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ORIGINAL PAPER

# High resolution mapping of Dense spike-ar (*dsp.ar*) to the genetic centromere of barley chromosome 7H

**Fahimeh Shahinnia · Arnis Druka · Jerome Franckowiak · Michele Morgante · Robbie Waugh · Nils Stein**

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**Abstract** Spike density in barley is under the control of several major genes, as documented previously by genetic analysis of a number of morphological mutants. One such class of mutants affects the rachis internode length leading to dense or compact spikes and the underlying genes were designated *dense spike* (*dsp*). We previously delimited two introgressed genomic segments on chromosome 3H (21 SNP loci, 35.5 cM) and 7H (17 SNP loci, 20.34 cM) in BW265, a $BC_7F_3$ nearly isogenic line (NIL) of *cv.* Bowman as potentially containing the dense spike mutant locus *dsp.ar*, by genotyping 1,536 single nucleotide polymorphism (SNP) markers in both BW265 and its recurrent parent. Here, the gene was allocated by high-resolution bi-parental mapping to a 0.37 cM interval between markers SC57808 *(Hv_SPL14)*–CAPSK06413 residing on the short and long arm at the genetic centromere of chromosome 7H, respectively. This region putatively contains more than 800 genes as deduced by comparison with the collinear regions of barley, rice, sorghum and *Brachypodium*, Classical map-based isolation of the gene *dsp.ar* thus will be complicated due to the infavorable relationship of genetic to physical distances at the target locus.

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F. Shahinnia · N. Stein (✉)
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany
e-mail: stein@ipk-gatersleben.de

*Present Address:*
F. Shahinnia
National Institute of Genetic Engineering and Biotechnology (NIGEB), 14965/161 Tehran, Iran

A. Druka · R. Waugh
The James Hutton Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK

J. Franckowiak
Agric-Science Queensland, Department of Employment, Economic Development and Innovation, Hermitage Research Station, 604 Yangan Road, Warwick, QLD 4370, Australia

M. Morgante
Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine, 33100 Udine, Italy

## Introduction

The grass family (*Poaceae* or *Gramineae*), contains many agronomically and economically important crops such as barley, rice, wheat and maize, and is an ideal system for studying the evolution of plant development (Malcomber et al. 2006). Grass inflorescences are developmentally distinct and inflorescence characters are closely associated with crop yield (Bommert et al. 2005). The basic unit of grass inflorescences is the spikelet, which is a short branch with leaf-like organs called glumes enclosing one or more florets (Gao et al. 2010).

In barley, spike morphology is associated with row type, grain density, spike length and grain number and is a target of central importance in crop improvement (Ayoub et al. 2002, Shahinnia et al. 2005). Indeed, breeding for ideal plant architecture (IPA) has been proposed as a means to enhance the yield potential of existing elite varieties (Jiao et al. 2010). However, the molecular mechanisms underpinning IPA and thus increasing the yield potential remain to be elucidated. To date, only a few genes controlling

barley plant architecture have been identified. These include genes controlling the barley spike and spikelet characteristics and include *SIX-ROWED SPIKE 1* (*vrs1*, Komatsuda et al. 2007), *INTERMEDIUM-C* (*int-c,* Ramsay et al. 2011), and *CLEISTOGAMY 1* (*cly1,* Nair et al. 2010). In each of these cases, molecular analyses of induced or natural mutants was key to the functional validation of the candidate genes, highlighting their value for the genetic decomposition of complex processes such as spike and spikelet development (Komatsu et al. 2001).

Several barley mutants with altered spike and internode lengths have been previously described (Forster et al. 2007). Internodes elongation can take place independently in different parts of barley mainly in vegetative structures such as the sub-crown region and the culm as well as reproductive structures such as the spike rachis. Rachis internodes can be extended in mutant classes like *accordion rachis* (*acr*) and *laxatum*/lax spike (*lax*) or shortened as described for *breviaristatum*/short awn (*ari*), *brachytic*/dwarf *(brh), erectoides*/short rachis internodes (*ert*), *Pyramidatum*/pyramide spike shape *(pyr) and Zeocriton*/short culms *(Zeo)* mutants. The class of mutants affecting rachis internode length leading to dense or compact spikes has been designated as dense spike (*dsp)* (Takahashi 1972). The dense spike trait may be an escape mechanism in barley because its characteristic is more common in cultivars grown in relatively humid post-anthesis environments. The mechanism involved may benefit from the smaller volume of moisture (dew) retained by shorter more compact spikes. Rapid drying in such environments could reduce grain discoloration and post-fertilization fungal infection.

Several genes controlling inflorescence morphology have already been characterized in rice. For example, the genes *DENSE AND ERECT PANICLE 1* (*DEP1*) and *3* (*DEP3)* control panicle density (Huang et al. 2009, Qiao et al. 2011). Although rice has a racemose inflorescence structure in contrast to barley, inflorescence organs both in barley and rice share the same evolutionary origin. Thus, based on the extensive conserved synteny of the barley and rice genomes (Mayer et al. 2011), the regulating factors still may be conserved between both species. This is certainly the case in dicots where floral organ identity is under control of highly conserved genes (Malcomber et al. 2006). Since the genomes of barley, wheat, rice, maize, sorghum and *Brachypodium* share a high degree of conserved synteny (Moore et al. 1995; Salse et al. 2009) a reasonable hypothesis is that genetic factors controlling the inflorescence development are also located at conserved syntenic positions. This is supported by the fact that positional cloning in barley has often involved exploiting conserved synteny with model genomes (for example: *RESISTANCE TO YELLOW MOSAIC 4* (*rym4)*, Pellio et al. 2005; *PHOTOPERIOD-H1* (*Ppd-H1)*, Turner et al. 2005; *SIX-ROWED SPIKE 1* (*vrs1)*, Pourkheirandish et al. 2007; *INTERMEDIUM-c* (*int-c)*, Shahinnia et al. 2009; *SEMI-DWARF 3* (*sdw3)*, Vu et al. 2010). In the absence of a reference barley genome sequence, it will continue to be routine to survey the orthologous regions in model genomes to either identify candidate genes or to assist in the development of new genetic markers that can be used to refine the genetic interval containing the target gene (Stein and Graner 2004).

In the present study we report the high-resolution mapping of a mutant locus designated dense spike-ar (*dsp.ar*) that controls spike internode length in barley. Using $F_3$ crosses between BW265 and barley cultivars Bowman, Morex and Barke, respectively, *dsp.ar* was assigned to the genetic centromere of chromosome 7H. Conserved synteny to rice, sorghum and *Brachypodium* was thoroughly exploited for marker development.

