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Detection of Variation in Egyptian Isolates of *Agrobacterium tumefaciens*

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Crown gall disease caused by *Agrobacterium tumefaciens* (Conn), results in significant economic losses to the stone and pome fruit trees in nurseries. In this investigation, ten isolates were isolated from soft galls of different hosts, *i.e.* almond, peach, apricot, pear, grapevine, rosella, rose, lupine and pepper, on selective D1 medium. Pathogenicity, morphological, physiological and biochemical tests proved that the 10 isolates belonged to *A. tumefaciens*, biovar 1. These isolates had different effects on some host plants, whereas, some hosts were highly susceptible to the pathogen, such as almond, peach, castor-bean and tomato and some hosts were less susceptible such as pepper. Also, variation among *A. tumefaciens* isolates was not related to their host plants, but mostly related to variation in the bacterial genome and to variation of virulence on different hosts. The RAPD-PCR technique indicate that variation in bacterial genome refer to the variation on bands appeared whereas, it was found some bands on all isolates except one of the isolates and also one isolate (Ac1 from almond) has bands doesn't exist in the other isolates. Also, isolates from the same host (Pp4&Pp5from peach) have band on the same location but with different amount or the band was on the different location. On the other hand, *A. radiobacter* isolate has a band with high amount but this band doesn't exist in some isolates and appeared in the other but with a very weak amount.

Keywords: *Agrobacterium tumefaciens*, almond, apricot, DNA-polymerase chain reaction, grapevine, host range, lupine, peach, pear, pepper, rose and RAPD- PCR technique.

Malignant swellings comparable to human cancer are caused in plants mostly by the bacterium *Agrobacterium tumefaciens*. Whereas, *A. tumefaciens* is the causal agent of crown gall disease that affect most dicotyledonous plants and is characterized by growth of tumors at the region between the stem and root (crown). Also, crown gall results in significant economic losses to the stone and pome fruits because galls can weaken or kill the host plant. Economical losses from the disease occur primarily at nurseries where galled plants should be discarded and damaged. So, all isolates from nurseries were virulent strains, their pathological, morphological and physiological characteristics proved to be identical to *A. tumefaciens*. The host range of this bacterium is extremely wide including more than 600 plant species of dicotyledonous plants were susceptible to attack by *A. tumefaciens* with no correlation between the taxonomic position of plant family and its susceptibility (Taha *et al.*, 1975 and Cleene and Ley, 1976). Some host plants

were used to determine the host range and host specificity of 62 strains of *A. tumefaciens* such as cauliflower, capsicum, chickpeas, melons, soybeans, medicago, petunia ,peas, radishes and spinach All strains showed a tumourigenic response on host plants selected except the isolate Pg1.Some studied refer to the susceptibility of seeded vegetable crops to *A . tumefaciens* biovar 3 strains with different types of opine utilization (octopine, nopaline and vitopine) using 24 species and 7 carrot cultivars and the tested vegetable crops differed in their susceptibility to *A. tumefaciens* strains (Qazi and Shahida, 1997 and Novak, 1999). Also, a molecular system of *Agrobacterium* strains based on the (RAPD-PCR) procedure was developed. The procedure allowed rapid identification of isolates recovered from tumors by comparison of their band patterns with band patterns of strains used as inoculums. This system can be applied for rapid screening of *Agrobacterium* the colonies isolated from plant tumors for genetic diversity studies Llop *et al.* (2003). In Egypt, El-Wakil and Kemp (1979) found the variation among *A tumefaciens* isolates in Egypt refer to the ability of these isolates to utilize unusual amino acid derivatives such as octopine or nopaline. So, this research was designated in order to find out the variation among the common isolates of crown gall pathogen collected from different host plants in Egypt. Also, to find out if this variation is due to the virulence of isolates or to the variation in the genome of isolates or to their host origin from which they were isolated.

