

Mapping of the *eibi1* gene responsible for the drought hypersensitive cuticle in wild barley (*Hordeum spontaneum*)

Guoxiong Chen^{1,2}, Takao Komatsuda^{*1}, Mohammad Pourkheirandish¹, Mohammad Sameri¹, Kazuhiro Sato³, Tamar Krugman⁴, Tzion Fahima⁴, Abraham B. Korol⁴ and Eviatar Nevo⁴

¹ Plant Genome Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8603, Japan

² Ecology and Agriculture Department, Cold and Arid Regions Environmental and Engineering Institute, Chinese Academy of Sciences, Lanzhou 730000, China

³ Research Institute for Bioresources, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan

⁴ Institute of Evolution, University of Haifa, Mount Carmel, Haifa 32905, Israel

Segregation analysis showed that *eibi1*, a drought hypersensitive cuticle wild barley mutant, was monogenic and recessive, and mapped in two F₂ populations, one made from a cross between the mutant and a cultivated barley (cv. Morex), and the other between the mutant and another wild barley. A microsatellite marker screen showed that the gene was located on barley chromosome 3H, and a set of markers already assigned to this chromosome, including both microsatellites and ESTs, was used to construct a genetic map. *eibi1* co-segregated with barley EST AV918546, and was located to bin 6. The synteny between barley and rice in this region is incomplete, with a large discrepancy in map distances, and the presence of multiple inversions.

Key Words: wild barley, *Hordeum spontaneum*, *eibi1* mutant, genetic mapping, rice, synteny.

Introduction

A waxy cuticle covers the aerial surfaces of the leaf and stem of many plants. The cuticle consists of two layers, the outer, translucent cuticle proper and an inner, opaque cuticular layer (Jeffree 1996). One of the cuticle's most important functions is to control water loss, a property which is especially important for plants growing in drought-prone environments (Riederer and Schreiber 2001, Jenks *et al.* 2002). The imposition of drought stress can affect both the thickness and the composition of the cuticular wax formed (Shepherd and Griffiths 2006). Current understanding of how this process is controlled is emerging from studies of cuticle mutants such as *eceriferum* (*cer*) in *Arabidopsis thaliana* and barley, and *glossy* (*gl*) in maize and various Brassicaceae; in these mutants, the synthesis of cuticular wax is blocked at various points, leading to the accumulation of intermediates (Jenks *et al.* 2002).

An increase in cuticle permeability has been associated both with a decrease (Jenks *et al.* 1994, Sturaro *et al.* 2005) and an increase in cuticular thickness (Chen *et al.* 2003), a change in cuticle polymer type (Xiao *et al.* 2004) or in the composition of the wax (Aharoni *et al.* 2004, Vogg *et al.* 2004). The metabolism responsible for the formation of the cuticle appears to be finely tuned, with only limited plasticity available to the plant to compensate for genetic changes.

This may explain why such a large number of different cuticle mutants have been identified.

At least 21 genes involved in cuticle formation have been isolated to date. Of these, seven encode regulatory loci (*CER2*, *CER7*, *GL15*, *RESURRECTION1*, *SHIN*, *WAX2/YRE/FLP1/CER3*, *WIN1*), ten encode enzymes (*ATT1*, *BODYGUARD*, *CER4*, *CER6/CUT1*, *CER10*, *FIDDLEHEAD*, *GL2*, *GL8*, *KCS1*, *LACS2*) and the remaining four (*CER1*, *CER5*, *GL1*, *AtWBC11*) are thought to be involved in the transport of wax compounds (Bird *et al.* 2007, Jenks *et al.* 1995, Kunst and Samuels 2003, Pighin *et al.* 2004, Rowland *et al.* 2007, Shepherd and Griffiths 2006). In barley, 1,890 *cer* mutants have been identified following mutagenesis (Lundqvist and Lundqvist 1988), and the genetic basis of 1,580 of these has been assigned to 79 *cer* loci, all but one of which act as recessive genes. A total of 27 distinct *cer* loci are distributed over all seven barley chromosomes, although only one maps to chromosome 6H (Franckowiak 1997). None of the barley *cer* genes has yet been either fine mapped or cloned.

