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ORIGINAL PAPER

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Approaching the self-incompatibility locus *Z* in rye (*Secale cereale* L.) via comparative genetics

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Abstract Using barley and wheat expressed sequence tags as well as rice genomic sequence and mapping information, we revisited the genomic region encompassing the self-incompatibility (SI) locus *Z* on rye chromosome 2RL applying a comparative approach. We were able to arrange 12 novel sequence-tagged site (STS) markers around *Z*, spanning a genetic distance of 32.3 cM, with the closest flanking markers mapping at a distance of 0.5 cM and 1.0 cM from *Z*, respectively, and one marker cosegregating with *Z*, in a testcross population of 204 progeny. Two overlapping rice bacterial artifical chromosomes (BACs), OSJNBa0070O11 and OSJNBa0010D21, were found to carry rice orthologs of the three rye STS markers from the 1.5-cM interval encompassing *Z*. The STS-marker orthologs on these rice BACs span less than 125,000 bp of the rice genome. The STS marker *TC116908* cosegregated with *Z* in a mapping population and revealed a high degree of polymorphism among a random sample of rye plants of various origin. *TC116908* was shown via Southern hybridization to correspond to gene no. 10 (OSJNBa0070O11.10) on rice BAC OSJNBa0070O11. Reverse transcription-PCR with a *TC116908*-specific primer pair resulted in the amplification of a fragment of the expected size from the rye pistil but not from leaf cDNA. OSJNBa0070O11.10 was found to show a highly significant sequence similarity to *AtUBP22*, a ubiquitin-specific protease (UBP). *TC116908* likely represents a putative UBP gene that is specifically expressed in rye pistils and cosegregates with *Z*. Given that the ubiquitination of proteins is emerging as a general mechanism involved in different SI systems of plants, *TC116908* appears to be a promising target for further investigation with respect to its relation to the SI system of the grasses.

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Introduction

Self-incompatibility (SI) is considered to be one of the most important strategies developed by flowering plants to promote outcrossing and maintain heterozygosity. As a result of its SI, rye (*Secale cereale* L.) is the only outbreeding cereal species among the Triticeae. As in other grasses, the recognition specificity of the SI reaction in rye is governed by the complementary action of two gametophytically expressed genes, *S* and *Z* (Lundqvist 1956), which are located on chromosomes 1R and 2R, respectively (Wricke and Wehling 1985; Gertz and Wricke 1989). An SI response occurs if both the *S* and *Z* allele of a haploid pollen grain are expressed in the diploid stigmatic tissue. As a unique feature among the gametophytic SI systems, the grass system reveals a pattern of physiological pollen and stigma characters, such as trinucleate pollen grains with a high respiratory rate, "dry-type" stigmas and a relatively rapid SI reaction near the stigma surface, which are otherwise correlated with sporophytic SI systems (Brewbaker 1967). While considerable progress has been achieved in our understanding of the molecular mechanisms acting in both gametophytic and sporophytic SI model systems (for reviews see Franklin-Tong and Franklin 2003; Hiscock and McInnis 2003), the molecular mechanism of the bifactorial grass SI system is still unknown.

Gene tagging in rye is hampered by its large genome size of approximately 9,120 Mbp (Bennett and Leitch 1995), high amounts of repetitive DNA, and a limitation in the genomic resources that have been developed for this species. The high level of conservation in gene content and gene order within the grass

family (Moore et al. 1995; Gale and Devos 1998; Dunford et al. 2002; Feuillet and Keller 2002), however, does allow for comparative approaches by using the wealth of genomic information presently developed for rice, wheat and barley. Gene coding sequences from closely related plant species are particularly suited for comparative approaches as they evolve at a slower rate than non-coding DNA. The sequence information accumulated on an expressed part of the Triticeae genomes as well as the almost completely sequenced rice genome [International Rice Genome Sequencing Project (RGP) http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/irgsp-status.cgi; Goff et al. 2002; Yu et al. 2002] has opened up new perspectives for genome research even in species like rye where limited genomic resources are available.

The genomic regions carrying the SI loci in rye have scarcely been addressed with molecular markers. Voylokov et al. (1998) located two genomic restriction fragment length polymorphism (RFLP) markers from wheat and rye on chromosome 1R as well as one barley cDNA on chromosome 2R that cosegregated with the *S* and *Z* loci, respectively. In the present paper we report on the directed development of markers for the *Z* locus on chromosome 2RL using an orthologous genomic region on rice chromosome R4 as a blueprint.

Materials and methods

Development of defined SI genotypes

We used two rye genotypes, 7092-16 and 7107-8, both of which had known genetic constitutions at the *S* and *Z* loci. These genotypes were obtained by the pseudo-compatible selfing of two self-incompatible plants from a population of the Carsten and Petkus genepool, respectively (G. Wricke, personal communication), under high temperature during anthesis (Wricke 1978). The two rye *S*/*Z* genotypes were crossed, with the resulting F1 being heterozygous at the *S* and *Z* loci. An individual F1 plant was selfed again by exploiting pseudo-compatibility, resulting in the selfed S1 family BAZ-033. Individuals of this family were genotyped and pre-grouped using the *S*-linked isozyme marker *Prx7* (Wricke and Wehling 1985) and the *Z*-linked cDNA marker *bcd266* (Voylokov et al. 1998). The SI genotypes of individual plants were assessed by in situ test pollinations and microscopic assessment of pollen-compatibility classes according to Lundqvist (1961). To simplify the rating and thereby increase its accuracy, we excluded plants heterozygous at both marker loci from the test pollinations. Consequently, only three out of the four possible phenotypic classes – 0, 50% and 100% compatible pollen grains – were expected to occur in individual pollen/stigma combinations, while the 75% class was excluded. Of 41 *S*/*Z*-marker genotypes of BAZ-033, 27 individual plants were *S*/*Z* genotyped in this way.

S/*Z* genotypes defined by the test pollinations and microscopy were used as parents to produce a total of 46 testcross families. Two of these were testcross families BAZ-530 and -534, which were used for mapping purposes in the present study. The gametic selection caused by SI in the testcrosses was used for the mapping of *Z* relative to markers (Wricke and Wehling 1985; Leach and Hayman 1987). Briefly, in a testcross of the type $S_1\ S_1\ Z_1\ Z_1 \times S_1\ S_1\ Z_1\ Z_2$ (or abbreviated, $S_{11}\ Z_{11} \times S_{11}\ Z_{12}$), the SI mechanism will prevent fertilization of all pollen grains except those carrying the Z_2 allele, which is not expressed in the stigma of the female parent. Consequently, seed set is possible only on the female parent, and legitimate offspring will be heterozygous at the *Z* locus. Likewise, a marker locus *M* is expected to be heterozygous (M_{12}) among all *N* testcross offspring individuals in the case of cosegregation with *Z*, while the occurrence of M_{11} offspring would indicate recombination between *M* and *Z*, with the recombination fraction $p = M_{11}/N$ and standard deviation $s = \sqrt{p(1-p)/N}$ (Leach 1988).

In addition to recombination with *Z*, the occurrence of M_{11} genotypes among testcross offspring may also result from illegitimate self-fertilization of the female parent caused by, for example, pseudo-compatibility or by pollen grains carrying a mutant *Z* allele not present in the stigma. Self-fertilization may be distinguished from recombination when a testcross of $S_{11}\ Z_{11}\ M_{11} \times S_{11}\ Z_{12}\ M_{12}$ also involves one or several markers *X*, with *X* being located in any genomic region and being homozygous with different alleles present in the testcross parents, for example, $S_{11}\ Z_{11}\ X_{11} \times S_{11}\ Z_{12}\ X_{22}$. In such a situation, X_{11} offspring kernels produced on the female parent would indicate self-fertilization and be excluded from the linkage analysis with respect to *M* and *Z*.

