Use of ISSR markers for cultivar identification in durum wheat

Pasqualone A., Lotti C., Bruno A., De Vita P., Di Fonzo N., Blanco A.

in

Royo C. (ed.), Nachit M. (ed.), Di Fonzo N. (ed.), Araus J.L. (ed.).
Durum wheat improvement in the Mediterranean region: New challenges

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 40

2000
pages 157-161

Use of ISSR markers for cultivar identification in durum wheat

A. Pasqualone*, C. Lotti, A. Bruno**, P. De Vita***, N. Di Fonzo*** and A. Blanco****
*Institute of Agricultural Industries, University of Bari, Via Amendola, 165/A, I-70126, Bari, Italy
**Institute of Plant Breeding, University of Bari, Via Amendola, 165/A, I-70126, Bari, Italy
***Experimental Institute for Cereal Research, sez. Foggia, S.S. 16 km 675, I-71100, Foggia, Italy

SUMMARY – Current methods for varietal identification of durum wheat, based on seed protein analysis, have a limited effectiveness since protein polymorphism is not so high. Subsequently, new more polymorphic analytical methods are required to avoid the possibility of seed mixture or substitution of high quality varieties with poor quality ones in commercial transactions. The inter simple sequence repeats (ISSR) are a new kind of molecular marker involving PCR amplification of DNA by a single primer composed of a repeated sequence anchored at the 3' or 5' end by 2-4 arbitrary nucleotides. The aim of this work is to test the efficiency of ISSR markers to distinguish a set of 30 Italian durum wheat cultivars and 22 breeding lines. The efficiency was found very high and two primers were found sufficient to distinguish all the durum wheat cultivars examined.

Key words: PCR, ISSR markers, durum wheat, cultivar identification.

RESUME – *"Utilisation de marqueurs ISSR pour l'identification des cultivars chez le blé dur". Les méthodes actuellement utilisées pour l'identification variétale du blé dur, basées sur l'analyse des protéines du caryopse, ont une efficacité limitée car le polymorphisme des protéines n'est pas très élevé. Conséquemment, de nouvelles méthodes analytiques sont nécessaires pour éviter la possibilité de mélanger des variétés de bonne qualité avec celles de faible qualité pendant des transactions commerciales. Les marqueurs ISSR (inter simple sequence repeats) sont un nouveau type de marqueur moléculaire nécessitant une amplification de l'ADN par PCR en utilisant un oligonucléotide d'amorce composé d'une séquence répétée liée, en position 3' ou 5', à 2-4 nucléotides arbitraires. L'objectif de cette étude est d'évaluer l'efficacité des marqueurs de type ISSR pour distinguer un groupe de 30 cultivars italiens de blé dur et de 22 lignées en homozygotie avancée. L'efficacité de la méthode a été très élevée vu que seulement deux amorces furent nécessaires pour distinguer tous les types de blé dur examinés.*

Mots-clés : *PCR, marqueurs ISSR, blé dur, identification des cultivars.*

Introduction

Current methods for varietal identification of durum wheat are based on seed protein analysis by electrophoresis or by HPLC, both providing a seed protein profile as an electrophoretic pattern or a chromatogram. However, the effectiveness of protein analysis for varietal identification is limited since protein polymorphism is not so high. Thus, closely related cultivars may be indistinguishable (Pasqualone *et al.,* 1998). Subsequently, seed mixture or substitution of high quality varieties with poor quality ones in commercial transactions would still be possible.

New more polymorphic analytical methods like those based on DNA analysis are under investigation. Previous work demonstrated the applicability of DNA microsatellite analysis to varietal identification in durum wheat lots of both seed and semolina (Pasqualone *et al.,* 1999). However, the need of several assays to achieve complete identification with microsatellites induced in searching for more informative methods requiring a minor number of analysis.

The inter simple sequence repeats (ISSR) are a new kind of molecular marker involving PCR amplification of DNA by a single primer 16-18 bp long composed of a repeated sequence anchored at the 3' or 5' end by 2-4 arbitrary nucleotides (Zietkiewicz *et al.,* 1994). They are easy to handle, highly informative and repeatable. Since repeated sequences are abundant throughout the genome, SSR primers anneal in several regions typically giving a complex amplification pattern in which fragments are often polymorphic between different individuals.

