Introduction of Genetic Diversity into Cereals from their Wild Relatives

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Abstract: Although numerous wild *Triticeae* species are potential gene sources for cereal breeding, introduction of genes from these species into cultivated crops are limited due to lack of relevant information. *Hordeum vulgare* L. is a self-pollinated, cultivated diploid species for which there is considerable information and materials are preserved. By using cultivated barley as a template genome, we try to share the genomic information among *Triticeae* species. We examined the application of more than one thousand non-redundant barley EST markers to twelve *Triticeae* species including cultivated barley as a control. Three *Hordeum* species showed higher number of amplified fragment(s) with the barley primer pairs compared to the non-*Hordeum* species. Many of these markers were polymorphic to wheat, indicating that they can be used as markers to identify alien chromosome segments added in wheat genetic background. Application of barley genomic information to wild species will promote efficient introduction of alien genes into the cultivated species, one of the fundamental breeding strategies using homologies of genomes and dissimilarity of genes among *Triticeae*.

Keywords: *Triticeae*; barley; EST; marker; chromosome addition lines

Triticeae is considered to be monophyletic and is a good example of a plant group with a high degree of biological diversity including several genomes, various levels of polyploidy, versatility in life forms, reproductive and dispersal patterns (Bothmer *et al*. 2003). Three major cereal crops, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare*. L.), rye (*Secale cereale* L.), and several important forages belong to the tribe (tribus) *Triticeae*. This tribe represents a highly successful evolutionary branch in the grass family (*Poaceae*) and comprises a vast number of species and genera.

Barley is a model genome system for *Triticeae*. The self-fertile, diploid genetic system has advantages for studies of phenotypic gene expression and development of homozygous material. Barley chromosomes are homoeologous to those of cultivated wheat and rye, which are polyploids and out-breeding, respectively. However, the primary genepool of barley comprises the single species of *H. vulgare*, with cultivated barley as ssp. *vulgare* and the wild form as ssp. *spontaneum*, which is assumed to be the progenitor of the domesticated subspecies. The barley secondary genepool also includes a single species, *H. bulbosum*, that shares the basic H genome with barley. All the remaining species of *Hordeum* are classified into the tertiary genepool. They cross with barley with difficulty and backcrossing to the crop is even more difficult (Bothmer *et al*. 1983; Bothmer & Linde-Laursen 1989). The wild South American species *H. chilense* belongs to the tertiary genepool of barley. Amphiploids with wheat as well as chromosome addition and substitution lines can be used as a bridge for

transfer of useful genes from *H. chilense* into wheat (Person-Dedryver *et al.* 1990).

Barley diversity is well represented in genebanks. According to the FAO Report on the State of the World's Plant Genetic Resources for Food and Agriculture (FAO 1996), barley is the second largest crop. With 8% of all six million accessions worldwide, barley comes second to wheat (13%) and is followed by rice (7%) and maize (5%) (Hintum & Menting 2003). FAO counts 485 000 barley accessions. The Barley Core Collection is a selected and limited set of accessions from these huge numbers of the world barley holdings (Knüpffer & Hintum 2003). It optimally represents the genetic diversity of cultivated barley and the wild species of *Hordeum*, covering the three genepools. The core should include as much as possible of the genetic diversity of the crop and its allies.

The objectives of crop genebank are changing, among other things due to the demand by the recent advancement of molecular genetics. For example, the Japanese government has a national bio-resource management program for major organisms including human cells, animals and plants. The barley and wheat genebanks are involved in the project and responsible for the distribution of DNA clones as well as seed samples under the web based database system (http://www.nbrp.jp/). These new functions of genebanks include genomic resources on model species, which may provide tools in crop diversity analysis. The project managers and databases tend to treat genes and accessions similarly among species but there is a fundamental difference in the approaches between the diversity analysis and genomic information, represented by a fairly small number of accessions.