The spontaneous *eibi1* mutant (named after Eibi, Eviatar Nevo) was identified in wild barley (*Hordeum spontaneum* Koch), and is hypersensitive to drought (Chen *et al.* 2004). Its relative water loss rate is higher than that of other wilted mutants. Compared to the wild type, treatment of *eibi1* leaves with 80% ethanol results in a five fold higher rate of chlorophyll efflux. The high transpiration rate of *eibi1* is not related to an abnormal stomatal density. The defective *eibi1* cuticle not only enhances the plant's sensitivity to drought stress, but also is associated with the rapid loss of water from

Communicated by K. Noda

Received September 16, 2008. Accepted November 29, 2008.

*Corresponding author (e-mail: takao@affrc.go.jp)

detached leaves, a twisted and dark green leaf phenotype, shorter plant stature, a twisted peduncle, a high tiller number, reduced fertility, smaller spikes, smaller grains, delayed germination, and a reduced germination rate (Chen *et al.* 2004).

We report here the genetic mapping of *eibil*, achieved by applying microsatellite assays to segregating populations. We have taken advantage of the established synteny between barley and rice to provide sufficient molecular markers in the *eibil* region to identify a locus that co-segregates with *eibil*.

Materials and Methods

Plant materials

Two wild barley accessions (23-19 and 23-73) were selected from the Wadi Qilt population maintained at the Gene Bank of the Institute of Evolution, University of Haifa, Israel. The *eibil* mutant arose from 23-19 (Chen *et al.* 2004). Seed of the barley cultivar Morex was obtained from Washington State University, Pullman, USA. Dehusked caryopses of these lines were germinated on wetted filter paper and kept at 4°C in the dark for 5d, and then at 25°C for 3d. The seedlings were then transplanted to 2.5 l soil-filled pots, and grown to maturity in a greenhouse. Crosses were made between *eibil* (as male) and either Morex, 23-19 or 23-73, and the resulting F₁ plants used to derive three independent F₂ populations. F₁ and F₂ seedlings were allowed to grow for an additional 3d beyond the normal germination time in Petri dishes in a transparent box, to obtain the material required for a leaf-drying test. Thereafter, the seedlings were grown in a greenhouse as normal. The leaf-drying test used a ~2 cm long leaf segment taken from the tip of the first expanded seedling leaf. This was held abaxial side up on tissue paper at room temperature for 1 h, following the procedure described by Chen *et al.* (2004).

DNA isolation, PCR conditions, and genetic map construction

DNA was extracted from fresh seedling leaf following Komatsuda *et al.* (1998). Each PCR mixture (10 µl) contained 20 ng genomic DNA, 300 nM of each primer, 200 µM dNTP, 25 mM TAPS (N-tris (hydroxymethyl) methyl-3-amino-propanesulphonic acid, pH 9.3), 50 mM KCl, 1 mM 2-mercaptoethanol, 1.5–4.0 mM (primer dependent) MgCl₂ and 0.25U ExTaq DNA polymerase (Takara, Tokyo). The PCR began with an incubation at 94°C for 5 min, followed by 30 cycles of 94°C/30 s, 55°C to 60°C (primer dependent)/30 s and 72°C/60 s, and ended with an incubation for 7 min at 72°C. Amplicons were electrophoresed through 1–2% (w/v) agarose (Iwai Kagaku, Tokyo) gels or, for the separation of fragments smaller than 60 bp, through 3–4% (w/v) Metaphor agarose (Cambrex, Rockland, USA) gels. Linkage maps were constructed using MAPMAKER v3.0 software (Lincoln *et al.* 1993). Kosambi's mapping function (Kosambi 1944) was used to convert recombination frequencies into map distances.

