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Genetic Diversity among Transconjugants of *Bacillus thuringiensis* Used for Controlling Tomato Borer *Tuta absoluta*

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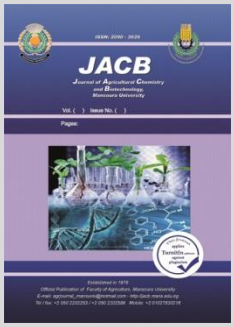

ABSTRACT

This study aimed to chacterize transconjugants of *Bacillus thuringiensis* on the basis of genomic DNA patterns, plasmid curing and bioassay of toxicity against tomato borer, *Tuta absoluta* in the open field. Eight transconjugants generated from four matings were used in this study. The shortening of extrachromosomal genetic material lead to loss of antibiotic resistance genes in some *Bt* strains and their transconjugants , as well as, loss of chitin hydrolysis as a consequence. In contrast, some *Bt* transconjugants were not affected by elevated temperature because they are still harboring antibiotic resistance genes and encoded chitinase. The extrachromosomal genome of *Serratia* was more stable than *Bacillus licheniformis* which lost *Cf* resistance gene at elvevated temperature. *Bt* strains and their transconjugants were genetically different because some bands appeared and or disappeared between them. Two transconjugants out of eight generated additional bands than the other ones which showed a higher toxicity index in relation to the mid-parent and the other transconjugants. Tomato plants treated with bioinsecticide formulations showed significant increase in photosynthetic pigments. Furthermore, some *Bt* transconjugants significantly increased shoot dry weight compared to the negative and the positive controls. This study indicated that mixing of dissimilar toxin genes in addition to chitinase genes in the same transconjugant led to increase toxicity and delay of resistance in insect pest than single - toxin gene carried by single strain.

Keywords: DNA patterns, *Bt* transconjugants, plasmid curing, chitinase gene, *Tuta absoluta* , Tomato.

INTRODUCTION

The tomato borer, *Tuta absoluta* is one of the most important lepidopteron pests associated with tomato crops, where crop losses range from 60 to 100% (Cristina *et al.* 2008). The larvae of *T. absoluta* can significantly reduce yield and fruit quality by direct feeding and the secondary pathogens which may enter through the wounds caused (Silva *et al.* 2011). Feeding damage is caused by all larval instars and the larvae feed on the mesophyll tissue, forming irregular leaf mines which may later become necrotic. Larvae also can form extensive galleries in the stems and attack fruits. Damage caused reduction of photosynthetic capacity and reduced plant growth and yields in both protected and open-field (Urbaneja *et al.* 2012).

One of the most successful microorganism used as a biopesticide is *Bacillus thuringiensis* (*Bt*), which is characterized by bearing a variety of plasmids encoding insecticidal proteins, the so-called δ-endotoxins (Hofte and Whiteley 1989). These proteins are expressed and assembled into parasporal crystalline inclusion bodies during the stationary phase of its growth cycle. The δ -endotoxins are natural insecticides for a number of agronomicaly important pests (Aronson *et al.* 1986). These toxins are considered to be environmentally safe and harmless to other life forms, including humans. Theoduloz *et al*. (2003) reported that expression of *B. thuringiensis* toxin in other *Bacillus* species that naturally colonize the phylloplane of tomato plants showed that these plant-associated microorganisms could be useful as a delivery system of toxins from *B. thuringiensis*, which would allow a reduction in pesticide applications.

Furthermore, Cabrera *et al.* (2011) postulated that the damage of *T. absoluta* can be greatly reduced by spraying only *B. thuringiensis* with no need for chemical insecticides.

Pulsed field gel electrophoresis (PFGE) has been widely used for the molecular typing of bacteria because it is a very powerful technique in differentiating microorganisms below the species level (Rivera *et al*. 2003), and for the analysis of microbial genomes (Carlson and kolsto 1993). Pulsed-field gel electrophoresis (PFGE) is a sensitive method providing valuable information on the genetic diversity and relationships between closely related microorganisms. The technique generates reproducible and highly discriminatory DNA fingerprints and gives information about genome complexity and topology (Arakawa *et al.* 2000). It was therefore used to analyze the chromosomal DNA patterns of environmental *B. thuringiensis* strains. Studying chromosomal polymorphism with PFGE has been accomplished for a limited number of *Bacillus* species (Zahner *et al*. 1998). Furthermore, few reports are available on PFGE typing for *B. thuringiensis* only (Ankarloo *et al*. 2000). Pulsed-field gel electrophoresis can be used to analyze large DNA fragments of the bacterial chromosome with high resolution, high repeatability, good comparability, and is recognized as the technique of choice for bacterial typing (Johnson *et al.* 2007). PFGE typing can be used to evaluate the clonal relatedness among bacterial isolates and to investigate outliers (Tenover *et al* . 1995).