Related wild and progenitor species represent a rich reservoir of useful genetic variation that can be exploited for crop improvement. Interspecific and intergeneric hybridization in the tribe Triticeae has been successfully used to facilitate the transfer of genes from wild relatives to cultivated species. Many amphiploids between wheat and other species and alien chromosome addition, substitution or translocation lines have been produced and maintained for this purpose. Agronomically important traits, including resistance to diseases and pests and abiotic stresses, have been transferred from these genetic stocks into wheat (cf. Friebe *et al.* 1996).

Although the numerous wild species are potential gene sources for cereal breeding, introduction of genes from these species into cultivated species is limited by lack of relevant information. In order to understand the entire genetic system in *Triticeae*, development of genetic markers across the genomes is necessary.

Expressed Sequence Tags (ESTs) seem to be the most powerful genetic marker available in *Triticeae*. EST is a partial sequence of a gene and is mapped on the respective gene position on the genome either by PCR based marker or RFLP probe. Mapping information can be compared among genomes in different species by the comparison of genetic sequences.

To demonstrate the advantage of sequence based markers in Triticeae, applicability of EST-based barley PCR primer sets to the genomes of three crops (barley, wheat and rye) and nine alien *Triticeae* species were examined in this study. We also try to adopt these markers to characterize the genome of *H. chilense* to the genetic background of wheat. Information obtained from the barley EST markers can be linked with the genomic information of rice and wheat. The potential uses of these genetic markers to improve the understanding of the whole genetic feature of characters existing among Triticeae species are discussed.

MATERIALS AND METHODS

*Plant material*s. A total of 12 species of the tribe *Triticeae* were included in the present investigation (Table 1). The common wheat (*Triticum aestivum*) cv. Chinese Spring was used as the recipient cultivar of most chromosome addition lines and barley (*Hordeum vulgare*) cv. Betzes was used as the chromosome donor of the addition lines. Moreover, *H. chilense* addition and substitution lines of Chinese Spring wheat were included.

EST marker generation. Four series of barley EST primer sets were examined (Hagras *et al.* 2005a). Series 1 consisted of 384 primer sets chosen randomly from the pool of barley EST primer sets. While series 2, 3 and 4 were pre-screened in the previous study (Sato unpubl.). Series 2 consisted of 651 primer sets that showed a clear single PCR product in barley but not in wheat, and might be effective to detect alien chromosome(s) in wheat genetic background. To develop markers showing PCR-product size difference between wheat and the alien species, series 3 and 4 consisted of 24 and 88 primer sets respectively showing a clear, single product both with barley and wheat with different and the same product size(s), respectively, were

selected. The four series originated from 10 336 markers produced on the basis of the EST information of barley cultivars Haruna Nijo, Akashinriki and wild barley *H. vulgare* ssp. *spontaneum* strain H602. More information is available at http://www.shigen.nig.ac.jp/barley/.

DNA isolation and PCR. Total genomic DNA was extracted from young plant leaves using CTAB method (Murray & Thompson 1980). PCR was performed on a 96-well plate as described by Hagras *et al.* (2005a). Electrophoresis of the PCR products was performed on 1.5% agarose gel. The presence of a clear band was scored as one unit and its absence or the presence of complicated types of amplifications scored as zero.

RESULTS AND DISCUSSION

Applicability of barley EST primers to other *Triticeae* species

The number of amplified fragments was different depending on the species and the primer series. A considerable number of single, clear bands was produced in all the species using the primer sets of series 1 (Figure 1 and Table 1). The barley cultivar Betzes showed the highest number (364) of bands. Barley-related species (*H. bulbosum* and *H. chilense*) and polyploid species (*Leymus mollis, Triticum aestivum, L. racemosus,* and *Elymus ciliaris*) showed more bands (293, 216, 209, 200, 196 and 193 bands, respectively) than the other species. An exception was *H. bogdanii,* also a diploid member of the barley genus *Hordeum,* but with a considerably lower number of amplified bands (165).

