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BRIEF COMMUNICATION

Genetic diversity of durum wheat as determined by AFLP in fluorescence

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Abstract

The DNA of fifteen Italian cultivars of durum wheat (*Triticum turgidum* L. ssp. *durum*) were analyzed by in fluorescence amplified fragment length polymorphism (fAFLP) in order to obtain the characteristic fingerprintings of genotypes and assess their genetic relatedness. Among 64 combinations of fluorescence labelled primers, three different combinations were chosen as producing a total of 6630 AFLP fragments, 2277 (34.3 %) of them being polymorphic. By using this fAFLP methodology a DNA fingerprinting of each durum wheat cultivar was generated for genotype identification. Analysis of the genetic relationships show the low variability among durum wheat cultivars.

Additional key words: genetic relationship, genotype identification, pattern analysis, *Triticum turgidum* L. ssp. *durum*.

The diversity of wheat cultivars and breeding lines has been conventionally estimated on the basis of morphological (Beuningen *et al*. 1997, Palumbo *et al.* 2006) and quantitative traits (Spagnoletti Zeuli and Qualset 1987), disease resistance (Sawhney *et al*. 1992) and storage proteins (Boggini and Pogna 1989, Pogna *et al.* 1990). However, the kind of traits do not often reliably portray genetic relationships among genotypes because of environmental and epistatic interactions. Random amplified polymorphic DNA (RAPD) markers, microsatellites, simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP) are routinely employed for genetic diversity studies in different plant systems (Dikshit *et al.* 2007, Joshi and Dhawan 2007, Yao *et al.* 2008). RAPD analysis has been widely used for the detection of genetic polymorphisms in *Triticum* species and a great numbers of loci linked to useful markers have been identified (Devos *et al*. 1992). Recently a range of specific SSR primer pairs for *Triticum* have been made available (Gupta *et al*. 2003, Song *et al*. 2005). AFLP technique has been widely used for genotype description of plant species and to assess the degree of biodiversity within populations (Vos *et al*. 1995, Christopher and Domini 1999). AFLP is the most promising technique compared to the other available methods as a tool for the identification of wheat cultivars and several studies indicate that AFLPs offer a high level of utility (Powell *et al*. 1996, Bohn *et al*. 1999, Fichera *et al.* 2006).

A previous study identified 461 wheat chromosome-specific AFLP markers in 21 accessions of *Triticum turgidum* ssp. *durum,* and then converted in sequence-specific PCR primers for many genetic linkage applications (Shan *et al*. 1999). A successive research studied 15 durum wheat genotypes with 18 primer combinations and revealed 189 polymorphic bands (Incirli and Akkaya 2001). A further study detected 89 polymorphic markers among 130 durum wheat genotypes resulting in an average of 8.9 polymorphic bands per primer pair (Soleimani *et al*. 2002).

Automated fluorescence dye-labelled AFLP technique (fAFLP) delivers the required resolution, precision and analytical power for large-scale genomic fingerprinting, with the advantage of being more highly reproducible and faster than plain gel slabs (Schwarz *et al*. 2000, Hayashi *et al*. 2005). The aim of this study was to characterize durum wheat cultivars by fAFLP and to determine their genetic relationships.

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Abbreviations: EtBr - Ethidium bromide; fAFLP - in fluorescence amplified fragment length polymorphism; HMM - high molecular mass; LMM - low molecular mass; SSR - simple sequence repeat; TBE - Tris-borate EDTA; UPGMA - unweighted pair group method with arithmetic average.
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Seeds of *Triticum turgidum* L. ssp. *durum* were collected from experimental farm of CRA-ACM in Catania, Siciliy, maintained at 4 °C for 24 h and then germinated in Petri dishes for 6 d at room temperature. Genomic DNA was isolated from 100 mg of fresh leaf tissue by the *DNeasy* plant mini kit (*Qiagen*, Hilden, Germany). Extracted DNA was quantified by spectrophotometric measurement of UV absorption at 260 nm (*Biophotometer*, Eppendorf Scientific, Hamburg, Germany). Selective amplifications were performed with 64 fluorescence labelled primer combinations in order to determine polymorphic profiles, according to the *Beckman-Coulter* protocol (*A-2015A*, Hayashi *et al*. 2005).

