Evaluation of Potato Tuber Moth (Lepidoptera: Gelechiidae) Resistance in Tubers of *Bt-cry5* Transgenic Potato Lines

AHMED MOHAMMED,[1] D. S. DOUCHES,[2] W. PETT,[3] E. GRAFIUS,[3] J. COOMBS,[2] LISWIDOWATI,[2] W. LI,[2] AND M. A. MADKOUR[1]

J. Econ. Entomol. 93(2): 472–476 (2000)

ABSTRACT The potato tuber moth, *Phthorimaea operculella* (Zeller), in tropical and subtropical countries, is the most destructive pest of potato, *Solanum tuberosum* L. The larvae attack foliage and tubers in the field and in storage. The purpose of this study was to evaluate the efficacy of a *Bt-cry5* transgene to control the potato tuber moth in tuber tissues. Tuber bioassays using stored (11-12 mo old) and newly harvested tubers of *Bt-cry5*-Lemhi Russet and *Bt-cry5*-Atlantic potato lines showed up to 100% mortality of 1st instars. Mortality was lowest in the newly harvested tubers of *Bt-cry5*-Atlantic lines (47.1–67.6%). Potato tuber moth mortality was 100% in the *Bt-cry5*-Spunta lines that were transformed with *Bt-cry5* gene controlled by the CaMV 35S promoter (pBIML5 vector) and in 2 of 3 lines transformed with *Bt-cry5* gene controlled by the Gelvin super promoter (pBIML1vector). The transgenic Spunta lines expressing *Bt-cry5* controlled by the patatin promoter (pBMIL2 vector) showed the lowest tuber moth mortality (25.6 and 31.1%). The *Bt-cry5* transgenic lines with high tuber expression of *B. thuringiensis* have value in an integrated pest management system to control potato tuber moth.

KEY WORDS *Phthorimaea operculella*, *Solanum tuberosum*, *Bacillus thuringiensis*, transgenic potato, genetic engineering

POTATO IS ONE of the most important vegetable crops in Egypt with total production up to 2 million metric tons annually. It is also the leading export vegetable in Egypt, with ≈225,000 metric tons exported to the United Kingdom and western European countries. Potato is cultivated in the Nile delta region on ≈72,000 ha, most in Behira, Menofya, and Garbiya, where yields range between 17.5 and 23.8 metric tons per hectare (Hasan 1991).

The primary insect pest of Egyptian potatoes, like many other countries in the Middle East, is the potato tuber moth, *Phthorimaea operculella* (Zeller). In the field, the moths lay their eggs on the potato foliage and the larvae mine the foliage and the stems. This feeding damage leads to irregular transparent tunnels in the leaves and weakening of the stem. Larvae attack the tubers through infected stems, or larvae hatch from eggs oviposited on exposed tubers in soil cracks and enter the tubers directly. Larvae mine the tubers in the field and in storage, reducing potato quality and increasing the potential for pathogen infection. Potato tuber moth may go through several generations in storage. In India, tuber damage in storage ranges from 25 to 100% (Nirula 1960; Saxena and Rizvi 1974) and tubers are 100% damaged in 1.5–2 mo in Tunisia (Essamet et al. 1988). In Sudan, ≈30–40% of the potatoes are stored in underground pits and can be completely destroyed by tuber moth within 2 mo (Ali 1993).

Bacillus thuringiensis kurstaki (Bt), an aerobic soil bacterium, produces an insecticidal crystal protein during sporulation (McGaughey and Whalon 1992, Barton et al. 1987). These insecticidal crystal proteins have to be ingested by the insect where they bind to specific receptors in the midgut of target pest species and cause death (Gill et al. 1992). These Bt Crystal proteins are nontoxic to humans, animals, birds, and most beneficial insects and have been used as biological insecticides to control agricultural insect pests for >30 yr (Koziel et al. 1993). The genes (*cry* genes) which encode Bt crystal proteins have been cloned, sequenced, and transformed into various crops and are now available commercially (Barton and Miller 1993).

