ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization 7(1); 72–79
doi:10.1017/S1479262108994235

# Aphid feeding response and microsatellite-based genetic diversity among diploid *Brachypodium distachyon* (L.) Beauv accessions

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**Received 23 October 2007; Accepted 12 April 2008 – First published online 14 May 2008**

## Abstract

False brome grass, *Brachypodium distachyon* (L.) Beauv, has been proposed as a new model species to bridge rice and temperate cereal crops for genomics research. However, much basic information for this species is still lacking. In this study, six diploid *B. distachyon* ($2n = 2x = 10$) accessions (Bd1-1, Bd2-3, Bd3-1, Bd18-1, Bd21 and BD29) were evaluated for their response to infestation by two cereal aphid pests of common wheat (*Triticum aestivum* L.): the greenbug, *Schizaphis graminum* Rondani, and the Russian wheat aphid (RWA), *Diuraphis noxia* Mordvilko. Through database mining of *B. distachyon* expressed sequence tag (EST) and genomic DNA sequences, 160 EST- and 21 genomic microsatellite markers were developed and used to evaluate genetic diversity among the *B. distachyon* accessions. All six accessions were resistant to RWA biotype RWA1 but showed distinct responses to feeding by greenbug biotypes C and E, as well as RWA2 RWAs. Although microsatellite-based genetic diversity among different accessions was generally low, Bd1-1 and BD29 were the most diverged from the other four lines. The genetic divergence was correlated with geographical distances between the *Brachypodium* accessions. Comparison of simple sequence repeat polymorphisms in three inbred lines (Bd2-3, Bd3-1 and Bd18-1) with their respective original parental lines revealed no effect of inbreeding on genetic diversity. Phylogenetic analysis suggested that *Aegilops tauschii* (Coss.) Schmal., the D genome donor of common wheat, was closer to *B. distachyon* than to rice. The greenbug - *B. distachyon* system seems to be a model of choice for plant–aphid interaction studies in the grass genome.

**Keywords:** aphid resistance; *Brachypodium distachyon*; *Diuraphis noxia*; genetic diversity; microsatellite; *Schizaphis graminum*

## Introduction

False brome grass, *Brachypodium distachyon* (L.) Beauv, has been proposed as a bridge between rice (*Oryza sativa* L.) and temperate cereal crops for genomics research (Draper *et al.*, 2001). *B. distachyon* has a number of advantages as a model species such as annual growth habit, self-fertility, short generation time, small genome (~355 Mb) (Bennett and Leitch, 2005) and five pairs of readily identifiable chromosomes. Some biological features in *B. distachyon* that are not found in rice but are important for temperate cereal

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crops include freezing tolerance, vernalization requirement and host resistance to specific pathogens or insect pests (Draper *et al.*, 2001). The genomic infrastructure of this species has continued to develop (Hasterok *et al.*, 2004, 2006; Vogel *et al.*, 2006a, b; Huo *et al.*, 2006, 2008). Whole genome sequencing of *B. distachyon* is underway (http://www.brachypodium.org/). Nevertheless, much basic information about this species is still lacking. For example, no linkage map of *B. distachyon* is available. In addition, although *B. distachyon* accessions have varying vernalization requirements and different reactions to several plant pathogens (Draper *et al.*, 2001; Routledge *et al.*, 2004; Allwood *et al.*, 2006), genetic variation in response to other biotic or abiotic stresses has not been reported. Such information could help facilitate utilization of *B. distachyon* resources in crop breeding.