Molecular marker selection

A set of genome-wide SSR markers (Ramsay *et al.* 2000) was applied to a sub-sample of 43 mutant phenotype F₂ segregants from another Morex × *eibil* population to establish the chromosomal location of *eibil*. Then, SSR assays defining loci on the candidate chromosome (3H) were used to map *eibil* in other F₂ populations derived from both Morex × *eibil* (155 individuals) and 23-73 × *eibil* (108 individuals). These F₂ populations were different from the F₂ populations used in segregation analysis (Table 1) and the chromosomal location of *eibil* (Table 2). Eleven CAPS markers found in the Haruna Nijo × OUH602 map (K. Sato, unpublished) were developed and used for further genotyping of the Morex × *eibil*, 23-73 × *eibil* and Azumamugi (AZ) × Kanto Nakate Gold (KNG) (Komatsuda and Mano 2002). Twenty barley ESTs, predicted as mapping close to AV922032 (a barley EST located on chromosome 3H; N. Senthil and T. Komatsuda, unpublished) on the basis of synteny with rice chromosome 1, were also used for mapping.

Rice in silico map

The rice homologue of each barley EST was identified by BLAST analyses against sequences deposited in the NCBI (<http://www.ncbi.nlm.nih.gov>), Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>), and Rice Annotation Project (RAP, <http://rapdb.dna.affrc.go.jp/>). Barley ESTs homologous with predicted rice genes were searched in GRAMENE (<http://www.gramene.org/>) and RAP-DB (<http://rapdb.dna.affrc.go.jp/>) using “G-integra” function. MapChart software (Voorrips 2002) was used to draw both the rice physical map and the barley genetic maps.

Table 1. Segregation of plants with the *eibil* phenotype in the F₂

Crosses	Wild type	Mutant type	χ^2 for 3:1
Morex × <i>eibil</i>	117	41	0.076
23-19 × <i>eibil</i>	157	55	0.101
23-73 × <i>eibil</i>	159	60	0.671

Table 2. Linkage between *eibil* and SSRs in a F₂ sub-population of individuals showing the mutant *eibil* phenotype of the cross between Morex (A) and *eibil* (B). The population was different from the Morex × *eibil* population of Table 1.

Chromosome	SSR	Genotype			χ^2 for 1:2:1
		BB	AB	AA	
1H	Bmag579	11	21	10	0.05
2H	Hvm36	11	25	6	2.71
3H	Hvm60	24	15	4	22.53***
3H	Bmag603	38	4	0	96.29***
4H	Hvm67	11	17	14	1.95
5H	Hvm68	12	18	11	0.66
6H	EBmac684	10	21	12	0.21
7H	EBmag757	12	23	6	2.37

*** Significant at the 0.1% probability level.

Results

The eibil is a single recessive nuclear mutation

While wild-type seedlings at the five-leaf stage wilted after water was withheld for 5 d, *eibil* seedlings had wilted within 3 d. After re-watering, the leaf tips of *eibil* seedlings remained wilted, but those of the wild type recovered (Fig. 1A). The genotypes differed substantially in response to the leaf-drying test (Fig. 1B), with *eibil* leaf segments losing 43.2% of their initial fresh weight within 1 h, and the wild-type only 4.6%. The appearance of the former leaf segments was dry and shrunken, but that of the latter were still fresh, and this phenotype proved reliable for scoring the F₂ segregants. In each of the three mapping populations, the trait segregated according the Mendelian expectation of a monogenic recessive gene (Table 1), indicating that the *eibil* mutation was caused by a single recessive nuclear mutation.

The eibil gene is located on chromosome 3H

Of the 40 SSR markers used for the genome-wide screen, 34 were informative between *eibil* mutant and Morex. When one SSR per chromosome was used to genotype the 43 F₂ plants with mutant phenotype of the Morex × *eibil* population, which were selected from a population different from the population in Table 1, only Hvm60 and Bmag603 (chromosome 3H) showed a linkage with *eibil* (Table 2). In the full Morex × *eibil* F₂ population, Bmag603 and Bmag828 were both closely linked with *eibil* (6.2 cM and 1.0 cM, respectively) (Fig. 1C). In the 23-73 × *eibil* F₂ population, the Bmag603–*eibil* distance was 7.7 cM.