Materials and Methods

1- Isolation, pathogenicity and identification of the causal pathogen:

Samples of different hosts showing typical symptoms of crown gall disease on the crown region and main roots were collected from different locations. The isolation was carried out using the technique described by Dowson (1957). Soft galls were washed carefully, then crushed in few drops of sterile water. The resulting suspension was streaked on selective medium D1 formulated by Kado and Heskett (1970), then the plates were incubated at 28°C for 3 days. The distinguished bacterial colonies resembling *Agrobacterium tumefaciens* were picked up and transferred to nutrient glucose agar (NGA) to verify colony morphology and to prepare inoculums for pathogenicity test. Pure cultures of all isolates were tested for their pathogenicity and ability to induce gall formation in tomato plants, carrot slices and squash fruits. Tomato plants, 50 day-old, were used as test plants and the pathogenicity of the isolates were studied by soil inoculation technique in sterilized clay sand soil. Inocula were prepared in NG broth medium with cell density of 10^8 cfu/ml (Abd-El-Aziz, 2004). The plants were wounded at the region between stem and root (crown). Then, each pot (20cm-diam.) contained 3 plants was inoculated with 100ml cell suspension. Plants showing typical galls on the wounded sites were considered as pathogens. Four pots were used as replicates/ isolate. Also, carrots were cut into slices (1cm) then surface inoculated (0.5ml/slice) with cell suspension (10^8 cfu/ml) and incubated in a Petri dish lined with moistened tissue paper and incubated at 28°C for 10 days. Whole mature squash fruits (*Cucurbita pepo*) were surface sterilized with ethanol 75%, small wells (3mm deep) were cut on the surface of fruit and 50 µl of the cell suspension was introduced into the well and

incubated at 28°C in a moist chamber and the progress of infection was daily examined. Each plate contained 3 slices, and 4 plates were used as replicates / isolate. Host reaction was recorded 3 days after inoculation.

Identification of *A. tumefaciens* was carried out according to Schaad (1988) and Lelliott and Stead(1987). The following tests were determined such as production of 3-ketolactose, gelatin liquefaction, nitrate reduction, starch hydrolysis, indol production, growth on congo-red medium, oxidase and catalase tests and utilization of carbon source from different compounds. The isolates which proved to be *A. tumefaciens* were designated as the number of isolate and its source.

2-Biovar characterization of A. tumefaciens isolates:

When pathogenicity was confirmed, a representative number of strains from each host were identified as biovars using the following criteria : production of 3-ketolactose, growth in 2% NaCl, growth at 35°C, formation of acid from erythritol (Furuya *et al.*, 2004 and Sandeep *et al.*, 2005), growth on ferric ammonium citrate medium, citrate utilization test and oxidase test (Schaad, 1988 and Fahy and Persley, 1983).

3- Susceptibility of some economic plants to the disease (host range):

In this study, different isolates were used for soil inoculation to investigate the susceptibility of different plants to the disease. The study was made on tomato, peach, almond, apricot, castor-bean, cucumber, eggplant and pepper. The inoculum was prepared in NG broth that contained 10^8 cfu/ml of each isolates. The plants were wounded in the crown region, then adding the bacterial suspension at the rate of 100 ml/pot (20cm diam.-having 4 kg loamy soil) for each host. Each pot contained 3 plants and 4 pots were used as replicates / isolate. The results were recorded after 40 days from inoculation. The degree of susceptibility was measured by using the root gall – index (RGI) as follows Abd-El-Aziz(2004):

$$RGI = \frac{\text{Average diameter of gall}}{\text{Average diameter of stem}} \times 100$$

Whereas: Diameter of gall = diameter of stem with gall - diameter of stem

4- Random amplified polymorphic DNA-polymerase chain reaction (RAPD- PCR) technique for Agrobacterium strains:

This test was done with the assistance of the Central Lab. of Biotechnol., Plant Pathol. Inst., Agric. Res. Centre. Ten isolates of *A. tumefaciens* (for designation and host plant, refer to Table 1) and one isolate of *A. radiobacter* (from Dr. A.E. Tawfik, Plant Pathol. Inst., ARC.) were used in this technique.

4-1- DNA isolation and RAPD technique:

DNA was isolated from 50 mg of bacteria using Qiagen kit for DNA extraction. The extracted DNA was dissolved in 100 µl of elution buffer. The concentration and purity of the obtained DNA was determined by using "Gen qunta" system-Pharmacia Bio-tech. The purity of the DNA for all samples was between 90-97% and the ratio between 1.7-1.8. Concentration was adjusted at 6ng/ µl for all samples using TE buffer (pH 8).

4-2- Random amplified polymorphism DNA technique (RAPD):

Thirty ng from the extracted DNA were used for amplification reaction. The polymerase chain reaction mixture contained PCR beads manufactured by Amessham Pharmacia Biotech Germany, which contained all of the necessary reagents. The kits also included five microliter of the primer (10 mer) were added. The sequence of the used primer was d_5 $(CCCGTCAGCA)^{-3}$

The total volume was completed to 25 µl using sterile distilled water. The amplification protocol was carried out as recommended by the manufacturer using PCR unit II biometra which include 7 µl of 6 x tracking buffer (manufactured by Qiagen Kit) added to 25 µl of the amplification product .

