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Taxonomic affinities of 5 Cultivars of *Vitis vinifera* L. as Aided by Serological Analysis of Pollen Proteins[1]

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Abstract. **The Ouchterlony immuno-double diffusion and crossed immuno-electrophoresis tests each identified one specific pollen protein in each of 5 grape cultivars. Additional proteins that were common provided evidence for 'White Roumi' being closely related to 'Red Roumi' and 'Italia' and distantly related to 'Thompson Seedless'. An intermediate but close serological relationship was also identified between 'Italia' and both 'Bez-El-Nakah' and 'Thompson Seedless'.**

The field of chemotaxonomy has rapidly extended and added additional chemical characteristics to contribute to investigations of plant taxonomy (3, 5, 7, 9, 11, 22, 23, 24). Acceptance of DNA as the source of the genetic message for protein synthesis, and of proteins as the enzymes that catalyze all biochemical activities, makes protein structure an important chemical characteristic of plants (6, 16, 18). Comparative study of primary protein structure, therefore, is a sensitive technique for demonstrating genetic difference or relationship among plant taxa. Among methods giving significant results are the serological techniques (1, 12, 15, 16, 17, 19, 27).

Application of serology to fruit taxonomy was begun only recently. Drawert and Gorg (10) identified grape cultivars according to the antigenic properties of their leaf proteins. Samaan (26) and Abd-El-Rehim et al. (1) also used serological analysis of leaf proteins to identify the degree of genetic relationship among *Citrus* species and cultivars.

The objective of this study was to test whether serological analyses of pollen proteins accurately reflect intervarietal relationships among 5 cultivars of *Vitis vinifera* of world wide importance.

Materials and Methods

Five grape cultivars grown in Egypt, 'Red Roumi', 'White Roumi', 'Italia', 'Bez-El-Nakah' and 'Thompson Seedless', were used. These cultivars are grown in many countries of the world (France, Italy, Spain, United States, etc.) under various synonyms: ('Thompson Seedless' = 'Sultanine blanche' = 'Sultanina' = 'Sultana'; 'White Roumi' = 'Muscat Romano' = 'Muscat of Alexandria'; 'Red Roumi' = 'Red Muscat'; 'Italy' = 'Ideal' = 'Pirovano 65' = 'Muscat Italia') (20, 28).

Vines of these cultivars were grown in the experimental vineyard, College of Agriculture, University of Alexandria. From May 5 to 20, 1980 pollen from the 5 cultivars was collected from flowers that were just opening. The pollen was quickly frozen in glass vials (25 ml) immersed in a dry ice-acetone mixture. The pollen was kept frozen on dry ice until used in the laboratory of the Department of Plant Breeding and Biometry, College of Agriculture, Cornell University.

Production of antisera. New Zealand White rabbits (3–4 kg) were used. The antigens were protein extracts from the pollen (18). One gram of pollen of each cultivar was air dried, defatted with 25 ml ethyl ether for 20 min and ground dry in a mortar and then in 10 ml of 0.1 M Na monophosphate-buffered physiologic saline (pH 6.7) until homogeneous. The antigen for injection was prepared using the incomplete adjuvant technique (14). One half ml of the antigen suspended in physiological saline was added slowly drop by drop with constant stirring to an equal volume of incomplete adjuvant. A 0.25 ml aliquot of this was injected intramuscularly in each thigh, for 12 weekly intervals. Prior to the first injection, 5 ml of blood was drawn from the lateral ear vein of each rabbit. The obtained antisera gave entirely negative results with all the described antigens. Ten days after the 8th and 10th injections rabbits were bled (5 ml blood) to check the activity of antisera using the precipitation ring method (21). The last bleeding was 10 days after the 12th injection. The obtained blood was first incubated for 3 hrs at room temperature, refrigerated overnight, and then the antisera were separated as recommended by Carpenter (8) and stored at –10°C until used.

Antigen for precipitation reactions. The extraction method used to obtain antigen for injection was adopted, except that no adjuvant was added. The antigen suspension was kept at 4°C for 24 hr before centrifugation at 20,000 g at 1° for 1 hr. To prevent microbial growth 0.1% sodium azide was added. This yielded approximately 6 ml of 3.5% protein solution.

