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Inheritance of Resistance against Phytophthora Infestans in Wiled Tomato Genotype (*Lycopersicon hirsutum* l03684)

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ABSREACT

Many varieties commercial's tomatoes have been grown in Egypt but most of them susceptible to the disease of late blight, which are very important and most dangerous fungal diseases that destroy the tomato crop in all its parts caused by the disease Phytophthora infestans. The L03684 wild tomato genotype was obtained from the Asian Development Center for Vegetables. The inter specific cross between L03684 and Edkawi species was done to obtained the six populations and study resistance of Late blight heredity, and assessment of genetic parameter for resistance. Results showed that, Many genes control the heredity of resistance to *P. Infestans* and resistant is inherited as a quantitative trait. While, susceptibility to late blight is a dominant characteristic of resistance. The heritability in broad ($H_{b.s}$%) estimates were 71.8 and in narrow sense ($H_{n.s}$%) estimates were 39.35% for severity detect the proportion of the environmental factors on the total variation. the inheritance of resistance to P. infestans greatly affected by dominance gene effects. While that, gene effects of estimations were low. So the effects of gene refers to epistasis more important than the additive. This study showed that, the inter actions between additive and dominance were highly significant in the inheritance of resistance to *P. infestans*. One of the best methods to breeding to improve this resistance by the reciprocal recurrent selection.

INTRODUCTION

Tomato (*Solanum Lycopersicon L.*) has a place with a family have a widest vegetables growing with ability to survive in diverse environmental conditions so it's fruit is considered to be fairly high in the tropic (Rice *et.al.*, 1987).

Economically, tomato one of four most important vegetable crops of food with worldwide creation of 162 million tons assessed an estimation of in excess of 62 billion $ in 2014 According to (FAO 2017)*. One of the first Arab countries in the production of tomatoes is Egypt and fifth in the world, with a production volume of more than 8.5 million tons annually estimated at value of more than 1.7 billion$.

In the same trend, all area planted in season 2015 - 2016 was (440233 /feddan) and average yield (16607 kg /feddan), all of this area need to (2.2 billion) seed / seedling according to Ministry of Agricultural Economic Affairs Sector (2018).* Data bases. http://faostat.fao.org/2017.

Number of factors cause a low tomato yield include insects, pests, diseases and weeds environmental conditions. It's well known that diseases remain and pose the biggest challenges in tomato creation and evaluated that there are in excess of 200 kind of realized diseases influencing tomatoes around the world. This disease cause 15 - 95% crop loss from total yield (Jarvis and McKeen, 2013). *P. infestans* is the most major fungal diseases on tomato, which known as late blight, pathogen of this disease is a diploid, heterothallic oomycete and hemibiotrophic oomycete that poses a real and potential threat.

The pathogen assaults all over the ground portions of the plant including leaves, petioles, stems and organic product at any growth stage, causing scourges, corruption, blotches and spoils that lessen yield and natural product quality (Lievens *et al*, 2004). The disease can spread and execute plants quickly when ideal natural states of high moistness and low temperature (18°C) win (Haq Italic., 2008 and Stroud *et al*., 2016).

The utilization of cultivars conveying obstruction qualities against Late blight is along these lines considered an all the more ecologically benevolent, financially savvy choice to control this ailment. Studies with respect to hereditary protection from P. infestans have been focus of different tomato reproducing programs for a long time. As indicated by these examinations, protection from LB in tomato plants is mind boggling and may include the declaration of one or couple of qualities (subjective opposition) or even the statement of different qualities (quantitative resistance).The protection from LB alludes to a characteristic of quantitative legacy constrained by around 28 genes(Abreu *et al*. 2008).

There are three genes of late blight resistance were identifying. one of them located on chromosome 7 has a single dominant allele it's called Ph-1,but it was overcome by new races of the pathogen. The second gene, Ph-2, was located on chromosome 10 , has a partially dominant allele. The second gene, Ph-2, was located on chromosome 10 , has a partially dominant allele for resistance to some of P. infestans isolates, But rarely resistance occurs with the aggressive isolates. The third gene Ph-3, was located on chromosome 9 , has a single dominant allele for resistance to some aggressive isolates of P. infestans like Pi-16 from Taiwan that can overcome Ph-1 and Ph-2 (Rzhansky and Cohen, 2006). Also, (Kim and Mutschler, 2006; De Miranda *et al.*, 2010 and Chen *et al.,* 2014) they are found that, can overcome on Ph-3 gene by some isolates. Also, Ph-3 has incomplete dominance resistance gene an it's consider an effective against a wide range of P. infestans isolates., Dariva, *et al.* (2018).