4-3- Amplification product analysis:

The amplified DNA for all samples were electrophoresed (15 µl) using electrophoresis unit (WIDE mini – sub cell GT Bio – RAD) on 1.5% agarose containing ethedium bromide (0.5 µg/ ml). At 100 constant volt, and determine with UV transilluminator.

4-4- Gel analysis:

All kinds of gels (protein, isozyme, and DNA) were scanned for band R_f using gel documentation system (ABB Advanced American Biotechnology 1166E. Valencia Dr. Unit 6C, Fullerton CA 92631). The different M.W. of bands were determined against PCR marker am Resco K 180 100 bp ladder by unweighted pair-group method based on arithmetic mean (UPGMA).

Results

Isolation, pathogenicity and identification of the causal pathogen:

Different infected plants, showing typical symptoms of crown gall disease collected from different locations were used for isolation. About 20 isolates were isolated from typical tumors of different hosts. Among these isolates only 10 showed typical symptoms on the test plant materials (tomato seedlings, carrot slices and squash fruits). Symptoms appeared as tumors on the region between the stem and root (crown) of tomato plants after 30 days from inoculation, and swelling appeared on the surface of carrot slices after about 10 days from inoculation, but on squash fruit gall developed after 3-8 days from inoculation. Considerable variation among isolates in their effect on tested plant material was observed and recorded in Table (1). Isolates Ac1from almond and Pp4 from peach gave the higher gall diameter of 14mm, while isolates Pp5 from peach and Pc10 from pear produce 12mm galls. Isolates Pa7 from apricot and Lt20 from lupine gave 11mm galls, while isolates Vv8 from grapevine and Hs19 from rosella produce 10mm galls. The least gall diam. (9mm) was produced by isolates Ro14 from rose and Ca11 from pepper.

Reaction on carrot slices was different, where gall development on slices was produced after 10 days from inoculation by isolates Pp4, Pp5, Vv8 and Lt20 and after 15 days by isolates Ac1, Pa7, Pc10, Ro14, Hs19 and Ca11. However, on squash fruits galls were produced after 3 days from inoculation by isolates Pp5 and Lt20, after 5 days by isolates Ac1andPp4 and after 8days by isolates Pa7, Pc10, Vv8, Ro14, Hs19 and Ca11.

Table 1. Virulence of isolates produced from soft galls collected from different infected plants on tomato plants, carrot slices and squash fruits

Designation of isolates	Host	Location	Tomato		Galls appeared after (days) on:	
			Gall diameter (mm)	Average weight of gall (g)	Carrot slices	Squash fruits
Ac1	*Amygdalus communis* (Almond)	Korashiya	14	2.6	20	5 – 8
Pp4	*Prunus persica* (Peach)	Nubariya	14	3.0	10 - 15	5 – 8
Pp5	*Prunus persica* (Peach)	Elkhatatba	12	2.4	10 - 15	3 – 5
Pa7	*Prunus armeniaca* (Apricot)	Qaha	11	1.7	20	10
Pc10	*Pyrus communis* (Pear)	Nubariya	12	2.1	20	10
Vv8	*Vitis vinefera* (Grapevine)	Wady El-Mallak	10	1.6	10 - 15	10
Ro14	*Rose* sp. (Rose)	Qaliobiya	9	1.4	20	10
Hs19	*Hibiscus sabdariffa* (Rosella)	Qena	10	2.0	20	10
Lt20	*Lupinus termis* (Lupine)	Kafr Elsheikh	11	2.0	10 - 15	3 – 5
Ca11	*Capsicum annuum* (Pepper)	Gizert El-Dahab	9	1.5	20	10

In addition, average weight of galls on tomato for each isolate was variable as isolate Pp4 from peach gave the highest weight 3.0g then isolate from almond 2.6g. After that isolates from peach Pp5, pear, rosella & lupine, apricot, grapevine, pepper and rose gave gall weight of 2.4, 2.1, 2.0, 1.7, 1.6, 1.5 and 1.4g, respectively.