Ouchterlony immuno double-diffusion test. The method described by Ouchterlony (25) was carried out in 1% agarose on 5x5 cm^2 glass slides. Agarose was prepared by dissolving 1 g of agarose (standard low-mv) in 98 ml of 0.1 M Na monophosphate-buffer red physiologic saline (pH 6.7) and 2 ml 0.1% sodium azide. This was boiled until the agarose dissolved. The hot solution was then clarified by filtration through glass wool, and stored in the refrigerator at 4°C until needed. Four ml of hot agarose was pipetted onto each glass slide. After it solidified, wells were formed using 8 mm and 4 mm diameter stainless steel assay cylinders for the central and the peripherals wells, respectively. Distance from the central antiserum well to the peripheral antigen wells was 5 mm.

The wells were charged by pipetting 100 µl of antiserum and 30 µl of antigen. The slides were kept at 35°C in an incubator for 48 hr. The bands were then recorded with pencil sketches and photographed. As a measure of the serological relationship among the 5 grape cultivars, the number of precipitation bands for a given heterologous reaction was converted to % of the total number of corresponding bands for the homologous reaction.

Crossed immuno-electrophoresis test. The methodology is from Axelsen et al. (4). In the first dimension, 20 µl of each antigen was electrophoresed in 15 ml of 1% agarose without antibody on a 100 × 100 × 1.5 mm glass plate. In the second dimension the

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electrophoresis was in agarose with absorbed antiserum (1:11 v/v). The first dimension electrophoresis was for 1 hr with 290 V at 10°C, the second was overnight (19 hr) with 70 V at 10°C. After electrophoresis the gel was pressed, washed with saline, dried at 37°C, stained with 50 ml of 0.5% Coomassie brilliant blue R-250 solution and destained with a 50 ml mixture of ethanol 95%, glacial acetic acid and distilled water 4.5:1.0:4.5 v/v/v, respectively.

Absorbed antiserum was prepared by adding to 1.5 ml of antiserum specific to one cultivar an 0.5 ml aliquot of each of the other 4 heterologous antigens. The 1.5:0.5 ratio was used since this quantity of homologous antigen removed all homologous antibodies. The antigen-antibody system being absorbed was incubated at 37°C for 1 hour, then stored overnight in the refrigerator. The precipitate was centrifuged down at 2000 g and 0°C for 20 min. The supernatant was used to test for unabsorbed antibodies.

Quantitative determinations of cultivar specific antigen-antibody systems, remaining in the absorbed antisera, were obtained from the crossed immuno-electrograms. Two methods (4) were used to measure the area enclosed by the precipitation band: 1) the height (distance from the base line to the top of the peak in mm) was multiplied by the width (at the point which bisected the height); 2) an outline of the precipitation band was drawn on paper, cut out, weighed, and converted to area. Because of near identify of the relative measurements from these 2 methods, the average of the 2 is given in the text as the concentration of the precipitation bands.

Results

The Ouchterlony double diffusion and the crossed immun-electrophoresis tests each identified 1 specific antigen from the pollen of each of the 5 tested grape cultivars. For 'Red Roumi', 'White Roumi', 'Italia', 'Bez-El-Nakah', and 'Thompson Seedless', respectively, these specific antigens were precipitation bands 3, 3, 7, 3, and 3 in the Ouchterlony test with unabsorbed antisera (Figs. 1-5, respectively). These respective antigens were bands 1, 2, 3, 4, and 5 in the crossed immuno-electrophoresis test with absorbed antisera. (Fig. 6) Thus, the serological specificity of both tests identified 1 pollen-protein that was specific to each cultivar. Each of the 5 cultivar specific antigens was further confirmed as being a different protein by its different movement in the first dimensional electrophoresis, in the absence of antibodies (Fig. 6). The second dimensional electrophoresis, in the presence of antibodies, quantified these antigens. 'Red Roumi' (105) and 'Bez-El-Nakah' (88) had the highest relative quantities of specific antigen (Fig. 6). The lowest was in 'White Roumi' (25) with intermediate quantities in 'Thompson Seedless' (49) and 'Italia' (33).