## Materials and methods

### Plant material and population development

A nearly isogenic line for *dsp.ar* (BW265) originated from backcrossing mutant 7114 (induced by X-ray in the genetic background of cultivar Volla, accession number GSHO 1726, USDA-ARS Aberdeen) seven times to the cultivar Bowman followed by three rounds of selfing (Davis et al. 1997; Druka et al. 2011). Three $F_2$ populations were derived from crossing of BW265 to cultivars Morex (6-rowed), Barke (2-rowed) and Bowman (2-rowed), respectively. $F_2$ plants were grown under greenhouse conditions and the mutant phenotype was scored as a single Mendelian trait at plant maturity based on a dense arrangement of kernels (short rachis internodes) or an overall dense spike phenotype. The $F_2$ genotype at locus *dsp.ar* was tested by phenotyping 16 $F_3$ progeny for all recombinants which were identified by screening with flanking molecular markers.

### Preparation of genomic DNA

Genomic DNA of barley was extracted using a previously described Cetyl-trimethyl Ammonium Bromide (CTAB)-based method (Stein et al. 2001). $F_2$ plants were grown in 96 Quick-pot plates in a greenhouse and DNA samples were taken at the two-leaf stage. For high-throughput screening for recombinants, a matrix extraction method (MagAttract 96 DNA Plant Kit, Qiagen, Hilden, Germany) was used to obtain PCR-ready DNA by applying manufacturer's instructions.

## Molecular marker development

The initial set of markers was based on those described previously (Druka et al. 2011). EST unigene sequences underlying SNP markers mapping to the introgressed segments were extracted from HarvEST barley (Version 1.68, Assembly # 35, http://harvest.ucr.edu/) and used for new marker assay design. Furthermore, published genetic maps of barley (Stein et al. 2007; Sato et al. 2009) provided additional SSR-, SNP- and STS-marker information for the target region (Table 1, Supplemental table 1). Finally, Next generation sequencing (NGS) survey sequence information of chromosome 7H that has been converted into a linear gene order model (genome zipper) built on synteny between barley, rice, sorghum and *Brachypodium distachyon* (Mayer et al. 2011) was also consulted.

PCR primers for new marker assays were designed using Oligo5 software (W. Rychlick, National Bioscience, Plymouth, MN, USA) and BatchPrimer 3 (http://probes.pw.usda.gov/cgi-bin/batchprimer3/batchprimer3.cgi). PCR was performed for 30 cycles (initial denaturation at 95°C/15 min, followed by 30 cycles of 94°C/30 s, primer pair dependent annealing temperature/30 s, 72°C/0.5–2 min for extension, final extension step of 72 C/7 min) in 10 µl 1× PCR buffer containing 1.5 mM MgCl2, 0.625 U of HotStar Taq DNA polymerase (Qiagen, Hilden, Germany), 250 µM of each dNTP, 10 µM of each primer, and 20–25 ng of genomic DNA. PCR products were resolved by agarose gel electrophoresis (UltraPure Agarose™, Invitrogen GmbH, Germany) and visualized after ethidium bromide staining of gels.

## Marker analysis

In order to determine sequence polymorphisms, purified PCR products (QIAquick PCR purification kit, Qiagen, Hilden, Germany) were subjected to cycle sequencing using Big Dye chemistry (Applied Biosystems, Foster, CA, USA). Sequence polymorphisms were assayed either by cleaved amplified polymorphic sequences (CAPS) analysis or pyrosequencing of PCR products. The developed markers were named after the corresponding HarvEST barley #35 unigene identifier (ID) or after the 454 sequence read code obtained from the barley genome zipper in cases where there was no related HarvEST #35 ID available. Each marker obtained a prefix indicating the assay type (CAPS = CAPS marker or IP = pyrosequencing marker), respectively (Table 2 and Supplemental table 2). Restriction digestion of PCR products for CAPS-analysis was carried out (incubation at the appropriate temperature for a

**Table 1** SSR (GBM) and SNP (GBS and CAPS) markers used for fine mapping of *dsp.ar* in Bowman × BW265 population

| Marker | HarvEST barley #35 unigene ID | Primer forward (5′–3′) | Primer reverse (5′–3′) | Restriction enzyme |
|---|---|---|---|---|
| GBM1516 | 5828 | CCCTCTCCTTTCCCTATCGT | GTGGGGTTGATGTTCCTGTT | Size |
| GBM1359 | 2085 | ATAGCAAACACGCGGAAAAA | CGCCTACGAGCAAGGTTC | Size |
| GBM1464 | 17903 | ATAGCCGTGCTCTTGCTCAT | CAAGACCACCATTTGCATTG | Size |
| GBM1033 | 1243, 14731 | AGCAAATGTTGAGCAACGG | TGCTTGCTCACTCACTGCTT | Size |
| GBS0591 | 15071 | TAAATGTGGAGCAATCGGACC | CAAGAAGGCTATGGTTGAGCTTG | Size |
| GBS0356 | 3468 | AGATCGACTTCTTCGGCGAG | TTCCTTTACACGAACACGCTAAA | *Ava*I |
| GBS0360 | 14599 | CATGCCGAAGAACAAGGGTA | GACTCCCTCGTTGAGGCG | *Hha*I |
| GBS0460 | 17612 | AATACGATAAATTCCGGGCG | GGATGAAGACCTGCTGTTTCC | *Bsm*AI |
| GBS0573 | 14731 | GTCCAAGTCCAGCATCACCC | GCGTAACGAGTACCTTGGCG | *Bsr*I |
| CAPS5174 | 5174 | GTCCGGTGTACAAGGCTGTAA | TTGAGACCTGACTTCCTTCCA | *Sml*I |
| CAPS26182 | 26182 | TGCGTTCTAGAGAGCTTGTCA | CTTCAGTGCATGGACTTCTCC | *Dra*I |
| CAPS5509 | 5509 | CAAATAGCAGGGACACTCAGG | AGCAGCAGCCCAAATCTAAAC | *Cla*I |
| CAPS2722 | 2722 | CAGAGCTTATTCGCACACTTG | TCCAGGAGCTTTGTTATCTTG | *Hinf*I |
| CAPSk04439 | 7916 | AGGACAGAGGCACTTCTCCA | CGTGTGCCATAACATCCTTG | *Alw*NI |
| CAPSk06413 | 1814 | CCCTTCGGTTTATTCCGATT | ATCAGGATCAGAAGACGGCA | *Ava*I |
| CAPS22604 | 22604 | TACACCATCACCGCCTCTATC | GATCGAACCGCTCCTTACTCT | *Sna*BI |
| CAPS7251 | 7251 | TCCTGAGTCCAGTCAAAGGAG | TATGATGCACACAGGCATAGG | *Cac*8I |
| CAPSk09406 | 19528 | AGCCCACACTGTACCCAAAG | GACAGAGGAGATACCAGCGG | *Nci*I |
| CAPS1238_7H | 1238 | CCACTAGTCCAGCATGGTTGT | ATTGCATGCTACGCACAGAA | *Bsp*HI |
| SC57808 (*HvSPL14*)[a] | – | GCTTCAGGAGTTCGCATACCGTG | GCTCGTCCAACTGGTCCCTGTAG | SNP |

