Mapping QTLs for $\alpha$-amylase activity in rye grain

Piotr Masojé, Pawe³ Milczarski

Department of Genetics and Plant Breeding, University of Agriculture in Szczecin, Szczecin, Poland

Abstract. Genetic control of $\alpha$-amylase activity in rye grain was investigated by QTL mapping based on DS2 × RXL10 intercross consisting of 99 F$_{5\times6}$ families propagated at one location during four vegetation seasons. A wide range of variation in $\alpha$-amylase activity and transgression effects were found among families and parental lines. This variation was shown to be determined in 40.1% by 7 significant (LOD score not less than 2.5) and 2 putative QTLs ($2 \leq \text{LOD} < 2.5$) distributed on all rye chromosomes except 4R. Two significant QTLs located on 3RL and 5RL chromosome arms were expressed each year. The third significant QTL was detected in three years (1RL). The other four significant QTLs (2RL, 5RS, 6RL, 7RL) were found in one year of study. The number and composition of QTLs were specific for a given year varying from three to six. QTLs were not correlated with isoenzyme polymorphisms at the structural $\alpha$-Amy1 loci. A QTL associated with a region containing the $\alpha$-Amy3 locus was detected on chromosome 5RL. Both high- and low-activity QTL alleles were found in each parental line, which explains the appearance of transgressive recombinants in the segregating population.

Key words: $\alpha$-amylase activity, QTL mapping, Secale cereale L.

Introduction

Mature grain of rye may develop high $\alpha$-amylase activity even if it shows no external signs of sprouting. Both the genetic background of a given variety and weather conditions during grain maturation affect the enzyme production, which leads to starch hydrolysis and eventually to economic losses on the cereal market. Four possible ways of triggering $\alpha$-amylase synthesis were recognized (Kettlewell et al. 1996; Lunn et al. 1996). Firstly, $\alpha$-AMY2 isoenzymes encoded by chromosomes of group 7 are produced in the pericarp of green seed. This activity vanishes during grain desiccation, but in more humid weather conditions it may be partially retained in the pericarp (RPAA) of harvested seeds (Nakatsu 1999). Predominant $\alpha$-AMY1 isoenzymes, encoded by chromosomes of group 6, may be synthesized in the process of pre-maturity sprouting (PrMS) or pre-maturity $\alpha$-amylase accumulation (PMAA), which occurs independently of visible sprouting. PMAA, described also as late maturity alpha-amylase (LMA), is developed in cold and wet weather conditions during grain maturation (Mrva and Mares 1999, 2002), but can also be observed in grain ripening at higher temperatures and dry conditions (Nakatsu 1999). Finally, post-maturity sprouting (PoMS), induced often by dry, warm days at full ripeness followed by rainfall, may lead to an intensive synthesis of $\alpha$-AMY1 isoenzymes.

There is an accumulating amount of data on structural and regulatory genes underlying $\alpha$-amylase production, which are involved in metabolic pathways of GA and ABA synthesis or in tissue responses to these phytohormones (Masojé and Gale 1991; Spielmeyer et al. 2004; Gottwald et al. 2004; Holdsworth et al. 1999, 2001). However, polymorphisms found in these loci may not be related to variation in $\alpha$-amylase activity and sprouting, as it was demonstrated for the $Fp1$ homologue gene in wheat (Osa et al. 2003). Thus, from a prac-
tical breeding perspective it is more useful to search for QTLs, the polymorphism of which leads to substantial variation in α-amylase activity. Mapping of such polymorphic QTLs proved to be the most effective method of detecting numerous loci affecting sprouting and α-amylase activity in cereals (Anderson et al. 1993; Marquez-Cedillo et al. 2000; Cui et al. 2002; Flintham et al. 2002; Gale et al. 2002).

This paper presents results of a four-year study aimed at the detection of QTLs underlying high α-amylase activity in rye kernels.