The greenbug, *Schizaphis graminum* Rondani, and Russian wheat aphid, *Diuraphis noxia* Mordvilko (RWA hereinafter), are the two most important aphid pests of wheat in the southern Great Plains of the USA. They are especially notorious for periodic changes of prevailing biotypes in the field, rendering currently deployed host resistance genes useless. Over 30 greenbug biotypes (Burd and Porter, 2006) and five RWA biotypes (RWA1–RWA5) (Burd *et al.*, 2006) have been identified. Greenbug biotypes E and I and RWA biotypes RWA1 and RWA2 currently dominate wheat fields (Burd and Porter, 2006; Puterka *et al.*, 2007). Although a number of host resistance genes (R genes) against the two aphids have been identified in wheat, none have been cloned. Map-based cloning of genes in wheat is still a formidable task because of its very large genome size (~16,000 Mb). Our long-term goal is to elucidate the molecular mechanisms of host resistance against aphid pests in cereal crops. Because wheat is phylogenetically closer to *B. distachyon* than to rice (Gaut, 2002), we are exploring the potential of *B. distachyon*-greenbug as a model system for plant–aphid interactions in the grass genome. In this study, we examined the reaction of *B. distachyon* diploid accessions to infestation by four different greenbug and RWA biotypes. Through database mining of *B. distachyon* expressed sequence tag (EST) and genomic DNA sequences, we developed microsatellite or simple sequence repeat (SSR) markers and used them to evaluate genetic diversity among six *B. distachyon* accessions.

## Materials and methods

### Plant materials

Seeds of four diploid accessions, BD2 (PI185133), BD3 (PI185134), BD18 (PI245730) and BD29 (PI639818) were acquired from the USDA Small Grain Collection at Pullman, WA (http://www.ars-grin.gov/). Five inbred lines, Bd1-1 (PI170218), Bd2-3, Bd3-1, Bd18-1 and Bd21 (PI254867), which had undergone five to six generations of selfing, were kindly provided by David Garvin (USDA-ARS, St Paul, MN). BD2, BD3 and BD18 were the respective parental lines of Bd2-3, Bd3-1 and Bd18-1. Three species in the grass genome: *Aegilops tauschii* (Coss.) Schmal. (accession AL8/78), rice (*O. sativa* L. cv. Nipponbare) and perennial ryegrass (*Lolium perenne* L. cv. Palmer) were also employed in phylogenetic analysis. Seeds of the ryegrass cultivar 'Palmer' were provided by Lloyd Nelson (Texas AgriLife Research at Overton, TX), and the *Ae. tauschii* AL8/78 seeds were supplied by Mingcheng Luo (University of California, Davis, CA).

### Aphid infestation procedure

Infestation procedure followed Weng and Lazar (2000). To study reactions of the six *B. distachyon* lines to aphid infestations, greenbug biotypes C (GBC) and E (GBE) and RWA biotypes RWA1 and RWA2 were used. GBC and GBE colonies were reared in an isolated room at Texas AgriLife Research, Bushland, TX. RWA1 and RWA2 were kindly provided by J. P. Michaud (Kansas State University, Hays, KS) and Gary Puterka (USDA-ARS, Stillwater, OK), respectively. Wheat cultivars or germplasm lines 'TAM 107', 'TAM 110', 'Halt' and '94M370' were used as resistant controls. Wheat cultivar 'TAM 105' was the susceptible control in all tests. Seeds of each line were germinated in 10.2 cm square plastic pots (Hummert International, Earth City, MO) filled with a commercial potting mix (Sunshine Mix LC1, BWI Companies, Dallas, TX). Seedlings were watered regularly. At the three-leaf stage, each pot was thinned to four seedlings per pot. In tests with each aphid biotype, all pots were arranged randomly in the growth chamber with five replicates per genotype. Feeding responses were recorded after 14 d of infestation for the greenbug and 3 weeks for the RWA when the susceptible controls were killed. The screening test for each biotype was repeated at least twice. Because the feeding response in all tested samples could be easily and clearly rated as either R (resistant) or S (susceptible), no statistical analysis of aphid screening data was applied.