Our aim was to test the efficiency of ISSR markers to distinguish both seeds and semolinas from a set of 30 Italian durum wheat cultivars and 22 breeding lines.

Materials and methods

Samples. Thirty durum wheat cultivars and 22 breeding lines, reported in Fig. 1, were used.

DNA extraction. DNA extraction from durum wheat seeds and from semolina was done as in Chunwongse *et al.* (1993).

Polymerase chain reaction. Primers of ISSR markers (Table 1) were synthesised by Biotechnology Laboratory of British Columbia University (Vancouver, British Columbia, Canada). Amplification reaction was performed as described by Zietkiewicz *et al.* (1994) in a Peltier Thermal Cycler PTC-200 (MJ Research).

Amplification product analysis and detection. Amplification products were separated by electrophoresis in 4% denaturing gels 0.4 mm thick, 50 cm long, containing 7M urea. PCR products were mixed with an equal volume of loading buffer containing 98% formamide, 0.1% bromophenol blue and 0.1% xylene cyanol. The mixture was denatured at 100ºC for 5 min and a 5 µl volume of each sample was analysed. Gels were run at 80 W for 4 hours in TBE 1x buffer (100 mM Tris-borate, pH 8.0, 2 mM EDTA) using a Sequi-Gen Sequencing Cell (BioRad). The amplification patterns were detected by silver staining of the gels as described by Bassam *et al.* (1991).

Results and discussion

In order to evaluate the efficiency of ISSR markers for durum wheat varietal identification 9 primers (Table 1) were tested against 30 cultivars and 22 breeding lines (Fig. 1). Primers, 16-18 bp long, were representative of all types of repeated sequence. Since during a first set up of the method the amplification patterns obtained using UBC-803 and UBC-872 primers resulted scarcely repeatable, due to a very low annealing temperature (ranging from 31 and 35ºC), only primers annealing to template DNA at 54ºC were considered afterward. All primers amplified several DNA regions giving a complex amplification pattern that made us able to analyse simultaneously many loci and to detect more polymorphic fragments with a unique amplification reaction.

Table 1. Primer names, sequences, annealing temperatures and polymorphism degree expressed as polymorphic/amplified bands ratio[†]

Primer name	Sequence (3'-5')	Annealing temperature (ºC)	Number of amplified bands	Number of polymorphic bands	Polymorphic/ amplified bands (%)
UBC 803	$(AT)_8C$	31	*	*	*
UBC-808	$(AG)_8C$	54	32	6	18.7
UBC-818	$(CA)_8G$	54	24	5	20.8
UBC 820	$(GT)_8C$	44	*	*	*
UBC-841	$(GA)_8YC$**	54	24	3	12.5
UBC-854	$(TC)_8RG$**	44	*	*	*
UBC-856	$(AC)_8YA$**	54	37	14	37.8
UBC 872	$(GATA)_4$	35	*	*	*
UBC-873	$(GACA)_4$	54	30	4	13.3
Mean value		*29.4*	*6.4*		*20.6*

[†]*Not repeatable; **type of degenerate nucleotide: Y = pYrimidine (C, T); R = puRine (A, G).

The polymorphism degree detected by each primer, expressed as polymorphic/amplified bands ratio, was calculated (Table 1). For the best evaluation, only well visible bands were considered. Primer UBC-

856 led to the greatest number of polymorphic bands thus having a high polymorphic/amplified bands ratio, equal to 37.8%. This is consistent with the findings of Nagaoka and Ogihara (1997) reporting $(AC)_n$ based primers as the most polymorphic ones.