With the Ouchterlony test (Fig. 1–5) the 3 to 6 antigen-antibody precipitation bands that were common between each pair of cultivars indicated the degree of genetic relatedness. Relatedness was quantified by expressing this number of common serological bands as its percentage of the total number of bands in the homologous antigen-antibody reaction (Table 1). 'Red Roumi', 'White Roumi' and 'Italia' were all closely related to each other; also, each was distantly related to 'Bez-El-Nakah' and more distantly to 'Thompson Seedless' (Table 1). 'Italia' was more closely related to 'Bez-El-Nakah' and to 'Thompson Seedless' than was either 'White Roumi' or 'Red Roumi'. Thus, averaged across all comparisons with the 4 other cultivars, 'Italia' had the most overall relationship, followed by 'White Roumi', 'Red Roumi', 'Ben-El-Nakah' and then 'Thompson Seedless'.

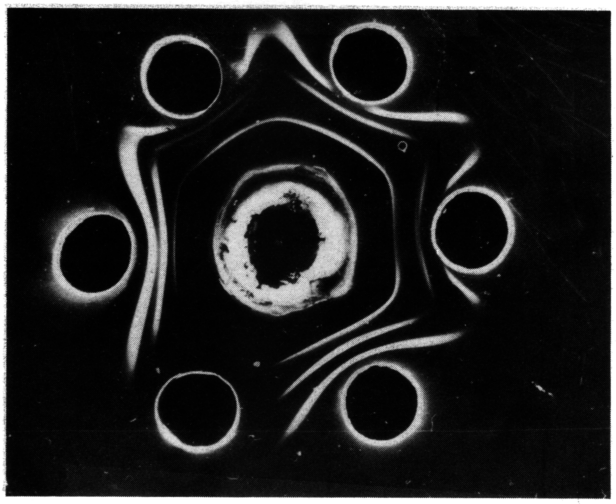
Discussion

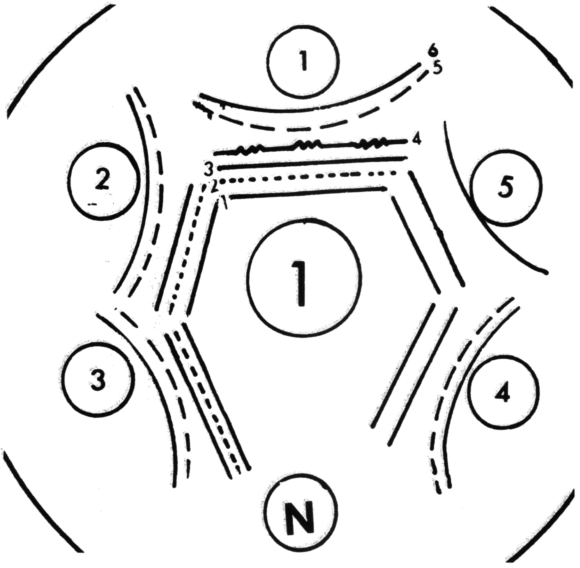
The successful measurement by serology of genetic relatedness between grape cultivars coincided with similar successes of Drawert and Gorg (10) with grapes and of Samaan (26) and Abd-

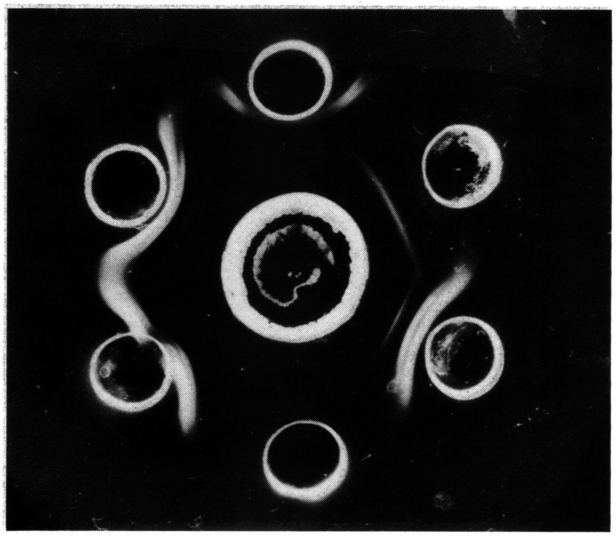
Table 1. Serological relationships among 5 *Vitis vinifera*.

