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RESEARCH ARTICLE

Identification and introgression of QTLs implicated in resistance to sorghum downy mildew (*Peronosclerospora sorghi* (Weston and Uppal) C. G. Shaw) in maize through marker-assisted selection

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Abstract

Sorghum downy mildew caused by *Peronosclerospora sorghi* is a major disease of maize and resistance is under the control of polygenes which necessitated identification of quantitative-trait loci (QTLs) for initiating marker-assisted introgression of resistant QTLs in elite susceptible inbred lines. In the present study, QTLs for sorghum downy mildew (SDM) resistance in maize were identified based on cosegregation with linked simple sequence repeats in 185 F_2 progeny from a cross between susceptible (CM500-19) and resistant (MAI105) parents. F_3 families were screened in the National Sorghum Downy Mildew Screening Nursery during 2010 and 2011. High heritability was observed for the disease reaction. The final map generated using 87 SSR markers had 10 linkage groups, spanning a length of 1210.3 cM. Although, we used only 87 SSR markers for mapping, the per cent of genome within 20 cM to the nearest marker was 88.5. Three putative QTLs for SDM resistance were located on chromosomes 3 (bin 3.01), 6 (bin 6.01) and 2 (bin 2.02) using composite interval mapping. The locus on chromosome 3 had a major effect and explained up to 12.6% of the phenotypic variation. The other two QTLs on chromosomes 6 and 2 had minor effects with phenotypic variation of 7.1 and 2%. The three QTLs appeared to have additive effects on resistance. The QTLs on chromosomes 3 and 6 were successfully used in the marker-assisted selection programme for introgression of resistance to SDM in eight susceptible maize lines.

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Introduction

Sorghum downy mildew (SDM) is a destructive systemic disease of maize worldwide and is caused by an obligate pathogen *Peronosclerospora sorghi* (Weston and Uppal) C. G. Shaw. The other maize downy mildew pathogens like *P. philippinensis* (Philippine DM), *P. maydis* (Java DM), *P. sacchari* (sugarcane DM) and *Sclerophthora rayssiae zeae* (brown stripe DM) (Sharma *et al.* 1993), cause severe disease symptoms in southeast Asia (Frederiksen and Renfro 1977). SDM occurs on maize and sorghum in warm, humid areas of the world causing significant yield reduction. This disease became a prominent disease of maize globally during the years when rapid spread of sorghum as grain and forage crop. SDM is prevalent in the peninsular India, in the states of Karnataka, Tamil Nadu and Andhra Pradesh causing yield losses of 30% and higher (Payak 1975; Krishnappa *et al.* 1995). Though conventional disease management practices (Frederiksen and Renfro 1977; Williams 1984; Craig and Odvody 1992) have been successful in controlling the downy mildews in most of the maize growing regions of the world, SDM of maize remains a serious problem. The disease appears in severe form on maize crop treated with metalaxyl under severe infestation (Raymundo 2000). Added to this, a recent report of metalaxyl resistant *P. sorghi* (Isakeit *et al.* 2003) suggests the need for alternative control methods like host resistance.

Genetic analysis of host resistance to SDM in maize has indicated that resistance is under polygenic control

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and additive effects contribute predominantly to resistance (Borges and Orange 1987; Geetha and Jayaraman 2002; Nallathambi *et al.* 2010). Three quantitative-trait loci (QTLs) that contribute resistance to *P. sorghi* were identified in a population of recombinant inbred lines derived from a cross between inbred lines G62 (resistant) and G58 (susceptible) (Agrama *et al.* 1999). Two of the loci map close together on chromosome 1, while the third one was on chromosome 9. George *et al.* (2003) reported six QTLs on five chromosomes (1, 2, 6, 7 and 10) in a RIL population from the cross, Ki3 (resistant) × CML139 (susceptible) based on tests in India, Indonesia, Thailand and Philippines. Nair *et al.* (2005) detected SDM resistance loci on maize chromosomes 2, 3 and 6 in the Indian maize line NAI116 and verified that the locus on chromosome 6 also contributed resistance to diverse downy mildews. In another study, three putative QTLs were detected in different environments with one locus on chromosome 2 had a major effect and explained up to 70% of the phenotypic variation in Thailand where disease pressure was highest and the other two QTLs on chromosomes 3 and 9 had minor effects; each explained not more than 4% of the phenotypic variation (Sabry *et al.* 2006).

