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MARA

IDENTIFICATION OF SOME TURKISH AEGEAN TOBACCO GENOTYPES BY ISOZYME BANDING PATTERNS

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ABSTRACT*: In this study, band designs of Esterase, Peroxidase and Malate dehydrogenase (MDH) isoenzymes were investigated in some selected Turkish Aegean tobacco genotypes by using polyacrilamide gel electrophoresis(PAGE) . Genotypes belonging to* ***N. tabacum*** *L. generated different band designs by esterase isoenzyme. Likewise same genotypes generated different band designs by MDH isoenzym. Whereas, according to peroxidase isoenzyme, similar and different bands were observed. Regarding to the isoenzymes; different band patterns were observed in morphologically similar genotypes. It was observed that when studied with at least two isoenzymes, morphologically similar Aegean tobacco genotypes could be identified.*

Keywords*: Aegean tobacco,* ***N. tabacum*** *L., isoenzymes, polyacrylamide gel electrophoresis.*

BAZI EGE TÜRK TÜTÜN GENOTİPLERİNİN İZOENZİM BANTLARINA GÖRE TANIMLANMASI

ÖZ: *Bu çalışmada, sekiz Ege Tütün çeşit ve ıslah hatlarında poliakrilamid jel elektroforezi kullanılarak Esterase, Peroksidase ve Malate dehydrogenase (MDH) isoenzimlerinin bant desenleri araştırılmıştır.* ***N. tabacum*** *L. türüne ait Ege tütün çeşit ve ıslah hatları esterase isoenzimine göre farklı bant desenleri oluşturmuştur. Benzer olarak ayni genotipler MDH isoenzimine göre de farklı bant desenleri oluşturmuştur. Peroksidase isoenzimine göre ise çeşit ve ıslah hatları arasında benzer ve farklı bantlar gözlenmiştir. Morfolojik olarak benzer olan tütün çeşit ve ıslah hatlarında, isoenzim bantlarına göre farklılıklar gözlenmiştir. En az iki enzim ile çalışıldığı takdirde, morfolojik olarak benzer Ege tütün çeşit ve ıslah hatlarının tanımlanabileceği saptanmıştır.*

Anahtar Sözcükler*: Ege tütünü,* ***N. tabacum*** *L., isoenzim, poliakrilamid jel elektroforezi.*

INTRODUCTION

Aegean tobacco genotypes show some similarity for morphological and physiological properties. Ege - 64 and Karabaglar tobacco groups, which are recognizable as different phenotypes at first glance, are grown in Aegean region. Ege - 64 group of tobacco genotypes, which have good quality characteristics, are grown in small amounts because of their low yields. Karabaglar group of tobacco genotypes is cultivated intensively because of their high yield. The cultivars of each group look same phenotypic ally.

Enzymes are used for identifying genotypic differences and individuals. Individual genotypic differences could not be always observed phenotypically. Since the individuals, which have no differences phenotipically, might be different genotipically, it's better to analyze what's behind of the phenotypic structure. Enzymes are primer products of the genes and it's possible that analysis of enzymes could give healthy information about them. For this reason separation of enzymes with electrophoresis gives the opportunity to obtain more detailed information to researchers (Turgut, 1983).

Since proteins and enzymes are primer products of the genes, they play important role of the evaluation of genetic systems. Proteins and enzymes are certain factors that would not be effected by environmental conditions, therefore they may be called fingerprints banding patterns to characterize the genotype and to reflect the genetypic structure (Cooke, 1986)

Isozymes are separated in a supporting medium such as polyacrylamide, starch etc. under the influence of an electric field by their electrical charge and stained with specific dyes. Then, they appear as visible bands (Simpson and Withers, 1986). Since gel electrophoresis is a rapid and reliable technique, it's widely used for testing of the varieties as well as breeding of new varieties (Cooke, 1986).

