Theor Appl Genet (2002) 104:868–873
DOI 10.1007/s00122-001-0816-x

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The molecular genetic characterization of the 'Bobwhite' bread wheat family using AFLPs and the effect of the T1BL.1RS translocation

Received: 8 November 2000 / Accepted: 27 April 2001 / Published online: 6 February 2002
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Abstract Bobwhite is a generic name that refers to all sister lines derived from the cross CM 33203 with the pedigree Aurora//Kalyan/Bluebird/3/Woodpecker made by the CIMMYT bread wheat program in the early 1970s. Individual sister lines can be distinguished by their unique selection history. One of the parents, Aurora, contains the T1BL.1RS translocation from rye, and approximately 85% of the sister lines have inherited the translocation. The sister lines demonstrate great variability for agronomic traits such as maturity, height, grain color, reaction to leaf rust, stem rust, yellow rust, septoria leaf blotch and powdery mildew. Certain groups of sister lines derived from particular F_1 plants can be distinguished by their phenotype. One hundred and one Bobwhite sister lines were fingerprinted using four AFLP enzyme/primer combinations. Following multivariate analysis, two main and very distinct clusters were found, which reflected the presence or absence of the T1BL.1RS translocation. Within these clusters, lines clustered together, for the most part, with other sister lines sharing a common selection history. Removal of the AFLP markers that were correlated with the presence or absence of the translocation caused lines to cluster based on pedigree alone. Therefore, the presence of translocations in wheat could bias genetic diversity studies using unmapped markers such as AFLPs that are located on the translocated segment(s), with the result that the resulting clusters will not reflect the true degree of relatedness.

Keywords Molecular diversity · AFLPs · Wheat Translocations · T1BL.1RS

Communicated by P. Langridge

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Introduction

The Bobwhite sister series are wheat lines developed by the CIMMYT bread wheat program in the early 1970s. The sister lines were generated from the top cross CM 33203 with the pedigree Aurora//Kalyan/Bluebird/3/Woodpecker. This cross resulted in a segregating F_1 population, from which further generations were selected from experiment stations in the Yaqui Valley, Sonora and Toluca in Mexico. By the late 1970s, these sister lines entered the CIMMYT International Nurseries and were distributed around the world. They have become important germplasm in many different programs, and between 1984 and 1991, 16 of the sister lines were released as commercial cultivars in Africa, South America and the Middle East. Because of their excellent regeneration ability, some Bobwhite selections have had great utility in transgenic work (Weeks et al. 1993). The sister lines are derived from a top-cross (F_1 × fixed line) and were selected from seven different F_1 plants. Therefore, the sisters demonstrate great variability for agronomic traits such as maturity, height and grain color; for reaction to leaf rust, stem rust, yellow rust and septoria leaf blotch; and for morphological traits such as leaf curling and awnedness. Certain groups of sister lines derived from particular F_1 and F_2 selections can be distinguished by their phenotype. One of the parents, Aurora, also carried the T1BL.1RS translocation from Petkus rye (Rajaram et al. 1983).

The use of molecular markers in the study of the diversity within domesticated species has become routine; in particular, the use of simple sequence repeats (SSRs) and amplified fragment length polymorphism (AFLPs) are promising methods for the fast and efficient estimation of relationships between lines and populations of a species. In wheat, AFLPs have been used for this purpose in a number of studies (Barrett and Kidwell 1998; Bohn et al. 1999; Ridout and Donini 1999). Because AFLPs are not routinely mapped, it is not possible to know the chromosomal location of each marker. It is assumed that the markers are randomly spaced throughout

the genome; however, evidence of clustering of AFLP markers has been seen in barley (Hayes et al. 1997). Furthermore, because only polymorphic AFLP markers are generally included in a diversity study, the incidence of high polymorphisms in a particular section of a chromosome will greatly bias the effect this section has on the study because a disproportionate number of the markers used in the study will come only from this region.