Sequence tagged site (STS) polymorphism was assessed on a random sample of plants from the landrace Lungauer Tauernroggen and from cv. Carokurz as well as on the two self-compatible inbred lines L301 and L2053, the latter of which were kindly provided by Dr. H. Wortmann, Hybro Saatzucht, Kleptow.

Development of STS markers

DNA from individual plants of the rye materials as well as of wheat cv. Chinese Spring and barley cv. Igri was extracted using the CTAB method (Saghai-Maroof et al. 1984). Rice genomic data was obtained from the rice database provided by The Institute for Genomic Research (TIGR), Rockville, Maryland, USA (http://www.tigr.org). Selected cDNA anchor markers located on the long arms of chromosomes 2R in rye (Voylokov et al. 1998) and 2H in barley (Qi et al. 1996) were aligned against TIGR assemblies [tentative consensus (TC) sequences] of barley and wheat expressed sequence tags (ESTs) to obtain maximum sequence information. Subsequently, the nucleotide sequences were aligned against all rice bacterial artificial chromosome (BAC)/

P_1-derived artificial chromosome (PAC) sequences in GenBank using the TIGR rice BLAST tool. Using a stringency cut-off of ≥80% identity with the marker sequence over a minimum of 100 bases, we parsed out the BAC and PAC clones that contained rice genes orthologous to the anchor markers. To enrich the *Z*-genomic region in rye with novel markers, we aligned sequence information from rice BACs within a 3-cM interval defined by OSJNBa0043L09 and OSJNBa0015K02 on chromosome R4 as well as from individual rice BACs on chromosomes R1, R9, and R11 against the TIGR barley (HvGI) and wheat (TaGI) Gene Index to identify orthologous sequences.

Primers suitable for cross-species amplification in rye were developed by carrying out multiple sequence alignments between barley, wheat and rice in order to identify conserved nucleotide sequence stretches for selected cDNAs. The software package CLUSTAL W (Higgins et al. 1994) and a www service provided by the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw/) were used for this purpose. Primers were designed using the software package PRIMER3 (Rozen and Skaletsky 2000) and a web site provided by the Whitehead Institute for Biomedical Research (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi).

STS-marker assays

For each STS assay, 50–100 ng of genomic DNA was used in a solution containing 1× reaction buffer (Qiagen, Hilden, Germany), 1.5 m *M* $MgCl_2$, 200 μM of each dNTP, 5 pmol of primers and 0.5 U *Taq* DNA polymerase (Qiagen) in a 25-µl volume. For cleaved amplified polymorphic sequence (CAPS) analysis, a 5-µl aliquot of each PCR was digested with 1 U of either *Dde*I, *Hin*fI, *Mse*I, *Hin*PII, *Rsa*I or *Taq*I (New England Biolabs, Frankfurt, Germany) to search for restriction fragment length polymorphisms among amplicons from various rye plants and *Z* genotypes. Restriction patterns of cleaved amplicons were visualized on agarose gels by means of ethidium bromide staining.

Mapping of STS markers

Chromosomal localization of the STS markers was determined using disomic wheat-rye addition lines kindly provided by S.M. Reader (Department of Crop Genetics, John Innes Centre, Norwich). Multipoint linkage analysis of markers in the S1 family BAZ-033 was performed using the software package JOINMAP ver. 3.0 (Van Ooijen et al. 2001) with a LOD score of 3.0. The Kosambi function was applied to convert recombination values to genetic distances (centiMorgans). For the mapping in testcross families BAZ-530 and BAZ-534, the backcross model with co-dominant markers and a unique paternal genotype and Kosambi's mapping function were selected in MAPMANAGER QTX (Manly et al. 2001) to calculate the genetic distances in the selected families. Graphical presentations of genetic linkage data were produced using the computer package MAPCHART (Voorrips 2002).

Reverse transcription-PCR

mRNA was isolated from rye leaves, pistils (stigmas together with the ovaries), anthers and pollen using paramagnetic beads (Dynal, Hamburg, Germany). First-strand cDNA synthesis on approximately 1 µg of mRNA was performed using an oligo-dT primer and the Omniscript reverse transcriptase (Qiagen) according to the manufacturer's recommendations. The primers Pc3046 (TCC TGG TGT GGG CCA TG) and Pc3714 (CTC AGG CTT GTT GGC GCC AAC GAG CTT GTC) were derived from the catalytic domain of a single-copy thioredoxin gene from the grass *Phalaris coerulescens* (GenBank Acc. AF159388). These primers allowed us to amplify transcripts from each of the investigated tissues and, thus, served as a positive control for the expression studies. Assay conditions for RT-PCR on diluted cDNA populations were identical to those used on genomic DNA.

Southern analysis

BAC clones were obtained from the Clemson University Genomics Institute and grown in LB medium supplemented with chloramphenicol. BAC DNA was isolated using an alkaline lysis method (Zhou et al. 1990) and digested with either *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I or *Xba*I (New England Biolabs). Restriction fragments were separated on 1% agarose gels and transferred to nylon membranes. Subgenomic fragments from rye were directly labeled with digoxigenin (DIG; Roche Diagnostics, Mannheim, Germany) using the indicated PCR conditions for all primers except *TC101821* and *TC116908*, which were labeled using HotStarTaq polymerase (Qiagen). Hybridization was performed overnight at 42°C in DIG Easy Hyb (Roche Diagnostics). Following hybridization, two low-stringency washes were performed for 15 min each in 2× SSC containing 0.1% sodium dodecyl sulphate (SDS) at room temperature. Subsequently, two high-stringency washes were performed for 15 min each in 0.5× SSC containing 0.1% SDS at 65°C. Chemiluminescent detection of the DIG-labeled probes was performed according to the manufacturer's recommendations.

Results

Establishing Defined SI Genotypes

Twenty-seven plants of family BAZ-033 were submitted to diallelic in situ test pollinations. Based on 265 pollen/stigma combinations, we were able to determine the

genetic constitution at both SI loci for 25 of these plants. Recombination between the *Z* locus and marker *bcd266* was observed for two plants that were homozygous at the *Z*-marker locus but displayed pollen/stigma interactions indicating heterozygosity at the *Z* locus. Thus, evidence was obtained via direct assessment of *Z* genotypes for recombination occurring between *Z* and the marker *bcd266* in family BAZ-033.

Since the mapping of *Z* via testcrosses relied on a functional SI system, we tested whether family BAZ-033 had retained SI after the two successive steps of pseudo-compatible selfing. Of the 25 plants mentioned above, in situ self-pollinations were assessed microscopically for 23 of them; a distinct SI response with 0% compatible pollen grains was observed for all 23. In addition, ears from all 41 plants of the BAZ-033 family were separately bagged under normal temperature conditions; no seed set was observed. Functionality of the SI system as well as *S*/*Z* genotyping of the testcross parents was also tested for all testcrosses via reciprocal crossing. Seed sets on the female and male cross parents in 43 of 46 (93%) pairwise testcrosses were in accordance with the *S*/*Z* genotyping of the testcross parents. Thus, the *S* and *Z* alleles present in the testcross parents selected from family BAZ-033 could be shown to be functional under normal temperature conditions and genotyped correctly. This was also true for the testcrosses giving rise to mapping families BAZ-530 and BAZ-534 used in the present study (Table 1).