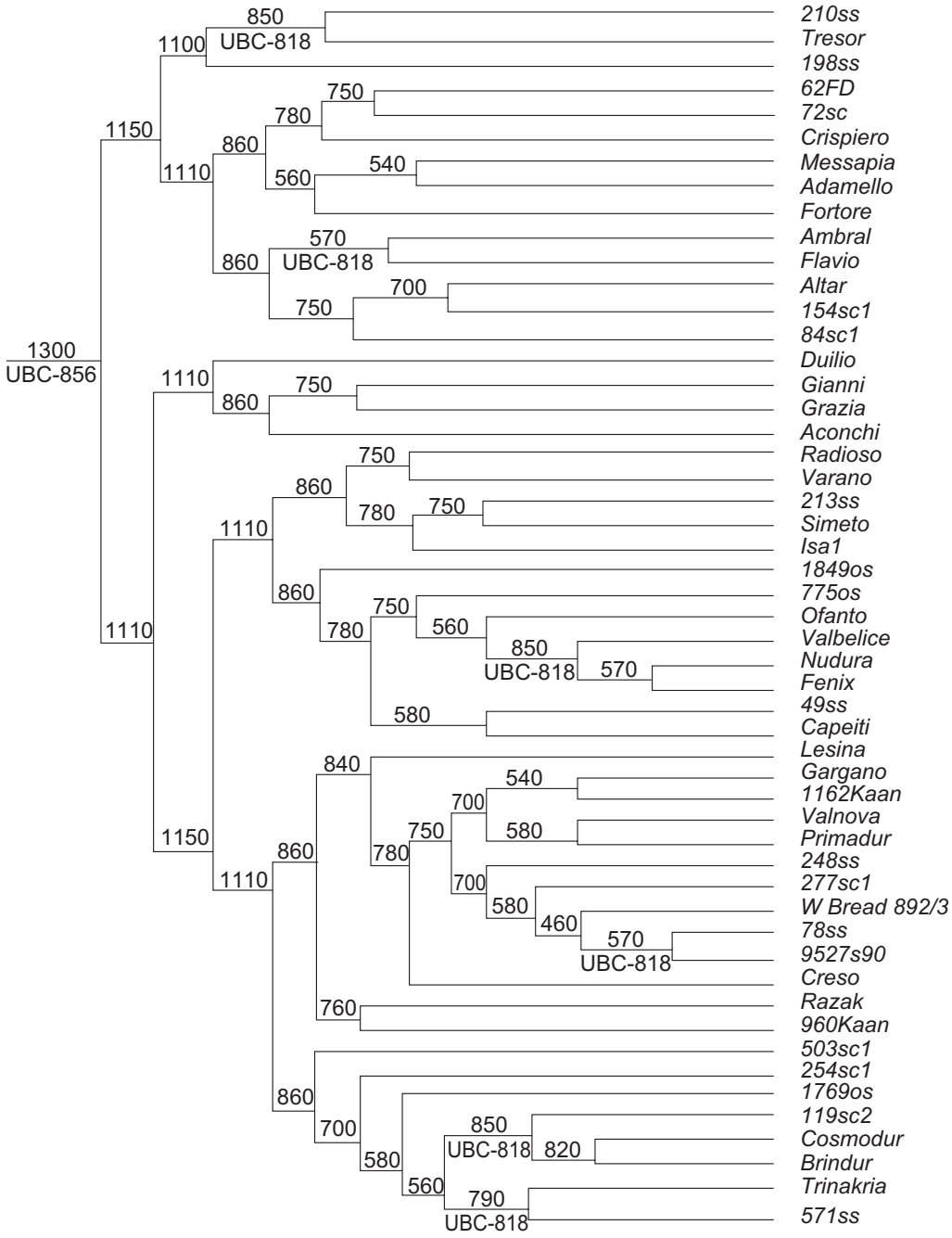

Fig. 1. Identification key of examined durum wheat cultivars and breeding lines, based on primers UBC-856 and UBC-818. Each branch corresponds to a different marker, whose dimensions in bp are reported, with the upper part corresponding to presence and the lower to absence of band.

Table 2 reports the values of Diversity Index (DI) calculated for all ISSR polymorphic bands, e.i. markers, analysed. Diversity index value permits to evaluate the discriminating ability of each marker and is determined as follows: $DI = 1 - \Sigma\, p_{ij}^2$, being p_{ij} the frequency of the *i*th pattern for *j*th marker (Botstein *et al.,* 1980). Since ISSR markers are of dominant type, for each polymorphic band only two patterns were possible: either presence or absence of band itself among the examined cultivars. For each polymorphic

