Field and Storage Testing *Bt* Potatoes for Resistance to Potato Tuberworm (Lepidoptera: Gelichiidae)

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ABSTRACT Potato tuberworm, *Phthorimaea operculella* (Zeller), is the most serious insect pest of potatoes worldwide. The introduction of the *Bacillus thuringiensis* (*Bt*) toxin gene through genetic engineering offers host plant resistance for the management of potato tuberworm. We report on the field and storage studies to evaluate *Bt-cry5* potato lines for resistance to potato tuberworm in Egypt under natural infestations and their agronomic performance in both Egypt and Michigan. From 1997 to 2001, field experiments were conducted at the International Potato Center (CIP) Research Station, Kafr El-Zyat, Egypt, and/or Agricultural Genetic Engineering Institute (AGERI), Giza, Egypt, to evaluate resistance to tuberworm. A total of 27 *Bt*-transgenic potato lines from six different Bt constructs were evaluated over a 5-yr period. After harvest and evaluation of the agronomic trials, storage evaluation of potato tuberworm damage was done at the CIP Research Station. The 1997 field trial was the first field test of genetically engineered crops in Egypt. Field tests to assess potato tuberworm resistance in Egypt were able to differentiate between the *Bt*-transgenic lines and the nontransgenic lines/cultivars in 1999, 2000, and 2001. The *Bt-cry5*-Spunta lines (Spunta-G2, Spunta-G3, and Spunta-6a3) were the most resistant lines in field with 99–100% of tubers free of damage. In the 2001 storage study, these lines were also over 90% free of tuberworm damage after 3 mo. NYL235–4.13, which combines glandular trichomes with the *Bt-cry5*/*gus* fusion construct, also had a high percentage of clean tubers in the field studies. In agronomic field trials in Michigan from 1997 to 2001, the *Bt*-transgenic lines in most instances performed similar to the nontransgenic line in the agronomic trials; however, in Egypt (1998–1999), the yields were less than one-half of those in Michigan. Expression of the *Bt-cry5* gene in the potato tuber and foliage will provide the seed producer and grower a tool in which to reduce potato tuberworm damage to the tuber crop in the field and storage.

KEY WORDS *Bacillus thuringiensis*, transgenic plant, *Solanum tuberosum*, *Phthorimaea operculella*

THE CULTIVATED POTATO, *Solanum tuberosum* L., is one of the world's most important food crops, following rice, wheat, and maize (Ross 1986). Potatoes are widely grown over many latitudes and elevations in over 130 countries. In Egypt, potatoes are grown on ≈80,000 ha per year, and ≈2.2 million tons are produced annually (Ali 1993). Three crops of potatoes are grown in Egypt per year (≈32,000–36,000 ha in summer and 20,000–24,000 ha each in fall and winter). Of this, ≈90% is consumed locally, and 10% is exported to Europe and other Arab countries (FAO 2002). Local consumption is important for subsistence and for nutritional balance, while export is an important source of foreign currency.

The potato tuberworm, *Phthorimaea operculella* (Zeller), is the most serious insect pest of potatoes worldwide. It probably originated in South America, where the potato also originates (Goldson and Emberson 1985). The potato tuberworm is of greatest importance in subtropical and tropical latitudes. Insecticide use is the most common means of potato tuberworm control in both field and storage. As many as 12–20 insecticide applications may be used in Egypt to control potato tuberworm and other insects during the spring growing season. Three to four insecticide sprays or direct applications are often applied in summer storage for potato tuberworm control (Madkour 1999).

The potato tuberworm attacks the potato by mining the leaves and tubers. Damage is caused only by the larval stage (Raman 1980, Goldson and Emberson 1985, Trivedi and Rajagopal 1992). Larvae tunnel in leaves, leaf veins, and stems of the plant. This damage causes loss of leaf tissue, death of growing points, and

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weakening or breakage of stems (Bald and Helson 1944, Raman 1980). In the field, the tubers may be infested by larvae from eggs deposited on the surface of the soil near the base of the plant, or moths may move down through cracks in the soil and oviposit directly on tubers. In storage, eggs are laid near the eyes of the tubers. The larvae mine into the tuber causing irregular tunnels both near the surface and deep inside the tuber, rendering them unfit for human consumption and susceptible to pathogens. The potato tuberworm is not strictly confined to potatoes. It may feed on numerous Solanaceae including tobacco (*Nicotiana tabacum* L.), tomatoes (*Lycospersicon esculentum* Mill.), and eggplants (*Solanum melongena* L.) (Goldson and Emberson 1985).

