Egypt. J. Hort. **Vol. 36**, No. 1, pp. 163- 178 (2009)

Clone Production from *Pelargonium graveolens* L'Herit. Tolerant to Wilt Through Tissue Culture

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THIS WORK was carried out in the plant tissue culture laboratory of the Vegetable and Medicinal Plant Research Department, Horticulture Research Institute, Ministry of Agriculture, during the period of 2004 to 2008 to produce geranium (*Pelargonium graveolens* L,Herit.) clones that tolerate to fusarium wilt through tissue culture. The study included three items:

1- Callus induction, callus differentiation and acclimatization.
2- Fungal infection of fusarium in the pots of the tested clones.
3- Evaluation of clones compared with mother plants.
- Wilt percentage after the plants were infected with fusarium fungal.
- Morphological characteristics for clones and mother plants and essential oil percentage & constituents.
- DNA and protein isolation for clones and mother plants

The obtained results were as follow:-
- Obtaining of two clones from *Pelargonium graveolens* L,Herit. tolerate to fusarium wilt
- There were true significant different between two clones and mother plant in leaves area characteristics and oil percentage
- There were variation in DNA isolation between two clones and mother plant

As such we recommended to propagate this clones and increase it's cultivated area to come over geranium wilt disease.

Pelargonium graveolens L,Herit. has a major part in the production of medicinal and aromatic plants in Egypt and cultivated in many Governorates such as Beni Seuf, El Gharbia, El Kalubeya and El Fayoum.

The genus *Pelargonium* is a member of the family Geraniaceae in which also includes the genera *Geranium, Erodium, Monsonia* and *Sarcocaulon.* The vast majority of the 250 or so natural species of *Pelargonium* derive from South Africa, although a few species are native to Australia, East Africa and Syria. The genus is subject to large morphological diversity and for descriptive purposes, has been subdivided into 15, or sometimes 16 sections (or subgenera) based on leaf and flower characteristics and on habitat. The leaves of many species and

numerous artificial hybrids are scented, and members of the subgenera *Pelargonium, Cortusina* and *Polyactium* appear to be especially rich in essential oil (Webb, 1984).

The essential oil is obtained by steam distillation of the green herb either directly after harvesting, or after 24 h in order to reduce the volume and to release the oil from the glycosidal form. Yields of oil vary according to the time of harvesting and, of course, environmental factors. Maximal oil content of up to 0.4 % may be obtained from herbage harvested just before and during flowering (Fleisher and Fleisher, 1985).

-Commercial geranium oil is a complex mixture of over 120 mono-and sesquiterpenes and low molecular weight aroma compounds, the main components are geraniol, citronellol, linalool, menthol, eugenol and other esters, and together these components constitute 60-70 % of the total oil (Vernin *et al.*, 1983).

Geranium plant has been observed to be generally affected by four fungal pathogens. *Rhizoctonia solani* and *Fusarium oxysporum* var. *redolens* are highly prevalent in the southern hilly tracts, while *Alternaria alternata, Colletotrichum gloeosporioides* and *Colletotrichum acutatum* cause severe leaf blight in the northern Indian plains (Alam *et al.*, 2007). Disease losses (54.9 & 58.4 %) and (63.5 &75.0%) in geranium herb and oil yield for plants grown in rows and plots, respectively (Arafa *et al.* 1998).

Because the crop is polyploid and highly sterile, traditional breeding cannot be carried out. Somatic mutation and genetic engineering techniques appear to be most suitable for incorporating disease resistance in such vegetatively propagated plants (Van den Bulk, 1991 and Bi *et al.*, 1999).

Skirvin and Janick (1976) mentioned that, the calliclones of scented *Pelargonium spp* showed high variability comparing to mother plant and calliclone variation was dependent upon clone and age of callus. Also Gauri *et al* (2000) obtained calliclones from *Pelargonium graveolens* Indian cultivar Hemanti which some of them was resemble to mother plant and the other differed in several herb related agronomic characteristics.

Somaclonal breeding programmers have proved extremely successful in developing disease-resistant cultivars in a number of crop species Thakur *et al.*, (2002). It has either been attempted at the plant level where a large population of plants, raised through *in vitro* callus cultures, have been screened for resistance in the field directly or via a more targeted approach of regeneration of disease-resistant plants through resistant callus cultures selected against fungal toxin Hammerschlag, (1992).

