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Full Length Research Paper

Quantitative trait loci for resistance to maize streak virus disease in maize genotypes used in hybrid development

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Maize streak virus disease is an important disease of maize in Kenya. In this study, we mapped and characterized quantitative trait loci affecting resistance to maize streak virus in maize populations of S4 families from the cross of one resistant MAL13 and one susceptible MAL9 recombinant inbred lines. Resistance was evaluated in replicated field trials under artificial inoculation while selecting using microsatellite markers. The method of composite interval mapping was employed for QTL detection with a linkage map based on 13 polymorphic microsatellite markers. Phenotypic variances for maize streak virus resistance were highly significant in the population, where the three families were selected. The three families expressed acceptable levels of resistance (1.0 - 1.5) with virus pressure. The three QTLs were for umc2228 with LOD score of 27.7 and explained 93% of the variance, umc2229 with LOD of 13 and explained 78.9% of the variance, and bnlg1832 with LOD of 18.7 and explained 71.3% of the variance. A total of 95% of the lines in the three families were found to be resistant to maize streak virus disease.

Key word: MSVD, QTLS, resistance, maize, SSR markers.

INTRODUCTION

Maize streak virus disease (MSVD) is widespread in Kenya but is more serious in mid-altitude areas and mid-highland zones where epidemics have been experienced (Bock et al., 1974; Theuri and Njuguna, 1988), contributing up to 100% yield loss. Disease severity depends on the age at which the plant is infected, the maize genotype and climatic conditions. Generally when maize plants are infected at younger stages of growth (2 - 8 leaves), the damage is higher than when they are infected at later stages (above 8 leaves). In susceptible genotypes this results in stunting and bareness, interveinal necrosis, chlorosis and death of affected plants. Epidemics resulting in economic loses have been reported in at least 20 African countries including Nigeria, Ghana, Sudan, Cameroon, Zimbabwe, Tanzania, Togo, Benin, Bukina Faso, Sao Tome, Uganda and Ethiopia.

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MSVD is caused by maize streak virus (MSV) which is a type member of mastrevirus genus of the family geminiviridae. It has geminate, isometric particles measuring 20 nm in diameter occurring in pairs of 30 x 20 nm with a sedimentation coefficient of 54 and 76S and the particles contain single-stranded, predominantly circular, DNA with a molecular weight of $0.7x10^6$ daltons and exists as a single component (Bock et al., 1974; Bock, 1974). The particles accumulate in the nucleus of the host cell producing large aggregates. MSV is known to exist in different strains, all in the family gramineae. It has been recorded on plants in the following families: Hordeae (*Triticum*), Drapogoneae (*Cymbopogon*, *Imperata*, *Saccharum*, *Rottboelia*), Eragrosteae (*Dactyloctenium*, *Diplachne*, *Eleusine*, *Eragrostis*, *Leptochea*, *Setaria*), Paniceae (*Digitaria*, *Panicum*, *Paspalum*), Sporoboleae (*Sporobolus*), Zoysieae (*Tragus*), Maydeae (*Zea*, *Euchlaena*,) and Avenae (*Avena*) (Storey and McClean, 1930; McClean, 1947). The strains differ greatly in host range and virulence (host adapted variants of one virus and referred to as strains of MSV).

Traditionally, the disease has been controlled through cultural, chemical and physical measures. Chemical sprays can only kill the insect vector found within a maize field within a given time, although application of Furadan and other soil fumigants that are systemic, during planting kills leafhoppers before they infect the plants. Since the leafhoppers are migratory insects and can travel several distances, the use of chemicals becomes very uneconomical, particularly because the resource poor farmer cannot afford to buy the chemicals. In addition, use of chemicals has been classified as environmentally unsafe and requires continuous monitoring. On the other hand, use of cultural measures such as crop rotation, early planting, intercropping, among others are not sustainable due to diminishing land sizes, uncertain climatic and weather sequences and changing farming systems. With regard to physical measures, rouging of diseased plants at time of notice does not guarantee non-infection thereafter. This is because the insects fly in and out of a maize field and, therefore, can re-infect healthy maize plants at any time. Therefore, the most feasible method of controlling the disease is through resistance breeding, since with resistant genotypes, yields are higher than for susceptible and tolerant genotypes.