We should study nature of inheritance and gene action of resistance from S. hirsutum before insertion it to local some of tomato cultivars to use in breeding programs. So this study aims to determined the inheritance and genetic parameters related with resistance to late blight in the (*S. hirsutum* and *L. esculentum*) cross.

MATERIALS AND METHODS

Genetic material was obtained through an interspecific crosses between a wild cultivars of tomato (*Solanum hirsutum*): L03684 (P1) (Ecuador) has Ph-3 gene and cultivars of tomato (*Solanum lycopersicum L.*): Edkawy (P2) (Egypt). Plants from each variety were self pollinated for three generations to get up inbred line from each one, were used from the breeding program against late blight resistance in tomato, Horticulture Research

Institute (HRI). These selected lines were provided by Ramadan and Kamel (2014).

Experimental Procedures:

Seeds of the chosen F_1 hybrids and their parents were sown on 10th September 2016 in seedling trays, after 45 days from seed sowing the seedling were transplanting under plastic green house at El-Bramoon Experimental Station. To increase F_1 seeds, some flower from each parent used to do crosses between the two parents. To produce F_2 generation seeds, F_1 plants were selfed to increase seeds from there parental. Some of F_1 plants were back crossed to their parents in order to obtain BC_1 and BC_2 generation.

On 15th February 2017 seeds of the parental (P_1 and P_2) there F_1, F_2, $BC_1$and BC_2 generations of the two crosses were sown in seedling trays as described earlier.

Transplanting were set into the field after 45 days. Experimental was conducted in the Bramoon Experimental Farm of Horticultural Research Institute, Dakahlia Governorate for this studies. The field experiment was arranged in three replications with Randomized Complete Blocks Design.

Culture of fungal and the zoospore production:

Mating type of isolate of *P. infestans* is A1 clonal lineage is (23-A1) was obtained from naturally infected tomato var GS Beheira Chat Conversation. (Plant Pathology Research Institute, (El-Ganainy 2018)

By transferring the late blight-infected tissues to medium (rifampicin +pimaricin + penta chloro nitro benzene agar + ampicillin)The culture was obtained. Also zoospore production was obtained from the middle of older leaves after six week from a susceptible plants.

Genotype Gs were put onto moistened filter paper in 140 mm Petri plates. The axial surfaces of these leaves were injured at the centre using a sterile 10 µl micro pipette tip and a 5 µl sporangial suspension, collected from PARP medium was placed on the wound of each leaf for 24 hrs at 18°C in darkness. Then 15 ml of sterilized distilled water was added to the plates and they were further incubated for 2-3 days at 18°C in darkness. The suspension was then filtered through four layers of sterile muslin cloth to remove other fragments. The zoospore suspension was adjusted in sterilized distilled water to a concentration of 5000 zoospores per ml using a haemocytometer.

Detached-leaf assay:

From the middle of plants and after six week old from planting, fully expanded leaves were detached to tested it in a greenhouse. In glass Petri plates (140 mm dia.) four leaves per genotype were placed in a axial side up on moistened filter paper and each of them was inoculated with a 20 µl drop of zoospore suspension at the centre on the axial surface after injuring with a sterile 10 µl micropipette tip. For three times, each of genotypes was replicated and inoculated at 18°C with a 16 hr photoperiod. After 24 hr of inoculation the experimental unit was examined seven days post inoculation. The estimation of leaf area occupied by late blight lesions by using descriptive scales by Rzhansky and Cohen (2006) after some modifications (Table 1).

Table 1. Tow tomato cultivars used in this study were presented in

No.	Genotypes	F.S*	Maturity	Origin	Resistance
P_1	L03684	Small	Moderate	Ecuador	Resistance to late blight
P_2	Edkawy	Large	Late	Egypt	Resistance to salinity

*** F. S : Fruit size.**

For each genotype the individual leaf ratings were added and calculated means to generate the corresponding severity index (SI). The Inoculated plants were kept in the incubation of plant pathology laboratory and seed production at the Faculty of Agriculture Mansoura University. There were for each genotype have three replications each of them contain 30 plants of two parents and F_1 but 45 plants of each of BC_1 and BC_2 , While F_2 it contains 60plants .

Data regarding the proportion of plant blighted and leaf were visually estimated by using a 0-5 scale to calculate percent disease index (DI %) in (Table 2).

Table 2. Key of scale of the rating disease for tomato late blight.

