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# A doubled haploid rye linkage map with a QTL affecting α-amylase activity

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**Abstract** A rye doubled haploid (DH) mapping population (Amilo × Voima) segregating for pre-harvest sprouting (PHS) was generated through anther culture of $F_1$ plants. A linkage map was constructed using DHs, to our knowledge, for the first time in rye. The map was composed of 289 loci: amplified fragment length polymorphism (AFLP), microsatellite, random amplified polymorphic DNA (RAPD), retrotransposon-microsatellite amplified polymorphism (REMAP), inter-retrotransposon amplified polymorphism (IRAP), inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers, and extended altogether 732 cM (one locus in every 2.5 cM). All of the seven rye chromosomes and four unplaced groups were formed. Distorted segregation of markers ($P \leq 0.05$) was detected on all chromosomes. One major quantitative trait locus (QTL) affecting α-amylase activity was found, which explained 16.1% of phenotypic variation. The QTL was localized on the long arm of chromosome 5R. Microsatellites SCM74, RMS1115, and SCM77, nearest to the QTL, can be used for marker-assisted selection as a part of a rye breeding program to decrease sprouting damage.

**Keywords** Microsatellite · Pre-harvest sprouting · Retrotransposon · *Secale cereale* L. · Segregation distortion · Sequence-related amplified polymorphism

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Pre-harvest sprouting (PHS) leads to significant economic losses worldwide in cereal production, especially in wheat and rye (e.g., wheat: Humphreys and Noll 2002). In areas such as Finland, where crops must be harvested during rainy seasons, PHS is especially common, because cool, wet weather favors germination (Nyachiro et al. 2002; Chono et al. 2006). The most widely used parameters for PHS determination are Hagberg falling number and α-amylase activity, which are inversely correlated (Hagberg 1960; Perten 1964). Several quantitative trait loci (QTLs) for α-amylase activity as well as other QTLs involved in controling sprouting resistance have been found in rye (Masojć et al. 1999; Masojć and Milczarski 2005; Twardowska et al. 2005; Masojć et al. 2007; Masojć and Milczarski 2009; Masojć et al. 2009).

Doubled haploid (DH) populations are excellent material for genetic mapping and QTL studies (Forster and Thomas 2004), and they have been used especially in self-pollinating species. The present study was carried out to construct the first linkage map of out-crossing rye using DHs, and to characterize QTL(s) affecting α-amylase activity.

A rye DH population (89 individuals) derived from the cross of two DH parents, the sprouting-resistant Polish cv. Amilo and the susceptible Finnish cv. Voima, was used for mapping studies. The population was developed from $F_1$ plants through anther culture, as explained by Tenhola-Roininen et al. (2006).

Several various marker types were used in the mapping studies (Table 1). Amplified fragment length polymorphism (AFLP) analyses were performed with minor modifications according to the procedure developed by Vos et al. (1995). Random amplified polymorphic DNA (RAPD) amplifications were carried out as described by Tenhola-Roininen and Tanhuanpää (2010). Sixty-nine microsatellites from rye

**Table 1** DNA markers used in rye linkage mapping

| Marker type | Name[1] | Polymorphic markers | |
|---|---|---|---|
| | | Analyzed (%) | Located (%) |
| AFLP | e*xxx*_m*xxx* | 175 (43.75) | 127 (43.9) |
| ISSR | ISSR*X* | 2 (0.5) | 2 (0.7) |
| Microsatellite: | | | |
| Rye | SCM*X*, REMS*X*, RMS*X* | 66 (16.5) | 57 (19.7) |
| Wheat | WM410 | 1 (0.25) | 1 (0.35) |
| Barley | BMS64, HVM4 | 2 (0.5) | 1 (0.35) |
| RAPD | AA*X*, AB*X*, AC*X*, AD*X*, AF*X* | 28 (7.0) | 21 (7.3) |
| IRAP | IR*X* | 16 (4.0) | 12 (4.2) |
| REMAP | IR*X*RE*X* | 32 (8.0) | 20 (6.9) |
| SRAP | me*X*em*X* | 78 (19.5) | 48 (16.6) |
| Total | | 400 (100.0) | 289 (100.0) |

