Mapping of QTLs for Yield and Alpha Acid Content in Hop

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Abstract

Hop breeding is a lengthy process due to the dioecious nature of hop, producing highly heterozygous offspring and only female plants are of commercial interest. Molecular approaches have therefore been developed to support conventional hop breeding programmes. We studied the effects of quantitative trait loci (QTLs) and determined map locations for alpha-acid content and yield in hop, using amplified fragment length polymorphism (AFLP) and microsatellite markers (SSRs). Genetic linkage maps were constructed from a mapping population consisting of 111 progeny from a double pseudo-testcross. A total of 194 markers were located on the 20 linkage groups (LGs) of the maternal and paternal maps, covering total map lengths of 706 and 616 cM, respectively. Due to the presence of common biparental SSR markers, homology of LGs between parental maps could be inferred. The progeny segregated quantitatively for alpha-acid content and yield determined in the years from 2002 - 2006. Several putative QTLs were determined and the results are discussed from the point of view of implementation of a marker-assisted selection (MAS) programme in hop.

INTRODUCTION

Hop growing in Slovenia has a tradition of over 130 years and has consistently been an important agricultural activity in Slovene hop growing regions. Slovenia produces mainly traditional European aroma hops, with domestic hop varieties covering approximately 3% of world hop production. The orientation in hop production is also towards high alpha varieties (high alpha-acid content) following the demands of the brewing industry.

The Slovenian Institute of Hop Research and Brewing (SIHRB) was established in 1952 to assist hop growers and, in particular, to develop new hop cultivars suitable for Slovenian growing conditions and to follow the demands of the brewing industry. The first cultivars, released in 1971, were ‘Ahil’, ‘Apolon’, ‘Aurora’ and ‘Atlas’, which were hybrids between the high alpha cultivars ‘Brewer's Gold’ and ‘Northern Brewer’ and Slovene wild genotypes. At the beginning of the 1980s, three cultivars, ‘Bobek’, ‘Buket’ and ‘Blisk’, with improved aroma and higher resistance to hop diseases, were developed. In 1990, the triploid cultivars, ‘Celeia’, ‘Cerera’, ‘Cekin’ and ‘Cicero’, combining the quality of ‘Savinjski golding’ and higher yield, were released. The most recent cultivar, ‘279D112’, released in 2007, was bred for higher alpha acid content and better adaptability than its mother plant, the German cultivar ‘Magnum’, by including parental components of the Slovene male hop. The results of the current breeding efforts are 3 hybrids, which are at present in official variety trials, following by new materials with included wilt resistance. Today, 1557 ha of hop fields are planted with 95% of Slovenian cultivars, the most established being ‘Aurora’, which is grown on over 63% of hop fields,

followed by ‘Savinjski golding’ (12%), ‘Bobek’ (10%), ‘Celeia’ (7%) and others.

Molecular breeding has been shown to be very useful in the construction of genetic maps for mapping monogenic traits and dissecting polygenic traits, and thus facilitating gene(s) incorporation or introgression or in linking genetic markers with traits of interest, which can then be used in breeding programs (MAS), thereby limiting the difficult and very time and space-consuming screening procedures in identifying desired individuals at an early stage. Our current breeding programme, combining classical and molecular approaches, is aimed at developing hop cultivars with improved quantity (yield), quality (high alpha acid content, aroma) and better resistance to fungal diseases (wilt, downy mildew, powdery mildew) and pests (damson hop-aphid).

This article describes the current results of studies related to mapping of quantitative traits (yield, alpha acid content) in hops. For alpha acid and yield improvement analysis of inheritance and linkage of molecular markers, a genetic map has been constructed using the F_1 family characteristic for the variability of both traits over 5 years.

MATERIALS AND METHODS

Materials

For the determination of markers associated with alpha acid content and yield, 111 progeny from the family ‘Magnum’ × ‘2/1’ were used. This family was selected on the basis of observed high differences in detected alpha acid content and yield. The mapping population was selected on the basis of observations made in the breeding programme carried out by the Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia.

DNA Isolation

Total genomic DNA was extracted from young leaves using a modified CTAB method according to Kump and Javornik (1996).

AFLP Analysis

The AFLP protocol was carried out as described by Čerenak et al. (2006). The progeny of the family were selectively amplified with 18 *Eco*RI/*Mse*I and 18 *Pst*I/*Mse*I primer pair combinations chosen after screening parental DNA samples. PCR fragments were separated and detected by ALFexpress II automated sequencer (Amersham Biosciences). Results were analysed by the software package ALFwin™ Fragment Analyser 1.03 (Amersham Biosciences) and visually. The AFLP markers were designated according to the selective primer set used and the number of detected bases.

SSR Analysis

Forty-six SSR markers, expressing polymorphism in the parents, were screened on the F_1 crossing family. Amplification of 17 SSR markers, STS marker HlBrapd and four gene derived markers (*chs2*, *chs3*, *chs4*, *hch1*) was carried out according to Čerenak et al. (2006). The remaining SSR markers were analysed as described by Jakse et al. (2008).