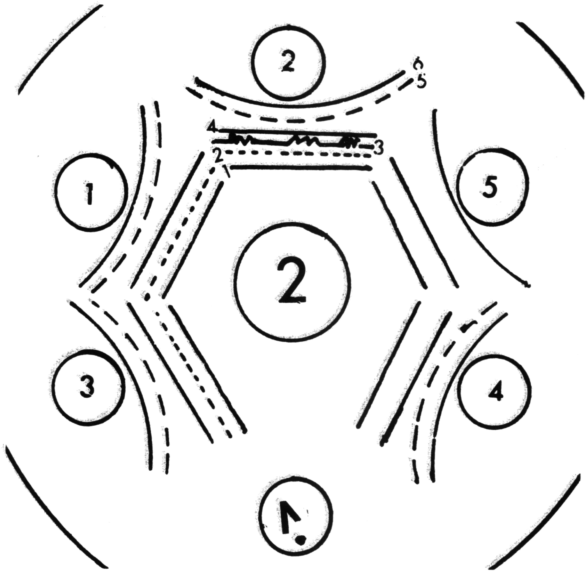
Antiserum	Antigen	Serological relationship[z]		Serial no.
		No. of bands	%	
Red Roumi	Homologous	6	100	1
	White Roumi	5	83	2
	Italia	5	83	3
	Bez-El-Nakah	4	67	4
	Thompson Seedless	3	50	5
White Roumi	Homologous	6	100	2
	Red Roumi	5	83	1
	Italia	5	83	3
	Bez-El-Nakah	4	67	4
	Thompson Seedless	3	50	5
Italia	Homologous	7	100	3
	Red Roumi	5	71	1
	White Roumi	6	86	2
	Bez-El-Nakah	5	71	4
	Thompson Seedless	5	71	5
Bez-El-Nakah	Homologous	7	100	4
	Red Roumi	5	71	1
	White Roumi	5	71	2
	Italia	6	86	3
	Thompson Seedless	4	57	5
Thompson Seedless	Homologous	6	100	5
	Red Roumi	3	50	1
	White Romi	3	50	2
	Italia	4	67	3
	Bez-El-Nakah	3	50	4

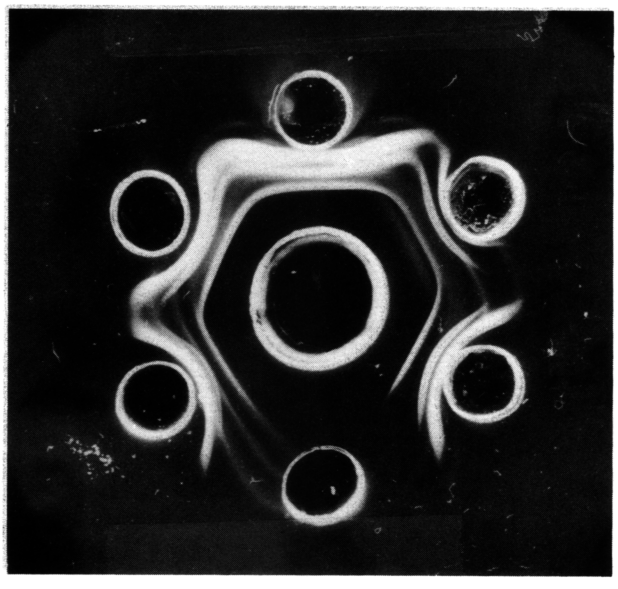
[z]Number of precipitation bands for a given heterologous reaction was converted to a percent of the total corresponding bands homologous reaction.