PCR condition for GBM and GBS markers is based on Stein et al. 2007

[a] This marker has been mapped by sequencing

**Table 2** Pyrosequencing markers for fine mapping of *dsp.ar* region in Bowman × BW265 population

| Marker | HarvEST barley #32 unigene ID | Primer forward (5′–3′) | Primer reverse (5′–3′) | Sequencing primer (5′–3′) |
|---|---|---|---|---|
| IP7810 | 7810 | TGCAGCTGGTGCTAAGATGG | **AGGAGCAAGACGGCCAAG** | GCTAAGATGGGTTCCG |
| IP2429 | 2429 | **TCTGAGATACCGGACAACGTGA** | GCGGTTGACAAAGGAAACCA | CTTATTAGAGATCCTCATCG |
| IP9820 | 9820 | CTGGTCGCTGCCTTCAGT | **CTGGTCGCTGCCTTCAGT** | CTTGCACTCCCATTTT |
| IP1674 | 1674 | CGAGGTCCTGAAAACTCCTGTATC | **TCGGAGGGCCATTATCAATAGG** | CGCTTGATATGCTTGG |
| IP4589 | 4589 | **CGTGCCGGTGGAATGGTAC** | CGTGCCGGTGGAATGGTAC | CGACGGAAACCATGAC |
| IP3731 | 3731 | ACGGCAGCACAGTAGAGCG | **ACGGCAGCACAGTAGAGCG** | ACCGAAGTACGCCAC |
| IP4850 | 4850 | **CGTTCATGAGGCCGTCGA** | ACACGCGCTACATGAGCTCC | GACGAGGGAAGGCGG |
| IP1286_7H | 1864 | CGTGTCGGTTTATTTTGATGC | **AAAAATGTGCCATTGACAATCA** | GTCAACTCCGAGTCTGT |
| IP1058 | 1058[a] | **GCTTCCGGCAGTTGTGGC** | ACCCCAAGGCGTCCAAGT | GGCGTCCAAGTCCTC |
| IP15313_S2 | 15313[a] | GGTGCAGCTTGGCAATCG | **GAGTCCAAGCTCCAGGTACGC** | CAGCTTGGCAATCGA |
| IPABC05818 | 4202 | CCAACCCGACATACAGTGC | **AGGCGCTCTCGGTAAGGA** | AGTGCACCGCTGACG |
| IP5289 | 5289 | CCCACAACCTCTGTCGTCATCT | **CCAAGGTTGCTGGGTTTATTCG** | CTCTTCGCCAGCACG |
| IP1549 | 1549 | TTCAGCTTTCTCTTTGCTTGTAAC | **ATAAAGCATCGCAAGGTTTTG** | TGCTATTTGTTGTATGGTTC |
| IP2792 | 2792 | **CGATTCGCAAGATACAACGTAGAG** | CGATGCCGATAACCAAACTGA | ACAGTAAGAGGTGGCAG |
| IP8097 | 8097 | **ACGCAACAAGGTATCATCCC** | GCTACCCGGCTCACTGTAGAT | TCTGTTTAGGATATGATTGG |
| IP1578 | 1578 | CAATATTTACAATGGACCGGAGTT | **GGTTTCCTCCATCCATCCTATACA** | TAGGAATTAGTAGAAGCCAT |
| IP2924 | 2924 | **ACGCCATCAAGGTCATGTTCA** | CGGCCAGAATCTGAATGCTT | AGTAGCAGTCTCTGGAGTT |
| IP3232 | 3232 | **CCTGGATCCGGTTCTTCTT** | GCTAGGGGAGAGATGGAGGA | GCCGTGACGGAGCCGC |
| IP16970S1 | 16970[a] | GTCCATGAGTGCGGTGAAT | **CATCTCCTGGAATGGCAATTT** | TGAGTGCGGTGAATC |
| IP1108 | 1108[a] | GGGATGGGACTATTCTTTTCAATT | **GGCCATTAGGTTCTGTATTCTGTT** | GTAAGAATACGATCACTATAAACT |
| IP15403 | 15403[a] | CACATAGAAGGCGTCCATCCTGTA | **GGCAAAGGAAAATCCTAACTTGTG** | CGTCCATCCTGTAGCAA |
| IP10869 | 10869[a] | GGTACGGATAGCTCATTCACATG | **AAGGTGCCAGAAAGTTAGAAATGC** | CATGGTCACTAACTGTTAATAC |
| IP43684 | 43684[a] | CGGTGAAGTGGCAAATCAT | **GCAGGACAACTTGCCAAAAT** | TTCAAATGGCTGAAGAT |
| IP668 | 668[a] | TCCAAAATGCGCCTGTAGC | **GAAAACCCGACACCAGAAGG** | CCTGTAGCATCATCGGT |
| IP16366 | 16366[a] | CTAGCAGTGCTCGGAGGTTC | **GCCGGGTAAGATTGGTATGC** | AGGTTCCTGGGCACT |
| IP948 | 948[a] | CGTCTGTGCTGCATTCAAATTATT | **CAAAGATCCTTTTACAGGGAATCA** | TTACCTGTCAGATTTCTTTT |
| IP15320 | 15320[a] | AGGCATACCTAGCATGAAGTTTA | **AGGAACACCACATGAAGGTTTACA** | AGGTTTACAAGATACTATGTTCTA |
| IP4460 | 4460[a] | **GGATTGATTTAAGCTGCTGTTTTT** | AGGCTTGGATAAATTTGAAGCA | CATTATTCAGGGCCAA |
| IP22253 | 22253[a] | **GCTGGTGGCCAAAAAACTAATA** | GCTGGAGCTTGAAATGGAGAC | ATGGAGACGTTCCCG |
| IP15055 | 15055[a] | TGCCTGTCCTGTTATATGTTCAA | **ATTCCAAGATAGCAAACAGAGATG** | TTTGTTCCCAACTGC |
| IP22967 | 22967[a] | ATGCCGTCATGTTTTCTTTTGTT | **AGCTTTTCTGAGCCTTGATTCCT** | AAATCTCCCTTGGATCTAT |
| IP1243_7H[b] | | CCTCAGGGAGCATCTCTCAAAA | **CGTTTTTCACTTTGTTGGGATGT** | GGTCCAGCATCCAGA |