Bacillus thuringiensis (*Bt*) is an aerobic, gram-positive, soil bacterium that accumulates high levels of insecticidal crystal proteins during sporulation (McGaughey and Whalon 1992, Barton and Miller 1993). These crystalline protein inclusions, or δ-endotoxins, are the principle active ingredients in Bt pesticide formulations currently in use (McGaughey and Whalon 1992) to control insect pests. The advantage of Bt over conventional insecticides is target specificity. The original Bt insecticidal protein is specific for Lepidoptera and has no known detrimental effects on beneficial insects, mammals, or birds (McGaughey and Whalon 1992).

The introduction of the *Bt* toxin gene through genetic engineering offers host plant resistance for management of potato tuberworm. The major advantages to this delivery system are increased efficacy, reduced application costs, and minimal scouting needs (Lambert and Peferoen 1992) compared with a conventional insecticide spray strategy. Our transformations in potato with a codon-modified *Bt-cry5* gene (effective against both lepidopteran and coleopteran insects) have produced high levels of Bt expression, with 80–100% tuberworm mortality in detached leaf tests (Douches et al. 1998, Li et al. 1999). The development of this germplasm was supported under the Agricultural Biotechnology Support Project (ABSP; http://www.iia.msu.edu/absp), funded by the U.S. Agency for International Development (USAID). ABSP supports the use of biotechnology to develop crops with improved agronomic traits for developing countries, and in this case, collaborative research between MSU and the Agricultural Genetic Engineering Research Institute (AGERI) in Egypt.

We report on field and storage studies to evaluate *Bt* potato lines for resistance to the potato tuberworm in Egypt under natural infestations. We also report on the agronomic performance of these lines in both Egypt and Michigan.

Materials and Methods

The *Bt-cry5* gene constructs used to produce these lines are described in Table 1. All *Bt-cry5* and *Bt-cry1Ac1* transgenic potato lines were developed at Michigan State University. Table 1 lists the potato lines evaluated in these trials. All *Bt-cry5*-Lemhi Russet and *Bt-cry5*-NYL235–4 lines were previously described in Douches et al. (1998).

The *Bt-cry5*-Spunta transgenic lines are described in Li et al. (1999). The glandular trichome-based insect resistant line, NYL235–4 (Plaisted et al. 1992), was obtained from Cornell University.

Seed tubers for the field studies were grown at the Michigan State University Lake City Experiment Station, Lake City, MI, the seed production site for the MSU Potato Breeding project. All seed was either one or two generations removed from disease-free tissue culture-derived greenhouse tubers. Field-grown seed tubers were shipped to Egypt in January for the winter field trials. Trials were planted in late January to late February and harvested in June. The timing of the field trials coincides with maximum potato tuberworm infestation in Egypt. Both insect control and agronomic trials were conducted concurrently.

From 1997 to 2001, field experiments were conducted at the International Potato Center (CIP) Research Station, Kafr El-Zyat, Egypt, and/or AGERI, Giza, Egypt, to evaluate resistance to tuberworm. The AGERI field trial in 1997 was artificially infested with potato tuberworms, and the trial was enclosed in row covers to maintain insect pressure. At AGERI, two potato tuberworm–infected tubers were placed in each row, whereas at Kafr El-Zyat, natural infestation of potato tuberworm occurred. From 1997 to 2000, each trial had 10-plant (85.4 cm spacing) one-row plots that were replicated four times using a randomized complete block design. A row of a nontransgenic cultivar was planted between each plot. Normal agronomic practices were followed, except that no insecticides were applied. Row hilling was done before row closure by the potato vines. Flood irrigation was applied at least once per week up to vine senescence. At harvest, each tuber was visually examined for potato tuberworm damage, and the percent tubers free from tuberworm damage and larvae were recorded. In 2001, plots were eight rows of 20 plants each with a row of a susceptible cultivar between each plot. The plots were replicated three times using a randomized complete block design.

From 1999 to 2001, after harvest and evaluation of the agronomic trials in June, storage evaluation of potato tuberworm control was done at the CIP Research Station. In 1999 and 2000, at least 100 undamaged tubers from each line from the CIP field trials were placed in a traditional, above ground, nonrefrigerated potato storage building (Nawalla), which will store potatoes up to 3 mo before fall planting or marketing. In 2001, three replications of 100 tuber samples were placed in the Nawalla after harvest. The tubers were examined for tuberworm damage monthly for 3 mo.