### Database mining and SSR marker development

Database mining and SSR marker development followed Weng *et al.* (2007). The *B. distachyon* EST and

bacterial artificial chromosome (BAC) end genomic DNA sequences were downloaded from NCBI (National Center for Biotechnology Information, Bethesda, MD; http://www.ncbi.nlm.nih.gov). In total, 20,449 *B. distachyon* ESTs (12,798 kb) and 2,185 genomic DNA sequences (~1,222 kb) were examined. Briefly, the DNA sequences were scanned for simple perfect SSRs using the SSR Primer Discovery Program (http://hornbill.cspp.latrobe.edu.au/cgi-binpub/), in which the minimum length of SSRs was set as 10 bp, and only SSRs with repeat units of 2–5 bp in the motif were reported. The SSRs with mono- and hexanucleotide repeats were not counted and were not included in the final statistics. For a 10 bp SSR, one occurrence may comprise a repeat of five dinucleotides, four trinucleotides, three tetranucleotides or two pentanucleotides. The SSR motifs include both strands of the DNA sequence. CGG, for example, also includes GCC and the reverse complements GGC and CCG. The total lengths of each repeat type were used to estimate abundance of SSRs in the genome. The output was downloaded into Microsoft Excel for further analysis.

Over 6,000 EST-SSRs were identified from database mining. Only SSRs with a minimum length of 20 bp (for EST-SSRs) or 16 bp (for genomic SSRs) were selected for phylogenetic analysis. SSRs in this subset were subjected to a Basic Local Alignment and Search Tool (BLAST) (Altschul *et al.*, 1990) search against the NCBI *B. distachyon* database to remove primers with redundant sequences. This resulted in 160 EST- and 21 genomic DNA-derived SSRs in this study. Detail information on all 181 SSRs is provided in Supplementary Table 1, available online only at http://journals.cambridge.org

**Table 1.** Responses of six *Brachypodium distachyon* accessions and the wheat controls to infestation of greenbug biotypes C (GBC) and E (GBE), and Russian wheat aphid biotypes RWA1 and RWA2[a]

| Samples | GBC | GBE | RWA1 | RWA2 |
|---|---|---|---|---|
| *Brachypodium accessions* | | | | |
| Bd1-1 | R | S | R | S |
| Bd2-3 | R | R | R | R |
| Bd3-1 | R | S | R | R |
| Bd18-1 | S | R | R | R |
| Bd21 | R | S | R | S |
| BD29 | S | R | R | M |
| *Wheat controls* | | | | |
| TAM 107 | R | S | S | S |
| TAM 110 | R | R | S | S |
| Halt | S | S | R | S |
| 94M370 | S | S | R | R |
| TAM 105 | S | S | S | S |

[a] R, resistant; S, susceptible; M, intermediate resistance.

### PCR amplification

Genomic DNAs were extracted from young leaves by the CTAB method (Murray and Thompson, 1980). Each PCR contained 20 ng template DNA, 0.5 μM each of two primers and 1 × PCR master mix (Promega Inc., Madison, WI) in a total volume of 10.0 μl, which was performed in a PTC-200 thermocycler (Bio-Rad, Hercules, CA). A single touch-down PCR program was used (Weng *et al.*, 2005).

For most SSR markers, the variation in the size of the PCR products allowed the patterns to be resolved unambiguously in 4% high-resolution agarose gels stained with ethidium bromide. For SSR markers generating DNA fragments of very similar size from different DNA templates, PCR products were size-fractioned in non-denaturing polyacrylamide gels and banding patterns were visualized by SYBR Gold staining (Molecular Probes, Eugene, OR). For quality control, markers detecting null alleles in PCR were repeated at least once to rule out the possibility of the failure of PCR amplification. For PCR products amplified by genomic SSRs, only fragments similar to the expected sizes were scored for data analysis.

### Data analysis

Data from 160 EST-SSRs and 21 genomic SSRs were analysed. PCR products from each SSR marker (locus) were scored and the polymorphic information content (PIC) value (Botstein *et al.*, 1980) was estimated to assess the informativeness of each marker. PIC was calculated using the formula $\mathrm{PIC} = 1 - \sum(P_i)^2$, where $P_i$ represents the frequency of the $i$th allele. Null and non-polymorphic alleles were not included in computing PIC.