[1] *x* nucleotide; *X* number

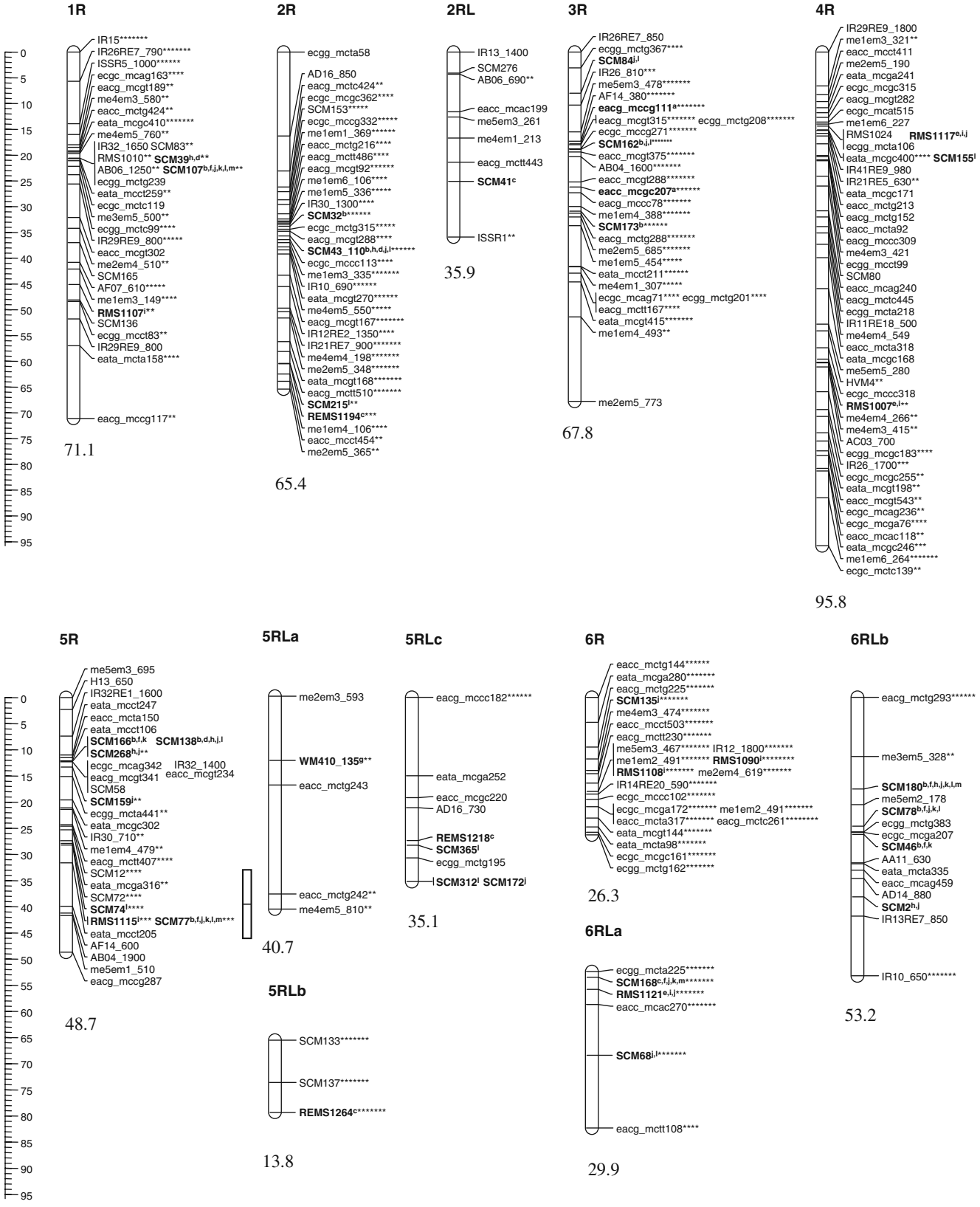
(mostly developed in BAZ, Germany, or in Lochow-Petkus GmbH and Hybro GmbH & CoKG, Germany; Saal and Wricke 1999; Hackauf and Wehling 2002b; Khlestkina et al. 2004, 2005; Hackauf et al. 2009), wheat (Röder et al. 1998), and barley (Saghai Maroof et al. 1994; Ramsay et al. 2000), which were polymorphic in the DH parents, were analyzed in the DH mapping population using various polymerase chain reaction (PCR) programs (Tenhola-Roininen and Tanhuanpää 2010). Retrotransposon-microsatellite amplified polymorphism (REMAP; Kalendar et al. 1999; Kalendar and Schulman 2006), inter-retrotransposon amplified polymorphism (IRAP), and inter-simple sequence repeat (ISSR) markers were produced using the PCR profile described by Schulman et al. (2004) with minor modifications (primers available on request). Sequence-related amplified polymorphism (SRAP) markers were mainly amplified according to Li and Quiros (2001). AFLPs, microsatellites, and SRAPs were detected with DNA sequencers (GE Healthcare, Buckinghamshire, UK).

