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A study of leaf and callus antigens in Euro-Asian and American grapevines

by

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S u m m a r y : Immunochemical reactions of absorbed antisera with leaf and callus antigens of American and European grapevine species revealed species-specific antigens. Immunochemical reactions of extracts from grape leaves and callus were identical. The presence of species-specific antigens in the undifferentiated callus indicated that they are not linked to cell specialization. Leaf and callus antigens with molecular weights of approximately 100 kDa are specific for *V. vinifera* L. Antigens with molecular weights of more than 200 kDa are specific for American species of grapevine.

K e y w o r d s : callus, antigen, grapevine, immunochemistry.

Introduction

It is well known that American grapevine species are resistant while Euro-Asian cultivars (*Vitis vinifera* L.) are susceptible to some pests and diseases. A number of scientists (Paludetti and Galo 1988: Tedesco *et al.* 1989) fused electrophoretical patterns of proteins to identify cultivars and species of grapevine. Khavkin *et al.* (1971), Galfre and Butcher (1986) and Kovaleva *et al.* (1975) have shown that immunochemical approaches are highly efficient to study proteins of differentiated plant tissues. Cell and tissue culture has become a model for the investigation of specific proteins of tobacco callus tissue (Butenko and Volodarski 1967). To study protein components of grapevine immunological approaches have scarcely been used.

This paper reports an attempt to analyse antigens of grape leaf and callus in *Vitis* spp. with different disease resistance.

Material and methods

Leaves of American species (*V. rupestris* Scheele, *V. riparia* Michx., *V. monticola* Buckley) and *V. vinifera* cultivars (Chasselas blanc, White Muscat, Rkatsiteli and Black Serexia) were collected during the flowering period when they had the highest protein content. Leaf mesophyll with large veins was cultured on agar medium (Schenk-Hildebrandt 1972; containing 2 mg/l αNAA and 0.5 mg/l 6-BAP) in climatic chambers at 26 °C in the dark. The callus was transferred to fresh media with intervals of 21 d. Proteins were extracted from the callus at its highest protein content, 9-21 d after a transfer.

P r e p a r a t i o n o f a n t i g e n s : A pooled fraction of soluble protein of grape leaf and callus was used as a source of antigens. Proteins were extracted using 0.02 M phosphate buffer (pH 9.0) containing 0.2 % ascorbic acid, 0.1 % 2-mercaptoethanol and 0.1 EDTA as protective additives (extraction buffer).

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Small amounts (5-10 g) of freshly washed grape leaves were frozen in liquid nitrogen and ground in a mill. Polyclar AT (leaves: Polyclar = 1:1 v/v) for binding phenolic compounds (CREMER and VAN DE WALLE 1985; MASA 1985) and then the extraction buffer (leaves:buffer = 1:5 v/v) were added. As phenolic synthesis in callus is weak, Polyclar was not used during protein extraction from the callus (callus:buffer = 1:1 v/v). The callus homogenate was treated in a MSE ultrasonic disintegrator (3 times, duration of treatment 1 min), kept for 1 h at 4 °C, pressed in a piece of silk cloth. The extraction was repeated once. After centrifugation at 12000 rpm the supernatant was gel-filtrated on a Sephadex G-25 column, and equilibrated with the extraction buffer which was also used for protein elution. The extracts were then concentrated in an Amicon device (Model 402) equipped with a molecular filter RM 10. The amount of protein was determined according to the method of BRADFORD (1976). This method is sensitive and phenol-tolerant. All procedures were done in a refrigeration chamber at 0-4 °C. 0.5-1 ml of antigen solutions were poured in test tubes and stored at -30 °C. They were used for rabbit immunization, biochemical and immunochemical analyses.

Preparation of immune sera: Chinchilla rabbits (2-3 animals for each variant) were immunized four times with weekly intervals. Antigens containing the Freund adjuvant were injected into the popliteal lymph nodes and hypodermically in the breast and belly without the adjuvant. Each rabbit received a total of 120 mg protein. A week after the last injection, the serological titer was checked. With its value being not less than 1:128, samples of blood were taken from the auricular and the marginal veins and a serum was raised. The serum was thiomersal-conserved, poured into sterile test tubes and stored at -30 °C. Antisera were absorbed with a heterologous antigenic material (initial protein concentration > 30 mg/ml) the neutralizing dose of the heterologous antigen having prefixed. Each antiserum of American origin was absorbed with the antigens of *V. vinifera* cultivar and those of an American species. Antisera of Euro-Asian origin were absorbed similarly. As a result, narrow specificity antisera against individual grape species were obtained and used to test leaf and callus antigens of different grapevine cultivars and *Vitis* spp. by the double-immuno-diffusion method of OUCHTERLONY (1953).

Results and discussion

Comparative immunological analysis of the antigens (ZILBER and ABELEV 1962) revealed that leaf and callus antigens of the *V. vinifera* cultivars were identical yet absent in the American species. Similarly, the identical antigens of American origin were not found in the *V. vinifera* cultivars. Thus, antigens specific for the American species were not present in the leaves and in the callus of the *V. vinifera* cultivars and vice versa. This study indicates that immunochemical differences among species of grapevine can be revealed by analysis of vegetative organs (leaves in this case) and their callus using test systems (Figure). No differences were observed between leaf and callus antigens of one species. Reactions of the Euro-Asian antisera with the callus antigens of the same origin showed weaker precipitation lines as leaf antigens, some of which seem to be absent in the callus. Species-specificity of antigens of vegetative plant organs has been shown by other workers (GAVRILIUK 1986; KLOZ *et al.* 1962) in legumes.