Plasmid curing is the process of obviating the plasmid encoded functions such as antibiotic resistance, virulence, degradation of aromatic compounds, etc. in bacteria. However, no plasmid curing agent can eliminate all plasmids from different hosts (Rajashree *et al.* 2018). Most of studies dealing

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with *B. thuringiensis* plasmid curing gave much importance to the lost plasmids and the genes they carry. Indeed, mutant strains cured of one or more plasmids are frequently no longer able to synthesize delta-endotoxins encoded by *cry* genes that are localized on high molecular weight plasmids (Gonzalez and Carlton 1984). In addition, plasmid curing converts the antibiotic resistant bacterial cells into sensitive ones (Molnar 1988). Thus, this study aimed to evaluate genome shortening in the hydrolysis of chitin , as well as , the role of *Bt* in biological control of *Tuta absoluta* in the open – field of tomato plots.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacterial strains and their transconjugants used in this study are listed in Tables (1 and 2).

Table 1. Bacterial strains and their sources .

Strains	Source or reference	Designation
Serratia marcescens	Microbial Activity Unit, Microbiology Dept., Soil, Water and Environmental Research Institute, Agricultural Research Center (ARC).	*Sm*
Bacillus licheniforms	National Center for Agriculture Utilization Research, USA.	*BL*
Bacillus thuringiensis 4A7	Daniel R. Zeigler, Ph.D., Bacillus Genetic Stock Center, Biochemistry Dept., Ohio University, Columbus, USA.	Bt_1
Bacillus thuringiensis 4Q1	Daniel R. Zeigler, Ph.D. Bacillus Genetics Stock Center, Biochemistry Dept., Ohio University, Columbus, USA.	Bt_2

Table 2 . Transconjugants used in this study.

Transconjugant designation	Genotype	Source or reference
Tr_1 Tr_2	Ap^+ $Eryth^+$	*Sm X* Bt_1
Tr_3 Tr_4	Ce^+ $Eryth^+$	*Sm X* Bt_2
Tr_5 Tr_6	Cf^+ Gm^+	*BL X* Bt_1
Tr_7 Tr_8	Cf^+ Gm^+	*BL X* Bt_2

Bacterial culture conditions

Luria broth medium (LB broth) was used for *Bacillus thuringiensis and Bacillus licheniforms* according to Sambrook *et al*. (1989). However, peptone glycerol medium (PGM) was used for the maintenance of *Serratia marcescens* strain according to Harris *et al*. (2004). Meanwhile, M9 minimal medium was used for preparing isolates and strains for electrophoresis (Sambrook *et al*. 1989).

Plant material

Tomato seeds were obtained from Seeds Technology Department, Sakha Agricultural Research Station, Agricultural Research Center , Giza, Egypt. The seeds were germinated as described by Asaka and Shoda (1996). After seven weeks the seedlings were transferedin june 2015 to the open field .

Plasmid curing

To determine the resistance to antibiotic is encoded by a plasmid or chromosomal genes, elevated temperature treatment at 45° C was applied. Cultures were inoculated into LB broth medium and subjected to elevated temperatures for 3 h and then plated on LB medium. Plates were incubated at 30° C for three days. Single colonies appeared were picked up and rechecked for the same antibiotic resistance pattern to ensure the occurrence of stability of resistance (Bastos *et al.* 1980).