JoinMap® (Van Ooijen and Voorrips 2001) was used for map construction (a logarithm of odds [LOD] value of 6 to 14) using the Kosambi mapping function (Kosambi 1944). The map contained 17 linkage groups (≥ 3 markers, ≥ 10 cM) with 289 loci. The map length was 732 cM (one locus in every 2.5 cM). Previously mapped rye microsatellites were used as anchor markers to identify all seven rye chromosomes (Fig. 1), of which some were comprised of several parts. The kinship of parents of the DH mapping population (both have the same cultivar, Kungs II, in their pedigree) explains the low polymorphism on certain areas of chromosomes possibly causing this fragmentation.

Fifty-seven percent of the mapped markers showed distorted segregation ($P \leq 0.05$, analyzed in JoinMap, Fig. 1) on all chromosomes mainly due to tissue culture, self-incompatibility system, and inbreeding depression of out-crossing rye. The most severely distorted region ($P \leq 0.0001$) was detected on chromosome 6R with alleles inherited from Amilo in excess. Also, chromosomes 2R (Amilo in excess) and 3R (Voima in excess) were extremely distorted. Highly inbred rye lines suffer from distortion too (Hackauf et al. 2009).

The DH parents and the DH progeny (DHs from $F_1$ plants) were crossed with the PHS-susceptible Finnish cv. Riihi (several individuals and 1–3 pollinators per one DH) to obtain grains for α-amylase activity measurements (Tenhola-Roininen et al. 2006). Due to the low fertility of DH plants and undeveloped grains (Tenhola-Roininen et al. 2006), α-amylase activity could be measured only from 68 DH plants (Tenhola-Roininen et al. 2006). In addition, α-amylase activity (Ceralpha U/g) was measured from one grain only (except several grains from the DH parents and Riihi) with adjusted volume by the α-amylase assay procedure (Megazyme, Ireland), but repeated 2–4 times when possible (depending on the quality and number of grains). The results from one grain correlate with those from several grains (results not shown). α-amylase activity in the DH progeny varied from 49 to 540 U/g (mean 214 U/g) after 4 days of germination (Fig. 2). The mean α-amylase activity of the DH parents, Amilo