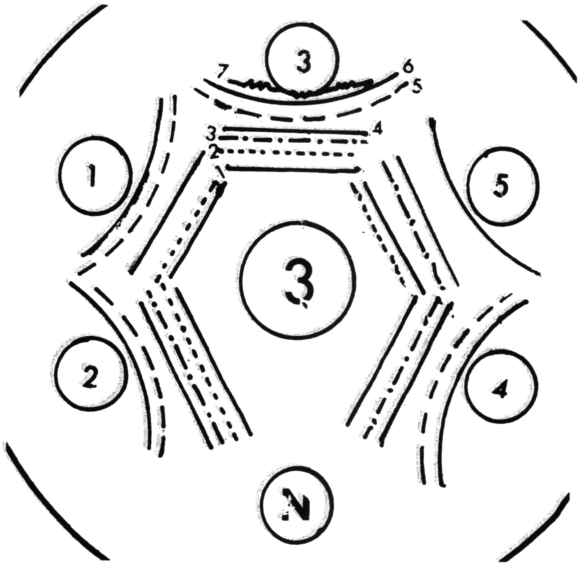
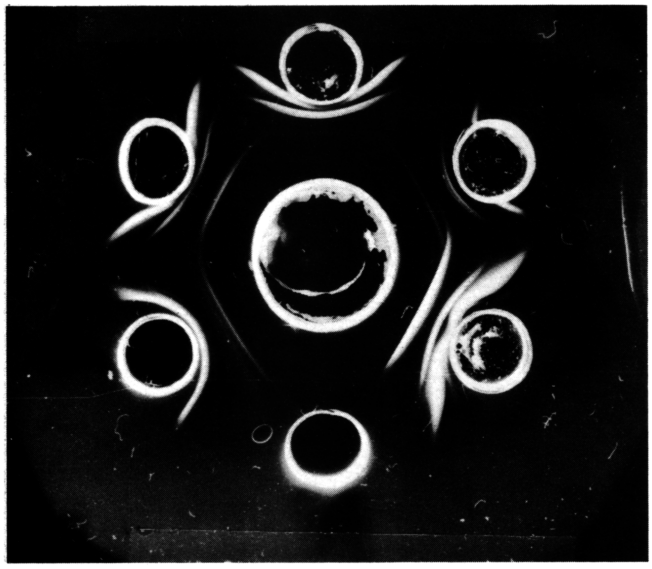
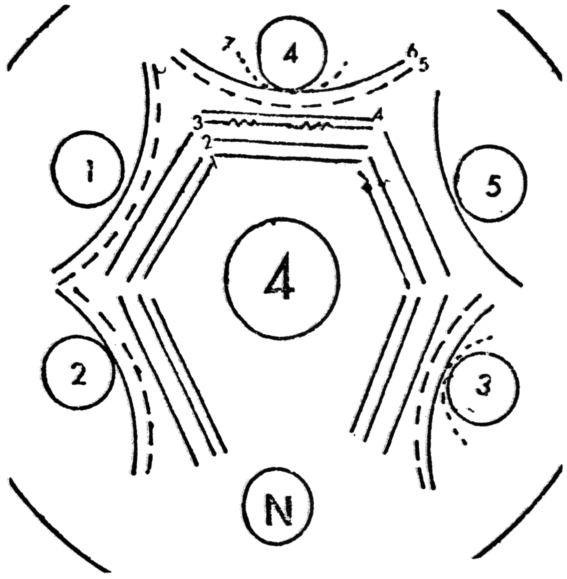
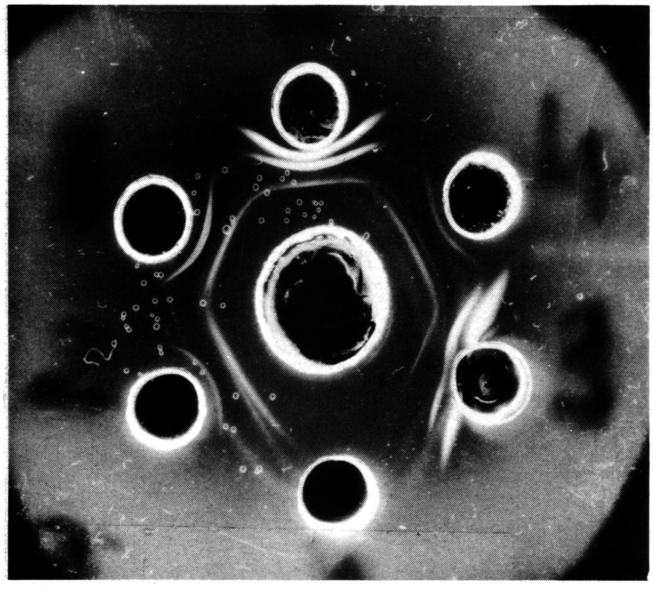
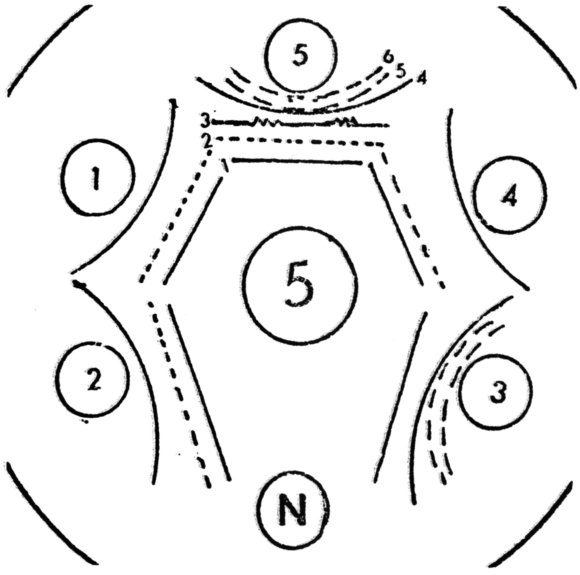


