Semi-Specific PCR-Marker as Valuable Tools for Genetic Analyses in Alfalfa (*Medicago sativa* L.)

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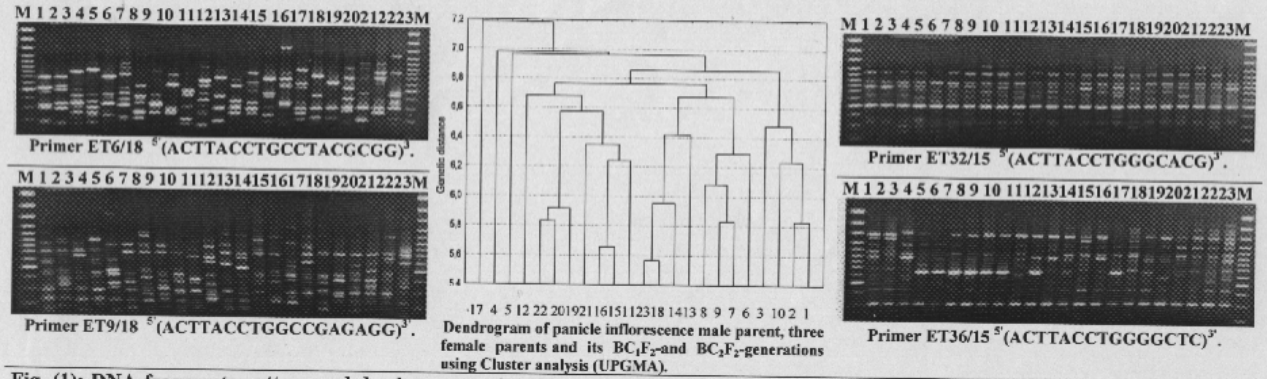
- Semi-specific PCR and use of primers with partial homology to sequences of intron-exon junctions seems to be an alternative to RAPD and other tedious and expensive methods such as RFLP and AFLP. The core sequences of semi-specific primers are based on existence conserved sequences 9 and 7 bases in length. The additional bases were added at random to extend the primer length and for generating the variability of primers. This approach enables to create the series of exon targeting (ET) or intron targeting (IT) primers from 10 to 18 bases in length. Although, the RAPD system is useful for many crops, is not suitable for genetic analysis of large and complex genomes such as alfalfa. The proposed system is as cheap and fast as RAPD, but unlike the latter, the semi-specific primers generate more complex and much more polymorphic band patterns. The increased length of the majority of primers and elevated annealing temperature during all amplification steps resulted in good reproducibility of PCR profiles. The high usefulness of semi-specific primers was proved by evaluation results of genetic diversity among open-pollinated varieties of rye **(Rafalski, *et al*., 2002)** and wheat and triticale **(Gaweł, *et al*., 2002)**.
- Most of investigated genotypes were formed a distinct branch on the dendrogram (Fig.1) and were clustered from a single branch point showing more than 66% of genetic similarity. Four primers showed 131 bands out of which 125 polymorphic. Furthermore, the amplification products obtained with four primers allowed to discriminate some normal- and *pi*-types such as three Egyptian accessions (normal-type) and mutant plant *pi*.2fr/1x72/11 at 1000 bp with primer ET9/18, at 1120 bp and 940 bp with primer ET6/18.
- The values at the different nodes of the dendrogram (Fig. 2) indicated that frequencies of occurrence of the corresponding nodes in the crosses involved long raceme peduncle parent (*lp*-type) and three female parents. This study, only, used four primers which showed 125 bands out of which 116 polymorphic. This limited number of bands reduces the possibility of identifying and selecting the chromosome fragments associated with *lp*-type.
- The female variance may be a major source of variation for characters that can be selected and used in the maintenance of heterotic groups and indicated maternal effect.
- The presented results can be treated as a preliminary study and indicated on the possibility of identification of markers of *pi*- and *lp*-types using semi-specific PCR techniques. Therefore, we can concluded that semi-specific PCR-markers may be profitably used in the genus *Medicago* to identify and select some chromosome fragments associated with some traits such as *pi*- and *lp*-types. These results should, also, indicate semi-specific PCR-markers as valuable tools for genetic analyses in species like alfalfa, where, there are a lack of suitable genetic markers and, where, significant time and labor may be involved in obtaining populations amenable to genetic analysis.

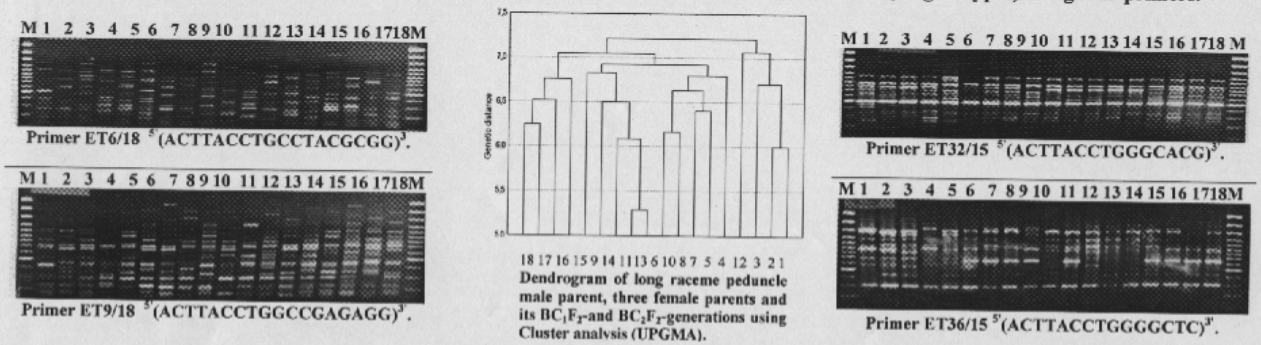
References:

1. **Gaweł, M.; I. Wiśniewska and A. Rafalski (2002):** Semi-specific PCR for the evaluation of diversity among cultivars of wheat and triticale. Cellular & Molecular Biology Letters; 7: 577-582.
2. **Rafalski, A.; L. Madej; I. Wiśniewska and M. Gaweł (2002):** The genetic diversity of components of rye hybrids. Cellular & Molecular Biology Letters; 7: 471-475.



Dendrogram of panicle inflorescence male parent, three female parents and its BC_1F_2-and BC_2F_2-generations using Cluster analysis (UPGMA).

Fig. (1): DNA-fragments pattern and dendrogram using Cluster analysis (UPGMA-unweighted pair-group arithmetic average method) generated from *pi*-type male parent, three female parents and its BC_1F_2- and BC_2F_2-generations (23-genotypes) using four primers.



Dendrogram of long raceme peduncle male parent, three female parents and its BC_1F_2-and BC_2F_2-generations using Cluster analysis (UPGMA).

Fig. (2): DNA-fragments pattern and dendrogram using Cluster analysis (UPGMA-unweighted pair-group arithmetic average method) generated from *lp*-type male parent, three female parents and its BC_1F_2- and BC_2F_2-generations (18-genotypes) using four primers.