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IDENTIFICATION OF WHEAT BY MOLECULAR MARKERS

Document prepared by experts from Belgium

Identification of wheat by molecular markers.

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Introduction

The CRA (Ministry of small trades and Agriculture) is in Belgium in charge of the official trials of small grains cereals for the inscription of new varieties on the National list. In this particular research activity, we have evaluated several plant DNA fingerprinting techniques for wheat material discrimination. Different kind of molecular markers are available for DNA fingerprinting in wheat: RFLP, microsatellites, AFLP. We used and compared those different techniques

A major problem with wheat fingerprinting is the lack of polymorphism displayed by this crop, specially between commercial varieties. Therefore an evaluation of the polymorphism detected by the different kind of markers is a preliminary step of this work.

In this document we present results on the use of these different markers in wheat discrimination and preliminary results on the DUS criteria. Distinctness was studied by several molecular techniques; uniformity and stability were mostly analysed with AFLP technique. All the tests were done on a collection of wheat varieties and lines for official inscription.

Materials and Methods

Plant material.
Seeds of *Triticum aestivum* varieties were from a reference collection maintained in Gembloux. The different lines are from material in official trials in Belgium.

DNA isolation
DNA was extracted from leaves using chloroform/phenol method as described (Mingeot and Jacquemin 1997) or with a CTAB protocol (Doyle and Doyle, 1988)

RFLP procedure and microsatellites.
Southern blotting and hybridisation were performed as described by Mingeot and Jacquemin (1999)
For microsatellite analysis, GWM publicly available primer pairs (Röder et al. 1998) were used. PCR amplifications were performed as described by Röder et al.. Fragment sizes were calculated using the computer program AlleleLinks (Pharmacia Biotech).

AFLP
The AFLP protocol was from Vos et al (1995) with minor modifications and was performed on an ALFexpress (Pharmacia Biotech) with CY5 labelling. The primers pairs employed were from Law et al (1998) and personal communication from R. Koebner. Gels were scored with ImageMaster (Pharmacia Biotech)

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Results and discussion

I. Molecular methods

A. Capacity for discrimination of varieties

A.1 RFLP
In the experiments reported we applied mostly these type of markers with the view of genetic mapping and QTL localisation.

We have produced several RFLP markers that we have mapped on the reference ITMI population issued from a cross between Opata85 and a synthetic wheat (Nelson et al, 1995). These markers have been characterised for their polymorphism on 13 commercial varieties by estimated the PIC (Polymorphism Information Content; Botstein et al 1980) value ($PIC_i = 1 - \Sigma\ p_{ij}^2$). The percentage of the different PIC values are respectively: between 0.1 - 0.19 (14%), 0.20 - 0.29 (6%), 0.30 - 0.39 (1%), 0.40 - 0.49 (28%), 0.50 – 0.59 (13%), 0.60 – 0.69 (11%), 0.70 – 0.79 (11%), 0.80 – 0.89 (7%)

We noticed that 41% of probes have a PIC between 0.40 and 0.59, corresponding to a probe able to distinguish 2 different profiles between the 13 varieties. 72% of the RFLP probes were able to distinguish the parents of the population Opata85 X synthetic wheat and if we consider only probes with PIC value different from 0.0, the proportion reach 86%.

Polymorph probe : gbx 3832
We characterised further the probe gbx 3832 with a very high PIC (0.88) (Mingeot and Jacquemin 1997). It could distinguish with DraI, EcoRV et HindIII digestion: 29 profiles / out of 45 varieties (DraI digestion), 25 profiles / 45 varieties (EcoRV) and 21 profiles / 46 varieties (Hind III). The 29 distinguished profiles with DraI enzyme are presented in Fig. 1.

A.2 Microsatellites
We have tested a number of publicly available microsatellites markers (gwm) (Röder et al. 1999) on a set of 7 varieties used in the construction of populations developed for mapping and QTL analysis.

Out of 133 pairs of primers employed, the percentage of polymorphism ranged, according to the population considered, from 70 % to 51% of microsatellites able to distinguish the 2 parents of the population. All the microsatellites were polymorph on the parents of the ITMI population (including a synthetic wheat).

