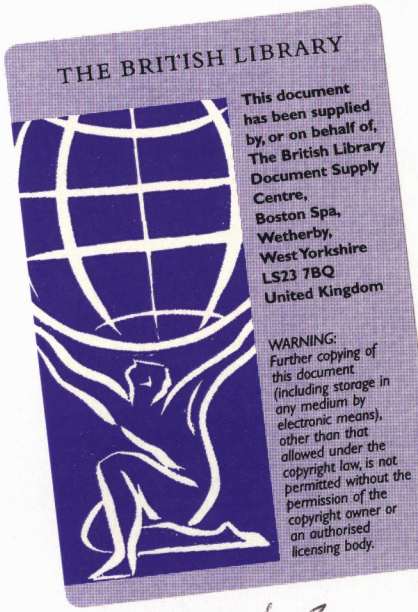

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MICROBIAL SURVEY OF THE GENUS *AGROBACTERIUM* IN GRAPEVINE NURSERIES IN JORDAN

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ABSTRACT: A microbial survey of total bacteria and Agrobacteria of seven study fields in three Jordan Valley nurseries during 11 months showed significant differences between cultivated and non-cultivated fields throughout the study period at the same nursery, between cultivated fields in different nurseries, between non-cultivated fields in different nurseries and between cultivated and non-cultivated fields within the same nursery at the same monthly interval. 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 *Agrobacterium* cultures were isolated, only nine of them were pathogenic. Twenty-three of them belonged to biotype I, 18 to biotype II and 31 to biotype III.

Key Words: Grapevine; Agrobacterium; Survey; Jordan

INTRODUCTION

The genus *Agrobacterium* is found abundantly in the soil as gram negative rod shaped bacteria, aerobic, mesophilic and it can survive in soil for many years (De Boer, 1982), and form galls in plants (Kerr, 1969). Species of *Agrobacterium* are abundantly present in the rhizosphere than nearby soil (New and Kerr, 1972; Bouzar and Moore, 1987). Three plant diseases, crown gall, cane gall, and hairy root, all characterized by host cell proliferation, are recognized to be caused by different species of the genus *Agrobacterium* (Lippincott and Lippincott, 1975). Four species of *Agrobacterium* have been recognized based on their pathogenicity, three of them are plant pathogens, *Agrobacterium tumefaciens*, the causal agent of crown gall disease, *A. rhizogenes* the inducer of hairy root and *A. rubi* which provokes cane gall, whereas, *A. radiobacter* is nonpathogenic (Allen and Holding, 1974). *Agrobacterium* plant interaction leads to the formation of galls which exhibit different morphologies, depending upon the strain of *Agrobacterium* that induces the tumor. The induced overgrowth differs from normal tissues in its ability to grow on hormone free medium and synthesize an unusual group of compounds called opines (Ma et al., 1987). The ability of Agrobacteria to induce tumor or neoplastic disease depends on the presence of an extra chromosomal DNA molecule designated as tumor inducing (Ti) plasmid (Zaenen et al., 1974). A pre-requisite for tumorigenesis is the wounding of the host plant. Infection can occur during various stages of life of the plants via wounds caused by growth, germination, subterranean insects or mechanical injuries (pruning, grafting, and replanting of trees) (Kersters and

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DeLey, 1984). Host range of *Agrobacterium* is very wide, at least 640 different plant species belonging to 93 different families of dicotyledonous and gymnosperms are susceptible to transformation by *A. tumefaciens*, few species of monocotyledonous plant are also susceptible (DeCleene and DeLey, 1976). Significant damage and economic loss has been reported on stone fruit in Australia and United States (peach, almond, cherries) and Vineyards in Bulgaria, Greece and Hungary (Kersters and DeLey, 1984). Taxonomy of the genus has been done using different criteria, like grouping, clustering and biotyping. (White, 1972; Kerr and Panagopoulos, 1977). In the present work the monthly variation of the mean of total viable bacterial count and Agrobacterial count were estimated and the biotypes of *Agrobacterium* were determined.

MATERIALS AND METHODS

Three grapevine nurseries were included in this study. They were divided into:

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

b) Rayyan Nursery; 1) Grape cultivated (Gg), 2) Control non-cultivated (Rc).

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

Sampling and Treatment

Monthly samples consisted of a mixture of nine Auger holdings collected from the top 20 cm (after removing the upper 2-3 cm) from fixed regions of the study fields. Soil mixture was dried at room temperature and sieved in 2mm x 2 mm sieve. One gram of sieved soil was suspended in 100 ml sterile distilled water and shaken at 190 rpm for 30 min. Serial dilution (10^{-1}-10^{-6}) was done and 0.1 ml of the appropriate dilution was spread by sterile L- shaped glass rod on standard agar plate (for total bacterial count) and on Kado and Heskett (1970) medium for Agrobacterial estimation. Plates were incubated at 27°C for 2-3 days for counting. From each sample three plates were inoculated and the average of their counts was the mean count.

Identification and Biotyping

Selected colonies were further purified (by having pure culture) and identification was followed to Bergey's of Bacterial Determination (Allen and Holding, 1974). For pathogenicity 24 h old bacterial culture was inoculated on young tomato, tobacco and kalanchoe stems, and results were recorded after 1-2 months. For the biotyping of the isolates, the procedure of Kerr and Panagopoulos (1977) was followed.

RESULTS AND DISCUSSION

Viable mean counts of total bacteria during the study period showed its maxmium in April for most of the study fields except D1 (Table 1). The mean count of most of the study fields decreased gradually till it reached its minimum count mostly in January. The

lowest mean count of Dc and Bc was in May, whereas D2 field was in November.

