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Horticulture Department, College of Agriculture, University of Mansoura, Egypt

Pollination and serological studies on Egyptian grapes

by

L. G. Samaan, M. W. Taha, A. H. Hassan and M. S. El-Boraey

Bestäubungsversuche und serologische Untersuchungen an ägyptischen Reben

Zusammenfassung. — Die Rebsorte White Roumi (*Vitis vinifera* L.) bringt allein gepflanzt nur niedrige Traubenerträge. Sowohl Bestäubungsexperimente als auch die serologische Prüfung zeigten, daß es sich hierbei um eine partielle Selbstinkompatibilität handeln dürfte. In Rebanlagen mit White Roumi ist Fremdbestäubung erforderlich, um reichliche Ernten zu erzielen und die Beerenqualität zu verbessern.

Zwischen den Blüten von White Roumi und vier Pollenspendern wurden unterschiedliche Grade der Fremdverträglichkeit festgestellt. Bei Bestäubung mit Pollen von Red Roumi und Thompson Seedless waren der Beerenansatz und Fruchtertrag am höchsten. Pollen von Bez-El-Nakah hatte eine frühe Beerenreife zur Folge; die Beeren dieser Kreuzung waren am schwersten und größten. Die Sorte Ghariby erwies sich als ungeeigneter Pollenspender für die White-Roumi-Blüten; der Beerenansatz und Traubenertrag waren vermindert und der Anteil der vor der Ernte abgefallenen Beeren erhöht. Überdies waren die Beeren von geringer Qualität.

Introduction

Production of grapes is one of the important fruit enterprises in Egypt. Total acreage has increased rapidly during the past 20 years. It may become the second most important fruit, exceeded only by *Citrus*. From statistics of the Ministry of Agriculture in 1977, grape vineyards occupied about 48,169 feddans and produced 247,887 metric tons (1 feddan = 4201 m^2).

Since expansion of the grape industry, much attention has been directed to improving yield and berry quality. Pollination requirements, a principle factor in this respect, have been the subject of several investigations (Cejtlin 1956; Iyer and Randhawa 1964; Sharples *et al.* 1965; Tulaeva 1971; Fainerman 1972; Weiss 1976).

Observations in Egypt showed that the yield of White Roumi (*Vitis vinifera* L.) was much less when planted alone than when grown with other grape varieties. Determining the pollination requirements of this variety was the aim of the present study. Seven pollination treatments and serological tests for genetic relatedness were performed to estimate the degree of self- and cross-compatibility for White Roumi flowers and four grape varieties as pollen sources.

Materials and methods

Pollination tests

Five *V. vinifera* L. grape varieties grown in the experimental vineyard, College of Agriculture, University of Alexandria, were used for the pollination studies:

White Roumi, Red Roumi, Bez-El-Nakah, Ghariby, and Thompson Seedless. White Roumi was used as the maternal parent, the other varieties as pollinators. 10 mature White Roumi vines, of uniform vigor, were chosen at random, and inflorescences were treated on each vine in 1977 and 1978, as follows:

1 — Bagged only
2 — Emasculated and self-pollinated with White Roumi pollen
3 — Emasculated and cross-pollinated with Red Roumi pollen
4 — Emasculated and cross-pollinated with Bez-El-Nakah pollen
5 — Emasculated and cross-pollinated with Ghariby pollen
6 — Emasculated and cross-pollinated with Thompson Seedless pollen
7 — Open-pollinated (control)

Hand pollinations (OLMO 1936; BARRETT and ARISUMI 1952) were started on May 12, 1977 and on May 5, 1978. 7 flower clusters on each vine were chosen and thinned to 80 flowers per cluster. 5 clusters were carefully emasculated and hand-pollinated. The other 2 clusters were left without emasculation for the bagging and open-pollination treatments. All treatments were performed on each vine at the same time and the pollinated inflorescences were enclosed in paper bags. The number of treated flowers was recorded.

2 weeks after pollination, the bags were removed and the percentage of berry set was recorded for each treatment. This value provided a measure of the degree of compatibility between White Roumi as female parent and each pollinator. After June drop, the percentage of remaining berries was again recorded to estimate the effect of the pollinators on preharvest drop of White Roumi berries.