Transformation of potato with a wild type *cryIA-Bt* toxin gene resulted in potato tuber moth mortality of only 20–60% (Hudy 1998). A *Bt-cry5* toxin gene, with activity against both Lepidoptera and Coleoptera (Tailor et al. 1992), was codon-modified to increase its expression level in the plant. Douches et al. (1998) transformed this gene into potato to obtain control of potato tuber moth. Detached-leaf bioassays with *Bt-cry5* potato lines demonstrated a high expression level of *Bt-cry5* protein in the leaves and up to 96% mortality of potato tuber moth with larvae in foliar assays (Westedt et al. 1998, Li et al. 1999).

W.L. (personal communication) produced a series of *Bt-cry5* gene constructs comparing promoters and transformed the cultivar Spunta. Detached leaf bioassays of these greenhouse-grown *Bt-cry5* transfor-

[1] Agricultural Genetic Engineering Research Institute, Giza, Egypt.
[2] Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824.
[3] Department of Entomology, Michigan State University, East Lansing, MI 48824.

mants revealed high tuber moth mortality levels. For these *Bt-cry5* transgenic lines to be a useful tool in an integrated pest management (IPM) program to reduce potato tuber moth damage, the tubers, as well as the foliage, must have some resistance. The objectives of this study were to evaluate potato tuber moth resistance in field-grown *Bt-cry5* transgenic potato tubers and stored transgenic tubers 11-12 mo old.

Materials and Methods

Potato Tuber Moth Rearing. A potato tuber moth population (obtained from University of California, Berkeley) has been maintained at Michigan State University since 1993. Field-collected potato tuber moth adults are added to the population biannually to prevent inbreeding depression. The culture was maintained at 25°C under a photoperiod 16:8 (L:D) h. Eggs were placed with whole tubers in a plastic container (29 by 23 cm, 9 cm deep) covered with cheesecloth. Corrugated cardboard stacks (5 by 5 cm each, 4 layers) were added to the container to serve as pupation chambers. The cardboard stacks, with pupae, were then transferred into a 3.8-liter glass jar and provided with 50% honey solution on a sponge for adult feeding. The jar was covered with nylon window screen (2-mm mesh), and a No.1 Whatman filter paper (Whatman, Hillsboro, OR) was placed on the screen for egg laying.

Plant Material. All *Bt-cry5* transgenic potato lines were developed at Michigan State University. All *Bt-cry5*- Lemhi Russet lines are previously described in Douches et al. (1998), and the *Bt-cry5*-Atlantic lines were developed in the laboratory (L., unpublished data). These lines contain the *Bt-cry5*/gus gene fusion (Table 1). The gene contructs used to produce the *Bt-cry5*-Spunta transgenic lines are described in Fig. 1.

Tubers for research were grown at the Michigan State University Montcalm Potato Research Farm, Entrican, MI. Tubers were planted in May (1997 for experiment 1, 1998 for experiment 2) and harvested in September, washed, graded, and placed in 4°C storage for 11-12 mo (experiment 1) or used immediately (experiment 2). For experiment 3, tissue culture plantlets were used to establish a field trial in 1998. These lines were transferred from tissue culture to the greenhouse. After 3 wk, the plantlets were transplanted to the field by hand in June. The tubers were harvested in September, washed, and a sample was taken for tuber moth bioassays.

Tuber Bioassay. Field-grown tubers from transgenic and nontransgenic control lines were assayed with potato tuber moth larvae. For each replication (4 per line), 1 tuber was placed in a Phytatray II box (Sigma, St. Louis, MO) with a vented lid for aeration. Neonate larvae were placed on the surface of each tuber (5 per tuber for tubers stored 11–12 mo, experiment 1; 10 per tuber for the newly harvested lines, Experiments 2 and 3). Tubers were kept in the dark at 25°C ± 2; the number of larvae and pupae were counted after 3 wk, and percentage mortality was calculated.