Fig. 1–5. (this and facing page) Photographs and diagrams of the precipitation bands resulting from the reaction antisera in the central wells with the homologous and heterologus antigens in the peripheral wells (1–5) and with physiological saline (N). Fig. 1, 'Red Roumi' antiserum in the central well; Fig. 2, 'White Roumi'; Fig. 3, 'Italia'; Fig. 4, 'Bez-El-Nakah'; Fig. 5, 'Thompson Seedless'.





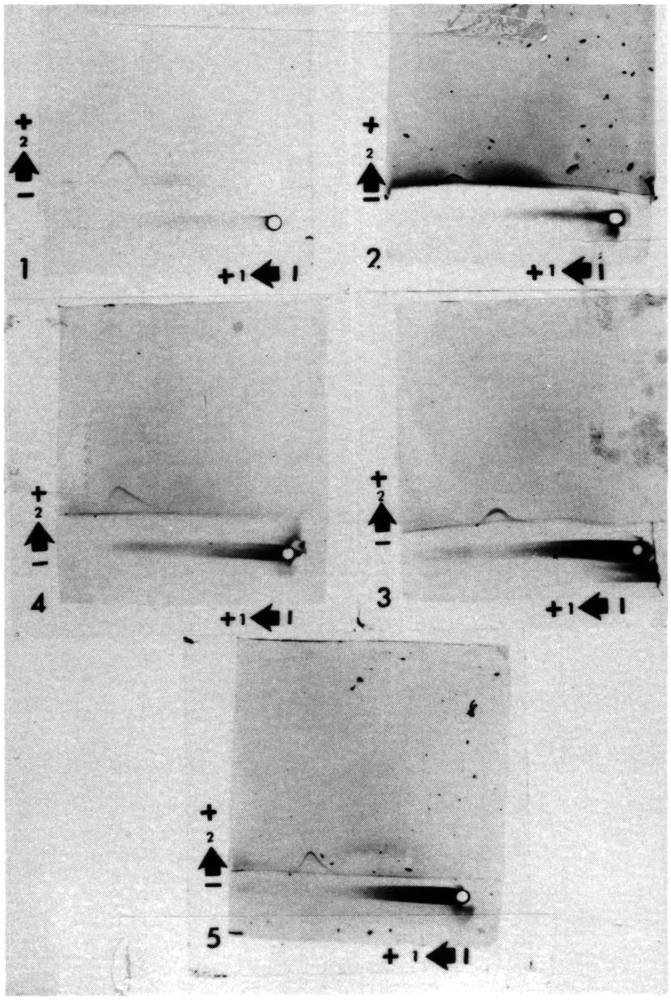




El-Rehim et al. (1) with *Citrus*. In other plants, serological techniques also identified the interspecific and intervarietal taxonomical relationships (2, 12, 13, 15, 16). Pollen grains were used as the antigen source because pollen protein are more often specific to the plant genotype (18). These proteins are controlled directly by the DNA code. Because the pollen is haploid, there is no interference by dominance.