Isolation of genomic DNA

Genomic DNA was extracted from bacterial strains and their transconjugants grown in 100 ml minimal medium at 30°C overnight and genetically analysized using 1.0 % agarose by agarose gel electrophoresis . Agarose was supplemented with 0.5 µg/ml ethidium bromide. Furthermore, five ml of the extracted DNA was loaded in agarose gel according to Barzegari *et al.* (2010) . After the electrophoresis was completed the gel was shown via Gel Documentation System under UV light according to Atashpaz *et al.* (2010)

Bioassay technique

This experiment was conducted on tomato plants growing in the open field during the summer seasone of 2015. One plant was grown in each plastic pot located in the experimental farm of Microbiology Laboratory , Sakha Research Station , Agriculture research Center, Kafr El-Sheikh Governorate. Nine treatments including control were distributed in a randomized complete block design with four replications. Endospores and crystals suspension of eight bioinsecticides were prepared with concentration of 20×10^6 spores / ml and sprayed on plants once weekly. Plants were infected with six days old larvae (mean body weight = 0.0039 mg) of *Tuta absoluta* larvae (ten larvae / replication). Plants were first sprayed with bioinsecticides after 48 hours of larval infection . Larvae were allowed to feed on treated leaves for four weeks through June and July of 2015. Plants from each replications were removed , weighted, as well as, chlorophyll concentrations was estimated after 45 days of infection with *Tuta absoluta* larvae.

Photosynthetic pigments

Chlorophyll contents (a, b and total chlorophyll) in tomato leaves were extracted in 80% methanol. The pigments were determined spectrophotometrically after storing the extracted solution for twenty four hours in a refrigerator according to Lichtenthaler and Wellburn (1983).

Statistical analysis

Data were subjected to the analysis of variance according to Snedecor and Cochran (1955). Least significant difference (L.S.D.) was used to compare between means.

RESULTS AND DISCUSSION

Plasmid curing

The relatively large genomes of *Bacillus thuringiensis* , *Bacillus licheniformis* and *Serratia* were shortened by provoking plasmid curing by raising temperature which affected the gene expression of both chromosomal and plasmid harboring genes as shown in Table (3). It was evident that the extrachromosomal genetic material shortening from Bt_1, Bt_2, Tr_3, Tr_4, Tr_7 and Tr_8 resulted in the loss of the antibiotic markers of resistance genes and chitin hydrolysis as a consequence. Indeed , it was evident that plasmid loss was a significant tool to obtain mutant strains with different expression of the genomic genes. Gonzalez *et al.* (1981) found that plasmid cured microorganism could lost several characteristics such as metals resistance, resistance and production of bacteriocins and synthesis of delta-endotoxins. Although complete curing was drastic for plasmid - harbouring gene expression . In fact, the genome of *Bt* was higher with about 2.4 – 5.7 millions base pairs, as well as, supported by extra chromosomal genetic material (Carlson *et al.* 1994) . These results agreed with Li *et*

al. (2000), who found that the parental strains of *B. thuringiensis* treated with elevating temperature to 42 ° C showed a crystalliferous mutants (cry$^-$).

Table 3. Effect of high temperaturs on plasmid curing .

Bacterial strains	Antibiotic resistance at 28°C	Antibiotic resistance at 40° C	Chitin analysis before plasmid curing	Chitin analysis after plasmid curing
Sm	*Eryth*$^+$	*Eryth*$^+$	1.7	1.7
Bt_1	*Ap*$^+$ *Gm*$^+$	*Ap*$^-$ *Gm*$^-$	2.3	0.0
Bt_2	*Ce*$^+$ *Gm*$^+$	*Ce*$^-$ *Gm*$^-$	1.9	0.0
Bl	*Cf*$^+$	*Cf*$^-$	2.0	2.1
Tr_1	*Ap*$^+$ *Eryth*$^+$	*Ap*$^-$ *Eryth*$^-$	1.7	1.8
Tr_2	*Ap*$^+$ *Eryth*$^+$	*Ap*$^+$ *Eryth*$^+$	1.6	1.6
Tr_3	*Ce*$^+$ *Eryth*$^+$	*Ce*$^-$ *Eryth*$^-$	4.0	0.0
Tr_4	*Ce*$^+$ *Eryth*$^+$	*Ce*$^-$ *Eryth*$^-$	3.2	0.0
Tr_5	*Cf*$^+$ *Gm*$^+$	*Cf*$^+$ *Gm*$^+$	2.1	2.1
Tr_6	*Cf*$^+$ *Gm*$^+$	*Cf*$^+$ *Gm*$^+$	2.0	2.0
Tr_7	*Cf*$^+$ *Gm*$^+$	*Cf*$^-$ *Gm*$^-$	2.9	0.0
Tr_8	*Cf*$^+$ *Gm*$^+$	*Cf*$^-$ *Gm*$^-$	2.1	0.0

