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ISOENZYMATIC POLYMORPHISM DIFFERENTIATION OF TURKISH GRAPEVINE CULTIVARS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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ABSTRACT

In this research isoenzymatic activity of Catechol oxidase (CO), Esterase (EST) and Peroxidase(PER) was studied in young leaves of 29 Turkish grape cultivars. Catechol oxidase, peroxidase and esterase isoenzyme banding patterns of 29 cultivars made it possible to distinguish between 29 Turkish grape cultivars. It was conculuded that all of the three enzyme systems provided the most usefull data as it was possible to distinguish all 29 grape cultivars.

Introduction

Scientists who have been concerned with the correct identification of *Vitis vinifera* L. cultivars for more than 80 years. Traditionally, identification techniques have involved the examination of vine and berry morphology (1). As it is known that these plant characters are relatively inconsistent and these are often not distinct in expression. This is especially true for leaf characters for which the greater share of morphological diversity is present. In addition, environmental factors and disease can affect the appearent values of leaf characters. Scientists have also tried to overcome these problems through the use of multivariete statistical analysis of a variety of qualified leaf characters. For cultivar identification, scanning electron microscopy of pollen has also been used (2).

Alternative approaches have been developed in recent years (3), including biochemical analysis of fruit compounds such as phenolic compounds, flavor components, berry isozymes (4), pollen isozymes(5).

The technique of electrophoresis is a useful tool for studying genetic variation and has been shown to have potential for discriminating among species, cultivar lineages and cultivars of grapes (3, 6, 7, 8). Enzyme extraction and preparation is simple and the method can be used to rapidly determine an organism's genotype independent of judgement based on phenotypic information (9). Polyacrylamide and starch gel electrophoresis have been used to determine differences between cultivars and species of grape, pear and peach (10, 11, 12, 13).

Viticulture is an ancient agricultural enterprises in Turkey and several cases of synonyms and homonyms have emerged among the different vine cultivars. Legislators and growers are concerned with the correct identification of cultivars when they purchase material to plant vineyards. Both morphological description which is also important but subjective and ampelometric methods (subjective) which is affected to environmental conditions should be complementary to other more objective and stable methods.

Different studies of the applicability of isoenzymatic activity for the characterization of grapevine cultivars have been developed since Wolfe (11) used this method for the identification of grape cultivars by isoenymes banding patterns.

Other researchers have reported that tissue source and the isoenzymatic systems used have a significant effect on the success of obtaining polymorphisms and on the reproducibility of the results (8, 11, 14, 15).

Young leaf and woody cane have been most widely used isoenzymatic sourses due to long time of span for collection and to the high isoenzymatic activity, although berries, adult leaves, pollen and roots have also been used as isoenzymatic sources (8, 11, 14, 15, 16).

The objective of this research was to determine the differentiation of 29 Turkish grape cultivars by using isoenzyme electrophoresis. In this research three enzymatic systems (Catechol oxidase, Peroxidase and Esterase) were studied.

Materials and Methods

Leaf Samples of Turkish grapevine cultivars used in this study were obtained from Regional Collection Vineyards planted in Ankara University, Agricultural Faculty, Viticultural Research and Experiment Station in Kalecik County.

Young actively growing leaves collected and placed in polyethylene bags in the morning and taken them to the laboratory and kept at 0 to 4 °C in the refrigerator till extraction for 1-2 days.

Two grams of young actively grown leaves were diced and transferred to a 50-ml centrifuge tube in ice containing 1 g of insoluble PVPP and 12 ml of cold extraction buffer. The contents of a tube were homogenized in a homogenizer for 15 seconds (24.000 rpm). Homogenized samples must be kept cold (0 to 4 °C) and preferably closed to prevent oxidation. The tubes then centrifuged to pellet the large debris or allowed to sit on ice until the debris settle (usually 15 minutes).Clear supernatants used immediately for electrophoresis (17). The rest of the samples were frozen at – 20 °C for future use.

Polyacrylamide gel concentration was %9.45 using in a pH 8.3 tris/glycine electrode buffer. The electrophoresis was performed at a constant voltage of 100 V until the tracking dye entered the stacking gel (after approximately 30 minutes) then the voltage was raised to 150 V. Finally when the tracking dye entered the resolving gel (approximately a furter 30 minutes) the voltage was increased to 350 V. The duration of electrophoresis at this final voltage changed between 2.5 –4 hours depending on the enzyme to be studied.

Each gel slice was asseyed by placing it into large petri dish with a buffered solution containing the appropriate substrates. Enzyme recipes were modified from those described by Wolfe (11) and by Arulsekar and Parfitt (17) as follows:

Catechol Oxidase (CO)

300 mg catechol and 50 mg P-phenylenediamine were dissolved in 100 ml 0.1 M acetate buffer (pH 4.2) and the gel incubated at 20 °C for 1 hour in the dark.