In silicio mapping of Triticeae markers in the rice genome

Marker sequences previously mapped on barley chromosome 2HL (Qi et al. 1996) were aligned to rice genomic sequences (Table 2). Sequence information was available for 30 of the 56 markers mapping within a 72-cM interval defined by *abc451* and *cMWG720* on 2HL. Of these, 24 markers (43%) could be mapped in silico to the rice genome. Collinearity of these markers between barley chromsome 2HL and rice chromosome R4 was given for the sub-interval of *abc451*–*bcd266* spanning positions 77.4–108.7 cM in the barley 2HL consensus map (Table 2). All sequences except for two cDNAs matched a sequence found solely on rice chromosome R4 under the given stringency conditions of the BLASTN search. For *abc451*, which represents a *S*-adenosylmethionine decarboxylase precursor, and for *His3C* as well as *abc152D*, both encoding a histone H3, sequences with significant similarity could be detected on rice chromosomes 2 and 9 and on chromosomes 1, 6 and 11, respectively (data not shown), which corresponds to the situation in barley where each of the probes detects multiple marker loci (Qi et al. 1996).

Alignment of cDNAs located distal of *bcd266*, namely *Crg3a*, *bcd453*, *cdo373*, *abc165*, and *abc252*, revealed rearrangements within a 26.5-cM interval of barley chromosome 2HL relative to rice (Table 2). These rearrangements include segments which appear to be orthologous to parts of rice chromosomes R1 (*Crg3a*, position 109.5 cM in the barley consensus map), R9 (*bcd453b*, 121.1 cM), and R11 (*abc252*, *cdo373*, *abc165*; 123.8–136 cM).

Development and mapping of STS markers in the rye genome

Based on the comparative sequence alignments, 33 genes from defined regions of the rice genome were selected to develop STS markers in rye (Table 3). The majority of the selected genes are located within a 3-cM interval on rice chromosome 4, and three display significant sequence similarities to the barley cDNA anchor probes *bcd266*, *bcd135*, and *Gln2*, respectively. We also included genes tagged by *Crg3A*, *bcd453*, *abc252* and *cdo373* and located on rice chromosomes R1, R9 and R11. The selected PCR conditions enabled us to amplify subgenomic fragments with 31 (94%) of the primer pairs deduced from the 33 rice genes (Table 4). Seven primers yielding multiple products were rejected from subsequent studies. Thus, 24 primers which generated single amplification products from rye-genomic DNA were included for mapping purposes. For 15 primer pairs, the observed fragment sizes were larger than expected from the underlying EST sequences (Table 4), indicating the presence of introns within the amplified regions. For three markers, namely *TC101821*, *TC116908* and *Xscm254*, an InDel polymorphism in family BAZ-033 allowed for a co-dominant scoring of the markers without additional processing. Sequence diversity between segregating genotypes in the selected plant materials was observed for 12 subgenomic fragments as a result of cleavage with individual restriction

Table 1 Testcross families used for the mapping of *Z* in rye

Testcross family	P_1[a]		P_2[a]		Seed set: $P_1 \times P_2$			Seed set: $P_2 \times P_1$		
	Plant no.	Genotype	Plant no.	Genotype	Ears	k[b]	k/ear	Ears	k	k/ear
BAZ-530	*033*/015	S_{22} Z_{11}	*033*/035	S_{22} Z_{12}	9	327	36.3	25	25	1.0
BAZ-534	*033*/041	S_{11} Z_{22}	*033*/013	S_{11} Z_{12}	36	488	13.6	39	23	0.6

[a] P_1 and P_2, Female and male testcross parents, respectively, of the F2 family BAZ-033
[b] k, Number of kernels

Table 2 BLASTN query of Triticeae anchor markers on chromosome 2HL in barley against barley and wheat ESTs as well as the rice genome (*n.s.* no significant sequence similarities found)

Marker	Position (cM)[a]	GenBank Acc.	HvGI[b]	TaGI[b]	O.s.[c] BAC	GenBank Acc.	Chromosome	Position (cM)[d]
abc451	77.4	L43952	*TC120143*	*TC167983*	OSJNBa0067K08	AL606627	R4	76.5
cdo588	77.4	L44002	*TC120143*	*TC167983*	OSJNBa0067K08	AL606627	R4	76.5
abg619	79.2	L44052	*TC114110*	n.s.	OSJNBa0004N05	AL606622	R4	78.2
MWG2081	79.9	AJ234762	*TC128791*	n.s.	n.s.	n.s.	n.s.	
MWG801	79.9	AJ234558	*TC128386*	n.s.	OSJNBa0038O10	AL663019	R4	78.2
cMWG699	79.9	AJ234426	*TC109813*	*TC144415*	OSJNBa0091D06	AL606459	R4	82.5
His3C	81.7	M34928	*TC120066*	*TC168195*	OSJNBb0108J11	AL606618	R4	60.2
abc152D	83.3	L43923	*TC120066*	*TC168203*	OSJNBb0108J11	AL606618	R4	60.2
MWG892	90.1	AJ234626	*TC117411*	*TC153359*	n.s.	n.s.	n.s.	
MWG581	91.3	AJ234520	*AJ476118*	*CA696701*	OSJNBa0093O08	AL606648	R4	102.7
MWG2123	92.6	AJ234786	*TC127966*	n.s.	OSJNBa0070C17	AL731610	R4	102.7
MWG882	92.6	AJ234614	*TC124314*	*TC175302*	OSJNBa0070C17	AL731610	R4	102.7
MWG503	93.5	AJ234472	n.s.	*CA741809*	OSJNBa0084K11	AL606687	R4	87.1
abg72	107.6	L44093	*TC125645*	*CK210684*	OSJNBa0058K23	AL662970	R4	107.4
bcd266	108.7	BE438789	*TC108778*	*TC147510*	OSJNBb0059K02	AL606692	R4	114
Crg3A	109.5	M60733	*TC109959*	*TC175026*	P0491F11	AP004669	R1	146.4
cdo680	113.6	AI978281	*TC109075*	*TC168563*	OSJNBa0071I13	AL606685	R4	114.3
bcd453B	121.1	BE438630	*TC121448*	*TC148500*	OSJNBb0066C12	AP005738	R9	0.8
abc252	123.8	L43937	*TC120370*	*TC146395*	OSJNBa0041C22	AC137752	R11	57.3
cdo373	124.5	BE439108	*TC120638*	*TC166718*	OSJNBa0041C22	AC137752	R11	57.3
Gln2	125.6	X16000	*TC109604*	*TC146954*	OSJNBa0015K02	AL606608	R4	Approx.114.3
abc165	136.0	L43932	*TC120638*	*TC166718*	OSJNBa0041C22	AC137752	R11	57.3
abc153	136.9	L43924	*TC109645*	*TC162604*	OSJNBa0043L09	AL606444	4	111.3
abg316E	139.4	L43975	*TC108922*	n.s.	n.s.	n.s.	n.s.	n.s.
abg609A	142.8	L44048	*AV835329*	n.s.	n.s.	n.s.	n.s.	n.s.
abg316D	143.8	L43975	*TC108922*	n.s.	n.s.	n.s.	n.s.	n.s.
Pcr1	144.4	X15869; X84738	*TC111069*	*TC169793*	OSJNBb0017I01	AL606456	R4	123.8
MWG949	147.0	AJ234667	*TC126723*	*TC177199*	OSJNBb0020J19	AL606656	R4	129.6
bcd410	148.8	BE438726	*TC121999*	n.s.	n.s.	n.s.	n.s.	
cMWG720	149.4	AJ234437	*TC121697*	*TC148828*	OSJNBa0043A12	AL606619	R4	122.9

[a]Position on barley chromosome 2HL according to Qi et al. (1996)
[b]HvGI, TIGR barley Gene Index; TaGI, TIGR wheat Gene Index
[c]O.s., *Oryza sativa*
[d]Position in the rice genome according to the TIGR Whole Automated Rice Genome Annotation Database

endonucleases. All except one digested amplicon displayed a co-dominant segregation pattern. For *TC87848*, the selected restriction enzymes led to a dominant/recessive inheritance. This marker was mapped in family BAZ-033 only. Fragments obtained with primers *TC108778*, *TC89869* and *TC35485* could be assigned to the long arm of chromosome 2R using disomic wheat-rye addition lines (Fig. 1).