Two methods were used to evaluate SSR-based genetic diversity. In the first method, similarity estimates of microsatellite alleles were obtained using the 'City Block' approach. Haplotypes were clustered with hierarchical clustering using the Cluster 3.0 program (http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/software.htm). In the second method, the marker data were analysed with the software package PHYLIP 3.66 (Felsenstein, 1989; http://evolution.genetics.washington.edu/phylip.html), in which 1000 bootstrapped datasets were created for construction of pairwise distance matrices (Nei and Li, 1979). The resulting datasets were subjected to neighbour-joining cluster analysis (Saitou and Nei, 1987), and a consensus tree was constructed with rice, *Ae. tauschii* and perennial ryegrass as outgroups.

## Results

### *Aphid feeding responses of six B. distachyon accessions*

The infestation test results with the RWA and the greenbug on six diploid *B. distachyon* accessions and the wheat controls are summarized in Table 1. On susceptible TAM 105 seedlings, RWA2 feeding caused leaf chlorosis and leaf rolling (Burd *et al.*, 1993). Symptom development in seedlings of *B. distachyon* lines Bd1-1 and Bd21 upon RWA2 aphid feeding was similar to the susceptible wheat controls. Thus, lines Bd1-1 and Bd21 were scored as susceptible to RWA2. Similar to the resistant wheat controls, no symptoms were observed in Bd2-3, Bd3-1 and Bd18-1 in response to RWA2 feeding. Thus, Bd2-3, Bd3-1 and Bd18-1 were scored as resistant to RWA2. All the *B. distachyon* lines appeared to be resistant to RWA1.

The *B. distachyon* responses to greenbug feeding were similar to those seen in wheat, resembling leaf senescence, which developed much faster in susceptible genotypes than in resistant ones (Weng *et al.*, 2004). Lines Bd1-1, Bd2-3, Bd3-1 and Bd21 gave resistant response to GBC. Lines Bd18-1 and BD29 gave susceptible response to GBC. Differential response was also observed in response to GBE feeding. Bd2-3, Bd18-1 and BD29 gave resistant response and Bd1-1, Bd3-1 and Bd21 gave susceptible response.

Because the initial aphid infestation was large (on average more than 50 per seedling), the symptoms caused by greenbug or RWA feeding developed quickly in both wheat and *B. distachyon* plants. Usually, susceptible *B. distachyon* and wheat seedlings were killed by greenbug feeding within 10 d after infestation, and 2–3 weeks in the case of RWA. Resistant *B. distachyon* plants with no vernalization requirement (Bd3-1 and Bd21) were able to grow to maturity, while the aphid populations on them continued to decline.

Overall, except for Bd1-1 and Bd21, which had similar responses, all other *B. distachyon* accessions gave differential responses to the infestation by GBC, GBE and RWA2 (Table 1). Feeding response in all lines could be clearly rated as either 'R' or 'S' except in BD29, which showed symptoms intermediate between typical resistant and susceptible lines upon RWA2 infestation. Symptoms appeared similar between the inbred lines Bd2-3, Bd3-1, Bd18-1 and their respective parental lines BD2, BD3 and BD18 indicating no effect of inbreeding on aphid feeding response in these genotypes. The distinct responses of different accessions to the four biotypes were consistent in repeated experiments.