The CIMMYT Wheat Program has been active in introgressing genetic material from other species into wheat. In wheat breeding lines from CIMMYT, introgressions from closely related species have caused an abundance of translocated segments on many of the chromosomes; for example, the T1BL.1RS, the T1AL.1RS and the T6BS.6RL (Freibe et al. 1996; Islam-Faridi and Mujeeb Kazi 1995) and the *Lr19* (Singh et al. 1998) and *Lr25* (McIntosh et al. 1995) chromosomal regions conferring rust resistance to bread wheat. These translocated segments are expected to be highly dissimilar from homologous segments on chromosomes of individuals lacking the translocation. The effect of these translocations on the estimation of genetic diversity using unmapped molecular markers has not been adequately studied in the past.

The objectives of the investigation reported here were to study the patterns of diversity within the sister line series Bobwhite and to study the effect of the T1BL.1RS translocation on this diversity.

Table 1 List of Bobwhite sister lines (which are named with a number) and their selection history. Generations are read from left to right. The first letter refers to the F_1 plant from which the selections were made. In subsequent number-letter combinations, the number identifies the individual plant, and the letter indicates the location of selection (from Skovmand et al. 1997). The zero-letter combinations are reserved for populations carried as bulk in that generation. Pedigrees designated by a number followed by SH are reselections that occurred outside of CIMMYT, and no data are available for these individuals for generations following the SH designation