Disease rating	Symptoms severity for detached-leaf assay	% Disease index	Disease response
0	Non a visible symptoms apparent.	0	Immunity
1	A few minute lesions to about 10% of the total leaf area is blighted.	0.01-10	Highly resistant
2	Around 25% of the total leaf area is blighted.	10.01-25	Resistant
3	Around 50% of the total leaf area is blighted.	25.01-40	Tolerant
4	Around 75% of the total leaf area is infected.	40.01-60	Susceptible
5	Leaves are fully blighted.	> 60.01	Highly susceptible

Data and statistical analysis

Heredity of resistance was studied by grouping plants into susceptible classes, resistant to moderate and resistant through Mendelian approach. classification of resistant were applied in three rating (Table 1) as (1) resistant 0-29%.; (2) moderate 30-69% and (3) susceptible 70-99%, that depending on the parent's interval rang.(Elsayed *et al*., 2012).

The statistical GENES software program(Cruz, 2013), were used to perform the Statistical procedures. By using the method of Mather and Jinks (1982) was estimate the minimum number of genes that determine the character by using the formula derived by Burton (1951).

The hypotheses of two recessive genes control the resistance were tested for goodness of fit theoretical ratios with segregation ratios. By using numerical data Chi-square (χ^2) test was performed on the segregating populations.

RESULTS AND DISCUSSION

Laboratory methods are among the most important evaluation method of the tomato leaf resistance to *P. infestans*, can be using the seedlings test in the resistance assay which are most effective and reliable methods ranks with the standard way consistent with field observations.

Evaluation of the tomato leaf resistance to *P. infestans*, using the seedlings test, ranks the standards in the way consistent with the field observations Michalska and Pazio (2002).

The inheritance of the resistance in two parents, F_1, F_2 and their backcrosses generations by The qualitative analysis using test X^2 showed that, the suitability of H_0 hypothesis of the genetic of qualitative model (9: 6: 1) to resist the late blight injury corresponds to the probability of 64.59% (Table 3).

Table 3. Goodness of fit (X^2 and P)of resistance to late blight (*P. infestans*) for qualitative genetic model in population of the crosses between the resistance (Ekdawy) and susceptible (L03684)

Generation	Total No. of plants	Min.	Max.	No. of plants per symptom class			Tow recessive genes (9:6:1)				
							Expected numbers/ratio of the F_2			Goodness of fit	
				S	M	R	S	M	R	X^2	p
P_1	30	16	32	-	-	30	-	-	-	-	-
P_2	30	50	80	30	-	-	-	-	-	-	-
F_1	30	32	60	19	11	-	-	-	-	-	-
F_2	60	35	90	35	23	3	37.8	22.5	3.8	0.87	64.59
BC_1	45	25	68	20	25	-	-	-	-	-	-
BC_2	45	25	65	36	9	-	-	-	-	-	-

Furthermore, the qualitative analysis of genetic model showed that, the inheritance of the resistance can't ignore on the basis of inherited genes in view of the genetic patterns (A-B-) as susceptible with presence of the susceptible parent of partial dominance. But when both alleles are (aabb) the resistance will appears, while the genetic patterns of (A-bb / aaB) are moderately, the resistant appears in (Table 4).

The repeated distribution of parents F_1 and F_2 showed that for Edkawy's sensitive parent, the intensity ranged between 50 and 80% with a majority (individuals) in the 85-99% category. Most individuals ranged from 16 to 32% of severity For the L03684 resistant parent. But, the individual generation of F_1 were in two classes with 19 sensitive resistance and 11 medium resistance. This distribution confirms the F_1 individuals' fact that hegemony is sensitive to resistance. These results agree with the results were obtained by Elsayed *et al.,* (2012) and Ramadan and Kamel (2014).

Table 4. A genetic model of qualitative resistance against *Phytophthora infestans* for the inheritance in (Edkawy x L03684).

Genotypes	Proportion	Phenotype
AABB	1	Susceptible
AABb	2	Susceptible
AaBB	2	Susceptible
AaBb	4	Susceptible
AAbb	1	Moderate resistance
Aabb	2	Moderate resistance
aaBB	1	Moderate resistance
aaBb	2	Moderate resistance
aabb	1	Resistance

F_2 population's segregation ratio S:M:R = 9:6:1

The result showed that, Edkawy cultivar was a susceptible and the severity at the end of epidemic mean was 68.63% , while 21.97% for the resistant cultivar L03684.