At harvest time, the mature berries of each treatment were picked, counted and weighed. A representative sample from each treatment was evaluated for characters of berry quality, as follows:

1 — Berry weight was recorded to the nearest gram.
2 — Berry size was determined by immersing 25 berries in 100 ml of water and recording the volume increase.
3 — The number of perfect (full and pear-shaped) seeds per berry, as averaged from 25 berries.
4 — Total soluble solids (T.S.S.) was measured with an Abbe hand refractometer, using the juice pressed from 25 ripe and fresh berries.
5 — Total acid content was determined by titrating 5 ml of clear juice with 0.158 n NaOH, using phenolphthalein solution as an indicator. The free acid was expressed as g of tartaric acid in 100 ml of juice.
6 — Soluble solids : acid ratio was calculated.

The data were statistically analyzed using the revised L. S. D. method (SNEDECOR and COCHRAN 1967).

Serological tests

Pollen from the five pollinator varieties and stigmas/styles of White Roumi flowers were used as antigen sources for the serological tests. Each antigen was tested against antiserum specific to White Roumi stigmas/styles. 1 g of pollen from each pollinator was air-dried, defatted with 25 ml ethyl ether for 15 min, air-dried again and ground in a mortar. 10 ml of 0.1 M Na-monophosphate buffered physiologic saline (pH 6.7) was added to the ground pollen and mixed until homogeneous. This mixture was kept at 4 °C for 24 h and then centrifuged for 1 h at 20,000 g at 0 °C. The resultant supernatant, with 0.1 % Na-azide solution added as a preservative, was the antigen source used in serological cross reactions. This procedure yielded approximately 6 ml of 13.55 % protein solution.

Proteins extracted from the stigmas/styles (MATTHEWS 1957; STEERE 1964) were also used as antigens. The proteins were precipitated by addition of $(NH_4)_2SO_4$ to the extract till saturation, stirred 10 min, then centrifuged at 14,000 *g* for 15 min at 0 °C. The precipitate was dialyzed against distilled water at 3—5 °C until no NH_4^+ or SO_4^{--} ions were detected. The dialyzate was used for immunization of two Boscat rabbits, 3—4 kg in weight. Each rabbit received 18 injections, twice a week, of 1—2 ml. The first 4 injections were intramuscular with free antigen, while the other 14 were intravenous with antigen to which 0.5 % Na-algenate was added to serve as an adjuvant.

Prior to the first injection, 5 ml of blood was drawn from the ear vein of each rabbit and the resultant antisera gave negative results with all the described antigens. 10 d after the last injection, the rabbits were bled and the antiserum was obtained as recommended by CARPENTER (1956).

Qualitative serology was carried out using the precipitation double diffusion test (OUCHTERLONY 1958) to study the location and number of precipitation zones resulting when the antiserum specific to stigmas/styles of White Roumi flowers was reacted with its homologous or heterologous antigens (ELLEN 1964).

For quantitative serology (BOYDEN *et al.* 1947; FREUND *et al.* 1948; KLEESE and FREY 1964). For each test quantities of antiserum and antigen solution were 0.7 ml and serum with its homologous antigen source using the ring precipitation test (ELLEN 1964). For each test quantities of antiserum and antigen solution were 0.7 ml and 1.4 ml, respectively. The precipitation reaction was measured by the apparent turbidity of the antigen-antibody system using a Pye Unicam sp. 6 series U. V. visible spectrophotometer, with a blank solution in the reference cell, and wave-length 375—535 nm. The reactants in each tube were mixed rapidly and the initial turbidity was recorded. Maximum turbidity was reached after 2 h (at 37 °C) and the magnitude of the precipitation reaction was the difference between the initial and final turbidities. A control tube containing antiserum alone was assayed for turbidity, and this value was subtracted from the reaction tube values. Turbidity for a given heterologous reaction was expressed as per cent of the turbidity for the corresponding homologous reaction.

Results and discussion

Pollination tests

Berry set percentage was used as an estimate of the degree of self- and cross-compatibility in and between the maternal variety and the tested pollinator varieties (CRANE and LAWRENCE 1929, DE VRIES 1968; FREY 1972). For both years of this study cross-pollination produced significantly higher percentages of berry set and preharvest drop than self-pollination (Table 1). Red Roumi and Thompson Seedless as pollinating varieties gave the highest set and least preharvest berry drop of White Roumi berries. Such findings were previously noticed in other grape varieties by OLMO (1936), SOSUNKO (1953), TKACENKO (1960), SHARPLES *et al.* (1965), TULAEVA (1971), FAINERMAN (1972), TSERTSVADZE (1973) and UPPAL *et al.* (1975). Thus, the low berry set from the bagged and hand-selfed flowers, in contrast to the high set of cross-pollination treatments, appears to be due to the presence of partial self-incompatibility in White Roumi flowers.