From these results, it was noticed that the variation among gall diameter, average weight of gall and gall formation on the susceptible test plants may be due to variation in virulence among *A. tumefaciens* isolates. Inasmuch as isolate from almond gave the highest gall diameter on tomato but didn't give the highest weight of gall and also gall appeared after 15 and 5 days on carrot and squash respectively. While, isolate from lupine gave moderate gall diameter 11mm and moderate weight of gall 2.0g and also gall appeared on carrot after 10 days but it gave the higher development of gall on squash where the gall appeared 3 days after inoculation. So, from results noticed, the variation may occur among the reaction of one isolate on the tested plants. Whereas, one of these isolates gave high gall diameter but did not give high weight of gall and the development of gall of this isolate on carrot and squash may be fast or not.

All isolates were gram negative, non spore-forming, single short rods. On nutrient glucose agar, colonies were white, circular, glistening. In nutrient glucose broth, they gave slight turbidity with thin pellicle.

Physiological and biochemical characteristics of these isolates are shown in Tables (2 and 3) where all isolates could utilize the tested carbon compounds except aesculin, forming acid only within 3days. However, acidity was delayed after 4-7 days in some isolates in media containing sucrose and lactose such as isolates Ac1from almond, Pp4and Pp5from peach, Pa7 from apricot, Ro14 from rose, Lt20 from lupine and Ca11 from pepper. Results indicate that all isolates could be identified as *A. tumefaciens*. The isolates which conform to *A. tumefaciens* were given designations to show the source of the isolates.

Table 2. Utilization of some carbon sources for the isolates which isolated from soft galls collected from infected plants

Tested isolate	Carbon source										
	Glucose	Fructose	Mannose	Arabinose	Sucrose	Galactose	Maltose	Lactose	Mannitol	Aesculin	Salicin
Ac 1	+	+	+	+	(+)	+	+	(+)	+	-	+
Pp 4	+	+	+	+	+	+	+	(+)	+	-	+
Pp 5	+	+	+	+	(+)	+	+	(+)	+	-	+
Pa 7	+	+	+	+	(+)	+	+	+	+	-	+
Pc 10	+	+	+	+	+	+	+	+	+	-	+
Vv 8	+	+	+	+	+	+	+	+	+	-	+
Ro 14	+	+	+	+	(+)	+	+	+	+	-	+
Hs 19	+	+	+	+	+	+	+	+	+	-	+
Lt 20	+	+	+	+	(+)	+	+	(+)	+	-	+
Ca 11	+	+	+	+	(+)	+	+	+	+	-	+

* For isolate designation refer to footnote of Table (1).
\+ Acid formation within 72hrs.
(+) Delayed reaction, after 4 -7days.
\- Negative reaction after 7 days.

Determination of Agrobacterium tumefaciens biovars:

Ten isolates of *A. tumefaciens* were collected from stone fruits, pomes, grapevine, rose, rosella, lupine and pepper growing in different localities in Egypt during 2005-2007. These isolates were subjected to different biochemical tests for biovar differentiation. All isolates causing crown gall on different studied hosts belonged to biovar I as they showed positive reaction towards(production of 3-ketolactose, tolerance to sodium chloride concentrations from 1-4%, growth at 35°C, growth on ferric ammonium citrate medium, could utilize citrate and oxidase positive (Table 4).

Table 3. Physiological and biochemical reactions for the isolates which were isolated from soft galls collected from infected plants

No. of Isolate	Test											
	Ketolactose production	Growth on congo red medium	Acetoin production (V.P)	Methyl red (MR)	H2S	Oxidase	Nitrate reduction	Catalase	KOH (3%)	Indol formation	Gelatin liquefaction	Starch hydrolysis
Ac 1	+	+	-	-	+	+	-	+	+	-	-	-
Pp 4	+	+	-	-	+	+	-	+	+	-	-	-
Pp 5	+	+	-	-	+	+	-	+	+	-	-	-
Pa 7	+	+	-	-	+	+	-	+	+	-	-	-
Pc 10	+	+	-	-	+	+	-	+	+	-	-	-
Vv 8	+	+	-	-	+	+	-	+	+	-	-	-
Ro 14	+	+	-	-	+	+	-	+	+	-	-	-
Hs 19	+	+	-	-	+	+	-	+	+	-	-	-
Lt 20	+	+	-	-	+	+	-	+	+	-	-	-
Ca 11	+	+	-	-	+	+	-	+	+	-	-	-