Previous studies on tobacco have indicated that differentiation of *Nicotiana sp*. by their isozymes profile might be possible while tobacco varieties might not (Bredemejier, 1982; Chien *et al*.,1986; Sheen, 1970; Trinh *et al*., 1981;). Recently, by using new techniques it's demonstrated that varieties in a species could be identified by electrophoresis (Abet *et al*., 1983; De Jong, 1995; Peksüslü and Sekin, 1998, 2000; and Wikinson *et al*., 1985).

The objective of this study was to find out the differences among Aegean tobacco genotypes by using polyacrilamide gel electrophoresis (PAGE).

Abet *et al*., have worked on enzyme polymorphism in tobacco and indicated that the enzyme of Esterase and peroxidase patterns might be used for identification of genetic types and the cultivars (Abet *et al*., 1984). Eighteen Burley tobacco cultivars were compared for peroxidase and malate dehydrogenase isozyme patterns in polyacrilamid gel electrophoresis (PAGE). Identification of Burley tobacco cultivars were performed according to those enzyme methods (Abet *et al.*, 1982, 1983). Wilkinson *et al*., compared Maryland, Burley, Flue cured and cigar tobacco cultivars by using PAGE for 4 isozyme profiles (Esterase, Peroxidase, Malate Dehydrogenase (MDH) and Catalase). They were able to identify the cultivars with these enzyme profiles except esterase (Wikinson *et al*., 1985).

In a research, *Nicotiana* spp. were investigated for MDH. Totally 12 MDH isozyme profiles were described, 7 of them were called C1,.....C7 and 5 of them that were called V1,.....V5, haven't been explained fully yet. And also two extra bands named C6x- C7x were observed. This work suggests that MDH isozyme system may be apart in the temperature adaptation process in tobacco (De Jong, 1995).

This study aimed to identify Aegean tobacco genotypes for esterase, peroxidase and malatdehydrogenase by using PAGE technique. Esterase and peroxidase and MDH banding patterns of each genotype were obtained in order to find out the possibilities of using use them as check method in certifications and in breeding stock material. It was also aimed to use them as molecular markers in the identification of the Aegean tobacco cultivars phenotypically similar.

MATERIALS AND METHODS

Plant materials: Tobacco seeds were taken from the Aegean Agricultural Research Institute (AARI). The tobacco leaves used in this study were matured and third priming leaves at the flowering stage.

Turkish Aegean tobacco types used: As Izmir type genotypes Karabaglar 6265, Izmir – Ozbas, Akhisar 97, Sarıbaglar 407, Ege 64, Sarıbaglar 402, Tekel Karabaglar and DH -204 .

Extraction and Electrophoresis: For each sample, the large midvein was cut out of the leaf and the remaining tissue was cut into small pieces and weighed. The tissue was ground with 2 ml/g tissue of chilled extraction buffer and small amount of insoluble polyvinylpyrolidone. Extraction buffer consisted of 5 mM Na_2 EDTA, 0.25 M NaCl, 0.05 M $NaHSO_3$ and 0.025 M β- Mercaptoethonol in a 0.05 M Tris - HCl buffer pH 8.0. Electrot buffer: Tris 5 mM, Glisin 38.5 mM pH 8.3. The

mixture was ground for 2 - 3 min. Filtered through cheesecloth, and centrifuged at 4000 rg for ten min (Moore and Collins, 1982).

Supernatant was analyzed by 7.5 % polyacrylamide gel electrophoresis by a vertical slab apparatus (Hoeffer 400 E) according to Davis 1964 (Davis, 1970).

Gel was run at 6 ^{o}C and 35 mA for 3 hr. Staining was for peroxidase, esterase and malate dehydrogenase. The peroxidase and MDH used were those of De Jong *et al* (De Jong and Çakir, 1991; Moore and Collins, 1982). The esterase stain used was that of Abet *et al*., (1984).

All gels were scored for number of bands, band migration and intensity. Number of bands and band intensity was scored visually.

RESULTS AND DISCUSSION

Eight Aegean tobacco genotypes were compared by three-ısozyme system Esterase, Peroxidase and MDH with PAGE and their zymogromes were obtained.