Given the complexity of quantitative traits, mapping populations must be carefully analysed over different years and environments to unravel important components of gene interaction. Identification of simple and accurately scored molecular markers for genes that contribute to SDM resistance of maize could greatly benefit future efforts to prevent disease losses, especially if there are differences in the pathogen populations or environment by genotype interactions in different locations. The major objective of this study was to validate the QTL information generated previously by using different mapping populations over years, to identify other major QTLs, if any, conferring resistance to SDM and introgression of major QTLs in breeding lines.

Materials and methods

Mapping population

Based on response to sorghum downy mildew in screening trials comprising of 57 lines originating from India and Mexico conducted over years, two inbred maize lines were selected for this study. MAI105 (yellow), which was consistently resistant, was selected as the resistant parent (RP) and CM500-19 (yellow) served as the susceptible parent (SP). The F_1 from the cross CM500-19 × MAI105 was selfpollinated and 185 F_2 individuals were produced. Leaf samples were collected from individual F_2 plants before they were selfpollinated to produce F_3 families. Healthy young leaves were collected from the parents and F_2 plants. Samples were frozen in liquid nitrogen and grounded using pestle and mortar. The fine powder obtained from each sample was used for DNA extraction.

Phenotyping of the mapping population

Seeds from 185 F_3 families and two parental lines were grown in the two field trials during 2010 and 2011 postmonsoon period to evaluate the responses to SDW in the National Sorghum Downy Mildew Screening Nursery, Mandya, Karnataka, India (12°N; 76°E; 695 m above mean sea level; average rainfall of 705 mm per year). The field experimental design was randomized block design with two replications, with one row per replication. The test entries were planted in 3 m long rows, with 15–20 plants per row and 0.75 m between rows. Seeds of SDM susceptible maize line CM500 were planted as spreader rows on all sides of the experimental block, 30 days prior to the planting of the test entries. One bed of spreader row was planted for every two beds of test entries. The conidial suspension of SDM was sprayed on the whorl of each seedling of the spreader rows (5–7 days after germination) between 3 and 4 am (Craig *et al.* 1977). The suspension was prepared by collecting conidia from SDM-infected plants at 2–3 am and suspended in water with a concentration of approximately 40,000–50,000 conidia per mL. The inoculation procedure was repeated for three consecutive days to ensure that no plant escaped artificial infection. Same procedure was repeated on test entries also for uniform disease expression. As a susceptible check, CM500 seeds were planted after every 10th row of test materials. Severe infection (97–100% DM incidence) in the check rows across the experimental block indicated uniform and strong pathogen pressure, leaving no possibility for 'disease escapes'. The disease reaction was assessed at 25 and 35 days after plant emergence by scoring for systemic infection in the individual plants. Percentage disease incidence in each test entry was determined. Inoculated plants that did not show systemic symptoms of DM (emergence of characteristic chlorotic leaves) were considered to be resistant (Rao *et al.* 1984).

SSR assay

A set of 384 simple sequence repeat (SSR) primers covering various 'bin' locations in the maize genome was selected from http://www.maizegdb.org/ssr.php. The primers were synthesized from M/s Sigma-Aldrich, Bengaluru, India. DNA used in the SSR analysis was extracted from the leaves of 1-week-old F_2 plants according to the procedure of Saghai-Maroof *et al.* (1984) and as modified by Hoisington *et al.* (1994). Approximately 40 ng of DNA was used as the template for PCR in a 20 μL reaction volume. PCR was carried out in the Eppendorf Thermal Cycler (Eppendorf, Hamburg, Germany) with the following cycling profile: an initial denaturation at 94°C for 2 min, followed by 40 cycles of amplification at 94°C for 1 min, 55–65°C (based on the annealing temperatures standardized for different SSR primers) for 2 min and 72°C for 2 min, with a final extension step at 72°C for 7 min followed by termination of the cycle at 4°C. The amplification products were separated on 3.5% superfine resolution (SFR) agarose gel. Electrophoresis was done at 100 V for 2–3 h.

Marker analysis

The program MAPMAKER ver. 2.0 (Lander *et al.* 1987) was used to establish linked marker groups and to create genetic map. A LOD score of 3.0 and a maximum recombination frequency of 0.40 were used to declare linkage between two markers. After linkage groups were determined, the recombination frequencies between marker loci were estimated by multipoint analysis.