### *Microsatellite abundance and distribution in B. distachyon EST sequences*

A total of 12,798 kb of the expressed portion (ESTs) of the *B. distachyon* genome was scanned for microsatellite abundance, and the results are summarized in Table 2. Of 20,499 ESTs screened, 6,977 (34.03%) SSR-containing ESTs with bi-, tri-, tetra- or pentanucleotide SSR motifs were identified. A significant number of ESTs (19.26%) harboured more than one SSR. Overall, approximately 0.80% of the 12,798 kb EST sequences contained SSRs motifs with on average one SSR in every 1.85 kb EST sequence. Because the mono- and hexanucleotide SSR repeats were not counted in this study, comparing the SSR abundance of *B. distachyon* with other grass species was difficult. If only the di-, tri-, tetra- and pentanucleotide SSR motifs were considered, the abundance of SSRs in EST sequences of *B. distachyon* (Table 2) was very similar to that found in several cereal crop species such as rice, wheat and barley (La Rota *et al.*, 2005).

Analysis of the distribution of the four repeat types revealed that trinucleotide repeats predominated the coding regions of the *B. distachyon* genome, accounting for 75.7% of all SSRs identified (Table 2). The other three repeat types had similar frequencies (dinucleotide 7.3%, tetranucleotide 9.8% and pentanucleotide 7.2%) in the EST sequences analysed.

Among the 2,185 *B. distachyon* genomic sequences (1,222 kb) examined, 281 were found to contain 312 SSRs. Because the sample size was too small (0.34% of the 355 Mb *B. distachyon* genome), the SSR abundance and distribution features of the genomic DNA sequences were not compared with those of the EST sequences. From the 281 genomic SSRs identified, 21 were selected and used in the genetic diversity study.

**Table 2.** Abundance and motif distribution of SSRs in the *Brachypodium distachyon* genome

| SSR source | Sequence examined | SSR length (%) | SSR density (# per kb) | # SSRs | Distribution of motifs (%)[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Di | Tri | Tetra | Penta |
| EST | 12,798 kb | 0.80 | 0.54 | 6,977 | 7.30 | 75.7 | 9.80 | 7.20 |
| Genomic | 1,222 kb | 0.34 | 0.22 | 312 | 17.70 | 36.60 | 26.60 | 19.90 |

[a] Di, dinucleotide repeats; tri, trinucleotide repeats; tetra, tetranucleotide repeats; penta, pentanucleotide repeats.

### *SSR-based genetic diversity among diploid B. distachyon accessions*

From database mining, 181 new *B. distachyon* SSR markers were developed including 160 EST- and 21 genomic SSRs (see Supplementary Table 1, available online only at http://journals.cambridge.org for details). Of the 160 EST-SSRs, 24 failed to amplify any product in any line, but all 21 genomic SSRs had successful amplification in at least one DNA template tested.

The 136 EST-SSR markers detected in total 289 alleles with an average of 2.13 alleles per marker. In contrast, the 21 genomic SSRs detected an average of 4.0 alleles per locus. The usefulness of these SSR markers was also evaluated by their PIC values, which are listed in supplementary Table 1, available online only at http://journals.cambridge.org. As many as 45.9% of the 157 SSRs were monomorphic among the six accessions, in which 63 were EST-SSRs and nine were genomic SSRs. The average PIC for the 85 polymorphic SSRs was 0.44, ranging from 0.06 (BDeSSR012) to 0.83 (BDgSSR12). In agreement with the low PIC values, the SSR-based polymorphisms among the different *B. distachyon* accessions were generally low, ranging from 11.7% between Bd2-3 and Bd3-1 to 37.8% between BD29 and Bd21. The average polymorphism for Bd1-1, Bd2-3, Bd3-1, Bd18-1, Bd21 and BD29 across the other five accessions was 31.2, 22.8, 22.2, 23.5, 26.0 and 33.8%, respectively.

The effect of inbreeding on genetic diversity was compared in three pairs of *B. distachyon* lines. The polymorphism between each of the three inbred lines and their respective parental lines based on 139 EST-SSRs was 4.5, 4.2 and 4.8% for BD2, BD3 and BD18, respectively. Interestingly, except for the marker BDgSSR17 (see Supplementary Table 1, available online only at http://journals.cambridge.org) which detected two polymorphic alleles between Bd3-1 and BD3, no polymorphism was found with the remaining 20 genomic SSRs between all three pairs of lines.

