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The SSR based linkage map construction and identification of QTLs for blast (*Pyricularia grisea*) resistance in pearl millet (*Pennisetum glaucum* (l.) r. br.)

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Abstract
Blast of pearl millet is one of the most important devastating diseases limiting pearl millet productivity. The DNA-based marker tools facilitate better understanding of the inheritance and expression of blast. To fulfill the objectives, two pearl millet inbred parental lines viz. J – 2537 x ICMB - 95444 were crossed to produce F_1 and the F_1 progenies were selfed to produce mapping population comprising of 36 segregating F_2 progenies for generating marker data using 55 SSR out of 100 markers exhibiting clear polymorphism between parental lines. The blast screening of segregating F_2 population progenies against Blast was done at JAU, Jamnagar.
R-QTL interval mapping method identified two blast QTLs on Linkage Group 1 and Linkage Group 6. This best single-QTL model detected by interval mapping on Linkage Group 1 (BRP11 marker) recorded a high LOD score of 8.2 and explained 46.4% phenotypic variation with map position 15.8 Linkage Group 6 (BRP37 marker) recorded a high LOD score of 1.6 and explained 36% phenotypic variation in blast incidence among 36 F_2 progenies against *Pyricularia grisea* pathogen.
The confidence interval detected that the position between 10-18 cM on linkage group 1 was responsible for the resistance against blast in pearl millet. The effect plot for marker BRP11 indicated that the resistance against the blast was governed by dominant inheritance and can be easily used for the transfer of trait through marker assisted selection using marker BRP11

Keywords: linkage, QTL, BLAST, linkage group, SSR, LOD

Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a monocot species belonging to the Poaceae family and has a relatively small diploid genome (2n=2x=14) with a DNA content of 1C =2.36 pg (Martel *et al.*, 1997). Pearl millet is an excellent organism for genetic research because of its low chromosome number (2n=14), short life cycle, high multiplication ratio (up to 1:1000), ratooning ability and the ease with which cross pollination can be done due to protogyny. It has also been found very suitable for molecular genetic studies. It is a highly cross-pollinated crop and possesses abundant phenotypic variation. It has a number of wild relatives (n=5, 7, 8 and 9) including a large group with 2n=14 with which it can be intercrossed (Jauhar, 1968; Jauhar, 1981; Jauhar and Hanna, 1998) [7, 8].
Crop losses due to plant diseases are economically important. Among the diseases affecting pearl millet, Blast is also known as leaf spot, is one of the most widely spread and destructive diseases of pearl millet, potentially resulting in devastating yield losses in India and Western Africa. It is caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*), has emerged as a serious disease affecting both forage and grain production in pearl millet in India. The pearl millet Blast pathogen reproduces asexually by means of sporangia that germinate to release motile zoospores and sexually to produce soil-borne oospores. Despite the wide host range of the pathogen, M. grisea populations mainly exist as host-specific (adapted) forms, capable of infecting a single host (Todman *et al.*, 1994) [21]. While some researchers have demonstrated successful infection of a host by an isolate from a different host under experimental conditions (Singh and Kumar, 2010) [20], others failed to confirm the results (Todman *et al.*, 1994) [21].
Pearl millet Blast is also known as leaf spot, caused by *Pyricularia grisea* (*teleomorph: Magnaporthe grisea*), has emerged as a serious disease affecting both forage and grain production in pearl millet in India. This disease causes substantial yield losses of grain and forage. Symptoms of the disease appear as gray, water-soaked foliar lesions that enlarge and become necrotic, resulting in extensive chlorosis and premature drying of young leaves. This disease becomes more severe during humid weather conditions, especially with dense plant stands. Leaf blast

on pearl millet has been found to be negatively correlated with green-plot yield, dry-matter yield, and digestive dry matter thus affecting the productivity and quality of the crop.

Poncet *et al.* (2002) [15] made a comparison of the locations and effect of QTLs controlling the morphological differences between domesticated and wild pearl millet with a focus on the organization of linkage groups LG 6 and LG 7. In their previous study (Poncet *et al* 2000) [13], they revealed that domesticated spikelet structure is mainly controlled by major genes located on LG 6 and LG 7. Poncet *et al.* (2000) [13] have analyzed another cross in which the domesticated parent differs in their geographical origin, agronomic characteristics and life cycle from wild parents in the studied population. Poncet *et al.* (2002) [15] further compared the level of polymorphism and constructed a linkage map consisting 22 RFLP loci distributed among the seven linkage groups covering 177 cM, which corresponded to 54.6% of the original pearl millet reference map (Liu *et al.,* 1994) [11]. This is due to both a strong reduction in recombination rate in their two crosses (wild x cultivated) relative to the cross between inbreds of cultivated pearl millet used to build the reference map (Poncet *et al.,* 2000) [13] and due to incomplete map coverage. Similar reductions in recombination rate and linkage map length in wild and cultivated crosses have been reported by Liu *et al.* (1996) [9].

Materials and Methods

Experimental Meterial

The investigation was carried out using the pearl millet genotypes. The parental seed of resistant genotypes J-2537 and susceptible genotypes ICMB- 95444 to blast were procured from the Research Scientist, Main Millet Research Station, Jamnagar, Junagadh Agricultural University, Gujarat.