The distance matrix of fragments was generated by the software *NTSYS-pc*, version *2.0* (*Applied Biostatistics*) according to the Jaccard index (1908). Based on the distance matrix, cluster analysis was performed by unweighted pair group method with arithmetic average (UPGMA) of the statistical package *NTSYS-pc* version *2.01*).

Amongst all tested primer combinations we selected three combinations able to induce the highest polymorphism levels as well as a uniform distribution of peaks in the region analyzed (50 - 600 bp). The selected combinations of primers generated a total of 6630 AFLP fragments, including 2277 polymorphic ones. The best combination, EcoRI+AAC/MseI+CTT, carried out a value of 36.53 % polymorphic fragments (Table 1).

Table 1. Polymorphism among the 15 *Triticum turgidum* ssp. *durum* cultivars obtained by fAFLP with selected primer combinations.

Primer combinations	Total number of bands	Polymorphic bands	Polymorphism [%]
AAC/CTT	1845	674	36.53
ACA/CTT	2100	650	30.95
AAC/CAG	2685	953	35.49
Total	6630	2277	

All polymorphic fragments were elaborated by *NTSYS-pc* to compute the distance matrix by using the Jaccard's index and a dendrogram was formed (Fig. 1). The 15 cultivars were arranged into 3 groups. Group 1 shows two sub-groups: the first consists of cvs Duetto, Claudio, Tiziana and Ciccio, with a mean similarity index (Si) 0.605; the second consists of cvs. Dylan, Iride, Duilio, Bronte and Creso with a mean Si 0.650. Group 2 consists of cvs Svevo and Colosseo with Si 0.549. Group 3 consists of cvs Sant'Agata, Simeto and Adamello with Si 0.455. Pairwise similarities among genotypes showed an average similarity among all cultivars of 0.548 ranging from 0.289 (Mongibello *vs*. Sant Agata) to 0.671 (Bronte *vs*. Creso). Mongibello is the most different from the other cvs. studied (Si 0.429).

In previous studies on durum wheat cultivars realized by conventional AFLP, Incirli and Akkaya (2001) observed 189 polymorphic markers in 15 cultivars, with a range from 4 to 24 per primer combination. Hayashi *et al*. (2005) obtained 89 markers in 13 cultivars, with an average of 8.9 per pair. In this work we observed a mean of 759 polymorphic peaks per primer pair. These results demonstrate that fAFLP represent a meaningful improvement compared to the conventional AFLP technique.

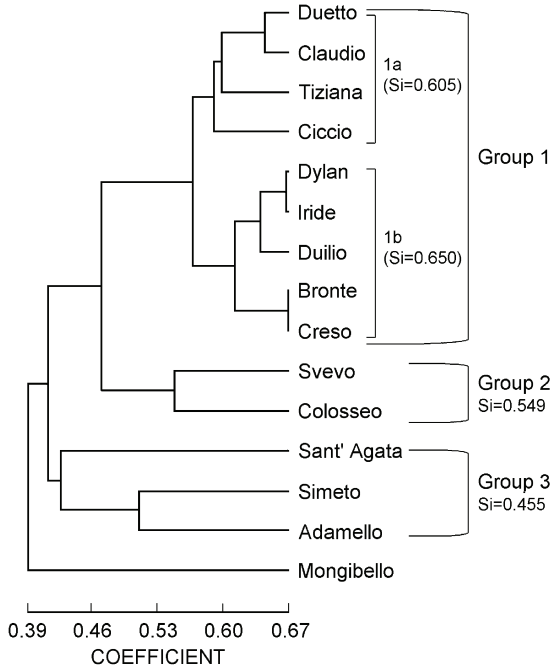

Fig. 1. UPGMA dendrogram showing genetic relationships among the 15 durum wheat Italian cultivars achieved by fAFLP analysis. The dendrogram was constructed using 2277 fAFLP polymorphic bands and was based on the genetic distance calculated by *NTSYS-pc* according to Jaccard's index.

In this study fingerprintings of 15 *Triticum turgidum* ssp. *durum* genotypes were generated by fAFLP for the first time. The genetic distance values show very low differences among cultivars studied. This fact is probably due to a high selection pressure associated with breeding practices (Soleimani *et al*. 2002). However, in line with previous reports, the results of this work demonstrate that a substantial level of genetic variation still exists within modern cultivars of durum wheat and that fAFLP analysis is sensitive enough to detect these low levels of variation, thus allowing discrimination among highly related cultivars such as Sant'Agata, Adamello and Simeto.

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