中图分类号: S532 **文献标识码**: A **文章编号**: 1672-3635(2007)02-0065-09

学术园地

Development and Evaluation of Codon-modified cryV Constructs in Cultivated Potato (Solanum tuberosum L.) for Control of Potato Tuber Moth

Guan Hongju[1], Li Wenbin[2*], Douches David[3], Lu Cuihua[2*]

(1. Huachuan Agrotechnique Extension Center, Huachuan, Heilongjiang 154300, China; 2. College of Agronomy, Northeast Agricultural University, Harbin, Heilongjiang 150030, China; 3. Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA)

Abstract: A cryV gene, specifically toxic to Lepidoptera and Coleoptera, was incorporated into binary vectors with different promoters and the presence or absence of the β-glucuronidase (gus) reporter gene. These constructs were integrated into potato cv. Spunta by Agrobacterium tumefaciens-mediated transformation. Highest expression of cryV gene, determined by mRNA levels and insect mortality, was obtained using the CaMV 35S promoter without the gus gene configuration. Detached leaf and tuber bioassays showed a mortality rate of up to 83% and 100%, respectively, for potato tuber moth (Phthorimaea operculella Zeller) in the transgenic lines. Our results demonstrated that the presence of the gus gene negatively affects the expression level of the cryV gene. Bt expression was also facilitated by using the $(ocs)_3$ mas super promoter, whereas the Bt expression regulated by the patatin promoter (tuber-specific) was too low to have any effect upon the mortality of potato tuber moth. These results represent significant improvement in the level of host plant resistance for the control of potato tuber moth via Bt transgenes.

Key Words: potato tuber moth; Bacillus thuringiensis; gus gene; promoter; transgenic plant

Insect damage can be a serious problem in potato production, and one of the most destructive pests is the potato tuber moth. The larvae mine the foliage, stems and tubers in the field and storage. Tuber infection causes dramatic losses, as damaged tubers can be attacked by different secondary pests and diseases. The annual losses in storage alone reach from 30% to 70% in India, and similar losses are encountered in the Middle East, North Africa and South America[1]. Effective control of the potato tuber moth is a challenge. Traditionally, chemical pesticides have been exploited to control potato tuber moth in both the field and storage by 12-20 applications during the growing season and 3-4 sprays during storage. The utility of insecticides, however, is limited by their high costs, persistence of residue in tubers and environment, development of insecticide resistant pests, and a negative public perception.

For over 30 years, crystalline inclusions, produced during sporulation of Bacillus thuringiensis (Bt) bacteria, have been used as biological insecticides to control agricultural pests. These inclusions dissolve in the midgut of the larvae, releasing one or more insecticidal crystal proteins, or δ-endotoxins. The activated proteins bind specifically to receptors in the midgut of the insect and bring about lysis of the cell by formation of pores[2]. The crystalline proteins are characterized by their high toxicity to specific insect pests. Hence, they are an ideal candidate to be introduced into crops for increasing the efficacy of insect control.

Numerous genes encoding Bt crystalline proteins

Received data: 2007-01-06

Foundation item: Tacking key scientific and technical problems in Heilongjiang (GB04B101)

Biography: Guan Hongju (1959-), male, senior agronomist. Research area: agrotechnique extension.

*Corresponding author: E-mail: wenbinli@yahoo.com for Li Wenbin; cuihualu@yahoo.com.cn for Lu Cuihua.

(Cry) have been cloned, sequenced, and transferred into agriculturally important plants through Agrobacterium tumefaciens-mediated gene transfer or particle gun technology. Earlier reports indicated that there was poor transcription of cry genes in transgenic plants compared to many other heterologous genes[3-6]. The insufficient production of toxic protein in situ has led to poor host plant resistance. Higher A-T content was found in native δ-endotoxin coding regions than in plant DNA sequences, reducing the stability of mRNA[7]. In recent years, Bt gene modifications focused on the truncation of A-T rich regions and codon-modification in native cry protein genes (e.g. cryI and cryIII), resulting in a distinct increase in Bt gene expression in certain crops[8-10].