Data analysis

Phenotypic data: The observations were recorded as the percentage of plants infected with SDM during both years. The percentage values ranged between 0 and 100. Analysis of variance was conducted on arcsine transformed phenotypic data (Little and Hills 1978) for individual environments using PROC GLM of SAS. Components of variance for the F_3 families in both experiments were computed. Transformed entry means were used to compute the combined analyses of variance (Bohn *et al.* 1996). Estimates of variance components of F_3 families were calculated as explained by Searle (1971). Broad sense heritability (H) on a F_3 family was estimated as described by Hallauer and Miranda (1981).

QTL mapping: The QTL analysis was carried out on the set of 185 $F_{2:3}$ individuals with phenotypic data for SDM using PLABQTL (http://www.uni-hohenheim.de/~ipspwww/soft.html), a computer program to map QTL (ver. 1.2). The genotypic data consisted of 87 SSR marker loci, and the phenotypic data comprised SDM percentage incidence. The program employs the interval mapping approach (Lander and Botstein 1989). Commands were designed following MAPMAKER/QTL (Lincoln *et al.* 1993). In contrast to this and other programs, we used a multiple regression approach with flanking markers according to the procedure described by Haley and Knott (1992). The method of composite interval mapping (CIM; Zeng 1994) as implemented in QTL CARTOGRAPHER ver. 2.0 (Wang *et al.* 2004) was used to map QTLs and estimate their genetic effects. The best estimate of QTL location was assumed to correspond to the position having the peak significance level. Additive effects of the detected QTLs were also estimated by the PLABQTL procedure. The R^2 values obtained through this analysis indicate the percentage phenotypic variance explained by each QTL. The QTL positions identified in the present study were compared with previously reported ones (Agrama *et al.* 1999; George *et al.* 2003; Nair *et al.* 2005; Sabry *et al.* 2006). QTLs within a marker interval size of less than 20 cM (falling in the same bin) are considered to be common across the experiments.

Marker-assisted introgression of QTLs implicated in resistance to SDM

To initiate marker-assisted selection (MAS), eight susceptible inbred lines were selected. These inbreds are parents of many experimental hybrids with high grain-yield potential. The inbreds CML212 and CML153 have high overall general combining ability. The flanking markers of two QTLs on chromosomes 3 and 6 were screened on this set of eight susceptible inbred lines and the resistant inbred MAI105 for initiating marker-assisted backcross breeding. During rainy season of 2012, the SDM-resistant parent MAI105 was crossed to eight susceptible inbred lines and the F_1s were raised during postrainy season, 2012–2013. These F_1s were backcrossed to susceptible parents. The first backcross generation (BC_1F_1) was planted during summer 2013 (table 1). We have screened 70–100 plants from all eight BC_1F_1 populations using flanking markers linked to QTLs on chromosomes 3 and 6. In total 13, 18, 15, 11, 17, 14, 9 and 24 plants were selected. The selected plants were selfed and BC_1F_2 plants were screened for SDM incidence during rainy season of 2013 and resistant plants were selfed to raise BC_1F_3 progenies during winter 2013 in the sorghum downy mildew screening nursery. Three-hundred BC_1F_3 progenies from the cross (CM500-19 × MAI105) × CM500-19 were evaluated for their response to SDM when both or either of QTLs from chromosomes 3 and 6 were present.

Results

Parental polymorphism

Of the 378 SSR primer pairs used, 108 were polymorphic between the parental lines. These SSRs were tried on entire F_2 population and 87 fit 1 : 2 : 1 Mendelian segregation ratio which were then used for linkage analysis. Thirteen markers showed deviation from 1 : 2 : 1 ratio and eight failed to show polymorphism. Hence, they were not used for linkage mapping and QTL analysis.

Linkage mapping

The final map generated using 87 SSR markers had 10 linkage groups, spanning a length of 1210.3 cM at an average marker interval of 31.0 cM. Although, we used only 87 SSR markers for mapping, the per cent of genome within 20 cM to the nearest marker was 88.5. The linkage map obtained in the present study was in agreement with published SSR maps (http://www.maizegdb.org/maizedb.php).