Table 1. Potato lines tested in potato tuber moth bioassay and their *Bt-cry5* gene constructs

Line	Construct[a]	Newly harvested	Stored[b]
Lemhi Russet	—	x	x
Lemhi Russet-Bt1	pSPUD12	x	x
Lemhi Russet-Bt7	pSPUD12	x	x
Lemhi Russet-Bt12	pSPUD12	x	x
Lemhi Russet-Bt14	pSPUD12	x	x
Lemhi Russet-Bt15	pSPUD12	x	x
Lemhi Russet-Bt21	pSPUD12	x	x
Lemhi Russet-Bt22	pSPUD12	x	x
Atlantic	—	x	x
Atlantic-Bt2	pSPUD12	x	x
Atlantic-Bt3	pSPUD12	x	x
Atlantic-Bt4	pSPUD12	x	x
Atlantic-Bt6	pSPUD12	x	x
Atlantic-Bt8	pSPUD12	x	x
Atlantic-Bt9	pSPUD12	x	x
Spunta	—	x	—
Spunta-P2	pSPUD2	x	—
Spunta-P6	pSPUD2	x	—
Spunta-G2	pSPUD5	x	—
Spunta-G3	pSPUD5	x	—
Spunta-G4	pSPUD5	x	—
Spunta-S1	pSPUD1	x	—
Spunta-S4	pSPUD1	x	—

[a] *Bt-cry5* vector constructs are described in Fig. 1.
[b] Tuber stored at 4°C for 11–12 mo.

Statistical Analysis. Arcsine transformations were performed on all percentage mortality data before analysis. Single-factor analyses of variance (ANOVAs) were conducted on data from each of the 3 experiments; however in experiments 1 and 2, the Lemhi Russet and Atlantic transgenic lines were analyzed separately. Two-factor ANOVAs were conducted to test the effect of stored tubers in experiment 1 versus newly harvested tubers in experiment 2 for both the Lemhi Russet and Atlantic transgenic lines. Mean comparisons were done using the Fisher least significant difference test at = 0.05. The general linear models procedure in SAS for Windows version 6.12 was used for statistical analysis (SAS Institute 1989).

Results

In experiment 1, potato tuber moth larval mortality on tubers 11-12 mo old ranged from 5.3 to 100% (Table 2). Tubers from all *Bt-cry5*-Lemhi Russet lines had significantly greater mortality (39–100%) than nontransgenic Lemhi Russet (5.3%). Although Lemhi Russet Bt-15 had only 39% mortality; larval moth development was severely delayed compared with larvae on the nontransgenic control tubers. These mortality data suggest that the tuber Bt concentration is not equal among the lines, and the tuber Bt concentration of Lemhi Russet Bt-15 is lower than that of the other lines. Nontransgenic Atlantic had only 11.3% potato tuber moth mortality, whereas mortality on (*Bt-cry5*-Atlantic lines ranged from 98.7 to 100% mortality (Table 2).

In experiment 2, mortality on newly harvested tubers ranged from 93.8 to 100% for the *Bt-cry5*-Lemhi

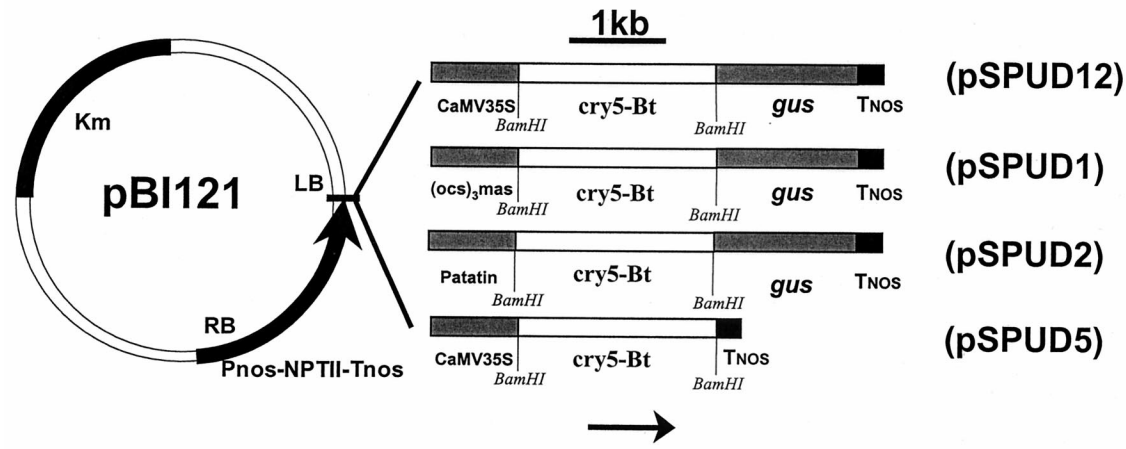

Fig. 1. Three plant transformation vectors containing cry5-Bt under the expression of different promoters: CaMV 35S promoter, $(ocs)_3$mas Gelvin-super promoter, patatin (tuber specific promoter). A 4th vector, pSPUD5, is the same as pSPUD12 except that the gus gene has been removed. The base vector is the binary pBI121 plasmid.