In this study, some transconjugants such as Tr_2,Tr_5 and Tr_6 were not affected by elevated temperature because of their stability for antibiotic resistance markers and encoded chitinase genes that may be localized at high molecular weight extrachromosomal DNA. Similarly, kamoun *et al.* (2009) found that *Bt* bacteriocin – encoding genes were a plasmid borne. However, the genome of *Serratia marcescens* was not shortened as it was still harboring *Eryth* resistance gene. The extrachromosomal genome of *Serratia* was still express chitin hydrolysis, this indicated that the extrachromosomal genome of *Serratia* was still stable at 40°C. Gonzalez and Carlton (1980) reported that *Bt* strains containing a set of plasmids varying in number from 1 to 11. Therefore, treated *Serratia* showed chitin hydrolysis as that of the wild type strain . It revealed that heat shock did have affect on chitinase expression .

Moreover, *Bacillus licheniformis* lost *Cf* resistance gene indicating that the extrachromosomal DNA was lost but it was stable for chitin hydrolysis . Transcojugant Tr_1, showed the same trend in relation to the loss of *Ap* and *Eryth* resistance genes and still express chitinase genes as in the wild type strain. In this case chitinase genes may be chromosomally located. This was in harmony with Driss *et al.* (2011), who noticed that complete cured *Bt* strains showed higher chitinolytic activity than the wild type or shortening cured ones . In addition, Jonathan *et al.* (1986) analysed clones from cosmid DNA library which showed that *Serratia* chromosome contains at least two chitinase genes, *chiA* and *chiB* encoding secreted forms of chitinase .

These results reflected that complete elimination of plasmid in future experiments was necessary to ensure obtaining chitinase - clearing phenotypes whether it was chromosomal or plasmid harboring. Although several microoranisms having plasmids that can naturally lose most of unstable ones because of environmental changes leading to altered metabolic properties (Aronson 1993). Genome shortened is the artificial method used by geneticists to obtain bacteria losing genetic material, mainly plasmid (Gonzalez *et al.* 1981) .

The appearance of chearing zones due to chitin hydrolysis by plasmid cured strains such as *Bl* and Tr_1 showed that there was an acquisition of chitinase synthesis . These result demonstrated that the loss of plasmid was in favour of the expression of chromosomal genes. This may be due to the loss of part DNA which makes the microorganism save the energy that will be required for the expression of other genes. Therefore, the use of totally plasmid – cured strains was important to show the express of chromosomal genes, as well as , the use of partially – cured ones to show the expression of plasmid- carried genes. This strategy was practical to show the expression of chromosomal genes like *chi* genes while *cry* genes were plasmid located (Driss *et al.* 2011) . All of these observations use antibiotic resistance genes to mark cured strains. Therefore genome – shortening technique would be promising alternative to obtain mutant strains for testing plasmid – harboured gene expression.

DNA profiles of *Bt* transconjugants

PFGE was used in this study to analyze the chromosomal DNA patterns of *Serratia, Bacillus lichenformis* , *Bacillus thuringiensis* and their transcojugants, in addition to investigate the clonal relatedness between *Bt* transconjugants with their parental strains to demonstrate the relationship between genotypes with their outbreaks of *Bt* toxicity. The donar strains including *Serratia marcescens* and *Bacillus lichenformis* yielded profiles differed from each other Figure (1). The number of DNA fragments observed in the DNA patterns varied between the donar stains in their size and density within a range of three bands which appeared to differ in their density and intensity.