In testcross family BAZ-530, one individual (0.8%) displayed a maternal genotype for every marker in the interval around the *Z* locus. In situ test pollinations identified this plant as an illegitimate offspring that probably derived from self-pollination (not shown). Offspring of this origin may appear as a result of fertilization by mutant pollen grains carrying a self-compatibility (sc) allele at either the SI locus, as has been demonstrated for the grass *Phalaris coerulescens* for which sc mutants were recovered using a "pollen trap" (Hayman and Richter 1992). Likewise, in testcross family BAZ-534 nine (10.1%) plants were identified as being homozygous at each of the *Z*-linked marker loci. All of these nine plants proved to be also homozygous for the female-parent allele at marker locus *X*, which in the present case was represented by *TC16395*, which maps distal of *TC35485* in family BAZ-033 (not shown). The genetic constitution of the parents (S_{11} Z_{11} $X_{11} \times S_{11}$ Z_{12} X_{22}) of this family allowed us to use *TC16395* to identify the nine plants as illegitimate offspring.

The settled segregation data sets of BAZ-530 and -534 with 125 and 89 plants, respectively, were first tested for statistical homogeneity, then pooled and used to map 12 STS markers relative to *Z* and to each other (Table 5). The marker *TC116908*, which originated from OSJNBa0070O11, was found to cosegregate with the *Z* locus in 204 plants ($s = 0.015$; Table 5). The remaining markers around *Z* could be arranged within a 32.3-cM interval, including *bcd266* (= *TC108778*), which mapped 3.5 cM from *Z*. No differences in the order of markers was observed when they were compared with the order obtained with the single data sets of the two testcross families and with the F2 multipoint data established with the 41 individuals of BAZ-033 (not shown).

Within a 18.4-cM interval encompassing *Z*, which was defined by markers *TC17178* and *TC108778*, collinearity was seen between chromosomes 2RL in rye and R4 in rice at the genetic map level (Fig. 2). In

Table 3 BLASTN query of rice BACs against barley and wheat ESTs for the development of cross-species STS markers in the *Z*-genomic region of rye chromosome 2RL (*n.s.* no significant sequence similarities found)

2HL Marker	*Oryza sativa*				HvGI	TaGI
	BAC	Chromosome	CentiMorgans	Locus		
	OSJNBb0015D13	R4	109.9	9481.t00012	*TC19780*	n.s.
	OSJNBa0043L09	R4	111.3	8334.t00019	*TC17178*	*TC30934*
	OSJNBa0033G05	R4	Approx. 111.3	8280.t00006	*TC32601*	*TC77775*
	OSJNBa0033G05	R4	Approx.111.3	8280.t00008	n.s.	*CA498418*
	OSJNBa0033G05	R4	Approx.111.3	8280.t00018	*TC91015*	*CA632968*
	OSJNBa0033G05	R4	Approx.111.3	8280.t00022	*TC102514*	*TC140135*
	OSJNBa0070O11	R4	113.2	5423.t00003	*TC101821*	*TC109091*
	OSJNBa0070O11	R4	113.2	5423.t00006	n.s.	*TC157579*
	OSJNBa0070O11	R4	113.2	5423.t00009	*TC123839*	*TC175864*
	OSJNBa0070O11	R4	113.2	5423.t00016	*TC116908*	n.s.
	OSJNBa0010D21	R4	113.2	8283.t00001	*TC89057*	*TC126225*
	OSJNBa0010D21	R4	113.2	8283.t00011	*TC101136*	n.s.
	OSJNBa0010D21	R4	113.2	8283.t00016	n.s.	*TC121349*
bcd266	OSJNBb0059K02	R4	114	8206.t00009	*TC108778*	*TC122017*
	OSJNBb0059K02	R4	114	8206.t00011	*TC90590*	*TC130087*
	OSJNBb0059K02	R4	114	8206.t00012	*TC59456*	n.s.
	OSJNBb0059K02	R4	114	8206.t00015	*TC90081*	*TC130901*
	OSJNBb0059K02	R4	114	8206.t00016	*TC99680*	*TC125205*
	OSJNBb0059K02	R4	114	8206.t00017	*CA006574*	*TC141719*
	OSJNBb0059K02	R4	114	8206.t00024	*TC100152*	*TC131503*
Crg3A	P0491F11	R1	146.4	4365.t00013	*TC88389*	*TC110077*
bcd453B	OSJNBb0066C12	R9	0.8	5972.t00013	*TC88701*	*TC126996*
cdo373	OSJNBa0041C22	R11	57.3	7164.t00007	*TC87848*	*TC101754*
abc252	OSJNBa0041C22	R11	57.3	7164.t00006	*TC98482*	*TC103276*
	OSJNBa0060D06	R4	114.3	8204.t00005	*TC16395*	*TC36344*
	OSJNBB0022F16	R4	Approx.114.3	2230.t00013	*TC35485*	*TC88626*
	OSJNBa0071I13	R4	Approx.114.3	5383.t00003	n.s.	*TC133483*
	OSJNBa0071I13	R4	Approx.114.3	5383.t00004	*TC31342*	*TC36695*
	OSJNBa0071I13	R4	Approx.114.3	5383.t00010	*TC97681*	*Zmlg-1*
cdo680	OSJNBa0071I13	R4	Approx.114.3	5383.t00011	*TC87436*	*TC124934*
	OSJNBa0071I13	R4	Approx.114.3	5383.t00018	*CD057956*	*TC113690*
bcd135	OSJNBa0011F23	R4	Approx.114.3	8199.t00011	*TC89869*	*TC110846*
Gln2	OSJNBa0015K02	R4	Approx.114.3	8126.t00003	*TC77238*	*TC85696*

contrast, rearrangements were observed distal of *Xbcd266*/*TC108778*. The positions of *TC98482* and *Xscm254* in rye and their orthologues in the rice genome (Fig. 2) indicated the presence of two segments related to rice chromosomes 9 and 11, respectively. Furthermore, a segment defined by *TC77238* and *TC35485*, which is located distal of *bcd266* on rice chromosome 4, revealed an inversion occuring on 2RL relative to rice (Fig. 2).

Relationship of the *Z* locus to the rice genome

Based on the extent of SI-induced gamete selection (Table 5), the *Z* locus would map within a 1.5-cM interval between *TC101821* and *TC89057* (Fig. 2), with cosegregation of *Z* and *TC116908*. The *TC101821* ortholog resides on the same BAC (OSJNBa0070O11) as *TC116908*, whereas the *TC89057* ortholog is located on an adjacent BAC (OSJNBa0010D21) that overlaps with OSJNBa0070O11 by 950 bp. Thus, rice BAC OSJNBa0070O11 (GenBank Acc. No. AL606445) , which represents less than 125,000 bp of total rice DNA, corresponds almost completely to the 1.5-cM rye-genomic interval encompassing *Z* (Fig. 2). Among the 12 genes identified on OSJNBa0070O11, nine exhibited significant sequence similarities to known or putative proteins, while the remaining three coding regions represented retrotransposons (Fig. 2).