Alpha-Acid Content

The lead conductance value (LCV) method (Analytica EBC 1998) was used for measuring alpha-acid content in 2002, 2003, 2004, 2005 and 2006. The dried hop cones of each female hop plant were ground and extracted with toluene. After shaking and filtration, the LCV of hop was determined by conductometric titration with lead acetate in a methanolic solution. Determination of the equivalent point gave the LCV, which is proportional to the percentage content of alpha-acids.

The distribution of alpha-acid content in all 5 years was tested with the Kolmogorov-Smirnov test. Phenotypic data from each year were used in the QTL analysis.

Dry Cone Weight and Harvest Index

The cone weight (yield) per plant was determined by picking cones by hand. The moisture in the fresh cones was determined with scales. The opened vessels filled with cones with known weight were dried at 103-104°C for 5 hours. The vessels were transferred to an exicator, cooled to room temperature and weighed on scales. The harvest index is the ratio of cone weight to total plant weight at harvest.

The distribution of dry cone weight and harvest index in all 5 years was tested with the Kolmogorov-Smirnov test for normality. Phenotypic data from each year were used in the QTL analysis.

Data Analysis

Dominant AFLP loci were used for map construction and separated into three segregation patterns (Čerenak et al., 2006). Monoparental AFLP markers and all SSR markers were used to construct separate genetic linkage maps for the female ('Magnum') and male ('2/1') parents, using the two-way pseudo-testcross strategy (Grattapaglia and Sederoff, 1994). A hypothetical *M* marker linked absolutely to the male plants was added to mapping data.

Linkage analysis was carried out using the JoinMap 3.0 program (Van Ooijen and Voorrips, 2001). The map was constructed using an LOD of 5.0 for the grouping of markers. The Kosambi mapping function was used to convert recombination data to map distances.

MapQTL version 4.0 (Van Ooijen et al., 2002) was used to identify and locate QTLs associated with alpha-acid content, dry cone weight or harvest index, by performing the Kruskal-Wallis non-parametric test, as well as both interval mapping (Lander and Botstein, 1989) and multiple-QTL mapping (MQM; Jansen and Stam, 1994).

Maps were drawn using MapChart version 2.1 software (Voorrips, 2002).

RESULTS AND DISCUSSION

Genetic Mapping

Hop breeding is a lengthy process due to the dioecious nature of hop and the highly heterozygous offspring. Marker assisted selection with markers linked to loci or genes of agronomically important traits is therefore invaluable in hop breeding programmes. Genetic maps based on a comparison of recombination frequencies between pairs of markers are well known in many crops and used to map monogenic traits and dissect polygenic traits. Male and female linkage maps of hop have been previously reported by Seefelder et al. (2000), Koie et al. (2004), Čerenak et al. (2006) and Jakše et al. (2007).

Our population, derived from a cross between 'Magnum' × '2/1', consisted of 97 female and 14 male plants. The population was selected from a Slovenian hop breeding programme because of the wide range of variability of alpha-acid content and cone weight. The yield (dry cone weight), harvest index (the ratio of cone weight to total plant weight) and alpha acid content were established at harvest time when the plants reached technological ripeness. Only female plants were thus included in the evaluation of all quantitative traits, since the cones are female inflorescences rich in bitter substances (alpha acids), which are not present in male plants.

The published map (Čerenak et al., 2006) was improved using new 24 SSRs (Jakše et al., 2008) while AFLP markers were used in the same way as in reported research. Both types of polymorphic markers (AFLPs, SSRs) were tested for their inheritance pattern in the mapping population. Segregation ratios were tested using the chi-square test and, as expected, a large proportion of markers did not fit the assumed Mendelian ratios ($p<0.01$).

Map Construction

A total of 116 markers were placed on the 'Magnum' map, forming 8 major

linkage groups, 2 triplets and 4 doublets, which are assumed to be part of other groups, defining 706 cM of total map distance. A total of 94 markers were placed on the '2/1' map, defining 616 cM of total map distance. The number of LGs for both maternal and paternal maps exceeded the haploid number of hop (n=10), but due to the presence of common biparental SSR markers, a homology of seven LGs between parental maps could be inferred. The marker distribution in the obtained maps seemed fairly even.

QTL Analysis

QTL analyses were carried out using data of alpha acid content obtained in the years 2002, 2003, 2004, 2005 and 2006. The plants were grown in the same place in the experimental field in all 5 years. An LOD score of 3.0 was used to declare the presence of a QTL linked to all 3 quantitative traits. Several putative QTLs linked to alpha acid content were determined in all 5 years.

Dry cone weight at harvest was determined. Several QTLs for dry cone weight in the segregating family were determined in all 5 years.

The progenies also varied in vigour and yield, so dry cone weight and total plant weight at harvest were determined. Again, several QTLs for the harvest index were detected in the years 2002-2006, half on each of parental maps.

QTLs related to yield in hops were detected for the first time, two parameters – dry cone weight (yield) and harvest index (ratio between dry cone weight and green matter) per plant were calculated in all five years. However, the most promising QTLs for marker assisted selection (MAS) are those that are stable in different environments. This research identified several QTLs controlling alpha acid content and yield under different environmental conditions.

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