Of the barley ESTs from Haruna Nijo × OUH602 map to this region of chromosome 3H, four were mappable in Morex × *eibil*, and five in 23-73 × *eibil* (Fig. 1C, blue letters). Barley EST AV922032 (green letters in Fig. 1C) co-segregated with *eibil* in 23-73 × *eibil*, but was non-informative in the Morex × *eibil* population (Fig. 1C). As a rice homologue (Os01g0188100) of barley AV922032 is located on rice chromosome 1, synteny was used between this region and barley chromosome 3H to identify a further 20 barley ESTs as potential markers for *eibil*. Four and three of the 20 ESTs were mappable in, respectively, the Morex × *eibil* and 23-73 × *eibil* populations (Fig. 1C, red letters). In the 23-73 × *eibil* populations, all three co-segregated with *eibil*, while in the Morex × *eibil* populations, the four loci delimited a 6.5 cM region spanning *eibil*. EST AV918546 co-segregated with *eibil* was found newly in both populations (Fig. 1C).

Discussion

SSR marker assisted identification of the chromosomal location of eibil

A procedure which allows the rapid assignment of mutant alleles to linkage group was described by Castiglioni *et al.* (1998), and was applied to a set of homozygous recessive F₂ segregants to map *branched*, *calcaroides*, and 29 other de-

velopmental mutants (Castiglioni *et al.* 1998, Pozzi *et al.* 2000, Pozzi *et al.* 2003). The same approach was taken by Pratchett and Laurie (1994) to locate the recessive liguleless locus to chromosome 2H, based on the genotyping of just 36 F₂ segregants. Once the monogenic nature of the *eibil* had been established by segregation analysis, a genome-wide SSR scan of a small subset of F₂ plants was sufficient to identify the likely chromosomal location of the gene responsible for the mutant phenotype. The linkage of *eibil* with Hvm60 indicated by the coarse-resolution scan suggested that *eibil* was located on chromosome 3H, and this location was confirmed by full population mapping using other SSRs and EST loci known to map to this chromosome or chromosome region.

eibil maps to the pericentromeric region of chromosome 3H

Bmag828 and Bmag603 flanked *eibil* in the Morex × *eibil* map (Fig. 1C). The former locus maps in position 92.1 cM close to the centromere of chromosome 3H in the Steptoe × Morex SSR map (Li *et al.* 2003). Bmag603 maps to 43.5 cM close to the centromere on chromosome 3H in the 2003 barley consensus map (Karakousis *et al.* 2003). This pericentromeric region contains at least eight barley ESTs in the region between 37.8 cM and 58.3 cM of Haruna Nijo × OUH602 map (K. Sato, unpublished data), and some of these are linked to *eibil* (Fig. 1C).

Synteny between rice and barley in the chromosome 3H pericentromeric region

Comparative mapping has revealed extensive conservation of gene content and order among the genomes of cereal crops (Devos and Gale 2000). Synteny between barley chromosome 3H and rice chromosome 1 is well documented, with gene order largely maintained (Smilde *et al.* 2001). Over 69% (91 out of 131) of barley ESTs on chromosome 3H are homologous to the coding sequences of the corresponding genes on rice chromosome 1 (Stein *et al.* 2007). A similar proportion of unigene sequences (34 out of 50, 68.0%) on barley chromosome 3H matches the unigenes on rice chromosome 1 (Rostoks *et al.* 2005). These proportions were maintained in the present study, where seven out of ten barley ESTs were well matched with sequences on rice chromosome 1. However, collinearity was disturbed within the syntenic region defined by these ten barley ESTs, and genetic distances were also discrepant (Fig. 1C). The genetic distance between CB859971 and AV928420 was 19.9 cM in the Morex × *eibil* map (Fig. 1C), whereas the genetic distance of this region (Os01g0193500–Os01g0719100, Fig. 1C) in rice spans 97.2 cM (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice>, "Rice Genetic Markers"). Smilde *et al.* (2001) observed a 30-fold suppression of recombination in barley chromosome 3H pericentromeric region compared to rice. Han *et al.* (1998) estimated a 6- to 15-fold suppression in recombination rate in the pericentromeric region of barley chromosome 7H compared to rice orthologous region. The different degrees of suppression reported in the literature