Esterase (EST)

100 mg α-napthyl acetate was dissolved in 2 ml acetone and 100 mg Fast blue RR salt was dissolved in 100 ml 0.1 M phosphate buffer (pH 6.5). The two solutions were mixed and the gel stained for 45 minutes.

Peroxidase (PER)

100 mg 3-amino-9-ethyl carbazole was dissolved in 5 ml dimethyl formamide. This was added to 100 ml 0.10 M acetate, pH 5.0 containing 2 ml 0.1 M $CaCl_2$ and 0.5 ml 30% hydrogen peroxide.

The gel was stained for 30 – 60 minutes in the dark.

Rf values were calculated for the banding positions dividing the distance a given band moved by the distance to the leading front of the bromophenol blue dye. Patterns were assigned to these isozyme profiles by consolidating similar profiles so that a given pattern could represent several very similar isozyme profiles.

TABLE

Presence (+) and absence (-) of Catechol Oxidase banding patterns obtained in 29 Turkish grape cultivars

	0,18	0,20	0,21	0,22	0,23	0,24	0,25	0,26	0,27	0,28	0,30	0,31	0,32	0,33	0,35	0,36	0,38	0,39	0,40	0,41	0,42	0,45
Amasya	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-
Ata sarısı	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Besni	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
Boğazkere	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-
Çalkarası	-	-	+	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-
Çavuş	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-
Dökülgen	-	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-
Emir	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Erenköy beyazı	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	+	-	-
Ergin Çekirdeksizi	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-
Gül üzümü	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-
Hafızali	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+
Hasandede	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-
Hönüsü	-	+	+	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-
Kabarcık	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-
Kadın parmağı	+	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	+	-	-	-	-	-
Kalecik karası	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-
Karagevrek	-	-	+	+	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-
Karasakız	-	+	-	-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-
Kozak beyazı	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-
Kozak siyahı	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-
Müşküle	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-
Narince	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Papaz karası	-	+	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-
Razakı	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-
Sultani Çekirdeksiz	-	+	-	-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-
Tahannebi	-	+	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-
Yalova İncisi	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	-	+	-	-	-	+	-
Yapıncak	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-

Results and Discussion

Well-resolved and consistent isozyme banding patterns were obtained with the methods described previously for Catechol oxidase (CO), Peroxidase (PER) and Esterase (EST).

Catechol Oxidase (CO)

In this study, four to seven catechol oxidase isozyme bands were detected in the investigated 29 grape cultivars. The Rf values were changed between 0,18-0,45. The tested cultivars did not share the same

isozyme banding patterns at all. Therefore, it would be possible to discriminate 29 cultivars by using CO isozyme system (Fig.1).

There was only one cultivar namely Besni cvs. showed 4 CO bands. Hafızali, Kalecik karası, Karagevrek, Karasakız, and Kozak siyahı showed 7 bands. But all of them could be seperated succesfully due to different Rf values of the bands.

Wolfe (11) found 3 to 6 CO bands in 55 cultivars examined.

Ağaoğlu et al. (19) also found 4 to 6 catechol oxidase bands which were between 0,16-0,34 Rf values bands of table and wine grape cultivars grown in Turkey.

Esterase(EST)

Esterase gave between 6 and 14 bands in 29 cultivars. Except Besni and Boğazkere, all cultivars had a band with 0,08 Rf value. Esterase isozymes showed more than 40 different Rf values in all cultivars.

In esterase isozyme system, Hafızali, Hasandede showed 6 bands, Amasya, Hönüsü and Papaz karası showed 7 bands; Yapıncak showed 8 bands; Ata sarısı, Dökülgen, Erenköy beyazı, Karasakız, Kozak beyazı, Narince and Razakı cvs. Showed 9 bands, Boğazkere, Çavuş, Ergin Çekirdeksizi, Kadın parmağı, Müşküle and Sultani Çekirdeksiz showed 10 bands; Emir, Gül üzümü showed 11 bands; Besni, Kabarcık, Kalecik karası showed 12 bands and Yalova incisi showed 13 bands.

It was also possible to distinguish all 29 cultivars with esterase enzyme system.

Peroxidase (PER)

The last enzyme system analysed in this study was peroxidase. All cultivars examined in this research gave between 5 to 10 bands. Rf values of the bands changed between 0,12 and 0,58.

Peroxidase showed 6 bands in Amasya , Ata sarısı, Kabarcık, Yapıncak cvs.; 7 bands in Besni, Boğazkere, Çalkarası, Ergin Çekirdeksizi, Hasandede, Hönüsü, Karasakız, Kozak beyazı, Papaz karası, Tahannebi; 8 bands in Emir, Gülüzümü, Hafızali, Kadın parmağı, Sultani Çekirdeksiz; 9 bands in Çavuş, Dökülgen, Kalecik karası, Kozak siyahı, Müşküle and Narince cvs.

In conclusion, it was possible to distinguish all cultivars containing same peroxidase band numbers with a diffrerent Rf values of the bands observed.

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