Bold letters indicate biotinylated primers

[a] Unigene ID refers to HarvEST barley assembly #35

[b] Marker was designed on the basis of 454 sequence read FUYK5KB01COB97_7HL (Mayer et al. 2011)

minimum of 3 h) in a 15 µl volume using 10 µl of PCR product, 2 U of the respective restriction endonuclease (New England Biolabs, USA; Fermentas, St. Leon-Rot, Germany). For pyrosequencing, primers were developed using the Assay Design Software, version 1.0.6 (Biotage AB, Uppsala, Sweden). PCR amplification was done for initial denaturation at 95°C/15 min, followed by 45 cycles of 94°C/30 s, 58°C annealing temperature/30 s and 72°C/0.5 min for extension and final extension step of 72°C/7 min (Supplemental table 2). Pyrosequencing of PCR products was carried out according to manufacturer's instructions with Pyro Gold Reagents (Biotage AB) on a Pyrosequencer PSQTM 96HS96A 1.2 (Biotage AB). Genotype information was analyzed with the manufacturer's software (PSQTM 96HS96A 1.2).

### Sequence analysis

Alignment of nucleotide sequences was performed with the software packages Sequencher TM version 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA) and/or ClustalW (http://www.ebi.ac.uk/clustalw/). Restriction site polymorphisms were identified by software tools like Mapper (http://arbl.cvmbs.colostate.edu/molkit/mapper/) and SNP2CAPS (Thiel et al. 2004).

Sequence homology search was performed using BLAST tools (Altschul et al. 1990) from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) under default parameter settings. Repetitive elements were identified through BLAST against the Triticeae repetitive elements database (TREP, http://wheat.pw.usda.gov/ITMI/Repeats). Corresponding orthologous sequences of rice and Brachypodium were identified using the rice annotation project database (RAP-DB; http://rapdb.dna.affrc.go.jp/tools/converter/run) and *Brachypodium distachyon* project (http://mips.helmholtz-muenchen.de/plant/brachypodium/searchjsp/blast.jsp) respectively.

### Genetic mapping

The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequencies into centiMorgans (cM). Linkage mapping was performed using JoinMap® v4.0 (Kyazma B.V., Wageningen, The Netherlands).

## Results

### Phenotypic analysis of the backcross derived line BW265

The genotype BW265 is a nearly isogenic line (NIL) derived by backcrossing seven times the original mutant 7114 (in 'Volla') to genotype 'Bowman' followed by several rounds of selfing (Davis et al. 1997; Druka et al. 2011). The NIL exhibited a typical dense arrangement of kernels and short rachis internodes at the heading stage (an average of 2.4 mm for BW265 vs. 3.7 mm for Bowman, Fig. 1). At maturity, the kernels were slightly shorter (8.5 vs. 9.8 mm) and wider (4.0 vs. 3.8 mm) with lower kernel weights (5.1 vs. 5.8 mg) and plants were about 10% shorter. A 3:1 segregation ratio was observed for the dense spike phenotype in BW265 crosses indicating that a single gene is responsible for the phenotype.

### Chromosomal assignment of the dense spike phenotype of mutant NIL BW265

The dense spike mutant locus of BW265 was previously assigned the identifier *dsp.ar* and was allocated to chromosome 3H at low genetic resolution by correlation to co-selected phenotypic characters (J. Franckowiak, unpublished data). Genotyping of BW265 and its recurrent parent 'Bowman' with 1,536 previously described single nucleotide polymorphism (SNP) markers (BOPA1, Close et al. 2009) identified contiguous intervals of polymorphic markers extending over 35.5 cM and 20.3 cM on chromosome 3H (21 SNP loci) and chromosome 7H (17 SNP loci), respectively. This was consistent with the original


**Fig. 1** Altered spike length in the mutant stock BW265. **a** Phenotype of a normal two-rowed barley spike (cultivar Bowman) in comparison to its *dense spike.ar* NIL, BW265. BW265 shows shorter inflorescence internode length (**b**) and has smaller sized grains (**c**, *right panel*)

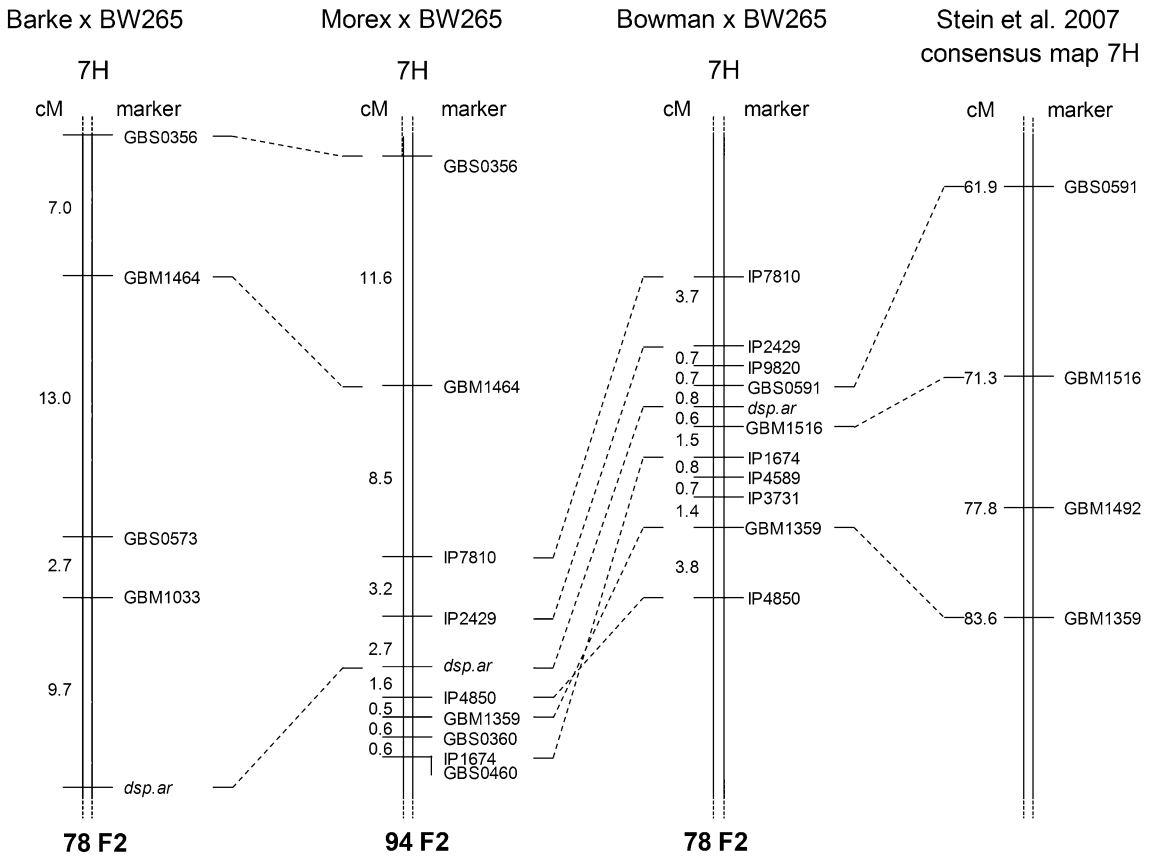


**Fig. 2** Low-resolution mapping of *dsp.ar*. *dsp.ar* was mapped on chromosome 7H using three independent small F2 populations (Bowman × BW265, Morex × BW265 and Barke × BW265). The relative position of the *dsp.ar* locus in context of the entire 7H chromosome is illustrated by a comparison to a segment of a 7H consensus map published previously (Stein et al. 2007)