Mapping Population

The experimental material comprised of single cross of pearl millet involving two genetically diverse parents ICMB-95444 and J-2537 for Blast, collected from Main Millet Research Station JAU, Jamnagar. Hence the experimental material was consisted of four basic generations, *viz,* P_1, P_2, F_1 and F_2.

Crossing programme

The seeds of inbred lines ICMB-95444 and J-2537 were used as parents and sown at Main Millet Research Station JAU, Jamnagar during summer 2015, and the cross was made between two parents to obtain F1 hybrids.

Field sowing

Seeds of all the entries were sown and individual plant was raised in 10 x 30 meter sick plot at Main Millet Research Station JAU, Jamnagar during *Kharif* season of 2015. This crop was raised to record disease incidence. The seeds were placed at a depth of 2-2.5 cm. The recommended doses of fertilizers were given to the crop. The row to row distance was 45 cm. The crop was well irrigated regularly to maintain high humidity condition. Thinning and weeding was done 20 and 40 days after sowing keeping plant to plant spacing of 20 cm. Highly susceptible genotype 7042S was sown after every fifth rows and 45 individual F_2 plants were sown in four intervening rows of every fifth highly susceptible genotype's row.

Disease Incidence

The severity record was taken on a 1-5 rating scale for reaction categories (Singh *et al*., 1993) [19].

Where,

1 = No disease symptom

2 = Disease only on the nodal tillers of a plant infected

3 = Less than 50% of the basal tillers of a plant infected

4 = More than 50% of the basal tillers of a plant infected and

5 = No productive panicle produced

Disease incidence was recorded 30 and 60 days after sowing whereas, disease severity was recorded 60 days after sowing.

DNA extraction and amplification

The DNA was isolated from dark-grown, young leaf tissues by following standard DNA extraction protocol as described by Sharp *et al.* (1989) [18] with minor modifications. DNA concentration was determined by Picodrop PET01 using software v2.08 (Picodrop Ltd., Cambridge U.K).

The parents were screened for molecular marker polymorphism with a total of 98 primers (Table 1). SSR primer pairs developed by Allouis *et al.,* 2001 and Qi *et al.,* 2001 were evaluated on the mapping population. Primers were excluded from the study if banding patterns were difficult to score or if the primers failed to amplify consistently in all individuals.

The PCR reaction mixture (25 µL total) consisted of 10X PCR buffer, 2.5 mM each dNTPs, 10 pmoles/l Primer, 25ng/l genomic DNA and 0.5 unit of *Taq* polymerase. Amplification was performed in gradient master cycler (Applied Biosystem and Eppendorf) using a program that was suitable for marker. The program for SSR consisted of denaturation for 5 min at 94ºC followed by 35 cycles of denaturation at 94 ºC for 1.5 min, annealing 45 sec. at Tm ± 2ºC and final extension for 7 min at 72 ºC. The amplified products of SSR were analyzed on 3% agarose gel and the presence of each band was scored as '1' and its absence scored as '0'. Faint bands were not considered. Only those markers that were highly reproducible and based on clear presence or absence of polymorphism without any intensity variation were included in the analysis.

Linkage map construction

The banding patterns obtained from PCR amplification of SSR primers in the F_2 individuals were scored as follows:

A = Homozygote for allele a from parental strain P_1 at this locus

B = Homozygote for allele b from parental strain P_2 at this locus

H = Heterozygote carrying alleles from both P_1 and P_2 parental strains i.e. genotype comparable to the F_1

C = Not a homozygote for allele a (i.e. either B or H)

D = Not a homozygote for allele b (i.e. either A or H)

- = Missing data for the individual at this locus

Linkage map was developed for the molecular markers used in the analysis of pearl millet F_2 generation. The linkage map was developed using R software with mapone programme. The distance between the markers have been demonstrated in the linkage map in cM unit. The data generated by mapone programme were used for QTL mapping.

The "sequence", "group" and "map" command were performed for linkage mapping and "build" command to place new markers from genotypic data set in the most appropriate position within the identified linkage group. Then software Mapchart was used to draw all linkage groups of the genetic linkage map.

Development of QTL map

The data obtained from the screening of F_2 population by

marker and sick plot screening were compared using R software for QTL mapping for the disease resistant. Trait data from F_2 were averaged for each entry and sorted to correspond with the progeny order of the genotypes (marker data). The total number of progeny individuals from the cross with trait and genotype information was 39. The QTL mapping was performed using the R software for QTL mapping. The R Software calculates additive and dominant effect from the change in phenotype resulting from the substitution of B parent alleles for A parent alleles. In the cross under study, susceptible to disease was scored as 'A' and the resistant to disease was scored as 'B', however the heterozygote was scored as 'H'.

Observations and measurements in field

Time to bloom (TB): Time to 50% flowering was recorded as the number of days from sowing until 50% of the plants in each plot produced stigmas on their main stem panicles.

Plant height (PH): Plant height (cm) was measured from the base of the main stem to the tip of the panicle at maturity.

