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Microbial Survey of the Genus *Agrobacterium* in Grapevine Nurseries in Jordan

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ABSTRACT. An eleven months survey of the total bacteria and *Agrobacterium* of seven fields in three Jordan Valley nurseries showed significant differences between cultivated and non-cultivated fields at the same nursery, between cultivated fields in different nurseries and between non-cultivated fields in different nurseries. The total bacterial count had its highest value in April for most of the study fields (except a sterilized one); also the highest mean count of agrobacteria was in April at Baqura and Rayyan fields. Seventy-two strains of *Agrobacterium* were isolated; twenty-three of them belonged to biovar I, eighteen to biovar II, and thirty-one to biovar III; however only nine of them were pathogenic; seven of the pathogenic strains belong to biovar II, and two to biovar III.

The Gram negative, rod shaped, aerobic, mesophilic bacteria of the genus *Agrobacterium* are found abundantly in soil and can survive there for many years (De Boer 1982), they form galls in plants (Kerr 1969). Species of *Agrobacterium* are more abundantly present in the rhizosphere than in the nearby soil (New and Kerr 1972, Bouzar and Moore 1987). A pre-requisite for gall formation is wounding of the host plant. Infection can occur during various stages of the life of the plants via wounds caused by growth, germination, subterranean insects or mechanical injuries (pruning, grafting, and replanting of trees). The microflora of the rhizosphere differs, both quantitatively and qualitatively, from that in the soil beyond the influence of the root (Deavin *et al.* 1981), however the rhizosphere effect depends on the type and age of plants as well as the type of microorganisms present in that region; it may have a stimulatory or inhibitory effect.

The taxonomy of the genus *Agrobacterium* has been treated by using different approaches, *e.g.* clustering, grouping, and biotyping (White 1972, Kerr and

Panagopoulos 1977). In recent years failure in grafting vine in Jordan increased tremendously (pers. comm. of the Ministry of Agriculture). Since an investigation of the soil microflora and in particularly of pathogenic bacteria has not been carried out in Jordan as yet, we started a project to survey plant pathogenic bacteria in Jordanian soil. In the present work the monthly variation of the total viable bacterial count and agrobacterial count were determined, and the biovars of *Agrobacterium* were identified. No significant correlation was found respectively between the viable count of total bacteria, agrobacteria and some environmental factors: temperature, humidity, pH and organic matter content of soil.

Materials and Methods

Seven areas of grapevine nurseries were included in this study:

(a) *Baqura Nursery:* (1) Grape cultivated (Bg), (2) Control non-cultivated (Bc).

(b) *Rayyan Nursery:* (1) Grape cultivated (Rg), (2) Control non-cultivated (Rc).

(c) *Deiralla Nursery:* (1) Grape cultivated and sterilized by methylbromide just before cultivated (D1), (2) Grape cultivated non-sterilized (D2), (3) Control non-cultivated (Dc).

Sampling and treatment of soil: Monthly samples consisted of a mixture of nine Auger holdings collected from the top 20 cm (after removing the upper 2-3 cm) at selected areas from each of the study fields. The soil mixtures were dried at room temperature, sieved in 2×2 mm sieve and one gram was suspended in 100 ml sterile distilled water and shaken at 190 rpm for 30 min. After serial dilution 0.1 ml of the dilution 10^{-3} was spread with a sterile L-shaped glass rod on standard plate count agar for total bacterial count and on the medium of Kado and Heskett (1970) medium for agrobacterial estimation. Plates were incubated at 27°C for 2-3 days. From each sample three plates were inoculated and the average of their counts was taken as the mean count.

Identification and biotyping: Suspected colonies of Gram-negative agrobacteria were further purified and identified according to Cowan and Steel (1965), Kersters and deLey (1984), and Sûle (1978). For testing pathogenicity 24 h old bacterial cultures were inoculated into a young stem of tomato, tobacco and kalanchoe and results recorded after 1-2 months. For biotyping of the isolates the procedure of Kerr and Panagopoulos (1977) was followed.

Results

As shown in Fig. 1, the viable mean count of total bacteria during the study period showed its maximum in April for most of the study fields except D1. The mean count then decreased gradually until it reached its minimum mostly in January.

Viable mean counts of agrobacteria on Kado and Heskett medium showed its maximum in April for Baqura and Rayyan fields, in November for D1, in December for Dc and in February for D2 as shown in Fig. 2.

