Genetic variability analysis of a collection of old Portuguese bread wheat using ISSRs

Carvalho A., Lima-Brito J., Maçãs B., Guedes-Pinto H.

in

Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.).
Cereal science and technology for feeding ten billion people: genomics era and beyond

Zaragoza : CIHEAM / IRTA
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81

2008
pages 35-38

Genetic variability analysis of a collection of old Portuguese bread wheat using ISSRs

A. Carvalho*, J. Lima-Brito*, B. Maçãs and H. Guedes-Pinto***
*Centre of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, (CGB-UTAD), Apt. 1013, 5001-801 Vila Real, Portugal
**National Plant Breeding Station, 7350-951 Elvas, Portugal

SUMMARY – Forty-eight bread wheat cultivars of an old Portuguese collection using ISSR markers were analysed. We used 18 ISSR primers that amplified a total number of 245 ISSR *loci*, being 233 of them polymorphic. Considering the overall analysis, the percentage of ISSR polymorphism was lower, 40.6%, since different primers amplified ISSR fragments with the same molecular weight in all the cultivars. After clustering analysis, we verified that most cultivars belonging to the same botanical variety were clustered in the same main group. However, an intra-variety ISSR polymorphism was also observed.

Introduction

In the last few decades, the outcome of DNA molecular markers based in PCR, such as inter-simple sequence repeats (ISSRs; Zietkiewicz *et al*., 1994), became an excellent tool for plant breeders. ISSRs amplify a DNA region between two adjacent microsatellite regions using one arbitrary primer. These markers have been used for genetic variability analysis, cultivars and genotype identification, phylogenetic relationships determination and genomic fingerprint. Although ISSRs are dominant markers, they present advantages like being less time and cost consuming, reproducible and highly polymorphic. With this work, we analysed a collection of 48 old Portuguese bread wheat cultivars, that constitutes an excellent germplasm, using ISSRs in order to determine their phylogenetic relationships since they belong to different botanical varieties.

Material and methods

Total genomic DNA was extracted, using the Plant DNeasy kit (Qiagen), from young leaves of forty-eight bread wheat cultivars representing 9 botanical varieties. We used the botanical names from material passport. For the amplification reactions, we used: 20ng/µl genomic DNA; 1µl primer 0.5 µM (set 100/9; UBC); 10µl Taq-PCR-Master Mix (Qiagen) and 8µl distilled ultra-pure water (Qiagen). Amplifications were made on a Biometra thermocycler UnoBlock II under standard PCR conditions, except for primer annealing temperature that was 52ºC. The amplification products were loaded on agarose gels 1.5% stained with ethidium bromide and exposed to ultraviolet light. Each ISSR band was considered an ISSR *locus*. Reactions were repeated twice. Only reproducible bands were considered for the presence/ absence analyses. For clustering analysis we used the NTSYS PC 2.02 software.

Results and discussion

Table 1 presents the percentage of ISSR polymorphism obtained with each primer and the total mean percentage of polymorphism.

The total mean percentage (95.1%) was high and the mean number of ISSR bands amplified per primer was 13.6. Lowest ISSR polymorphism percentages were produced by primers 849 and 850, probably due to their sequences (GT repeats with 3'-Y anchored nucleotides; Table 1). Highest percentages of polymorphism (100%) were obtained with the dinucleotide repeats AG, GA, AC, CA and TC. Pentanucleotide 880 also produced 100% of polymorphism and it was previously reported as

a successful primer (Fernández *et al*. 2002). Similar results were previously obtained by our group, in durum and bread wheat DNA fingerprinting with ISSRs (Carvalho *et al*. 2005), and by Sarla *et al*. (2003) in different accessions of *Oryza nivara*. The high percentages of ISSR polymorphism could be explained by the variability detected among botanical varieties and among cultivars of the same botanical variety (Fig. 1).

Table 1. Total (T) and polymorphic (P) number of ISSR *loci*. Percentage of polymorphism per primer.

Primer (5'→3')	T	P	% P
807 (AG)8T	22	22	100
808 (AG)8C	22	22	100
810 (GA)8T	10	10	100
811 (GA)8C	10	7	70
812 (GA)8A	15	15	100
817 (CA)8A	13	13	100
823 (TC)8C	12	12	100
825 (AC)8T	12	11	91.7
826 (AC)8C	10	9	90
834 (AG)8YT	24	23	95.8
841 (GA)8YC	12	12	100
846 (CA)8RT	18	18	100
849 (GT)8YA	11	8	72.7
850 (GT)8YC	10	8	80
855 (AC)8YT	9	8	88.9
856 (AC)8YA	11	11	100
880 (GGAGA)3	9	9	100
888 BDB(CA)7	15	15	100
TOTAL	245	233	95.1

Y = C, T;
R = A, G;
B = C, G, T;
D = A, G, T.