Panicle length (PL): Length of the panicle (cm) was measured for main stem of the plants.

Results and Discussion

Disease reaction by parental entries

The parental lines of the mapping population (For Blast: J-2537 X ICMB - 95444) was screened under field conditions at Main Millet Research Station, JAU, Jamnagar. Parental line J-2537 was highly resistant and exhibited no symptoms of infection against Blast. Very high diseases were observed on susceptible parental line ICMB - 95444. So, these lines were selected for the purpose developing F_2 generation to be screened against the Blast resistance. For the identification of resistance sources, a pearl millet mini-core comprising 125 F_2 mapping population was evaluated under greenhouse conditions against three *M. grisea* isolates (Pg118, Pg56, Pg45) representing the three pathotypes.

Parental polymorphism for blast

Pearl millet mapping F_2 population from parental lines J-2537 X ICMB - 95444 were screened against 100 SSR (microsatellite) markers to identify polymorphic combinations. Out of 100 markers screened approximately 55% showed polymorphism between these two parental lines of Pearl millet for Blast resistant. A total of 55 SSR markers were polymorphic between two parental lines and were used to screen the mapping population of F_2 (36 lines) developed for blast resistance.

Genetic linkage map

Genotypic data generated for a total of 27 markers loci were used to construct a linkage map of the pearl millet mapping population of 36 F_2 progenies based on the cross J-2537 X ICMB - 95444 for blast. A linkage map of seven linkage groups with a total map length of 248.37 cM was constructed using data from 27 marker loci for 36 F_2 progenies. The map lengths of individual linkage groups ranged from a minimum of 24.64 cM (LG2) to a maximum of 81.23 cM (LG6), as shown in Figures 1.

R QTL interval mapping method identified two blast QTLs on Linkage Group 1 and Linkage Group 6 (Figure 2). This best single-QTL model detected by interval mapping on Linkage Group 1 (BRP11 marker) recorded a high LOD score of 8.2 and explained 46.4% phenotypic variation with map position 15.8 and Linkage Group 6 (BRP37 marker) recorded a high LOD score of 1.6 and explained 36% phenotypic variation in blast incidence among 36 F_2 progenies against *Pyricularia grisea* pathogen.

The confidence interval detected that the position between 10-18 cM on linkage group1 was responsible for the resistance against blast in pearl millet (Figure 3). As per the effect plot for marker BRP11 as shown in fig.4.16, the trait was govern the dominant gene and so the breeding strategy involving dominant gene action/heterosis may be beneficial; since the trait is govern by dominant gene with large effect, the marker can be used for positional cloning to identify genes involved in the resistance to blast.

Blast is a menace to pearl millet production in SAT (Semi arid Tropics) regions of the world. It causes devastating losses and last century has witnessed pearl millet blast epidemics many times in India. The parental lines ICMB – 95444 (susceptible to blast) and J-2537 (resistant to blast) and their F_2 mapping population progenies were screened against blast in sick plot in the present study. Parental line J-2537 was found to be highly resistant and exhibited no symptoms of infection against blast. At the same time, parental line ICMB – 95444 recorded very high blast incidence in screens against pathogen populations. Blast inheritance results among 36 F_2 progenies based on cross J-2537 x ICMB – 95444 are presented in Annexure 1. In present study, about 41% of the population inheritance as per the expected ratio of 1:2:1 among the 36 F_2 populations. The remaining 45% population exhibited significant segregation distortion in this mapping population (as shown in Table 2).

Genetic linkage map for cross J-2537 X Icmb – 95444

The inclusion of polymorphic SSR markers located on both, upper and lower distal ends of several linkage groups, quite far from putative centromeric regions has resulted in the increase in total map distance of the cross J-2537 x ICMB – 95444, under study. Such marker loci have been mapped in this course of study on top of both ends of LG3 and LG6 as shown in Figures 4 and Figures 5. Large inter marker distances (>50 cM Haldane) have been recorded for several of these distally located maker loci leading to enhancement of total map length. This expectation has been strengthened by subsequent mapping studies in pearl millet using different parental combinations. This increase in map length is because of adding new RFLP markers from pearl millet and other cereal crops (Devos *et al.,* 2000) [5] along with AFLP and SSR markers. Qi *et al.* (2004) from John Innes Centre, UK has recently reported an update consensus map of pearl millet.

Conclusions

The present study was based on a cross of parental lines J-2537 and ICMB – 95444. This study was designed to construct a skeleton linkage map, based on J-2537 x ICMB – 95444, to identify and map QTLs controlling blast resistance, to study inheritance of blast resistance. To fulfill these objectives, the two pearl millet inbred parental lines were crossed to obtain F_1, and the F_1 progenies were selfed to produce 36 segregating F_2 mapping population progenies for generating marker data using 27 SSR markers exhibiting clear polymorphism between parental lines. The F_2 mapping population progenies were screened against pathogen. The blast screening of segregating F_2 population progenies based on J-2537 x ICMB – 95444 against blast pathogen population was done at JAU, Jamnagar.