Matsumoto (1995) investigated an increased tolerance of the selected Maca banana plants to the race 1 fungus in greenhouse tests. It is concluded that in vitro selection by fusaric acid is a useful method for obtaining fusarium disease tolerance, although the tolerance mechanism of the selected plants may be different from that of the existing tolerant cultivars.

Saxena (2008) demonstrated that, the induction of disease resistance in rose-scented geranium plants was found at the cellular level. This approach could be successfully exploited in raising new disease-resistant cultivars in geranium against various fungal pathogens.

These reviewed results confirm the need to produce the wilt tolerant clones. So this work aimed to produce and evaluate of some clones from callus formation through tissue culture technique which promising as fusarum wilt tolerant of high productivity of herb and oil as well as oil of good quality.

Material and Methods

This work was carried out on *Pelargonium graveolens* L,Herit. plant to produce clones tolerant to fusarium wilt through tissue culture, during the period from 2004 to 2008, in the plant tissue culture laboratory of the Vegetable and Medicinal Plant Research Department, Horticulture Research Institute, Ministry of Agriculture

In vitro and acclimatization stage

Callus induction

The explants (internodes segments of 1cm length and 0.5cm thickness) were taken from terminal cuttings and washed under running tap water for one hour, then surface sterilized by immersing in sodium hypochlorite 0.8% and 2 drops of tween 20 emulsifier for 20 minutes, then rinsed three times in sterile distilled water containing 0.5 g/l PVP for 5 minutes to prevent browning. After surface sterilization, the external parts of explants were removed and the explants of 3mm length placed onto the nutrient medium. After preparation the medium and prior to agar addition, the pH was adjusted to 5.8. According to the stages of this work, the medium was poured into either culture tubes of size 2.5 x 15cm or jars (175ml) where each tube contained 10ml and each jar contained 15ml of the medium. The tubes were covered with aluminum foil while the jars were capped with polypropylene closures. The culture vessels were autoclaved at 121^{O}C and 1.2 Kg/cm^{2} air pressure for 20 minutes.

Murashige and Skoog (MS, 1962) basal medium was used in this experiment of this study. The medium contained 30g/l sucrose and 6g/l agar. The used growth regulators for this purpose was 2.5 mg/l BA + 0.5 mg/l NAA, Hamouda, (2003).

The experiment are divided into five treatments from fusaric acid 0.0, 0.1, 0.5, 1.0 and 1.5 mg/l.

The explants were cultured on the medium at November 2005 aiming to callus induction.. Each treatment was represented by 12 tubes, each tube contained one explant, then the cultured tubes were kept at 25 ± 2 °C with light intensity 1500 lux for 16 hr. photoperiod provided by cool white fluorescent lamps. Data were recorded after six weeks including percentage of callus induction and callus weight.

Callus differentiation (organogenesis)

Fifty tubes were cultured on the best concentration callus differentiation (MS basal medium supplemented with 10 mg/l Kin), Hamouda (2003) this concentration divided into five treatment from fusaric acid 0.0, 0.1, 0.5, 1.0 and 1.5 mg/l. for obtaining callus differentiation Then, data were recorded as, differentiation percentage (cultures showed shoots only, roots only or both), plant length, leaf number and root length.

Acclimatization

The acclimatization of the produced plantlets (four plantlets from 0.1 mg/l fusaric acid and four plantlets from 0.5 mg/l fusaric acid) planted in plastic pots (7.5 x 10.5cm) containing peatmoss: vermiculite (1:1 v/v), every pot contained one plantlet covered with plastic bag to maintain high relative humidity in the greenhouse. The plastic bags were bored by needle twice every day in order to get rid of excess humidity. The bags were removed after one month. The obtained seedlings were transferred into sand: clay mixture (1:1 v/v) in 30cm pots and kept to grow for six months, then they were cut and the obtained cuttings were cultured in pots to make tow groups (clone 1 and clone 2) and kept to grow for six months to infect with fusarium fungal

Source of F. oxysporum isolate

The virulent isolate of *F. oxysporum* of *Pelargonium graveolens* L'Herit. used in this study was gently obtained from the stock of the isolated fungi of Ornamental, Medicinal and Aromatic Plant Diseases Department, Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt. The fungus was grown on potato dextrose liquid media (200 g. potatoes and 20 g. dextrose per liter of medium) in 500 ml glass bottles. The inoculated bottles were incubated at 25°C ± 1 for 15 days Hasan, Wafaa F. E. (2006).