A.3 AFLP
In a first survey, we have tested several AFLP primers (from a BRL kit) after a digestion with EcoRI /MseI, using the published protocol, but the observed polymorphism was low. In order to increase the last one, we compared several types of digestion (EcoRI, PstI, SseI respectively combined with MseI) (Fig. 2) and adapted the protocol with 2 additional bases (Law et al 1998; Koebner R. personal communication). Several primer combinations were tested and in order to select some potential pairs, we took in consideration the pattern which could be readable and gave a high polymorphism. Between 100-200 amplified DNA fragments could be visualised dependant on the primer used. Cluster analysis were performed using UPGMA method.

According to the tests of the different molecular markers, we can conclude that:

- In agreement with the different publications, microsatellites display greater polymorphism than RFLP markers. Moreover, comparing the technical facilities, the last ones are more laborious to handle.
- The knowledge of the PIC value enable the preselection of markers in order to increase the polymorphism detected among the varieties.
- AFLP establish a powerful technique since many polymorph bands can be visualised in a single assay. Nevertheless, AFLP markers are less easy to handle than microsatellites in results examination.

II. DUS Issues

A) Distinctness

This type of study was performed more recently with the AFLP technique. A set of varieties was analysed after SseI/MseI digestion and amplification. The observed profiles of a subset of these varieties is showed in Fig. 3 and molecular distance calculation using the UPGMA method and Jaccard indice on some varieties is presented in Fig. 4. All the varieties are distinguishable.

B) Uniformity

With the view to evaluate the uniformity within the same variety or line, we have analysed 20 to 50 plants from the same origin; DNA was extracted and amplified with the 2 primers S12/M24. Fig. 5 showed the results for line A842. We observed some individuals with minor different bands; this difference is about 5 percent and the degree of uniformity is usually high. We never found in material in trials, a very heterogeneous variety. Moreover, it seems that in a variety the same shift appears in the analysed profiles.

C) Stability

The study of the variation of data from several seed generation and source was performed. Leaf DNA was extracted from material in official trials during 2 successive years (1997-1998). AFLP was performed with primer S12/M24 and results were analysed by UPGMA algorithm. We saw that most of the varieties from 97/98 were classified altogether but some exceptions appeared like A837/A835/A827 which were more distantly classified (Fig. 6).

References

Botstein D, White R, Skolnick M and Davis R (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of human genetic.32:314-331.

Law j, Domini P, koebner R, Reeves J and Cooke R (1998) DNA profiling and plant variety registration. III: The statistical assessment of distinctness in wheat using amplified fragment lenght polymorphisms Euphitica 102: 335-342

Mingeot D, Jacquemin JM (1997) A wheat cDNA coding for a thaumatin-like protein reveals a high level of RFLP in wheat. Theor Appl Genet 95:822-827

Mingeot D and Jacquemin J (1999) Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98:1132-1137.

Nelson JC, Van Deynze AE, Autrique E, Sorrels ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995b) Molecular mapping of wheat. Homoeologous group 2. Genome 38:516-524

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P and Ganal MW (1998) A microsatellite map of wheat. Genetics 149: 2007-2023
Vos P, Hogers R, Bleeker M, Reijans M, Lee T V d, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research.23:4407-4414.

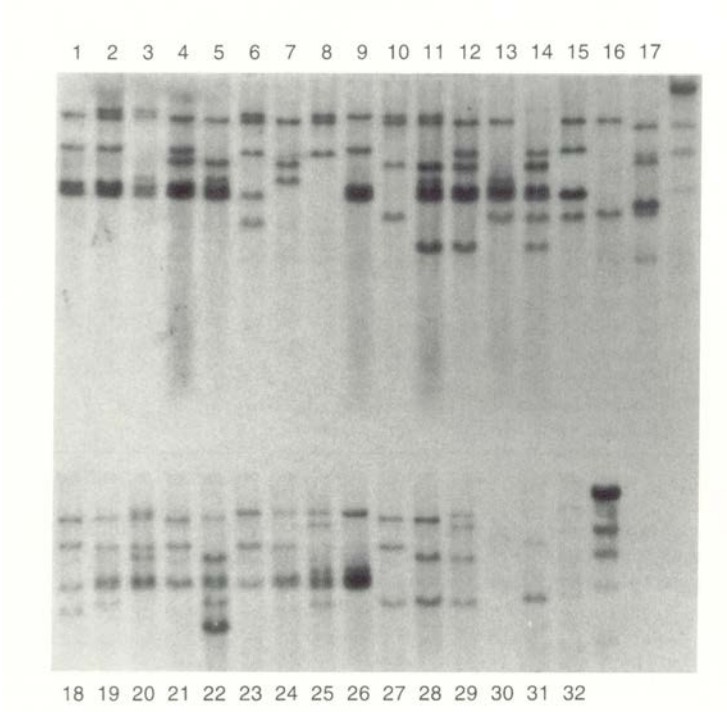


Fig.1. Southern blot obtained by hybridization of gbx3832 to DraI digested DNA from 29 wheat varieties.