Agronomic field trials were conducted at the Montcalm Research Farm, Entrican, MI, from 1997 to 2001, to measure total and marketable yields, tuber size distribution, and specific gravity, and to evaluate tuber appearance and incidence of external and internal defects. Trials were planted in May, arranged in a randomized complete block design with four replica-

Table 1. Potato lines tested in potato tuberworm and agronomic field trials and their *Bt*-gene constructs

Potato line	Construct name	Construct[a]	Parent clone
FL1607	None-NT[b]	None-NT	NA[c]
FLBT-11	pWB139Bt	*CaMV35S/cry1Ac1*	FL1607
FLBT-35	pWB139Bt	*CaMV35S/cry1Ac1*	FL1607
NYL235-4	None-NT	None-NT	NA
NYL235-4.13	PSPUD12	*CaMV35S/cry5/gus*	NYL235-4
'Lemhi Russet'	None-NT	None-NT	NA
LRBT-1	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
LRBT-7	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
LRBT-12	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
LRBT-14	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
LRBT-15	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
LRBT-21	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
LRBT-22	pSPUD12	*CaMV35S/cry5/gus*	'Lemhi Russet'
'Atlantic'	None-NT	None-NT	NA
ATBT-1	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-2	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-3	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-4	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-5	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-6	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-7	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-8	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
ATBT-9	pSPUD12	*CaMV35S/cry5/gus*	'Atlantic'
'Spunta'	None-NT	None-NT	NA
Spunta-P2	pSPUD2	*Class I Patatin/cry5/gus*	'Spunta'
Spunta-P6	pSPUD2	*Class I Patatin/cry5/gus*	'Spunta'
Spunta-G2	pSPUD5	*CaMV35S/cry5*	'Spunta'
Spunta-G3	pSPUD5	*CaMV35S/cry5*	'Spunta'
Spunta-G4	pSPUD5	*CaMV35S/cry5/gus*	'Spunta'
Spunta-S1	pSPUD1	*ocs_3mas/cry5/gus*	'Spunta'
Spunta-S4	pSPUD1	*ocs_3mas/cry5/gus*	'Spunta'
Spunta-6a3	pSPUD6	*CaMV35S/cry5//CaMV35S/PVY*	'Spunta'
'Nicola'	None-NT	None-NT	NA
'Diamant'	None-NT	None-NT	NA

[a] Transgene construct information: *CaMV35S:* 35S Cauliflower Mosaic Virus promoter; *ocs_3mas:* trimer octopine synthase and mannopine activator and promoter; *Class I Patatin:* tuber-specific promoter; *cry1Ac1* and *cry5: Bt-cry1Ac1* and *Bt-cry5* are lepidopteran and coleopteran-specific insecticidal crystal protein genes; *PVY:* confers resistance to potato virus Y; *gus: β galacturonidase* reporter gene.
[b] None-NT: No gene construct, nontransgenic potato line.
[c] NA: not applicable.

tions, and harvested in September. The plots were 7 m long with 31 cm spacing between plants in-row; between-row spacing was 86 cm. Normal agronomic practices were followed, and supplemental irrigation was applied as needed. Imidacloprid (Bayer, Kansas City, MO) was applied at the standard rates at planting to control Colorado potato beetle (*Leptinotarsa decemlineata* Say) and aphids. The soil type at the Montcalm Research Farm is a McBride sandy loam.

All data from Egypt field experiments, the Egypt Nawalla storage trial, and the Michigan agronomic performance trials were analyzed by analysis of variance (ANOVA) as a randomized complete block design using SAS general linear models procedure (SAS Institute 2000). All percent tuberworm-free tuber data were arcsine transformed before statistical analysis. Mean comparisons were done using Fisher least significant difference (LSD; $\alpha = 0.05$) for agronomic trials and Dunnet's test ($\alpha = 0.05$) for insect control trials.

Results

Potato Tuberworm Field Trials. From 1997 to 2000 at the AGERI, Egypt location, only the 1999 results showed differences in potato tuberworm tuber damage between the *Bt*-transgenic lines and the nontransgenic clones. In 1997, the tuber damage level was high, and only 8 and 2% of the 'Atlantic' and 'Lemhi Russet' tubers, respectively, were free of potato tuberworms. Some of the *Bt*-transgenic lines had less damage, but none were even 40% potato tuberworm–free. In 1999, the infestation levels were much lower than 1997, but differences were observed between some *Bt-cry5* lines. Of the 14 lines tested, ATBT-3, Spunta-G3, and Spunta-S1 were free of potato tuberworm mining. In the 2000 trial, no significant differences for percent tuberworm-free tubers was observed.