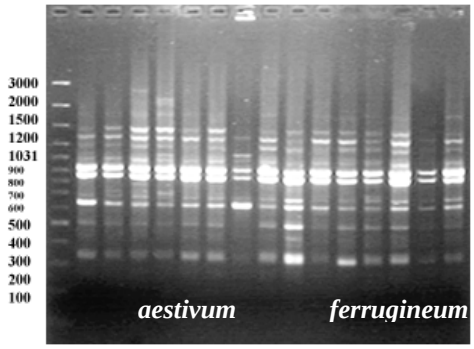

Fig. 1. ISSR products amplified by primer 846 and visualised on agarose gel 1.5% stained with ethidium bromide. Each lane represents a bread wheat cultivar and brackets are grouping cultivars of the same variety. First lane: Gene Ruler 100 bp DNA Ladder Plus (Fermentas).

In a second approach, we considered the overall ISSR dataset. Thus, the 18 primers amplified 32 ISSR fragments with molecular weights ranging from 3025 to 100 bp in all the 48 cultivars. Among these, only 13 ISSR *loci* were polymorphic, resulting in a total mean percentage of 40.6%. The mean number of ISSR *loci* amplified per cultivar was 25.4. All ISSR dataset were used for the construction of a dendrogram using the NTSYS software and the Unweighted Pair Group Mean Average method (UPGMA; Fig. 2). Clustering revealed two main groups: Group 1 with 29 cultivars and Group 2 with 19, while cultivar 31 (*T. aestivum* var. *ferrugineum* cv. 'Funchal') was clustered separately. Group 1 cultivars, mainly from var. *aestivum*, were clustered in two sub-groups, the smallest one constituted by 4 cultivars (23, 33, 4 and 82) from var. *milturum* and *lutescens*. Except for cultivar 14, all from var. *ferrugineum* were clustered in Group 2, as well as the varieties represented only by one cultivar: 36, 49, 87 and 100. Cultivars 94 and 96 belong to variety *plenoerythrospermum* but were placed in different clusters. Cultivar 96 was clustered with 74 and 84, which were grouped as being the closest: distance =2.0 (Group 1). Most cultivars belonging to same botanical variety were clustered in same main group, with few exceptions, supporting their original botanical classification. These markers were successful in the detection of inter-variety and intra-variety ISSR polymorphism.

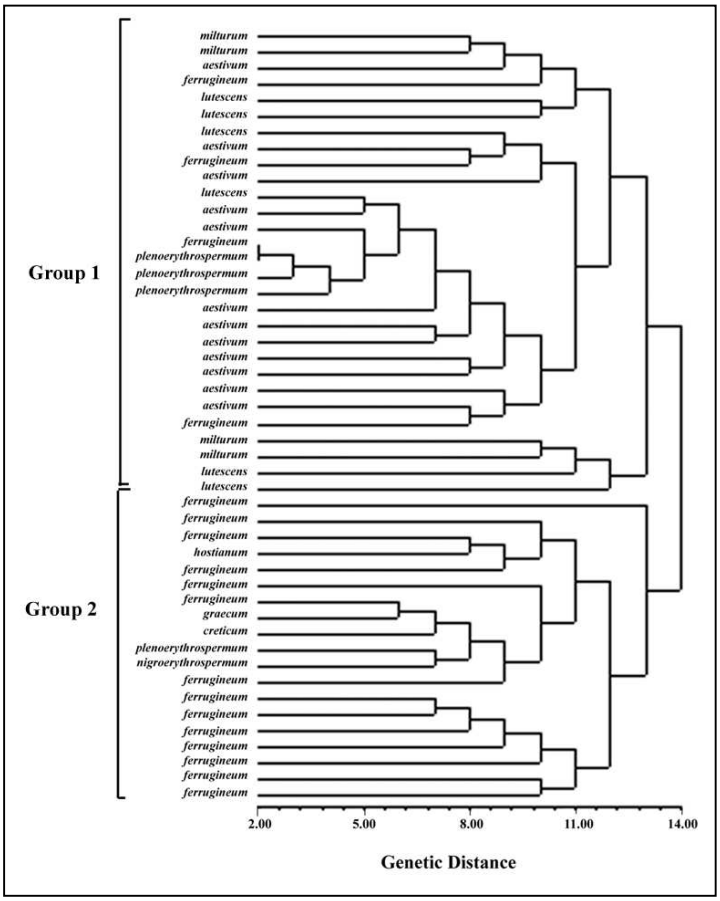

Fig. 2. UPGMA dendrogram of 48 bread wheat cultivars based on ISSR dataset. (Names of the nine botanical varieties are identified in the figure).

Acknowledgements

This work was supported by POCTI/AGR/35107/2000 project and research grant SFRH/BD/17348/2004, financed by the Portuguese Foundation of Science and Technology.

References

Carvalho, A., Matos, M., Lima-Brito, J., Guedes-Pinto, H., and Benito, C. (2005). *Euphytica,* 143: 93-99.

Fernández, M.E., Figueiras, A.M. and Benito, C. (2002). *TAG,* 104: 845-851.

Sarla, N., Bobba, S. and Siddiq, E.A. (2003). *Current Science,* 84(5): 683-690.

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). *Genomics,* 20: 176-183.