Material and methods

Ninety nine $F_{5,6}$ families derived from individual $F_2$ plants of the original $DS2 \times RXL10$ intercross being a mapping population (Devos et al. 1993) were analysed. Parental lines of this cross originated from a hybrid between $S. cereale \times S. dighoricum$ (DS2 line) and a Polish variety Zeelendzkie (RXL10) and represented the 21st generation of inbreeding. Each $F_2$ family, consisting of 20–50 plants representing 10–15 inbred lines derived from one $F_2$ plant, was grown in one mini-plot and propagated in isolation in 1996, 1997, 1998 and 2003 on the field of the University of Agriculture in Szczecin. A bulk of $F_6$ seeds developed on bagged $F_5$ family constituted a representation of the initial $F_2$ plant genotype. Spikes were harvested at full maturity and bulked $F_6$ seeds of each family were stored in paper bags at room temperature.

Alpha-amylase of each line or family was extracted from 1g sample of milled kernels in 4 mL of distilled water as described previously (Masojć and Larsson-RaŸnikiewicz 1991). Activities of α-amylase were assessed, using a gel diffusion method (Masojć and Larsson-RaŸnikiewicz 1991), in which the diameters of diffusion circles show a linear relationship with the logarithm of the enzyme activities given in U mL$^{-1}$ (1U liberates 1 mg of maltose from starch in 3 min at pH 6.9 at 20°C). Tests were carried out in three replications for lines and in two replications for $F_{5,6}$ families.

QTL mapping was performed at the critical LOD value of 2.5 (assessed by the permutation test), using MAPMAKER/QTL v.1.1 software package (Lander and Botstein 1989; Lincoln et al. 1993) and a rye map developed by Devos et al. (1993), Masojć et al. (2001) and Bednarek et al. (2003). Peaks of the LOD curve reaching a level between 2 and 2.5 were interpreted as putative QTLs, the existence of which needs to be confirmed in further studies. LOD peaks equal or higher than 2.5 were considered as significant QTLs, even if they were observed only in one year. QTLs were characterized in respect to their most probable map position (interval between marker loci), LOD value, variance explained ($V_e$) and additive value of the allele derived from line RXL10 (Add.). The confidence intervals for the QTL position were determined by drawing a line at $LOD_{max} – 1$ value, limited by the LOD curve.

Results

A wide range of variation of α-amylase activities in sound grain among 99 $F_{5,6}$ families and parental lines was found in each year of study (Table 1). Line RXL10 showed c. 5 times lower α-amylase activity than line DS2. Differences between parental lines in alpha-amylase activity were statistically significant in each year. The variation range found among $F_{5,6}$ families in particular years always exceeded by far that of parental lines, which may be attributed to transgression. Total variation range (0.1–247.3 U mL$^{-1}$) shows that there may be

<table>
<thead>
<tr>
<th>Year</th>
<th>Line DS2 (SD)</th>
<th>Line RXL10 (SD)</th>
<th>$p^*$</th>
<th>Means of $F_{5,6}$ families (SD)</th>
<th>Variation range among $F_{5,6}$ families</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>21.0 (6.5)</td>
<td>3.4 (1.0)</td>
<td>0.05</td>
<td>17.8</td>
<td>0.4 – 247.3</td>
</tr>
<tr>
<td>1997</td>
<td>4.4 (1.4)</td>
<td>1.2 (0.2)</td>
<td>0.05</td>
<td>4.3</td>
<td>0.2 – 71.0</td>
</tr>
<tr>
<td>1998</td>
<td>8.9 (1.7)</td>
<td>2.7 (0.4)</td>
<td>0.01</td>
<td>14.3</td>
<td>0.8 – 181.0</td>
</tr>
<tr>
<td>2003</td>
<td>2.9 (0.5)</td>
<td>0.4 (0.1)</td>
<td>0.01</td>
<td>2.0</td>
<td>0.1 – 20.4</td>
</tr>
<tr>
<td>Mean</td>
<td>9.3 (8.1)</td>
<td>1.9 (0.7)</td>
<td></td>
<td>9.6 (7.6)</td>
<td>0.4 – 130.0</td>
</tr>
</tbody>
</table>