In 1998, at the CIP in Egypt, potato tuberworm infestation was very low, and no differences were observed between lines (data not shown). Tuberworm damage was high enough in 1999 to separate many of *Bt-cry5* transgenic lines from the susceptible check clones. Both 'Atlantic' and 'Spunta' had similar percentages of tuberworm-free tubers (Table 2). Many of the *Bt-cry5*-Spunta lines were significantly less damaged than 'Spunta'. Spunta-G2, Spunta-G3, Spunta-S1, and Spunta-S4 were free of potato tuberworm mining, while Spunta-P2 was 96% potato tuberworm–free. NYL235–4.13, a line that combines *Bt-cry5*

Table 2. Potato tuberworm field trial results from the International Potato Center (CIP) in Kafr-El-Zayat, Egypt

Potato line	1999		2000		2001	
	% Potato tuberworm-free	Total no. tubers	% Potato tuberworm-free	Total no. tubers	% Potato tuberworm-free	Total no. tubers
'Atlantic'	74	69	—	—	—	—
ATBT-2	85	72	95[a]	263	—	—
ATBT-3	92	78	—	—	—	—
ATBT-4	81	89	—	—	—	—
ATBT-5	—	—	98[a]	166	—	—
ATBT-6	87	102	—	—	—	—
ATBT-8	91	67	—	—	—	—
'Diamant'	—	—	79	317	—	—
NYL235-4	88	162	—	—	—	—
NYL235-4.13	100[a]	213	99[a]	309	—	—
'Spunta'	73	153	87	97	71	618
Spunta-6a3	—	—	100[a]	195	100[a]	1733
Spunta-G2	100[a]	155	100[a]	151	100[a]	1356
Spunta-G3	100[a]	189	100[a]	191	100[a]	2068
Spunta-G4	88	171	—	—	—	—
Spunta-P2	96[a]	197	96[a]	159	—	—
Spunta-P6	71	130	88	188	—	—
Spunta-S1	100[a]	165	100[a]	209	—	—
Spunta-S4	100[a]	135	100[a]	157	—	—

[a] Means within year are significantly different from corresponding nontransformed variety as determined by Dunnett's Test at $\alpha = 0.05$.

expression with glandular trichomes, was also free of potato tuberworm tuber damage.

In 2000, less potato tuberworm infestation was observed than 1999. However, significant differences in percent tuberworm-free tubers were observed overall, and 'Spunta' had 87% potato tuberworm–free tubers. Similar to 1999, the *Bt-cry5*-Spunta lines, Spunta-G2, Spunta-G3, Spunta-S1, Spunta-S4, and Spunta-6a3 were free of potato tuberworm mining (Table 2). The only *Bt-cry5*-line that was not better than 'Spunta' was Spunta-P6, similar to the results in 1999. In 2002, all three *Bt-cry5* lines were free of potato tuberworm damage, despite 'Spunta' having only 71% potato tuberworm–free tubers (Table 2).

In the 1999 storage evaluation, the *Bt-cry5*-Spunta lines, Spunta 6a-3, Spunta-G2, and Spunta-G3 were effective in reducing potato tuberworm mining over a 3-mo storage period (data not shown). In 2001, after 44 d of storage, only 19% of the 'Spunta' tubers were free of potato tuberworm damage, whereas the three *Bt-cry5*-Spunta lines were greater than or equal to 96% clean (Table 3). By the second evaluation (74 d), all 'Spunta' tubers were damaged by the potato tuberworm, while the three *Bt-cry5*-Spunta lines were greater than or equal to 92% clean. Of the three *Bt-cry5*-Spunta lines, Spunta-G2 was the least damaged by mining after the second and third evaluations (74 and 105 d) of storage (Table 3).

Table 3. Cumulative percent of tuberworm damage-free *Bt-cry5* potato tubers in Nawalla storage over 3-mo period at the International Potato Center (CIP), Kafr El Zayat, Egypt

Potato line	Date		
	16 July 2001	15 August 2001	15 September 2001
Spunta-G2	99 a	98 a	96 a
Spunta-G3	98 a	94 b	91 b
Spunta-6a3	96 a	92 b	90 b
'Spunta'	19 b	0c	0 c

Means within sampling date with the same letter are not significantly different as determined by Fisher's LSD at $\alpha = 0.05$.