The construction of genetic linkage maps and QTL mapping for economical traits in fields crops are very important tools

for studying genome structure, identifying introgression between genomes and localizing genes of interest in genomic regions. Co dominant markers such as RFLPs and SSRs have simple genetic segregation patterns and are potentially abundant in number. The parental lines J-2537 and ICMB – 95444 were screened against a set of 100 SSR markers following standardized protocols and 55% of this exhibiting clear and scorable polymorphism was used to generate the mapping population marker data.

Table 1: List of SSR primers used in the present study

S. No	Markers		Sequence 5' – 3'	Tm (^{0}C)	GC (%)
1	Pyrms 7 and 8	F	GCAAATAACATAGGAAAACG	51.2	35.0
		R	AGAAAGAGACAAAACACTGG	53.2	40.0
2	Pyrms 15 and 16	F	TTCTTCCATTTCTCTCGTCTTC	56.5	46.9
		R	CGATTGTGGGGTATGTGATAG	57.9	47.6
3	Pyrms 33 and 34	F	CATTTGTTCAAGGGGATTTC	53.2	40.0
		R	CTCGGGAGGTTGCTAACG	58.2	51.1
4	Pyrms 37 and 38	F	ACCCTACCCCCACTCATTTC	59.4	55.0
		R	AGGATCAGCCAATGCCAAGT	57.3	50.0
5	Pyrms 39 and 40	F	CGCATACAGGAAAGCCAAGA	57.3	50.0
		R	CTGACGAGGGACTCCTGTGT	61.4	60.0
6	Pyrms 41 and 42	F	AACGTGACAATGTGAGCAGC	57.3	50.0
		R	GCCATGTTCTAAGGTGCTGAG	59.8	52.4
7	Pyrms 43 and 44	F	TCAGTAGGCTTGGAATTGAAAAA	55.3	34.8
		R	CTTGATTGGTGGTGGTGTTG	57.3	50.0
8	Pyrms 45 and 46	F	CCACTTTATAGCCCACCCAGT	59.8	52.4
		R	CTCTTTTCTCGCAGGAGGTG	59.4	55.0
9	Pyrms 47 and 48	F	TCACATTTGCTTGCTGGAGT	55.3	45.0
		R	AGACAGGGTTGACGGCTAAA	57.3	50.0
10	Pyrms 59 and 60	F	TTCTCAGTAGGCTTGGAATTGA	56.5	40.9
		R	CTTGATTGGTGGTGGTGTTG	57.3	50.0
11	Pyrms 61 and 62	F	GAGGCAACTTGGCATCTACC	59.4	55.0
		R	TGGATTACAGAGGCGTTCG	56.7	52.6
12	Pyrms 63 and 64	F	TTGGGATCTTCGGTAAGACG	57.3	50.0
		R	GCCGACAAGACACTGAATGA	57.3	50.0
13	Pyrms 67 and 68	F	AGCAAGCAGGAGATGCAGAC	59.4	55.0
		R	GTTTGGCTGGCAAGACAGTT	57.3	50.0
14	Pyrms 77 and 78	F	GAAGTATTGCACACAAACAC	53.2	40.0
		R	GCTTTCGGCAAGCCTAATC	56.7	52.6
15	Pyrms 81 and 82	F	CCTTGTTTTCCCCCTGTGTA	57.3	50.0
		R	TAGCCAAATGCCCATTATCC	55.3	45.0
16	Pyrms 83 and 84	F	GTCTGCCTCGACTCCTTCAC	61.4	60.0
		R	AGCCCAAAAACAGAAAGCAA	53.2	40.0
17	Pyrms 87 and 88	F	AGACTTGTTACTCGGGTCTTGA	58.4	45.5
		R	CCAGATGTCACTCCCCTGTA	59.4	55.0
18	Pyrms 93 and 94	F	CCTCGACTCCTTCACCAAAA	57.3	50.0
		R	CGGAGAGCTCAGGAAGAGG	61.0	63.2
19	Pyrms 99 and 100	F	CACCACTTTATGGCGCAGT	56.7	52.6
		R	ACCTAGGTAGGTATACATGTTGTT	57.6	37.5
20	Pyrms 101 and 102	F	CTGCGTTCAACATGCCTCTA	57.3	50.0
		R	CTTGATCTGCGGTATGAGCA	57.3	50.0
21	Pyrms 107 and 108	F	GCAGCAAGCAGCAATATCAG	57.3	50.0
		R	GTGGATATCGAAGGCCAAGG	59.4	55.0
22	Pyrms 109 and 110	F	TACAGTGGGAGGGCAAAGAG	59.4	55.0
		R	CCAGATCGAGAAGGGGGTAT	59.4	55.0
23	Pyrms 115 and 116	F	TTCGTTCACCTTTTGGCTCT	55.3	45.0
		R	TTGTTAAGTGAGCGGACGTG	57.3	50.0
24	Pyrms 125 and 126	F	CTCTCCGGCCAAGATTGA	56.0	55.6
		R	GGTTGTTGGGAGAAAGAACG	57.3	50.0
25	*Xpsmp2031*	F	CACATCCGCAAGAGACACCAAAT	60.6	47.8
		R	TTTGGGGGTGTAGGTTTTGTTG	58.4	45.5
26	*Xpsmp2231*	F	TTGCCTGAAGACGTGCAATCGTCC	64.4	54.2
		R	CTTAATGCGTCTAGAGAGTTAAGTTG	60.1	38.5
27	*Xpsmp2089*	F	TTCGCCGCTGCTACATACTT	57.3	50.0
		R	TGTGCATGTTGCTGGTCATT	55.3	45.0
28	*Xpsmp2251*	F	TCAAACATAGATATGCCGTGCCTCC	63.0	48.0
		R	CAGCAAGTCGTGAGGTTCGGATA	52.4	52.2
29	*Xpsmp2225*	F	CCGTACTGATGATACTGATGGTT	58.9	43.5
		R	TGGGAGGTAAGCTCAGTAGTGT	60.3	50.0
30	*Xpsmp2255*	F	CATCTAAACACAACCAATCTTGAAC	58.1	36.0
		R	TGGCACTCTTAAATTGACGCAT	56.5	40.9

