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Mapping of QTL for intermedium spike on barley chromosome 4H using EST-based markers

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The lateral spikelets of two-rowed barley are reduced in size and sterile, but in six-rowed barley all three spikelets are fully fertile. The trait is largely controlled by alleles at the *vrs1* locus on chromosome arm 2HL, as modified by the allele present at the *I* locus on chromosome arm 4HS. Molecular markers were developed to saturate the 4HS region by exploiting expressed sequence-tags, either previously mapped in barley to this region, or present in the syntenic region of rice chromosome 3. Collinearity between rice and barley was strong in the 4.8 cM interval *BJ468164–AV933435* and the 10 cM interval *AV942364–BJ455560*. A major QTL for lateral spikelet fertility (the *I* locus) explained 44% of phenotypic variance, and was located in the interval *CB873567–BJ473916*. The genotyping of near-isogenic lines for *I* placed the locus in a region between *CB873567* and *EBmac635*, and therefore the most likely position of the *I* locus was proximal to *CB873567* in a 5.3 cM interval between *CB873567–BJ473916*.

Key Words: lateral spikelet fertility, row-type, mapping, rice genome, synteny.

Introduction

Barley (*Hordeum vulgare* ssp. *vulgare*) has one central and two lateral spikelets at each rachis node. In the two-rowed barley the lateral spikelets remain small and are sterile, but in six-rowed barley all three of these spikelets are fully fertile. As the spike of the wild-type progenitor (*H. vulgare* ssp. *spontaneum*) is also of the two-rowed type, it has been suggested that this spike type must be more ancient. The six-rowed spike gene (*vrs1*) is genetically recessive which originated from a mutation in a homeobox gene (Komatsuda *et al*. 2007). The selection by pioneering agriculturalists of a six-rowed spike plant, which has the potential to set three times more grains per spike than the two-rowed type, is thought to have established barley as a founder crop for the Near Eastern Neolithic civilization (Zohary and Hopf 2000).

However, full development of the lateral spikelets in six-rowed barley needs the additional action of the *intermedium* gene (*I*). The *I* gene naturally and commonly occurs in six-rowed barley (Gymer 1978) and increases the size of lateral spikelets. Fertility of lateral spikelet is considerably enhanced by *I* in combination with *Vrs1vrs1* heterozygotes (Lundqvist and Lundqvist 1987). The *I* gene was located on the short arm of chromosome 4H (Marquez-Cedillo *et al*. 2000, Komatsuda and Mano 2002, Hori *et al*. 2005). Alleles at the *intermedium spike-c* (*int-c*) also alter the size of lateral spikelets (Lundqvist and Lundqvist 1987) and all mutant lines for *int-c* were artificially induced in two-rowed barley (Lundqvist *et al*. 1997). The *int-c* gene is recessive for intermedium spike, therefore the directions of actions of dominance of the *I* and *int-c* genes were opposite and it is not clear whether the two genes are alleleic, although the location of *I* and *int-c* are both in the short arm of chromosome 4H.

Gene product encoded by *I* is unknown and molecular mechanism of the interaction between *I* and *Vrs1*. Although the *I* locus was located in molecular maps (Marquez-Cedillo *et al*. 2000, Komatsuda and Mano 2002, Hori *et al*. 2005), its status is far from the molecular cloning. In this paper we present a comparative map of the region of barley 4H containing the locus, with an emphasis on its synteny with rice chromosome 3 (Stein *et al*. 2007). This has been combined with a QTL analysis for lateral spikelet fertility.

Materials and Methods

Plant materials

Azumamugi (AZ) is a standard six-rowed cultivar, and Kanto Nakate Gold (KNG) is a two-rowed cultivar. AZ is

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homozygous for *vrs1* and *I* and KNG for *Vrs1* and *i* (Komatsuda and Mano 2002). We use the *I-i* gene designation in this paper because it is not clear whether the *I* and *int-c* genes are alleleic, and parental cultivars carry alleles of natural variation (but not the mutational alleles). A set of 99 F_{12} recombinant inbred lines (RILs) were developed from the AZ × KNG cross by single-seed descent. An *I/i* pair of near-isogenic lines (NILs) was generated from a recurrent back-crossing programme between AZ (donor) and KNG (recurrent parent). In each BCn generation, six to ten randomly selected plants of heterozygous *Vrs1/vrs1* (these plants have developed lateral spikelets and tip-pointed lemma) were pollinated with KNG pollen. At the maturity, plants showing 35% to 50% fertility of lateral spikelets (by self-fertilization) in the spikes on the remaining tillers, the indication of *I*, were selected and its hybrid grains were taken for the next back-crossing. Finally, a single BC_5F_1 plant (AZ/6*KNG 2-3-3-s4) was established and self-pollinated to generate NILs. Fertility of lateral spikelets was documented previously (Komatsuda *et al*. 1999). A set of wheat-barley chromosome addition lines (CALs) (kindly provided by Dr. A.K.M.R. Islam, University of Adelaide, Australia) were used to allow the chromosome location of marker loci. Each CAL represents a wheat plant carrying a single pair of barley chromosomes, and all seven barley chromosomes are represented, except for 1H (Shepherd and Islam 1981).