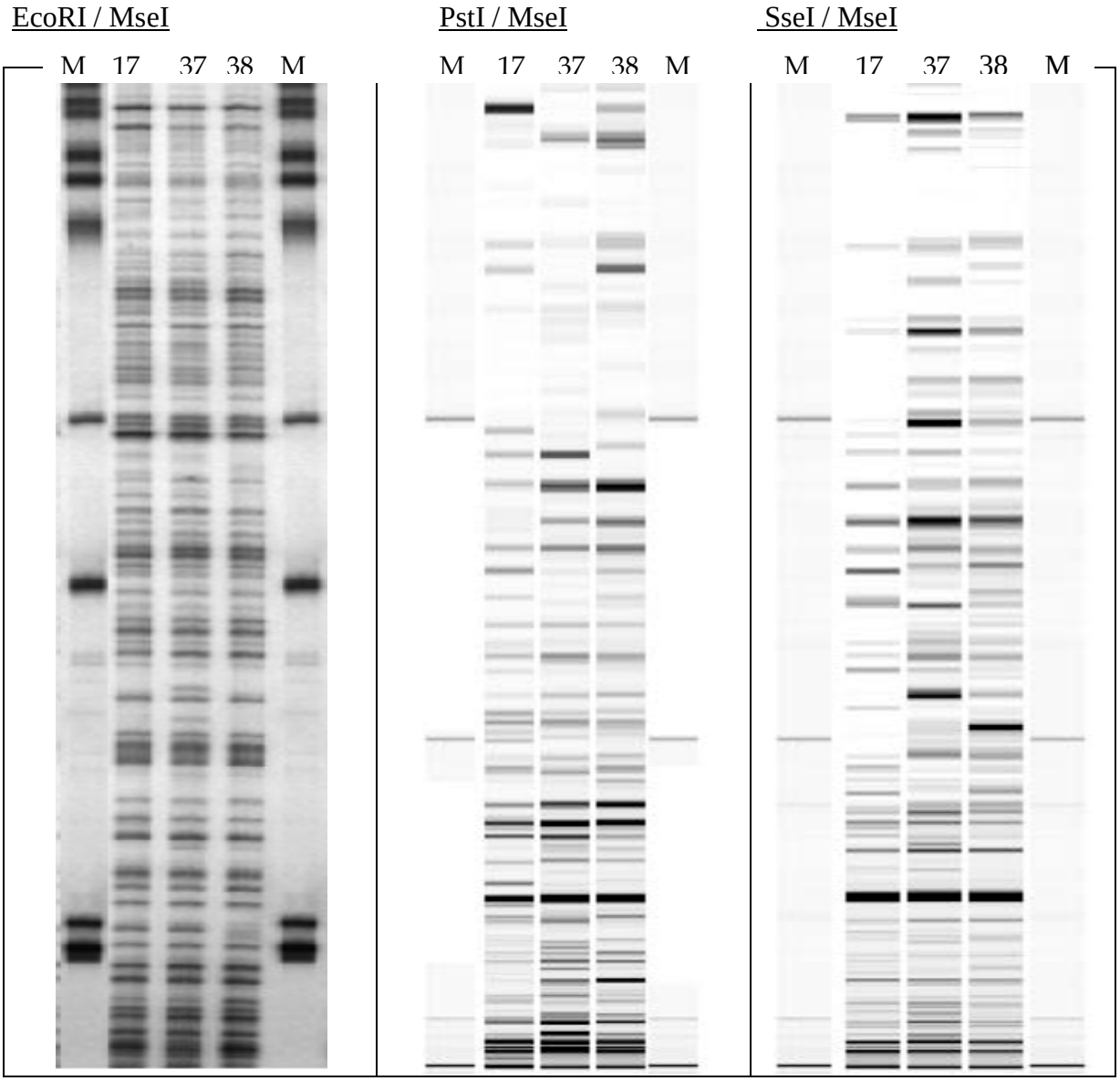
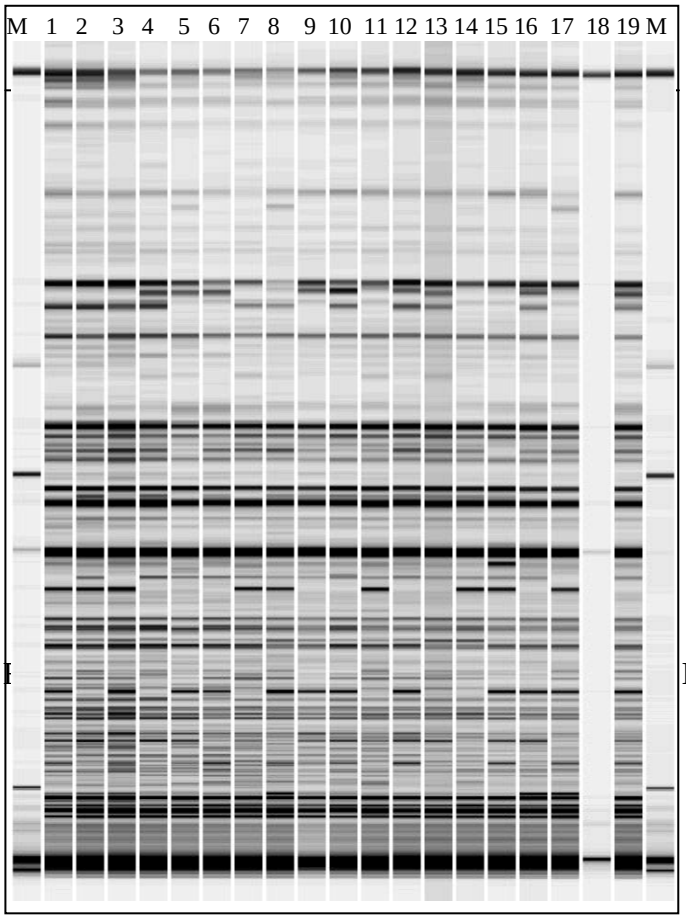


