Mapping genomic regions associated with Maize Lethal Necrosis (MLN) using QTL-seq

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Project summary

Maize lethal necrosis (MLN) is caused by the co-infection of maize with maize chlorotic mottle virus (MCMV) and any of the cereal viruses in the Potyviridae family, including sugarcane mosaic virus (SCMV), maize dwarf mosaic virus or wheat streak mosaic virus (Cabanas et al., 2013). SCMV was reported in Kenya over three decades ago (Louie, 1980) and is known to cause a minor losses. The introduction of MCMV in Kenya (Wangai et al., 2012) and its ability to co-infect with SCMV leads to the development of MLN, posing a serious threat to maize productivity and livelihoods of the farming communities in eastern Africa. The first sign of MLN was reported in Kenya in September 2011 near Bomet. There is an urgent need to identify MLN resistance source germplasm, map the genomic regions associated with MLN resistance, and introgress MLN resistance loci from suitable donors into the genetic backgrounds of widely used inbred lines and hybrids.

CIMMYT has undertaken discovery studies to identify putative genomic regions associated with MLN disease resistance. Two association mapping panels and six bi-parental populations were evaluated for MLN disease severity under artificial inoculation (Gowda et al. 2015). All lines were genotyped with 156 to 289 polymorphic SNPs using the Kompetitive Allele Specific PCR (KASP) assay (Semagn et al., 2014) and genotyping by sequencing (GBS, Elshire et al., 2011). The analysis of the data from the bi-parental populations revealed three major QTL on chromosomes 3 and 6, and a few minor QTL across other chromosomes.

Currently, CIMMYT is working to introgress MLN resistance into adapted germplasm using both conventional backcrossing and marker-assisted backcrossing (MABC). Based on results from the QTL mapping studies, it is currently being introgressed few major genomic regions using 7 MLN donor lines to improve MLN tolerance level across 16 highly popular but MLN susceptible inbred lines widely grown in Africa. Based on the result of the initial QTL mapping studies, it became necessary to conduct additional MLN QTL mapping studies using bulk segregant analysis and QTL sequencing, which has been proposed as a rapid method for QTL detection (Lu et al., 2014; Takagi et al., 2013). For this purpose, five F2 mapping populations were developed and phenotyped under artificial inoculation. Based on the MLN disease score, the most resistant and susceptible progenies were selected for QTL seq, each population represented by 31-71 resistant and 29-67 susceptible progenies.

The overall aim on the project was to: (i) deliver improved versions of the elite African lines having 0.6 to 1.0 point lower MLN severity score and contributing at least 10% higher grain yield (GY) advantage to hybrid combinations under MLN disease pressure as compared to the near isogenic hybrids involving the recurrent parent; and (ii) having less than 5% GY reduction in near isogenic hybrid combinations in the absence of MLN disease pressure.

Outputs

- Six bi-parental populations were evaluated in three seasons under artificial inoculation using a combination of MCMV and SCMV (Fig. 1) and genotyped with 156 to 289 polymorphic SNPs using the KASP assay (Semagn et al. 2014) and GBS (Elshire et al. 2011)
- Five additional F_2 mapping populations were developed, genotyped with DART SNPs and phenotyped under artificial inoculation.
- Three major QTLs on chromosomes 3 and 6 and a few minor QTL across other chromosomes were identified (Fig. 2)
- Major MLN disease resistant QTL on chromosome 6 was validated by QTL seq and confirmed (Fig. 3)

Figure 1: Farmer fields in Kenya (left) and Babati, Tanzania (right) under natural MLN disease pressure