**Fig. 1** The genetic linkage map of rye constructed using doubled haploids (DHs). The rulers show map distances in cM. The total lengths (cM) of chromosomes or linkage groups are shown beneath. Anchor markers are printed in **bold**. The marker names include the size of the amplified DNA fragment if more than one fragment was amplified with the primer(s) used. Asterisks inform the segregation distortion (**$P \leq 0.05$, *** $P \leq 0.01$, **** $P \leq 0.005$, ***** $P \leq 0.001$, ****** $P \leq 0.005$, ******* $P \leq 0.0001$). The quantitative trait locus (QTL) affecting α-amylase activity is shown as a vertical bar, with the cross-line indicating the location of the QTL peak, with a confidence interval with a logarithm of odds (LOD) fall-off of 1.0. *a* Bednarek et al. (2003), *b* Hackauf and Wehling (2002a, 2003), *c* Khlestkina et al. (2004, 2005), *d* Korzun et al. (2001), *e* Kubaláková et al. (2003), *f* Masojć et al. (2007), *g* Röder et al. (1998), *h* Saal and Wricke (1999, 2002), *i* information provided from Lochow Petkus GmbH and Hybro GmbH & CoKG, Germany, *j* Bolibok et al. (2007), *k* Masojć and Milczarski (2009), *l* Hackauf et al. (2009), m Masojć et al. (2009)


1R
IR15*******
IR26RE7_790*******
ISSR5_1000*******
ecgc_mcag163****
eacg_mcgt189**
me4em3_580**
eacc_mctg424**
eata_mcgc410********
me4em5_760**
IR32_1650 SCM83**
RMS1010** SCM39h,d**
AB06_1250** SCM107b,f,j,k,l,m**
ecgg_mctg239
eata_mcct259**
ecgc_mctc119
me3em5_500**
ecgg_mctc99****
IR29RE9_800*****
eacc_mcgt302
me2em4_510**
SCM165
AF07_610******
me1em3_149****
RMS1107i**
SCM136
ecgg_mcct83**
IR29RE9_800
eata_mcta158****
eacg_mccg117**
71.1
2R
ecgg_mcta58
AD16_850
eacg_mctc424**
ecgc_mcgc362****
SCM153******
ecgc_mccg332******
me1em1_369*******
eacc_mctg216****
eacg_mctt486****
eacg_mcgt92*******
me1em6_106****
me1em5_336******
IR30_1300****
SCM32b******
ecgc_mctg315******
eacg_mcgt288****
SCM43_110b,h,d,j,l*******
ecgc_mccc113****
me1em3_335********
IR10_690*******
eata_mcgt270*******
me4em5_550******
eacg_mcgt167********
IR12RE2_1350****
IR21RE7_900********
me4em4_198********
me2em5_348********
eata_mcgt168********
eacg_mctt510********
SCM215l**
REMS1194c***
me1em4_106****
eacc_mcct454**
me2em5_365**
65.4
2RL
IR13_1400
SCM276
AB06_690**
eacc_mcac199
me5em3_261
me4em1_213
eacg_mctt443
SCM41c
ISSR1**
35.9
3R
IR26RE7_850
ecgg_mctg367****
SCM84j,l
IR26_810***
me5em3_478********
AF14_380********
eacg_mccg111a********
eacg_mcgt315******* ecgg_mctg208********
ecgc_mccg271********