Greenhouse experiment

Soil infestation was carried out by mixing the pathogen inoculums thoroughly with formalin sterilized soil at the rate (3%) of soil weight .The infested soil was then placed in formalin sterilized pots (25 cm in diam.) . Five pots were used for each treatment, and each pot was considered as one replicate .Commercial variety of pelargonium cuttings were obtained from The Horticulture Research Institute, MAPs Department and surface sterilized using sodium hypochlorite (1%) solution for 3 min. and planted at the rate 3 cuttings/pot. Percentage of wilt infection were recorded 90 days after the planting date Dhingra & Sinclair (1995) and Domasch *et al.* (1980).

Evaluation of clones compared with mother plants

Vegetative growth

These clones were compared with mother plant seedlings propagated by cuttings after infection in the pots at the same time. The evaluation of two cuts/year included wilt percentage after fusarium infection, plant height, branch number, stem fresh weight, leaves number, leaves fresh weight, leaf area cm^2 and plant fresh weight.

Oil percentage and constituents

Oil percentage of the fresh herb was determined according to the method described in the British Pharmacopoeia (1963). Gas liquid chromatography (GLC) method was used to analyze the essential oil for the main components.

DNA isolation

Polyacrylamide gel electrophoresis: Fresh leaf samples from *Pelargonium graveolens* L'Herit. were homogenized in 200 ul buffer containing 1M Tris-Hcl pH 8.8 and 0.25 M EDTA. After centrifugation at 10,000 rpm for 10min., 100ul supernatant containing water soluble proteins were transferred to a new eppendorf tube and mixed with 800 ul acetone and then kept in freezer for 15 mion., after centrifugation at 10000 rpm for 10min., the pellets were suspended in 80 ul buffer containing 1M Tris –Hcl pH 8.8, 0.25M EDTA, 10%SDS and 10%glycerol. Mercaptoethanol was added to each tube and boiled in water bath for 10 min. Polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate was performed on 12.5% (w/v) polyacrylamide gels according to Sambrook *et al.*, 1989. After electrophoresis, gels were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 (Sigma). A value of the relative molecular mass (Mr) of the protein band was estimated by comparison with the molecular weight marker (Bio Rad).

Protein measurement

The protein content of the samples was measured according to Lowry *et al.*, (1951) with bovine serum albumin as standard protein. Measurements were carried out at 595 nm.

Plant DNA extraction

Leaves of *Pelargonium graveolens* L'Herit. were collected, quickly frozen in liquid nitrogen, and ground to a fine powder. Total genomic DNA was extracted and purified from 1 g of freeze-dried powder as described by Dellaporta *et al.*, (1983). DNA present in the supernatant was precipitated according to the described protocol, re-dissolved in sterile, distilled water and quantified by spectrophotometry.

Amplification of genomic DNA using polymerase chain reaction (PCR)

PCR was performed with final volume of 25-µl according to Williams *et al.*, (1990). Four random DNA oligonucleotide primers were independently used in the PCR reactions (UBC University of British Colombia, Canada) the primers

with the following sequence UBC21 5'ACCGGGTTTA3' UBC30 5' CCGGCCTTAG3', UBC75 5'GAGGTCCAGA3' and UBC82 5'GGGCCCGAGG3'

The amplification was carried out as follows, one cycle 94°C for 4 min, 37 cycles 94 °C for 45 sec., 36 °C for 1 min., 72 °C for 2 min., and one cycle at 72 °C for 12 min., then 4 °C infinitive. To check the PCR products, agarose gel electrophoresis was employed a DNA marker (100 ladder (Roche)) Electrophoresis took place in 1X TBE buffer for 30 min., at 100 volts. After staining with ethidium bromide, the gel was photographed.

Gels analysis

All gels resulted from the two genetic criteria, protein and DNA Fingerprints, were scanned and analyzed with Bio-Rad video densitometer model 620.

The following data were recorded on geranium clones and mother plants:

Data in tissue culture laboratory:

1- Percentage of callus formation.
2- Callus weight (gm).
3- Callus differentiation percentage.
4- Shoot length (cm).
5- Root length (cm).
6- Leaves number.