band the frequency of presence was calculated as well as that of the absence and, on their basis, DI was calculated. The higher is DI value, which ranges, for dominant markers, from 0 to 0.5, the higher is the marker informativeness. DI values obtained for ISSR markers among the 52 cultivars and breeding lines examined were found to be acceptably high, with an overall mean value of 0.38. Five markers resulted to be extremely polymorphic: UBC-808_{840}, UBC-808_{750}, UBC-818_{570}, UBC-818_{850} and UBC-856_{750}, all having DI value of 0.5. On the contrary, a very low value was found for markers UBC-841_{960}, UBC-856_{840} and UBC-856_{1200}, due to their poorly informative distribution among examined cultivars. These markers, infact, showed the same pattern in almost all different samples, being identical in 48 cultivars over 52 in case of UBC-841_{960} and UBC-856_{1200} and even in 50 cultivars in case of UBC-856_{840}. Anyway, their low discriminating ability may be compensated by other markers genereted by the same primer, as in case of primer UBC-856.

Table 2. Diversity index of ISSR markers

ISSR marker	DI	ISSR marker	DI	ISSR marker	DI
UBC-856_{1300}	0.39	UBC-808_{885}	0.37	UBC-873_{830}	0.49
UBC-856_{1200}	0.14	UBC-808_{875}	0.49	UBC-873_{740}	0.47
UBC-856_{1150}	0.44	UBC-808_{840}	0.50	UBC-873_{610}	0.48
UBC-856_{1110}	0.49	UBC-808_{810}	0.44	UBC-873_{550}	0.41
UBC-856_{860}	0.49	UBC-808_{765}	0.43		
UBC-856_{840}	0.07	UBC-808_{750}	0.50	UBC-818_{850}	0.50
UBC-856_{780}	0.31			UBC-818_{820}	0.49
UBC-856_{750}	0.50	UBC-841_{960}	0.14	UBC-818_{570}	0.50
UBC-856_{700}	0.31	UBC-841_{710}	0.20	UBC-818_{650}	0.37
UBC-856_{580}	0.33	UBC-841_{530}	0.31	UBC-818_{790}	0.20
UBC-856_{560}	0.46				
UBC-856_{540}	0.39				
UBC-856_{460}	0.45				
UBC-856_{430}	0.23				

By combining the amplification patterns from two primers only, namely UBC-856 and UBC-818 – that were the most polymorphic one in terms of polymorphic/amplified bands ratio – it was possible to distinguish seeds and semolinas from all cultivars and breeding lines examined.

Finally, an identification key (Fig. 1), based on these two primers, was obtained. The key was built distinguishing in a dichotomic way, for each marker, cultivar groups showing presence of band from those where the band was absent. Cultivars presenting the same amplification pattern, when amplified with UBC-856 primer, resulted distinguishable by using UBC-818 primer.

Conclusions

As few as two primers resulted sufficient to distinguish 52 durum wheat cultivars and breeding lines indicating a very good discriminating ability of ISSR technique, even higher than microsatellite analysis. The method, however, like all molecular marker based methods, is expensive. It is possible to suggest its application for cases resulting not distinguishable with more economic analysis, above all to settle legal conflicts of a certain relevance.

References

Bassam, B.J., Caetano-Anolles, G. and Gresshoff, P.M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.,* 196: 80-83.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.,* 32: 314-331.

Chunwongse, J., Martin, G.B. and Tanksley, S.D. (1993). Pre-germination genotypic screening using PCR amplification of half-seeds. *Theor. Appl. Genet.,* 86: 694-698.

Nagaoka, T. and Ogihara, Y. (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet*., 94: 597-602.

Pasqualone, A., Lotti, C. and Blanco, A. (1998). Situazione attuale dell'identificazione varietale in frumento duro e potenzialità offerte dall'analisi dei microsatelliti. In: *Proc. VI Int. Meeting on Durum Wheat*, Trocoli, A., De Vita, P. and Di Fonzo, N. (eds), Foggia, Italy, 30 April-2 May 1998, pp. 225-240.

Pasqualone, A., Lotti, C. and Blanco, A. (1999). Identification of durum wheat cultivars and monovarietal semolinas by analysis of DNA microsatellites. *Eur. Food Res. Technol.*, 210: 144-147.

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeats (SSR)-anchored polymerase chain reaction amplification. *Genomics,* 20: 176-183.