Tubers were placed in storage 2 June 2001.

Agronomic Trials. In the 1997 agronomic trials in Michigan, all *Bt*-transgenic lines had similar emergence, vine vigor, vine maturity, tuber size distribution, and internal and external defects compared with the nontransformed lines (data not shown). However, of the six *Bt-cry5* Atlantic lines, ATBT-2, ATBT-8, and ATBT-9 were significantly lower in U.S. #1 yield than 'Atlantic', while ATBT-9 was also lower in total yield (Table 4). There were no differences between FL1607 and the two *Bt-cry1*-FL1607 lines and no differences between 'Lemhi Russet' and the seven *Bt-cry5* Lemhi Russet lines.

In 1998, the six *Bt-cry5*-transgenic lines of 'Atlantic' and seven 'Lemhi Russet' lines were equivalent or better for total or U.S.#1 yield and specific gravity to the respective nontransformed cultivar. An advanced selection from Cornell University that has type A glandular trichomes (NYL235–4) was also evaluated. Agronomic performance of NYL235–4 was similar to 'Atlantic', 'Lemhi Russet', and 'Spunta'.

In 1999, six *Bt-cry5*-Atlantic lines were compared with 'Atlantic'. No differences were observed. The yields in 2000 were the highest observed over the 5-yr period (Table 4). In 2000, seven *Bt-cry5*-transgenic lines of 'Atlantic', seven of 'Spunta', and one of NYL235–4 were compared with their nontransformed cultivar. Only ATBT-3 had a lower yield, but no difference for specific gravity was observed (Table 4). Two *Bt-cry5*-Spunta lines (Spunta-S4 and P1) were lower in yield, and Spunta-S4 had a lower specific gravity. No differences were observed between the three *Bt-cry5*-Spunta lines and 'Spunta' in 2001.

In 1998 and 1999, at the CIP Egypt location, in general, the yields were less than one-half of the yields

Table 4. Agronomic performance trials of the *Bt-cry5* lines at the Michigan State University Montcalm Research Farm, Entrican, Michigan