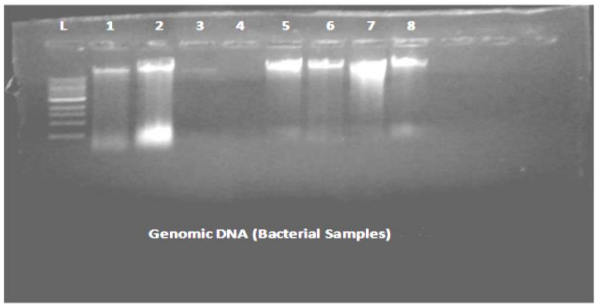

Fig. 1. PFGE analysis of total DNA on 1% agarose gel. Lanes 1–8 represent *Serratia marcescens* , *Bacillus licheniformis*, Bt_1, Bt_2, Tr_1, Tr_2, Tr_3 and Tr_4 , respectively.

The two donar strains showed less similarity in the number of bands . These indicated that both donar strains are genomically diverse species and the DNA patterns providing additional evidence of heterogeneity of both strains. The patterns of these strains reflected that they have not the same clonal origin.

The two recipient strains of *Bt* were genetically dissimilar with each other and with the donar strains in their intensity and the density of the bands in the donar strains showed different patterns from the recipient ones. This agreed with Benintende *et al.* (1996) , who found that *Bt* is an ecologically, phenotypically and genomically diverse species. In this study DNA patterns submit additional evidence of genetic diversity between the donar and recipient strains. The patterns of *Bt* transconjugants generated more number of bands than either one of the parental strains. This could be attributed to *Bt* transconjugants aquisted and generates a higher number of DNA fragments and gave more informative patterns than that of the parental strains.

On the other hand, close similarities were noted among transconjugants. Therefore, genetic conjugation can lead to genomic changes between transconjugants and their parents .These phenomenon is generally described as genetic plasticity as *Bt* transconjugants aquired DNA from both parents leading them to genome diversity with the parental strains (Kolsto 1997). Meanwhile, the genome of *Bt*

transconjugants may be slightly higher in relation to their parents which appeared a smaller number and lower intensity of bands. Interestingly, *Bt* transconjugants may differ significantly in the sizes of their genomes. This suggested that the population structure of *Bt* transconjugants was similar with the donar strains. Nevertheless, some diversity between the clones of *Bt* transconjugants was found. PEGE offers a clear differentiation of *Bt* and their transconjugants harboring DNA from other sources through a conjugation induced in the laboratory, which is not common in the environment.

It is evident that PEGE was a useful technique for use in distinguishing *Bt* spread and genotypic variability in the environment. This technique was found to be discriminatory and reproducible method for molecular studies of bacterial isolates. It facilitates the evaluation of the clonal relationships of *Bt* strains and the identification of the common sources of outliers.

The PFGE patterns showed two to four band varied which possibly leading to single genetic event . The larger DNA fragments were widely separated and clearly shown in donar strains, as well as, their transconjugants. However smaller DNA fragments did not show clear bands as shown in *Bt* recipient strains used in this study. However, the resolution of the smaller genomic fragments of *Bt* recipient strains is still less than that of the donar and their transconjugants. This may attributed to frequent cutting of the DNA in the smaller fragments which may lead to difficulty of interpretation. Additional restriction enzymes will be required to validate discriminatory power and reproducibility of DNA patterns. Rivera and Priest (2003) found correlation between PFGE type and *cry* gene composition using PCR primers designed specificity toward *cry* genes. The *cry* genes are generally plasmid - borne and the domain shuffling within cry proteins by recombination of *cry* genes from different sources is the route to the vast range of extant toxins (De Maagd *et al*. 2001).

In this study the toxicity index ranged between 0.18 (control) to 0.66 (Tr_8 - presented in lane 8) . Meanwhile, feeding deterrence index (FDI) ranged between 0.0 (control) to 73 % (*Bl*) (Data not shown herein) . This suggested that *Bt* strains and their transconjugants were genomically diverse genotypes . In the present study the DNA patterns for two transconjugants in the lane number 6 (Tr_4) and number 8 (Tr_8) providing additional evidence than other transconjugants. These transconjugants providing higher toxicity index in relation to the mid - parents, as well as, in relation to the other transconjugants derived from other crosses. The additional band evidence in both transconjugants may reflected the increase in the value of genome size than their parents. The relation between toxicity index correlated with *cry* gene content and DNA patterns suggested that successful combinations of chromosomal host background and *cry* gene complement was emerged (Rivera and Priest 2003). For example , a new cry protein emerges by recombination of plasmid - borne *cry* genes. If that noval gene resides in the bacterial transconjugants it may lead to some physiological and maximum toxicity and become superior entomopathogen.

