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# Simple sequence repeat (SSR) polymorphism in the tropical Asian maize inbred lines differing in resistance to banded leaf and sheath blight (*Rhizoctonia solani* f. sp. *sasakii*)

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**Abstract**

**A set of 23 tropical maize inbred lines, including 18 from the CIMMYT-Asian Regional Maize Program (CIMMYT-ARMP) and 5 inbred lines developed under the All-India Coordinated Maize Improvement Project (AICMIP), were selected for this study. These lines, with distinct differences in responses to Banded Leaf and Sheath Blight (BLSB; *Rhizoctonia solani* f. sp. *sasakii*) at different locations in India (Delhi, Pantnagar and Udaipur), were analyzed using 49 polymorphic simple sequence repeat (SSR) markers (179 SSR alleles). The study aided in genotype differentiation as well as analysis of genetic diversity among the lines. Besides revealing high genetic diversity among the tropical/sub-tropical inbred lines, cluster analysis using SSR dataset clearly demonstrated the genetic distinctness of the CIMMYT-ARMP lines *vis-a-vis* maize inbreds developed in India. The study also aided in identifying suitable lines with phenotypic contrast (BLSB resistance) and genetic divergence. The information would be helpful in undertaking detailed genetic and molecular analyses of BLSB resistance, besides planned utilization of promising genotypes in breeding for BLSB resistance in the tropical maize germplasm.**

**Key words:** BLSB, SSR, polymorphism, inbred, *Zea mays* L.

## Introduction

The Banded Leaf and Sheath Blight (BLSB) disease, caused by *Rhizoctonia solani* f. sp. *sasakii* Exner (teleomorph) *Thanatephorus sasakii* (Shirai), is considered as one of the most important diseases of maize in south and south-east Asia [1] and has the potential to cause significant yield reduction and loss in fodder quality [2]. The fungus *Rhizoctonia* is soil inhabitant, highly pathogenic, and causes serious diseases in many crops, like sheath blight in maize, rice, wheat and sorghum, stem rot in mungbean and soybean, sheath rot in sugarcane, black scurf and sprout canker in potato [3]. In maize, the pathogen spreads from the first and second leaf sheaths above the ground and then spreads upwards to infect the ear [2].

BLSB became increasingly severe and widespread in the recent decades [2], and is potentially a major threat, particularly in areas where rice-maize cropping system is growing in importance. India and China have reported yield losses as high as 88%, and severe effects are also common in Indonesia, the Philippines, Vietnam, Nepal, Indonesia and Thailand [4]. In India, Lal *et al.* [5] estimated grain yield losses of 23 to 39% for ten cultivars, whereas Singh and Sharma [6] reported yield loss ranging from 12 to 41% for artificially inoculated maize inbred lines, hybrids and OPVs. Major emphasis is currently being laid on host-controlled resistance to BLSB in several countries in Asia, including India. In a recent study, we analyzed the genetic variability for resistance in the tropical Asian maize germplasm to BLSB in India, by undertaking field experiments during 2002- 2004 under artificial inoculation conditions at Delhi, Pantnagar and Udaipur [7].

The microsatellite or SSR markers are PCR-based, genetically codominant, robust, reproducible, hypervariable, informative and reasonably easy-to-use [8]. These markers, which are available in abundance in maize (http://www.maizegdb.org), offer significant advantages in DNA fingerprinting, genetic diversity analysis, gene/QTL mapping, and molecular marker-assisted breeding in crops like maize [9]. SSR profiling of the elite inbred lines aids in unambiguous differentiation of genotypes and assessment of genetic diversity. Such information, coupled with phenotypic data, would aid in planned utilization of genotypes in breeding programmes. Therefore, the present study was undertaken with the specific objective of molecular profiling and analysis of genetic diversity in a selected set of lines with distinct differences in resistance to BLSB isolates in India.

## Materials and methods

Based on their responses to BLSB at three different

locations in India (Delhi, Udaipur and Pantnagar) during field experiments conducted during 2002-2004 [7], a set of 23 inbred lines were selected for this study. These lines comprised 18 genotypes from the CIMMYT-Asian Regional Maize Program (CIMMYT-ARMP), four CM (Coordinated Maize) lines developed by various public sector institutions in India, and one LM (Ludhiana Maize) line. The pedigree, seed sources and responses of these lines to BLSB are given in Table 1.

Genomic DNA from each of the genotypes was extracted from the leaves of three-week old maize seedlings using modified CTAB procedure [10]. A set of 49 SSR markers covering the maize genome were utilized for analysis of SSR polymorphisms in the selected genotypes. Primers for these SSR loci were synthesized through Research Genetics Inc., USA, using sequence information available in public domain (http://www.maizegdb.org).