Phenotypic data analysis and heritability estimates

The parental lines differed significantly in their reaction to SDM disease incidence. The F_1 was also found to be susceptible. The genotypic component of variance was highly significant for infection in both environments and over environments. This is indicative of the presence of relatively high genetic variance for resistance to SDM in the population (table 2). The genotype × environment interaction was also significant. Broad-sense heritability (H) values were estimated to be 67.13% and 66.31% during 2010 and 2011,

Table 1. Backcrosses made for transfer of resistance to SDM in eight inbreds.

Crosses	No. of plants screened with flanking markers in BC_1F_1	No. of plants selected with both QTLs present
CML132 × MAI105	78	13
CML212 × MAI105	100	18
CML153 × MAI105	100	15
CML169 × MAI105	86	11
CML439 × MAI105	100	17
CML304 × MAI105	100	14
CML326 × MAI105	70	9
CML335-B-B-# × MAI105	100	24

Table 2. Means, ranges, genetic variance and heritabilities of systemic infection by *P. sorghi* for 185 $F_{2:3}$ population.

Environment	SP mean	RP mean	$F_{2:3}$ mean	Variance components			Heritability (%)
				Vg	Vge	Ve	
Kharif 2010	93.96	0.00	50.44	249.36		122.12	67.13
Kharif 2011	89.36	0.00	32.95	543.04		275.84	66.31
Combined	89.96	0.00	41.70	403.43	84.80	249.49	61.79

SP, susceptible parent; RP, resistant parent; Vg, genotypic variance; Vge, variance due to genotype x environment interaction; Ve, environmental variance.

Table 3. Parameters associated with QTLs significantly affecting the percentage of SDM incidence in the $F_{2:3}$ plants analysed using CIM.

	Chromosome bin location	Position	SSR marker interval	LOD at QTL position	Additive effect	R^2
LG2	2.02	34.8	umc2363–umc1165	2.1	−1.44	2
LG3	3.01	66.7	umc2255–bnlg1144	3.4	−8.609	12.6
LG6	6.01	25.5	phi077–bnlg107	2.8	2.8	7.1
Comparison with QTLs reported earlier						
George *et al.* (2003)	6.05	89	bnl8.23–bnl5.47		−5.4	20
	2.06	158	umc55–csu154		4	3.4
Sabry *et al.* (2006)	2.09		bnlg1893–umc36		4.26	70
	3.05		Phi073–bnlg1350			<4.0
Nair *et al.* (2005)			bnlg1018–bnlg371			
	2.04–2.05	0.01		2.37	−4.98	5.4
	3.04–3.05	55.6	umc1223–bnlg420	4.22	−7.13	14.9
	6.05	21.1	mmc0241–umc1859	3.82	−6.41	12.8

respectively. The F_3 families were not normally distributed with respect to their responses to SDM. The distribution for SDM was skewed towards susceptibility. The distribution was made near normal to make the means and variances independent and normally distributed using the arcsine transformation.

QTL mapping

Three QTLs for SDM resistance were discovered in the mapping population by marker regression analysis (table 3). These QTLs were located on chromosomes 2 (bin 2.02), 3 (bin 3.01) and 6 (bin 6.01) (figures 1 and 2). Additive effects were significant for all the QTLs detected. The QTL for SDM resistance detected on chromosome 3 had the largest effect, with the MAI105 allele at this locus decreasing the percentage of disease incidence by 12.6%. The QTL on chromosome 6 contributed 7.1% to phenotypic variance in SDM, while the one on chromosome 2 contributed 2%.

Marker-assisted introgression of QTLs in eight susceptible inbreds

The BC_1F_3 progenies recorded resistance to moderate resistance reaction confirming the presence of resistant QTLs in the selected plants. The progenies with QTL on chromosome

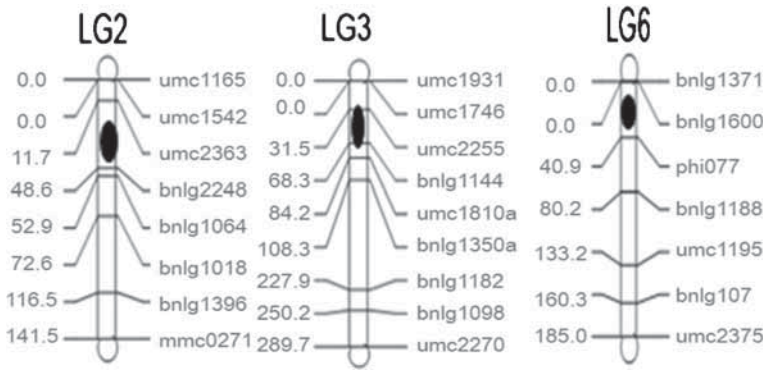

Figure 1. Linkage map showing the chromosomes 2, 3 and 6. The solid mark indicates the marker intervals with QTLs. The numbers to the left of the chromosomes indicate the distance in cM relative to the first marker. The SSR marker names are given to the right of each chromosome.