Fig.2. AFLP markers polymorphism: comparison of the enzyme pairs EcoRI/MseI, PstI/MseI and SseI/MseI.



M : Marker
1 : Shango
2 : Audace
3 : Ormil
4 : Haven
5 : Eureka
6 : Tribun
7 : Oracle
8 : Hereward
9 : Valais
10 : Génial
11 : Mobil
12 : Versailles
13 : Torfrida
14 : Ritmo
15 : Rialto
16 : Génésis
17 : Apostle
18 : Hussar
19 : Castor
M : Marker

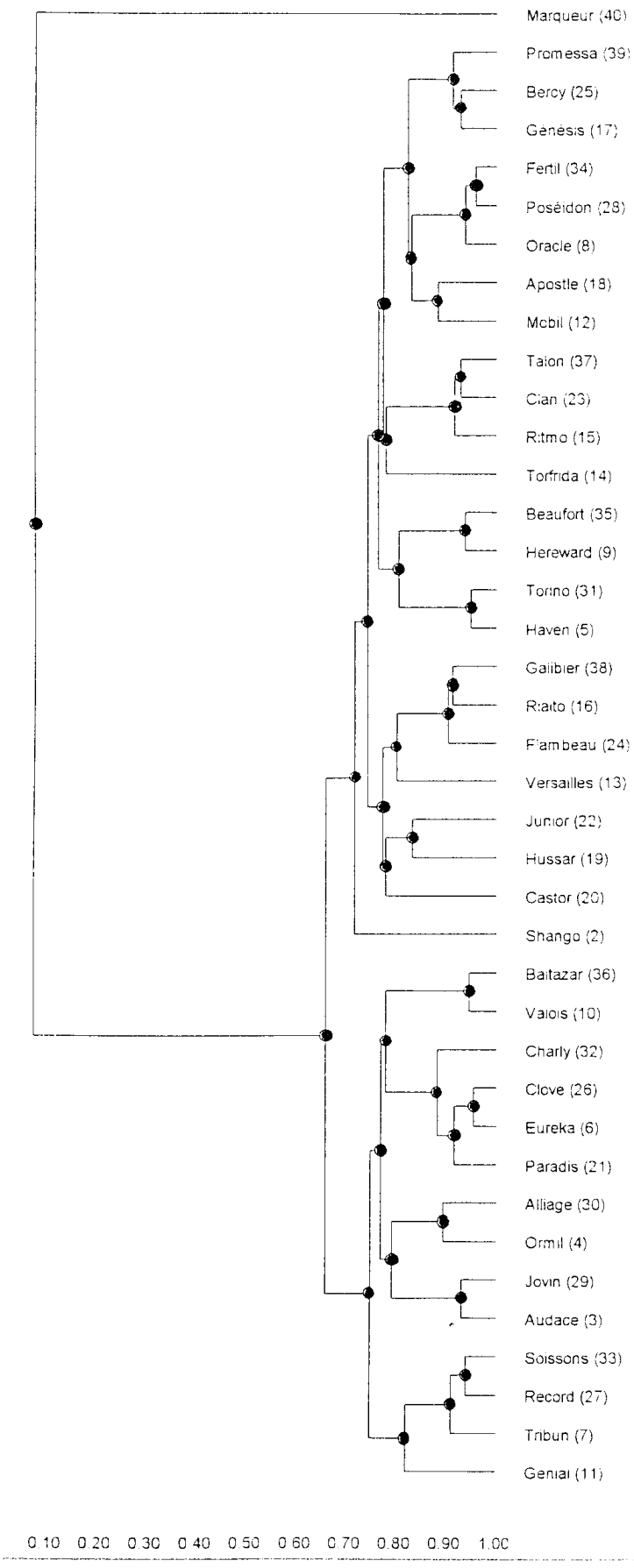


Fig. 4. UPGMA classification of wheat varieties (AFLP marker).

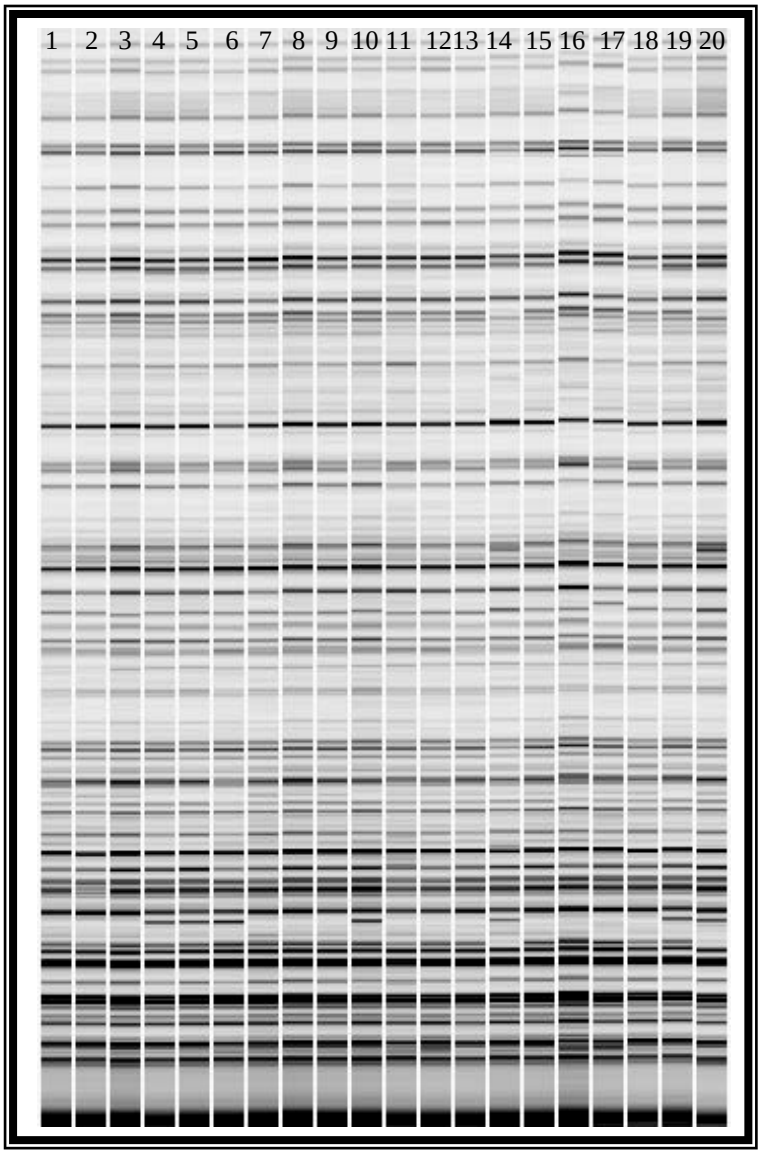


Fig. 5. Uniformity: AFLP analysis (primers S12/M24) on 20 plants from A842 wheat line.