Data in open field plants:

1- Wilt percentage after fungal infection.
2- Plant height (cm).
3- Number of branch.
4- Number of leaves.
5- Leaves fresh weight (gm).
6- Stem fresh weight (gm).
7- Plant fresh weight (gm).
8- Leaf area (cm^2).
8- Oil percentage.
9- Oil yield ml/plant.
10- Oil constituents.
11- DNA isolation.
12- Protein isolation.

Data were subjected to analysis of variance and Lest significant different Test was used to compare the obtained means at 1% and 5% levels l Snedecor and Cochran, (1980).

Results and Discussion

Callus formation

Data in Table 1 and Fig. 1 indicated that, control and control + 0.1 mg/l fusaric acid showed successful effect on callus formation of the cultured internodes.

The higher percentage of callus formation (80 %) and callus weight (0.425 gm) were obtained when MS medium was free from fusaric acid, while the other treatments which contain different concentrations from fusaric acid (0.5 , 1.0 and 1.5 mg/l) had no effect on callus formation of the cultured internodes. The exception was found in one concentration 0.1 mg/l fusaric acid which gave 40 % callus formation and callus weight (0.371 gm).These data were in accordance with Matsumoto *et al* (1995).

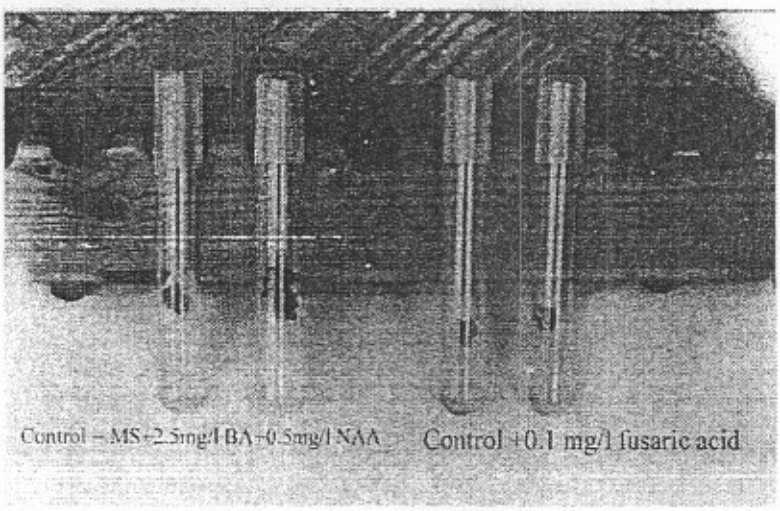

Fig. 1. Callus formation.

TABLE 1. Effect of different concentrations of fusaric acid on the percentage of callus induction and weight of formed callus/explant (gm) from internodal explants of *Pelargonium graveolens* L'Herit.

Treatments	Callus formation %	Callus weight (gm)
Control	80.0	0.425
Control + 0.1 mg/l F. acid	40.0	0.371
Control + 0.5 mg/l F. acid	00.0	0.0
Control + 1.0 mg/l F. acid	0.0	0.0
Control + 1.5 mg/l F. acid	0.0	0.0

Control = MS+2.5mg/l BA+0.5mg/l NAA

Callus differentiation (Organogenesis)

Data in Table 2 and Fig. 2 showed that, differentiation percentage reached the highest value (90%) when callus was cultured on MS medium containing 10.0 mg/l Kin without supplemented fusaric acid (control), while differentiation percentage achieved 40 % at the low concentration from fusaric acid (control + 0.1 and 0.5 mg/l fusaric acid).

Date clearly indicated that, fusaric acid affected negatively in callus differentiation *i.e.* the lower concentration (control + 0.1 and 0.5 mg/l) exerted an inhibiting effect on shoot length (6.17 and 5.33 cm) in comparison with control (9.34 cm).

It was also showed that, fusaric acid at the highest concentration (1.0 and 1.5 mg/l) showed the greatest inhibiting effect in callus differentiation.

Moreover the root length and leaves number showed an inhibiting effect due to fusaric acid supplement. In this concern, the inhibiting effect of fusaric acid was found to be parallel with its concentration, *i.e.* increasing the concentration of fusaric acid showed an increase of its inhibiting effect as illustrated in Table 2.