assignment of *dsp.ar* to chromosome 3H. However, another dense spike mutant locus, *dsp1*, was previously allocated to chromosome 7H of barley (Franckowiak and Konishi, 1997). Thus genetic mapping was required for definitive chromosomal allocation of the *dsp.ar* mutant gene in BW265.

## Low-resolution genetic mapping

For low-resolution bi-parental genetic mapping, three different F2 mapping populations (Bowman × BW265, Morex × BW265 and Barke × BW265) were established. Initial segregation analysis with markers from barley chromosome 3H did not reveal any linkage between the dense spike phenotype of BW265 and the introgressed segment on 3H (data not shown). Instead, the phenotype was reliably linked to chromosome 7H in all three mapping populations (Fig. 2). Between four and nine markers (Tables 1, 2) from previously published maps of chromosome 7H (Stein et al. 2007; Close et al. 2009) were mapped in one or more of the three populations (Fig. 2). The three populations differed in their suitability for mapping *dsp.ar*. Phenotyping was complicated in the Morex × BW265 population most likely due to the segregation of a natural allele of a second dense spike gene (*Zeo2*) from Morex located on chromosome 2HL (J Franckowiak, unpublished data). The highest recombination frequency was observed distal to *dsp.ar* (Fig. 2) in the combination Barke × BW265, which was, however, accompanied by very low level of polymorphism between both parental genotypes making marker development inefficient. However, the dense spike phenotype was very clear in the Bowman × BW265 combination (Fig. 1). Due to the preceding 1,536 SNP survey between Bowman and BW265 (Druka et al. 2011) marker saturation of the target interval was initially greatly facilitated. We focused on saturation mapping of *dsp.ar* in the Bowman × BW265 population and were rapidly able to narrow down the original 20.3 cM introgression of chromosome 7H containing *dsp.ar* to a 1.4 cM interval between markers GBS0591 and GBM1516 (Fig. 2). This position was largely coincident with a previously reported locus called *dsp1* (Franckowiak and Konishi 1997; Taketa et al. 2011). Five molecular markers could be jointly mapped in Morex × BW265 and Bowman × BW265. The three markers IP4859, IP1359 and IP1674 mapped proximal to *dsp.ar* in the two populations

but exhibited an inverted order and the genetic distance between these markers was condensed by a factor of 4 in Morex × BW265.

## High-resolution mapping of the locus *dsp.ar*

1,993 $F_2$ plants (3,986 meiotic events) of the Bowman × BW265 population were screened by four co-dominant flanking markers (IP2429, GBS0591, GBM1516, IP1674) leading to the identification of 392 recombinant plants between IP2429 and IP1674. The order of the four loci remained consistent with that previously determined at low genetic resolution. *dsp.ar* resided in a 2.2 cM interval between markers GBS0591 and GBM1516. All recombinants (82) were re-evaluated for their spike phenotype in their $F_{2:3}$ progenies and were subsequently used for marker saturation in the *dsp.ar* target interval between markers GBS0591 and GBM1516.

Thirty-six EST-based markers from a previously published transcript map (Sato et al. 2009) were selected initially (Supplemental table 1). Sequencing of the respective amplicons revealed in three of them (k06413, k04439, k09406) at least one single nucleotide polymorphism (SNP) between BW265 and Bowman (Table 1) which could be converted into CAPS markers (Fig. 3).

For further marker saturation we relied on collinearity information from the sequenced grass genomes of rice, *Brachypodium* and sorghum. The region around the genetic centromere of barley chromosome 7H exhibits highly conserved synteny with part of rice chromosomes 6 and 8, sorghum chromosomes 7 and 10 and *Brachypodium* chromosomes 1 and 3 (Gale and Devos 1998; Stein et al. 2007; Thiel et al. 2009; Mayer et al. 2011). This information was previously utilized along with NGS survey sequence from flow-sorted chromosome 7H, to develop a virtual linear gene order map ("four way genome zipper") comprising 3,204 genes of barley chromosome 7H (Mayer et al. 2011). Since markers GBS0591 and GBM1516 were not yet integrated into that resource the underlying barley ESTs were used to identify the orthologous genes of *Brachypodium distachyon* by sequence comparison at PlantsDB (http://mips.helmholtz-muenchen.de/plant/brachypodium/searchjsp/blast.jsp). GBS0591 identified gene Bradi1g44460.1 anchored at position '1034' of the 7H genome zipper model, while no homologous *Brachypodium* or rice gene was identified for GBM1516. In a consensus transcript map of barley chromosome 7H (Stein et al. 2007) GBM1516 was located in the vicinity of marker GBM1492. A BLASTn search of GBM1492 against the rice genome sequence revealed homology to gene Os06g0338900 anchored at position '2092' in the virtual gene order model of barley chromosome 7H. Three-hundred and seventy nine of the 1,058 genes putatively located between the markers GBS0591 (Bradi1g44460.1) and