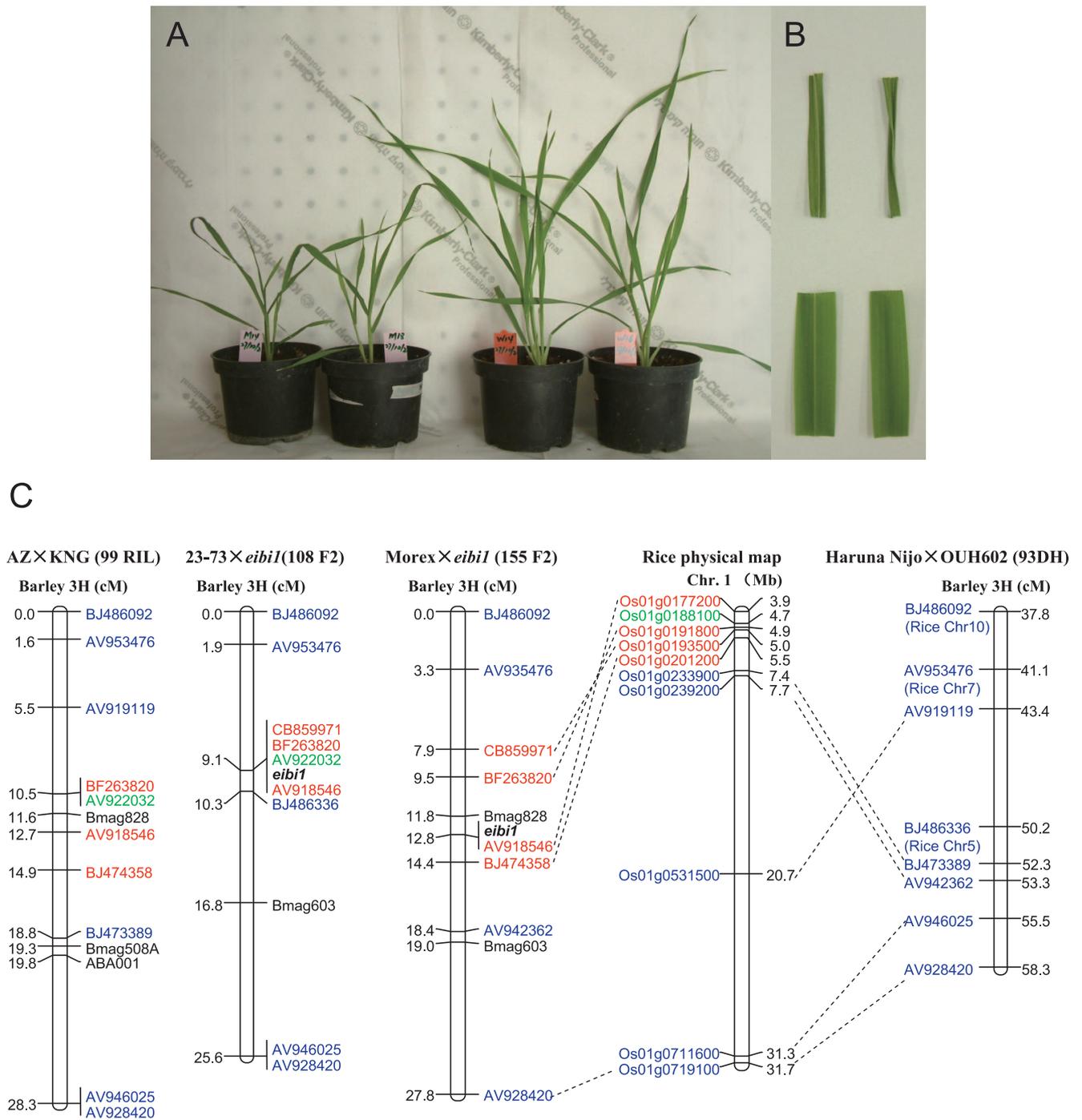


Fig. 1. The drought sensitivity of the *eibi1* mutant. (A) Five-leaf stage seedlings rehydrated after a drought stress applied by withholding water for 5d. Left hand pair of plants are *eibi1*, and the right hand pair are wild type. (B) Leaf segments cut from *eibi1* (above) and wild type (below) plants after drying for 1h. (C) Genetic maps of the *eibi1* gene in barley chromosome 3H and the syntenic region of rice chromosome 1. The barley EST map was adapted from Haruna Nijo×OUH602 map (blue letters) and the conserved order of markers was shown in four barley mapping population. AV922032 (green letter) was a barley EST located on chromosome 3H (N. Senthil and T. Komatsuda, unpublished). EST markers in red were developed based on barley-rice synteny. Dashed lines join barley ESTs with their rice homologues. Genetic distance of barley is shown in centimorgans (cM) and the physical map distance of rice is shown in mega-bases (Mb). These F₂ populations were different from the F₂ populations used in segregation analysis (Table 1) and the chromosomal location of *eibi1* (Table 2).

may merely reflect chromosome-to-chromosome or chromosome region-to-region variation. The genetic background can also affect estimates of map distance, as for example the

CB859971 to AV918546 interval which spanned 4.9 cM in the Morex×*eibi1* map, but 0 cM in the 23-73×*eibi1* map. This may suggest that the fine mapping of *eibi1* would be

more effective in the former cross.

Barley chromosome 3H and rice chromosome 1 have a collinear organization over almost their entire length (Rostoks *et al.* 2005, Smilde *et al.* 2001, Stein *et al.* 2007). However, we have observed in this study that the pericentromeric syntenic region is disrupted by two inverted blocks and one transposition (Fig. 1C). Transpositions are particularly problematical for synteny-based cloning. In the case of map-based cloning of the *Vrs1* gene, extensive gene sequence conservation existed between barley chromosome 2H and rice chromosome 4 (Pourkheirandish *et al.