Since *TC116908* cosegregated with *Z*, rice BAC OSJNBa0070O11 was digested with six restriction enzymes and the separated restriction fragments probed with *TC116908*. A hybridization pattern comprising eight fragments was obtained (Fig. 3) which, based on restriction site analysis of OSJNBa0070O11, was the only pattern expected, with parts of gene no. 10 in all fragments. The sizes of the expected restriction fragments were 16,686 bp (*Bam*HI), 6,285 bp (*Eco*RI), 26,999 bp (*Eco*RV), 1,430 bp as well as 20,456 bp (*Hin*dIII), 10,318 bp (*Pst*I) and 6,007 bp as well as 14,207 bp (*Xba*I). Thus, Southern analysis verified that the 2.2-kbp *TC116908* fragment amplified from rye-genomic DNA corresponded to gene no. 10 (OSJNBa0070O11.10) on OSJNBa0070O11. Southern analysis indicated a single-copy nature of *TC116908* in rye. As expected, a RFLP was observed that corresponds to the underlying *Z* genotypes (not shown). This observation is in accordance with the BLASTN search of

Table 4 Development of STS primers from barley, wheat, and maize cDNAs for mapping in rye

Identifier	Barley, wheat, and maize cDNAs	STS-marker assays					
		Forward (F) and reverse (R) primer sequences (5′–3′)		Fragment size (bp)		T_a[a]	Cycles
	Tentative annotation			Expected	Observed		
TC19780	Organic anion transporter-like protein	F	GCCTCCTCACGGGCTTCAG	1,083	Approx. 1,100	55	35
		R	TCCAGGCCGGCCATGGTGT				
TC17178	Probable diacylglycerol kinase	F	TGCTGGTGGTGATGGCACgG	941	Approx. 600	55	35
		R	ATACACGGTGGAATGCATGCA				
TC32601	Beta-ketoacyl-acyl carrier protein synthase III	F	ACGGACTGGGATTCGGAACAG	691	750	55	35
		R	AAAGAGAACAAGGTCAACATC				
CA498418	Cyclic nucleotide and calmodulin-regulated ion channel	F	AACAAGGTCCAGCTAAAGGAG	461	480	55	35
		R	GGCTTCGGCACTCTCACAA				
TC91015	Hydrolase, alpha/beta fold family, putative	F	TCGGCCAGATGCTCTACA	853	700[c]	55	35
		R	ACCTTCTCTGGCCAGTCCTC				
TC102514	Ca2 + -dependent lipid-binding protein, putative	F	CTTCCTTGGAGAGGACAATGA	459	750	55	35
		R	ATTGTGGCGACCACTTCAGA				
TC101821	TPR domain, putative	F	AGGGTGATCAGAGACCGGAAG	1,098	1,800	55	35
		R	AAGCTCAAGGGAAGCAAAATC				
TC157579	Expressed protein	F	TGGCCAAGATTCCATAAATGC	566	1,800	50	35
		R	CGAACCCAGAAAATACCTGGACA				
TC123839	G10 protein	F	TGGATGCCAAAATGAGAGAAG	542	No amplicon		
		R	GTCAGGAAACCGGATAAGAGG				
TC116908	Ubiquitin-specific protease 22	F	GCTCAACAACCTCGGCAAC	700	2,200	60	35
		R	CCTCCGAATGGACATTTGCT				
TC89057	Glycerol kinase	F	TGGAGCAAATCCACAGGCCTTC	814	900	55	35
		R	GCACAAAGTATATGCCACCTG				
TC101136	Zinc finger, C3HC4 type (RING finger), putative	F	AAATGATTCAGAGAGGGGAGAATG	708	300[c]	50	40
		R	CAAAGTGATGGCAACAAGTCA				
TC121349	Transcription factor AHAP2-related	F	GTCTACTTGGGGCTGTTCGAC	404	1,100[c]	60	35
		R	GGACTGAAGGGCATGTGG				
TC108778	Similar to oryzain alpha chain precursor	F	AAGAGCTTGTTGACTGTGACACTT		1,500	55	35
		R	TGCAGATGGGATAGTTATGAGG				
TC90590	Glycosyltransferase family 43, putative	F	GTCGTCTTCCTCCTCTTCCTG	786	Multiple amplicons	50	40
		R	GTGGTAGCCCACCAGCTTC				
TC59456	UDP-glucoronosyl and UDP-glucosyl transferase	F	CACCTCGTCTCCCTCGTCACC	889	Multiple amplicons	55	35
		R	CACGAACCCGGCCCACTCCAG				
TC90081	Asparaginase	F	CACACTCCTACCTCGCCTTC	446	Multiple amplicons	55	35
		R	CAGCGTAGAGCGGATGATG				
TC99680	Phosphoglycerate dehydrogenase-like protein	F	CCAACGTCGACTGCTCCTAC	860	380	55	35
		R	TGCAAGCTCCCCTTTCAGAG				
CA006574	Putative alpha-*N*-acetylglucosaminidase -related	F	AACTGGTGGTGCACAATTATC	438	1,900	55	35
		R	CAGCAGGGATGTTACCAGAAA				
TC100152	Sulphate transporter protein -related	F	AAGGGGTAGCAGTTGGTAGGA	813	1,300	55	35
		R	TCGCTCGTTGTATTTTCTCCA				
TC88389	XRN 5′–3′ exonuclease *N*-terminus, putative	F	TTAGCAATGGCAAAGTGTCTC	436	400[c]	50	40
		R	GATAAATACAGACTCCCATCAAGG				
Xscm254[b]	Beta-fructofuranosidase {*Triticum monococcum*}	F	TGAGCAGAGCTCAAGCCTATT	105	220	[d]	[d]
		R	ATCCAATCGGCCGTAGAAAT				
TC87848	Glycine hydroxymethyltransferase	F	GAACTGACAACCA(C,T)CTTGTTC	622	1,200	50	35
		R	TTGGCAAATACTC(G,A)ACATTCC				
TC98482	AdoHcyase	F	AGACTGACCGCTGGGTCTTC	511	600	55	35
		R	CCAACCCTCACAAGGCTCA				
TC16395	Similar to sulphate transporter protein	F	GCTCCCTTGGCATCCGTcATC	884	950	55	35
		R	GTTGTATGCAGCAGGGAGATC				
TC35485	JUN-activation-domain-binding protein homolog-rice	F	TGGTTATGGATGCTGGCTgTC	615	600	55	35
		R	ATATCGACTGTGTGCCAGTTG				
TC133483	Putative lipase	F	ATGAGCAAGCTCGACTACTGC	1124	No amplicon		
		R	TTGTAGGAAACGTCGAAGTGG				
TC31342	Farnesyl pyrophosphate synthetase	F	TGGACTACAACGTGCCTGGAG	1287	1,500	55	35
		R	GAGGGTCACCGAAGCAATCCA				

Table 4 (Contd.)

Identifier	Barley, wheat, and maize cDNAs	STS-marker assays				
		Forward (F) and reverse (R) primer sequences (5′–3′)	Fragment size (bp)		T_a[a]	Cycles
	Tentative annotation		Expected	Observed		
Zmlg1	Similar to maize liguleless1 protein	F TCAGCTGCTGGGCTACAAC R ACTGCTGGCAGAA(C,T)CTCTG(G,A)T	548	650	55	35
TC87436	Plasma membrane P-type proton pump ATPase	F CTGCAAGGAGGATGTGAAGAG R TTTCCCTGATTGACCTGACAC	944	1,700	50	40
CD057956	Lectin (probable mannose binding), putative	F ATCAACCACATGAACCTCGTC R CAGCTTCACAAGACCGAAGTC	303	300	50	40
TC89869	AT5g20600/F7C8_190	F AGTGATGGAGGGGATAGCAGT R AAAGGTAGCAAGCCAAGAGTG	662	600	55	35
TC77238	Glutamine synthetase, catalytic domain, putative	F CAAGATCATCGCCGAGTACAT R GATTTCAATTCCAGCGTAAAGG	508	1,500	55	35

[a] T_a, Annealing temperature
[b] This rye-SSR marker was developed based on the rye EST BE438508 and is orthologous to *BCD453B*
[c] Plus additional fragment
[d] PCR conditions for the EST-derived microsatellite are as described by Hackauf and Wehling (2002)

OSJNBa0070O11.10 against the rice genome data, which revealed the single-copy nature of this particular gene in rice.