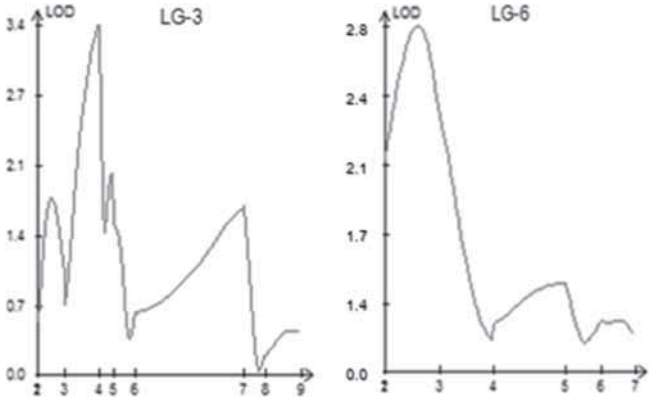

Figure 2. QTL likelihood maps indicating LOD scores for SDM incidence on chromosomes 3 and 6.

3 alone recorded moderate resistant reaction (0–25% disease incidence) and with both QTLs recorded resistant reaction. The progenies with QTL on chromosome 6 were found to be moderately susceptible (table 4). These QTLs segregated as nine (no QTLs); three (QTL from chromosome 3); three (QTL from chromosome 6) and one (QTLs from chromosome 3 and 6) in the BC_1F_3 population generated from the cross (CM500-19 × MAI105) × CM500-19. The resistant progenies from eight BC_1F_3 populations will be advanced to develop SDM-resistant inbreds with better combining ability.

Discussion

SDM disease cause severe yield losses in maize despite the use of chemical control measures. Host resistance is more effective in the SDM management and resistance has been reported to be under polygenic control necessitating identification of resistant QTLs. In this study, resistant inbred MAI105 was crossed with susceptible line CM500-19 and the F_3 progenies were screened for response to SDM. There was absence of a normal distribution in the phenotypic values of the mapping population and this kind of absence of normal distribution is frequent, particularly for diseases such as SDM. Agrama *et al.* (1999), George *et al.* (2003), Nair *et al.* (2005) and Sabry *et al.* (2006) also reported the absence of a normal distribution in phenotypic data, with the distribution also skewed towards the susceptible parent. Heritability values were high and differences in heritability values measured in the different seasons can be attributed to differences in disease pressure. High heritability for SDM was reported earlier (Singburaudom and Renfro 1982; Sabry *et al.* 2006). The genetic map was constructed using 87 SSRs spanning a length of 1210.3 cM. Although, 108 SSRs were polymorphic between parents, eight produced monomorphism in the mapping population and the possible reason for this is that these alleles might have been fixed due to sampling error. Thirteen markers showed deviation from expected Mendelian segregation ratios of molecular markers and this kind of distortions have been reported in maize (Bentolila *et al.* 1992; Gardiner *et al.* 1993; Murigneux *et al.* 1993; Pereira and Lee 1995), as well as in many other plant species. These distortions may result from a selection process during gametogenesis, fertilization or germination (Lyttle 1991). The region on chromosome 3 (bin 3.01) was associated with downy mildew resistance in both test environments suggests a presence of major resistance gene complex. This region from the resistant parent contributes resistance to SDM that affect maize. It is interesting to note that Nair *et al.* (2005) detected a QTL on the same chromosome (bins 3.04–3.05) that contributed resistance to SDM in a recombinant-inbred population derived from NAI116 (resistant) × CML139 (susceptible). But the percentage phenotypic variance explained by this QTL was lower in the present study than in that of Nair *et al.* (2005) in which a different resistant parent was investigated. Second major QTL was detected in the bin 6.01, whereas George *et al.* (2003) and Nair *et al.* (2005) identified the QTL in the bin 6.05. The use of CIM also revealed one more QTL on chromosome 2 (bin 2.02) with less effect. The fact that George *et al.* (2003), Nair *et al.* (2005) and Sabry *et al.* (2006) also found a QTL for downy mildew resistance

Table 4. Measurement of QTL effects in the 300 BC_1F_3 progenies generated from the cross (CM500-19 × MAI105) × CM500-19.