SCM162b,j,l*******
eacc_mcgt375********
AB04_1600********
eacc_mcgt288********
eacc_mcgc207a*******
eacg_mccc78********
me1em4_388********
SCM173b*******
eacg_mctg288********
me2em5_685********
me1em5_454******
eata_mcct211*******
me4em1_307******
ecgc_mcag71**** ecgg_mctg201****
eacg_mctt167****
eata_mcgt415********
me1em4_493**
me2em5_773
67.8
4R
IR29RE9_1800
me1em3_321**
eacc_mcct411
me2em5_190
eata_mcga241
ecgc_mcgc315
eacg_mcgt282
ecgc_mcat515
me1em6_227
RMS1024 RMS1117e,i,j
ecgg_mcta106
eata_mcgc400**** SCM155l
IR41RE9_980
IR21RE5_630**
eata_mcgc171
eacc_mctg213
eacg_mctg152
eacc_mcta92
eacg_mccc309
me4em3_421
ecgg_mcct99
SCM80
eacc_mcag240
eacg_mctc445
ecgg_mcta218
IR11RE18_500
me4em4_549
eacc_mcta318
eata_mcgc168
me5em5_280
HVM4**
ecgc_mccc318
RMS1007e,i**
me4em4_266**
me4em3_415**
AC03_700
ecgg_mcgc183****
IR26_1700***
ecgc_mcgc255**
eata_mcgt198**
eacc_mcgt543**
ecgc_mcag236**
ecgc_mcga76****
eacc_mcac118**
eata_mcgc246***
me1em6_264********
ecgc_mctc139**
95.8
5R
me5em3_695
H13_650
IR32RE1_1600
eata_mcct247
eacc_mcta150
eata_mcct106
SCM166b,f,k SCM138b,d,h,j,l
SCM268h,j**
ecgc_mcag342 IR32_1400
eacg_mcgt341 eacc_mcgt234
SCM58
SCM159j**
ecgg_mcta441**
eata_mcgc302
IR30_710**
me1em4_479**
eacg_mctt407****
SCM12****
eata_mcga316**
SCM72****
SCM74l****
RMS1115j*** SCM77b,f,j,k,l,m***
eata_mcct205
AF14_600
AB04_1900
me5em1_510
eacg_mccg287
48.7
5RLa
me2em3_593
WM410_1359**
eacc_mctg243
eacc_mctg242**
me4em5_810**
40.7
5RLc
eacg_mccc182*******
eata_mcga252
eacc_mcgc220
AD16_730
REMS1218c
SCM365l
ecgg_mctg195
SCM312l SCM172j
35.1
5RLb
SCM133*******
SCM137********
REMS1264c********
13.8
6R
eacc_mctg144*******
eata_mcga280********
eacg_mctg225********
SCM135j********
me4em3_474********
eacc_mcct503********
eacg_mctt230********
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me1em2_491******** RMS1090j********
RMS1108l******** me2em4_619********
IR14RE20_590********
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ecgc_mcga172******** me1em2_491********
eacc_mcta317******** eacg_mctc261*********
eata_mcgt144********
eata_mcta98********
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ecgg_mctg162********
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eacc_mcac270********
SCM68j,l********
eacg_mctt108*****
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6RLb
eacg_mctg293*******
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ecgc_mcga207
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SCM2h,j
IR13RE7_850
IR10_650*******
53.2

