Diploid *Brachypodium distachyon* of Turkey: Molecular and Morphologic Analysis

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Abstract. Brachypodium distachyon is a model species for the grass family, Poaceae, which includes major cereal crops such as wheat and barley. The aim of this study were to assess morphological and phylogenetic relationships among diploid accessions of Brachypodium representing diverse geographic regions of Turkey based on Sequence related Amplified Polymorphism (SRAP) analyses. The similarity matrix indicated close relation among species used in the section using SRAP primer combinations, produced 156 fragment bands, of which 120 were polymorphic. Genetic distance ranged from 0.03 to 0.62. Plant genotypes were grouped into two major clusters based on SRAP analysis. There was a high level of diversity among the native diploid Brachypodium genotypes. These genotypes can be used for a better understanding of grass genomics.

Introduction

The genome sequences of the *Arabidopsis* (The Arabidopsis Genome Initiative 2000), and rice (Khan and Stace 1999) are already available and are a major resource for functional genomics. However, neither of these species serve as model for temperate grasses. *Arabidopsis* as a dicot species, it does not share with grass crops most of the biological features related to agricultural traits. Although rice would be a better alternative, the rice plant itself does not fulfill requirements such as the short size,

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rapid life cycle or ease of transformation. As a tropical species, it does not display all the agronomic traits that are relevant for temperate grasses, as resistance to temperate grass pathogens, freezing tolerance, vernalization or postharvest biochemistry of silage. Rice is also phylogenetically distant from the *Pooidae* subfamily that includes wheat, barley and temperate grasses (Catalan et al. 1995). Additionally molecular phylogenetic analysis has demonstrated that the genus *Brachypodium* diverged from the ancestral *Pooidae* clade immediately prior to the radiation of the modern 'core pooids' (*Triticeae*, *Bromeae* and *Avenae*) which includes the majority of important temperate cereals (Catalan and Olmstead 2000).

Brachypodium accessions have chromosome numbers ranging from 10 to 30 (Martín A and Sánchez-Monge-Laguna 1980). The reported size of the diploid *Brachypodium* ($2n = 2x = 10$) genome varies from 172 to 355 Mbp (Anamthawat-Jónsson et al. 1997; Draper et al. 2001), and, given that the former value may be an underestimate (Anamthawat-Jónsson et al. 1997), it is assumed to be approximately 355 Mbp. Its genome size is between the sizes of *Arabidopsis thaliana* with 157 Mbp, (Bennett and Leitch 2004) and rice with 490 Mbp (Bennett and Leitch 2004). GISH analysis of somatic chromosomes has shown the preponderance of repetitive DNA in the pericentromeric regions, reflecting the compactness of its genome (Sharma and Gill 1983).

The aim of this study was to assess morphological and phylogenetic relationships of diploid *Brachypodium* accessions sampled diverse geographic regions of Turkey based on sequence-related amplified Polymorphism (SRAP).

Materials and Methods

Plant Materials

A total of 500 *Brachypodium* individuals collected from diverse geographic regions of Turkey were firstly stratified at 4°C for 7–10 days in dark between the wetted filter papers in the petri plates. After cold treatment, they were put under light at room temperature. The germinated seeds were first transferred to peat-soil mixture in the viols and then grown in soil-pots under a 16/8-h (light/dark) photoperiod at the greenhouse.

DNA Extraction

DNA was extracted from leaves of 2-week-old seedlings using 1 ml of extraction buffer (50 mM Tris–HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1 mM 1, 10-phenathroline, and 0.15% 2-mercaptoethanol). The extract was incubated at 60°C for 1 h, and then mixed with equal volume of chloroform: isoamyl alcohol (24:1). After centrifuging at 12,000 rpm, the supernatant was transferred to a new tube and isopropanol was added and then incubated for 30 min at room temperature to precipitate the DNA. The pellet was dried, resuspended in 200 µl of TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) plus 20 µg of RNase, and then incubated at room temperature overnight. The DNA concentration was quantified by spectrophotometry (TKO100 Fluorometer, Hoefer Scientific Instruments, San Francisco).