Table 4. Detection of *Agrobacterium tumefaciens* biovars

Isolates Designation	Test					
	Production of 3-ketolactose	Tolerance to sodium chloride from (1–4%)	Growth at 35°C	Growth on Ferric ammonium citrate medium	Citrate utilization	Oxidase test
AcI	+	+	+	+	+	+
Pp4	+	+	+	+	+	+
Pp5	+	+	+	+	+	+
Pa7	+	+	+	+	+	+
Pc10	+	+	+	+	+	+
Vv8	+	+	+	+	+	+
Ro14	+	+	+	+	+	+
Hs19	+	+	+	+	+	+
Lt20	+	+	+	+	+	+
Ca11	+	+	+	+	+	+

Susceptibility of some economic plants to the disease (host range):

Tomato, castor-bean, peach, almond, apricot, cucumber, eggplant and pepper were used to determine the host range and host specificity spectrum of 10 isolates of *A. tumefaciens* (Table 5). All isolates were tumourigenic on more than 90% of the host plants tested. The isolates from almond and from peach had a wide host range and could induce tumors on all host plants, while isolates from apricot, lupine and grapevine couldn't infect pepper. However, pear isolate (Pc10) couldn't infect cucumber and isolates from rose and rosella couldn't infect eggplant plants. However, peach isolate Pp4 couldn't infect eggplant and pepper. While, pepper isolate couldn't infect cucumber, eggplant and pepper plants.

Table 5. Host range of some tested isolates of *Agrobacterium tumefaciens* from different hosts

Tested isolate	Root gall index (%) *							
	Tomato	Castor bean	Peach	Almond	Apricot	Cucumber	Eggplant	Pepper
Ac1	300	220	333	300	366	112	133	50
Pp4	250	240	433	275	333	125	-	-
Pp5	225	220	400	250	300	175	100	125
Pa7	225	180	333	250	366	50	100	-
Pc10	250	200	333	225	300	-	83	100
Vv8	200	180	266	200	300	38	100	-
Ro14	200	140	200	150	166	100	-	150
Hs19	200	200	300	225	266	63	-	150
Lt20	225	200	266	225	300	75	75	-
Ca11	225	140	233	150	200	-	-	-

* For explanation refer to the relevant section in Materials and Methods.

Results in Table (5) indicated that all *A. tumefaciens* isolates were virulent. However, these isolates were different in their virulence and in their effect on different host plants. Whereas, variation and diversity to these isolates as well as similarities in tumor sizes were observed and reflected on their RGI which can be taken as a criterion of the severity of infection. On the other hand, variations in the RGI and host susceptibility to each isolate were indicative to the variation in *Agrobacterium tumefaciens* isolates.

Genome variation using RAPD-PCR technique:

A molecular typing system for *Agrobacterium* isolates based on the polymerase chain reaction – random amplified polymorphic DNA (RAPD-PCR) procedure was employed. Using one primer, the band patterns obtained for each of the eleven *Agrobacterium* isolates were different (Fig. 1). Eight host plants were inoculated with ten *Agrobacterium* isolates and the isolates obtained from the resulting tumors

(ten isolates were *A. tumefaciens* and one isolate of *A. radiobacter*. In the RAPD analysis, variability was observed among the isolates which clustered into two major groups. The first group included the almond isolate (Ac1) and the second include the other isolates. From (Fig. 2) it is shown that the similarity cluster divided the eleven isolates into two main sub clusters. The first included Ac1 isolate from almond, while the second sub cluster included the remaining isolates.

The overall similarity among isolates in this subcluster was 76.28%. Grouping of isolates within this sub cluster was not related to their host plants. For example, isolates Pp4 and Pp5 from peach, however, were included in two remotely related subgroups and similarity was 78.28%. Isolate Vv8 from grapevine and isolate Lt20 from lupine showed very high similarity level being 94.84 % however, they were isolated from taxonomically unrelated hosts .On the other hand, isolate Ac1 from almond have a great variation in its genome with other isolates, so, its lies in a separate cluster with very low similarity level with other isolates being 1.83%. Also, isolates Cal1from pepper and A84 the non pathogen (*A. radiobacter*) have similarity of 96.86% and the isolate Ca11 couldn't infect all plants in the host range and also, it gave lowest RGI on the tested plants. So, this isolate Ca11 from pepper was similar to the non pathogenic isolate A84 and have a high similarity with it 96.86%. Isolates Vv8from grapevine and Lt20 from lupine have similarity 94.84%, the two isolates couldn't infect pepper plants while infecting the other host plants.