The serological identification of 1 pollen protein that was specific for each of the 5 grape cultivars provided specific identification for each cultivar. Serological search for such cultivar specific pollen proteins should be extended to a large number of grape cultivars. This would determine the extent to which all grape cultivars have 1 or more specific pollen proteins, or alternatively the extent to which all can be classified according to a smaller number of such pollen proteins. Because grape cultivars are propagated by cuttings, such information would help reconcile the conflicting names given to the same grape cultivars in various countries and locations.

Our results from the Ouchterlony test suggested a close genetic relatedness between 'Red Roumi', 'White Roumi' and 'Italia'. 'Thompson Seedless' appears to be distantly related to 'Red Roumi' and 'White Roumi' and intermediately related to 'Italia'. These findings agree with the ampelography of *Vitis vinifera* grapes reported by Viala (29). He indicated that 'Red Roumi' ('Red Muscat') and 'White Roumi' ('White Romain', 'Muscat Romano' or 'Muscat of Alexandria') were originated from the same region (North Africa). Such results also agree with the phenotypical classification key for vinifera cultivars (20). Based on similarities in morphological features, they placed 'White Roumi' and 'Italia' in the Downy growing tip group and 'Thompson Seedless' in the cobwebby growing tip group. The distant relationship between 'Thompson Seedless' and other



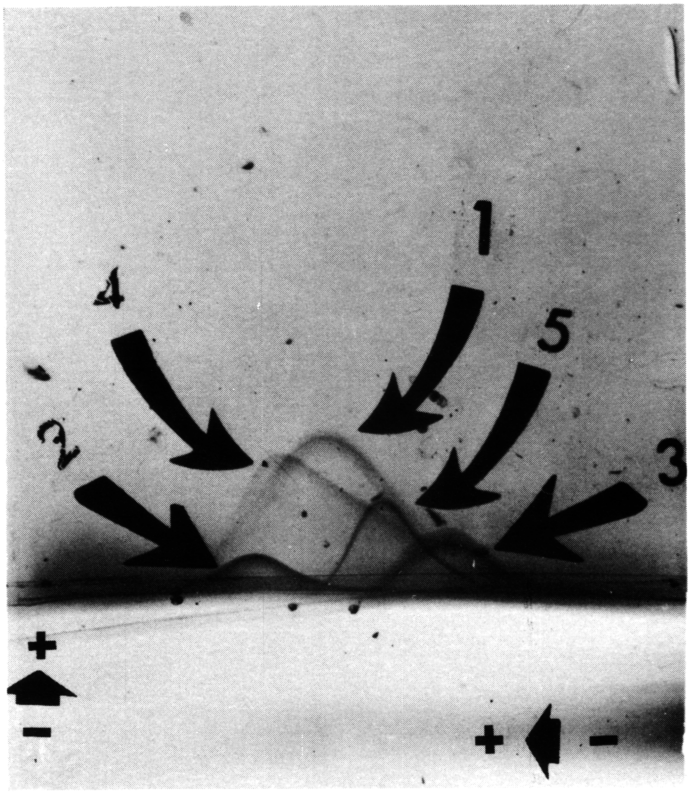


Fig. 6. Crossed immunoelectrophoresis of pollen proteins from 'Red Roumi' (1), 'White Roumi' (2), 'Italia' (3), 'Bez-El-Nakah' (4), and 'Thompson Seedless' (5) grape cultivars against their homologous absorbed antisera.
(a) Separated immunoelectrograms.
(b) Immunoelectrograms collected above each other.

tested cultivars was also apparent from the morphological description of *Vitis vinifera* cultivars made by Winkler et al. (29) and Leucie and Adams (20). They indicated differentiation by both berry and vine characteristics.