Resources of molecular marker development

Various consensus genetic maps of barley chromosome 4H (http://wheat.pw.usda.gov/GG2/ index.shtml) were exploited to provide a set of RFLP loci mapping within the telomeric region of 4HS (Table 1). The DNA sequences of oat and wheat clones mapping to this region were BLASTed against the set of barley ESTs present in GenBank (http://www.ncbi.nlm.nih.gov/) to obtain their barley orthologues, and the sequences of these clones were used to design 21nt PCR primers using Oligo5 software (W. Rychlick, National Bioscience, Plymouth, MN, USA) and synthesized commercially (Bex, Tokyo, Japan). Additional barley ESTs were obtained from a set which have been directly mapped to chromosome 4H (Sato *et al*. 2009) (Table 2). An third set of barley ESTs were obtained by selecting those with high homology (E value $<10^{-15}$ or a score value >300) to the genomic sequence of rice chromosome 3 (*japonica* chromosome 3 pseudo-molecule AP008209) (Table 3). High copy number sequences were identified in TIGR (http://tigrblast.tigr.org/euk-blast/index.cgi?project=plant.repeats). In opposite the rice regions most highly homologous to these barley ESTs were searched in TIGR database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) and RAP-DB (http://rapdb.dna.affrc.go.jp/tools/converter/run) to confirm their orthology.

Molecular marker analysis

Plant DNA was extracted as described by Komatsuda *et al*. (1998). PCRs were carried out in a volume of 10 µl, containing 0.25 U Ex*Taq* polymerase (Takara, Tokyo, Japan), 0.3 µM of each primer, 200 µM dNTP, 1.0–4.0 mM (primer pair dependent, see Table 2 and Table 3) $MgCl_2$, 25 mM TAPS pH 9.3, 50 mM KCl, 1 mM 2-mercaptoethanol and 20 ng genomic DNA. The PCR programme consisted of a denaturation step of 94°C/5 min, followed by 30 cycles of 94°C/30 s, 50–72°C (primer pair dependent, see Table 2 and Table 3)/30 s and 72°C/0.5–2 min, and a final incubation step of 72°C/7 min. Reaction products were electrophoresed through either agarose (Agarose ME, Iwai Kagaku, Tokyo, Japan) or MetaPhor agarose (Cambrex Bio Science Rockland Inc., Rockland, MA, USA) gels, depending on amplicon size, and were visualized by ethidium bromide staining. Prior to sequencing, the PCR products were purified using the QIAquick PCR purification kit (Qiagen, Germantown, MD, USA) and subjected to cycle sequencing using a Big Dye kit (Applied Biosystem, Foster, CA, USA). Sequencing reactions were purified by Sephadex G-50 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and analysed with an ABI Prism 3100 Genetic Analyzer (Applied Biosystem). Sequence data were aligned using ClustalW software (http://www.ebi.ac.uk/clustalw/). Restriction site polymorphisms were identified by Mapper software (http://arbl.cvmbs.colostate.edu/molkit/mapper/) applying the 'Restriction Maps' option.

Linkage and QTL analysis

Newly developed chromosome 4H markers were incorporated into the AZ × KNG base map (Mano *et al*. 2001, Mano and Komatsuda 2002, Sameri *et al*. 2009). Linkage analysis was performed using MAPMAKER/EXP v3.0 (Lander *et al*. 1987), and recombination frequencies were converted to genetic distances into cM by the Kosambi (1944) function. The lateral spikelet fertility data described by Komatsuda and Mano (2002) were used to detect QTL. A combination of regression models (forward, backward and forward and backward), walk speed (1–2), window size

Table 1. RFLP markers obtained from the barley consensus genetic map of chromosome 4H

Marker	Primer Upper	Primer Lower	Anneal. (°C)	Ext. (min)	Cycle	$MgCl_2$ (mM)	Amplicon (bp)	Polymorphism
MWG2282	CTTTCGCCATCACCATAGTGG	AAGATTAGAGGCCAGACATTGC	62	1	30	2.5	313	Dominant[a]
MWG634	GTGCTGGGTGGATTAAAAAAGAGGG	GAACTAAAGATAGGCGGGAGTACTG	64	1	30	4.0	832	Monomorph
WG622	TTCACCTTGCCATGACGA	CTGCCTGTTGATTTTCCATG	62	1	30	2.5	161	Dominant[a]

[a] Dominant allele present in AZ.