From data in Table (6) it was noticed that isolate Ac1 from almond has two bands between 500-600bp comprising over 50% of the total amount of amplified DNA compared with the other isolates. Also, this isolate has three bands between 1800-1900bp, 2500-2600bp and 3400-3500bp while these bands weren't found in other isolates. On the other hand, isolates Pp4 and Pp5 from the same host (peach) but isolate Pp5have one band between 300-400bp amount of it was 30.4% compared with isolate Pp4 which has two bands in the same location having a little amount 0.2% and 0.3%. Also, between 600-700bp isolates Pp4 and Pp5 have one band but the amount of it was high in Pp4 (30.3%) and in Pp5 was low (3.4%). Also, isolate Pp4 have one strong band between 900-1000bp with amount of 26.5%. This band doesn't exist in isolate Pp5, while isolate Pp5 have a band between 1000-1100bp with amount of 25.7% this band disappeared from isolate Pp4. However, *A. radiobacter* isolate has a band between 800-900bp with high amount of 22.5%, while this band doesn't exist in some isolates and appeared in the other, but it was found in a weak amount from 1.2 to 6.1%. Isolate Ca11 from pepper and isolate A84 has one band between 900-1000bp of amount as high as 22.1% and 17.4% respectively. Isolate Pc10 from pear have highly amount band of 21.6% between 2300-2400bp this band not found in the other isolates. Also, from results found that band between 300-400bp appear in all isolates except isolate Ca11 also band between 400-500bp appeared in all isolates except isolates Ac1 and Pp5, and between 500-600bp appeared in all isolates except in isolate Pp4. While a band between 600-700bp was common in all isolates of *Agrobacterium*.

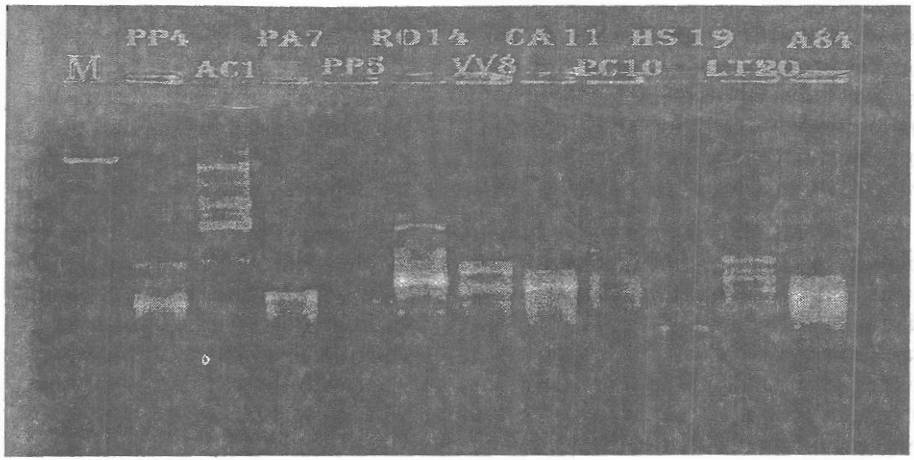

Fig. 1. Differentiation between isolates of *Agrobacterium tumefaciens* and *A. radiobacter* isolates by RAPD-PCR technique.

Lane1: the marker (M)
Lane 2: isolate Pp4 from peach
Lane 3: isolate Ac1 from almond
Lane 4: isolate Pa7 from apricot
Lane 5: isolate Pp5 from peach
Lane 6: isolate Ro14 from rose
Lane7: isolate Vv from grapevine
Lane8: isolate Ca11 from pepper
Lane9: isolate Pc10 from pear
Lane10: isolate Hs19 from rosella
Lane11: isolate Lt20 from lupine
Lane12: isolate A84 non pathogen