### *Phylogenetic relationships of B. distachyon with other grass species*

Of the 373 alleles detected by 157 SSR markers, 258 (69.2%) were specific to the *B. distachyon* genome. In total, 59, 40 and 31 alleles were detected in the perennial ryegrass, *Ae. tauschii* and rice, respectively. It seems that there were no significant differences in cross-species transferability of EST versus genomic SSRs in this study. For example, of the 54 alleles detected in the ryegrass, 39 and 15 were identified by 32 EST (1.2 alleles per SSR) and 10 genomic (1.5 alleles per SSR) markers, respectively. Each EST and genomic SSR was able to detect, on average, 1.2 (28/24) and 1.7 (12/7) alleles, respectively, in the *Ae. tauschii* genome.

The phylogenetic relationships between the six *B. distachyon* accessions and three other grass species (rice, *Ae. tauschii* and perennial ryegrass) were evaluated using the City Block distance matrix-based hierarchical clustering and the genetic distance-based neighbour-joining clustering methods. The phylogenetic tree generated by the City Block approach is shown in Fig. 1, which was the same as that produced with the neighbour-joining method (data not shown). The genetic distance matrix of Nei and Li (1979) based on 1,000 bootstrapped datasets is presented in Table 3, which agreed very well with the phylogenetic relationships of the nine tested samples as revealed by the City Block-based analysis (Fig. 1). For example, from Table 3, the genetic distances of *Ae. tauschii* to *B. distachyon* (average over all accessions) and rice were 0.4623 and 0.7303, respectively, suggesting that *Ae. tauschii* was closer to *B. distachyon* than to rice.

## Discussion

In this study, six *B. distachyon* accessions were tested for their response to infestation by two cereal aphids: the greenbug (biotypes GBC and GBE) and Russian wheat aphid (biotypes RWA1 and RWA2). Different reactions

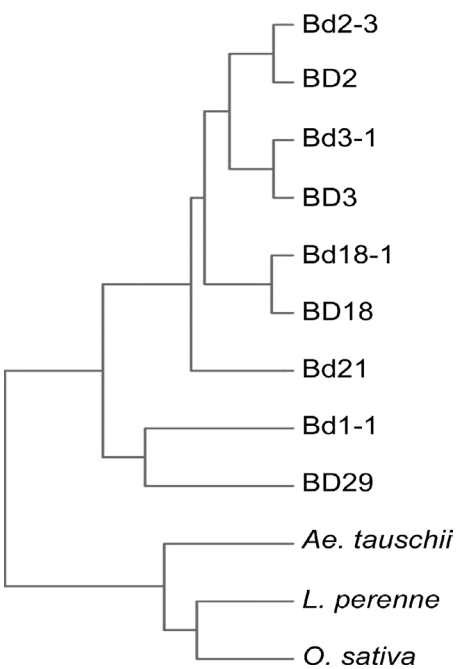


**Fig. 1.** Cluster analysis of microsatellite marker haplotypes of six *Brachypodium distachyon* (BD or Bd) accessions, rice (*Oryza sativa* cv. Nipponbare), perennial ryegrass (*Lolium perenne* cv. Palmer) and *Aegilops tauschii* (accession AL8/78). Haplotype clustering was based on similarity estimates using the 'City Block' approach.

**Table 3.** Genetic distance matrix (Nei and Li, 1979) among six *B. distachyon* accessions, rice (cv. Nipponbare), perennial ryegrass (cv. Palmer) and *Ae. tauschii* (accession AL8/78)