The percentage of agrobacterial count in relation to total bacterial count which was set as 100% is shown in Fig. 3. Lowest values were in April for most of the study fields. The percentage varied from 0.005% at Bg in April to 16.66% at D2 in December. Variations in these results may be due to species composition in these fields. It seems that agrobacteria respond differently from total bacteria to changes in the soil environment; they prefer a lower temperature.

Seventy-two isolates were identified as strains of *Agrobacterium*. Twenty-three of them belonged to biovar I, eighteen to biovar II and thirty-one to biovar III. However only nine of them were pathogenic at least on one of the tested hosts (Table 1). The biochemical characteristics of these isolates is shown in Table 2. Only slight differences (not significant) were observed in the values for humidity, organic matter content, pH and temperature in these fields.

Discussion

Analysis of variance (ANOVA) of the mean counts of total bacteria and agrobacteria showed significant differences between the fields of the three nurseries, between the fields of each nursery and between the monthly interval samples within the same nursery.

The reproduction of soil microorganisms is influenced by many factors such as plant type, plant age, plant exudate, soil type, soil fertility, soil moisture and the presence and influence of other microorganisms (De Boer 1982). On one occasion one factor, on another occasion another factor acquires the main importance. In this study, the variations in the bacterial counts could be due to uncontrolled fertilization, removing of grasses and irrigation of these fields. They were treated randomly and not at the same day.

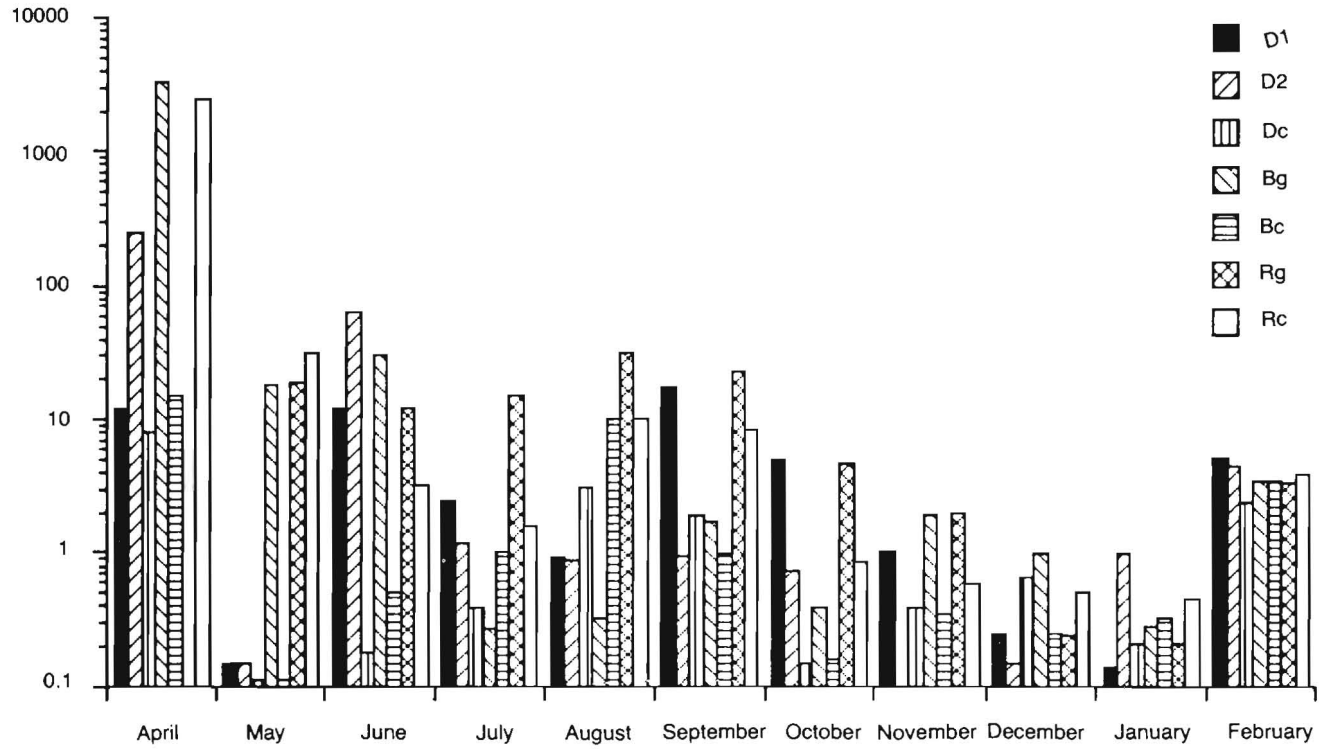

Fig. 1. The mean viable count of total bacteria on standard plate count agar of the different fields per gram of dried soil.