Bioassy of toxicity in the field

Tomato plants were infested with many pests. *Tuta absoluta* was one of the most serious pests of tomato in Egypt. This pest management in Egypt has been based on chemical application. Microbial insecticides offer an alternative tool to chemical insecticides as environmentally safe strategy with increased specificity which is being used recently in integrated pest management programmes. *Bacillus thuringiensis* may be a good alternative, as they have been used to control other insect pests successfully (Pena and Schaffer, 1997).

Photosynthetic efficiency depends on plant chlorophyll content (Engel and Poggiani 1991), as well as, the damage caused by insects can alter the chlorophyll content of plants (Cardenas and Gallardo 2016). The results presented in Table (4) showed significant increase in total chlorophyll in the plants treated with most bioinsecticide strains in relation to positive control (artificially infected with the larval stage of *Tuta absoluta*) and chemical insecticide (Pestban). The results indicated a growth inhibitory effects of bio- insecticides on the larval stage of *T. absoluta*. These results agreed partially with Gonzalez-Cabrera *et al*. (2011) who reported that *B. thuringiensis* is highly efficient in controlling *T. absoluta*. Giustolin *et al*. (2001) found that *B. thuringiensis* var. *kurstaki* (*Btk*) can cause mortality in all *T. absoluta* instars and the use of *Bt* has a synergistic effect or additive effects when applied to tomato tolerant genotypes. Furthermore, Niedmann and Meza-Basso (2006) performed bioassay screens of native *B. thuringiensis* strains from Chile and found that two strains were more toxic against *T. absoluta* than the strain isolated from the formulate Dipel (Abbott Laboratories, Chicago, IL, USA). Hafsi *et al*. (2012) decided that *B. thuringiensis* had an impact on *T. absoluta* and could be used instead of synthetic insecticides. Therefore, the integration of *B. thuringiensis* may provide a safe strategy to manage this pest, as it kills larvae and the eggs (Urbaneja *et al*. 2009).

Table 4. Effect of *Bt* formulations on some traits of plants infected by *Tuta absoluta*.

Treatment	Shoot dry weight	Chlorophyll concentration (mg/g)		
		Chl.a	Chl.b	Total Chl
Control (-)	3.27	4.2	2.1	6.3
Control (+)	3.37	1.1	5.0	6.1
Pestban	5.13	11.2	3.8	14.9
Sm	3.60	8.0	2.6	10.6
Bt_1	6.47	5.6	1.0	6.6
Tr_1	4.87	5.95	7.58	13.53
Tr_2	4.57	9.04	2.65	11.69
Bt_2	4.20	5.54	7.08	12.63
Tr_3	4.03	13.65	2.57	16.22
Tr_4	5.10	7.87	3.99	11.86
BL	4.60	5.64	5.04	10.68
Tr_5	4.93	5.46	3.43	8.89
Tr_6	3.73	3.18	3.96	7.14
Tr_7	4.57	5.28	2.49	7.77
Tr_8	4.37	7.49	3.12	10.61
F-test	*	**	IS	*
LSD 0.05	1.27	4.10	-	5.14
LSD 0.01	1.85	5.96	-	7.47

***,** = Significant at 0.05 and 0.01 probability levels, respectively.**
IS=Insignificant differences.
Control (-)= Plants without artificially infected, Control (+) =Plants artificially infected with the larval stage, Pestban 48%EC (chemical insecticide).

The data of shoot dry weight revealed that Bt_1 recorded significant increase in shoot dry weight at 45 days plant-old above the negative control, as well as, the positive control and chemical insecticide. On the other hand. transconjugants Tr_1, Tr_2 and Tr_4 showed significant increase in shoot dry weight above the negative and the positive controls at 45 days plant- old . These results are in partial agreement with Bandopadhyay (2015), who found that *Bacillus thuringiensis* increased the root length, shoot height, leaf area, fruit weight, fresh weight and dry weight of *Amaranthus viridis*, *Capsicum annuum*, *Abelmoschus esculentus* and *Ocimum tenuiflorum* in pots condition.

