Genomics based marker saturation of a BaYMV/BaYMV-2 resistance gene located on barley chromosome 5H

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Introduction

Soil-borne Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) are the causal agents of barley yellow mosaic virus disease (Fig.1), which seriously threatens winter barley production with yield losses up to 50 percent in Asia and Europe. Chemical measures to ensure barley production are neither effective, due to the transmission of the viruses by the plasmloidiophor Polymyxa graminis, nor acceptable for ecological reasons. Thus breeding for resistance is the only way to avoid high yield losses. Recently a new gene was discovered being only effective against BaYMV and BaYMV-2 located on chromosome 5H (Perovic et al. 2014). The aim of this study is to isolate this gene via a map based cloning approach in order to get information on the function and structure of this gene. This approach turned out to be successful already in the isolation of the Rym4/Rym5 and recently the Rym11 locus (Stein et al., 2005).

Material and Methods

For the construction of a high-resolution mapping population, 5085 F2-plants (resolution 0.0098 % recombination) of the cross 'HHOR4224' (resistant) x 'Igor' (susceptible) were analyzed with two co-dominant flanking markers, i.e. HVACL_1 and Bmag337. All in all 691 homozygous segmental recombinant inbred lines (RILs) have been obtained and used for marker saturation of the target interval and phenotyping (Fig. 2).

Results

The target interval between the flanking markers HVACL_1 and Bmag337 was estimated at 12.06 % recombination based on the analyses of 5085 F2-plants. Marker saturation was conducted by mapping of the flanking markers in published high density maps (Stein et al., 2007, Sato et al. 2009, Thiel et al. 2009), employing the ‘GenomeZipper’ (Mayer et al., 2011) and including next generation sequencing data available for barley, followed by mapping of respective markers located in the interval in the high resolution mapping population. All in all 96 markers were selected for the analysis of polymorphisms between the parental lines including 16 markers out of the ‘Genome Zipper’ and 27 markers derived from ‘Morex’ contigs. Up to now 27 markers turned out to be polymorphic and were mapped in a population of 691 segmental RILs (Fig. 3) developed out of 3369 F2-plants resulting in a resolution of 0.015 % recombination. In order to integrate the resistance locus in this high resolution map, all segmental RILs were grown on two BaYMV/BaYMV-2 infected fields in the growing periods 2012/2013 and 2013/2014. The observed segregation ratio of 340 resistant to 351 susceptible segmental RILs shows a good fit to the expected segregation ratio of 1r:1s (Chi²= 0.14). The results of these phenotypic analyses revealed that the BaYMV/BaYMV-2 resistance gene is located between markers k0xx1 and Bradi4gxx2 comprising an interval of 0.31 % recombination (Fig. 3).

Outlook

In order to identify additional markers an exome capture approach of the parental lines was conducted. A high number of markers were used to map the resistance gene in BaYMV/BaYMV-2 resistant lines. This approach turned out to be successful already in the isolation of the Rym4/Rym5 and recently the Rym11 locus (Stein et al., 2005).