Viable mean counts of estimated Agrobacteria on Kado and Heskett medium (1970) showed its maxium in April for Baqura and Rayyan fields, in November for D1 and Dc and in February for D2 (Table 2)

Estimated percentage of Agrobacteria to the total bacterial mean counts revealed lowest percentage in April for most of the study fields (Table 3). Bc showed lowest percentage in August. Estimated percentage varied from 0.005% at Bg in April to 16.66% at D2 in December.

Table 1. Mean viable count of total bacteria on standard plate count agar of the different fields per gram of dried soil

Month	D1 10^7	D2 10^7	Dc 10^7	Bg 10^7	Bc 10^7	Rg 10^7	Rc 10^7
April	12.0	250.0	7.9	3300.0	15.0	1600.0	2500.0
May	0.15	0.15	0.11	18.0	0.11	19.0	31.6
June	12.0	63.0	0.18	30.0	0.5	12.0	3.16
July	2.5	1.2	0.39	0.72	1.0	15.0	1.6
August	0.95	0.87	3.12	0.32	10.0	31.0	10.0
September	17.7	0.93	1.9	1.7	0.97	23.0	8.5
October	5.0	0.74	0.15	0.39	0.16	4.7	0.85
November	1.0	0.1	0.39	1.9	0.35	2.0	0.58
December	0.25	0.15	0.66	0.97	0.25	0.42	0.51
January	0.14	0.97	0.21	0.28	0.33	0.21	0.46
February	5.1	4.4	2.4	4.3	3.4	3.3	3.9

Table 2. Mean viable count of estimated Agrobacteria on Kado and Heskett medium from the different fields per gram of dried soil

Month	D1 10^5	D2 10^5	Dc 10^5	Bg 10^5	Bc 10^5	Rg 10^5	Rc 10^5
April	0.33	1.58	2.3	16.9	15.0	50.1	19.0
May	1.25	1.17	1.34	15.8	0.3	33.8	13.0
June	1.3	1.9	0.16	6.3	2.3	8.5	0.34
July	1.3	7.5	0.79	7.9	1.4	15.8	0.34
August	0.15	1.04	1.25	1.25	0.61	16.9	11.0
September	1.5	6.3	6.7	0.95	0.22	12.5	0.89
October	4.07	7.9	1.58	0.97	0.31	1.02	0.5
November	7.2	1.58	1.69	1.58	3.3	6.1	0.6
December	2.9	2.5	7.9	7.9	2.51	2.8	1.25
January	1.2	10.0	2.01	3.1	3.3	1.4	1.3
February	6.6	12.8	5.6	9.3	8.6	6.6	6.1

Seventy-two isolates were identified as *Agrobacterium* cultures and only nine of them were pathogenic at least on one of the tested hosts. Twenty-three of them belonged to biotype I, 18 to biotype II 31 to biotype III (Table 4).

Analysis of variance (ANOVA) of the mean counts of total bacteria and es-

Table 3. Estimated percentage of Agrobacteria in different fields of the study

Month	D1	D2	Dc	Bg	Bc	Rg	Rc
April	0.03	0.007	0.29	0.005	1.0	0.03	0.007
May	8.3	7.8	12.2	0.87	2.7	1.7	0.41
June	0.1	0.3	0.9	0.21	4.5	0.7	0.1
July	0.52	6.3	2.0	10.9	1.4	1.05	0.1
August	0.16	1.2	0.4	3.9	0.06	0.51	1.1
September	0.08	6.8	3.5	0.56	0.22	0.54	0.1
October	0.81	10.6	10.3	2.5	1.97	0.21	0.58
November	7.1	15.8	4.3	0.88	9.3	3.01	1.1
December	11.6	16.66	12.0	8.1	10.0	6.6	2.3
January	8.57	10.3	9.6	10.9	9.7	6.5	2.79
February	1.3	2.8	2.3	2.16	2.5	2.01	2.29

Table 4. Number of samples, number of Agrobacteria, number of pathogenic isolates and number of isolates in each biotype

Soil	Number of tested samples	Number of identified Agro-bacteria	Number of pathogenic isolates	No. of isolates in each biotype I	II	III
D1	11	6	0	1	1	4
D2	11	7	1	2	2	3
Dc	11	3	1	0	2	1
Bg	11	16	1	6	4	6
Bc	11	10	1	5	2	3
Rg	11	16	5	5	3	8
Rc	11	14	0	4	4	6
Total	77	72	9	23	18	31

timated 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. Least significant difference (LSD) of the mean counts of both total bacteria and estimated Agrobacteria at $P>0.05$ showed significant difference within cultivated fields, non-cultivated fields and between cultivated and non-cultivated fields in the same nursery. These variations may be due to many factors such as plant type, plant age, plant exudate, soil type, soil fertility, microbial interaction, moisture, pH, organic matter , nitrogen content and soil minerals (De Boer, 1982).

The estimated Agrobacterial percentage in Jordan nurseries was higher than what was 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 (1972), Bouzar and Moore (1987) or it may be due to best range specificity as reported by Yanofsky et al. (1985). It was higher than what was reported by Schroth et al. (1965). Biotype III was dominant over other biotypes, which may be due to the reason that the fields were previously cultivated by grape, and as reported by Kerr and Panagopoulos (1977), Perry and Kado (1982), Ma et al. (1987), biotype III was dominant in grapevine tumors.

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