31	*Xpsmp2266*	F	CAAGGATGGCTGAAGGGCTATG	62.1	54.5
		R	TTTCCAGCCCACACCAGTAATC	60.3	50.0
32	*Xpsmp2208*	F	GAAAGAGCAAACTGAACAATCCC	58.9	43.5
		R	ACTTTGCCCTGGATGATCCTC	59.8	52.4
33	*Xpsmp2248*	F	TCTGTTTGTTTGGGTCAGGTCCTTC	63.0	46.0
		R	CGAATACGTATGGAGAACTGCGCATC	64.8	50.0
34	*Xpsmp2236*	F	ATAAGTGGGACCCACATGCAGCAC	64.4	54.2
		R	CGAAAGACTAGCAAAATTGCGCCTTC	63.2	46.2
35	*Xpsmp2249*	F	CAGTCTCTAACAAACAAACACGGC	61.0	45.8
		R	GACAGCAACCAACTCCAAACTCCA	62.7	50.0
36	*Xpsmp2275*	F	CCAGTGCCTGCATTCTTGGCCC	65.8	63.6
		R	GCATCGAATACTTCATCTCA	53.2	40.0
37	*Xpsmp2270*	F	AACCAGAGAAGTACATGGCCCG	62.1	54.5
		R	CGACGAACAAATTAAGGCTCTC	58.4	45.5
38	*Xpsmp2261*	F	AATGAAAATCCATCCCATTTCGCC	59.3	41.7
		R	CGAGGACGAGGAGGGCGATT	63.5	65.0
39	*Xpsmp2227*	F	ACACCAAACACCAACCATAAAG	56.5	40.9
		R	TCGTCAGCAATCACTAATGACC	58.4	45.5
40	*Xpsmp2219*	F	ACTGATGGAATCTGCTGTGGAA	58.4	45.5
		R	GCCCGAAGAAAAGAGAACATAGAA	59.3	41.7
41	*Xpsmp2273*	F	AACCCCACCAGTAAGTTGTGCTGC	64.4	54.2
		R	GATGACGACAAGACCTTCTCTCC	62.4	52.2
42	*Xpsmp2080*	F	CAGAATCCCCACATCTGCAT	57.3	50.0
		R	TGCAACTGAGCGAAGATCAA	55.3	45.0
43	*Xpsmp2069*	F	CCCATCTGAAATCTGGCTGAGAA	60.6	47.8
		R	CCGTGTTCGTACATGGTTTTGC	60.3	50.0
44	*Xpsmp2085*	F	GCACATCATCTCTATAGTATGCAG	59.3	41.7
		R	GCATCCGTCATCAGGAAATAA	55.9	42.9
45	*m13_Xpsmp2237*	F	TGGCCTTGGCCTTTCCACGCTT	64.0	59.1
		R	CAATCAGTCCGTAGTCCACACCCCA	66.3	56.0
46	*m13_Xpsmp2232*	F	TGTTGTTGGGAGAGGGTATGAG	60.3	50.0
		R	CTCTCGCCATTCTTCAAGTTCA	58.4	45.5
47	*m13_Xpsmp2229*	F	CCACTACCTTCGTCTTCCTCCATTC	64.6	52.0
		R	GTCCGTTCCGTTAGTTGTTGCC	62.1	54.5
48	*Xicmp3027*	F	ACACCATCACCGACAACAAA	55.3	45.0
		R	AGTGACCTGGGGTACAGACG	61.4	60.0
49	*Xicmp3088*	F	TCAGGTGGAGATCGATGTTG	57.3	50.0
		R	TTACGGGAGGATGAGGATG	56.7	52.6
50	*Xicmp3050*	F	ATGTCCAGTGTTGACGGTGA	57.3	50.0
		R	CGGGGAAGAGACAGGCTACT	61.4	60.0
51	*Xicmp3032*	F	AGGTAGCCGAGGAAGGTGAG	61.4	60.0
		R	CAACAGCATCAAGCAGGAGA	57.3	50.0
52	*Xctm10*	F	GAGGCAAAAGTGGAAGACAG	57.3	50.0
		R	TTGATTCCCGGTTCTATCGA	55.3	45.0
53	*Xctm12*	F	GTTGCAAGCAGGAGTAGATCGA	60.3	50.0
		R	CGCTCTGTAGGTTGAACTCCTT	60.3	50.0
54	*Xctm25*	F	GCGAAGTAGAACACCGCGCT	61.4	60.0
		R	GCACTTCCTCCTCGCCGTCA	63.5	65.0
55	*Xpsms2*	F	TGATGATCAATTGATTCATCCG	54.7	36.4
		R	TATTCAGCTGGACAATGTGCG	57.9	47.6
56	*Xpsms6*	F	TGTCCCACTCTCTACAGATTC	57.9	47.6
		R	TATACACCACTCAACTTACTCA	54.7	36.4
57	*Xpsms17*	F	CCCTTCATGGTGAGGATGAG	59.4	55.0
		R	GACAGAGAAGCTTATCCTGC	57.3	50.0
58	*Xpsms18*	F	TGTGCCATCATCATTCTTGG	55.3	45.0
		R	CGAGATAGCATCTATGGTGC	57.3	50.0
59	*Xpsms29*	F	CCCTGCGTCAGCATCTCCTG	63.5	65.0
		R	GGTGGAGGACATCCTCAAAG	59.4	55.0
60	*Xpsms31*	F	ACGAGACCTTCATCTTCACTG	57.9	47.6
		R	CTTGACGACTGGGTGAGCTG	61.4	60.0
61	*Xpsms32*	F	TGGTAAGGCCAAGAAGATGG	57.3	50.0
		R	AAATCCGTCCATGTTCACGC	57.3	50.0
62	*Xpsms39*	F	CCTGAACGATGTCTCAATACC	57.9	47.6
		R	ATCAATGAGCCAGAGCTTGC	57.3	50.0
63	*Xpsms41*	F	TGAGGAGCATTTGTACAGGC	57.3	50.0
		R	CCATCGATGAGCTTCAGTTC	57.3	50.0
64	*Xpsms58*	F	GTTTCATGTCTGATCTCGACG	57.9	47.6
		R	AGACTCTTTCTGCCGTTGCG	59.4	55.0
65	*Xpsms59*	F	CTTTCACGTGTCTGCCAAGC	59.4	55.0