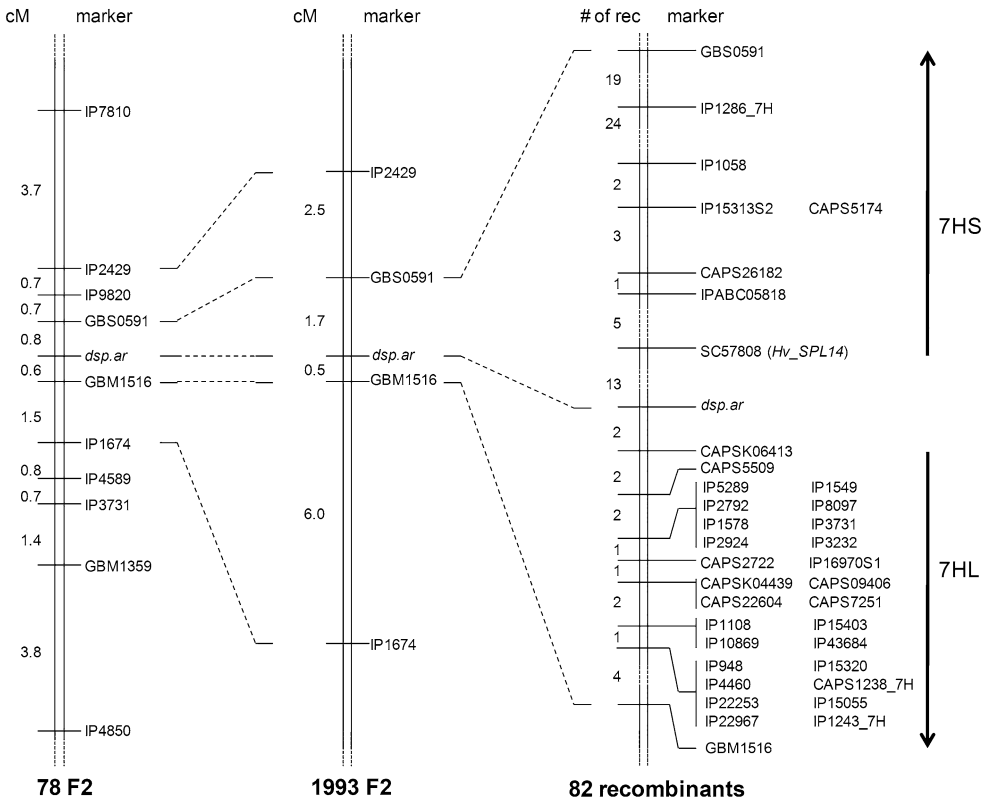


**Fig. 3** High-resolution mapping of *dsp.ar*. *dsp.ar* was fine mapped in a population of 1993 F2's from a Bowman × BW265 cross. The target interval was narrowed down from 20.34 to 0.37 cM (15 recombinants) between markers SC57808 (*HvSPL14*) and CAPSK06413. *Arrows* indicate which markers of the right panel map reside on the short and the long arm of chromosome 7H, respectively

**Table 3** Conserved synteny of barley genes mapped at the *dsp.ar* locus and model grass genomes

| Marker[a] | Putatively orthologous gene[b] | | |
|---|---|---|---|
| | *Brachypodium* | Rice | Sorghum |
| IP7810 | Bradi1g45710.1 | Os06g0218600 | – |
| IP2429 | Bradi1g43770.1 | Os06g0270900 | Sb10g009790.1 |
| IP9820 | Bradi1g44530.1 | – | Sb10g008780.1 |
| GBS0591 | Bradi1g44460.1 | Os06g0247500 | Sb10g008850.1 |
| IP1286_7H | Bradi3g37680.1 | Os08g0460000 | Sb09g004970.1 |
| IP1058 | Bradi3g39850.1 | Os08g0504700 | Sb07g027940.1 |
| IP15313_S2 | – | – | – |
| CAPS5174 | Bradi1g43580.1 | Os06g0283300 | Sb10g010440.1 |
| CAPS26182 | Bradi1g42760.1 | – | Sb07g028690.1 |
| IPABC05818 | Bradi1g42707.1 | Os06g0318600 | Sb10g012160.1 |
| SC57808 (*HvSPL14*)[d] | Bradi3g40030.1 | Os08g0509600 | – |
| *dsp.ar* | | | |
| CAPSk06413 | Bradi1g36840.1 | Os06g0598900 | Sb10g023300.1 |
| CAPS5509 | Bradi3g18190.1 | Os08g0556000 | Sb07g024500.1 |
| IP1549 | Bradi3g22330.1 | Os08g0345800 | Sb07g012320.2 |
| IP2792 | Bradi3g16187.1 | – | Sb07g004240.1 |
| IP8097 | Bradi1g45620.1, *Bradi3g15380.1*[c] | Os08g0180300 | Sb07g005035.1 |
| IP5289 | Bradi3g12830.2 | Os12g0641500 | Sb07g023430.1 |
| IP1578 | Bradi3g10620.1, *Bradi3g38960.1*[c] | Os02g0290500, *Os06 g0340600*[c] | Sb10g019340.1 |
| IP2924 | Bradi3g42030.1 | Os08g0545200 | Sb07g025220.1 |
| IP3232 | Bradi1g36700.1 | Os06g0602600 | Sb10g023460.1 |
| IP16970S1 | Bradi1g37250.1 | Os06g0588900 | |
| CAPS2722 | Bradi3g14000.1 | Os08g0120500 | Sb07g002090.1 |
| CAPSk04439 | – | – | – |
| CAPSk09406 | Bradi3g12960.1 | – | – |
| CAPS22604 | Bradi1g37977.1 | Os06g0556200 | – |
| CAPS7251 | Bradi1g41947.1 | – | – |
| IP43684 | Bradi3g41920.1 | Os08g0543600 | Sb07g025350.1 |
| IP10869 | Bradi3g41830.1 | Os08g0541500 | Sb07g025480.1 |
| IP15403 | Bradi3g41600.5 | Os08g0538600 | Sb07g025770.1 |
| IP1108 | Bradi3g41480.3 | Os08g0536000 | – |
| IP15320 | Bradi3g18820.1 | Os12g0152700 | Sb01g022260.1 |
| IP22253 | Bradi3g17040.1 | Os08g0152800 | Sb07g003500.1 |
| CAPS1238_7H | Bradi3g16240.1 | Os08g0162200 | – |
| IP4460 | Bradi3g14090.1 | Os08g0122000 | Sb07002150.1 |
| IP15055 | Bradi3g13800.1 | Os08g0117300 | Sb07g001620.1 |
| IP22967 | Bradi3g13330.1 | Os08g0109100 | Sb07g000950.1 |
| IP1243_7H | – | Os08g0161800 | – |
| IP948 | Bradi1g36830.1 | Os06g0600100 | |
| GBM1516 | – | – | – |
| IP1674 | Bradi1g37582.1 | Os06g0568200 | Sb10g022500.1 |
| IP4589 | Bradi1g37630.1 | Os06g0567000 | Sb10g022410.1 |
| IP3731 | Bradi1g35780.1 | Os06g0354700 | |
| GBM1359 | Bradi1g37360.1 | Os06g0574500 | Sb10g022710.1 |
| IP4850 | Bradi3g43020.1 | Os08g0560500 | Sb07g024010.1 |

The gene with second best sequence identity fitted the conserved collinear linkage block

[a] Order according to Fig. 3

[b] According to Mayer et al. (2011) and individual sequence comparisons (BLASTN) for genes not represented in the linear gene order model of chromosome 7H

[c] Two homologs with almost similar sequence identity were present in regions of segmental genome duplication in Brachypodium and rice