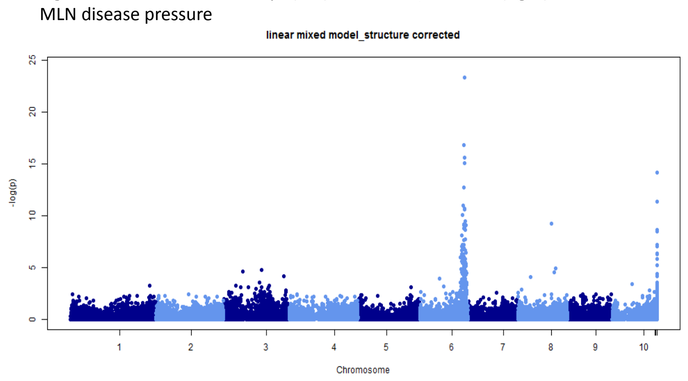

Figure 2: Major and minor MLN QTLs detected across chromosomes 3, 6, 8 and 10

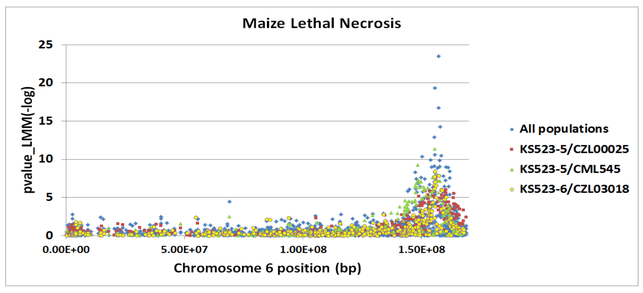

Figure 3: A major MLN QTL detected and validated on chromosome 6 association

Outcomes

- QTLs associated with MLN resistance through whole genome sequencing of MLN contrasting phenotypes tagged, mapped and characterized
- Improved elite African lines having MLN tolerance and associated QTLs identified and used for marker assisted introgression breeding
- Improved versions of the elite African lines having 0.6 to 1.0 point lower MLN severity score and contributing at least 10% higher grain yield advantage to hybrid combinations under MLN disease pressure are available.
- Less than 5% grain yield reduction in near isogenic hybrid combinations in the presence of MLN pressure are achieved

Partnerships

biosciences eastern and central africa – ILRI International Livestock Research Institute

CIMMYT International Maize and Wheat Improvement Center

Diversity Arrays Technology – IT'S A BULLSEYE WITH DArT

Way forward

- Whole genome sequencing of both resistant and susceptible bulk population along with their parents
- Development of Maize Lethal Necrosis resistant lines that will be used in hybrid breeding program and ultimately released to farmers

References:

Cabanas D., Watanabe S., Higashi C.H.V., Bressan A. (2013) Dissecting the mode of maize chlorotic mottle virus transmission (Tombusviridae: Machlomovirus) by Frankliniella williamsi (Thysanoptera: Thripidae). Journal of Economic Entomology 106:16-24. DOI: 10.1603/ec12056.

Elshire R.J., Glaubitz J.C., Sun Q., Poland J.A., Kawamoto K., Buckler E.S., Mitchell S.E. (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:e19379.

Lu H., Lin T., Klein J., Wang S., Qi J., Zhou Q., Sun J., Zhang Z., Weng Y.Q., Huang S. (2014) QTL-seq identifies an early flowering QTL located near Flowering Locus T in cucumber. TAG Theoretical and Applied Genetics 127:1491-1499.

Semagn K., Babu R., Hearne S., Olsen M. (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Molecular Breeding 33:1-14. DOI: 10.1007/s11032-013-9917-x.

Gowda M., Das B., Makumbi D., et al. (2015) Genome-wide association and genomic prediction of resistance to maize lethal necrosis disease in tropical maize germplasm. *Theoretical and Applied Genetics* ;128(10):1957-1968.

Takagi H., Abe A., Yoshida K., et al. (2013) QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant Journal 74:174-183. DOI: 10.1111/tpj.12105.

Wangai A.W., Redinbaugh M.G., Kinyua Z.M., Miano D.W., Leley P.K., Kasina M., Mahuku G., Scheets K., Jeffers D. (2012) First report of Maize chlorotic mottle virus and maize lethal necrosis in Kenya. Plant Disease 96:1582-1583. DOI: 10.1094/pdis-06-12-0576-pdn.

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