Table 1

Effect of different pollination treatments on berry-set, pre-harvest berry drop and fruiting per cluster in White Roumi grape variety in 1977 and 1978

Der Einfluß unterschiedlicher Bestäubungsverfahren auf Beerenansatz, Beerenverluste vor der Ernte sowie Beerenausbeute je Traube bei der Sorte White Roumi (1977 and 1978)

Treatments[1])	Berry-set				Pre-harvest drop		Fruiting	
	1977		1978		1978		1978	
	No.	%	No.	%	No.	%	No.	%
Bagged only	6.4	8.0	7.2	9.0	1.1	1.3	6.1	7.7
Self pollinated	5.3	6.6	6.4	8.0	1.4	1.7	5.0	6.3
Cross-pollinated with Red Roumi pollen	29.3	36.6	33.4	41.7	1.1	1.3	32.3	40.4
Cross-pollinated with Bez-El-Nakah pollen	21.6	27.0	24.2	30.3	1.8	2.3	22.4	28.0
Cross-pollinated with Ghariby pollen	13.9	17.3	15.0	18.7	2.6	3.3	12.4	15.4
Cross-pollinated with Thompson Seedless pollen	25.1	31.3	26.6	33.3	0.8	1.0	25.8	32.3
Open-pollinated (control)	9.6	12.0	11.0	13.7	0.8	1.0	10.2	12.7
L.S.D. at 5 %	—	5.67	—	9.27	—	0.94	—	8.83
1 %	—	7.70	—	12.89	—	1.31	—	12.34

[1]) 80 flowers were treated per cluster and the others were removed.

Table 2

Effect of different pollination treatments on some characters affecting fruit quality in White Roumi grapes (1978)

Der Einfluß unterschiedlicher Bestäubungsverfahren auf Merkmale der Beerenqualität bei der Sorte White Roumi (1978)

Treatments	Weight of 25 berries (g)	Size of 25 berries (cm^3)	No. of perfect seeds per berry	T.S.S. (%)	Acid (%)	T.S.S./Acid
Bagged only	120.7	113.5	1.00	21.0	0.98	21.3
Self pollinated	124.7	114.0	1.00	21.0	0.98	21.6
Cross-pollinated with Red Roumi pollen	145.7	139.5	1.33	21.4	0.81	26.3
Cross-pollinated with Bez-El-Nakah pollen	150.7	140.5	1.34	20.5	0.77	26.5
Cross-pollinated with Ghariby pollen	135.2	132.0	1.25	20.8	1.09	19.1
Cross-pollinated with Thompson Seedless pollen	144.7	140.0	1.34	20.4	0.97	21.0
Open-pollinated (control)	153.7	141.0	1.40	20.3	0.84	24.4
L.S.D. at 5 %	1.56	1.10	0.15	1.46	0.03	NS
1 %	2.06	1.49	0.20	NS	0.04	NS

The cross-pollinations were superior in giving highest weight, size and number of perfect seeds per berry (Table 2). The most effective pollinator was Bez-El-Nakah followed by Red Roumi and Thompson Seedless. The variation in such berry characters can be related to genetic differences of the pollinators.

Our results for the cross-pollination effect on berry quality are in line with DARNAY (1954) and IYER and RANDHAWA (1964) who found in all cross-pollinations an increase in berry weight and size and a reduction in number of small berries. Moreover, the latter investigators observed that the changes in berry characters were mainly due to the influence of pollen on the seed. This was then manifest in the final berry characters. UPPAL and MUKHERJEE (1968) and WEISS (1976) also reported that cross-pollinated vines produced larger berry weight, seed number and berry number per bunch than self-pollinated ones.