In addition to the achievement of engineering potatoes resistant to specific insect pests, enhancing expression of Bt genes or their expression in specific organs still needs to be examined. The level of gene expression is, in part, a function of the promoter to which the coding region of the gene is fused. The most popular promoter, CaMV 35S (35S), had been employed for Bt gene expression in transgenic potatoes[11]. This promoter confers high constitutive gene expression in a wide variety of tissues during most stages of development[12]. Westedt et al.[13] explored the feasibility of cryV protein gene in engineering potato by the regulation of 35S promoter. This gene is active against both Lepidoptera and Coleoptera. In the present report, we incorporate a novel super promoter and a class-I patatin promoter into cryV constructs. The $(ocs)_3$mas super promoter has been tested in arabidopsis, tobacco, cassava and cowpea and shows up to a 156-fold stronger effect than the 35S promoter[14]. The class-I patatin gene element (a tuber-specific expression) promoter operates the production of 30%~40% soluble protein in potato tubers[15]. This research was undertaken to evaluate the impact of these promoters and the flanking gus sequence on regulating cryV gene expression in transgenic potato.

1　Materials and Methods

1.1　Bacterial strains and plasmid constructs

E. coli strains were cultured at 37℃ with LB medium[16]. For plasmid amplification, 50 μg·mL^{-1} kanamycin or 100 μg·mL^{-1} ampicillin was added. Agrobacterium tumefaciens strain LBA4404 was incubated at 28℃ in Ty medium containing 50 μg·mL^{-1} kanamycin and 10 μg·mL^{-1} rifampicin[17].

The basal skeleton of all constructs in this experiment was the binary vector pBIN19[18]. The Bluescript plasmid harboring a codon-modified cryV gene, was supplied by ICI Seed/Zeneca (Berkshire, UK). Initially, the cryV gene was cut from the Bluescript and inserted into the BamHI site of pBI121, creating pBICryV (Fig.1). Subsequently, the cryV fragment from pBICryV was subcloned into the SmaI/XbaI site of pE1120 under control of a super promoter, compounded by the triple octopine synthase activators and the manopine synthase activator and promoter[14], yielding the plasmid pBIML1 (Fig.1). The cryV fragment from pBICryV was

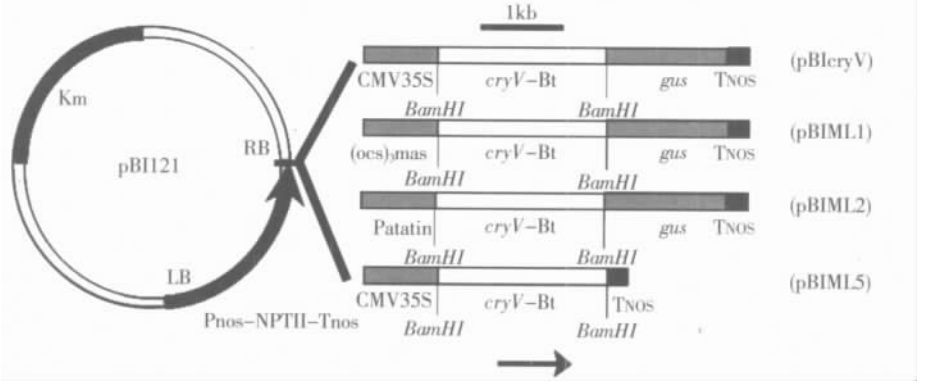

Figure 1　Plasmid constructs used in this study

also inserted into the BamHI site of pS20A-G containing the class-I patatin promoter[15], resulting in the plasmid pBIML2 (Fig.1). Meanwhile, pBI121 was digested with SmaI and EcoICRI, and self ligated. The resulting plasmid pBIML4, was then cleaved with BamHI and ligated with the cryV fragment, resulting in the plasmid pBIML5 (Fig.1). The constructed plasmids were consequently mobilized into the A. tumefaciens strain LBA4404 by triparental mating[18].