Russet lines (Table 2). In general, tuber moth mortality on newly harvested Lemhi Russet lines was similar to mortality on stored tubers except for Lemhi Russet Bt-15 (93.8% on newly harvested tubers compared with 39% mortality on the stored tubers). Potato tuber moth mortality in the *Bt-cry5*-Atlantic lines ranged from 47.1 to 67.6%, whereas mortality on nontransgenic Atlantic tubers was 19.5%. In all cases, tuber moth mortality in the *Bt-cry5*-Atlantic lines from fresh tubers was lower than tuber moth mortality on the stored tubers.

In experiment 3, larval mortality on newly harvested *Bt-cry5*-Spunta tubers ranged from 25.6 to 100% compared with 6.2% mortality on the nontransgenic Spunta tubers (Table 3). Mortality on the 2 lines with the constitutive Gelvin super promoter (S1 and S4) was 100%, mortality on the 3 lines with the constitutive 35S promoter (G2, G3, and G4) was 66.9–100%, whereas mortality on the 2 *Bt-cry5* lines with the patatin promoter were least effective (25.6 and 31.1% mortality). Although the patatin promoter is tuber specific, the tuber Bt concentration in these lines were the lowest found in this study.

Table 2. Percentage mortality of potato tuber moth neonate larvae on stored or newly harvested tubers of nontransformed and *Bt-cry5*-transgenic potatoes of culivors Lemhi Russet and Atlantic

Line	% mortality		
	Stored[a]	Newly harvested[b]	*P* value $\mu_s = \mu_n$
Lemhi Russet	5.3	5.7	NS
Lemhi Russet-Bt-1	93.8	100	0.03
Lemhi Russet-Bt-7	100	100	NS
Lemhi Russet-Bt-12	98.7	100	NS
Lemhi Russet-Bt-14	100	100	NS
Lemhi Russet-Bt-15	39	93.8	0.0001
Lemhi Russet-Bt-21	100	100	NS
Lemhi Russet-Bt-22	100	100	NS
$LSD_{0.05}$	7.6	3.5	
Atlantic	11.3	19.5	NS
Atlantic-Bt-2	100	66.4	0.0001
Atlantic-Bt-3	100	52.5	0.0001
Atlantic-Bt-4	98.7	52.9	0.0001
Atlantic-Bt-6	98.7	52.5	0.0001
Atlantic-Bt-8	100	67.6	0.0001
Atlantic-Bt-9	100	47.1	0.0001
$LSD_{0.05}$	7.9	7.2	

[a] Tuber stored at 4°C for 11–12 mo (experiment 1).
[b] Experiment 2.

Discussion

The mortality from the *Bt-cry5*-Lemhi Russet lines for both stored and newly harvested tubers is generally consistent with results of detached leaf bioassays on the same lines (Westedt et al. 1998). However, the transgenic tubers caused a higher mortality among larvae than caused by detached leaves. *Bt-cry5* expression, driven by the CaMV 35S promoter, may be higher in the tubers than in leaves or that feeding on tuber tissue may be more challenging to the larvae than feeding on leaf tissue, or a combination of both factors. Larval mortality was higher in tubers because of bioassay duration. The foliar assays were a 48- to 72-h period compared with the 3-wk tuber assay. Li et al. (1999) observed that many larvae that survived a 72-h detached-leaf test displayed reduced development and died after a few additional days of feeding.