Table 2. Public EST markers already known to be located on barley chromosome 4H

Rice chrom.	Rice BAC accession	Position (bp)		*E*-value	Barley EST accession[a]	Primer Upper	Primer Lower	Anneal. (°C)	Ext. (min)	Cycle	$MgCl_2$ (mM)	Amplicon (bp)	Alleles	Restriction enzyme
		start	end											
Chr. 3	AP146581	4072902	4098191	e-17	BJ459896	TCCCGACATTTACTTTTGAACC	TGGTGCGGAAAGTCCTATCT	60	1	30	2.5	>1114	Monom.	–
	AP146581	4088716	4092085	e-150	BJ468196	TGCGAGAGCGTAATGAAATG	ACCTCTCATCCTTGCTGTGC	60	1	30	2.5	500	SNP	*Scr*FI
	AP146581	5302268	5304536	e-30	AV929366	AGTTGAACGCTGGCTAGGAA	CCTGAGGTGATTGGAAAGGA	60	1	30	2.5	360	SNP	not found
	AC146702	5375590	5379287	e-69	BJ458824	CGACTGGATAAAATCCCAGG	CTGACAGTTGGTGGCCTGTA	60	1	30	2.5	400	SNP	*Hah*I
	AC105346	6396781	6401040	e-62	AV921260	ATTCAATCGCCTCACCTCTG	ATCCTGCAGATGGAGCTTGT	60	1	30	2.5	700	SNP	*Msp*I
	AC139168	8972054	8974100	e-162	BJ459309	CTTCGAAGAAACAGCGTGTG	GGGACGACAAGCTCAAGAAG	60	1	30	2.5	387	Monom.	–[b]
	AC109602	23096769	23098486	e-32	AV834611	TTTGCTCTATGCCGTGACTG	ATCACCATCCAAAGGTTCCA	60	1	30	2.5	900	Monom.	–
	AC133860	23587610	23593201	e-28	AV833030	ATGCCTGTCCAGTATCCACC	ATCGGTTAGAGCTGGCACAC	60	1	30	2.5	400	SNP	*Mwo*I
	AC092390	24685792	24694367	e-31	AV928044	TAGCAGCCCACCCTAAGCTA	GACTTTCGAGGAGGCAGACA	60	1	30	2.5	800	SNP	*Tsp*4cI
	AC087851	28039871	28043880	e-16	BJ459709	AGGCCGGTTCGTCAACTTAT	TATAACATGTTCGTCGCCCA	60	1	30	2.5	377	SNP	not found
	AC097277	28269037	28273046	e-23	AV933435	TACGAGAGGGCGCTGTTCCGG	CCAGCAAAGGGAAAGCGAGCA	65	2	30	2.5	760	SNP	*Dra*I
	AC091670	28930241	28934680	e-8	BJ461837	CTGATATAACCGGGGTCACG	CGACAACACAGACGCATACC	60	1	30	2.5	393	Monom.	–
	AC087181	29371907	29376346	e-55	AV942364	ACATCATCACCACGCCTACA	TCTGGATGAGCTTCCAGGTC	60	1	30	2.5	1500	SNP	*Taq*I
	AC091532	29785795	29790234	e-32	BJ473916	GCCCTTTGGCATATGTTTCT	TGCACAAAGAATGGAATGGA	60	1	30	2.5	375	SNP	*Sac*II
	AC146936	30046905	30051344	0.010	BJ455560	CGTCTCCTGGTGTTCCAAAT	GCCTCAACTTCCAGGACATC	60	1	30	2.5	>1114	Size polym.[c]	–
	AC135228	30241349	30245788	e-4	BJ486606	GCAGGTCCCTACGTACCAAA	TGAAAGGTCAGCATGAGCAG	60	1	30	2.5	550	SNP	not found
	AC135228	30287946	30290829	e-27	BE438696	AAAACAAACACCACAAAGCAA	GGTATGGAGGAGGGAGAGTTC	62	1	30	1.5	234	Monom.	–
	AC096689	30848599	30853291	e-17	BJ468365	CTCGGACAGTGTGGTGAATG	CGGCCTGGTAGTGATGGTAT	60	1	30	2.5	550	SNP	*Pst*I
Chr. 6	AP003635	28601500	28605318	e-59	BJ479484	TGATGAGCAATTATCGTCGG	AGCCCATAGGTCTTCGGTTT	60	1	30	2.5	>1114	Monom.	–
Chr. 9	AP008215	22553577	22555234	0.003	BJ460446	TCCATGCAACCTCACGAATA	CCTCCTTGTCGTCTCTTTGC	60	1	30	2.5	500	SNP	*Tsp*45I
Chr. 10	AP008216	5006157	5010565	e-6	BJ476948	CCGTCAGTTTCCAACAAACC	AAGCGGAGTTCAAGAAGCTG	60	1	30	2.5	300	Monom.	–
Chr. 11	AP008217	4954529	4958253	e-18	BJ552418	AAATCTGGCGTTGGAATCTG	GCAAGAAGCTAGCACCCATC	60	1	30	2.5	382	Monom.	–
	AP008217	8020358	8026663	e-10	AV912076	TACACACAGCACGTGCAAAA	TACCACCACCAAAACAGCAA	60	1	30	2.5	395	Monom.	–

[a] Previously assigned to chromosome 4H by Sato *et al.* (2009) except for AV933435 (CDO669) and BE438696.