2=G-9M-5Y-0M-3SH
4=H-8M-1Y-0M-2SH
5=N-1M-2Y-0M-3SH
6=J-16M-1Y-0M-1SH
7=K-8M-1Y-0M-2SH
9=S-1M-1Y-0M-3SH
10=K-9M-24Y-0M-3SH
11=F-4M-4Y-1M-1Y-0M-3SH
15=F-9M-2Y-501M-500Y-0M-1SH
16=G-9M-2Y-501M-501Y-0M-3SH
17=J-24M-1Y-1M-1Y-0M-3SH
18=K-8M-1Y-1M-1Y-0M-2SH
19=K-9M-1Y-1M-1Y-0M-1SH
20=K-9M-1Y-1M-3Y-0M-2SH
21=K-9M-9Y-4M-1Y-0M-1SH
22=K-9M-9Y-4M-4Y-0M-1SH
23=K-9M-12Y-1M-0Y-1SH
24=K-9M-15Y-1M-4Y-0M-1SH
25=K-9M-33Y-1M-2Y-0M-2SH
26=K-12M-1Y-5M-5Y-0M-1SH
30=G-9M-5Y-1M-1Y-3M-3Y-0M-1SH
31=G-9M-5Y-1M-3Y-1M-0Y-2SH
32=G-9M-5Y-1M-3Y-2M-0Y-3SH
33=G-9M-5Y-1M-1Y-4M-2Y-0M-1SH
34=K-9M-1Y-6M-4Y-0M-2SH
36=K-9M-9Y-4M-1Y-1M-1Y-0M-1SH
37=K-9M-9Y-4M-1Y-1M-2Y-0M-2SH
39=K-9M-10Y-1M-5Y-2M-0Y-1SH
40=K-9M-14Y-1M-1Y-1M-1Y-0M-2SH
41=K-9M-14Y-1M-2Y-2M-0Y-3SH
42=K-9M-14Y-4M-1Y-1M-0Y-1SH
43=K-9M-14Y-4M-1Y-2M-0Y-3SH
44=K-9M-15Y-1M-4Y-2M-0Y-1SH
45=K-9M-15Y-1M-4Y-3M-1Y-0M-1SH
46=K-9M-19Y-3M-3Y-0M-1SH
47=K-9M-19Y-3M-3Y-1M-1Y-0M-1SH
48=K-9M-23Y-5M-1Y-2M-1Y-0M-1SH
49=K-9M-23Y-12M-1Y-1M-0Y-2SH
51=K-9M-24Y-1M-1Y-1M-1Y-0M-2SH
52=K-10M-7Y-3M-1Y-1M-0Y-3SH
53=K-9M-33Y-1M-2Y-2M-0Y-1SH
54=K-9M-33Y-1M-500Y-500M-500Y-0M-1SH
55=K-10M-7Y-3M-1Y-3M-0Y-1SH
56=K-12M-5Y-1M-0Y-2SH
57=K-12M-14Y-3M-0Y-3SH
58=K-12M-14Y-3M-0Y-1PZ-0Y-2SH
59=N-1M-2Y-500M-0Y-1SH
60=S-1M-5Y-4M-0Y-0PZ-0Y-1SH
63=H-4M-1Y-0M-74B-0Y-1SH
64=H-4M-1Y-0M-95B-0Y-1SH
65=H-4M-1Y-0M-153B-0Y-1SH
66=H-4M-1Y-0M-161B-0Y-1SH
67=H-4M-1Y-0M-164B-0Y-3SH
68=H-8M-1Y-2M-3Y-1M-0Y-3SH
69=K-9M-15Y-1M-4Y-3M-0Y-1SH
70=K-9M-33Y-1M-1Y-1M-0Y-2SH
71=H-8M-8Y-1M-1Y-1M-0Y-1SH
72=K-9M-2Y-1M-1Y-1M-0Y-1SH
73=K-9M-2Y-1M-1Y-2M-0Y-2SH
75=K-9M-9Y-4M-4Y-1M-1Y-0M-3SH
76=K-9M-19Y-3M-3Y-2M-1Y-0M-3SH
78=K-10M-7Y-3M-2Y-1M-0Y-1SH
79=K-9M-24Y-0M-15Y-0B-3SH
81=H-4M-1Y-0M-81B-0Y-3SH
83=H-8M-8Y-1M-1Y-2M-0Y-1SH
85=K-9M-24Y-0M-15Y-0B-0PZ-2SH
87=K-9M-33Y-1M-500Y-0M-1J-0J-1SH
88=K-9M-2Y-1M-1Y-1M-0Y-0PZ-2SH
89=K-9M-2Y-1M-1Y-2M-0Y-1PZ-2SH
91=K-9M-9Y-4M-4Y-1M-0Y-1PZ-1SH
92=K-9M-9Y-4M-4Y-1M-0Y-2PZ-1SH
93=K-9M-15Y-1M-4Y-3M-0Y-0PZ-1SH
94=K-9M-33Y-1M-500Y-0M-1J-0J-0PZ-3SH
95=K-10M-7Y-3M-2Y-1M-0Y-3SH
96=K-9M-9Y-4M-1Y-2M-0Y-2SH
97=K-9M-24Y-1M-1Y-1M-2Y-0M-1SH
98=K-9M-2Y-1M-1Y-2M-0Y-0PZ-0Y-2SH
99=K-9M-19Y-3M-3Y-2M-1Y-0M-0PZ-0Y-1SH
100=K-9M-19Y-3M-4Y-1M-0Y-1PZ-0Y-3SH
101=K-9M-24Y-1M-1Y-1M-1Y-0M-0YD-3SH
103=K-9M-1Y-1M-3Y-0M-100R-3SH
104=4SH
106=K-9M-15Y-1M-4Y-2M-0Y-0HL-3SH
107=?-1SH
109=42Y-1M-5Y-1M-4Y-5Y-0A-1SH
110=6A-4A-0A-1SH
111=K-10M-7Y-3M-2Y-1M-0Y-1B-2SH
112=M-8M-8Y-1M-1Y-1M-0Y-1T-2T-0ARG-3SH
113=K-9M-33Y-1M-500Y-0M-1J-0J-0ARG-1SH
114=11SH
116=H-8M-8Y-1M-1Y-1M-0Y-1PZ-0Y-2SH
117=K-9M-33Y-1M-500Y-0M-1J-0J-0MO-1SH
119=16SH
120=19SH
121=22SH
122=H-8M-8Y-1M-2Y-2M-0Y-?-1SH
124=25SH
125=H-8M-8Y-1M-1Y-1M-0Y-0ARG-1SH
126=30SH
127=H-8M-8Y-1M-2Y-2M-0Y-0PRY-2SH
128=M-8M-8Y-1M-1Y-1M-0Y-1T-2T-0ARG-2Y-05XM