The differences between the two generation in terms of resistance to *P. infestans* were presented in this study. The mean values of the F_1 individuals for Late blight severity showed value 30.0 of severity (Table 5). While the mean performance of F_2 population increased to 54.77 compared to their F_1 generation, But BC_1 and BC_2 generations showed (56.23) and (63.50) respectively (Table 5). This result could be assign to the effect of dominance to the susceptibility, similar finding was reported by Ramadan and Kamel (2014). The variances obtained for each generation are presented in (Table5).

Table 5. The means and variances of estimation for the severity of *P. infestance* caused by Phytophthora infestans in the parental (P_1, P_2), filial (F_1, F_2) and back crosses (BC_1, BC_2) generations of tomato cross (Edkawy x L03684).

Generations	S. V.				
	No. of plant	Mean	Variance	V (m)	1/V (m)
P_1	30	21.97	27.34	0.91	1.09
P_2	30	68.63	63.07	2.11	0.48
F_1	30	30.00	28.14	0.94	1.07
F_2	60	54.77	202.42	3.37	0.31
Bc_1	45	56.23	156.71	3.87	0.26
Bc_2	45	63.50	168.48	3.42	0.29

The estimates Both of additive genetic variance, mean dominance degree, variance due to dominance deviation, heritability in broad and narrow sense and the number of genes that control character were presented in (Table 6).

The estimated dominance variance (89.08) was higher than the variance due to additive deviations (76.76) and represented approximately (165) of the genotypic variance (Table 6). Heritability in broad sense ($H_{b.s}$ %) was (71. 8%) and in narrow sense ($H_{n.s}$ %) was (39.35%), for severity detect the magnitude of the environmental factors on the total variation.

The heritability of resistance for late blight ranged from (65% to71%).The environmental factors is a highly affect on the low heritability for resistance severity. This results according to (Foolad *et al.,* 2018). Also, (Ramalho *et al.,* 2000) observed that the low heritability, that associated with quantitative traits attributed to the environment factors. The minimum number of genes controlling resistance was 4.93 genes According to current model, estimated by Burton, (1951) minimum effective factors calculated with F_2 generation (Table 6).

These outcomes according to the outcomes were acquired by Kim and Mutschler (2003) of Cornell tried a few lines of L3708 from AVRDC and found that they were fixed for their degree of resistance. Frary *el al.,* (1998) had sign that L3708 contains extra qualities for protection from late blight. They tried the resistance of a F2 populace from susceptible tomato × L3708 to California disconnects of P. infestans under field conditions and discovered three QTLs related with this obstruction, all situated in chromosome 6. Marker-helped molecular mapping of the resistance genes of L3707 is required so as to clarify their associations with other resistance genes in tomato . Thus, with the subjective investigation of legacy the outcomes acquired don't concur with the quantitative examination for obstruction. while, these outcomes can't help contradicting the past finding came about because of the subjective investigation exhibit two latent qualities controlling the obstruction in L03684, maybe this is because of at least one of these elements; multiplier impacts coming about because of polygenes and real qualities, the conceivable job of the significant qualities in kinds of polygenes and relationship between's the polygenes and the real qualities. Along these lines, Marker-helped sub-atomic mapping of the opposition qualities of L3708 is required so as to explain their associations with other obstruction qualities in tomato and potato (Irzt and Cohen 2006).

Table 6. The final severity of genetic parameters for the parental varieties (F_2) generations of tomato cross (Edkawy x L03684).

Parameters	Estimates
Phenotypic variance	202.42 ± 14.22
Environmental variance	39.33 ± 6.27
Genotypic variance	165 ± 12.84
Additive variance	76.76 ± 8.76
Variance of the dominance deviation	89.08 ± 9.44
Broad-sense heritability %	71.8 ± 8.47
Narrow - sense heritability %	39.35 ± 6.27
Heterosis (M.P) %	- 33.77
Average degree of dominance (based on variances)	1.57
Maximum value in the F_2 generation	90
Minimum value in the F_2 generation	35
Number of genes (Based on variances)	4.93

Heterosis value has showed by susceptibility to late blight, as witnessed by the fact that although the values of F_1 hybrids had a severity at the end of epidemic were intermediary between those for the resistant and susceptible, the values for Edkawy susceptible genitor were closer (Table 6).

Estimation of the mean dominance degree was 1.57, indicated that over dominant of the gene action. But when the estimation of the mean dominance degree was based on the means of the mean dominance, gene action indicated a partially dominant . While, the results of analysis of variance showed that the dominance deviations more important than additive variance. The positive sign of dominance more important, wich due to indicate that was not predominant for resistance but for susceptibility. (Kearsey and Pooni, 1996, and Flávia *et al*., 2008).