PCR amplification using SSR primers were carried out as per the procedure described by Kassahun and Prasanna [11]. The amplified products were resolved on a 3.5% super-fine resolution agarose (Amresco, USA) gel, using a submarine gel electrophoresis system (Sunrise 96) at 100-125 V for 1.5-2 h. The gel images were captured using a CCD camera (Sony XC-75CE) attached to a gel documentation system with the Alpha Imager software (Vilber Courmet, France). Allele designations were made and approximate size range for the amplification products for each SSR locus was determined based on the positions of the bands relative to the 100 bp molecular size standard (Bangalore Genei, India) (Fig. 1).

Polymorphism Information Content (PIC) at each SSR locus was determined as described by Smith *et al.* [12]. PIC is equal to $1 - \Sigma f_i^2$, where $f$ is the frequency of ith allele. Pair-wise genetic similarity matrix between the genotypes based on SSR data was computed using Jaccard's [13] genetic similarity coefficient, and the similarity matrix was further subjected to an agglomerative hierarchical classification by employing UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering algorithm, using NTSYS-pc Ver. 2.11a [14]. The robustness of the clusters was assessed by bootstrap analysis using the WINBOOT software [15]. The analysis was undertaken

**Table 1.** Pedigree, salient characteristics and seed sources of maize inbred lines used in this study

| S. No. | Inbred | Pedigree[a] | Responses to BLSB[b] | Origin | Type[c] | Source[d] |
|---|---|---|---|---|---|---|
| 1 | CA00102 | EY-DMR-C5-S2-B-B-3-1-B-BBB-B | S (U,P,D) | Asia | TY | CIMMYT |
| 2 | CA00106 | EY-DMR-C5-S2-B-B-2-1-B-BBB-B | MR (U,P,D) | Asia | TY | CIMMYT |
| 3 | CA00310 | AMATLCOHS71-1-1-2-1-1-1-B-6 | MR (U,D); HS (P) | Asia | TY | CIMMYT |
| 4 | CA00332 | KTX3752F2-7-1-1-1-B-BBB-B | S (U,P,D) | Asia | TY | CIMMYT |
| 5 | CA00344 | Pac777F2-6-1-1-BBBB | S (U); MR (P,D) | Asia | TY | CIMMYT |
| 6 | CA00370 | (AMATLCOHS133-1-F/R)-1-3-1-2-5-BBB-B | S (U); MR (P,D) | Asia | TY | CIMMYT |
| 7 | CA00396 | PIO3011F2-3-4-1-BBB | MR (U,D); S (P) | Asia | TY | CIMMYT |
| 8 | CA03106 | P31C4S5B-85-#-#-10-B-B-B-B-B-B | S (U,P,D) | Asia | TY | CIMMYT |
| 9 | CA03131 | P31C4S5B-6-#-#-B-B-B-B-B | S (U,P); MR (D) | Asia | TY | CIMMYT |
| 10 | CA03134 | AMATLCOHS44-1-1-2E-2-2-1-B-B | MR (U); S (P,D) | Asia | TY | CIMMYT |
| 11 | CA03149 | P31C4S5B-43-#-#-9-BBBB-B | S (U,P); MR (D) | Asia | TY | CIMMYT |
| 12 | CA14509 | SW92145-IP2S2-#-#-3-B-B-BB-B | S (U,P,D) | Asia | TY | CIMMYT |
| 13 | CA14510 | SW91145-2P3S2-#-#-3-B-B-BB-B | MR (U,P); S (D) | Asia | TY | CIMMYT |
| 14 | CA14518 | SW92145-2EV-108-2-BBB-B | MR (U); HS (P) | Asia | TY | CIMMYT |
| 15 | CA34506 | P34C5S1B15-4-2-12-1-2-B-BB-B | MR (U); S (P,D) | Asia | TY | CIMMYT |
| 16 | CA34516 | SW91345-1EV-100-3-BB-B | MR (U); S (P,D) | Asia | TY | CIMMYT |
| 17 | CA049Y04 | P49 (Y)S5B-124-#-6-B-BBB-B | S (U,P,D) | Asia | TY | CIMMYT |
| 18 | CML425 | P31C4S5B-6-#-#-BBBB | MR (U); S (P,D) | Asia | TY | CIMMYT |
| 19 | CM104 | Derivative of Theo-21 (B) | MR (U,P); S (D) | Colombia | TYF | DMR |
| 20 | CM105 | Derivative of Peru 330 | S (U,D); MR (P) | Colombia | TYF | DMR |
| 21 | CM139 | Derivative of (Tarun × MS1)-Y63 | S (U,D); MR (P) | India | TYF | PAU |
| 22 | CM300 | Derivative of EtoP1-13-1 | S (U,P,D) | Colombia | TYSF | DMR |
| 23 | LM5 | Derivative of Tux. Pool C21C2 | MR (U); S (P,D) | India | TYF | PAU |