Materials and methods

Sample preparation

Sister lines included in this study are shown in Table 1, along with their selection history. Four lines were analyzed twice, independently, to serve as internal controls in the study in order to provide an approximation of error rate; however, these lines were only included once in the final analysis. Leaves were harvested from each 8-week-old plant grown in the greenhouse and freeze-dried. Tissue was then ground and stored at –20 °C. DNA was extracted using the CTAB extraction method of Saghai-Maroof (1984) with minor modifications, according to Hoisington et al. (2000). Following RNAse digestion, an estimate of the quantity of DNA was obtained using a spectrophotometer (Beckman), and each sample was diluted to 0.3 µg/µl for storage at 4 °C.

T1BL.1RS translocation assay

Five seeds from each of the sister lines were subjected to cytological and biochemical analyses to diagnose the presence of entries with the T1BL.1RS translocation. Each seed was cut in two and the endosperm portion used first for biochemical analysis. The test involved the glucose phosphate isomerase assay (GPI) of Chojecki and Gale (1982). The corresponding embryo halves were germinated, and the root tips were sampled, prepared for analysis (Mujeeb-Kazi et al. 1994) and cytologically evaluated by Giemsa C-banding (Jahan et al. 1990). Some selected samples were also checked by fluorescent in situ hybridization (Islam-Faridi and Mujeeb-Kazi 1995) for their T1BL.1RS status.

Marker analysis

AFLP analysis was done according to Vos et al. (1995) but using a chemiluminescent protocol as described in Hoisington et al. (2000). Briefly, 1 µg DNA was digested serially with 5 u *Mse*I and 10 u *Pst*I, and double-stranded adaptors were ligated to the ends of the resulting fragments. Pre-amplified DNA was amplified using the following primer combinations: *Mse*I-CTA with *Pst*I-ACC and *Pst*I-AAG, and *Mse*I-CAA with *Pst*I-AAG and *Pst*I-ACC. The *Mse*I primers were labelled with digoxigenin (Operon). Resulting fragments were separated on a Bio-Rad (Bio-Rad, Hercules, Calif.) sequencing gel apparatus using 6% acrylamide:bisacrylamide (19:1) gels. Gels were blotted onto a non-charged nylon membrane, and the digoxigenin-labeled products were detected with anti-Dig AP (Boehringer Mannheim, Indianapolis, Ind.) and CSPD (Tropix). The membrane was used to expose X-ray film for 4–8 h, after which the film was developed and read by manual scoring of fragments.

Data analysis

Polymorphic AFLP fragments were read from the gels, and data was entered into a matrix of observations scored as either present (1) or absent (0) for each marker/Bobwhite line combination. The data was transformed to a matrix of similarity coefficients using the Jacaard, Dice, and Simple Matching methods. All matrices were compared using the Matrix Comparison function of NTSYS. Since all similarity matrices were highly similar ($R^2 \geq 0.95$), only the Simple Matching coefficient was used for further calculations. The resulting similarities between the lines were visualized in a dendrogram using the UPGMA clustering algorithm. All statistical calculations were done using NTSYSPC 2.02 (Rohlf 1999).

Results and discussion

Variation for AFLP markers

From the four primer/enzyme combinations scored in this study, a total of 273 AFLP bands were found to be reliably detected. Of these, 40 were polymorphic, which leads to a 14.7% polymorphism rate. This is lower than the rates reported in other studies of wheat diversity (Bohn et al. 1999; Ridout and Donini 1999) but reflects the high relationship of the sister lines in the study. The data for the four duplicated pairs of Bobwhite lines were identical at all 40 polymorphic markers except for one fragment in one pair. This indicates an overall error rate in gel reading and data input of $1/(4 \times 40) = 0.625\%$.