		R	TCAATCCTCTTGCTCGCAAC	57.3	50.0
66	*Xpsms61*	F	CTGGCTTCACACCTAGAGATG	59.8	52.4
		R	GGATAGCATTGCGAATGGTG	57.3	50.0
67	*Xpsms68*	F	AGGAGGTGGAGTCGATAAGG	59.4	55.0
		R	CTTTGCTCCTCTCGTTGTACG	59.8	52.4
68	*Xpsms73*	F	TTCACTTGCAAGCAAGATGG	55.3	45.0
		R	CTTGTATCCAGAGCTAAGACC	57.9	47.6
69	*Xpsms74*	F	TTCTGACACTGTGCCTTTAGC	57.9	47.6
		R	AGACCCAGCATGCACTCAAC	59.4	55.0
70	*Xpsms75*	F	AAGAGGGCCTTGAACTGTTG	57.3	50.0
		R	CAGATCTTTCAGGCTGTCTCC	59.8	52.4
71	*Xpsms76*	F	CAACCATGCTACTCTATCTGG	57.9	47.6
		R	GCAATGTCTGTCATGAACTG	55.3	45.0
72	*Xpsms77*	F	GGATGCTACCTTCTCCTTCAC	59.8	52.4
		R	AACCTTCTACAGCTTCGCTG	57.3	50.0
73	*Xpsms78*	F	GCGCGATCTTGAACCACTCG	61.4	60.0
		R	GCCATCTTCCTTGACCGCATC	61.8	57.1
74	*Xpsms80*	F	GTACAAGGAGATCGAGAACG	57.3	50.0
		R	GACGGAAGGTGTCAACAATG	57.3	50.0
75	*Xpsms86*	F	CGTACAAGGAGATCGAGAAC	57.3	50.0
		R	AATGTCGACATCAACAGCTC	55.3	45.0
76	*Xpsms88*	F	AATGCACTAGTCCACCGTCC	59.4	55.0
		R	CCTACACCACACGCTTCCTC	61.4	60.0
77	*Xpsms89*	F	AGGGACACGCGAATACAAGC	59.4	55.0
		R	CTTGAGAAGGAGAGTTGTCTTC	58.4	45.5
78	*Xpsms92*	F	TGGTGATGCTGCTGCTTTAG	57.3	50.0
		R	CGACCGAGTACATCTTCTGG	59.4	55.0
79	*Xpsmp2027*	F	AGCAATCCGATAACAAGGAC	55.3	45.0
		R	AGCTTTGGAAAAGGTGATCC	55.3	45.0
80	*Xpsmp2064*	F	ACCGAATTAAAGTCATGGATCG	56.5	40.9
		R	TTGATTCTTCTGACACAAATGAG	55.3	34.8
81	*Xpsmp2068*	F	CAATAACCAAACAAGCAGGCAG	58.4	45.5
		R	CTTCACTCCCACCCTTTCTAATTC	61.0	45.8
82	*Xpsmp2078*	F	CATGCCCATGACAGTATCTTAAT	57.1	39.1
		R	ACTGTTCGGTTCCAAAATACTT	54.7	36.4
83	*Xpsmp2084*	F	AATCTAGTGATCTAGTGTGCTTCC	59.3	41.7
		R	GGTTAGTTTGTTTGAGGCAAATGC	59.3	41.7
84	*Xpsmp2203*	F	GAACTTGATGAGTGCCACTAGC	60.3	50.0
		R	TTGTGTAGGGAGCAACCTTGAT	58.4	45.5
85	*Xpsmp2222*	F	TGGCTTCCAGACTAATCATCAC	58.4	45.5
		R	TTATTTTAGCGGCGAGATTGAC	56.5	40.9
86	*Xicmp3017*	F	CACCAAACAGCATCAAGCAG	57.3	50.0
		R	AGGTAGCCGAGGAAGGTGAG	61.4	60.0
87	*Xicmp3063*	F	TCCGGTAGAGACCGTAATGG	59.4	55.0
		R	GGCACTCCCTAGCAAAATGA	57.3	50.0
88	*Xicmp3073*	F	GCACGAGGGCCAGATTCTA	58.8	57.9
		R	TACACGGTGATGACACGACA	57.3	50.0
89	*Xicmp3081*	F	ACGCCGTTTTCGTGTAGTCT	57.3	50.0
		R	TCCACAAGGTGACCTCACTG	59.4	55.0
90	*Xicmp3086*	F	ACCAAACGTCCAAACCAGAG	57.3	50.0
		R	ATATCTCTTCGCTGCGGTGT	57.3	50.0
91	*Xpsm37*	F	AAAGGTGTCGTTGTTGTGCC	57.3	50.0
		R	GACTGCTGGTCGGTCACG	60.5	66.7
92	*Xpsm345*	F	CTGGGGGAGAGAGAAGGG	60.5	66.7
		R	AAAAGGCTGGGAGAGTAGGC	59.4	55.0
93	*Xpsm592*	F	GCCACAGAAACACTGAAGATG	57.9	47.6
		R	GGAAGGCATCCAAGAGCC	58.2	61.1
94	*Xpsm669*	F	TAATGGGTAGGAAAACCTCGC	57.9	47.6
		R	GAAAAAGAGGCAGGCAAATG	55.3	45.0
95	UGTP001	F	GAACGACACAATTCAAAGTAGATTA	56.4	32.0
		R	CGGCTTTTCTGTATGTATTGTAGG	59.3	41.7
96	UGTP002	F	AGTTGCTCCGGGTTTGTTGTT	57.9	47.6
		R	GCATCCTAATCAGTCACTTTCA	56.5	40.9
97	UGTP003	F	AGGTTGCTAAAGCTACTGATGTTA	57.6	37.5
		R	GCCTCTGTGTGATATGTTATTTGTC	59.7	40.0
98	UGTP004	F	TGTAGGCTATCAATATTATGAGTGG	58.1	36.0
		R	AACGACAAACACTCTTCATTCAT	55.3	34.8