Potato line	1997			1998			1999			2000			2001		
	Yield (mt/ha)		Specific gravity	Yield (mt/ha)		Specific gravity	Yield (mt/ha)		Specific gravity	Yield (mt/ha)		Specific gravity	Yield (mt/ha)		Specific gravity
	US#1[a]	Total		US#1	Total		US#1	Total		US#1	Total		US#1	Total	
Atlantic	26.5	30.2	1.092	32.4	37.9	1.078	33.0	42.1	1.090	47.0	51.1	1.090	—	—	—
ATBT-1	—	—	—	—	—	—	—	—	—	42.5	48.7	1.091	—	—	—
ATBT-2	21.1	25.2	1.090	40.7	47.4	1.080	30.6	38.4	1.087	43.5	49.3	1.090	—	—	—
ATBT-3	26.0	30.4	1.090	31.7	37.6	1.076	31.5	39.5	1.087	35.9	40.8	1.086	—	—	—
ATBT-4	21.8	25.8	1.087	33.7	39.6	1.077	31.9	38.5	1.089	50.2	57.0	1.091	—	—	—
ATBT-5	—	—	—	—	—	—	—	—	—	50.8	57.8	1.089	—	—	—
ATBT-6	22.4	26.7	1.087	33.5	40.1	1.077	33.1	40.7	1.088	41.5	47.0	1.091	—	—	—
ATBT-8	20.1	24.6	1.091	34.0	40.7	1.078	31.8	39.6	1.088	36.5	41.2	1.087	—	—	—
ATBT-9	21.1	24.6	1.087	29.0	39.2	1.077	27.2	34.7	1.088	—	—	—	—	—	—
FL1607	19.2	22.0	1.091	—	—	—	—	—	—	—	—	—	—	—	—
FLBT-11	25.3	33.7	1.098	—	—	—	—	—	—	—	—	—	—	—	—
FLBT-30	15.6	22.7	1.091	—	—	—	—	—	—	—	—	—	—	—	—
Lemhi Russet	19.1	28.0	1.078	31.1	41.0	1.071	—	—	—	—	—	—	—	—	—
LRBT-1	20.0	26.5	1.080	21.5	33.5	1.072	—	—	—	—	—	—	—	—	—
LRBT-7	22.0	29.3	1.080	29.2	42.3	1.075	—	—	—	—	—	—	—	—	—
LRBT-12	16.0	24.9	1.080	23.5	35.6	1.072	—	—	—	—	—	—	—	—	—
LRBT-14	20.6	28.1	1.078	29.6	41.3	1.073	—	—	—	—	—	—	—	—	—
LRBT-15	16.9	23.6	1.079	26.3	39.0	1.075	—	—	—	—	—	—	—	—	—
LRBT-21	15.0	23.3	1.078	19.1	29.5	1.072	—	—	—	—	—	—	—	—	—
LRBT-22	16.2	25.6	1.078	22.5	30.3	1.070	—	—	—	—	—	—	—	—	—
NYL235-4	25.7	31.6	1.074	32.9	52.6	1.070	—	—	—	61.1	67.7	1.084	—	—	—
NYL235-4.13	—	—	—	—	—	—	—	—	—	54.0	62.6	1.084	—	—	—
Spunta	—	—	—	—	—	—	—	—	—	51.4	60.5	1.065	39.4	50.0	1.058
Spunta-6a3	—	—	—	—	—	—	—	—	—	62.1	71.9	1.062	41.5	51.5	1.056
Spunta-G2	—	—	—	—	—	—	—	—	—	54.2	62.7	1.063	42.2	52.7	1.059
Spunta-G3	—	—	—	—	—	—	—	—	—	47.9	58.2	1.062	47.8	59.4	1.058
Spunta-G4	—	—	—	—	—	—	—	—	—	53.3	60.6	1.062	—	—	—
Spunta-P1	—	—	—	—	—	—	—	—	—	40.5	49.9	1.061	—	—	—
Spunta-P5	—	—	—	—	—	—	—	—	—	48.0	59.9	1.063	—	—	—
Spunta-S4	—	—	—	—	—	—	—	—	—	36.5	45.3	1.056	—	—	—
$LSD_{0.05}$	4.9	5.6	0.004	9.9	9.8	0.004	4.5	4.5	0.003	10.3	10.4	0.005	NS	NS	NS

[a] US#1: tubers, ≥41.3 mm; ≥113 g, excluding culls.

at the Michigan field trials (Table 5). ATBT-6 was the only *Bt-cry5*-Atlantic line with significantly lower yield than 'Atlantic', whereas two *Bt-cry5*-Lemhi Russet lines had lower total yield than 'Lemhi Russet'. In the 1999 agronomic trial at CIP, the yields were below U.S. averages; however, the *Bt-cry5*-Atlantic lines performed comparably with their nontransgenic cultivar. Spunta-G4 line was higher yielding and Spunta-S4 was lower yielding than 'Spunta'.

Discussion

The objective of these field trials was to examine agronomic performance and field and storage resistance to potato tuberworm among 26 Bt-transgenic potato lines over a 5-yr period. Six different Bt constructs were evaluated that were transformed into five different potato lines/cultivars (Table 1). The first field test of genetically engineered potatoes in Egypt occurred in January 1997 at AGERI after the establishment of Egyptian biosafety regulations.

Potato tuberworm trials were planted at two locations in Egypt, one at AGERI and the other at CIP. The AGERI location is outside the potato production region. In 1997, the field trial was artificially infested with potato tuberworms, and the trial was enclosed in row covers to maintain insect pressure. This enclosure of the trial led to severe potato tuberworm damage in some plots (Table 2) and considerable heat stress in the later stages of the trial. In the following years, the row covering was discontinued. The AGERI trials were still artificially infested, but the tuberworm damage was much lower, with 1999 being the only year differences between the *Bt-cry5*-transgenic lines and the nontransgenic check clones were observed. Trials at AGERI were discontinued after the 2000 trial.

The CIP location was added in 1998 for both potato tuberworm and agronomic field trials. This location is within the Nile delta region where potato production is centered in Egypt. Natural tuberworm infestations occur each spring season (Ali 1993). In 1998, like the AGERI trial, no differences were observed between the lines in the trial (data not shown). The mediocre level of resistance of the *Bt-cry5* lines ('Atlantic' and 'Lemhi Russet' lines expressing the pSPUD12 construct) may have also contributed to the lack of differences in 1998. The 1999–2001 trials at CIP all showed differences between some of the Bt-cry5 lines and 'Spunta' (Table 2).