Markers showing polymorphism between wheat and the alien species should detect alien chromosome(s) in wheat genetic background. The primer series 2, pre-screened by PCR with positive amplification in barley and negative in wheat (Sato unpubl.), was used to test this. Most of the 651 primer sets of series 2 produced a single clear band in certain species and multiple faint bands in the others. From 39 to 651 markers for each species

Table 1. Numbers of amplified and polymorphic EST markers to wheat in four barley EST primer series (1165 primers in total)

Species	No. of amplified markers in alien species but not in wheat		No. of size polymorphic markers to wheat		Total no. of polymorphic markers to wheat (%)
	series 1[a]	series 2[b]	series 3[c]	series 4[d]	
Hordeum vulgare (barley)	166 (364)[e]	651 (651)[e]	42 (42)[e]	– (88)[e]	859 (75.0)[f]
H. bulbosum	119 (293)	414 (414)	31 (39)	8 (74)	572 (69.8)
H. chilense	62 (216)	198 (198)	22 (32)	10 (64)	292 (57.3)
H. bogdanii	38 (165)	150 (150)	11 (17)	6 (58)	205 (52.6)
Dasypyrum villosum	30 (152)	91 (91)	9 (11)	2 (63)	132 (41.6)
Elymus ciliaris	42 (193)	123 (123)	10 (14)	4 (63)	179 (45.5)
Psathyrostachys huashanica	50 (209)	147 (147)	14 (27)	13 (61)	224 (50.5)
Leymus racemosus	46 (196)	122 (122)	10 (14)	4 (57)	182 (46.8)
L. mollis	36 (153)	96 (96)	10 (17)	7 (42)	149 (48.4)
Secale cereale (Rye)	26 (152)	47 (47)	6 (9)	3 (50)	82 (31.8)
Thinopyrum elongatum	29 (172)	39 (39	4 (10)	6 (50)	78 (28.8)
Triticum aestivum (wheat)	– (200)	– (–)	– (42)	– (88)	– (–)
Average	59 (205)	189 (189)	15 (23)	6 (63)	268.5 (56.3)

[a]Primer series 1 consists of 384 barley primer sets that were chosen randomly; [b]Primer series 2 consists of 651 barley primers that showed a single band in barley but not in wheat (a previous study); [c]Primer series 3 consists of 42 barley primers that showed a single band with different size in both barley and wheat (a previous study); [d]Primer series 4 consists of 88 barley primers that showed a single band with the same size in both barley and wheat (a previous study); [e]Number in parentheses is number of amplified markers; [f]Number in parentheses is percentage of number of the polymorphic markers (to wheat) to number of amplified markers for each species
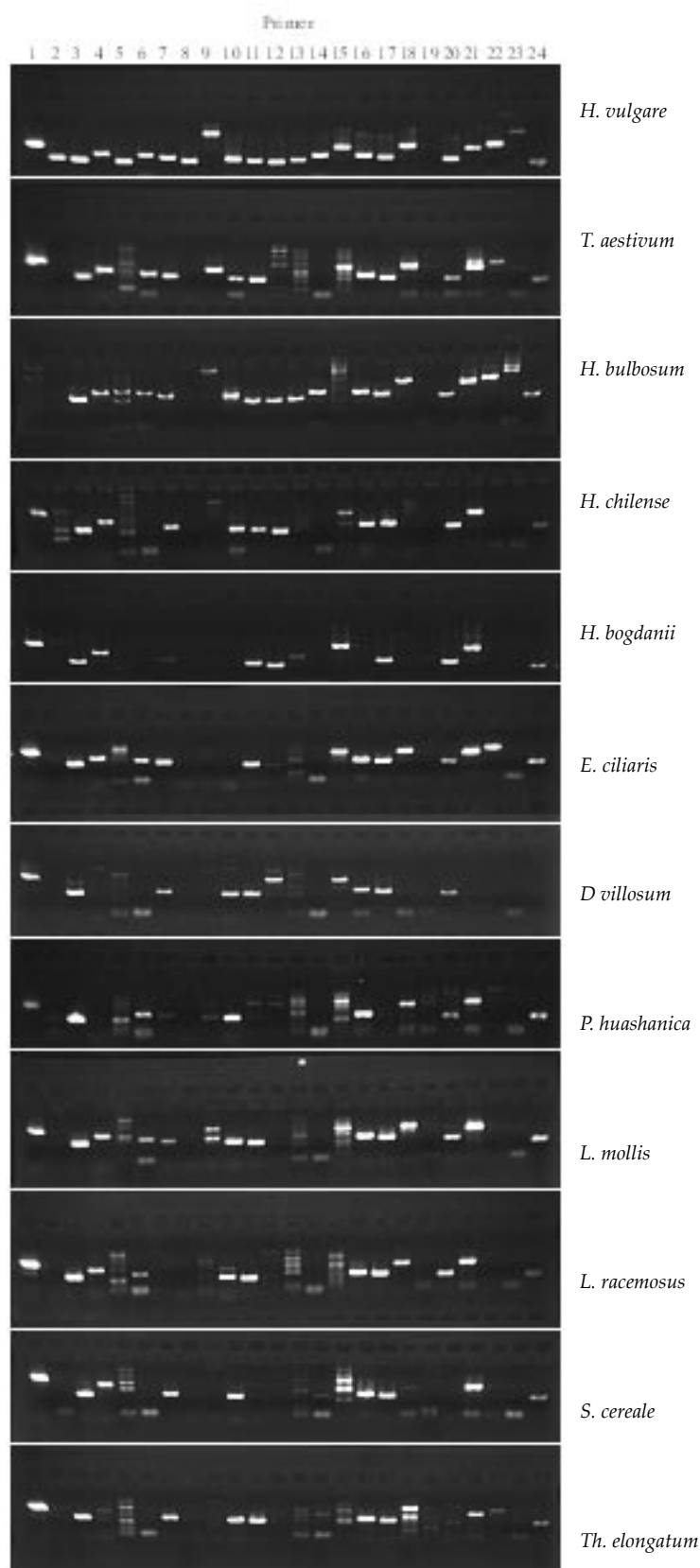
Figure 1. Representative images of DNA amplification patterns of the 12 *Triticeae* species using 24 barley EST primer sets.species