Table 3. Percentage mortality of potato tuber moth neonate larvae in tuber bioassay with newly harvested tubers

Line	% mortality
Spunta	6.2
Spunta-P2	25.6
Spunta-P6	31.1
Spunta-G2	100
Spunta-G3	100
Spunta-G4	66.9
Spunta-S1	100
Spunta-S4	100
$LSD_{0.05}$	6.1

The results from stored Lemhi Russet and Atlantic tubers indicate that the *Bt-cry5* in the tuber tissues is still effective against larval feeding after 12 mo storage. These results increase the value of the *Bt-cry5* transgenic tubers in reducing potato tuber moth damage. In the field, tubers are infected by the larvae before harvest, but the most significant damage of the tubers occurs during storage through infection by moths emerged from infested tubers (Ali 1993). Only on Lemhi Russet-Bt -1 and Lemhi Russet-Bt-15 was there less mortality on the stored tubers than on newly harvested ones (Table 2); however, survivors were observed to be severely restricted in growth and development (data not shown). It may be that in these 2 lines that the tuber Bt protein concentration in the stored potatoes dropped below a concentration threshold that is necessary for tuber moth mortality.

The potato tuber moth mortality results in *Bt- cry5*-Atlantic lines are problematic. Higher mortality was observed among larvae that were assayed on the stored tubers of the *Bt-cry5* lines than on the newly harvested *Bt-cry5*-Atlantic tubers. Mortalities observed with the fresh tubers parallel results observed in field trials and from nawalla (traditional masonry storage buildings) storage experiments in Egypt (unpublished data). The Bt levels in the Atlantic tubers may be marginal for control of the larvae. Thus, small changes in Bt concentration (e.g., increased concentration because of tuber drying) or a small increase in feeding rate could increase mortality.

The class I patatin element (Wenzler et al. 1989) was used to develop a *Bt-cry5* construct with expression targeted to the tuber. Use of this promoter to express Bt mainly in tubers was not successful; only 25.6 and 31.1% mortality were observed in the 2 transgenic lines with the patatin promoter. W.L. (personal communication), in detached-leaf bioassays, observed no control of potato tuber moth as expected. This result is consistent with analyses of greenhouse tubers by W.L. (personal communication) where only a faint transcription signal was detected via northern analysis of the tubers from these lines. Low expression of the *Bt* gene may be caused by the specific transformation event or to the *Bt-cry5*/*gus* gene fusion (Westedt et al. 1998). We will be conducting additional transformations with a patatin/*Bt-cry5* construct in the future to test whether greater levels of Bt protein can be generated in the tuber with the patatin promoter.

Our results showed a difference in mortality between Spunta lines transformed with 35S/*Bt-cry5* gene construct. Potato tuber moth mortality in the Spunta G4 line was 66.9%, whereas the mortality was 100% in the other 2 lines (G2 and G3). The tuber results parallel the foliar results (W.L., personal communication). The Gelvin super promoter/*Bt-cry5*/*gus* construct was tested in 2 lines (S1 and S4); both lines had 100% potato tuber moth mortality. These results support a constitutive high efficiency of the Gelvin super promoter. Ni et al. (1995) reported that a super promoter is 156 times stronger than the 35S promoter in *Arabidopsis thaliana* L. Our results support the use of this promoter as a substitute for the CaMV 35S promoter for constitutive *Bt* gene expression.

In this study, we have shown that *Bt-cry5* expression can be obtained in the tubers at a high enough level to significantly control potato tuber moth. The results of these *Bt-cry5* potato lines demonstrate a higher mortality among potato tuber moth larvae than those caused by lines transformed with a codon-modified *Bt-cryIA* gene (Jansens et al. 1995). The Gelvin super promoter and CaMV 35S were both effective promoters. With the exception of the newly harvested tubers of *Bt-cry5*-Atlantic lines, the various *Bt-cry5* gene constructs were effective for the control of potato tuber moth within tuber tissues except with the pBIML2 construct (Class I patatin element). The expression of the *Bt- cry5* protein within the tuber tissues, the main target of potato tuber moth infestation, indicates that *Bt-cry5* lines can be an important tool in an IPM program.

Acknowledgments

This publication was made possible through support provided by the Office of USAID/CAIRO/AGR/A, under cooperative Agreement No. 263-0152 A-00-3036-00. The research also was supported by the Michigan Agriculture Experiment Station. A UNESCO Short-Term Fellowship in Biotechnology supported A.M. at Michigan State University.

References Cited

Ali, M. 1993. Effects of cultural practices on reducing field infestation of potato tuber moth (*Phthorimaea operculella*) and greening of tubers in the Sudan. J. Agric. Sci. Cambr. 121: 187–192.