| | Bd1-1 | Bd2-3 | BD2 | Bd3-1 | BD-3 | Bd18-1 | BD18 | Bd21 | BD29 | Palmer | AL8/78 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bd1-1 | – | | | | | | | | | | |
| Bd2-3 | 0.0795 | – | | | | | | | | | |
| BD2 | 0.0740 | 0.0065 | – | | | | | | | | |
| Bd3-1 | 0.0735 | 0.0231 | 0.0222 | – | | | | | | | |
| BD3 | 0.0698 | 0.0237 | 0.0206 | 0.0064 | – | | | | | | |
| Bd18-1 | 0.0812 | 0.0234 | 0.0225 | 0.0300 | 0.0284 | – | | | | | |
| BD18 | 0.0878 | 0.0260 | 0.0262 | 0.0341 | 0.0336 | 0.0072 | – | | | | |
| Bd21 | 0.0874 | 0.0401 | 0.0363 | 0.0334 | 0.0341 | 0.0363 | 0.0381 | – | | | |
| BD29 | 0.0619 | 0.0769 | 0.0747 | 0.0697 | 0.0693 | 0.0753 | 0.0765 | 0.0779 | – | | |
| Palmer | 0.4088 | 0.3681 | 0.3727 | 0.3859 | 0.3851 | 0.3977 | 0.4293 | 0.3996 | 0.3923 | – | |
| AL8/78 | 0.5911 | 0.4894 | 0.4950 | 0.4936 | 0.4927 | 0.5191 | 0.4850 | 0.5566 | 0.5007 | 0.8044 | – |
| Nipponbare | 0.4045 | 0.4110 | 0.4160 | 0.4150 | 0.4140 | 0.4313 | 0.4493 | 0.4130 | 0.4037 | 0.2991 | 0.7303 |

among the six accessions to aphid feeding of GBC, GBE and RWA2 were observed (Table 1). Resistant and susceptible phenotypes could be easily identified. The feeding response in *B. distachyon* was similar to that observed in wheat. In addition, because no difference in feeding response was found between each of the three inbred lines (Bd2-3, Bd3-1 and Bd18-1) and their respective parental lines (BD2, BD3 and BD18), host resistance in the *B. distachyon* accessions to different greenbug or RWA biotypes may be controlled by simply inherited genes. In earlier experiments, we found that *Arabidopsis thaliana* was not a host of the greenbug (non-host resistance), and the greenbugs infesting rice (cv. Nipponbare) were able to survive but performed very poorly (Weng *et al.*, unpublished data). Considering the closer phylogenetic relationship of wheat with *B. distachyon* than to rice (Draper *et al.*, 2001; also see discussion below), the greenbug–*B. distachyon* and RWA–*B. distachyon* host–insect systems may prove to be useful models to study the molecular mechanisms of R gene-mediated aphid resistance in the grass genome.

One objective of this study was to develop molecular markers in *B. distachyon* that can be used to evaluate genetic diversity among *B. distachyon* accessions. We conducted database mining of the *B. distachyon* EST and genomic sequences for SSRs. It was found that the *B. distachyon* genome was abundant in microsatellite sequences, and the distribution of microsatellites in the *B. distachyon* genome was similar to that in wheat and barley (Table 2). Among the four types of SSR motifs examined in the *B. distachyon* EST sequences, over 75% belong to trinucleotide repeats (Table 2). The predominance of trinucleotide repeats in coding regions seems to be common in cereal genomes such as rice and wheat (La Rota *et al.*, 2005; Peng and Lapitan, 2005), which is probably the consequence of negative selection against frame-shift mutations in the coding regions (Metzgar *et al.*, 2000). By exploring the genomic resources, it is possible to develop a large number of new SSR markers for *B. distachyon*. This has proven to be a robust and efficient strategy for marker development in the cereal crop genomes (Gupta and Varshney, 2000; Theil *et al.*, 2003; Nicot *et al.*, 2004; Zhang *et al.*, 2005).

