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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 59-61

Molecular characterisation of a Portuguese collection of durum wheat

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SUMMARY – Fifty-one cultivars of old Portuguese durum wheat (*Triticum turgidum* and *Triticum durum* sp.), belonging to 26 different botanical varieties, were analysed using ISSR markers. This collection constitutes an excellent germplasm repository for Portuguese durum wheat breeding programme. Amplified ISSR *loci* ranged from 150 to 3000 bp. The total mean percentage of ISSR polymorphism was 42.1%. All primers used allowed the detection of inter-variety and intra-variety ISSR polymorphisms.

Introduction

The Inter-Simple Sequence Repeats (ISSRS; Zietkiewicz *et al*., 1994) are dominant DNA markers widely used by plant breeders for genetic variability analysis, cultivars and genotypes identification, phylogenetics (Fernández *et al*., 2002) and DNA fingerprinting (Carvalho *et al*., 2005). These markers amplify the DNA region between two adjacent microsatellite regions inversely repeated with one arbitrary primer that could be 5'- or 3'-anchored. ISSRs are advantageous because they are less time and cost consuming, reproducible and present high levels of polymorphism. In the present work, we analysed a collection of 51 Portuguese durum wheat cultivars (grouped in 26 different botanical varieties) with ISSRs, in order to evaluate their genetic variability.

Material and methods

Plant material consisted on a Portuguese collection of 51 durum wheat cultivars representing 26 botanical varieties. We used the botanical names from material passport. Total genomic DNA was extracted from young leaves of each cultivar using the Plant DNeasy kit (Qiagen). For the amplification reactions, we used: 20ng/µl genomic DNA; 1µl primer 0.5 µM (set 100/9; UBC); 10 µl Taq-PCR-Master Mix (Qiagen) and 8 µl distilled ultra-pure water (Qiagen). Amplifications were made on a Biometra thermocycler UnoBlock II under standard PCR conditions, except for the primer annealing temperature, which was 52ºC. The amplification products were loaded on agarose gels 1.5% and visualised after staining 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. An UPGMA dendrogram was constructed using the NYSYS 2.02 PC version software.

Results and discussion

Most of the primers used here amplified 100% of polymorphic ISSR *loci* in the 51 durum wheat cultivars, resulting in a total mean percentage of ISSR polymorphism of 98.6%. The mean number of ISSR fragments amplified by primer was 15.7. However, if we considered all ISSR dataset, obtained with the 18 primers in the 51 cultivars, we verify that 38 ISSR fragments with different molecular weights, ranging from 3000 to 150 bp, were amplified. Among these, 16 ISSR fragments were monomorphic in all the cultivars and ranged from 2000 to 400 bp. Thus, the total mean percentage of ISSR polymorphism obtained in this work was 42.1%. All ISSR primers amplified polymorphic *loci* among the 26 botanical varieties and among cultivars belonging to the same botanical variety (Fig. 1).

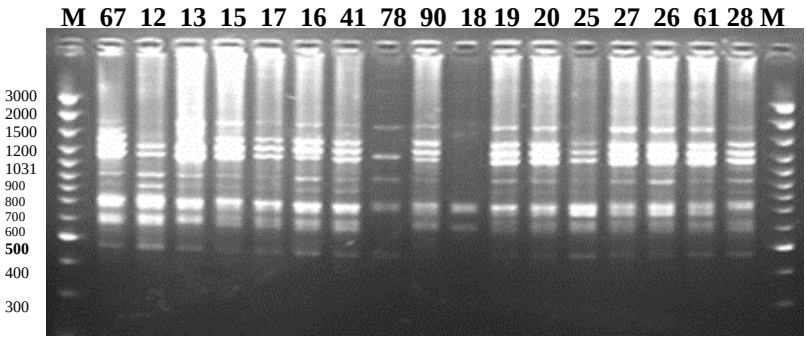

Fig. 1. ISSR products amplified with primer 817, visualised on an agarose gel 1.5% stained with ethidium bromide. Each lane contains a different durum wheat cultivar (identified by its code number). M – Molecular weight marker Gene Ladder 100 bp Plus (Fermentas).

Cultivars 16, 41, 78 and 90 belong to the botanical variety, *T. durum affine*, but they presented different band patterns revealing intra-variety ISSR polymorphism. The same was observed for cultivars 25 and 27 (both, *T. durum provinciale*). This intra-variety ISSR polymorphism was confirmed at the UPGMA dendrogram where cultivars of the same variety were clustered separately (Fig. 2). Inter-variety ISSR polymorphism was also detected.

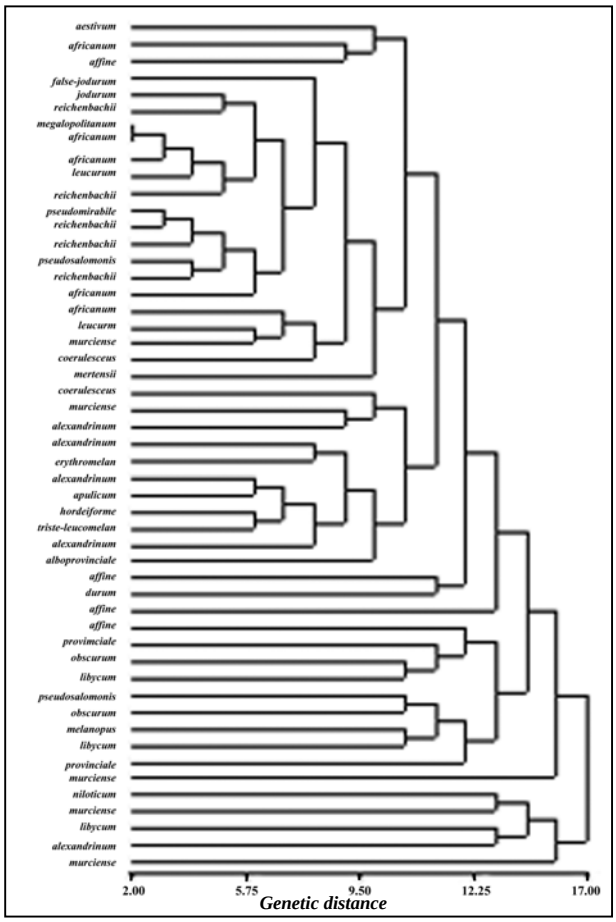

Fig. 2. UPGMA dendrogram of 51 durum wheat cultivars based on ISSRs dataset. (Names of the 26 botanical varieties are identified in the Figure).

ISSR-PCR technique revealed to be a powerful tool for detection of intra-variety ISSR polymorphism and, as we expected, it was also observed inter-variety polymorphism.

Acknowledgements

Work supported by POCTI/AGR/35107/2000 project and research grant SFRH/BD/17348/2004 financed by FCT.

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.

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