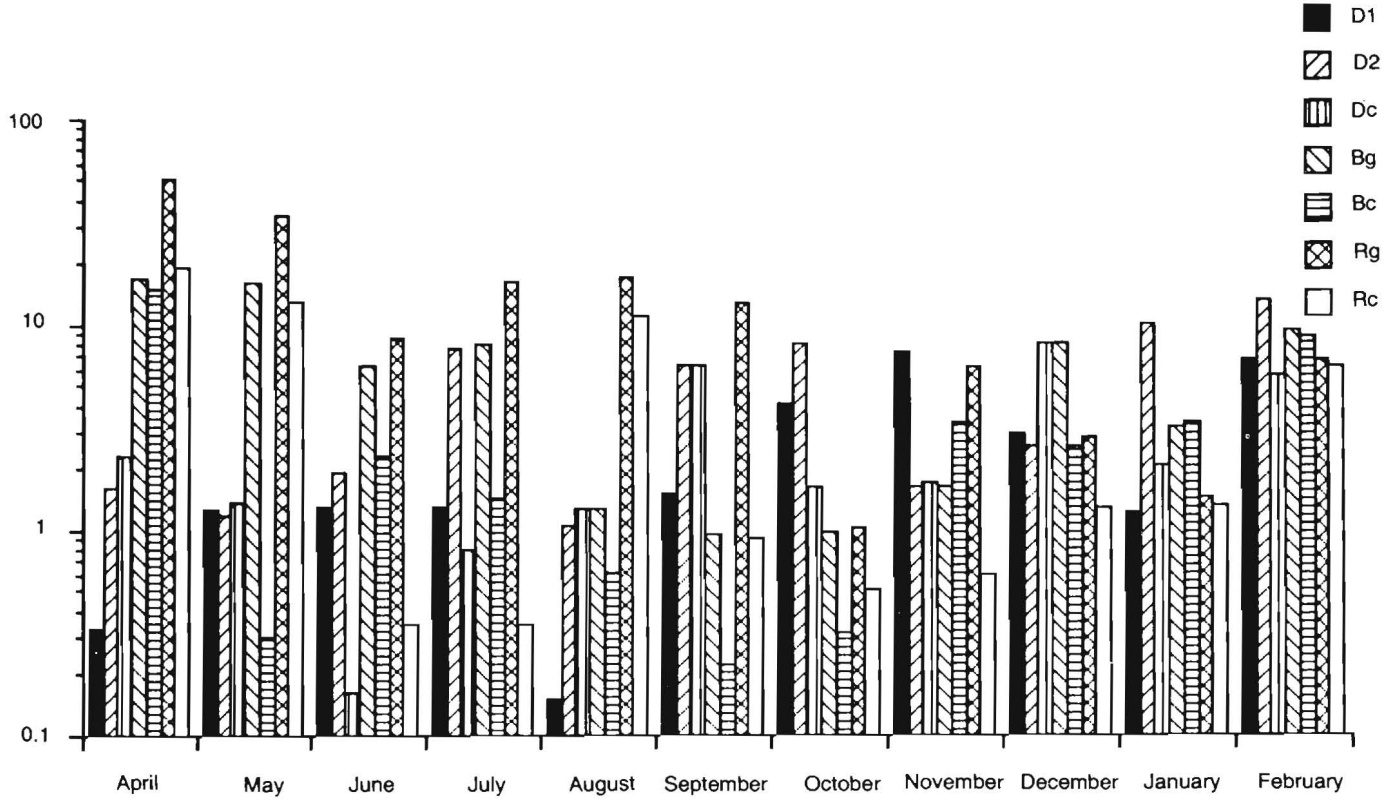

Fig. 2. The mean viable count of agrobacteria on Kado and Heskett medium from the different fields per gram of dried soil.