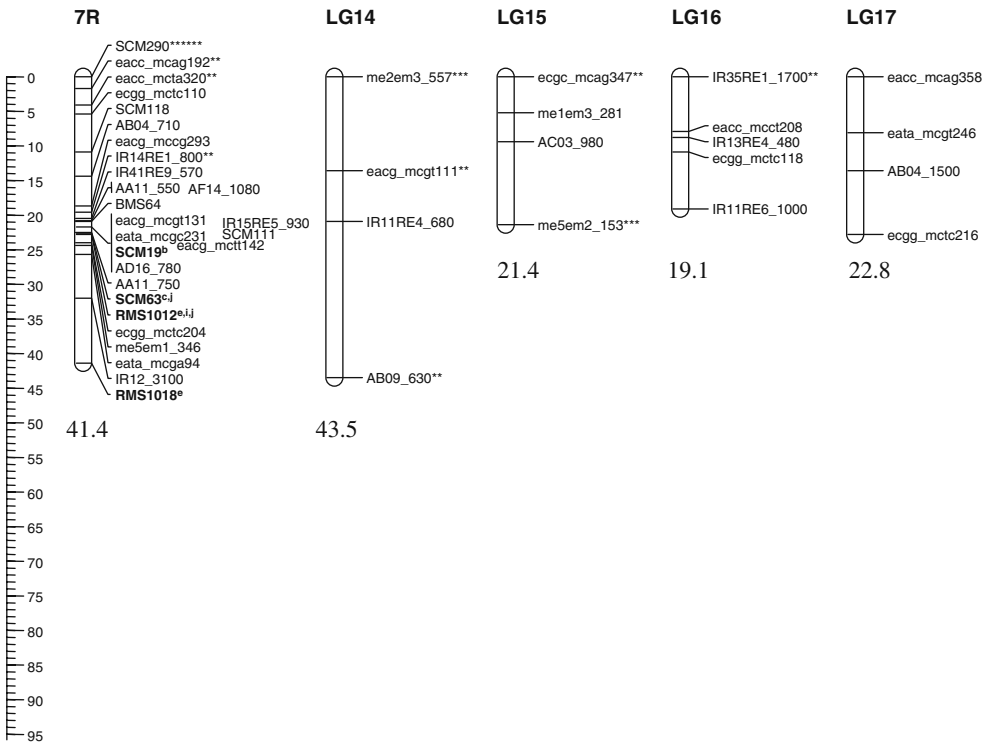


**Fig. 1** (continued)

and Voima, was 216 U/g and 334 U/g, respectively, and of the Riihi test cultivar, it was 348 U/g. QTL analysis was performed with NQTL software (Tinker and Mather 1995) with simple interval mapping. One thousand permutations were performed to estimate the threshold (test statistics 12.4=LOD score 2.7) with a type I error rate below 5%.

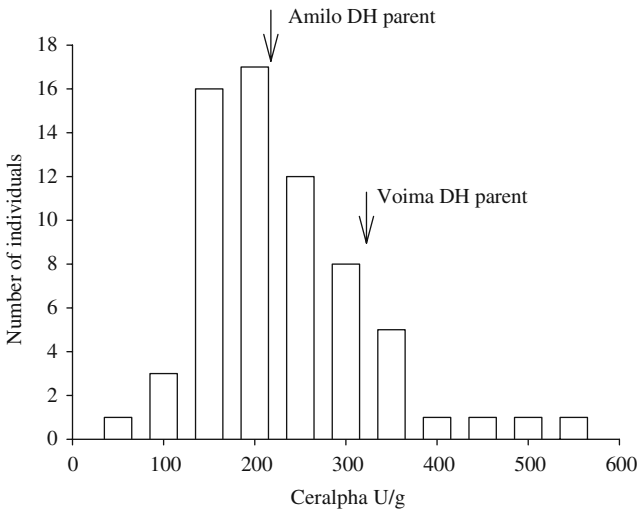


**Fig. 2** α-amylase activity (Ceralpha U/g flour) in the rye mapping population after four days of germination. The grains were derived from the testcrosses with cv. Riihi

One major QTL (test statistics 15.6=LOD score 3.4) controllig α-amylase activity was found on the long arm of 5R (Fig. 3), and it explained 16.1% of phenotypic variation. The QTL peak was located at the microsatellite loci SCM74, RMS1115, and SCM77 (Figs. 1 and 3).

Previously, three QTLs affecting α-amylase activity and three QTLs affecting sprouting have been found both on the short and on the long arm of 5R (Masojć et al. 1999; Masojć and Milczarski 2005; Masojć et al. 2007; Masojć and Milczarski 2009). From comparison with the existing rye QTL map (based on microsatellite SCM77), it can be concluded that our QTL is located near the PHS-enhancing locus (PHSE) on 5RL (Masojć et al. 2009). This locus probably represents a regulatory gene involved in the gibberellic acids (GA) signaling system (Masojć et al. 2009). As a consequence, the QTL found in the present study might be the same QTL as the one reported by Masojć et al. (2009) or is a nearby located modifying or regulatory gene.