Table 2: The mean Blast incidence percentage (BI%) reaction of 36 F_2 mapping population, parents and control entries against three isolates of pathogen, under greenhouse conditions at Jamnagar, Gujarat, 2015.

Parents and F_2 population	Blast incidence percentage			Comments
	Pg118	Pg56	Pg45	
P_1 (J-2537)	4.13	1.35	1.02	HR
P_2 (ICMB – 95444)	97.87	89.97	24.80	HS to MR
F_1 (P1 x P2)	55.15	76.60	1.00	S to R
JMSB 2091 (S)	99.02	100.00	94.38	HS
F_2 Population				
1	97.42	98.80	45.18	HS to MS
2	98.12	94.78	91.65	HS
3	96.95	97.55	92.32	HS
4	100.00	98.48	62.13	HS to S
5	97.87	84.97	14.80	HS to MR
6	11.95	5.42	2.22	MR to HR
7	97.42	98.80	45.18	HS to MS
8	99.07	99.40	35.37	HS to MS
9	84.95	51.32	51.92	HS to S
10	91.63	92.27	89.70	HS
11	55.15	76.60	0.00	S to R
12	94.38	92.48	91.28	HS
13	7.58	11.62	10.05	MR to R
14	38.32	39.37	2.90	MS to R
15	100.00	99.28	100.00	HS
16	23.60	12.92	1.63	MS to HR
17	8.87	1.28	46.15	MS to HR
18	82.27	82.75	1.38	HS to HR
19	32.82	23.93	1.05	MS to HR
20	8.63	0.83	1.33	R to HR
21	99.55	97.12	11.85	HS to MR
22	87.15	95.02	77.68	HS to S
23	98.88	99.43	7.30	HS to R
24	6.63	51.93	15.02	S to R
25	97.53	97.65	95.32	HS
26	10.72	36.38	7.67	MS to R
27	94.13	96.90	75.53	HS to S
28	86.43	92.35	63.78	HS to S
29	5.70	0.00	2.38	R to HR
30	4.13	1.35	0.00	HR
31	92.22	90.28	78.07	HS
32	13.72	32.38	4.67	MS to R
33	9.63	2.83	0.33	R to HR
34	78.88	39.43	5.30	HS to R
35	12.72	16.38	2.67	MS to R
36	82.22	70.28	68.07	HS
Mean	61.59	61.26	35.58	