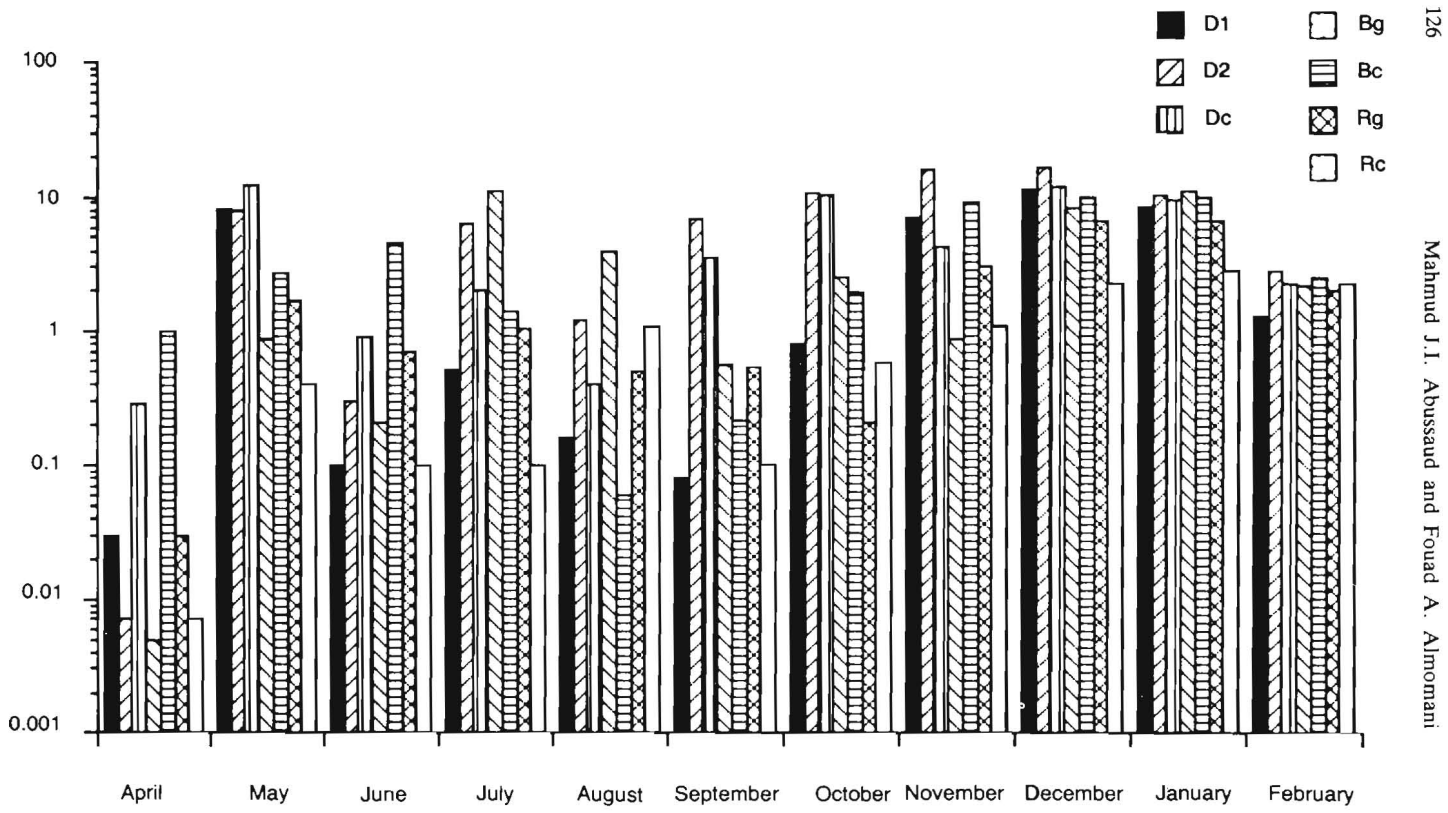

Fig. 3. Percentage of agrobacteria in different fields of the study.

The estimated agrobacterial percentage in this study was higher than that mentioned by Alexander (1982). Low pathogenicity of the isolates may be due to the fact that most of the soil isolates were saprophytic as reported by New and Kerr

Table 1. Number of samples, number of agrobacteria, number of pathogenic agrobacteria, and number of isolates in each biovar

Soil	Number of samples	No. of total agrobacteria	No. of pathogenic agrobacteria	No. of isolates in each biovar		
				I	II	III
D1	11	6	0	1	1	4
D2	11	7	1	2	2(1)	3
Dc	11	3	1	0	2(1)	1
Bg	11	16	1	6	4(1)	6
Bc	11	10	1	5	2(1)	3
Rg	11	16	5	5	3(3)	8(2)
Rc	11	14	0	4	4	6
Total	77	72	9	23	18(7)	31(2)

Number in parenthesis represents pathogenic isolates.