The serological analysis of pollen proteins gave genetic relatedness values that agreed with classifications based on morphological characters. Serology applied to grapes here for the first time, provides a new tool for grape breeders. More simply and rapidly than any other present techniques, serological anaylsis of pollen proteins can tell the breeder the genetic relatedness among his grape lines. This may enable him to predict the crosses that will give genetic advance in F_1. Strong associations have been identified between the magnitude of vigor in crosses and the degree of genetic relationship among the parents (1, 18, 26). Crosses between widely related genotypes not only produced greater vigor, they also gave a larger range of segregation than crosses between less diverse parents.

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Opening and Vase Life Extension of Peach Flowers on Detached Shoots with Sucrose and Ethanol[1]

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Additional index words. Prunus persica, water relations, flower opening, postharvest life

Abstract. **Detached shoots of double-flowered peach [*Prunus persica* (L.) Batsch] selections Fla. 6-1 and Fla. 0-5 were successfully opened in floral solutions containing 1 to 10% sucrose in deionized water. Addition of 8-hydroxyquinoline citrate (8-HQC), gibberellic acid (GA_3), or 6-benzylaminopurine (BA) to solutions did not extend vase life. Solution uptake rate decreased over the 8-day life of the shoots and was influenced by solution molarity. Xylem plugging by pectic-type materials increased with time in solution. Addition of 1% ethanol to the floral solution hastened time of first opening, decreased the extent of xylem plugging, and extended vase life. Ethanol at 2% extended vase life and increased solution uptake rate over solutions containing sucrose alone.**

Peach, almond, and Japanese apricot shoots have been used as cut flowers in Australia, Taiwan, and Chile, but their potential use has never been fully examined in the United States. Floral crops such as gladioli (16), chrysanthemums (18), and snapdragons (21) have been successfully opened from a tight bud stage by adding sucrose and a bactericide such as 8-hydroxyquinoline citrate (8-HQC) to the solution. Vase life of carnation was also extended by adding BA (6, 12), NAA and ABA (6), or kinetin (22), aminoethoxyvinylglycine, or sodium benzoate (2) to the holding solution.

Shoot water status is important to flower longevity. Reduced water uptake (19, 20), stem plugging (9, 15), and decreased fresh weight (20) have been associated with a decrease in vase life. Treatments that increase moisture retention by cut flowers generally increase flower longevity (26).

The University of Florida breeding program has developed double-flowered peach selections that have been opened as detached shoots (7); however, little is known about factors that affect flower opening and vase life. Our objectives were to study the influence of various floral solutions on flower opening, vase life, and shoot water relations, including xylem vessel plugging, during postharvest life.

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Materials and Methods

Shoots of double-flowered Fla. 6-1 and Fla. 0-5 peaches 30–50 cm in length were harvested from Jan. 31 to Feb. 9, 1979, and Jan. 19 to Feb. 26, 1980, when most buds were in the calyx green stage of development. Shoots were used within 24 hr, or stored in moist peat moss at 4 to 5°C for 7 to 10 days prior to use. Storage has no adverse effect on bud opening (7).

Flower opening studies. Three to 6 shoots per treatment were recut 1 to 2 cm above the base just prior to use and placed in the following solutions in 1979: 0.01 M citric acid (CIT); CIT + 200 ppm 8-HQC; CIT + 3% sucrose; CIT + 3% sucrose + 75 ppm BA; and CIT + 3% sucrose + 100 ppm GA_3 + 75 ppm BA. The same treatments were repeated in 1980, but no citric acid was added to the solution. Four additional treatments included 3% sucrose plus 1, 2, 4, or 8% ethanol, respectively. Deionized water was used in all solutions.

Shoots were weighed and stage of flower development and percent flower opening were monitored daily during 1980. Buds were opened under low light (50–100 $\mu E/m^2 \cdot sec$) and typical indoor temperatures (23 to 25°C) in the laboratory. Vase life was measured as time from first flower opening to flower fading.

Water uptake studies. Shoots were recut, weighed, and attached to suspended 10-ml pipets via rubber tubing for solution uptake studies. Daily uptake rates for 3.4 (0.1 M), 6.8 (0.2 M), 10.2 (0.3 M), or 13.6% (0.4 M) sucrose solutions were measured in 1979 and 1980. Duplicate samples were used at each sucrose