OSJNBa0070O11.10 is delimited on the BAC by positions 110453 and 113303 and is predicted to encode a 370-amino acid protein having a significant similarity to a ubiquitin-specific protease (UBP) 22 (UBP22) in *Arabidopsis thaliana* (BLASTP: 56% identities (208/367), 70% positives (259/367), expect = e-113). OSJNBa0070O11.10 is also identical with part of the 2,220 bp full-length cDNA clone J023143B02 (BLASTN: 100% identities (1113/1113), expect = 0.0) that has recently been isolated from rice flowers (Kikuchi et al. 2003).

Polymorphism of *TC116908* in rye

Subgenomic fragments were amplified using the *TC116908* primers (Table 4) in a random sample of individual rye plants. Subsequently, the amplicons were subjected to CAPS analysis. The patterns obtained revealed a high degree of polymorphism among the sample, with at least eight CAPS alleles of the *TC116908*-derived amplicon (Fig. 4). The nine plants of the landrace Lungauer Tauernroggen differed by seven CAPS alleles and the five plants of cv. Carokurz, by four alleles. While the two defined, homozygous *Z* genotypes (Z_1 Z_1, Z_2 Z_2) as well as the two inbred lines each displayed only one principle CAPS fragment, most of the remaining plants showed two main fragments, thus appearing to be heterozygous at the CAPS marker locus.

Transcript analysis

RT-PCR transcript analysis with cDNA populations from different rye tissues was performed for rye STSs deduced from five genes located on rice BAC OSJNBa0070O11 (Fig. 5). Under the given experimental conditions we were unable to amplify a fragment using the primers of *TC101821* with either of the cDNAs investigated. In contrast, amplicons of the expected size (Table 4) could be observed with the remaining primer pairs. Using primers of *TC157579* and *TC123893*, we were able to amplify fragments from leaf, pistil (i.e., stigmas and ovaries) and anther cDNA. *TC102514* gave amplification predominantly in the pistils, there were also weak signals in the anthers and leaves. With the primers of *TC116908* we observed amplification predominantly with cDNA from pistils and obtained two fragments approximately 600 bp and 700 bp in size. To a much lesser extent, a transcript was obtained from anther cDNA, but not from leaves.

Discussion

Due to the ever-increasing availability of detailed information on the barley and wheat transcriptomes and the almost complete sequence information on the rice genome, we were able to delineate the target region bearing the *Z* locus in rye more precisely than has been possible to date. The homoeologous group 2 chromosomes of the Triticeae consist of, in terms of an insertion, rice chromosome R7 into chromosome R4 (Van Deynze et al. 1995; Moore et al. 1995; Sorrells et al. 2003). Based on anchor markers mapped on barley chromosome 2HL (Qi et al. 1996) and their in silico localization in the rice genome, we were able to address the region-of-interest encompassing *Z* on rye chromosome 2RL via its ortholog on rice chromosome R4 and subsequently to derive 12 novel markers from that orthologous region. By mapping these 12 novel markers in relation to *Z*, we were then able to narrow down considerably the marker-defined genomic *Z* interval on chromosome 2RL.

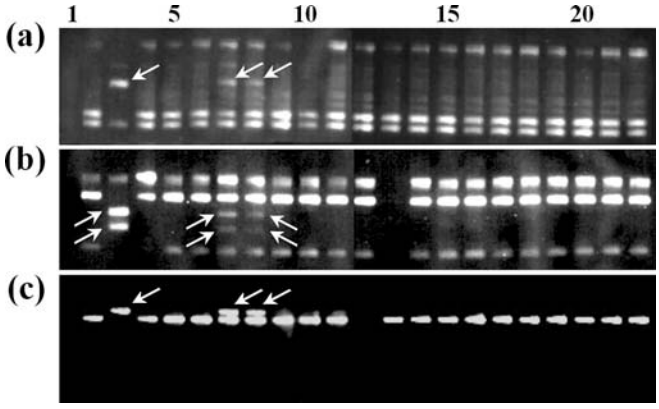

Fig. 1 Chromosomal localization of the STS markers *TC108778* (*bcd266*) (**a**), *TC35485* (**b**) and *TC89869* (*bcd135*) (**c**) using disomic wheat/rye addition and translocation lines. *Lanes*: *1* H_2O, *2* *T. aestivum* cv. Chinese Spring, *3* *S. cereale* cv. Imperial, *4* 21"+1R", *5* 21"+1RS", *6* 21"+1RL", *7* 21"+2R′/21"+2R′+2RL′, *8* 21"+2RL", *9* 21"+3R", *10* 21"+3RS", *11* 21"+4R", *12* 21"+4RS", *13* 21"+4RL", *14* 21"+5R", *15* 21"+5RS", *16* 21"+5RL", *17* 21"+6R", *18* 21"+6RS", *19* 21"+6RL", *20* 21"+7R", *21* 21"+7RS", *22* 21"+7RL". *Arrows* indicate the rye-specific STS fragments

A similar approach based on the sequence information of a region on rice chromosome 3 has recently been used for the development of two closely linked PCR markers flanking the aluminum tolerance gene *Alt3* on rye chromosome 4RL (Miftahudin et al. 2004). Collinearity between the Triticeae and rice has also been successfully exploited for marker saturation of target intervals in barley encompassing the stem-rust resistance gene *Rpg1* on chromosome 7HS (Kilian et al. 1995), the photoperiod-response gene *Ppd1-H1* (Dunford et al. 2002), the leaf-rust resistance genes *Rph7* on 3HS (Brunner et al. 2003) and *Rph16* on 2HS (Perovic et al. 2004) and the gibberellic acid-insensitive dwarfing gene *sdw3* on 2HS (Gottwald et al. 2004) as well as of a genomic interval on wheat chromosome 6B bearing the grain protein-content locus *Gpc-6B1* (Distelfeld et al. 2004).

It has been noted that despite the broad-scale collinearity of genes among the grasses (Gale and Devos 1998), the rice genome cannot serve as a general blueprint for directed gene isolation strategies. For instance, rearrangements have been demonstrated between rice chromosome 5S and wheat chromosome 1S which limit the use of the rice genome for positional cloning in this sub-genomic region of wheat (Guyot et al. 2004). In the present study, rearrangements on the macro-level relative to rice were found distal of *TC108778* on rye chromosome 2RL and its orthologous genomic region of the barley consensus map according to Qi et al. (1996). This finding is in line with comparative studies between rice and wheat, which suggest that in wheat the distal parts of chromosomes are more frequently affected by a disruption of microcollinearity (Gallego et al. 1998; Distelfeld et al. 2004).

Continuous collinearity with rice chromosome R4 on the macro-level was present in a marker interval extending 31.3 cM on barley chromosome 2HL. On rye

Table 5 Pooled segregation data of testcross families BAZ-530 and -534 for STS markers linked to the self-incompatibility locus *Z*. M_{11} represent the number of maternal marker genotypes and recombinants with *Z*; M_{12}, the number of paternal genotypes

Marker	M_{11}	M_{12}	Number of recombinants	Number of plants scored (*n*)	Genetic distance between adjacent loci (cM)	Standard error	95% Confidence interval		LOD[a]
TC17178	12	68							
			9	80	11.4	3.7	6.2	21.5	11.9
TC32601	3	77							
			2	80	2.5	1.7	0.8	8.8	20.0
TC101821	2	200							
			2	202	1.0	0.7	0.3	3.5	55.9
TC116908	0	204							
			0	204	0.0	0.0	0.0	1.5[1]	61.4
Z	0	204							
			1	204	0.5	0.5	0.1	2.7	58.7
TC89057	1	203							
			6	203	3.0	1.2	1.4	6.4	49.4
TC108778	7	196							
			7	203	3.5	1.3	1.7	7.0	47.9
Xscm254	15	189							
			1	203	0.5	0.5	0.1	2.7	58.4
TC98482	14	189							
			10	203	4.9	1.5	2.0	8.0	43.8
TC77238	24	180							
			4	204	2.0	1.0	0.8	5.0	52.9
TC89869	24	180							
			2	204	1.0	0.7	0.3	3.5	56.5
TC31342	26	178							
			4	203	2.0	1.0	0.8	5.0	52.6
TC35485	30	173							