*significance of differences between parental lines tested by C-Cochran-Cox test
Table 2. Characterization of QTLs and their effects on variation of α-amylase activity in DS2 × RXL10 mapping population of rye. QTLs with significant LOD scores (*) are in bold.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>QTL</th>
<th>Interval</th>
<th>1996 LOD</th>
<th>V_E [%]</th>
<th>Add. LOD</th>
<th>V_E [%]</th>
<th>Add. LOD</th>
<th>V_E [%]</th>
<th>Add. LOD</th>
<th>V_E [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1RS/1RL</td>
<td>Q. Amy. uas-1R.1</td>
<td>Xpsr161-APR1.7</td>
<td>ns</td>
<td>3.8*</td>
<td>20.7</td>
<td>1.14</td>
<td>4.4*</td>
<td>27.3</td>
<td>0.12</td>
<td>2.2</td>
</tr>
<tr>
<td>2RS/2RL</td>
<td>Q. Amy. uas-2R.1</td>
<td>Xpsr143-Xpsr107</td>
<td>ns</td>
<td>2.5*</td>
<td>11.1</td>
<td>-0.8</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>3RS</td>
<td>Q. Amy. uas-3R.1</td>
<td>APR3.7-APR3.1</td>
<td>2.2</td>
<td>11.4</td>
<td>-1.02</td>
<td>2.1</td>
<td>10.1</td>
<td>-0.86</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>3RL</td>
<td>Q. Amy. uas-3R.2</td>
<td>Xpsr78-Xpsr170.1</td>
<td>2.6*</td>
<td>12.1</td>
<td>-0.97</td>
<td>3.6*</td>
<td>17.7</td>
<td>-0.96</td>
<td>3.2*</td>
<td>16.1</td>
</tr>
<tr>
<td>5RS</td>
<td>Q. Amy. uas-5R.1</td>
<td>Xubp3-APR5.7</td>
<td>ns</td>
<td>3.2*</td>
<td>14.2</td>
<td>-0.96</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>5RL</td>
<td>Q. Amy. uas-5R.2</td>
<td>Xpsr79-αAmy3</td>
<td>2.2</td>
<td>16.0</td>
<td>0.58</td>
<td>3.6*</td>
<td>21.2</td>
<td>0.97</td>
<td>2.4</td>
<td>13.9</td>
</tr>
<tr>
<td>6RL</td>
<td>Q. Amy. uas-6R.1</td>
<td>Xpsr1203-Xpsr454</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>2.7*</td>
<td>15.1</td>
</tr>
<tr>
<td>7RL</td>
<td>Q. Amy. uas-7R.1</td>
<td>APR7.8-Xubp9</td>
<td>2.5*</td>
<td>11.6</td>
<td>-0.83</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>7RL</td>
<td>Q. Amy. uas-7R.2</td>
<td>APR7.5-Xpsr150</td>
<td>2.4</td>
<td>11.6</td>
<td>-0.79</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Total values for multiple QTLs</td>
<td></td>
<td></td>
<td>5.2*</td>
<td>30.6</td>
<td>13.0*</td>
<td>51.8</td>
<td>8.3*</td>
<td>41.8</td>
<td>8.6*</td>
<td>38.4</td>
</tr>
</tbody>
</table>
Figure 1. QTLs for a-amylase activity in grain, localized on the genetic map of rye. Black and white vertical bars represent confidence intervals for significant and putative QTLs, respectively.
almost 2.5 thousand-fold differences in α-amylase activity between sound kernels of rye. From 1.3 to 8.5-fold differences in the mean enzyme activities of each parental line were found between years, which can be attributed entirely to environmental variation. The analysis of variation (ANOVA) showed that genotypes, years and genotype × year interaction are significant components of total variation at p < 0.001. Broad sense heritability of α-amylase activity in rye assessed on a four-year set of data was $h^2_b = 0.66$.

Generally, four classes of α-amylase activity may be discerned in rye grain: low – with activity lower than or equal 1.7 U mL$^{-1}$, medium – activity between 1.7 and 8.0 U mL$^{-1}$, high – between 8.0 and 32 U mL$^{-1}$, and very high – above 32 U mL$^{-1}$. Line DS2 is a representative of a medium class with the exception of the year 1996, when it showed a high enzyme level. On the other hand, line RXL10 belongs rather to a low activity class, but in some years it can reach the lower limit of the medium activity class. A majority of $F_{5,6}$ families are divided into medium, low and high activity classes. Only few families belong to the high activity class.