[b] Not tested.

[c] A size polymorphism between parents AZ and KNG.

Table 3. Barley ESTs detecting orthologous loci on rice chromosome 3 used to enrich the genetic interval *WG622–BJ455560* on barley chromosome 4H

Rice BAC accession	Position (bp)		*E*-value	Barley EST accession	Primer Upper	Primer Lower	Anneal. (°C)	Ext. (min)	Cycle	$MgCl_2$ (mM)	Amplicon (bp)	Location by CALs	Alleles	Restriction enzyme	Location by RILs
	start	end													
AC146718	27205680	27205718	e-18	BG415913	GACCTGGACGGCCGTAACATC	GTCACATCTACCGGAAGCCAG	n.d.[a]	n.d.	n.d.	n.d.	m.b.[b]	–[c]	–	–	–
	27205740	27209187	e-45	CB872182	TGGCCATGAAAGTTTACAAAG	CAAGAAACAATAGTGCCAGAG	58	1	30	2.0	900	n.d.	Monom.	–	–
AC116369	27307786	27309205	e-33	AV924028	CTGACGGTCGTATGAGGGAAC	TTGATCTCACGCCCTGTTTAG	56	1	30	1.5	900	n.d.	Monom.	–	–
	27287096	27291103	e-68	BJ468164	AGGAAAATTCTCGTAAAAAAG	AAAACATGCAACGACTTCTTC	50	1	30	1.5	638	4H	SNP	*Alu*I	4H
AC079889	27559846	27563353	e-30	AV942492	ACAGGAACTCCATGGACACTC	AAGGCCATTGTTGAAGAAGAC	52	1	30	1.5	518	n.d.	Monom.	–	–
AC133335	27651512	27655019	e-38	CA000177	GGTCTTGCAACATTCACTGGC	TGAGGCAGATGGTGGCAAGTC	56	1	30	1.5	590	n.d.	SNP	*Hin*dIII	3H
AC084406	27713483	27717492	e-52	AV944879	TGTACAAACCATTCCAATCTG	GGGATTGCTAGTGATGAAAGT	56	1	30	1.5	555	n.d.	Monom.	–	–
	27712487	27719430	e-137	BJ461534	ATATATCCTGCTTACCACCTC	TATCCTACAAAATCGCTGCTC	58	1	30	2.5	>1114	n.d.	SNP	*Msl*I	4H
AC079736	27712487	27719430	e-103	BG344928	GCTGAGATTTCTGCGGCTGAG	GAGAGGGAGTTTGAGTCGAGC	56	1	30	1.5	2020	n.d.	SNP	not found	–
	27871924	27876865	e-28	AL511667	ACAGTTGAGTTGATACAGGTG	GCTCCACAGGTTATATTTATG	54	1	30	4.0	454	n.d.	Monom.	–	–
AC087412	27894595	27899118	e-28	AL511105	CGCCTCCTCGTCTTCTCACAC	CCAGCACGGCCACACTCAAAG	n.d.	n.d.	n.d.	n.d.	m.b.	–	–	–	–
	27894595	27899118	e-44	BF626147	GCAAACTCACCGGCAACCTGG	GTAAGATCACCACGAGCAGGC	64	2	30	1.5	900	4H	Monom.	–	–
AC087851	27999306	28108830	e-32	CK568792	TCATGACGATCCACCCGAACC	GATCTGCGGAACAACACCAAG	58	2	30	1.5	>1114	n.d.	SNP	*Avr*II	4H
	28048282	28054785	e-25	BJ477036	TTCCTATTTTTCACTTGTCAG	GTGATGATATGGAATGTTCTG	54	2	30	2.5	>1114	4H	SNP	*Mwo*I	4H
	28074706	28076602	e-83	BJ468503	CCCCAGCAACAGTAAACATTC	ATTTCTGTGACCTCGGTGATG	54	1	30	1.5	1114	n.d.	SNP	*Bsp*l407I	4H
AC092779	28137093	28141102	e-38	CK565759	AGATGGCAAGAAAACAAACAG	TTTGCGAAGGAAGTGGTGAAG	54	1	30	1.5	502	n.d.	SNP	*Fnu*aHI	6H
	28173930	28179196	e-116	CK568251	CACCTCATTGTTGTTTCCTTC	GCATTCAACCTCATCAGCCAG	54	1	30	1.5	504	n.d.	SNP	*Nla*III	4H
AC097277	28269037	28273046	e-31	BJ477462	ACAATAGTTACACCCATACAT	ATGGCTCCTTGATTTACTTAT	50	2	30	2.5	>1114	n.d.	SNP	not found	–
	28306732	28310506	e-42	AV943484	ACCCATGTTACCAAAATTGCG	TCACATCGGAATCCCATATAC	52	2	30	2.5	2020	n.d.	SNP	*Taq*I	4H
AC105747	28382925	28386934	e-90	CX626750	AGAATCGGCAACGGGTCATAC	AACGACTTGATATTGGCATGG	54	1	30	2.5	378	n.d.	Monom.	–	–