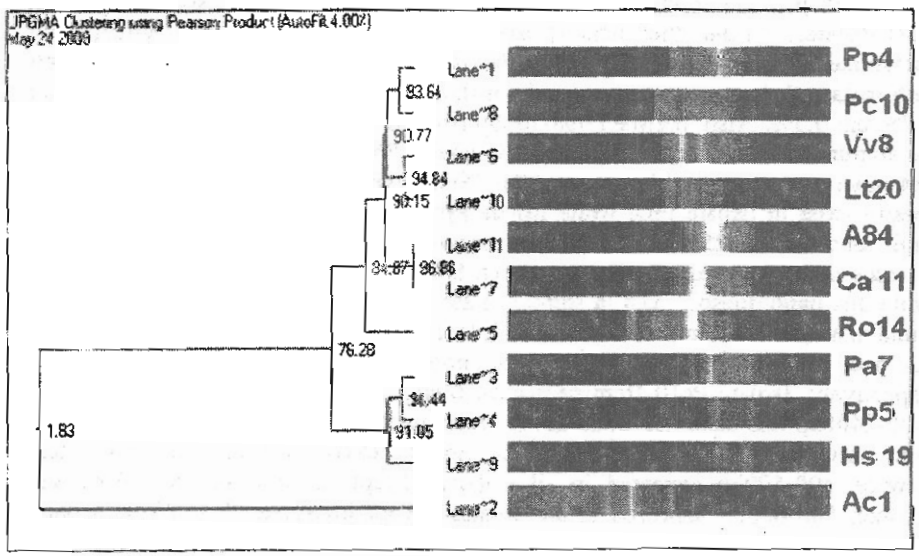

Fig 2. Dendrogram showing polymorphism of DNA of some *Agrobacterium* spp. isolates collected from different locations obtained by RAPD-PCR technique.

Table 6. Amount (%) of each band from whole amount of amplified DNA sequences for *Agrobacterium tumefaciens* and *A. radiobacter* isolates

Isolate/bp	Ac1	Pp5	Pa7	Pp4	Ro14	Vv8	Ca11	Pc10	Hs19	Lt20	A84
100/200		27.5	15.2 17.3	16.7 16.3	15.8		15.9				12.0
200/300		3.4		4.4		11.9	18.4	18.3	20.0	17.1	13.3
300/400	24.1	30.4	19.1	0.2 0.3	17.2 18.2	12.8 13.6		19.6	5.1	18.4	13.7 13.8
400/500			20.9	2.5 2.7	18.3	14.8	18.5	21.0	0.5 3.0	19.5	0.1 0.8
500/600	25.8 27.0	3.5	0.1 0.2		0 3 0.3	14.8	0.6	0.3	3.4	20.1 0.9	1.3
600/700	4.1	3.4	27.3	30.3	1.2	0.4 0.9 0.1	24.5	2.6 3.4	35.9	0.1	5.0
700/800					2.2 4.6			0.1 0.2		1.3 1.3	
800/900		6.1			2.3			1.2			22.5
900/1000	0.1			26.5	19.8	3.1 15.4	22.1	11.9			17.4
1000/1100		25.7							32.1	21.4	
1100/1200	1.9										
1300/1400	0.1 0.2					12.3					
1800/1900	2.2 3.7										
2300/2400								21.6			
2500/2600	5.1										
3400/3500	5.8										

Discussion

In this investigation, isolates of *A. tumefaciens* the causal agent of crown gall disease were isolated from different plants and were subjected to Koch's postulates to ascertain their pathogenicity. These isolates were identified and their morphological and physiological characteristics were determined.

The results of pathogenicity test, physiological and biochemical characteristics of the isolates were revealed that isolates were capable of inducing galls on tomato, carrot slices and squash fruits, and all isolates were gram negative, non spore-forming, single short rods, ketolactose producers, oxidase and catalase positive, isolates couldn't hydrolyse starch or liquefy gelatin. These results conform with those recorded by Brenner *et al.* (2005) and with those reported by Taha *et al.* (1975) for some Egyptian *A. tumefaciens* isolates.

Isolates *of A. tumefaciens* were collected from different hosts and locations, the identification tests indicated that all isolates belong to biovar 1, so *A. tumefaciens* biovar 1 is considered as the widely prevalent isolate in Egypt. Whereas, *A. tumefaciens* biovar 1 is the widely spread in many countries of the world. These results are in agreement with those obtained by Gupta *et al.* (2005) and Furuya *et al.* (2004), in addition to Sandeep *et al.* (2005) who noted the similarity of Indian isolates.

On the other hand, isolates of *A. tumefaciens* have different effects on various hosts plants in inducing gall formation. It was also noticed that isolates vary in virulence and its ability to infect various plants. Thus, variation of susceptibility of different host plants to infection with *A. tumefaciens* isolates of different origin. It in apparent that stone fruit plant, were highly susceptible to the pathogen while pepper plant was less susceptible. So, *A. tumefaciens* have the widest host range of any bacterial plant pathogen and hosts with different susceptibility to the isolates. These data correspond with those reported by Hayward and Waterston (1965) and also with those reported by Ali-Rhouma *et al.* (2005) in greenhouse and field tests in Tunisia.