[a]Pedigree: Pop or P = population; C = Cycle; HS = Half Sibs; B = Selfed and Bulked; -1,-2,-3 = ear to row; # = sibbing; AMATL = Asia Mildew Acid Tolerant; EY = Early; DMR = Downy mildew resistant; SW = Suwan; Tux. = Tuxpeno; MS = Makki Safed; [b]Responses based on study undertaken by Garg *et al.* [7]: R - Resistant; MR - Moderately resistant; S - Susceptible; HS - Highly susceptible; U - Udaipur; P - Pantnagar; D - Delhi; [c]Type: T = Tropical; Y = Yellow grain; F = Flint; SF = Semi-flint; [d]Source: CIMMYT: CIMMYT-Asian Regional Maize Program; PAU: Punjab Agricultural University, Ludhiana, India; DMR: Directorate of Maize Research, New Delhi, India

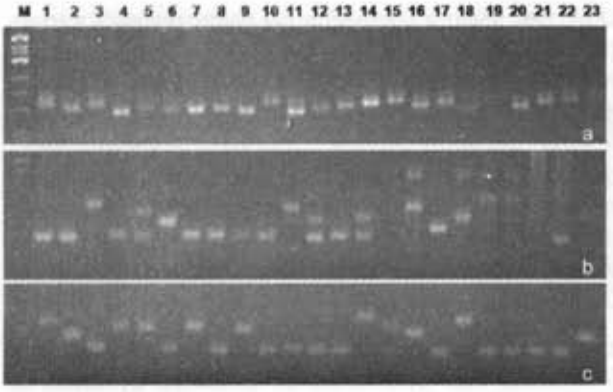


**Fig. 1. SSR polymorphisms in the selected maize inbred lines, illustrated by (a) *bnlg615*; (b) *bnlg371*; and (c) *bnlg1182*. 'M' indicates the molecular size standard. Lane information: 1: CA00106; 2: CA049Y04; 3: CA14509; 4: CA14518; 5: CA03106; 6: CA003134; 7: CA00370; 8: CA00102; 9: CA00396; 10: CA00310; 11: CA34506; 12: CA03131; 13: CA34516; 14: CA03149; 15: CA14510; 16: CA00332; 17: CA00344; 18: CML425; 19: CM104; 20: CM105; 21: CM300; 22: LM5; 23: CM139.**

with 500 repeated samplings with replacement, for >95% accuracy of the bootstrap [16].

## Results and discussion

In the present study, evaluation of a specific set of maize inbred lines against BLSB infection at Udaipur, Pantnagar and Delhi, which are considered as the 'hot spots' for BLSB in India [17], provided an insight into the differential responses of the tropical Asian maize genotypes to BLSB isolates; a summary of these responses is provided in Table 1. To ascertain the genetic divergence among these maize genotypes differing in resistance to BLSB, 49 polymorphic SSR markers, dispersed across the ten maize chromosomes and with at least 4 markers per chromosome (except in case of chromosomes 3, 6, 7 and 10) were employed. The analysis revealed a total of 179 SSR alleles for the 49 polymorphic SSR loci, at an average of 4.3 alleles per locus. Summarized data for the polymorphic SSR loci and their PIC values are presented in Table 2. The PIC values ranged from 0.21 (*umc1545*) to 0.89 (*bnlg127*). The mean PIC value, estimated across all the polymorphic SSR loci, was 0.59. Nine SSR loci revealed PIC values of more than 0.70. Among these, *bnlg127*, *bnlg238* and *bnlg1178* are noteworthy due to their high levels of polymorphism (6 alleles each) and PIC values of 0.89, 0.79 and 0.79, respectively. These SSR markers, with high PIC values, could be highly useful in genotype differentiation and genetic diversity analysis.

The level of polymorphism displayed by the SSR markers was considerably high in the present analysis, as reflected by the high mean PIC value (0.70), as compared to some earlier studies on US inbred lines [12, 18], wherein mean PIC values of 0.62 and 0.59, respectively, were obtained. Pushpavalli *et al.* [19] recorded a mean PIC of 0.50, with a range 0.24 to 0.72, in a study of 32 maize inbred lines developed under the AICMIP. In a study of 102 inbred lines (including lines from Asian breeding programmes, Mexico, USA and Germany) using 76 SSR markers, George *et al.* [20] recorded a mean PIC of 0.59, with a range of 0.14 to 0.83. The variation in the PIC values reported in diverse studies could be attributed to several reasons, including the germplasm analyzed, the nature and types of repeats in SSR loci, as well as methodology employed for allele detection.