[d] This marker has been mapped by sequencing

GBM1492 (Os06g0338900) in the 7H genome zipper (Mayer et al. 2011) were selected for marker development to yield amplicons in the range of 500 bp. For 241 the designed primer combinations produced robust and specific amplification suitable for amplicon sequencing (Supplemental table 2). After their initial failure the remaining 138 primer sets were not considered for further PCR optimization. Twenty-five of the 241 amplified gene fragments carried at least a single SNP if compared between genotypes BW265 and Bowman (Supplemental table 2). Seven were exploited for conversion into CAPS markers and the remaining eighteen were converted to PCR-based markers suitable for pyrosequencing (Tables 1, 2). In total, the 2.2 cM interval between markers GBS0591 and GBM1516 was enriched with 34 newly developed markers based on ESTs showing homology either to rice, sorghum or *Brachypodium*. After analyzing their segregation in the Bowman × BW265 recombinants, the gene *dsp.ar* could be allocated to a 0.5 cM interval between markers IPABC05818 and CAPSK06413 (Fig. 3). Based on the sequence evidence available for the corresponding genes in the genome zipper model of chromosome 7H (Table 3, Mayer et al. 2011) these *dsp.ar* flanking markers still resided on the short and the long arm of barley chromosome 7H, respectively. Overall conserved synteny between barley and the sequenced model grass genomes provided an excellent source of information for marker development/saturation at the *dsp.ar* locus. However, for identifying most closely linked markers collinearity was not a good predictor due to substantial reshuffling of segments of the conserved syntenic linkage blocks between barley and the other grass genomes (Table 3).

## Analysis of rice genes *DEP1*, *DEP3* or *IPA1* as candidates for *dsp.ar* of barley

Rice was originally introduced as a model for facilitating map-based cloning in the large genome cereal crop species because of conserved synteny of functional genes (Bennetzen and Freeling 1993; Moore et al. 1995). Since several genes related to rice inflorescence development and panicle architecture have recently been isolated, we tested whether any of these was a candidate for *dsp.ar*.

Down regulation by RNA interference (RNAi) of the homolog of *DENSE AND ERECT PANICLE 1* (*DEP1*, Huang et al. 2009) in *Triticum monococcum* induced an extended ear phenotype which implied a possible role of the orthologous gene on ear length also in barley. *DEP1*, however, is located on rice chromosome 9 in a region collinear with barley chromosome 5H. Thus the barley homolog of *DEP1* was not considered a candidate gene. A paralogous sequence of this gene, however, is present on rice and *Brachypodium* chromosomes 7 and 3 (adjacent to Bradi3g37600), respectively, in a region that shows conserved synteny with barley 7H. However, we could not find any orthologous sequence from barley in public EST data and we failed to amplify such a gene from barley with degenerate primers (data not shown). Neither could we find a sequence trace in the NGS chromosome shotgun sequence data obtained from 7H (Mayer et al. 2011). A barley ortholog (marker IP1286_7H, Table 2 and Supplemental table 2) of a neighboring Brachypodium CDS (Bradi3g37670.1) could be mapped 48 recombination events distal to *dsp.ar*. Based on this evidence we also excluded a putative paralog of *DEP1* on 7H as a potential candidate gene.

*DENSE AND ERECT PANICLE 3* (*DEP3*) regulates internode length in the panicle of rice and has recently been cloned (Qiao et al. 2011). Based on its genomic position on rice chromosome 6 its ortholog in barley can be expected to be located on chromosome 4H. It was therefore also rejected as a strong candidate at this stage. *IDEAL PLANT ARCHITECTURE 1* (*IPA1*, Jiao et al. 2010) is a semi-dominant quantitative trait locus (QTL) that changes rice plant architecture (semi-dwarf stature, reduced panicle internode length, more branches in the inflorescence) and enhances rice grain yield (Jiao et al. 2010). It is located on rice chromosome 8 (RAP ID Os08g0509600), orthologous to *Brachypodium* Bradi3g40030. Based on a barley full-length cDNA sequence NIAS2012N23 (GenBank: AK363115.1) representing a putative barley ortholog of the rice gene *IPA1* (*OsSPL14*) and a corresponding genomic sequence (contig_57808) obtained from eightfold whole genome shotgun sequence data of BW265 (Nils Stein, Burkhard Steuernagel, Stefan Taudien, Matthias Platzer: unpublished data available from corresponding author on request), specific PCR primers (SC57808, Table 1) could be designed to genotype a SNP (C/T change between Bowman and BW265) at bp position 652 after the stop codon of coding sequence (CDS) of the flcDNA. Sequencing of amplicons derived from the panel of informative recombinants mapped this gene to the vicinity but thirteen recombination events distal to *dsp.ar*. The genotyped SNP was not part of the coding sequence of the barley homolog of *IPA1*. We cannot rule out completely but consider it unlikely that *HvSPL14* represents a strong candidate for *dsp.ar* due to the high number of 13 recombination events found between the marker and the gene. Thus we rejected the gene as a candidate for the present study. Mapping of marker SC57808, however, reduced the size of the genetic interval containing *dsp.ar* to 0.37 cM. Given the corresponding positions of the orthologous *Brachypodium* genes of the two closest flanking markers SC57808 *and* CAPSK06413 (Table 3) in the 7H genome zipper (Mayer et al. 2011) the remaining interval at locus *dsp.ar* still may contain more than 800 genes.

## Discussion

Since the domestication of cereal crop species inflorescence morphology and architecture has been a trait under strong selection because variability of the inflorescence is frequently translated into higher yields of larger and plumper "good quality" grain. Here we report on the high resolution mapping of a mutation of a *DENSE SPIKE* gene (*dsp.ar*) which is involved in the determination of ear length in barley. The gene was allocated to the genetic centromere of barley chromosome 7H in almost 4,000 meiotic events. Due to conservation of synteny with rice, *Brachypodium* and sorghum, marker saturation was greatly facilitated. However, while the gene was assigned to a genetic window of less than 0.4 cM, the closest flanking markers still reside on opposing arms of chromosome 7H, illustrating the severe constraints on attempting classical map-based cloning of *dsp.ar*.