In the conservation of germplasm in ex situ genebank collections, a frequent consideration is the balance between conserving many accessions or bulking to decrease cost, at the possible risk of losing some of the diversity present in the bulked accessions. A considerable amount of genetic diversity was detected by the 40 AFLP markers (Fig. 1). Considering the range of genetic and phenotypic diversity that the Bobwhites display, it would not be recommended to bulk the sister lines in the genebank for convenience in storage and record keeping, despite identical pedigrees. Furthermore, considering the low cost of maintaining each line, there is no urgent financial need to do so at this time (Pardey et al. 2000). Many of the sister lines could not be distinguished based on the 40 markers (Fig. 1); these lines were generally related at the F_3 or greater (more similar) level. Therefore, it is not surprising that these lines could not be separated using only four AFLP primer/enzyme combinations, and more markers would be required to separate them.

Cluster analysis

When all of the markers were used, two very distinct clusters were formed (Fig. 1), which was not expected in a set of lines highly related by pedigree. These two clusters were distinguished in every case by the presence or absence of the T1BL.1RS translocation in the individuals belonging to each cluster. Because AFLPs are not generally located to chromosomal location, it is not possible to know in advance what chromosomal region they are illuminating in a genetic diversity study. Furthermore, only those AFLP fragments that were polymorphic in this study were included in the analysis. The region affected by the T1BL.1RS translocation is half a chromosome (1RS), which is not the majority of the genome; however, in a set of sister lines, (or in any self-pollinated species), the total amount of diversity as measured by molecular markers is usually quite low. Therefore, a small region of the genome that can be expected to be polymorphic in all markers of this region (such as a translocation from another species) can greatly influence the total amount of diversity in a study. The effect of the T1BL.1RS translocation is readily apparent in this data