Polymorphic microsatellite markers are one of the current marker systems of choice for marker-based genetic analysis and marker-assisted plant breeding (Akkaya et al., 1992). Microsatellite markers occur in many plants including maize (Senior and Heun, 1993), soybean (Akkaya et al., 1992), *Brassica* spp (Kresovich et al., 1995), rice (Wu and Tanksley, 1993) and Barley (Saghai-Maroof et al., 1984). DNA markers offer 2 main advantages: faster recovery of the recurrent genome and more efficient selection of genomes that have recombinant events close to the target gene. Simple sequence repeats (Jacob et al., 1991) are loci which are comprised of highly variable arrays of tandem repeats, 2 - 6 base pair long DNA sequences. The SSR can be amplified by the PCR (Saiki et al., 1988) using primers which are complimentary to the regions flanking the repeats. The resulting products, separated electrophorectically, are highly polymorphic and provide co-dominant genetic markers with Mendelian inheritance (Beckmann and Soller, 1990). This study was carried out to map quantitative trait loci (QTLs) for resistance to MSVD in one resistant source MAL13 crossed to one elite line, MAL9, using simple sequence repeat markers.

MATERIALS AND METHODS

Genetic materials

The genetic materials used were MAL13 (resistant) and MAL9 (susceptible). The mapping population MAL9/MAL13 was made by crossing the two inbred lines. Selective pedigree breeding was carried out conventionally through artificial inoculation with MSV up to S4 generation. Three families MAL9/MAL13-X-100, MAL9/MAL13-X-85 and MAL9/MAL13-X-130 were selected for screening with markers alongside their two parents.

DNA extraction

Eight young leaves were sampled from each maize seedling at 8-leaf growth stage and separately put in small perforated bags, transported on ice to the laboratory and stored at -80°C until ready for DNA extraction. The frozen leaves were placed in pre-chilled motor, liquid nitrogen was added to quickly freeze-dry the leaf material prior to grinding into very fine powder with a pestle. The ground material was put in a 15 ml polypropylene centrifuge tube and stored at -20°C. For DNA extraction, 300 mg of the ground material was weighed and put in a new 15 ml polypropylene centrifuge tube and 7 ml of extraction buffer (600 mM sodium chloride, 100 mM Tris Hcl, 5 0mM EDTA, 1% SDS, 140 mM BME in double distilled sterile water) was added. These were placed at 65°C while shaking for 20 min. To the homogenate, 4.5 ml chloroform/octanol mixture (24:1) was added into each tube and mixed on a shaker for 10 min. The homogenate was centrifuged at 3200 rpm for 10 min at room temperature. The upper aqueous layer was poured out into a new 15 ml polypropylene centrifuge tube, 4.5 ml of chloroform/octanol mixture added, rocked gently for 5 min, and centrifuged at 3200 rpm for 5 min. The upper aqueous layer was pipetted out with sterile plastic pasteur pipettes and transferred into a new 15 ml centrifuge tube. The tubes were then filled with ice-cold iso-propanol, rocked gently back and forth for 10 min. The DNA precipitated out of solution to form white cotton like threads. These were hooked out into1.5 ml micro centrifuge tubes, washed twice with 70% ethanol and dried in the fume hood for 30 min to evaporate excess ethanol. The dried DNA was re-suspended in 300 µl Tris-EDTA consisting of 1 mM Tris pH 7.5 and 0.1 mM EDTA pH 8.0, and 10 µl pre-boiled RNAse A (10 mg/ml) was added and mixed briefly before incubation at 65°C for 30 min while shaking gently to speed up the process. The reconstituted DNA was stored at -20°C.

DNA quality and purity

The quality of DNA was assessed using 0.8% agarose gel electrophoresis. The gel was prepared by weighing 0.8 g of agarose, placed in a 250 ml beaker containing 100 ml of 1X TAE buffer and swirled to mix. The mixture was boiled in a microwave oven and then allowed to cool to about 40°C. The molten gel was stained by adding 1 µl Ethidium bromide (10 mg/ml) solution before pouring into a midi gel mold with two 14 well combs. The gel was allowed to solidify until it turned cloudy and hard before being used. The solid gel was then placed in a gel box containing 1X TAE buffer ensuring the gel was completely submerged prior to removing the combs. Each DNA sample was mixed with 10X bromophenol blue loading dye at a ratio of 5 µl of DNA to 2 µl of dye. These were loaded into the wells, hooked up the electrodes of the gel box, and power turned on to 75 volts. After running for one hour, the gel was removed, visualized on a UV box and photographs taken. From the photographs, the quality of DNA was checked.

DNA purity

The concentration of the genomic DNA was determined on the basis of optical density readings. From each stock DNA sample, a 15 µl aliquot was removed and diluted in 735 µl of TE buffer, and its optical densities (ODs) read at wavelengths 260 and 280 nm on a spectrophotometer. The concentration of DNA in samples was determined as follows: 1 OD unit is approximately 50 µg double stranded DNA per ml, and 15 µl of sample in 750 µl cuvette is a dilution of 50 times. After calculating the concentrations in µg/µl, dilutions were made to a uniform stock concentration of 0.3 µg/µl for amplification.