	Presence of QTL on linkage group 3	Presence of QTL on linkage group 6	When both QTLs present	No QTL present
No. of plants	55	58	21	166
Disease incidence	10–25% disease incidence	26–50% disease incidence	0–10% disease incidence	>50% disease incidence
Phenotype reaction (BC_1F_3)	Moderate resistant	Moderately susceptible	Resistant	Highly susceptible

on the same chromosome but in different bins. Although our population and populations developed by George *et al.* (2003) and Nair *et al.* (2005) have parents from the Indian germplasm, the presence of QTLs in different bins might be due to the fact that our population had fewer recombination events (Balint-Kurti *et al.* 2008; Zwonitzer *et al.* 2010; Chung *et al.* 2011). It is also interesting to note that bin 3.01 has QTL influencing resistance to southern leaf blight (Jiang *et al.* 1999). An array of resistance genes are present on bin 6.01, including the gene *mdm1* which confers resistance to the maize dwarf mosaic virus (MDMV) (Simcox *et al.* 1995); wsm1, which confers resistance to a potyvirus, wheat streak mosaic virus (WSMV) (McMullen and Louie 1991); rhm1, which confers resistance to the fungal pathogen *Cochlilobus heterostrophus* (Zaitlin *et al.* 1993); a QTL for resistance to sugarcane mosaic virus (SCMV) (de Souza *et al.* 2008). The bin 2.02 also colocalized QTLs for resistance to gray leaf spot and northern leaf blight (Zwonitzer *et al.* 2010). Clustering of genes and QTLs for resistance against diseases and pests appears to be a widespread phenomenon in maize, being reported in all 10 chromosomes (McMullen and Simcox 1995). The QTLs, one located on chromosome 3 (bin 3.01) near the marker umc2255 and the other on chromosome 6 (bin 6.01) linked to the marker phi077, were constant in both environments. The estimated effects of QTL in the present study are inconsistent with earlier reports. Various reasons for the inconsistency of estimated QTL effects are (i) different QTL segregating in different mapping populations, (ii) QTL × genetic background interaction, (iii) QTL × environment interaction and (iv) the Beavis effect (Beavis 1994; Xu 2003). In a simulation study, Beavis (1994) showed that the average estimates of phenotypic variances associated with QTLs were greatly overestimated if the size of the population is less and fairly close to the actual magnitude when large progenies were evaluated.

MAS along with phenotypic selection for polygenically-controlled SDM disease resistance would not only be cost-effective and time-effective but could also aid in recovery of a large proportion of the recurrent parent genome along with favourable alleles from the donor parent with significantly reduced linkage drag (Ribaut and Bertran 1999). The major QTL on chromosome 3 is important as the BC_1F_3 progenies with this QTL exhibited moderate resistance to resistance reaction either alone or in combination with the QTL on chromosome 6. Validation of major QTLs on chromosome 3 (3.01) and 6 (bin 6.01) has considerable significance in the implementation of MAS for the transfer of resistance to SDM in elite, but DM-susceptible, maize germplasm. Hence, we have attempted MAS programme that lead to incorporation of SDM resistance in the susceptible inbreds CML132, CML212, CML153, CML169, CML439, CML335-B-B-#, CML326 and CML304. In the same line, molecular markers were successfully utilized in line conversions through a back-cross approach in maize at CIMMYT for the introgression of the opaque2 (o2) gene on chromosome 7 for the development of QPM lines, favourable QTL for earliness and grain yield (Bouchez *et al.* 2002) and transfer of a major QTL identified on the short arm of chromosome 1 that is associated with maize streak virus resistance (Prasanna and Hoisington 2003). The CIMMYT also conducted several experiments on QTL analysis and MAS for transfer of drought tolerance to tropical maize, and obtained encouraging results (Ribaut *et al.* 2002; Ribaut and Ragot 2007). Despite a wealth of published literature on QTL mapping, particularly in recent years, successful examples of effective utilization of QTL information in maize breeding through MAS are limited.

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