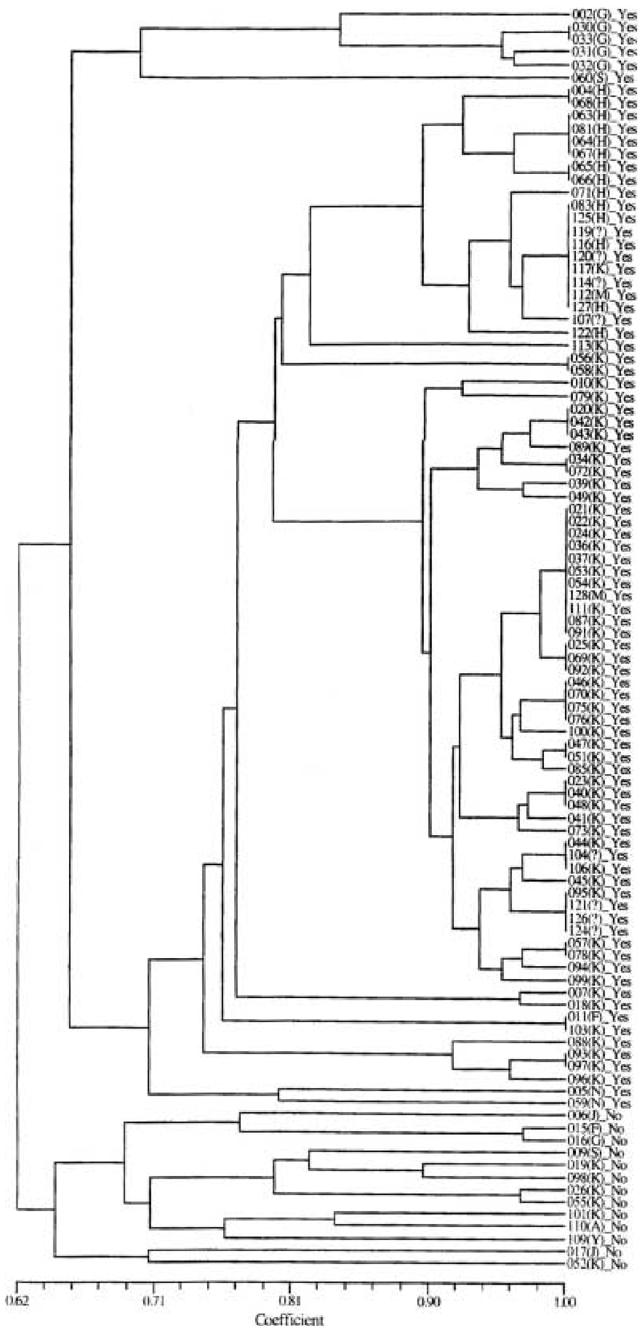

Fig. 1 UPGMA dendrogram of Simple Matching similarity coefficients produced from the 101 Bobwhite sister lines in the analysis. The Simple Matching pairwise matrix was generated using 40 polymorphic AFLP fragments

set, as it splits sister lines with identical pedigrees into two very distinct groups.

Within the two main clusters, the majority of the lines clustered with other lines sharing the same F_1 parent. At the 0.725 level of genetic similarity, four clusters formed among the lines carrying the translocation: one containing only lines derived from the F_1 plant known as G; one containing only lines derived from the F_1 plant known as S; one containing lines derived from the F_1 plants H, K, M, and F; one containing only lines derived from the F_1 plant N. At the 0.825 level of genetic similarity, the large cluster containing lines from four different F_1 plants could be broken into seven sub-clusters, each containing lines derived usually from the same F_1 plant. Similar results were found among the lines not carrying the translocation, although this group of lines tended to be more diverse. The fact that the lines tended to cluster based on pedigree is not surprising and will be useful for classifying those lines whose pedigree and selection history is not known. For example, there are eight Bobwhite lines for which there is no selection history, as these lines were re-selected outside of CIMMYT and returned to the CIMMYT Wheat Genetic Resources Center without information as to which F_1 Bobwhite plant had been used or how selection had taken place. In the majority of the cases, these lines fall directly into a cluster that has only (or very nearly so) lines derived from a single F_1 plant. In some cases, further generations can also be deduced using the AFLP data. We expect molecular markers to have an even greater utility in determining the pedigree for lines with no paternity data at all.

Effect of the translocation

Sister lines carrying the translocation were compared to those not carrying the translocation to find which markers were present in one group of lines but not the other. Four markers, or 10% of the total markers, were identified that were in only one group. The markers ACC/CTA:8 and AAC/CAA:12 were found exclusively in the lines carrying the T1B1.1RS lines, and markers AAG/CTA:7 and AAG/CTA:10 were found in the lines not carrying the translocation (with one exception). These markers can be considered diagnostic for the translocation itself and are being converted into STS markers. The effect of these four markers on the cluster analysis was checked by removing them from the analysis. The resulting dendrogram is shown in Fig. 2. As can be clearly noted, the lines no longer cluster on the presence or absence of the translocation. Most of the lines now cluster exclusively based on pedigree, as lines that had been separated out by the lack of the translocation now cluster quite close to other lines displaying a similar selection history.