Even though several QTLs affecting PHS have been reported, only one major QTL was found in the present study. Reasons for this include the mainly small sample sizes (Melchinger et al. 1998) in α-amylase analyses and the kinship of mapping parents leading to gaps in the map. Further, the use of heterogenous pollinator (cv. Riihi) with high α-amylase activity in testcrosses decreased the power of QTL mapping.

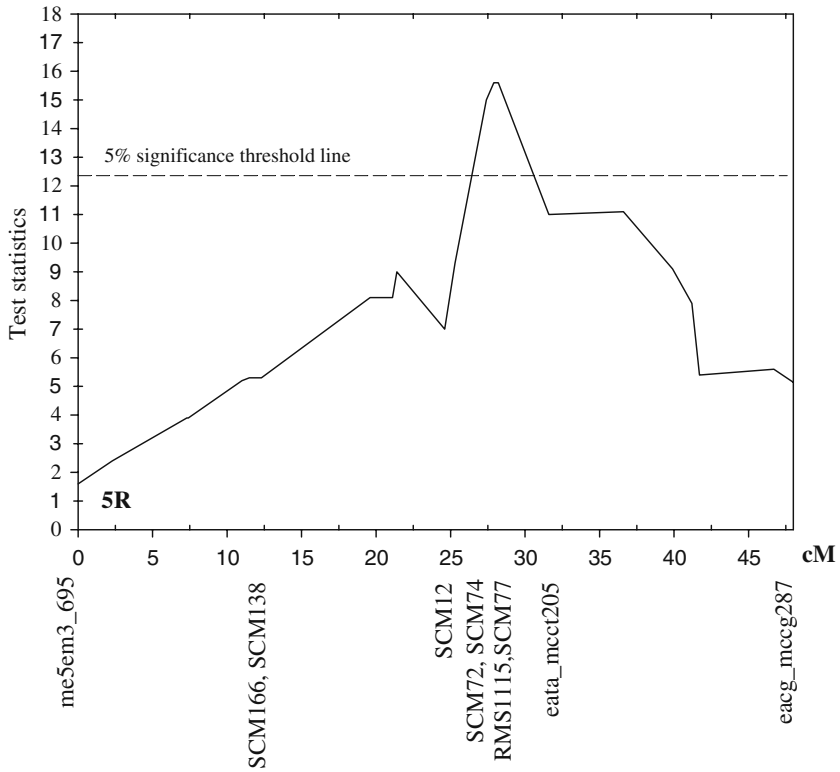


**Fig. 3** The profile of the QTL affecting α-amylase activity on chromosome 5R in the DH mapping population derived from the Amilo DH × Voima DH cross

When considering producing DHs in an out-crossing rye species, one should bear in mind that the development of DHs by anther culture is more problematic and time-consuming than in a self-pollinating species. Due to the low survival rate and the low fertility, 10–30% of regenerated plants at the most are suitable for further use (Tenhola-Roininen et al. 2006). In conclusion, DHs of out-crossing rye can be recommended to be used on a small-scale for special research and breeding purposes (for example, in purifying breeding material from undesirable recessive genes), but the benefits and costs need to be considered twice before DH production.

PHS resistance is a sum of many factors, and several QTLs associated with PHS (α-amylase activity, visible sprouting, or seed dormancy) have been found in cereals (Tenhola-Roininen 2009). It has been suggested that epistatic interactions mainly affect PHS resistance in rye (Masojć et al. 2009). An efficient way to develop a sprouting-resistant rye cultivar is via the pyramiding of several genes affecting α-amylase activity and PHS (especially directional and resistance loci, Masojć et al. 2009) using marker-assisted selection (MAS), as Twardowska et al. (2005) and Masojć and Milczarski (2009) have reported. In conclusion, in the present study, the first DH rye map was developed and one major QTL affecting α-amylase activity on 5RL was found. Three different robust microsatellite markers, SCM74, RMS1115, and SCM77, linked to the QTL can be used for MAS to decrease PHS as a part of the rye breeding strategy in Finland.

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