The isolate Vv8 produced from grapevine, could induce gall formation in the crown region and belongs to biovar 1. This result contrasted with those of Loubser (1978) and Burr and Hurwitz (1981) who found that another biovar causes galls to grapevine plants. However, this isolate was found on the trunk and was named *Agrobacterium vitis* biovar 3 as it can migrate systemically within grapevine trunk.

The RAPD – PCR technique was used to determine the differentiation among the isolates especially the difference among the genome. The similarity among *A. tumefaciens* isolates and the amount of each band based on RAPD-PCR indicated that the high variation among the isolates was not related to their host plant origin. Thus, isolates from peach (Pp4&Pp5) have low similarity and have bands on the same location but the amount of it was different. Also, isolate Pp4 has band which was not found on Pp5 and Pp5 has a band which was not found on Pp4. But variation was mostly related to variation in its virulence on different hosts. This is true since some isolates could infect all or some tested host plants and couldn't infected the others. *A. Radiobacter,* the non pathogenic isolate, has a band with high amount but which doesn't exist in some isolates and appeared in the others but with a very weak amount. On the other hand, isolates produced from almond (Ac1) and peach (Pp5) were aggressive isolates in pathogenicity test but the similarity between these isolates was very low. But this isolate could infect all plants tested in the host range and also having the highest gall index compared with the other isolates. Whereas, isolate from almond has three bands but these bands doesn't exist in the other isolates and has two bands comprising over 50% of the total amount of amplified DNA compared with the other isolates, so the genome has a high variation compared with the other isolates. So, results indicated that isolates vary in their genome similarity and the bands appeared were variable. So, some isolates have the ability to infect the same host plants and couldn't infected the others. These results confirm the results obtained by Sachadyn and Kur (1997) as they used the PCR technique to know the characteristics and virulence of *A. tumefaciens*. Also, from

these results it could be explained that virulence of *A. tumefaciens* refer to T-DNA from the Ti-plasmid and not by the whole genome. Lacroix *et al.* (2006) and Lai *et al.* (2006) recorded that *A. tumefaciens* could induce the crown gall disease in plants by transfer and integration a segment of Ti-plasmid (T-DNA) in to the genome of numerous plant species.

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الكشف عن الاختلافات فى العزلات المصرية للاجروباكتيريم تيوميفاشينز

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يسبب مرض التدرن التاجي المتسبب عن البكتيريا اجروباكتيريم تيوميفاشينز خسائر معنوية فى مشائل الفاكهة ذات النواة الحجرية و التفاحيات . و قد تم الحصول على 10 عزلات من عدد من العوائل المختلفة مثل اللوز, الخوخ , المشمش , الكمثرى , العنب , الكركديه , الورد , الترمس و الفلفل . وقد اثبتت اختبارات القدرة المرضية و الشكل المورفولوجي و الاختبارات الفسيولوجية والبيوكيميائية للعزلات انها اجروباكتيريم تيوميفاشينز الصنف الحيوى 1, وتختلف هذه العزلات فى تأثيرها على العوائل النباتية فهناك بعض العوائل النباتية ذات حساسية عالية للاصابة و تكوين الاورام مثل اللوز , الخوخ , الخروع و الطماطم و بعض العوائل منخفضة الحساسية للاصابة مثل الفلفل. وقد وجد ان هناك اختلاف ايضا بين عزلات الاجروباكتيريم و ترجع هذه الاختلافات ليس لاختلاف العوائل المعزولة منها و لكن الاختلاف بين العزلات يرجع للاختلاف فى الجينوم البكتيرى نفسه , و للاختلاف فى القدرة المرضية للعزلات على العوائل المختلفة. ومن اختبار RAPD-PCR يتضح ان الاختلاف بين الجينوم البكتيرى يرجع الى ظهور و وجود الباند (قطعة من DNA ذات وزن جزيئى معين) . حيث توجد بعض الباندز فى كل العزلات ماعدا عزلة واحدة , ايضا وجود عزلة تحتوى على عدد من الباندز لا توجد فى العزلات الاخرى مثل عزلة اللوز.وجد ان عزلتى الخوخ على الرغم من انهما من نفس العائل الا انهما تحتويان على نفس الباندز ولكن كميتها فى واحدة كبيرة وفى الاخرى قليلة او انهما تحتويان على باندز فى اماكن مختلفة. من ناحية اخرى العزلة غير الممرضة الاجروباكتيريم راديوباكتر تحتوى على باند كميتها عالية جدا هذه الباند لا توجد فى بعض العزلات والبعض الأخر وجدت به ولكن بكمية ضعيفة جدا.