13				
Interval mapping:											
Group2	P = 0.05		1:3/3	p:50/50		38 X	42.3	cM			
Locus	Mat	Het	Pat	X	N	Map	SE	low	hi	LOD	
n BKLSVD	37	0	12	22	49	24.2	6.2	16.0	38.0	6.8	
n BKLSVF	36	0	14	16	46	18.1	5.0	11.4	29.8	9.2	
n BKLSVG	34	0	12								

Statistical window

The terminology used:

Res: The number of progeny with the resistant genotype at this locus.

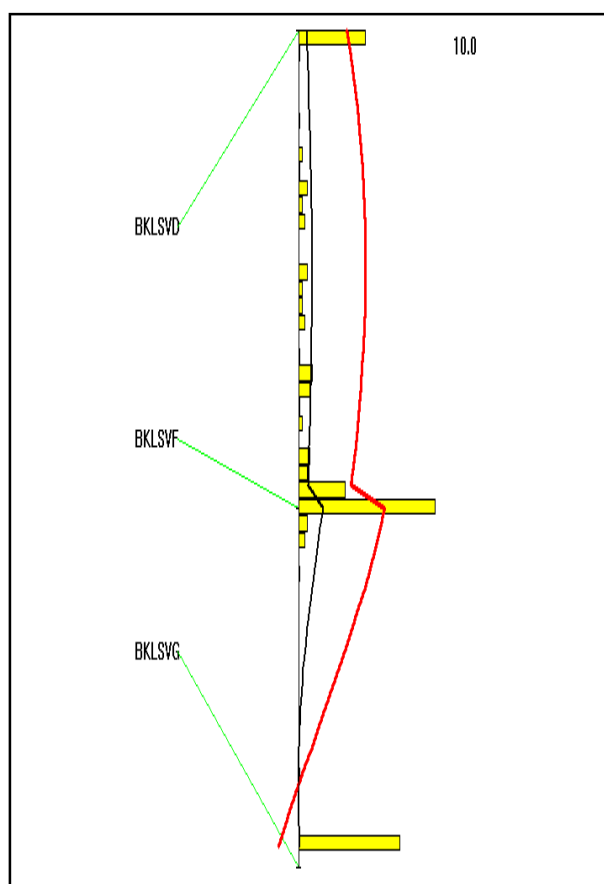
- Het: The number of progeny with the heterozygote genotype at this locus.
- Sus: The number of progenies with the susceptible genotype at this locus
- X: The number of crossovers for this interval (does not include crossovers whose position is ambiguous because of missing data).
- N: The number of informative loci for this interval.
- Map: The map distance for this interval calculated using the mapping function.
- SE: The standard error of the map distance for this interval.
- Low: The lower limit of the 95% confidence interval for the map distance.
- High: The upper limit of the 95% confidence interval for the map distance.
- LOD: The LOD linkage of the markers flanking this interval.
- Stat: The likelihood ratio statistic (LRS) for the association of the trait with this locus.
- %: The difference between the total trait variance and the residual variance, expressed as a percent of the total variance.
- P: The probability of an association this strong happening by chance.
- CI: An estimate of the size of a 95% confidence interval for a QTL of this strength, using the estimate of Darvasi and Soller (1997).
- Dom: The dominance regression coefficient for the association.

Fig. 4.6 Molecular map of QTLs/genes for disease tolerance.

### QTL analysis for disease resistance

In this study, Eighty three markers were screened and nine markers were found polymorphic among parents. The nine markers were used to screen F<sub>2-3</sub> population. Linkage among markers was analyzed and map distance among markers AB7, D1 and QLTY 30 was calculated as 43.9 cM. Interval QTL mapping was carried out using software programme map

manager QTX version 0.30. Molecular markers linked to disease bacterial blight were L19, AB7 and D1 and QLTY30. In the earlier studies, there was no report regarding molecular mapping of bacterial blight resistant gene in clusterbean. Boora (2003) tagged genes resistant to pathogens in sorghum using SSR markers. SSR markers Xtxp 212, Xtxp 274 and Xtxp 105 were found to be closely linked to the resistance gene for anthracnose, leaf blight and oval leaf spot,



Group 2:

respectively. A RAPD (random amplified polymorphic DNA) marker OPJ 01<sub>1437</sub> has been identified by Singh *et al.* (2006) closely linked to anthracnose resistance gene in sorghum by bulked segregant analysis of HC 136 x G 73 derived recombinant inbred lines (RILs) of sorghum. Goto *et al.* (2009) mapped bacterial blight resistance gene *Xa11* on rice chromosome by using RAPD, CAPS and SSR marker system in near isogenic lines (NILs). They identified an RAPD fragment L19<sub>1200</sub> linked to *Xa11* gene. Yang *et al.* (2003) mapped *Xa 26* gene of rice, resistant to bacterial blight caused by *Xanthomonas oryzae* in a region of about 1.68 cM on rice genome. Microsatellite markers (SSR) linked to a bacterial blight resistance gene *xa33* was identified by Korinsak *et al.* (2009) in rice cultivar 'Ba7'.

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