In this study, both EST-SSR and genomic SSR markers were developed and used in evaluating genetic diversity among the six *B. distachyon* accessions. As compared with EST-SSRs, each genomic SSR marker was able to detect twice as many alleles (on average 4.0 alleles per genomic SSR versus 2.13 alleles per EST-SSR). It has been well documented that EST-SSRs are less polymorphic than genomic SSRs in earlier studies in rice (Cho *et al.*, 2000), sugarcane (Cordeiro *et al.*, 2001), barley (Thiel *et al.*, 2003) and durum wheat (Eujayl *et al.*, 2002). Because EST-SSRs detected much fewer alleles, their usefulness in genetic mapping in *B. distachyon* is limited, and genomic DNA-derived SSRs should be employed. Large-scale development of these markers will be feasible soon as the whole genome sequence of *B. distachyon* is near completion.

From the number of alleles that each EST- or genomic SSR marker was able to detect, and the overall low PIC values of these markers (Supplementary Table 1, available online only at http://journals.cambridge.org), it is evident that the genetic diversity among the six *B. distachyon* diploid accessions tested in this study is generally low. This can also be seen from the SSR-based polymorphism levels among these lines, which varied from 11.7% between Bd2-3 and Bd3-1 to 37.8% between BD29 and Bd21. Calculation of the pairwise genetic distances among these *B. distachyo*n accessions (Table 3) indicated that BD29 was the most diverged from the other five lines. Our observations also showed that BD29 had the longest vernalization requirement (at least 6 weeks) among the six accessions. BD29 was originally collected from Ukraine, whereas the other

five lines were from either Iraq or Turkey (USDA Small Grain Collection, http://www.ars-grin.gov/). The high level of polymorphism in BD29 is correlated with its distinct geographical distribution separated over long distances from the other five lines.

The inbred line Bd21 was chosen for whole genome sequencing because of its small stature, no requirement for vernalization and short life cycle. In this study, the SSR-based polymorphism level between BD29 and Bd21 was the highest (37.8%) among all pairs of the six lines. The *B. distachyon* linkage map under development (Garvin *et al*., 2008) was from a cross between Bd21 and Bd3-1. However, the polymorphism level between these two lines (17.1%) was less than half of that between Bd21 and BD29. Therefore, it seems that either Bd1-1/Bd21 or BD29/Bd21 would be a good choice to develop segregating populations for linkage mapping in *B. distachyon*. Because both Bd1-1 and Bd21 are susceptible to the biotype E greenbug, which is the prevailing biotype in the field (Burd and Porter, 2006), the cross combination BD29/Bd21 seems to be the most promising for developing a mapping population based on genetic diversity, reaction to greenbug feeding and vernalization responses.

The phylogenetic relationships of *B. distachyon*, rice, *Ae. tauschii* and perennial ryegrass were also analysed using the SSR markers developed from the present study. The phylogenetic trees generated from the City Block approach (Fig. 1) and the neighbour-joining method were highly consistent suggesting that the SSR markers used in this study were robust for phylogenetic analysis. From the phylogenetic tree (Fig. 1) and genetic distance matrix (Table 3), it is evident that *Ae. tauschii*, the D genome donor of common wheat, is closer to *B. distachyon* than to rice. This result is consistent with conclusions from earlier studies (Shi *et al*., 1993; Hsaio *et al*., 1994; Catalán *et al*., 1995; Catalán and Olmstead, 2000; Vogel *et al*., 2006a; Bossolini *et al*., 2007) and supports the unique position of *B. distachyon* as a bridge between rice and temperate cereal crops for genomic research. Information from this study should be useful in genetic mapping and map-based cloning of greenbug or RWA resistance genes in *B. distachyon*.

## Acknowledgements

We thank Josh Evans for technical help. The authors are grateful to Drs David Garvin (USDA-ARS, St Paul, MN) for providing *B. distachyon* seeds, J.P. Michaud (Kansas State University, Hays, KS) and Gary Puterka (USDA-ARS, Stillwater, OK) for providing RWA used in this study. We also thank John Raupp for editing assistance in the preparation of this manuscript. This research was partially supported by the United States Department of Agriculture, National Research Initiative grant (CSREES 2006-35301-16892).

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