On average, the highest α-amylase production in rye grain was observed in 1996 and 1998, and the lowest – in 2003, when the summer season was characterized by high temperatures and a lack of rain. Alpha-amylase activities expressed in the logarithmic scale showed close to normal distribution among 99 families of the mapping population, which made the experimental data suitable for QTL analysis.

Seven chromosomal regions containing significant QTLs for α-amylase activity were found in the DS2 × RXL10 mapping population (Figure 1, Table 2). Chromosome 1R contains a QTL on the long arm, close to the centromeric region. Chromosome 2R has a single QTL near the centromeric region, where the endogenous α-amylase inhibitor locus $I_{sa}I$ is mapped. A significant QTL is located on the long arm of chromosome 3R. No QTL was found on chromosome 4R. Chromosome 5R contains two QTLs, one on 5RS and the other on 5RL in the vicinity of a structural $\alpha$-Amy3 locus. Chromosome 6R shows a single QTL, located on the long arm in a distance of about 40 cm from $\alpha$-Amy1 structural genes. A significant QTL found on chromosome 7R is localized on the proximal part of the long arm. Additionally, two putative QTLs, with LOD values close to but lower than 2.5, were found on chromosome arms 3RS and 7RL.

Only two out of nine QTLs, i.e. those from 3RL and 5RL chromosome arms, were detectable in each of the four years of study. Other loci were not stably expressed across the years, being found three times (1RL), twice (3RS) or only once (2RL, 5RS, 6RL and 7RL). Thus in a given year there were from three (1998) to six (1997) detectable QTLs, the composition of which seems to be specific to a particular vegetation season. Confidence intervals for a position of a given QTL detected in different years overlapped to a great extent. The sum of their lengths is presented on the map as a composite confidence interval (Figure 1).

For seven QTLs (2R, 3RS, 3RL, 5RS, 6RL, 7RL) the larger-value alleles originated from the DS2 line (Table 2). The two remaining QTLs (1RL, 5RL) have larger-value alleles from the RXL10 line. The existence of both larger- and lower-value alleles in each parental line explains the transgression effects observed within the DS2 × RXL10 mapping population. Variation explained by particular QTLs usually ranged from 10.1 to 17.7%. Only in 1997 (1RL, 5RL) and 1998 (1RL) individual $V_E$ values exceeded 20%. Total $V_E$ values, equivalent to the coefficients of determination ($R^2$), were obtained by means of a simultaneous analysis of multiple QTLs. As shown in Table 2, the observed variation of α-amylase activity was determined by detected QTLs in 30.6 (1996) to 51.8% (1997), with the mean value of 40.1%.

**Discussion**

There are some similarities between QTL distribution in rye, found in this paper, and that presented by Zanetti et al. (2000) and by Mrva and Mares (2002) in the wheat genome. Amylase QTLs were found in similar regions on chromosome 5AL ($\alpha$-Amy3 marker loci), 3AS, 3BL and 7BL (linked to the $\alpha$-Amy2 locus). A similar centromeric position as that on the 2R chromosome was reported on 2A, 2B and 2D for QTLs underlying sprouting in wheat (Anderson et al. 1993). The distribution of amylase QTLs in the rye genome is partially consistent with that found in barley (Marquez-Cedillo et al. 2000). Both cereals contain QTLs affecting α-amylase activity on chromosomes of homoeologous groups 1L, 2L, 5L and 7L. Due to chromosomal rearrangements found in rye (Devos et al. 1993), the observed similarities in QTL distribution need further verification. It is possible that rye has its own, genome
specific, mechanisms underlying variation in \(\alpha\)-amylase activity.

Instability of the QTL set across the years of study found in rye is understandable, considering a strong impact of weather conditions on \(\alpha\)-amylase synthesis and significant genotype × environment interactions (Froment et al. 1999; Major et al. 1999; Nakatsu 1999). It may be speculated that the expression of the QTL allele controlling high \(\alpha\)-amylase synthesis is triggered by a specific sequence of temperature and moisture conditions during grain maturation. It means that due to a lack of such conditions, the expression of both QTL alleles may be similar, which makes this QTL undetectable in a given year. Similar levels of QTL instability in different environments was reported in wheat (Zanetti et al. 2000) and in oilseed rape (Pilet et al. 2001). It seems that for quantitative traits, having a complex genetic background and high environmental component of variation, a considerable level of instability in the expression of QTL polymorphism is rather a common feature.