This detected inhibiting effect of fusaric acid on callus differentiation, shoot length, root length and leaves number may be due to that fusaric acid exert an harmful effect on the hormonal balance as well as the favorable balanced conditions of the callus differentiation and organs growth.

TABLE 2. Effect of different concentrations of fusaric acid on the percentage of callus differentiation (organogenesis), shoot length, leaves number and root length of *Pelargonium graveolens* L'Herit.

Treatments	Callus differentiation %	Shoot length (cm)	Root length	Leaves number
Control	90.0	9.34	4.89	8.45
Control + 0.1 mg/l F. acid	40.0	6.17	4.0	7.17
Control + 0.5 mg/l F. acid	40.0	5.33	3.67	5.67
Control + 1.0 mg/l F. acid	0.0	0.0	0.0	0.0
Control + 1.5 mg/l F. acid	0.0	0.0	0.0	0.0
L.S.D. 5%		1.73	N.S	N.S
L.S.D. 1%		2.62	N.S	N.S

Control = MS+10.0 mg/l Kin

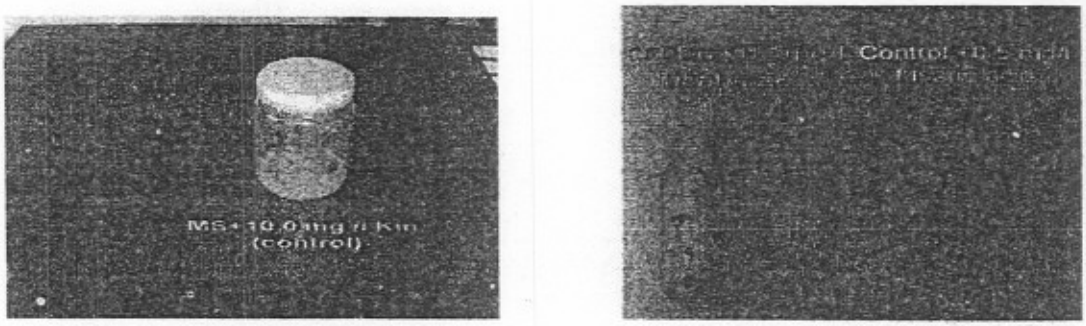

Fig. 2. Callus differentiation.

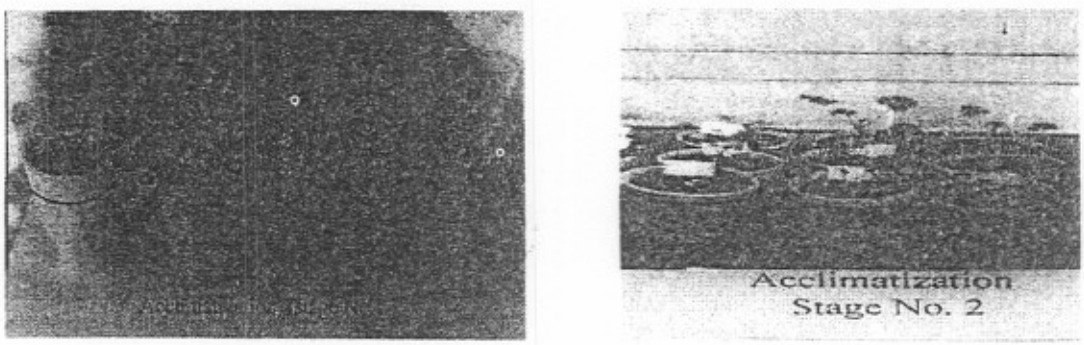

Fig. 3. Acclimatization.

Evaluation of the resultant plants under field conditions

Two clones were obtained from culturing of cuttings taken from the acclimatized plants which were previously regenerated from callus grown in MS media supplemented with fusaric acid. These clones were compared with mother plant seedlings cultured by cuttings at the same time in the same pots.

Survival percentage after artificial fungal infected

Data in Table 3 emphasized that, the survival percentage (100 %) of the two clones and (60 %) of mother plants after artificial fungal infection.

It is clear that, two clones were tolerant to *Fusarium oxysporum* infection more than mother plants.

TABLE 3. Survival percentage after fungal infection.

Clones	Survival percentage after fungal infection
Mother plants	60
Clone (1)	100
Clone (2)	100

The vegetative growth, oil percentage and constituents

Data in Table 4 showed the vegetative characteristics of the resultant clones, in the first cut, significant increase was found in plant height while, high significant decrease in leaves fresh weight was obtained in comparison with mother plants.