In the present study, the number of unique or rare SSR alleles (with frequencies <0.10) in the 23 maize inbreds was 39, with LM5 showing the highest number (7), followed by four each in CA049Y04, CA00396 and CA14510. The unique alleles were found in as many as 18 of the 23 inbreds for different SSR loci. Such alleles, either alone or in combinations, are valuable for DNA fingerprinting as they enable effective differentiation of the genotypes.

The utility of SSR markers in determining genetic divergence and relationships in elite maize germplasm has been effectively demonstrated in several studies [12, 18-20]. In the present study, SSR data was utilized to compute pair-wise genetic similarity (GS) values between various inbreds using Jaccard's similarity coefficient. The pair-wise GS values ranged between 0.08 (LM5-CA14510) and 0.87 (CM104-CM105), indicating least genetic similarity between LM5 and CA14510, and the highest genetic similarity between CM104 and CM105, among the inbred lines analyzed. The average GS value, across all the genotypes, was 0.26, indicating very high genetic divergence among the inbred lines analyzed in this study. Such high genetic diversity was also reported by George *et al.* [18], who found that lines from India, Indonesia, Philippines, Thailand, Vietnam and CIMMYT comprised seven indistinct clusters of tropical and subtropical maize (average GS = 0.29), thereby concluding that maize breeding activity in Asia has not caused a decline in the overall amount of diversity in the region.

The genetic relationships revealed by cluster analysis (Fig. 2), followed by bootstrap analysis, were largely in congruence with the pedigree and breeding history of lines (Table 1), The bootstrap values, reflecting the frequency with which each group is formed in repeated cycles of dendrogram construction, were used as a measure of the relative stability of the clusters [16]. The moderate to very high bootstrap values at most of the internal nodes in the dendrogram indicate the robustness of the SSR dataset used in this study.

**Table 2.** SSR loci analyzed and their polymorphism information

| S. No. | SSR locus | Bin | Allele no. | PIC* |
|---|---|---|---|---|
| 1 | *bnlg1178* | 1.02 | 6 | 0.79 |
| 2 | *bnlg2238* | 1.04 | 3 | 0.57 |
| 3 | *bnlg615* | 1.07 | 3 | 0.65 |
| 4 | *bnlg400* | 1.09 | 3 | 0.55 |
| 5 | *bnlg1297* | 2.02 | 3 | 0.32 |
| 6 | *bnlg1175* | 2.04 | 4 | 0.62 |
| 7 | *dup21* | 2.05 | 3 | 0.62 |
| 8 | *bnlg371* | 2.05 | 7 | 0.68 |
| 9 | *bnlg1045* | 2.07 | 6 | 0.76 |
| 10 | *bnlg198* | 2.08 | 5 | 0.69 |
| 11 | *phi127* | 2.08 | 3 | 0.47 |
| 12 | *bnlg1523* | 3.03 | 3 | 0.60 |
| 13 | *bnlg420* | 3.05 | 2 | 0.45 |
| 14 | *phi053* | 3.05 | 3 | 0.52 |
| 15 | *bnlg1182* | 3.09 | 4 | 0.62 |
| 16 | *umc1594* | 3.10 | 3 | 0.59 |
| 17 | *bnlg490* | 4.05 | 4 | 0.61 |
| 18 | *bnlg252* | 4.06 | 2 | 0.35 |
| 19 | *dup34* | 4.07 | 5 | 0.70 |
| 20 | *bnlg589* | 4.11 | 3 | 0.52 |
| 21 | *bnlg143* | 5.01 | 2 | 0.49 |
| 22 | *bnlg105* | 5.02 | 3 | 0.51 |
| 23 | *umc1225* | 5.08 | 4 | 0.68 |
| 24 | *bnlg389* | 5.09 | 3 | 0.65 |
| 25 | *bnlg1043* | 6.00 | 4 | 0.70 |
| 26 | *bnlg238* | 6.00 | 6 | 0.79 |
| 27 | *bnlg161* | 6.00 | 4 | 0.73 |
| 28 | *phi077* | 6.01 | 4 | 0.70 |
| 29 | *umc1014* | 6.04 | 5 | 0.47 |
| 30 | *phi078* | 6.05 | 2 | 0.30 |
| 31 | *nc013* | 6.05 | 3 | 0.57 |
| 32 | *phi089* | 6.08 | 2 | 0.47 |
| 33 | *umc1545* | 7.00 | 2 | 0.21 |
| 34 | *bnlg572* | 7.03 | 2 | 0.47 |
| 35 | *bnlg339* | 7.03 | 5 | 0.67 |
| 36 | *bnlg155* | 7.04 | 4 | 0.56 |
| 37 | *phi116* | 7.06 | 4 | 0.60 |
| 38 | *bnlg1065* | 8.06 | 4 | 0.66 |
| 39 | *bnlg240* | 8.06 | 3 | 0.54 |
| 40 | *phi015* | 8.08-8.09 | 4 | 0.71 |
| 41 | *phi080* | 8.08-8.09 | 4 | 0.66 |
| 42 | *bnlg1272* | 9.00 | 4 | 0.63 |
| 43 | *bnlg127* | 9.04 | 3 | 0.89 |
| 44 | *bnlg1810* | 9.10 | 4 | 0.67 |
| 45 | *phi059* | 10.02 | 3 | 0.49 |
| 46 | *bnlg1526* | 10.04 | 3 | 0.56 |
| 47 | *bnlg594* | 10.06 | 5 | 0.75 |
| 48 | *phi035* | 10.06 | 3 | 0.58 |
| 49 | *bnlg1839* | 10.07 | 5 | 0.60 |