Conclusion

It is essential to know in advance if a translocation is present in a set of lines for which genetic diversity will be measured using AFLPs. The translocation is certain to cause lines to look extremely different from other lines in the species, and the cause must be ascertained in these cases in order to avoid faulty conclusions about relatedness of these lines. In this case, 10% of the markers fell within the translocation, and in an analysis of sister lines where only one to two markers may separate lines, this is

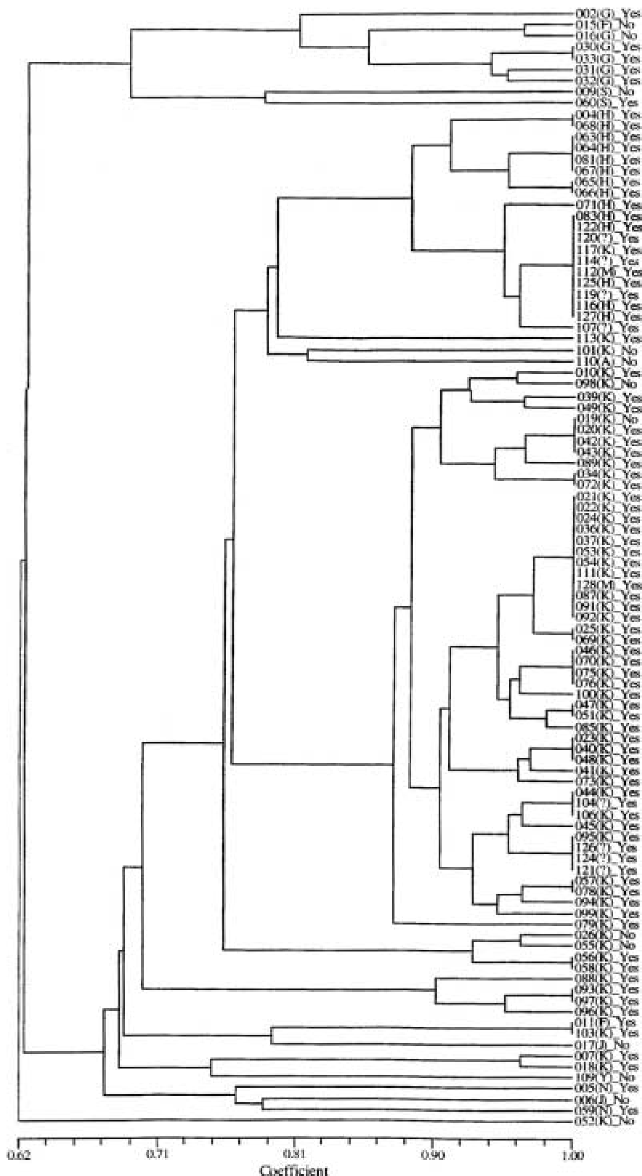

Fig. 2 UPGMA dendrogram of Simple Matching similarity coefficients produced from the 101 Bobwhite sister lines in the analysis. The Simple Matching pairwise matrix was generated using only the 36 AFLP fragments found to be unassociated with the T1BL.1RS translocation

a huge difference. Diagnostic markers can be developed to test for the presence of each class of alien translocation known in wheat. The CIMMYT Applied Biotechnology Center is currently working to identify such markers (both AFLP and SSR). These markers can be included as part of the set of markers used in routine genetic diversity studies, thus providing additional information on presence or absence of the translocations during the course of the study. Use of these markers would allow correct conclusions to be drawn regarding genetic diversity for lines containing these translocations.

The maintenance of very large germplasm collections, such as the wheat collection at the CIMMYT Wheat Genetic Resources Center (with over 152,000 Triticeae accessions) may be greatly aided by molecular marker analyses of genetic diversity. Markers have proven useful in determining relationships when no information on pedigree or origin is available, and in determining when not to bulk seeds from lines that appear to be highly similar based on pedigree, phenotype or origin. However, caution must be used when AFLP or other non-located markers are used to fingerprint wheat germplasm, as the presence of translocations from other species may bias the analysis and render an incorrect picture of genetic relatedness among the germplasm.

Acknowledgements The authors would like to thank Dr. S. Rajaram, Director, CIMMYT Wheat program, and Dr. D. Hoisington, Director, Applied Biotechnology Center, for their valuable input into the project; S. Ambriz and E. Villordo for their excellent technical assistance in the laboratory; and D. Poland for his review of the manuscript. Experiments conducted in this paper comply with all current Mexican laws.

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