On the other hand the rest of vegetative characters (number of branch, number of leaves, stem fresh weight and leave area) showed no significant differences between two clones and mother plant.

In the second cut data in Table 4 clear that, leaves area (cm^2) of the two clones showed insignificant difference than mother plant. On the same way, plant height, number of branch, number of leaves, leaves fresh weight (gm), stem fresh weight (gm) and plant fresh weight (gm) recorded insignificant differences between the two clones and the mother plant.

Essential oil yield

Oil percentage and oil yield (ml / plant)

Essential oil percentage of the two cuts is shown in Table (5). In the first cut, the highest oil percentage (0.133 & 0.13 %) was obtained from the clone (2 & 1) respectively, these values were significantly higher than mother plant (0.09%).

In the second cut, similar trend were recorded, the highest percentage (0.290 %) was recorded in case of clone (2) followed by clone (1) (0.287 %) compared with mother plants (0.113 %).

However, the oil yield (ml)/plant of the two cuts for the two clones were insignificantly higher than the mother plants.

TABLE 4. Vegetative characteristics of some clones which obtained from different tissue culture conditions compared with the mother plant of *Pelargonium graveolens* L'Herit.

Clones	Plant height (cm)	Number of branch	Number of leaves	Leaves fresh weight (gm)	Stem fresh weight (gm)	Leaf area (cm^2)	Plant fresh weight (gm)
			First cut				
Mother plant	59.03	13.33	221.67	245.0	116.67	16.00	362.67
Clone (1)	67.33	14.83	223.67	220.67	106.33	18.79	327.00
Clone (2)	68.5	9.67	228.0	183.33	91.67	21.34	248.00
L.S.D. 5%	**4.83**	**N.S**	**N.S**	**27.4**	**N.S**	**N.S**	**N.S**
			Second cut				
Mother plant	72.33	18.00	216.67	511.00	179.20	21.83	690.20
Clone (1)	71.00	14.67	220.00	327.00	148.97	29.73	475.97
Clone (2)	76.67	15.33	300.67	257.83	123.70	32.53	381.53
L.S.D. 5%	**N.S**	**N.S**	**N.S**	**N.S**	**N.S**	**3.60**	**N.S**

Oil constituents of geranium clones

Data in Table 5 showed the percentages of the main oil constituents of the obtained calliclones.

Concerning geraniol percentage, the clone (2) showed an increase in geraniol content (22.57 %) when compared with mother plant (20.95%). However, the lowest geraniol percentage (17.52%) was produced from the clone (1).

As for citronellol content similar trend was showed, the highest percentage was recorded in case of clone(2) (31.45 %)followed by mother plant (29.09 %)and clone(1) (26.85 %) respectively.

However, the least linalool percentage (5.00 %) was obtained from the oil of clone (1) and the highest percentage was recorded in case of mother plant (6.81 %).

As for eugenol percentage, the highest percentage was recorded at clone (1) (11.55 %), followed by clone (2) (8.15 %) and mother plant (8.11 %) respectively.

TABLE 5. Oil percentage, oil yield (ml/plant) and main constituents on geranium plants.

Clones	Oil percentage		Oil yield/ plant (ml)		Main constituents of geranium oil %			
	1st cut	2nd cut	1st cut	2nd cut	Geraniol	Citronellol	Linalool	Eugenol
Mother plant	0.090	0.113	0.34	0.80	20.95	29.09	6.81	8.11
Clone (1)	0.130	0.287	0.42	1.38	17.52	26.85	5.00	11.55
Clone (2)	0.133	0.290	0.31	1.07	22.57	31.45	5.68	8.15
L.S.D. 5%	0.013	0.036	NS	NS	--	--	--	--

These results indicated some differences in phenotype especially in leaf area in the two clones as well as their superiority in essential oil content in comparison with the mother plant. In this regard Gauri *et al* (2000) obtained calliclones of *Pelargonium graveolens* L'Herit. differed in several herb related agronomic characteristics such as plant height, herb yield, canopy size and number of branches per plant from the parental type as well as from the parent, which seems advantageous for commercial exploitation of such clones.