AC120508	28512092	28516101	e-59	AL503129	CATTTTAACTCCTCGCATTGG	GGTGGTGCGTGCCGGAGAGAC	50	1	30	1.0	430	n.d.	SNP	not found	–
AC087181	29371907	29376346	e-41	BJ475972	CTCGACGTAGGATTATTCAAG	CTTCTTCGTGCAGTACATGTG	60	1	30	2.5	900	4H	SNP	not found	–
	29431629	29439296	e-59	BF621639	CTCTTGCTTCTATGGCTGATC	TTCACATGCATTCTTCCACAC	n.d.	n.d.	n.d.	n.d.	m.b.	–	–	–	–
	29431629	29439296	e-83	CK569932	ACATTTCACAACCTCGTCAAG	GTGCACATTTCAAGCTAAGCC	60	0.5	30	1.5	>1114	4H	SNP	*Ssp*I	4H
AC082645	29509407	29513846	e-130	CB882711	TTCATATCTTCGCGCACTTGC	CCACAGACGACGAACGGATTT	60	1	30	2.5	661	n.d.	SNP	*Mae*II	4H
	29530392	29532549	e-25	AV920747	ACTGACGTTTTACAAGCCATG	CCTGCCTTAAAGTTCGGTATG	60	1	30	2.5	900	4H	SNP	*Mbo*II	4H
	29555852	29558017	e-96	BJ466365	GATGAAAAAAAGCCGACTCCG	TCGACTTCGCACGGCAATATC	65	1	30	4.0	715	n.d.	Monom.	–	–
	29574052	29580522	e-30	BJ455322	GTACCGGCAGCACAGCAGAGT	GCACTGGGGTACTTATGGTCG	62	1	30	1.5	>1114	4H	SNP	*Cvi*RI	4H
	29602006	29605277	e-74	BU978294	ATTGCTGCCTATGTGAAACGG	AATATTCATCCGGCCAACAAG	n.d.	n.d.	n.d.	n.d.	m.b.	–	–	–	–
AC090882	29607250	29612967	e-37	CB873567	GGATCATACAGGAGGCCAAAG	AACAATAACACTCCGGCCAAC	60	1	30	1.5	900	4H	SNP	*Mbo*II	4H
	29629494	29633624	e-34	BF255122	CGACACCGCCAAATTCACCAC	GACCTTGCCATGTTGAGTGCG	n.d.	n.d.	n.d.	n.d.	m.b.	–	–	–	–
	29657123	29665742	e-38	BG418523	AACAATTGGAAAACTACCTGG	AGACCCATCACTTTTTTGCAG	55	2	30	2.5	>1114	n.d.	Monom.	–	–
	29719530	29723888	e-41	CB874199	AATGAATTGTACAAAGCAGAC	TTGTCGAAGCAGATATTGAAT	52	1	30	4.0	700	5H	–	–	–
	29776407	29780472	e-62	AV946627	AATAGCAGCAGCACCGGGCAG	TGGGTCATGCCGTCAACGTGC	72	0.5	30	2.5	612	n.d.	Monom.	–	–
AC091532	29825420	29827983	e-85	BQ659801	TCAGCTGGACTCCTCAAATTC	AGTCTGTCAAGCCTTCCCGTC	60	1	30	1.5	676	n.d.	SNP	*Alu*I	4H
	29862461	29865414	e-29	AL501345	TCATGTGAGCTAATAACTACG	AGAGAGAGGTGAAGGTTAAAC	55	2	30	2.5	2500	n.d.	SNP	*Taq*I	4H
	29919785	29923776	e-49	BJ456881	CACAACACAGGGCATTTTTAG	ACGATTCTCCTGCGACATTAC	60	1	30	2.5	629	n.d.	SNP	*Scrf*I	4H
	29924581	29927354	e-71	AL501915	AATGAAATCAAAAACACCAGC	TAGGGAAGGGATCTGTAACCG	n.d.	n.d.	n.d.	n.d.	m.b.	–	–	–	–
AC147426	29928510	29929891	e-17	AL503174	TCACCAGCAAGCCAAAATCAC	CTCTTAAAGCTGGGGATGATC	62	1	30	1.5	603	4H	Monom.	–	–
	29930025	29937834	e-172	CB882815	AGGGTTATGGCTTACAAGTCC	CAGAGAGAATTGTTTGCATCC	60	1	30	2.5	646	n.d.	Monom.	–	–
	29946917	29952189	e-67	BE195973	GGAGGAATCGAACCAACTTAC	TTATCTCTCTCCCCTCCTTCC	51	1	30	1.5	942	6H	–	–	–
	29965463	29978123	e-103	CK567947	AATCCCGCCCCTTAAAAACCG	AATCCCGCCCCTTAAAAACCG	70	0.5	30	2.5	700	4H	Monom.	–	–
	30022358	30034056	e-16	BJ484931	TTTACATCAAGGGTCAGAGGG	GTGAAGCCTCTACGAAAACTC	60	1	30	1.5	>1114	4H	SNP	*Mae*I	4H
AC135228	30241349	30245788	e-24	BE438696	AAAACAAACACCACAAAGCAA	GGTATGGAGGAGGGAGAGTTC	62	1	30	1.5	234	n.d.	Monom.	–	–