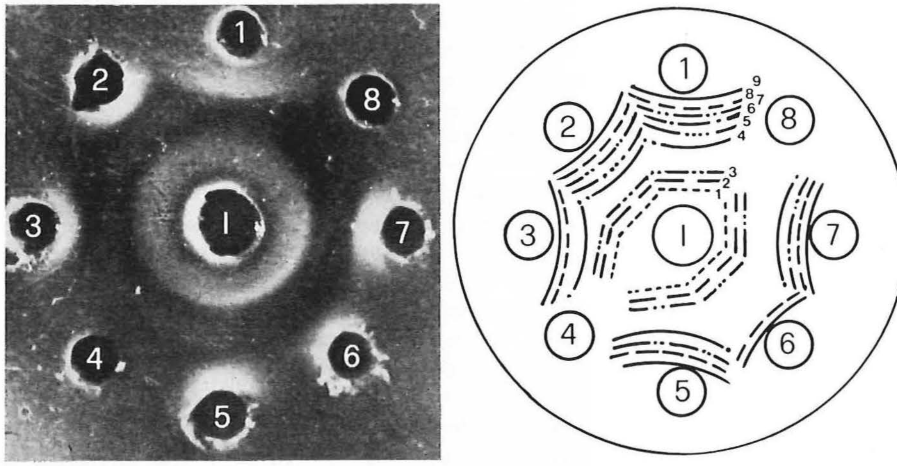
The positive effect of cross-pollination in this respect was also emphasized in other fruits by TETEREV and MEL NIKOVA (1959), WILLIAMS (1968), AL-DELAIMY and ALI (1969), STAFFORD (1970), VELLO and FERNANDO (1971), DE LANGE and VINCENT (1972), DE LANGE *et al.* (1973), KITAT *et al.* (1973) and SAMAAN and HASSAN (1976).

Effect of pollination treatments on White Roumi berries (Table 2) showed no significant differences among treatments in the percentage of T. S. S., a result observed previously by SHARPLES *et al.* (1965) with Cardinal grapes.

Acid content and the T. S. S. : acid ratio showed no consistency. Ghariby pollen increased the acid content and decreased the T. S. S. : acid ratio in the berries. On the other hand, the lowest acidity and the highest T. S. S. : acid ratio were found in the berries produced from Bez-El-Nakah pollen. These results suggest that among the tested pollinators Bez-El-Nakah variety produced the earliest ripening berries of White Roumi. Moreover, such results coincide with those of NEBEL (1930) and DZIECIOL (1961) who indicated that the sugar and acid content in apple juice were highly influenced by the male parent. Similar results were observed in grapes by DARNAY (1954). He found that time of ripening and other characteristics in the berries were greatly affected by cross-pollination. Also KITAT *et al.* (1976) working with pears, found no consistent effect of cross-pollination treatments on the chemical characteristics of the fruit juice.

Serological tests

Qualitative and quantitative serological tests were made of the degree of antigenic relationships for proteins in the stigmas/styles of White Roumi versus the pollen from the five pollinator varieties. These tests revealed that the greatest number of precipitation bands (Figure) and the strongest serological relationships (Table 3) were against the fully homologous White Roumi stigmas/styles antigens. The second strongest reaction was against pollen antigens of the same grape variety. The fully homologous reaction with the White Roumi stigmas/styles formed a specific precipitation band (No. 6) that was not found in cross-reaction with any pollens. The relationship of the degree of compatibility between pistil and pollen with the variability in the level of the self-incompatibility proteins that were present in the stigma was evidenced in the serological studies of LANDOVA and LANDA (1973), NASRALLAH (1974) and KOVALOVA *et al.* (1975). NASRALLAH (1974) indicated positive correlation between the concentration of an S-allele-specific protein in the stigmas and the degree of self-incompatibility. Accordingly, in our results, the highest number of precipitation bands and the strongest serological relationships which were present in the homologous reaction with White Roumi stigmas/styles antigens as



Immunodiffusion in agar showing precipitation bands between antiserum specific to stigmas/styles of White Roumi flowers in the center well (I) and homologous antigen (well 1) along with pollen antigens of White Roumi (2), Red Roumi (3), Bez-El-Nakah (5), Thompson Seedless (6) and Ghariby (7) and physiological saline in the wells (4) and (8).

Agar-Immundiffusion mit Präzipitationsbändern. In der Mitte befindet sich das gegen Narben bzw. Griffel der Sorte White Roumi spezifische Antiserum (I); die äußeren Löcher enthalten das homologe Antigen (1) sowie Pollenantigene von White Roumi (2), Red Roumi (3), Bez-El-Nakah (5), Thompson Seedless (6) und Ghariby (7), ferner physiologische Salzlösungen (4) und (8).