Genetic relationships were evaluated using a combination of SRAP markers as reported by Budak et al. (2004a,b). Nuclear genome amplifications were carried out as follows: for 32 cycles of 1 min at 94°C; 1 min at 47°C and 37°C for SRAP analyses; 1 min at 72°C; followed by a final extension at 72°C for 5 min before cooling to 24°C. The PCR products (25 µl) were fractionated on 12% polyacrylamide gel using a Hoefer vertical-gel apparatus (SE600). Amplified fragments were visualized using ethidium bromide staining and photographed using a Gel Doc 2000 (Bio-Rad) (Hercules, CA).

Scoring Gels and Data Analysis

Presence or absence of each fragment was coded as "1" and "0", where "1" indicated the presence of a specific allele and "0" indicated its absence. The distance matrix and dendrogram were constructed using the Population Genetic Analysis (POPGEN32) version 1.32 software package. Nei's gene diversity (He) was used to compute Nei's standard genetic distance coefficients (Nei and Li 1979). NTSYS-pc version 2.1 software package (Rohlf 2000) was used for PCA analysis. Additionally, regression analysis using PROC REG (SAS, Cary, NC) was performed to determine associations between pair wise genetic distance from nuclear DNA data sets.

Flow Cytometry Analysis

Flow cytometry analysis was performed as described by Arumuganathan and Earl (1991) to identify the ploidy levels of the accessions sampled from Turkey. Mean DNA content was based on analysis of 1,000 nuclei. Each genotype was analyzed by four separate extractions and flow cytometric runs.

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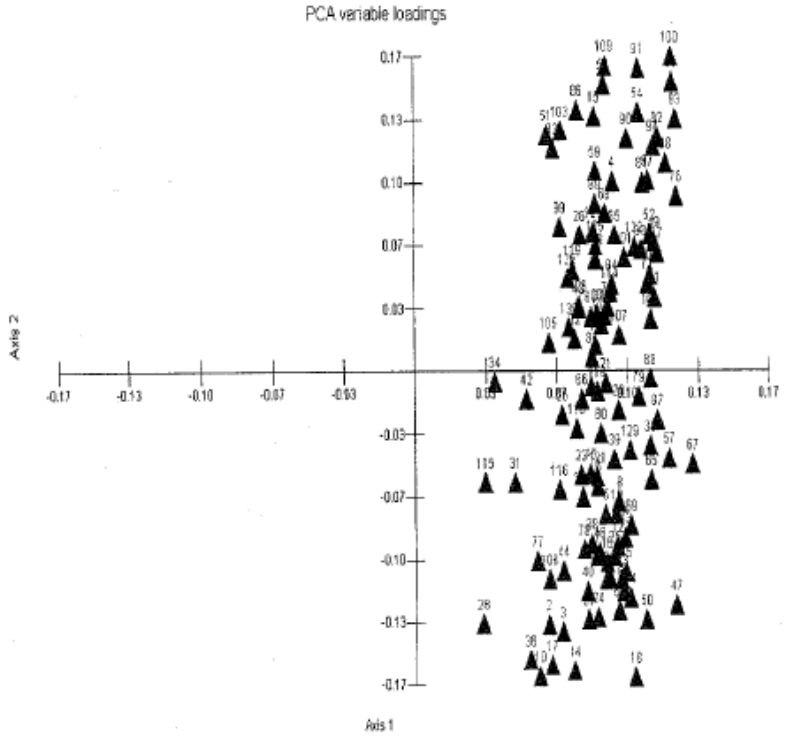

Fig. 2 Principal component analysis of diploid *Brachypodium* of Turkey

that germplasm from different geographical regions grouped together. The SRAP polymorphism detected in this study can be used to genetically classify *Brachypodium* genotypes However; morphological characteristics coupled with the nuclear DNA variation helped a better understanding of the classification and evolution of genotypes.

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