[a] not determined.
[b] multiple bands.
[c] not tested.

(5–15 cM), permutation test (1000–2000) and in and out probability (0.01–0.1) were considered, using Windows QTL Cartographer v2.0 (Wang *et al*. 2004). Composite interval mapping (CIM) employed a 5 cM window and a maximum of ten marker cofactors per model, at walk speed 1. Tests were performed at 1 cM intervals, and cofactors were selected by forward-backward stepwise regression (Model 6, $P_{in,out} = 0.05$). Genome-wide, trait-specific threshold values ($\alpha = 0.05$) of the likelihood ratio test statistic for declaring the presence of a QTL was estimated from a 2000 permutation test by random sampling of phenotypic data (Doerge and Churchill 1996). The phenotypic variation explained by a QTL (R^2) conditioned by the CIM cofactors was calculated at the most likely QTL position, along with the additive effect of an allelic substitution at each QTL. The LOD peak of each significant QTL was taken as the QTL location on the linkage map.

Results

Mapping the subtelomeric region of chromosome 4HS

The sequences of three RFLP probes detecting loci in the telomeric region of 4HS were used to convert the assays into a PCR format (Table 1). *MWG2282* and *WG622* produced a dominant assay for AZ, and the two loci were completely linked to one another, mapping 8.7 cM distal of *MWG2033* mapped in the same population previously (Komatsuda and Mano 2002) (Fig. 1). The *MWG634* sequences of AZ and KNG were identical. All 23 barley EST markers previously located to chromosome 4H generated a single PCR product (Table 2). One (*BJ455560*) generated a size polymorphism between AZ and KNG, and ten were converted to CAPS markers. *BJ460446* co-segregated with *WG622* and *MWG2282* at the end of the linkage map, and *AV933435* mapped between *BJ460446* and *MWG2033*. The others were evenly distributed across the chromosome (Fig. 1).

Enrichment of EST markers at the I region

A set of 21 barley ESTs homologous to rice chromosome 3 in the contig defined by BACs AC146718 and AC120508 was assembled (Table 3). Of these, 19 were amplifiable by PCR, and the sequences of 12 of the amplicons were polymorphic between AZ and KNG. Nine of these 12 polymorphisms could be exploited for conversion into a CAPS marker (Table 3), and seven of the CAPS markers were mapped within the interval *BJ460446–AV933435*, in the same order as they are present in rice (Fig. 1). The other two CAPS markers detected loci on chromosomes 3H and 6H (Table 3). The 20 cM interval between *MWG2033* and *BJ455560* was enriched with markers based on ESTs showing homology to the rice segment delineated by AC087181 and AC135228 (Table 3). Of the 23 ESTs tested, 19 produced a single PCR product, and 17 of these amplicons were sequenced (the other two detected loci on chromosome 5H and 6H). Ten of the 17 amplicons were polymorphic in sequence between AZ and KNG, and nine were convertible to a CAPS assay (Table 3). These nine CAPS loci mapped in the interval *MWG2033–BJ455560* (Fig. 1), and their order in barley was identical to that in rice.

QTL mapping of lateral spikelet fertility

CIM analysis using the enriched chromosome 4H map detected a major QTL (peak LOD 9.41) within the interval of 5.3 cM between *CB873567–BJ473916*. The location of this QTL at 23 to 28 cM distal to the centromeric marker *MWG058* (Fig. 1) is in good agreement with the location of *I* as reported in the literature (Franckowiak 1997, Hori *et al*. 2005, Kleinhofs 1997, Marquez-Cedillo *et al*. 2000). The QTL explained 44% of the phenotypic variance (data not shown), with the AZ allele increasing lateral spikelet fertility. The 90% confidence interval of the QTL (Lander and Botstein 1989) represented a wider region of 18 cM between *BJ468503* and *BJ473916* (Fig. 1C, shaded region). Outside chromosome 4H, two minor QTL (each explaining 9% of the phenotypic variance) were located—one close to *MWG882* (peak LOD 6.17) on chromosome 2HL, and the other to *MWG2230* (peak LOD 7.94) on chromosome 5HL (for the map location of these loci, see Mano and Komatsuda 2002). The AZ allele at the chromosome 2HL QTL had a positive effect on the trait, but the one at the chromosome 5HL QTL had a negative effect.