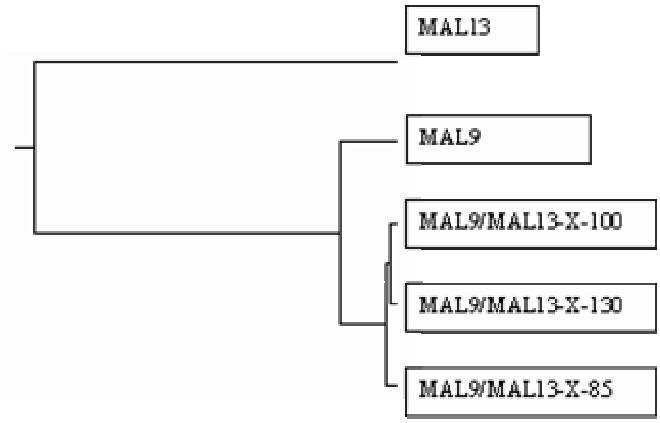

Figure 1. Clustering of the two parents and the three selected recombinant inbred families.

SSR analysis

The PCR reaction mixture and conditions were carried out as previously described (Danson et al., 2006). The primers used were 13 fluorescently labeled microsatellite markers contained between bin 1.04 and 1.05 of chromosome 1 of maize. The PCR products were resolved by capillary electrophoresis and data extracted and analyzed using gene mapper software version 3.7. Estimation of allele frequencies and detection of the presence of null alleles arising from amplification errors was done using macrochecker program. Adjusted allele frequencies of amplified alleles, was based on four methods of null allele estimation, Oosterhout, Chakraborty, Brookfield 1, and Brookfield 2. Genetic analysis was carried out using Popgene version 1.32 (Yeh and Boyle, 1999) as described previously (Danson et al., 2006). The expected homozygosity and heterozygosity statistics were computed using Levene (1949) and also Nei's (1972) expected heterozygosity. A mapping software MapQTL®5.0 (Van Ooijen et al., 2002) was used in identifying QTL positions for MSV resistance. Interval mapping model of MapQTL was used to identify the QTLs. In this method, background markers are selected to take over the role of the putative QTL as cofactors to reduce the residual variance (Yin et al., 2004). A LOD threshold of 3.0 was assumed so that any LOD above this threshold was presumed to be QTL.

RESULTS

Phenotypic analysis

In family MAL9/MAL13-X-85, the MSV scores from artificial inoculation ranged from 1.0 to 1.5, with 98% of the lines screened carrying a mean score of 1.0 in three replications and 2% carrying a mean score of 1.5. Family MAL9/MAL13-X-130 had scores ranging from 1.0 to 2.0, with 96% of the lines carrying a mean score of 1.0, 3% carrying a mean score of 1.5 and 1% carrying a mean score of 2.0. Family AL9/MAL13-X-85 had scores ranging from 1.0 to 3.0, with 92% of the lines carrying a mean score of 1.0, 4% scoring a mean of 1.5, 3% scoring a mean of 2.0, 2% scoring a mean of 2.5, and 1% scoring a mean of 3.0. There was no significant difference ($p < 0.05$) in the resistance levels among the three families. These results showed that with the aid of markers, it is possible to fix the MSV resistance at the S4 generation. All the primers produced amplification products ranging in size from 70 to 300 bp.