* 2007), allowing 22 EST-based STS markers to be placed within the critical region, and their linear order was identical in barley and rice. However, the rice *Vrs1* homologue mapped to rice chromosome 7, presumably as a result of a transposition. Such breakdowns in micro-collinearity emphasize the limitations of synteny-based cloning, and stress the importance of implementing genomic studies directly in the target species (Pourkheirandish *et al.* 2007).

Is eibil a new barley cuticle gene?

The *eibil* cuticle is defective, raising the possibility that it represents a new cuticle gene, alongside the 27 *cer* genes distributed throughout the genome (Franckowiak 1997). Four *cer* genes (*cer-zd*, *cer-r*, *cer-zn*, and *cer-b* (*gsh2*)) map within, respectively, bins 6, 7, 8, and 10 of chromosome 3H. Bin 6 also contains *eibil* because the interval defined by the two RFLP loci ABG462 and MWG2015 includes both Bmag828 and Bmag603—which flank *eibil*—and both RFLP loci map to bin 6. However, since *cer-zd* lies outside the ABG462–MWG2015 interval, *eibil* and *cer-zd* cannot be the same locus. Thus, as *eibil* is not one of the previously identified mapped *cer* genes, it appears to be a new barley cuticle gene.

Acknowledgements

We thank Dr. N. Senthil for supply of EST AV922032 marker, and Dr. A. Kleinhofs for the gift of cv. Morex seed. We are grateful to Dr. S.K. Nair for her constructive suggestions. This research was supported by the One Hundred Talents Project of the Chinese Academy of Sciences (O827751001), a JSPS post-doctoral grant of Japan (P05642), grant of the Genomics for Agricultural Innovation (TRC1004) of the Ministry of Agriculture, Forestry and Fisheries of Japan, and the Israel Discount Bank Chair of Evolutionary Biology.