Barton, K. A., and M. J. Miller. 1993. Production of *Bacillus thuringiensis* insecticidal proteins, pp. 297–315. *In* S. Kung and R. Wu [eds.], Transgenic plants, vol. 1. Engineering and utilization. Academic, San Diego, CA.

Barton, K. A., H. R. Whiteley, and N. Yang. 1987. *Bacillus thuringiensis*—endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. Plant. Physiol. 85: 1103–1109.

Douches, D. S., A. L. Westedt, K. Zarka, B. Schroeter, and E. J. Grafius. 1998. Transformation of potato (*Solanum tuberosum* L.) with the *Bt-cry5* transgene to combine natural and engineered resistance mechanisms to control tuber moth. Hortic. Sci. 33: 1053–1056.

Essemat, M., R. Von Arx, P. Ewell, J. Goueder, A. Bem Temine, and M. Cheikh. 1988. Aspects techniques et economiques du problem del a teigne et du stockage des pomnes de terre de sasion en Tunisia. Ann. Inst. Nat. Rech. Agron. Tunis 61: 1–50.

Gill, S. S., E. A. Cowles, and P. V. Pietrantonio. 1992. The mode of action of *Bacillius thuringiensis* endotoxins. Annu. Rev. Entomol. 37: 615–636.

Hasan, A. A. 1991. Potato. El-dar Alarabia Lilnashr we Eltawzea, Naser City, Cairo, Egypt.

Hudy, P. S. 1998. Analysis of engineered (*cryIA[c]-Bt*-transgenic) and natural resistance mechanisms in potato (*Solanum* spp.) for the control of potato tuber moth (*Phthorimaea operculella* Zeller). M.S. thesis, Michigan State University, East Lansing.

Jansens, S., M. Cornlissen, R. De Clercq, A. Reynaerts, and M. Peferoen. 1995. *Phthorimaea operculella* (Lepidoptera: Gelechiidae) resistance in potato by expression of the

Bacillus thuringiensis CryIA(b) insecticidal protein. J. Econ. Entomol. 88: 1469–1476.

Koziel, M. G., N. B. Carozzi, T. C. Currier, G. W. Warren, and S. V. Evola. 1993. The insecticidal crystal proteins of *Bacillus thuringiensis:* past, present and future uses. Biotechnol. Genet. Eng. Rev. 11: 171–228.

Li, W., K. Zarka, D. S. Douches, J. Coombs, W. Pett, and E. J. Grafius. 1999. Co-expression of potato PVY coat protein and cryV-Bt genes in potato. J. Am. Soc. Hortic. Sci. 123: 218–223.

McGaughey, W. H., and M. E. Whalon. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science (Wash. D.C.) 258:1594–1600.

Ni, M., D. Cui, J. Einstein, S. Narasimhulu, C. E. Verga, and S. B. Gelvin. 1995. Strength and tissue specificity of chimeric promoters derived from the octopine and mannopine synthase genes. Plant J. 7: 661–676.

Nirula, K. K. 1960. Control of *Phthorimaea operculella.* Indian Potato J. 2: 47–51.

Saxena, A. P., and S.M.A. Rizvi. 1974. Insect pest problems of potatoes in India. J. Indian Potato Assoc. 1: 45–50.

SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, NC.

Tailor, R., J. Tippett, G. Gibb, S. Rells, D. Pike, L. Jordan, and S. Ely. 1992. Identification and characterization of a novel *Bacillus thuringiensis* endotoxin entomocidal to coleopteran and lepidopteran larvae. Mol. Microbiol. 6: 1211–1217.

Wenzler, H. C., G. A. Mignery, L. M. Fisher, and W. D. Park. 1989. Analysis of a chimeric class-I patatin-GUS gene in tubers and sucrose-inducible expression in cultured leaf and stem explants. Plant Mol. Biol. 12: 41–50.

Westedt, A. L., D. S. Douches, W. Pett, and E. J. Grafius. 1998. Evaluation of natural and engineered resistance mechanisms in *Solanum tuberosum* for resistance to *Phthorimaea operculella* (Lepidoptera: Gelechiidae). J. Econ. Entomol. 91: 552–556.

Received for publication 11 May 1999; accepted 11 November 1999.