[1]Since there is no recombination between *TC116908* and *Z* the standard error was calculated by the method of Stevens (1942)

[a]LOD score according to MAPMANAGER

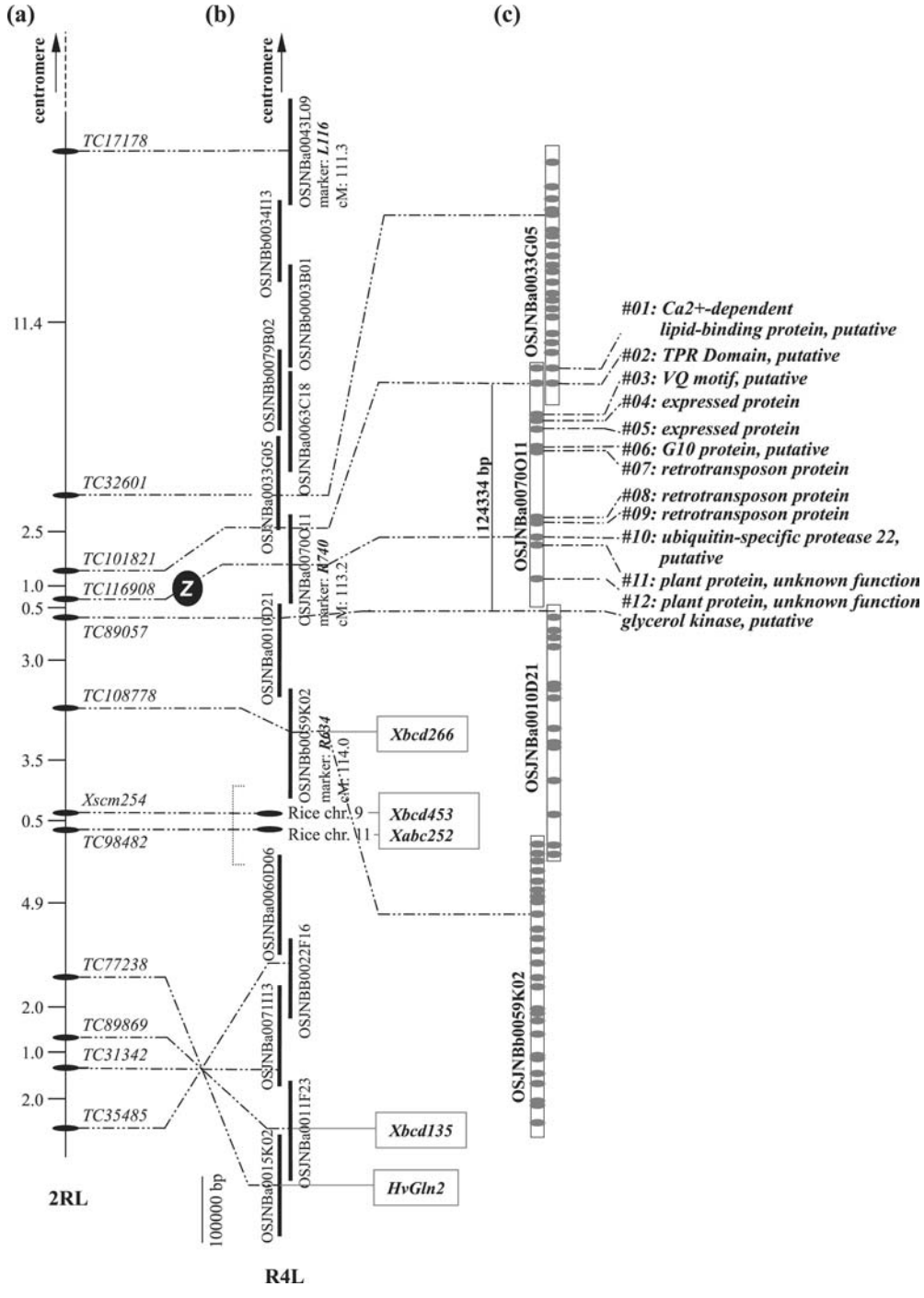

Fig. 2 Linking the genetic map of the rye genomic *Z* region with the physical map of rice. **a** Genetic linkage map of rye chromosome 2RL based on 204 individuals from testcross families BAZ-530 and BAZ-534. *Dotted lines* indicate the genes on rice BACs orthologous to the Triticeae-ESTs, which were used for STS marker development. **b** Order of rice BACs in a segment on rice chromosome 4. *Bold lines* representing BAC clones are proportional to the lengths and positions of the clones, which have been obtained from the TIGR rice genome annotation database (http://www.tigr.org). Localization of barley cDNA anchor markers (*boxed*) on the rice BACs was performed in silico (see Table 1). **c** Close-up of the rice-genomic region corresponding to a 7-cM interval encompassing *Z* in the rye genome. *Grey ellipses* indicate genes predicted on the rice BACs according to GenBank entries AL606454 (OSJNBa0033G05), AL606445 (OSJNBa0070O11), AL606635 (OSJNBa0010D21) and AL606692 (OSJNBb0059K02). The tentative annotation of OSJNBa0070O11.10 is given on the basis of BLASTN and BLASTP sequence similarity searches performed in this study

chromosome 2RL, the region orthologous to this interval contains the *Z* locus, which was mapped 3.5 cM proximal of anchor marker *bcd266*. Thus, there was no indication that our rice-based search for novel *Z* markers would have been compromised by rearrangements within this specific Triticeae-genomic region.

Voylokov et al. (1998) reported that the *Z* locus of rye cosegregates with *bcd266* on chromosome 2RL and is flanked by the markers *cMWG669* and *cMWG720* within a 32-cM interval. In *P. coerulescens*, comparative mapping by Bian (2001) placed *Z* within a 13.1-cM genetic interval between *ksuF2* and *bcd266*, the latter of which was mapped 0.9 cM apart from *Z*. In our study we also observed recombination between *bcd266* and *Z*, in both testcrosses and by direct assessment of the *Z* genotypes via test pollinations among segregating F2 individuals. Another comparison of mapping data is given by the marker *bcd135*. We mapped *Z* in the rye genome 14.5 cM proximal of *bcd135*. This position of *Z*

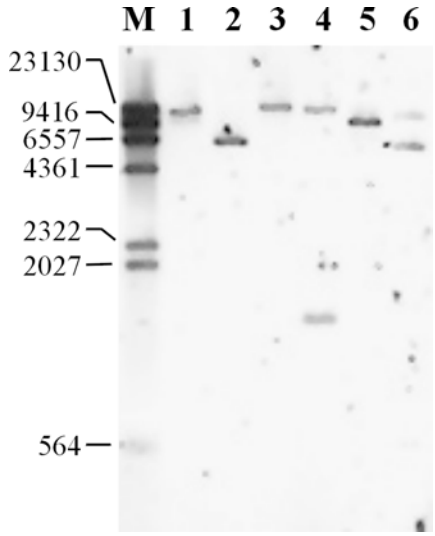

Fig. 3 Southern blot hybridization of OSJNBa0070O11 using a 2.2-kb rye-genomic amplicon of STS marker *TC116908* as a probe. OSJNBa0070O11 DNA was cleaved with *Bam*HI (*lane 1*), *Eco*RI (*2*), *Eco*RV (*3*), *Hin*dIII (*4*), *Pst*I (*5*), *Xba*I (*6*). *Lane M* Molecular weight marker

