Available online at www.sciencedirect.com

ScienceDirect

The Crop Journal

Transposon-based genetic diversity assessment in wild and cultivated barley

Surinder Singh[a,b,1,2], Prabhjot Singh Nandha[a,1], Jaswinder Singh[a,*]

[a]Department of Plant Science, McGill University, 21,111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec H9X 3V9, Canada
[b]Department of Plant Sciences, University of Saskatchewan, 51 campus drive, Saskatoon, SK S7N 5A8, Canada

ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form
12 January 2017
Accepted 20 January 2017
Available online 8 March 2017

Keywords:
IMP
REMAP
IRAP
Polymorphic information content (PIC)
Discrimination power (D)

ABSTRACT

Transposable element-based molecular markers can be utilized to investigate genetic diversity and to create genetic linkage maps. In this study, Class I and class II transposons were employed to obtain a comparative account of genetic diversity between wild and cultivated barley genotypes. Three types of PCR-based techniques were used: IMP (Inter MITE Polymorphism), IRAP (Inter-Retrotransposon Amplified Polymorphism) and REMAP (Retrotransposon-Microsatellite Amplified Polymorphism). Specific primer pairs for IMP, IRAP, and REMAP detected a total of 200 bands with an average of 20 bands per marker. The mean polymorphic information content (PIC) and discrimination power (D) values in all 47 genotypes from these three types of transposon-based polymorphisms were 0.910 and 0.935, respectively. Unweighted Pair Group Method with Arithmetic mean (UPGMA)-based cluster analysis classified all 47 genotypes, both wild and cultivated, into separate groups consistent with their geographical origins. Sequencing followed by chromosome location of polymorphic bands enables precise gene introgression from wild gene pool to cultivated barley. The highly polymorphic nature of these marker systems makes them suitable for use in varietal identification and MAS-based breeding programs in barley and other cereals.

1. Introduction

Multilocus transposon-based molecular markers have great potential for genotyping, fingerprinting, and establishing genetic relationships between cultivars and wild accessions [1]. Transposable elements are present predominantly in the interspersed repetitive region, which comprises a large fraction of the genome in the majority of eukaryotic organisms [2]. Transposons are divided into two major classes. Class I retrotransposons transpose through an RNA intermediate, so that each transposition event creates a new copy of the transposon while the original copy remains intact at the donor site [3], and are accordingly found in high copy numbers. Class II transposons transpose by a cut-and-paste

* *Corresponding author.*
E-mail address: jaswinder.singh@mcgill.ca (J. Singh).
[1] Both these authors contributed equally as first authors.
[2] Current address.
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

http://dx.doi.org/10.1016/j.cj.2017.01.003

mechanism as double-stranded DNA and are moderately abundant in the genome with the exception of miniature inverted repeat transposable elements (MITEs), which may be present in moderate to high copy numbers [4]. Retrotransposons are further separated into two major subclasses that differ in structure and transposition cycle. These are the long terminal repeat (LTR) retrotransposons and the non-LTR retrotransposons (*LINE*s, Long Interspersed Nuclear Elements and *SINE*s, Short Interspersed Nuclear Elements), which are distinguished by the presence, or absence, of LTRs at their ends. Some examples of class II transposons in plants are *Ac/Ds*, *En/Spm*, *PIF*, *Mutator*, and MITEs [5]. Transposons have been conveniently used as molecular markers for performing fingerprinting and genetic similarity studies in plants [1] and their utility as molecular markers for plant breeding has been recently reviewed by [6]. Each transposition event in a high copy number transposon can generate an insertion polymorphism and thus can create many molecular markers. Many retrotransposable elements have been shown to be highly polymorphic for their insertion locations in various plant species [5,7–9]. Both class I and class II transposons have been used as marker systems in barley [9–11]. DNA fingerprinting techniques based on *BARE*-1 retrotransposon in barley, including inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon microsatellite amplified polymorphism (REMAP) have been applied [9]. Other retrotransposon-based methods include retrotransposon-based insertional polymorphism (RBIP) [12] and sequence-specific amplified polymorphism (SSAP) [7,13]. Likewise, DNA-based transposons have also been used for genotyping and genetic diversity studies using MITEs [10,14].

The present study aimed to evaluate the genetic diversity between a collection of wild (*Hordeum vulgare* subsp. *spontaneum*) and cultivated barley (*H. vulgare* subsp. *vulgare*) genotypes using both class I and class II transposons in order to assess the potential application of these transposon-based marker systems in barley breeding programs. Polymorphic information content (PIC) and discrimination power (D) values were calculated for each type of the transposon-based markers. Furthermore, cluster analysis was performed to establish the relationships among different genotypes, and finally some of the polymorphic bands were sequenced and located on barley genetic and physical maps.

2. Materials and methods

2.1. Plant material and growth conditions

Plant material consisted of seeds of 47 barley genotypes, which included 27 wild and 20 cultivated accessions (Table 1), obtained from plant gene resources of Canada (PGRC) Saskatoon, Canada. Plants were grown in a plant growth chambers at McGill University, Montreal, Canada in a 16:8 photoperiod regime with a daytime temperature of 22 °C and a nighttime temperature of 18 °C. Prior to seeding, the seeds of the wild barley genotypes were vernalized at 4 °C in the dark for 4 weeks.

Table 1 – Accessions of wild and cultivated barley genotypes.

Hordeum vulgare subsp. *spontaneum* (wild)			*Hordeum vulgare* subsp. *vulgare* (cultivated)			
S. No.	Accession number	Origin	S. No.	Accession number	Name	Origin
1	CN 26509-W	Turkey	28	CN 771	Valkie	Ukraine
2	CN 26556-W	Turkey	29	CN2047	Jou Abi	Afghanistan
3	CN 26840-W	Turkey	30	CN2098	Juliaca	Peru
4	CN 28025-W	Morocco	31	CN2107	Mianwali	Pakistan
5	CN 28520-W	Turkey	32	CN2127	Quinn	Australia
6	CN 28547-W	China	33	CN2449	Anatolian black	Turkey
7	CN 48319-W	Jordan	34	CN2523	Ga Ta Mi	China
8	CN 48378-W	Jordan	35	CN2574	Caucasian	Russia
9	CN 48462-W	Jordan	36	CN3807	Coast	Germany
10	CN 48480-W	Jordan	37	CN3847	Kwan	India
11	CN 48489-W	Jordan	38	CN42485	Chalottetown 80	Canada
12	CN 48502-W	Jordan	39	CN51811	Brier	Canada
13	CN 48674-W	Syria	40	CN60065	Hakkoku	Japan
14	CN 48861-W	Cyprus	41	CN81044	Tamang-Ny	Nepal
15	CN 48873-W	Cyprus	42	CN106429	AC Alma	Canada
16	CN 48897-W	Cyprus	43	CN106456	CDC Candle	Canada
17	CN 48900-W	Cyprus	44	CN106458	Harrington	Canada
18	CN 48908-W	Cyprus	45	CN106469	AC Hawkeye	Canada
19	CN 48911-W	Cyprus	46	CN106478	CDC McGwire	Canada
20	CN 48919-W	Cyprus	47	CN113916	Binscarth	Canada
21	CN 64691-W	Israel				
22	CN 64762-W	Israel				
23	CN 64764-W	Israel				
24	CN 64774-W	Israel				
25	CN 64793-W	Israel				
26	Damon-W	Israel				
27	Shechem-W	Israel				