The low level of potato tuberworm–damaged *Bt-cry5* tubers in field and storage assays parallel laboratory tuber bioassays results of Douches et al. (1998), Li et al. (1999), and Mohammed et al. (2000). The high expression of the Bt in the *Bt-cry5*-Spunta lines may

Table 5. Agronomic performance trials at the International Potato Center (CIP) field station, Kafr El Zayat, Egypt

Potato line	1998		1999	
	Yield (mt/ha)		Yield (mt/ha)	
	US#1[a]	Total	US#1	Total
'Atlantic'	11.0	18.5	7.4	10.9
ATBT-2	—	—	5.8	8.5
ATBT-3	9.1	17.8	6.1	10.7
ATBT-4	10.4	18.6	8.5	12.1
ATBT-6	5.2	10.7	6.4	10.1
ATBT-8	8.7	16.5	5.8	8.1
ATBT-9	9.4	18.0	—	—
'Lemhi Russet'	9.3	17.8	—	—
LRBT-12	11.3	19.3	—	—
LRBT-14	10.1	17.0	—	—
LRBT-21	7.9	13.0	—	—
LRBT-22	8.2	11.9	—	—
NYL235-4	6.9	13.0	10.4	14.4
'Spunta'	—	—	7.4	10.7
Spunta-G2	—	—	9.9	14.3
Spunta-G3	—	—	9.8	12.7
Spunta-G4	—	—	10.7	16.4
Spunta-S4	—	—	6.6	9.7
$LSD_{0.05}$	3.5	4.0	3.1	3.9

[a] US#1: tubers, ≥41.3 mm; ≥113 g, excluding tubers with unmarketable defects.

have also made it easy to discriminate between susceptible and resistant lines compared with 'Atlantic', where Bt levels were lower. For example, in the 1999 trial, none of the five *Bt-cry5*-Atlantic lines were significantly different in potato tuberworm damage from 'Spunta', whereas most of the *Bt-cry5*-Spunta lines were different. In both the 1999 and 2000 trials, NYL235–4.13, the line that combines *Bt-cry5* expression and type A glandular trichomes, was also very resistant to potato tuberworm mining of the tubers. This observation was surprising because the *Bt-cry5* expression level is similar to the *Bt-cry5*-Atlantic lines, and the glandular trichome-based resistance is only found in the foliage. J. Kalazich (personal communication) observed partial resistance to the potato tuberworm in Chile using similar germplasm. These observations may suggest that the NYL235–4 may have additional insect resistance factors besides type A glandular trichomes.

Agronomic trials in Michigan and Egypt showed that many of the *Bt-cry5* transgenic lines perform similarly to their nontransformed cultivar (Tables 4 and 5). Dale and McPartlan (1992) reported evidence of somaclonal variation of transgenic potatoes expressing *gus* and *nptII* gene constructs. They also proposed that the *gus* expression may be influencing the plant performance. Some of our *Bt-cry5* transgenic lines did have low levels of *gus* expression, but agronomic performance was not compromised. Conner et al. (1994) suggested that phenotypic changes in transgenic potatoes also might result from a combination of somaclonal variation and insertional mutagenesis from the random integration of the gene construct. Felcher et al. (2003) reported many off-type transgenic lines when potato cultivars were transformed with a constitutively expressed glucose oxidase gene. The *Bt-cry5* transgenic lines chosen for our trials were previously tested against tuberworms in the laboratory using detached leaf tests (Westedt et al. 1998, Li et al. 1999) and were selected based on agronomic performance in preliminary field trials (data not shown); therefore, the comparable agronomic performance of these lines was not unexpected. Other field tests of *Bt-cry3A* transgenic lines in Michigan have been able to identify transgenic lines with agronomic performance similar to the nontransformed line (D. S. Douches and J. Coombs, unpublished data).

Expression of the *Bt-cry5* gene in the potato tuber and foliage will provide seed producers and growers a tool to reduce potato tuberworm damage to the tuber crop in the field and storage. The addition of host plant resistance to an integrated pest management (IPM) program to manage potato tuberworms will result in less pesticide use in the field and in storage. Benefits will be lower potential pesticide residues in and on tubers, potentially improved environmental quality and farm worker safety, increased yield, quality and storability of the tubers, and increased Egyptian seed production, which reduces need for foreign currency.

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