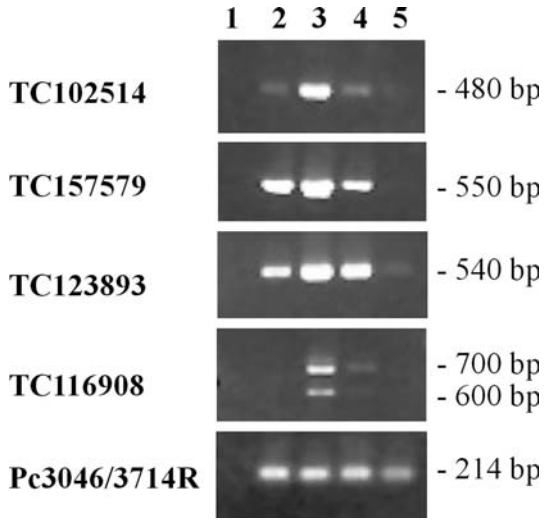

Fig. 5 RT-PCR of rye transcripts with primers derived from Triticeae cDNA orthologs. The primers of *TC102514*, *TC157579*, *TC123893* and *TC116908* were derived from Triticeae ESTs orthologous to genes on rice BAC OSJNBa0070O11. The primers Pc3046/Pc3714R served as a internal control. RT-PCR was performed on rye cDNAs from leaves (*lane 2*), pistils (*3*), anthers (*4*) and pollen (*5*). *Lane 1* Negative control (minus cDNA)

compares well with the position of *Z* reported for another grass species, *Lolium perenne*, in which *Z* was mapped near the proximal end of a 12.2-cM interval between the markers *bcd135* and *bcd1823* (Thorogood et al. 2002).

In our study, *Z* was found to be flanked proximally by *TC101821* and distally by *TC89057* while it cosegregated with *TC116908*. This suggests that rice BAC OSJNBa0070O11 delineates a rice-genomic region with orthology to the *Z* locus in rye. A subset of the gene repertoire of this BAC was analyzed in relation to *Z*. A rye gene represented by *TC102514* was found to be predominantly expressed in the pistils. On rice chromosome R4, the *TC102514* ortholog is located proximal of a *TC101821* ortholog, the latter of which gave rise to a rye marker recombining with *Z* (Fig. 2). Provided that the arrangement of the 12 genes on OSJNBa0070O11 is similar to that in rye, *TC102514* would, thus, not be expected to cosegregate with *Z*. The genetic distance across the marker interval of *TC17178–TC35485* on rye chromosome 2RL was about tenfold larger than the orthologous region on rice chromosome R4 (approx. 30 cM vs. approx. 3 cM, Fig. 2), indicating that DNA contents and/or recombination activities differ largely between these genomic regions of the two species. The question of whether there is collinearity within this region on a finer scale will have to be addressed by means of high-resolution mapping of the entire gene set within the OSJNBa0070O11 orthologous region of the Triticeae genome.

At the present time *TC116908* appears to be the most interesting marker with respect to mapping the SI locus *Z*. This marker cosegregated with *Z* in 204 testcross progeny derived from two testcross families that had been thoroughly eliminated of any obvious illegitimate offspring via test pollination and marker analysis. CAPS analysis revealed a high degree of polymorphism for the amplified *TC116908* sequence among a small sample of randomly chosen rye plants. This result was expected for a gene residing at a multiallelic locus. A multiallelic nature of the SI loci in rye has been demonstrated by Trang et al. (1982) who estimated the presence of 7 and 13 alleles, respectively, at the two SI loci among a population variety.

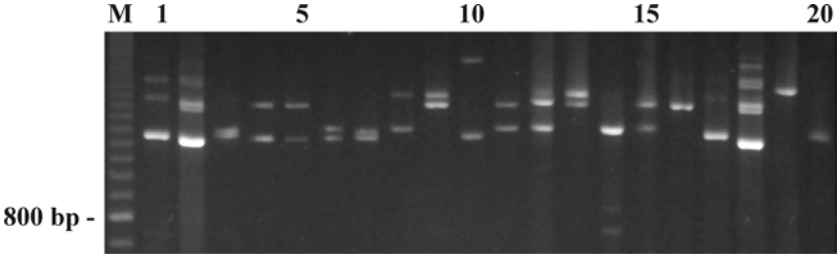

Fig. 4 CAPS polymorphism of the STS marker *TC116908* in rye. *Lanes*: *M* Size standard, *1* BAZ-033/8 (Z_{22}), *2* BAZ-033/15(Z_{11}), *3–11* Lungauer Tauernroggen, *12–16* Carokurz, *17* BAZ-033/10 (Z_{22}), *18* BAZ-033/15(Z_{11}), *19* L301, *20* L2053

TC116908 also displayed a tissue-specific transcription, predominantly in the stigmas and/or ovaries. Several reasons may clarify the presence of two RT-PCR products (Fig. 5). Multiple bands often do not correspond to actual, complete transcripts. These artifact RT-PCR products can be divided into two classes – incomplete or nonspecific (BD SMART RACE cDNA Amplification Kit User manual, Clonetech, Palo Alto, Calif.). The larger of the two rye RT-PCR fragments, which was with 700 bp (Fig. 5), corresponded to the size of the fragment expected on the basis of sequence information on the barley EST *TC116908*.

TC116908 has a highly significant similarity with a ubiquitin-specific protease gene (*UBP22*) isolated from *Arabidopsis thaliana*. In mammalian cells, UBPs are thought to be responsible for the maintenance of the mono-ubiquitin pool by releasing ubiquitin either from free polyubiquitin chains (Hadari et al. 1992) or from polyubiquitin chains still attached to degraded protein remnants (D'Andrea and Pellman 1998). As part of the ubiquitin-proteasome system in mammalian cells, de-ubiquitinating enzymes act by enzymatically shortening or removing the polyubiquitin chain from the targeted proteins, which may lead to both the rescue of such proteins from degradation as well as their targeted destruction (D'Andrea and Pellman 1998; Kim et al. 2003). Gewies and Grimm (2003) showed that the ubiquitin-specific protease UBP41 is directly involved in the apoptotic cell death of human cells.

Thomas and Franklin-Tong (2004) recently demonstrated that the SI response in the gametophytic SI system of *Papaver rhoeas* triggers programmed cell death (PCD) in the pollen, with the PCD response being dependent on a caspase-like activity. In *Brassica*, the sporophytic SI mechanism involves the ubiquitination of proteins via ARC1, an E3 ubiquitin ligase which is directed in its subcellular localization as a result of phosphorylation by the *S*-receptor protein kinase following the SI-specific recognition of the pollen-borne *S* ligand (Stone et al. 2003). In *Antirrhinum*, which together with members of the Solanaceae and Rosaceae represents a third genetic mechanism of SI among dicots involving *S*-RNases, evidence has accumulated that it is in compatible rather than incompatible pollination that ubiquitinated *S*-RNases are recruited for destruction via the 26S proteasome system. This recruiting of non-self *S*-RNases is thought to be mediated by the binding of non-self *S*-RNase to the SCF (Skp1/Cullin or CDC53/F-box) complex; this binding involves the *S*-pollen determinant. As well, an additional, yet unknown, factor has been postulated that would protect self *S*-RNase from ubiquitination in self-pollinations and, consequently, keep it active to inhibit pollen-tube growth (Qiao et al. 2004).

We have shown that the rice genome provides a valuable resource of molecular markers for enriching the *Z*-genomic region on rye chromosome 2RL in a directed approach. Using this approach, we were able to identify the *AtUBP22*-like ortholog of *TC116908* as a marker cosegregating with *Z*. Since the ubiquitination of proteins is emerging as a general mechanism that is active in different SI systems of plants, the question of whether *TC116908* represents a component of the SI system in the grasses appears to deserve further investigation. The fact that *Oryza sativa* is self-compatible does not preclude its potentiality as a carrier of remnant SI genes since in wild rice, the occurrence of SI has been reported (Nayar 1967; Chu et al. 1969).

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