Literature Cited

- Aharoni, A., S. Dixit, R. Jetter, E. Thoenes, G. van Arkel and A. Pereira (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. *Plant Cell* 16: 2463–2480.
- Bird, D., F. Beisson, A. Brigham, J. Shin, S. Greer, R. Jetter, L. Kunst, X. Wu, A. Yephremov and L. Samuels (2007) Characterization of Arabidopsis ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. *Plant J.* 52: 485–498.
- Castiglioni, P., C. Pozzi, M. Heun, V. Terzi, K. J. Müller, W. Rohde and F. Salamini (1998) An AFLP-based procedure for the efficient mapping of mutations and DNA probes in barley. *Genetics* 149: 2039–2056.
- Chen, G. X., M. S. Sagi, W. N. Song, T. Krugman, T. Fahima, A. B. Korol and E. Nevo (2004) Wild barley *eibil* mutation identifies a gene essential for leaf water conservation. *Planta* 219: 684–693.
- Chen, X. B., S. M. Goodwin, V. L. Boroff, X. L. Liu and M. A. Jenks (2003) Cloning and characterization of the WAX2 gene of *Arabidopsis* involved in cuticle membrane and wax production. *Plant Cell* 15: 1170–1185.
- Devos, K. M. and M. D. Gale (2000) Genome relationships: the grass model in current research. *Plant Cell* 12: 637–646.
- Franckowiak, J. D. (1997) Revised linkage maps for morphological markers in barley, *Hordeum vulgare*. *Barley Genetic Newsl.* 26: 9–21.
- Han, F., A. Kleinhofs, A. Kilian, M. Yano and T. Sasaki (1998) Synteny with rice: Analysis of barley malting QTL and *rpg4* chromosome regions. *Genome* 41: 373–380.
- Jeffree, C. E. (1996) Structure and ontogeny of plant cuticles. *In: Kerstiens, G. (ed.) Plant cuticles: an integrated functional approach.* Bios, Oxford, pp 33–82.
- Jenks, M. A., R. J. Joly, P. J. Peters, P. J. Rich, J. D. Axtell and E. A. Ashworth (1994) Chemically induced cuticle mutation affecting epidermal conductance to water vapor and disease susceptibility in *Sorghum bicolor* (L.) Moench. *Plant Physiol.* 105: 1239–1245.
- Jenks, M. A., H. A. Tuttle, S. D. Eigenbrode and K. A. Feldmann (1995) Leaf epicuticular waxes of the *eceriferum* mutants in *Arabidopsis*. *Plant Physiol.* 108: 369–377.
- Jenks, M. A., S. Eigenbrode and B. Lemeux (2002) Cuticular waxes of *Arabidopsis*. *In: Somerville, C. and E. Meyerowitz (eds.) The Arabidopsis book.* American Society of Plant Biologists, Rockville. DOI/10.1199/tab.0016, <http://www.aspb.org/publications/arabidopsis>
- Karakousis, A., J. P. Gustafson, K. J. Chalmers, A. R. Barr and P. Langridge (2003) A consensus map of barley integrating SSR, RFLP, and AFLP markers. *Aust. J. Agr. Res.* 54: 1173–1185.
- Komatsuda, T., I. Nakamura, F. Takaiwa and S. Oka (1998) Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*. *Genome* 41: 680–685.
- Komatsuda, T. and Y. Mano (2002) Molecular mapping of the intermediate spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 105: 85–90.
- Kosambi, D. (1944) The estimation of map distances from recombination values. *Ann. Eugen.* 12: 172–175.
- Kunst, L. and A. L. Samuels (2003) Biosynthesis and secretion of plant cuticular wax. *Progr. Lipid Res.* 42: 51–80.
- Li, J. Z., T. G. Sjakste, M. S. Röder and M. W. Ganal (2003) Development and genetic mapping of 127 new microsatellite markers in barley. *Theor. Appl. Genet.* 107: 1021–1027.
- Lincoln, S. E., M. J. Daly and E. S. Lander (1993) Constructing genetic linkage maps with MAPMAKER/EXP 3.0. Whitehead Institute for Biomedical Research Technical Report, 3rd edition, Cambridge.
- Lundqvist, U. and A. Lundqvist (1988) Mutagen specificity in barley for 1580 *eceriferum* mutants localized to 79 loci. *Hereditas* 108: 1–12.
- Pighin, J. A., H. Zheng, L. J. Balakshin, I. P. Goodman, T. L. Western,