Genetic mapping and map-based gene isolation in Triticeae species has taken advantage of conserved synteny among the grasses. Since the introduction of the concept of using the small genome species rice as a model for trait isolation in large genome crop species like maize, wheat and barley (Bennetzen and Freeling 1993; Moore et al. 1995) many studies have used rice as a genomic model. In most cases it was used to establish closely linked gene-based markers (for example: Perovic et al. 2004; Gottwald et al. 2004; Pellio et al. 2005; Turner et al. 2005; Pourkheirandish et al. 2007; Shahinnia et al. 2009). In the case of the barley genes *ELONGATION INITIATION FACTOR 4E* (*HveIF4E)* and *Ppd-H1* as well as *VERNALISATION 1* (*Vrn1*) of wheat this strategy even identified a candidate gene at the conserved position in rice (Stein et al. 2005; Turner et al. 2005; Yan et al. 2003). However, candidate genes are frequently missing at collinear positions as shown for *VRS1* (Komatsuda et al. 2007), and *VRN-H2* (Yan et al. 2004) in barley and *PAIRING HOMEOLOGOUS 1* (*Ph1* (Griffiths et al. 2006) in wheat, The use of genomic information from sorghum and *Brachypodium* largely complements rice helping bridge gaps in physical maps (*Vrn1*, Yan et al. 2003) and identifying candidate genes (*Ph1*, Griffiths et al. 2006). In the present study, we took advantage of a recently established "virtual gene order" resource ("barley genome zippers") constructed by integrating information from the sequenced rice, sorghum and *Brachypodium* genomes, a dense gene-based marker map of barley and sequence-evidence of genes from NGS shotgun sequences of individually purified barley chromosomes/chromosome arms (Mayer et al. 2011). All genes selected for marker development that were predicted to be contained in the *dsp.ar* target region and for which a sequence polymorphism could be identified between the parental genotypes of the mapping population were mapped genetically to the correct region. This allowed us to reduce the genetic target interval in a relatively small number of steps from 20.3 to 0.37 cM.

The virtual gene order model of barley chromosome 7H (Mayer et al. 2011) suggests high conservation of synteny between barley and the three sequenced model grass genomes at the *dsp.ar* region. Mapping markers derived from the virtual gene order around *dsp.ar* revealed numerous cases of different gene orders than expected from the "genome zipper" model. This can largely be attributed to the *dsp.ar* containing region being colocalized with the genetic centromere of 7H. The virtual gene order model predicts as many as 478 genes anchored to a single genetic position at the genetic centromere (cM 78.22, Mayer et al. 2011). These genes are present in two different linkage groups in *Brachypodium*, rice and sorghum. Therefore, it is not surprising that changes in marker order were observed, since the genetic resolution used for developing the virtual gene order model was far below that used here for *dsp.ar*. Despite the limitations of predicting gene order in centromeric regions, the integrated information provided by the genome zipper (Mayer et al. 2011) substantially aided synteny-based marker development for fine mapping.

Recently, *dense spike 1* (*dsp1*) has been roughly located to the centromeric region of 7H (Taketa et al. 2011) confirming previous results (Franckowiak and Konishi 1997). *dsp1* is a naturally occurring allele while *dsp.ar* is an induced-mutant allele in the cultivar Volla. Since we have now mapped both genes to the genetic centromere of 7H it is possible that both represent alleles of the same gene. However, to our knowledge appropriate allelism tests have not been performed yet. In the study of Taketa et al. (2011) the gene co-segregates with three genomic sequence-derived microsatellite markers which could not be related at highly significant sequence identity to any putatively orthologous gene of rice, sorghum or *Brachypodium*. The closest distal flanking marker (MWG511, http://wheat.pw.usda.gov) exhibits sequence identity to Bradi1g43310.1 and Os06g0292400 of *Brachypodium* and rice, respectively. This is consistent with the order of the markers IP2429 (Bradi1g43770.1), CAPS5174 (Bradi1g43580.1), CAPS26182 (Bradi1g42760.1) and IPABC05818 (Bradi1g42707.1) in the *dsp.ar* high-resolution genetic map as well as with the position of these genes in the 7H "genome zipper". This may support further the hypothesis that *dsp1* and *dsp.ar* could represent allelic mutations. The genetic distance between MWG511 and *dsp1* (5.8 cM) is however ten times bigger than the distance between CAPS5174 and *dsp.ar* (0.55 cM). A simple allelism test will resolve this issue and F1 crosses between *dsp1* and *dsp.ar* genotypes have been initiated.

Little is known about the regulation of spike development in barley. Genes have been isolated that control row-

type—a specific character of the inflorescence in the genus *Hordeum* (*Vrs1*, Komatsuda et al. 2007, *Int-c*, Ramsay et al. 2011). The genes underlying regulation of internode length in barley are not yet known although a plentiful of barley mutants like *dense spike*, *laxatum*, *erectoides*, and *brachytic* with altered spike length have been described (reviewed in Forster et al. 2007). Genes underlying development of inflorescence architecture and morphology have been isolated and characterized in rice. *DEP1* (*DEP1*, Huang et al. 2009) *DEP3* (Qiao et al. 2011) and *IPA1* (Jiao et al. 2010) exhibit reduced internode length making them candidate genes for regulators also of internode length in the barley inflorescence. Genetic mapping of the barley orthologs or neighboring genes however, rule these out as orthologs of *dsp.ar*. However, they still may represent candidates for any of the above mentioned alternate loci in barley controlling spike internode length.

High resolution genetic mapping allocated *dsp.ar* to a 0.37 cM interval at the 7H genetic centromere. Fifteen recombination events around the locus are still remaining for fine mapping of additional markers. For this task, a wealth of gene information from the 7H genome zipper is still unexploited, so map-based cloning of *dsp.ar* remains a realistic possibility. However, based on its location, and the unfavorable relationship between genetic and physical distances in these regions (Künzel et al. 2000), there remains the risk that positional cloning of *dsp.ar* will end in failure. Genetically resolvable distances in this region are likely to translate into enormous physical distances. This general skepticism was shared by Taketa et al. (2011) after mapping *dsp1* and is supported by the fact that the current *dsp.ar* flanking markers reside on opposing chromosome arms.

Does this mean that genes in the genetic centromeres of large genome *Triticeae* crops are not clonable? Work in *Arabidopsis* has shown that it is feasible to positionally clone mutant genes by shotgun sequencing of phenotypic pools. The approach, named SHOREmap (Schneeberger et al. 2009), identifies the target region/gene based on SNP frequencies at flanking loci from completely heterozygous towards homozygous mutant at the locus of interest. The method is essentially a higher resolution version of bulked segregant analysis and relies on having a reference genome sequence. However, it also relies on recombination. Target genes residing in regions with limited effective recombination like the genetic centromeres will therefore always be identified as one of a group that fulfill the same SNP frequency criteria. One potential solution to this problem may be obtained by increasing the genetic resolution in these regions through the use of natural populations in an association genetics approach (Waugh et al. 2009). Another is to expand the SHOREmap approach to mutant populations obtained by fast neutron mutagenesis, which provides the potential of generating single gene deletions (Li et al. 2001) that can then be directly associated to the functional gene of interest. Establishing an efficient approach for gene identification in genetically recalcitrant regions in barley remains a high priority.

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