Immunochemical properties of the antigens subject to heat treatment were studied and it was found that they were thermostable and could be boiled for three minutes. This did not induce any changes in immunochemical properties of the *V. vinifera* antigens. In the American antigens, the precipitation reaction even occurred only after their having been boiled, which seems to be due to the breakdown of factors preventing diffusion of the American antigens in the agar. The thermostability of the antigens of American origin suggests that grape proteins are glycoproteid complexes. This opinion is also shared by HSU and HEATHERBELL (1987) and SITTERS (1988).

Ultrafiltration with Amicon filters (x 300, x 100, PM 30, PM 10) showed that the antigens of Euro-Asian origin generally had molecular weights of approximately 100 kDa while those of American origin had molecular weights of more than 200 kDa. It should also be noted that protein fraction with high molecular weights were found in grape leaves and juice (Hsu and Heatherbell 1987; Sitters 1988). It is worth mentioning that in the American species, protein concentration of the antigens with molecular weights of more than 200 kDa was three times larger than that of the *V. vinifera* antigens with the same molecular weights (3.94 and 1.25 mg/ml, respectively). Protein concentration of the antigens of Euro-Asian origin with molecular weights of 100 kDa was 3.5 times higher compared to that of the American antigens (3.92 and 1.11 mg/ml, respectively). The double-immunodiffusion reaction of the leaf antigens of both origins (MW more than 200 kDa) with the antisera of American origin revealed qualitative differences. Precipitation lines were obtained only with antigens of American origin (Figure, E). The double-immunodiffusion reaction of the antigens of both origins (MW approximately 100 kDa) with the Euro-Asian antisera yielded precipitation lines only in the *V. vinifera* antigens. In the antigens of both origins with molecular weights of less than 100 kDa, protein concentration was much lower (0.42 mg/ml on the average). No precipitation occurred in the leaf and the callus antigens with molecular weights of less than 100 kDa. It suggests that the leaf and callus antigens with molecular weights of approximately 100 kDa are species-specific of *V. vinifera* while the antigens with molecular weights of more than 200 kDa are specific for the American species. The antigens with molecular weights of less than 100 kDa were non-immunogenic in the species of both origins.

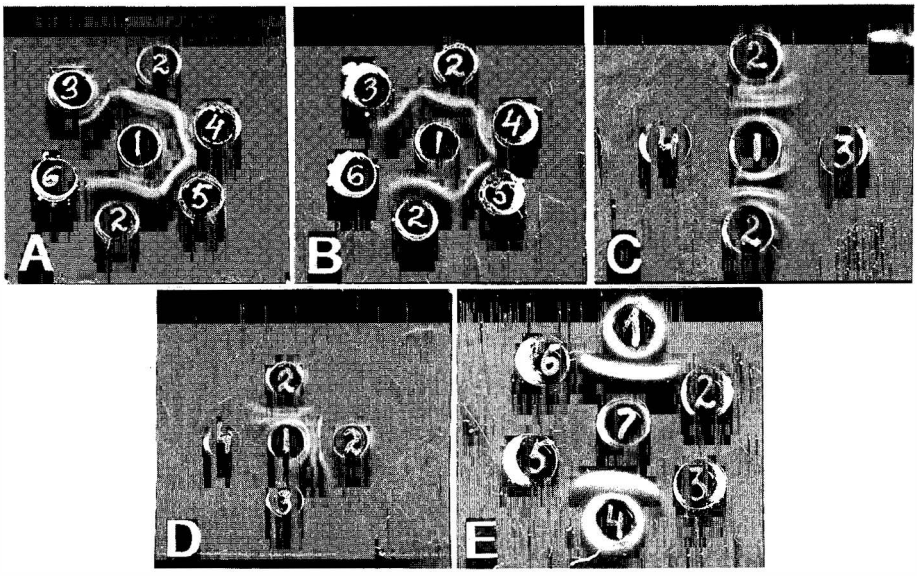

Figure: Comparative analysis of leaf and callus antigens of Euro-Asian and American origins. 1-2 components of the test system: (A) against leaf antigens of Chasselas blanc, 3 leaf antigens of Rkatsiteli, 4 leaf antigens of White Muscat, 5 leaf antigens of Black Serexia, 6 saline; (B) against callus antigens of Chasselas blanc, 3 callus antigens of Rkatsiteli, 4 callus antigens of White Muscat, 5 callus antigens of Black Serexia, 6 saline; (C) against leaf antigens of Chasselas blanc, 3 leaf antigens of *V. riparia* Michx., 4 saline; (D) against callus antigens of Chasselas blanc, 3 callus antigens of *Vitis riparia* Michx., 4 saline. (E) Double-immunodiffusion reaction of an American antiserum with grape leaf antigens of Euro-Asian and American origins (MW more than 200 kDa): 1 leaf antigens of *V. rupestris* Scheele; 2 leaf antigens of Chasselas blanc; 3 leaf antigens of White Muscat; 4 leaf antigens of *V. monticola* Buckley; 5 leaf antigens of Rkatsiteli; 6 saline; 7 absorbed antiserum against leaf antigens of *V. rupestris* Scheele.

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