Thus good agricultural practices may be needed to enhance herb yield of the wilt tolerant clone if chosen according to its high content of oil percentage. This may be of a great importance since some derived clones showed an increase in geraniol and linalool contents and all of the derived clones showed an increase in citronellol content compared to the mother plant.

Biochemical and molecular identification

The present investigation has been carried out to study the differences between mother plant and two clones and the similarities and genetic distance between the clone (1) and clone (2), which were collected from *Pelargonium graveolens* L'Herit. (mother plant) the goal of the present study has been achieved by employing to different criteria, *i.e.* protein banding patterns, and RAPD-PCR analysis.

Biochehemical genetic marker: Polyacrylamid gel electrophoresis (PAGE) technique was used to identify the three different breeds under investigation. Figure (4) shows the electrophoretic patterns of soluble protein of the plant samples.

The most important variation to be studied in plant breeds as detected by anodic polyacrylamide gel electrophoresis for acidic proteins the result agreement with Recorbet *et al.*, (1998) who detect acidic protein in tomato plant after inoculation with the strains of *F. oxysporum* f.sp. *lycopersici.*

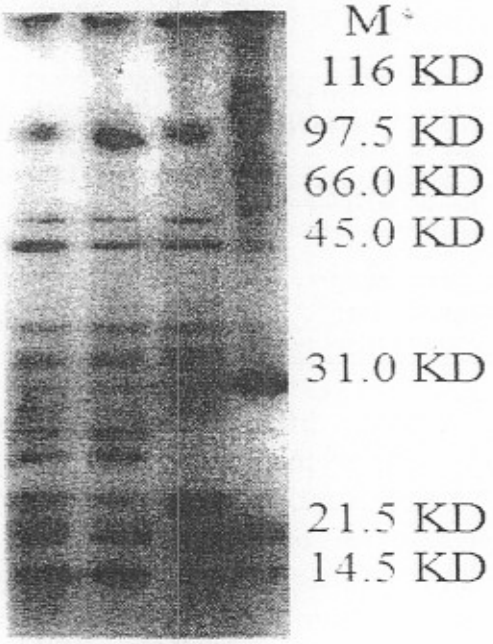

Fig. 4. SDS- Protein banding pattern of three *Pelargonium graveolens* L'Herit. lane M referees to the standard protein marker.

Electrophoretic SDS-protein patterns of fresh leaf samples for the three *Pelargonium graveolens* L,Her. (clone 1 & 2 and mother plant) are shown in Fig. 4 considering band's number, the three line showed high similarity , which suggesteds high genetic background similarity within these genotypes. However, interspecific differences in band's number between the treated and original were identified. No variations were detected with regard to band's number, and no differences in band's intensity. According to the presence of band at arrows (at approximately molecular weight 27.5, 26, 21.5 and 14.5KD) were detect in selected plant. These bands are low molecular weight and acidic proteins. These results are in agreement with Recorbet *et al.*, (1998) who detected by polyacrylamide gel electrophoresis for acidic proteins after inoculation, the strains of *F. oxysporum* f.sp. *lycopersici* .

DNA isolation: The isolated DNA from three line of *Pelargonium graveolens L* were tested against four 10-mer random primers. The results of RAPD analysis using four primers UBC 21, UBC30, UBC75 and UBC 82 fore three lines of are illustrated in Fig. (5). The molecular size of the PCR products generated by these primers are ranged from approximately 2500 to 250 bp. The primers UBC 21, UBC30, UBC75 and UBC82 generate unique bands at ~1580, ~506, ~470 and ~ 517 respectively in selected line and absent in original. According to these observations, RAPD analysis was completely discriminates among the three lines. The obtained data across SDS and RAPD, which indicates a high level of genetic background similarity studied lines.