Figure 1. Esterase isozyme patterns of Aegean tobacco genotypes in PAGE.

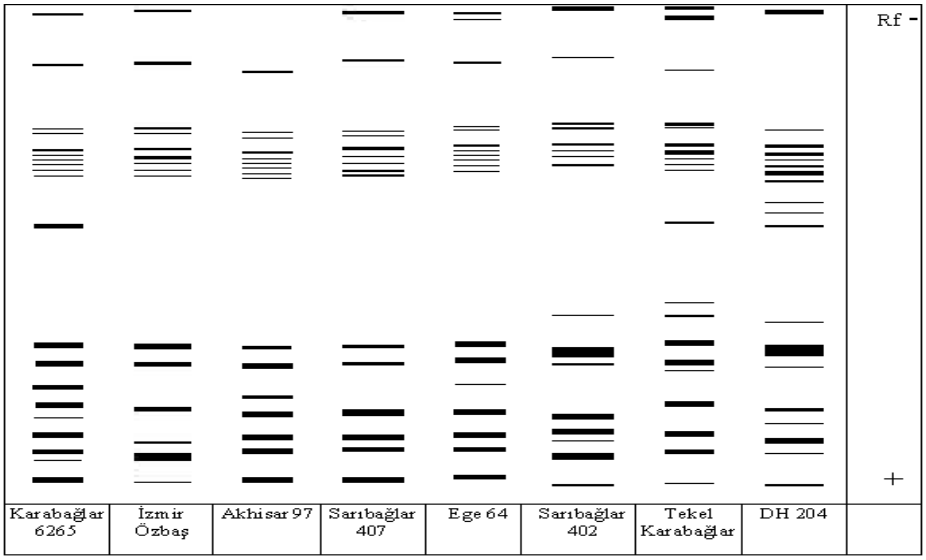

Figure 2. Representative patterns of Esterase isozyme of Aegean tobacco genotypes in PAGE.

Esterase, Peroxidase and MDH isozyme bands, which were used in comparison of genotypes, showed polymorphism.

Different band patterns were observed for Esterase isozyme between genotypes. In general, esterase band number of material varied from 15 to 21 and some of them had specific band patterns (Figure 1 and 2). Genotypes giving the highest number of bands were Sarıbaglar 407 and Akhisar 97. Genotype presenting the fewest number of bands was Karabaglar 6265.

Esterase Rf values showed a variation between 0.02 – 0.09 Karabaglar 6265 and Tekel Karabaglar genotypes of Aegean group, which are similar in origin, had the specific identical band with Rf value 0.5.

Similiar band patterns were observed in most varieties but some of them gave different unique band patterns for Peroxidase enzyme. Peroxidase isozyme band numbers of the material varied from 7 to 10 (Figure 3 and 4). Tekel Karabaglar was the genotype showing the highest band number. Rf values belonging peroxidase, showed a variation between 0.33 – 0.66. Almost all varieties had an identical bold band.

Figure 3. Peroksidase isozyme patterns of Aegean tobacco varieties (*N. tabacum*) and breeding lines in PAGE.