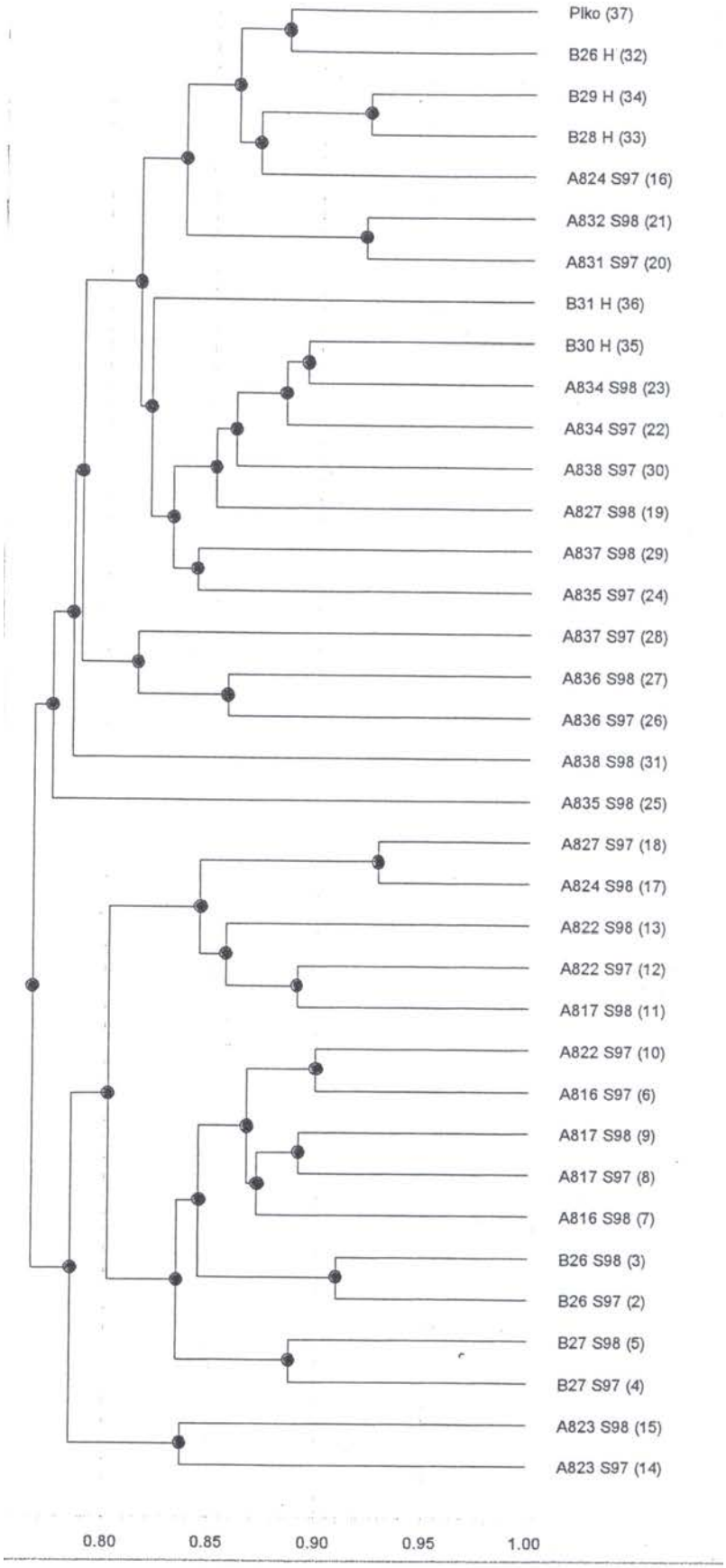


Fig. 6. Stability: UPGMA constructed according the AFLP patterns of wheat lines for 2 years. The names of the lines are followed by the year of seed production.

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