Since in each year of study, seeds were harvested at the stage of full ripeness, when there were no visible signs of sprouting, the majority of the developed \(\alpha\)-amylase should represent late maturity amylase (LMA), which was shown to be the \(\alpha\)-AMY1 group of isozymes (Mrva and Mares 1999, 2002). Interestingly, polymorphisms at the \(\alpha\)-Amy1 structural loci mapped on chromosome 6R had no effect on the enzyme activity. Also the \(\alpha\)-Amy2 locus was not localized within the QTL interval on 7RL, yet a linkage between these loci seems to be proven. On the contrary, the QTL from the 5RL chromosome arm coincided with the region containing the \(\alpha\)-Amy3 locus encoding the third group of \(\alpha\)-amylase isoenzymes, expressed only in the early stage of grain development (Daussant et al. 1987; Masojec and Gale 1991).

A lack of relationship between QTLs and polymorphisms in the \(\alpha\)-Amy1 loci in rye suggest that allelic differences in structural genes found in the DS2 × RXL10 cross do not affect \(\alpha\)-amylase activity. Other authors reported associations of QTLs for enzyme activity and \(\alpha\)-amylase structural genes in barley and wheat (Han et al. 1997; Marquez-Cedillo et al. 2000; Flintham et al. 2002; Gale et al. 2002). Also one out of two QTLs for \(\alpha\)-amylase activity found in rice coincided with the \textit{Amy2A} structural gene on chromosome 6 (Cui et al. 2002). Apparently, within the allelic sets in \(\alpha\)-amylase structural loci in cereals there are forms strongly affecting the enzyme activity. A part of this polymorphism, as shown in rye, is however irrelevant to \(\alpha\)-amylase activity.

The QTL region detected on chromosome 2RL contained a structural locus for endogenous \(\alpha\)-amylase and subtilisin inhibitor – \textit{Isa1} (Masojec et al 2001). \textit{Isa1} in rye was shown to be highly polymorphic and it is possible that some isoforms have stronger inhibitory effects than others (Masojec 1991). This QTL was also shown to underlie visible sprouting (Masojec et al. 1998) and therefore may represent a part of amylase activity that is produced via post maturity sprouting (PoMS).

The QTL for \(\alpha\)-amylase activity found in this study on the long arm of chromosome 6R, c. 40 cM apart from the \(\alpha\)-Amy1 structural loci, may be interesting for triticale breeders. This chromosome was shown to be connected with elevated \(\alpha\)-amylase levels in sound grain of wheat/rye addition lines (Flintham 1990; Gale et al. 1990) and a QTL from its long arm may be crucial for a high production of \(\alpha\)-amylase in triticale grain.

Nine QTLs identified in this study represent regulatory or modifying genes, the polymorphism of which altogether determines c. 40% of the observed variation. The complexity of this genetic system and its apparent interaction with weather conditions explain difficulties in breeding rye and possibly triticale varieties with stable, low levels of \(\alpha\)-amylase. QTLs reported in the DS2 × RXL10 mapping population most probably constitute a subset of loci underlying \(\alpha\)-amylase activity and preharvest sprouting in rye. More polymorphic loci affecting \(\alpha\)-amylase variation may exist in other genetic materials. This hypothesis needs to be verified through the analysis of different rye intercrosses, as it was performed for oilseed rape by Pilet et al. (2001). These authors showed medium to low stability of QTLs across different genetic backgrounds, which suggests that attempts to develop molecular markers useful in selection for low \(\alpha\)-amylase across a wide range of genotypes may be a difficult task. Since breeding material, collected for the purpose of development of a new variety, may contain a specific set of QTLs for \(\alpha\)-amylase activity, it will be necessary to adjust a marker system to the particular genetic pool if marker assisted selection is to be used.

On the basis of previous results on QTL mapping in the DS2 × RXL10 cross, possible effects of traits such earliness and thousand kernel mass (TKM) on \(\alpha\)-amylase activity seem to be unlikely. Namely, QTLs for earliness and TKM were found...
in different map intervals than those for α-amylase activity (Masojć and Milczarski 1999; Milczarski and Masojć 2003).

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REFERENCES