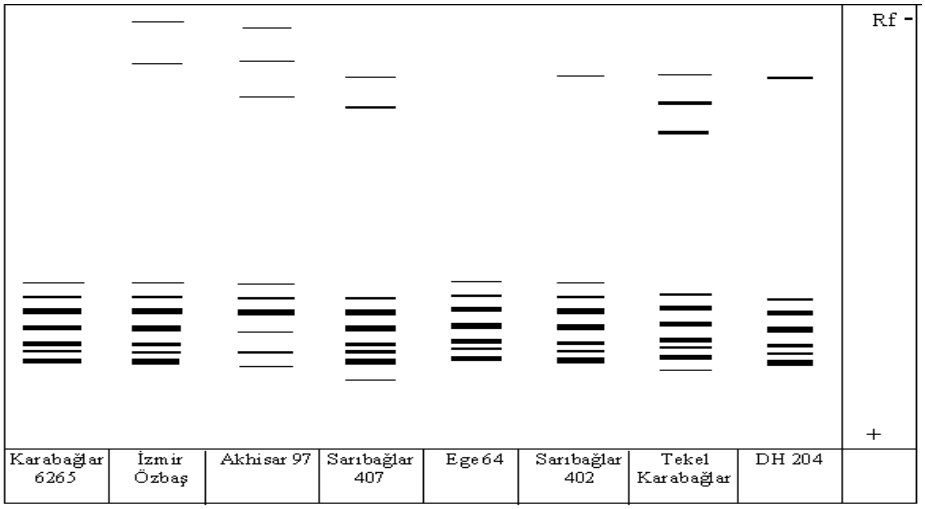

Figure 4. Representative patterns of peroksidase isozyme of Aegean tobacco genotypes in PAGE.

Differences were investigated for MDH among the genotypes. However, when working with enzymes it's necessary to work with enzymes as many as possible. Genotypic differences, which can not be found with an isozyme, can be found with another isozyme.

These were qualitative differences in the enzyme profiles of genotypes. Abet *et al.* (1982, 1983), found important differences by using cultivars, which were chosen from different tobacco types.

Different band patterns were observed for MDH isozyme (Figure 5 and 6). Variations between varieties were recorded. Akhisar 97, Sarıbaglar 407 and Tekel Karabaglar were found to have C6x – C7x bands. Field trials showed that these varieties were found to be resistant to temperature stress. MDH system might be a useful model for breeding new varieties, which were resistant to temperature stress (De Jong, 1995).

Figure 5. MDH isozyme patterns of Aegean tobacco genotypes in PAGE.

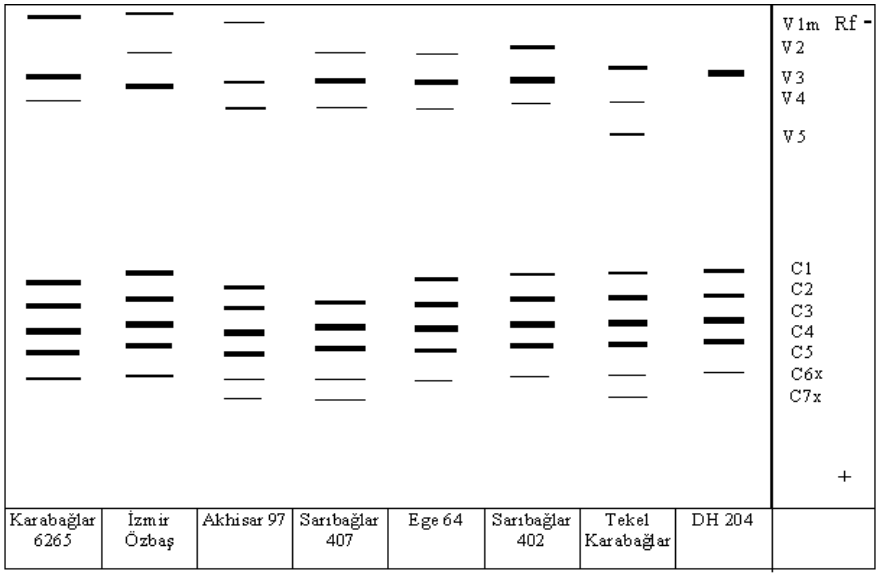

Figure 6. Representative patterns of MDH isozyme of Aegean tobacco genotypes in PAGE.

Polymorphism is essential in to use of isoenzymes as genetic marker. When considered EST, MDH and PEO isozymes, polymorphism of these isozymes and their coding capacity of the molecules with different velocity, they are convenient for identification of genotypical differences.

In this study, varietal identification was investigated by using three biochemical markers. Some different band patterns were observed between the varieties for Esterase. Some varieties had different band patterns while other had same band patterns for PEO.

It's conluded that band patterns of varieties could be used for varietal registration and also be helpful for tobacco breeders.

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