were obtained (Table 1). The number of markers was higher in *Hordeum* than in any of the other species.

To detect the size polymorphic markers between wheat and the alien species, the primer series 3 and 4 that produced a single band in both barley and wheat, with different and the same band size, respectively, in the pre-screening, were used. Prime series 3 produced from 9 to 42 markers and primer series 4 from 42 to 88 markers (Table 1).

In general, the average percentages of polymorphic fragments between wheat and the other species (Table 1) were higher in primers series 2 (100%) and 3 (67.3%) than that of series 1 (28.5%) and 4 (10.0%). Thus, the pre-selection was informative for generating diagnostic markers. A total of 2954 polymorphic markers between wheat and the other species were obtained showing a great variation among the species. *Hordeum vulgare*, *H. bulbosum* and *H. chilense* had the highest number of polymorphic markers with wheat and ranging from 859 to 292. *Leymus mollis*, *L. racemosus*, *Elymus ciliaris*, *Pseudoroegneria huashanica*, and *Dasypyrum villosum* showed 224, 182, 179, 149 and 132 markers, respectively. *Secale cereale* and *Thinopyrum elongatum* had the lowest number of markers (82 and 78 markers, respectively).

Allocation of barley EST markers to *Hordeum chilense* chromosomes

From the 209 markers of the primer series 2, 3 and 4 developed for *Hordeum chilense* (Table 1), we used 140 markers identified as unique to *H. chilense* chromosomes in addition lines (HAGRAS *et al.* 2005b). Out of these markers, 29 were on each of 1H^{ch} and 7H^{ch}, 26 were on each of 4H^{ch} and 5H^{ch}, 21 were on 6H^{ch}, and only 10 were on the short arm of 2H^{ch}. Seven markers were commonly allocated on two or three chromosome pairs with the same amplicon size. On average, 25 markers were allocated to each chromosome of *H. chilense*. Most of these markers were allocated to *H. vulgare* chromosomes us-

ing wheat-*H. vulgare* addition lines (Nasuda *et al.* 2005) and/or linkage map (Sato unpubl.). In this study, 90% of the EST markers were allocated to the same homoeologous pairs of *H. vulgare*, while the other markers were allocated to more than one pair or to different homoeologous pairs.