This agreed with the theoretical models of Roush (1998), who suggested that pyramiding two dissimilar toxin genes in the same transconjugant or in the same transgenic

plants has the potential to delay the onest of resistance much more effectively than single – toxin gene. In addition , Khan *et al*. (2013) found that incorporation of *cry1Ab* insecticidal crystal protein gene in large number of crop plants particularly rice, tomato, maize, sugarcane and cotton have shown considerable protection against different lepidopteran insects and significant enhancement in productivity .

In conclusion , genome shortening allows some *Bt* isolates to lose chitinase expression while some other isolates were still stable for chitinase expression. *Bt* transconjugants showed a close similarity on the molecular level but they were differ from their parents. PEGE offers a clear differentiation between transconjugants and their parents. Some transconjugants acquired additional bands providing higher toxicity index and high feeding deterrence index in relation to the mid -parents.

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الإختلافات الوراثية على مستوى الإتحادات الوراثية الجديدة من الباسيليس ثيرونجنسز المستخدمة فى المقاومة الحيوية لثاقبة الطماطم توتا أبسليوتا

ميرفت إبراهيم كمال[١]،على ماهر محمد العدل [١] ، خليفه عبد المقصود زايد[١] ، أحمد ابراهيم السيد [٢] و هبه حامد عطيه[٢]

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تهدف هذه الدراسة إلى توصيف المتحولات التزاوجية للباسيليس ثيرونجنسز على أساس التفريد الكهربى لأ نماط حزم المادة الوراثية والإستئصال البلازميدى وتقييم التأثير السمى ضد حشرة ثاقبة الطماطم توتا أبسليوتا على مستوى الحقل المفتوح . تم فى هذا البحث إستخدام ثمانية متحولات تزاوجية ناتجة عن أربع تهجينات مختلفة إستخدمت فيها السراتيا، الباسيليس ليشنفورمس كأباء معطية للمادة الوراثية بينما إستخدمت سلالات *Bt* كمستقبلات للمادة الوراثية .أدى إختزال حجم المادة الوراثية من البلازميدات إلى فقد بعض سلالات *Bt* والمتحولات التزاوجية لجينات المقاومة للمضادات الحيوية هذا بالإضافة إلى فقدها لقدرتها على تحليل الشيتين. وعلى النقيض من ذلك، فإن بعض المتحولات التزاوجية لل *Bt* لم تتأثر بدرجة الحرارة المرتفعة حيث ظل بها التعبير الجينى ثابت بالنسبة لصفات المقاومة للمضادات الحيوية وإنتاج إنزيم الشيتينيز. كان جينوم السراتيا كان أكثر ثباتا من الناحية الوراثية عند درجات الحرارة المرتفعة بالنسبة لجينوم الباسيليس ليشنفورمس والتى فقدت بدورها جين المقاومة للمضاد الحيوى Cefotaxime . أوضحت نتائج الفصل الكهربى للمادة الوراثية إختلاف جينوم سلالات *Bt* والمتحولات التزاوجية الناتجة عنها بسبب ظهور وإختفاء بعض حزم المادة الوراثية بين السلالات بعضها البعض . أظهرت إثنين من المتحولات التزاوجية الثمانية حزم إضافية بالمقارنة بباقى العزلات وقد اعطت نفس هذه المتحولات دليل سمية مرتفع ضد يرقات ثاقبة الطماطم بالمقارنة بباقى المتحولات الوراثية الأخرى وبمتوسط الآباء. نتج عن النباتات المعاملة بالمبيد الحيوى زيادة معنوية فى تركيز الكلورفيل فى الأوراق .هذا بالإضافة إلى أن بعض المتحولات التزاوجية لل *Bt* قد أنتجت زيادة معنوية فى الوزن الجاف لنباتات الطماطم بالمقارنة بالنباتات فى تجربة المقارنة السالبة والموجبة. تعكس النتائج المتحصل عليها من هذه الدراسة ان عملية خلط الجينات المنتجة للمادة البروتينية السامة مع تلك المنتجة لإنزيم الشيتينيز فى نفس التركيب الوراثى سوف يؤدى إلى زيادة السمية وسيؤخر من صفة المقاومة فى الآفة الحشرية المستهدفة من المبيد الحيوى وذلك بالمقارنة بتأثير جين واحد يتعلق بالسمية فى نفس السلالة.