- R.Jetter, L.Kunst and A.L.Samuels (2004) Plant cuticular lipid export requires an ABC transporter. *Science* 306: 702–704.
- Pourkheirandish, M., T. Wicker, N. Stein, T. Fujimura and T. Komatsuda (2007) The barley six-rowed spike gene *vrs1* reveals a breakdown of rice-barley micro collinearity by a transposition. *Theor. Appl. Genet.* 114: 1357–1365.
- Pozzi, C., P. Faccioli, V. Terzi, A.M. Stanca, S. Cerioli, P. Castiglioni, R. Fink, R. Capone, K.J. Muller, G. Bossinger *et al.* (2000) Genetics of mutation affecting the development of a barley floral bract. *Genetics* 154: 1335–1346.
- Pozzi, C., D.D. Pietro, G. Halas, C. Roig and F. Salamini (2003) Integration of a barley (*Hordeum vulgare*) molecular linkage map with the positions of genetic loci hosting 29 developmental mutants. *Heredity* 90: 390–396.
- Pratchett, N. and D.A. Laurie (1994) Genetic map location of the barley developmental mutant *liguleless* in relation to RFLP markers. *Heredity* 120: 135–139.
- Ramsay, L., M. Macaulay, S.D. Ivanisovich, K. MacLean, L. Cardle and J. Fuller (2000) A simple sequence repeat-based linkage map of barley. *Genetics* 156: 1997–2005.
- Riederer, M. and L. Schreiber (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. *J. Exp. Bot.* 52: 2023–2032.
- Rostoks, N., S. Mudie, L. Cardle, J. Russell, L. Ramsay, A. Booth, J.T. Svensson, S.I. Wanamaker, H. Walia, E.M. Rodriguez *et al.* (2005) Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Mol. Genet. Genomics* 274: 515–527.
- Rowland, O., R. Lee, R. Franke, L. Schreiber and L. Kunst (2007) The *CER3* wax biosynthetic gene from *Arabidopsis thaliana* is allelic to *WAX2/YRE/FLP1*. *FEBS Letters* 581: 3538–3544.
- Shepherd, T. and D.W. Griffiths (2006) The effects of stress on plant cuticular waxes. *New Phytol.* 171: 469–499.
- Smilde, W.D., J. Halukova, T. Sasaki and A. Graner (2001) New evidence for the synteny of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags. *Genome* 44: 361–367.
- Stein, N., M. Prasad, U. Scholz, T. Thiel, H. Zhang, M. Wolf, R. Kota, R.K. Varshney, D. Perovic, I. Grosse *et al.* (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. *Theor. Appl. Genet.* 114: 823–839.
- Sturaro, M., H. Hartings, E. Schmelzer, R. Velasco, F. Salamini and M. Motto (2005) Cloning and characterization of *GLOSSY1*, a maize gene involved in cuticle membrane and wax production. *Plant Physiol.* 138: 478–489.
- Vogg, G., S. Fischer, J. Leide, E. Emmanuel, R. Jetter, A.A. Levy and M. Riederer (2004) Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid beta-ketoacyl-CoA synthase. *J. Exp. Bot.* 55: 1401–1410.
- Voorrips, R.E. (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93: 77–78.
- Xiao, F.M., S.M. Goodwin, Y.M. Xiao, Z.Y. Sun, D. Baker, X.Y. Tang, M.A. Jenks and J.M. Zhou (2004) *Arabidopsis* CYP86A2 represses *Pseudomonas syringae* type III genes and is required for cuticle development. *EMBO J.* 23: 2903–2913.