A,B and C Scaling test values were used to testing the presence of epistasis for this studied traits. when any of A,B and C test presente significance this indicated that non-allelic interaction. But, when the test values present insignificance the additive - dominance model is enough to interpret gene effects. For this trait are presented in (Table 7), regarding this trait the values of scaling tests were significantly differed from zero, indicating to the presence of non-allelic interaction.

Results in (Table 7), showed that , the estimates of dominance gene effect (d) was positive significant and important than additive effect (a) for this trait. In addition the cross (Edkawy x L03684) showed significant (aa, ad, and dd) for the severity of late blight. The presence of significant non-allic interaction may hinder the progress of selection leading to losses of favorable genotypes during the early generation of selection. Therefore, the improving of this trait could be achieved through hybrid breeding method.

Table 7. Scaling tests (A, B and C), types of gene action and stander error for the severity of late blight caused by Phytophthora infestans in the parental varieties (P_1 and P_2), (F_1 and F_2) and back crosses(BC_1 and BC_2) generations of tomato cross (Edkawy x L03684). Scaling tests

Scaling tests parameters	Estimates	SD
A	60.49**	3.97
B	28.37**	4.24
C	68.47**	6.54
Types of gene action		
m	54.77**	1.50
a	-7.27**	2.69
d	5.09**	8.16
aa	20.39**	8.06
ad	16.06**	2.82
dd	109.25**	12.58

****,* Scaling factors significantly different from zero at P = 0.001 and 0.05, respectively**

When the additive effects have a minor importance in the total variation of this trait, the program of breeding will be made more rapid advance for the improvement of this traits. The reciprocal recurrent selection breeding procedure proposed by (Comstock *et al*. 1949) appears to be the best available to meet the requirements. The value of severity for late blight from resistance to susceptibility, in segregating generations derived from the cross between (Edkawy x L03684) lead to the conclusion that resistance to *P. infestans* is controlled by polygenic ally. The genetic parameters and analysis of variances suggests that this kind of resistance is quantitative inheritance.

The gene effects of dominance were more important for inheritance of resistance to late blight. Estimates of additive gene effects were of low, magnitude. In this cross was studied, gene effects of epistasis were considered to be more important than additive gene actions in the inheritance of resistance to *P. infestans*. The gene actions of this interactions additive x additive, additive x dominance and dominance x dominance were highly significant, so the best method to improve the resistance to *P. infestans* was reciprocal recurrent selection breeding program.

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وراثة المقاومة للندوة المتاخرة في التركيب الوراثي البري L03684 التابع للنوع *Lycopersicon hirsutum*

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[١]قسم الخضر والزينة - كلية الزراعة جامعة المنصورة – مصر

[٢]محطة بحوث بساتين الخضر بالمنصورة - معهد بحوث البساتين - مركز البحوث الزراعية- مصر

يوجد في مصر العديد من الأصناف التجارية للطماطم ولكنها غير مقاومة لمرض الندوة المتاخرة الذي يعد من أهم وأخطر الامراض الفطرية التي تدمر محصول الطماطم بجميع اجزائه والذي يسببه المرض البيضي Phytophthora infestans. وتم الحصول علي التركيب الوراثي البريمن الطماطم L03684 من مركز التنمية الأسيوي للخضر- وبالتهجين النوعي مع الصنف إدكاوي تم الحصول علي العشائر السته وهي (الأباء، الجيل الأول، والجيل الثاني، والهجين الرجعي الأول، والهجين الرجعي الثاني) وذلك لدراسة وراثة الندوة المتاخرة والقياسات الوراثية المرتبطة بها وكانت النتائج كما يلي: يتحكم العديد من الجينات في وراثة صفة المقاومة للندوة المتأخرهفي الطماطم. صفة المقاومة تورث كصفة كميةفي الطماطم. الحساسية للإصابة بالندوة المتاخره في الطماطم صفة سائدة علي صفة المقاومة. معامل التوريث لشدة الاصابة في مداه الواسع كان ٧١.٨% بينما في مداه الضيق كان ٣٩.٣٥% ويعبر ذلك عن التأثير الكبير للتباين البيئي. كان الفعل الجيني السائد ذو تأثير كبير في توريث المقاومة للندوة المتأخرة بينما كان الفعل الجيني المضيف قليل التأثير وكانت جينات التفوق اكثر أهمية. كان الفعل الجيني (الإضافي x الإضافي ، الإضافي x السيادي ، السيادي x السيادي) عالي المعنوية يعتبر الإنتخاب المتكررالعكسي او المتبادل من افضل طرق التربية لنقل صفة المقاومة للندوة المتأخرة في الطماطم.