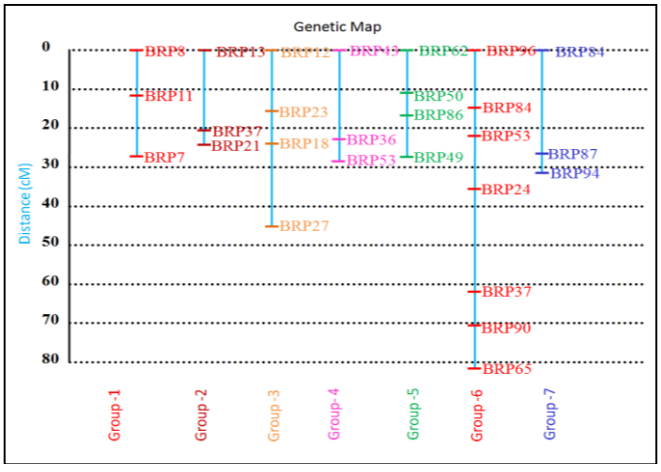


Fig 1: Genetic linkage map constructed using SSR markers in F_2 population derived from the cross J-2537 X ICMB – 95444

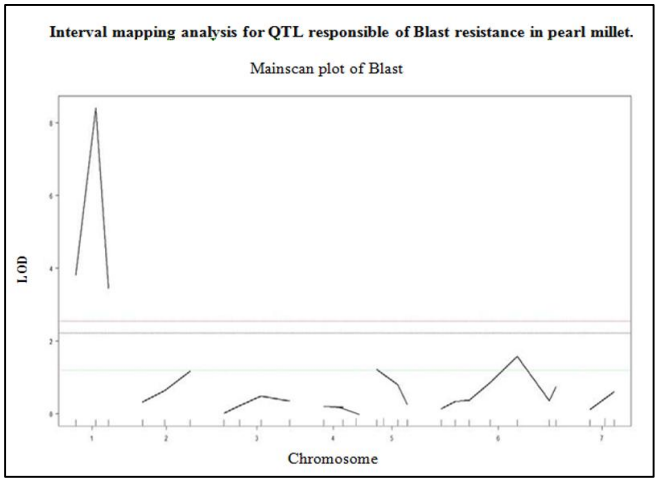


Fig 2: Showing Mainscan plot of BLAST

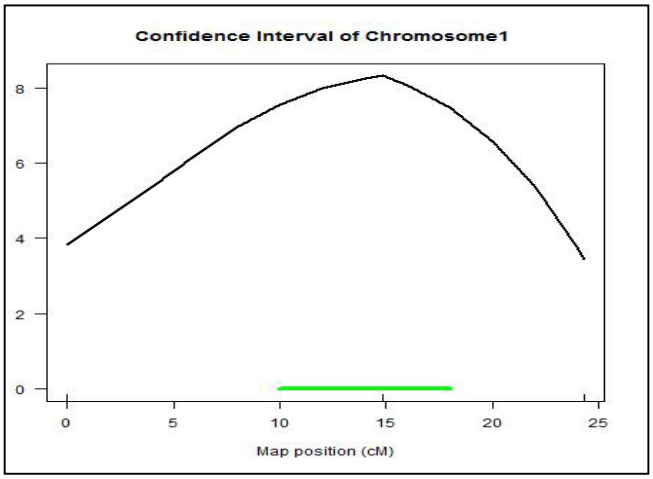


Fig 3: Confidence Interval of chromosome 1 for QTL responsible of Blast resistance in pearl millet

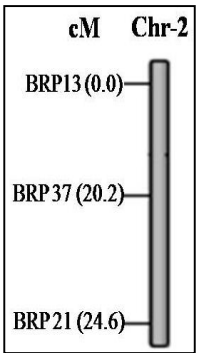
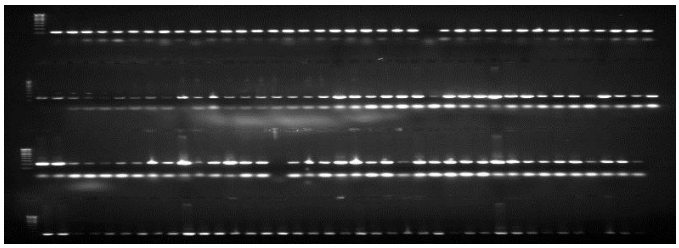


Fig 4: linkage group 2(LG2)



L P_1 P_2 H F_2 [1-36]

Fig 5: Agarose gel obtained from genotyping of the segregating F_2 mapping population progenies using SSR loci BRP12, BRP18, BRP23 and BRP27 Marker differing in size of PCR-amplified DNA of plant entries.

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