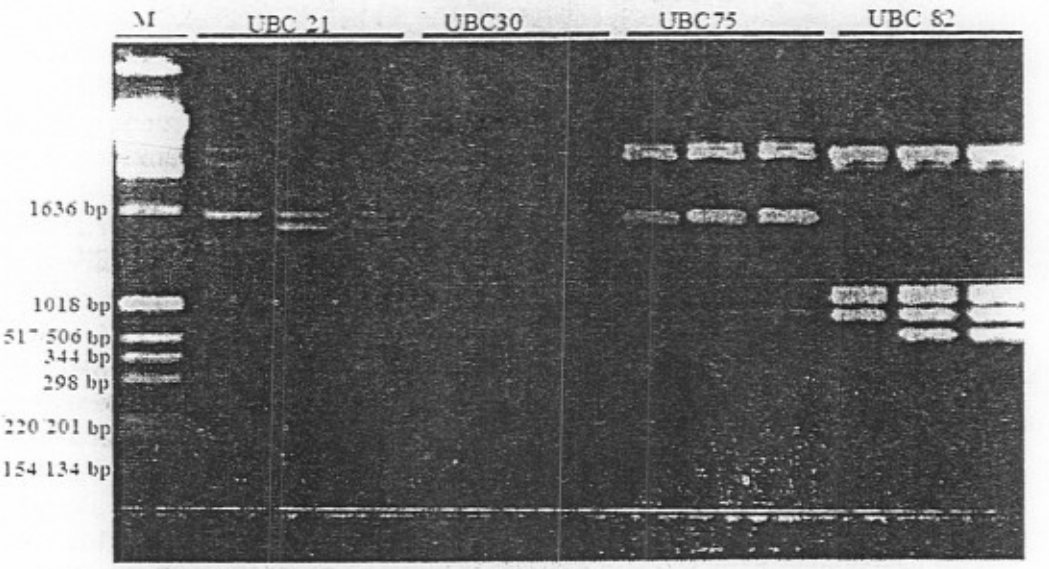

Fig. 5. DNA polymorphism based on RAPD analysis for three Pelargonium graveolens L'Herit. line.

Plants possess a broad spectrum of basic defense mechanisms, pre-established or induced, which render them resistant to most potentiai colonizers. The ability of *F. oxysporum* strains to tolerate or delay host defenses has been proposed as a trait that may reflect pathogenicity on a specific host. Olivain & Alabouvette (1997, 1999) demonstrated, on the basis of cytological observations, that whereas the colonization of the nonpathogenic GUS-marked strain was restricted to the superficial layers of cells by the defense reactions of the plant. Apart from cytological observations, few studies have adressed the expression of plant defenses in response to both pathogenic and nonpathogenic *F. oxysporum*. Among them, special attention has been paid to the secondary metabolites produced by plants, many of which have antifungal activity. Saponins, which are plant glycosides, have been implicated as preformed chemical barriers against pathogen attacks (Osbourn, 1996). α-tomatine, a phytoanticipin from tomato, is likely to have been the most studied saponin (Safe *et al.*, 1997).

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(*Received* 3/3/2009;
accepted 14/4/2009)

إنتاج سلالات من العتر تتحمل الذبول من خلال مزارع الأنسجة

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أجرى هذا البحث فى قسم بحوث النباتات الطبية والعطرية – معهد بحوث البساتين – مركز البحوث الزراعية خلال الفترة من ٢٠٠٤ -٢٠٠٧ ويهدف هذا البحث إلى إنتاج سلالات من العتر تتحمل الذبول الفيوزارمى من خلال مزارع الأنسجة وتمت الدراسة على النحو التالى:

- تم دراسة إستجابة الاجزاء النباتية لإنتاج كالوس العتر على بيئة MS تحتوى على حمض الفيوزريك وتكشف هذا الكالوس على بيئة تكشف MS وأقلمة النباتات الناتجة.
- دراسة تأثير عدوى النباتات فى الاصص بفطر الفيوزاريم.
- تقييم السلالات الناتجة ومقارنتها بالأم من حيث:
  - نسبة البقاء بعد العدوى الخارجية بفطر الفيوزاريم.
  - الصفات المورفولوجية للسلالات ونسبة الزيت.
  - عزل DNA والبروتين للسلالات والأم.

وكانت أهم النتائج المتحصل عليها الأتى:

١- الحصول على سلالتين من العتر البلدى تتحمل الذبول الفيوزارمى.
٢- توجد فروق حقيقية ومعنوية بين السلالتين والام فى صفة مساحة الورقة ونسبة الزيت دون باقى الصفات المختبرة.
٣- يوجد اختلاف فى تفريد DNA بين السلالتين والام وكذلك بين السلالتين بعضهما البعض.
٤- ظهور نوع جديد من البروتين فى السلالتين لم يكن موجود فى الأم.

ومما سبق نوصى بإكثار هاتين السلالاتين والتوسع فى زراعتهما للتغلب على مرض الذبول فى العتر البلدى.