Access to the genome database by barley ESTs

Each barley EST is localized on a rice linkage map by sequence homology comparisons between their original barley ESTs and the public rice genome information (http://earth.lab.nig.ac.jp/~dclust/cgi-bin/barley_map_pub/index.html). Via barley ESTs the genome information of *Triticeae* species can be linked to rice, a model plant, in which the genome sequence has been completed. The very limited information for genome research of wild *Triticeae* species can partly be overcome by using the barley EST markers. Many of these markers have already been marked on the high-density linkage map of barley (Sato unpubl.), allowing identification of the part(s) of alien chromosomes that are homoeologous to the barley chromosome. It may also elucidate chromosomal evolution by allocating the markers onto the alien chromosomes. Thus, introduction of barley genomic information to wild species will promote efficient introduction of alien genes into the cultivated species, which is one of the fundamental breeding strategies. It relies on knowledge of homologies of genomes and dissimilarity of genes among Triticeae, knowledge that can be obtained from barley ESTs.

References

Bothmer R. von, Flink J., Jacobsen N., Kotimäki M., Landström T. (1983): Interspecific hybridization with cultivated barley. Hereditas, **99**: 219–244.

Bothmer R. von, Linde-Laursen I. (1989): Backcrosses to cultivated barley (*Hordeum vulgare* L.) and partial elimination of alien chromosomes. Hereditas, **111**: 145–147.

Bothmer R. von, Sato K., Komatsuda T., Yasuda S., Fischbeck G. (2003): The domestication of cultivated barley. In: Bothmer R. von, Hintum T. van, Knüpffer H., Sato K. (eds): Diversity in Barley (*Hordeum vulgare*). Elsevier Science B.V., Amsterdam, 9–27.

FAO (1996): FAO State of the World's Plant Genetic Resources for Food and Agriculture. Rome.

Friebe B., Jiang J., Raupp W.J., McIntosh R.A., Gill B.S. (1996): Characterization of wheat-alien translocations conferring resistance to disease and pest: current status. Euphytica, **91**: 59–87.

Hagras A.A., Kishii M., Sato K., Tanaka H., Tsujimoto H. (2005a): Extended application of barley EST markers for the analysis of alien chromosomes added to wheat genetic background. Breeding Science, **55**: 335–341.

Hagras A.A., Kishii M., Sato K., Tanaka H., Tsujimoto H. (2005b): Genomic differentiation of *Hordeum chilense* from *H. vulgare* as revealed by repetitive and EST sequences. Genes and Genetic Systems, **80**: 147–159.

Hintum T. van, Menting F. (2003): Diversity in ex situ genebank collections of barley. In: Bothmer R. von, Hintum T. van, Knüpffer H., Sato K. (eds): Diversity in Barley (*Hordeum vulgare*). Elsevier Science B.V., Amsterdam, 247–257.

Knüpffer H., Hintum T. van. (2003): Summarised diversity – the Barley Core Collection. In: Bothmer R. von, Hintum T. van, Knüpffer H., Sato K. (eds): Diversity in Barley (*Hordeum vulgare*). Elsevier Science B.V., Amsterdam, 259–267.

Murray H.G., Thompson W.F. (1980): Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, **8**: 4321–4325.

Nasuda S., Kikkawa Y., Ashida T., Rafiqul Islam A.K.M., Sato K., Endo T. (2005): Chromosomal allocation and deletion mapping of barley ESTs. Genes and Genetic Systems (in press).

Person-Dedryver F., Jahier J., Miller T.E. (1990): Assessing the resistance to cereal root-knot nematode, *Meloidogyne naasi*. I: a wheat line with the added chromosome arm 1H^{ch}S of *Hordeum chilense*. Journal of Genetics and Breeding, **44**: 291–296.