NIL genotypes

The genotype of the NIL carrying the AZ allele at *I* was determined at the set of EST-based loci covering the entire length of chromosome 4H (Fig. 1). The initial BC_5F_1 plant (AZ/6*KNG 2-3-3-s4) was homozygous for KNG alleles in the regions *WG622–CB873567* and *EBmac635–HVM67*, and heterozygous between *BJ473916* and *EBmac775*, therefore AZ-derived segment stretched to a region between *CB873567* and *EBmac635*. Taking the 90% confidence interval of the QTL and the AZ-derived segment, the most likely position of the *I* locus was a 5.3 cM interval between *CB873567–BJ473916* or a 5.8 cM interval between *CB873567–BQ659801/AL501345* to be in more safe side.

Discussion

To date there have been few large-scale comparisons between rice and barley at the DNA sequence level (Brunner *et al*. 2003, Dubcovsky *et al*. 2001, Stein *et al*. 2007). Here we have shown that barley/rice collinearity has been maintained in a 58 cM genetic interval of chromosome 4H (equivalent to a physical interval of 30 Mbp on rice chromosome 3), and this is separated into a 35 cM (3 Mbp in rice) and a 23 cM region (27 Mbp in rice) of inverted collinearity (Fig. 1). The same interruption in collinearity has been documented by Stein *et al*. (2007). Gene level collinearity between rice and barley has been observed in several studies (Kilian *et al*. 1997, Caldwell *et al*. 2004, Rossini *et al*. 2006). The present data have been based on a single mapping population (in contrast to the commonly used integrated maps based on