Genotypic analysis

The DNA extracted and used for analysis was of good quality, and the mean concentration was 45 µg/ml. From PCR products, there was evidence for null alleles for a few markers in recombinant inbred lines of MAL9/MAL13-X-100. This family was possibly in Hardy Weinberg equilibrium with loci umc1144 and umc1243. However, there was no significant evidence for the presence of a null allele at other loci and families MAL9/MAL13-X-85 and MAL9/MAL13-X-130. The accuracy at 95% level of confidence indicated minimum errors due to stuttering and scoring errors. There were significant deviations from the expected Mendelian ratios for 7.7% of the loci in family MAL9/MAL13-X-85, 53.8% of the loci in family MAL9/MAL13-X-100, and 23%of the loci in family MAL9/MAL13-X-130. Allele frequencies were calculated for all families and markers, and computed exact tests revealed significant differences in the allele frequencies between the three families. From the results of allele frequencies, the susceptible parent was significantly higher, and was even detected in the phenotypically resistant genotypes. The effective number of alleles was assessed as a corollary to the expected heterozygosity. For any given number of alleles, the expected heterozygosity (gene diversity) is highest when all the allele frequencies are equal (Danson et al., 2006). All the three families had equal average heterozygosity (0.1890) and standard deviation (0.114) for all loci. The highest number of polymorphic loci was in family MAL9/MAL13-X-100 with 100% polymorphism with the parents, followed by family MAL9/MAL13-X-85 with 92.31% polymorphism and family MAL9/MAL13-X-130 with 69.23% polymorphism. Three QTLs were declared according to the threshold LOD scores of 3.0 with genome-wide false-positive rate of 5%, depending on chromosome map length (Van Ooijen, 1999). The three QTLs were for umc2228 with LOD score of 27.7 and explained 93% of the variance, umc2229 with LOD of 13 and explained 78.9% of the variance, and bnlg1832 with LOD of 18.7 and explained 71.3% of the variance. The possibility of diminishing resistance in the S4 families could also be seen in the way they clustered with the susceptible parent (Figure 1). The resistant parent clustered on its own. This was an additional explanation to the way conventional breeding skews parental alleles during pedigree selection. In addition, within the second cluster, the susceptible parent clusters alone, and the three families on another cluster.

DISCUSSION

The results from this study showed that there are more that one QTL controlling the resistance to MSV in a region between bin 1.04 and 1.05. They also showed that most QTLs are of different sizes and effects in different genotypes studied regardless of having the same parents. This is supported by the fact that during inbreeding there is diminished heterozygosity, where conventional breeding subjectively selects the allele for the recurrent parent during backcrossing. The major QTL was located at position 1.04, which corroborated with studies carried out elsewhere. The two loci umc2228 and umc2229 fell within this region. There was a minor one at locus bnlg1832. However, these results did not agree with the allele frequencies at these loci except for umc2229. For Locus umc2228, the allele frequency for the allele representing resistance was merely 0.0588 and that of the susceptible parent was 0.9412 for all families. Locus bnlg1832 was a third consistent allele which had a higher frequency than the parental alleles. In similar studies using recombinant inbred lines, Danson et al. (2006) identified three loci in one recombinant family. These studies suggest that there is the possibility of MSV rsistance being modified by several modifying genes.

These results agree with results carried out elsewhere. For example, a major quantitative trait locus (QTL) for MSV resistance was found to be on chromosome 1 in CML202 (Welz et al., 1998) a CIMMYT line, D211 (Rodier et al., 1995) a line from Réunion island and Tzi (Kyetere, 1995) a line from IITA. The major resistant gene was identified as *msv 1* in CML 202 and Tzi4. This study was designed to confirm the presence of this QTL and to discover any new QTLs, major or minor QTLs. The results from genotypic analysis were associated positively with phenotypic data. Similar efforts, using conventional methods were initiated as early as 1930s. These efforts resulted in the identification of the first source of resistance to MSV was in 1931 in the variety 'Peruvian Yellow' (Fielding, 1933). Later, an additional source was identified in the variety 'Arkells Hickory' which was subsequently used to develop resistant varieties (Rose, 1936). Several resistant varieties including 3 NA, 29-29A-5-4, Max 375, Vrg.54 and 'yellow Bounty' were also reported (Gorter, 1959). The genetics of resistance in these varieties was considered to be non-Mendelian (Gorter, 1959). The resistance in 'Peruvian yellow' x 'Arkells Hickory' was conditioned by a single incompletely dominant gene but whose potency was affected by the genetic background of the parents (Storey and Howland, 1967). The deviations from theoretical segregation ratios were attributed to modifying genes. In 1975, researchers at International Institute of Tropical Agriculture (IITA), Nigeria, detected resistance to MSV in the line Tropical Zea Yellow (TZY), which was improved through mass selection and transferred to the most productive varieties (Soto et al., 1982). In East Africa, some lines of Columbian and Mexican origin were identified as resistant and used to develop the variety 'White Star' which did not last long (Efron et al., 1933). Another variety 'La Revolution' was identified as resistant through cooperative efforts of the Institute de Recherche Agronomiques Tropical (IRAT) in France and the Kenya Agricultural Research Institute (KARI). This variety originated from the Reunion islands in the Indian Ocean (Etienne and Rat, 1973). It is from these sources of resistance and resistance from teosinte that breeders in CIMMYT, IITA, CIRAD, KARI and other maize research organizations have introduced MSV resistance genes in their breeding programs. Molecular marker assisted selection has the potential of reducing the time it takes breeders to fix the resistance trait. In addition, it is useful in identifying heterotic groups to be used in the development of hybrids.

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