*Polymorphism Information Content

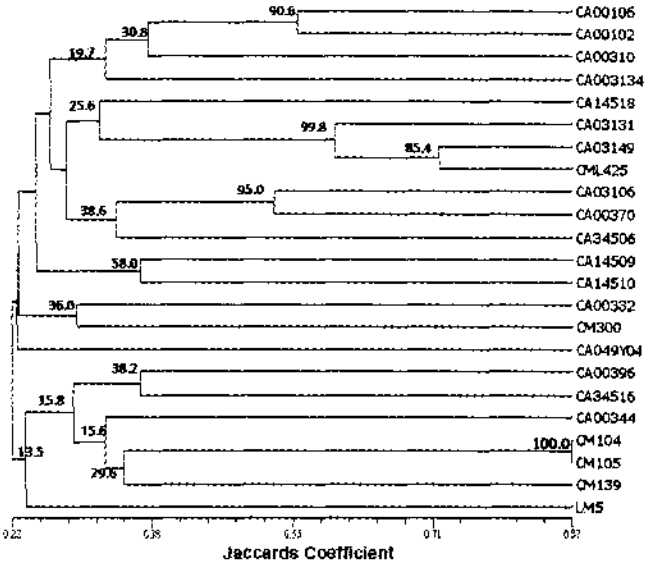


**Fig. 2. Dendrogram depicting the genetic relationships among the selected maize inbred lines based on cluster analysis of SSR data; bootstrap values are indicated at the major nodes in the dendrogram**

However, cluster analysis using SSR data could not differentiate inbred lines based on their specific responses to BLSB. For instance, CA00102, CA00332, CA03106, CA14509 and CA049Y04 showing susceptibility to BLSB isolates at Udaipur, Pantnagar and Delhi (Table 1) were found to be dispersed in different sub-clusters. Similarly, CM104 and CM105 with high genetic similarity (0.87) recorded differential responses to BLSB isolates in India [7]. Both these lines were introduced to India from Colombia and further adapted at the initiation of AICMIP, and were considered as sources of resistance to several foliar diseases of maize. The genetic heterogeneity, and consequent failure of the SSR data to group specific inbred lines based on BLSB disease resistance patterns, was as expected since the broad-based Asian maize germplasm was primarily selected for downy mildew resistance [20] and not for BLSB resistance.

The study showed moderate genetic similarity (0.53) between CA00106 and CA00102, which were 'sister' lines derived from an extra-early, downy mildew resistant population. CA03131, CA03149 and CML425 were found to cluster together, as these lines were derived from Pop.31 (Amarillo Cristalino-2) at CIMMYT. CA00396, CA00344 and CA00332 were inbred lines recycled from commercial maize hybrids; these genotypes, as expected, did not show close genetic similarity with Pop.31, AMATL or EY-DMR derivatives. All the inbred lines used in Indian maize breeding programmes, except CM300, were found to be genetically divergent from the CIMMYT- ARMP lines.

The SSR polymorphisms identified in the selected maize inbred lines differing in resistance to BLSB, coupled with information on genetic relationships, shall be useful in undertaking Quantitative Trait Loci (QTL) mapping experiments as well as for planned utilization of promising genotypes in breeding for resistance to BLSB. Further elucidation of the number, effect, and location of genes controlling resistance to BLSB will enhance the breeding efforts for protection of maize cultivars against BLSB.

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