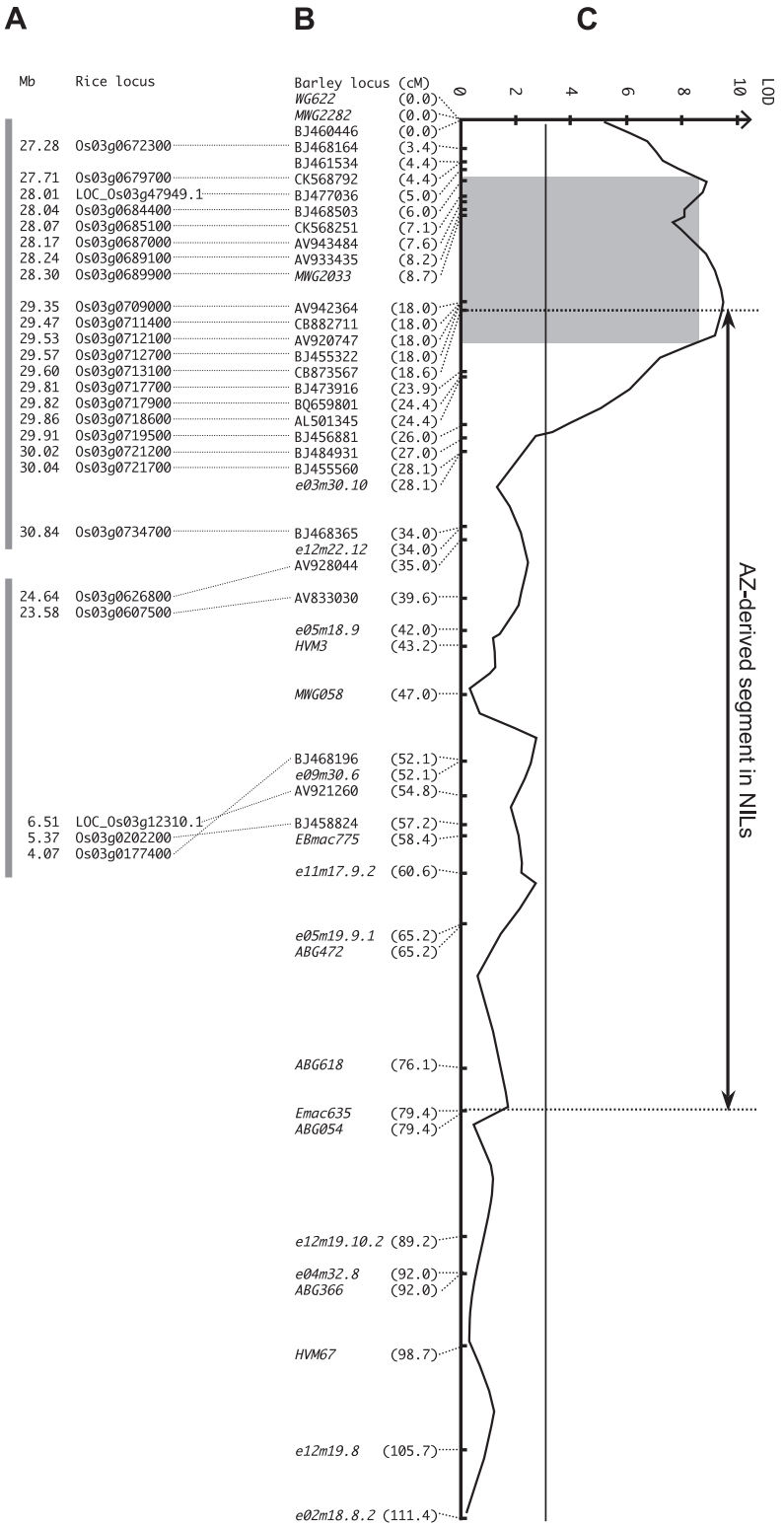

Fig. 1. Mapping of *intermedium spike* (*I*) as a QTL in barley chromosome 4H. The physical map of rice chromosome 3 (A) was alignment with the genetic map of barley (B). The portion of rice chromosome 3 collinear with the barley *BJ468164–AV933435* segment is represented by five BAC clones of rice. The next collinear region (*AV942364–BJ468365*) is represented by seven BAC clones. On the barley genetic map, markers in *italics* are either derived from RFLPs or are SSRs while AFLP markers carry the suffix "*e*" mapped previously (Mano *et al*. 2001, Sameri *et al*. 2009). *MWG058* is a centromeric marker, and EST markers appearing were mapped in this study. Dotted lines connect markers collinear between barley and rice. (C) The interval *AV942364–EBmac775* segregates between the *I* NILs derived from a single BC_5F_1 plant (AZ/6*KNG 2-3-3-s4) (arrow at right). A QTL LOD score plot for lateral spikelet fertility derived from CIM. Threshold LOD value estimated by permutation test was 3.0. The shaded region between *BJ468503* and *BJ473916* represented the 90% confidence interval of the QTL.

several mapping populations, eg. Rostok *et al*. (2005), Stein *et al*. (2007)), which may allow for a less speculative comparison between the two different genomes. In the present study a 10 cM barley region flanked by *AV942364* and *BJ455560* is collinear with only a 0.8 Mbp rice segment defined by AC087181 and AC135228 (Fig. 1). Breakdown of collinearity within telomeric regions was reported as a general trend (Caldwell *et al*. 2004). Location of the *BJ460446*, which is homologous with rice chromosome 9 at the telomeric region of barley chromosome 4H, demonstrated that the border of rice chromosome 3 linkage block corresponding with telomeric region is characterized by an extensive loss of synteny. This may happen by the insertion of one rice linkage block into another by the breakage and fusion (Kilian *et al*. 1999).

Komatsuda and Mano (2002) treated lateral spikelet fertility as a qualitative trait as well as a quantitative trait, where the *I* (gene symbol *int-c* was used in the report) was mapped at 8.2 cM distal to *MWG2033*. In the present study, the *I* locus was mapped proximal to *MWG2033* due probably to a number of molecular markers added to 4H in this study, which allowed accurate QTL mapping. Disagreement of the position may also be due to misclassification caused by QTLs located on chromosomes 2HL and 5HL, however, this would not be the case because the same trait data of the same 50 families presented in Komatsuda and Mano (2002) were used for the analysis in this study. It was a coincident that the location of the *I* locus was around the upper border of the segment inherited from AZ in the NIL, therefore the *I* gene is likely located immediately proximal to the border (Fig. 1). The rice segment syntenous with the barley *CB873567-BJ473916* region includes 18 expressed genes (ESM 1). Considering the wider region toward the centromere, there were several genes which could be related to formation of floral organs. One of which is a zinc finger homeodomain protein (ZF-HD, Os03g0718500). In *Arabidopsis thaliana*, the ZF-HD gene family members represent a group of transcriptional regulators which are expressed predominantly or exclusively in floral tissue, indicating their likely regulatory role during floral development (Tan and Irish 2006). The various members of the family all contain two highly conserved amino acids motifs in their N-terminal region, and these motifs are also present in rice homologues (Windhövel *et al*. 2001). Of course collinear regions are commonly separated by regions where rearrangement disturbs linear order (Mammadov *et al*. 2005, Pourkheirandish *et al*. 2007), and relationship between the annotated genes and the intermedium spike needs further evidences. Candidate gene for *int-c* was detectable by an association approach using 192 cultivars and 4600 SNPs and reportedly confirmed by re-sequencing the *int-c* mutant lines, but details about the gene were not described (Waugh *et al*. 2009). It remains unclear whether the *I* and *int-c* genes were alleleic, and why directions of dominance of the two genes were opposite each other.

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