Table 2. Biochemical characteristics of *Agrobacterium* isolates

Test	Biovar		
	I	II	III
(1) Catalase	100*	100	100
(2) Oxidase	95*	25	39
(3) Utilization of:			
Mannitol	100*	100	100
3-keto-lactose	100*	8	11
Melezitose	95*	0	100
Raffinose	95*	25	39
Malonate	9*	92	94
Tartrate	45*	92	88
Citrate	31*	83	88
(4) Urease	100*	100	100
(5) H2S production	100*	100	100

* Percent positive strains.

(1972), Bouzar and Moore (1987) or it may be due to host range specificity as reported by Yanofsky *et al.* (1985). It was higher than that reported by Schroth *et al.* (1965). Biovar III was dominant over the other biovars of *Agrobacterium* in this study; this may be due to the fact that these fields were repeatedly cultivated with grapevine during the last three years, and that biovar III is the most frequently isolated one from grapevine tumors (Kerr and Panagopoulos 1977, Perry and Kado 1982 and Ma *et al.* 1987).

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مسح ميكروبيولوجي لبكتيريا الاجروبكتيريوم في مشاتل عنب في وادي الاردن

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أجري مسح ميكروبيولوجي للتربة في ثلاثة مشاتل حكومية من أكبر المشاتل المستعملة في تجهيز وتنمية غراس العنب وذلك بهدف التعرف على مدى تواجد بكتيريا الاجروبكتيريوم ونسبتها إلى العدد الكلي لجميع البكتيريا وعلاقتها مع بعض العوامل البيئية. كانت هذه المشاتل هي: مشتل الباقورة، مشتل الريان ومحطة دير علاً الزراعية. تم تحديد سبعة حقول في هذه المشاتل، أربعة مزروعة وثلاثة غير مزروعة (شاهد)، لجمع العينات الترابية منها. أُخِذت عينات ترابية من تسع مناطق في كل حقل ثم خُلِطت مع بعضها لتكوِّن عينة واحدة لكل حقل. كُرِّرت هذه العملية على مدى أحد عشر شهراً بحيث بلغ عدد العينات الترابية الموحدة والمدروسة ٧٧ عينة خلال فترة الدراسة. كانت العينات الترابية تُجمع في أكياس بلاستيكية نظيفة ثم تؤخذ إلى المختبر حيث يتم تجفيفها على درجة حرارة الغرفة العادية وتنخيلها بمنخل أبعاده ٢ × ٢ ملم وإجراء الفحوصات الفيزيائية والبيولوجية عليها أول بأول. إستعملت طريقة التخافيف المعروفة في مراجع الاحياء الدقيقة لإجراء عملية العد للمستعمرات البكتيرية. أظهرت هذه الدراسة وجود فروق جوهرية في الأعداد البكتيرية الكلية وكذلك في أعداد بكتيريا الاجروبكتيريوم بين المشاتل المختلفة سواء في الحقول المزروعة أو غير المزروعة، كذلك كانت الفروق واضحة بين الحقول المزروعة وغير المزورعة في المشتل

الواحد، كما بينت هذه الدراسة أن هناك فرق في استجابة بكتيريا الاجروبكتيريوم والبكتيريا الكلية لبعض العوامل البيئية حيث بلغت نسبة الاجروبكتيريوم إلى البكتيريا الكلية أقل قيمة لها في شهر نيسان (أبريل). بلغ العد لبكتيريا الاجروبكتيريوم ولجميع البكتيريا أعلى قيمة له في شهر نيسان (أبريل) وأقلها في شهر كانون ثاني (يناير). تم عزل وتشخيص ٧٢ عزلة من بكتيريا الاجروبكتيريوم وعند دراسة خصائصها الكيماوية والفسيولوجية وقدرتها على إحداث المرض في نباتات التجربة (البندورة، الدخان والكلنشوة) تبين أنه يمكن توزيعها في ثلاث مجموعات :

الأولى : اشتملت على ٢٣ عزلة.

الثانية : اشتملت على ١٨ عزلة منها ٧ عزلات قادرة على إحداث المرض لأحد نباتات التجربة.

الثالثة : اشتملت على ٣١ عزلة منها اثنتان فقط قادرة على إحداث المرض.

تبين هذه الدراسة أن عزلات المجموعة الثالثة تشكل نسبة عالية وهذا يعود إلى إستعمال هذه المشاتل في زراعة أشتال العنب في صورة متكررة، حيث أصبح من المعروف أن عزلات المجموعة الثالثة تكاد تكون مختصة بعزلات العنب (انظر كيرستر وديلا ١٩٨٤، وكير وبناجوبلس ١٩٧٧).