Table 3

Serological relationship (i.e. heterologous/homologous) between White Roumi stigmas/styles antiserum and its homologous antigen along with antigens from pollen grains from five grape varieties

Die serologische Verwandschaft (heterolog oder homolog) des gegen Narben bzw. Griffel der Sorte White Roumi spezifischen Antiserums mit seinem homologen Antigen sowie mit Antigenen aus Pollenkörnern von fünf Rebsorten

Antiserum	Antigen	Reading Initial	Reading After 2 h	Serological relationship (%)[1]
White Roumi (stigmas/styles)	Homologous	0.27	0.58	100
" " "	White Roumi (pollen)	0.51	0.76	80.7
" " "	Red Roumi (pollen)	0.63	0.77	45.2
" " "	Bez-El-Nakah (pollen)	0.74	0.95	67.7
" " "	Ghariby (pollen)	0.46	0.69	74.2
" " "	Thompson Seedless (pollen)	0.97	1.15	41.9

[1]) Turbidity for a given heterologous reaction expressed as per cent of the turbidity for the corresponding homologous reaction.

well as pollen antigens in comparison to the heterologous ones show the presence of high levels of self-incompatibility proteins in White Roumi stigmas/styles, thus causing self-incompatibility in this variety. The presence of such proteins was indirectly indicated by pollination study.

The precipitation band No. 6 formed only in the fully homologous reaction, could be due to a genotype specific protein whose presence is attributed to the specific self-incompatibility S allele of White Roumi stigmas. This conclusion is in line with NASRALLAH and WALLACE (1966), NASRALLAH *et al.* (1970) and TAKESHI and HINATA (1977) who indicated that stigmas of a self-incompatible genotype have specific and unique antigens which cannot be detected in pollen or in other tissues of the same plant.

In the present study, the serological tests also showed that the heterologous reactions generally formed a smaller number of precipitation bands and showed weaker serological relationships than those of the homologous ones. The cross-reaction with Thompson Seedless and Red Roumi pollen antigens resulted in the lowest numbers of bands, the weakest serological relationships and the highest berry set percentage. These inverse relationships suggest a degree of cross-compatibility between the pollinating varieties and White Roumi flowers and that degree has differed further from one to another according to their serological behavior. The highest degree was found with Thompson Seedless and Red Roumi pollens followed by Bez-El-Nakah, whereas Ghariby was the least in this respect.

Referring to the aforementioned results, it seems clear that both pollination and serological studies show the presence of partial self-incompatibility and cross-compatibility in and between White Roumi flowers and the tested four grape pollinators. Cross-pollination is necessary in White Roumi vineyards in order to obtain abundant yield and improve quality of the berries. The most compatible pollinators to White Roumi were Red Roumi and Thompson Seedless followed by Bez-El-Nakah. All are acceptable pollinators. Each of them has some advantages. The former two pollinators lead to the highest berry set percentage and fruiting. The latter one resulted in early ripening berries possessing the highest weight and size.

On the other hand, Ghariby was the least as far as compatibility to White Roumi flowers. This pollinator proved to be unsuitable, since it decreased berry set percentage and fruiting and increased percentage of preharvest berry drop. Moreover, the berries were lighter in weight, smaller in size, higher in acid content and later in ripening when compared to those of the other pollinating varieties.

Summary

White Roumi grape (*Vitis vinifera* L.) produces low yield when planted alone. Both pollination and serological tests indicated that this problem appears to be due to the presence of partial self-incompatibility in its flowers. Cross-pollination is necessary in White Roumi vineyards in order to obtain abundant yields and to improve berry quality.

Different degrees of cross-compatibility were found between White Roumi flowers and four grape pollinators. Evaluating these pollinators indicated that Red Roumi and Thompson Seedless pollen gave the highest berry set and fruiting. Bez-El-Nakah resulted in early ripening berries possessing the largest weight and size. Ghariby proved to be an unsuitable pollinator of White Roumi flowers. It decreased

berry-set percentage and fruiting and increased percentage of preharvest berry drop. Moreover, the berries were of poor quality.

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Dr. L. G. SAMAAN
Associate Professor of Pomology
Horticulture Department
College of Agriculture
University of Mansoura
Mansoura
Egypt