1.2 Production of transgenic plants

The potato cv. Spunta was used for all transformations. Aseptic plants were micropropagated in GA-7 Magenta vessels with 25 mL of modified MS medium (MS salts, 3% sucrose, 2 mg·L^{-1} D-pantothenic acid, 0.25 mg·L^{-1} GA_3, pH=5.6). The tissue culture room was maintained at a 16 h photoperiod under fluorescent lights (30 μE·m^{-2}·s^{-1}) at 23-27℃. For transformation, leaves, with tip and petiole ends removed, were plated abaxial side down onto solidified step I medium containing MS salts, 0.5 mg·L^{-1} zeatin, 2 mg·L^{-1} 2, 4-D and cultured for 2-4 days[19]. The dedifferentiated tissues were then soaked for 5-10 min in a 10-fold dilution of A. tumefaciens LBA4404 derived from a 36 h culture at 28℃. The leaf explants were then dried on sterile paper and further cocultured for two days on solidified step I medium. After coculture, leaf tissues were transferred to solidified step II regeneration medium containing 3% sucrose, 0.5 mg·L^{-1} zeatin, 2 mg·L^{-1} GA_3, 200 mg·L^{-1} Timentin (Smith Kline Beechum, PA) and 50 mg·L^{-1} kanamycin. Explants were transferred onto fresh step II medium every 1-2 weeks. Regenerated green shoots were excised and transferred into 25 mm×100 mm culture tubes containing MS medium with 3% sucrose, 2 mg·L^{-1} D-pantothenic acid, 0.25 mg·L^{-1} GA_3, 200 mg·L^{-1} Timentin and 50 mg·L^{-1} kanamycin. Rooted plantlets were transplanted into pots in the greenhouse. The leaves and tubers from these clones were used for molecular analyses and bioassays.

1.3 Analyses of transgenic plants

1.3.1 PCR amplification

Genomic DNA was extracted from leaf tissue according to Mauricio[20]. The primers used for nptII amplification were 5'-CGC AGG TTC TCC GGC CGC TTG GGT GG and 5'-AGC AGC CAG TCC CTT CCC GCT TCA G. The primers used for cryV amplification were 5'-AAC TGG AGG TCG GTG GTG CTG GCG T and 5'-GGA CCA TCG GCG GCA CCC TCA ACA T. PCR was conducted by initial denaturation at 94℃ for 3 min, followed by 30 cycles of denaturation at 94℃ for 1 min, annealing at 65℃ for 1 min and extension at 72℃ for 2 min, and a final extension at 72℃ for 7 min.

1.3.2 Southern blots

Genomic DNA was extracted by the CTAB method of Saghai-Maroof et al.[21], modified by adding 2% beta-mercaptoethanol to the extraction buffer. DNA (20 μg) from each line was digested with BamHI or XbaI. The fragments were separated by electrophoresis through a 1.0% agarose gel, eluted onto a nylon membrane (Hybond N, Amersham, England) using a capillary transfer procedure[16]. As a DNA standard, 6 pg of cryV DNA (2.2 kb) was loaded on the gel, which was equal to the amount of a single copy of cryV gene in 20 μg of potato genomic DNA. Nucleic acids were fixed to the membrane by auto-crosslinking. Prehybridization was conducted for 2 h at 42℃ in the solution containing 5 X SSC, 1% skim milk, 0.1% N-lauroylsarcosine, 0.02% SDS, 50% formamide and 125 μg·mL^{-1} sheared salmon sperm DNA. Hybridization was performed at 42℃ overnight in fresh solution with a DIG-labeled probe (2.2 kb fragment of the cryV coding region amplified by PCR). Following hybridization, the membrane was washed twice in 2X SSC, 0.1% SDS for 15 min at room temperature, twice in 0.5X SSC, 0.1% SDS for 20 min at 65℃. For chemiluminescence detection, the manufacturer's instructions were followed (BMB, Indianapolis, IN). The membrane was exposed to X-ray film for 15-30 min.

1.3.3 Northern blots

Total RNA from young leaf was isolated by using the Qiagen RNeasy Plant Total RNA Kit (Qiagen, Chatsworth, CA). Total RNA (20 μg) was fractionated by formaldehyde gel electrophoresis through a 1.0% a-

garose gel in MOPS buffer(20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA, pH=7) , followed by blotting onto a Hybond N nylon membrane. Prehybridization, hybridization and detection conditions were as described above for DNA analysis, except hybridized at 52℃ and the cryV RNA probe was synthesized by in vitro transcription of DNA(BMB, Indianapolis, IN) .

1.4　Potato Tuber Moth Bioassays

Potato tuber moth rearing was performed according to Westedt et al.[13]. For the leaf bioassay, a young, fully-expanded leaf was collected and the petiole of the detached leaf was cut off under water using a new single-edged razor blade. The petiole with the leaf was inserted through a pre-moistened sponge(1 cm × 1 cm) and inserted into a 3.5 mL glass vial full of water. The unit was then placed in a Petri dish(150 mm × 20 mm) with a pre-moistened 150 mm diameter 3MM Whatman paper. Ten newly-hatched larvae were placed near the mid-rib of each leaf. The Petri dish was covered and incubated in a chamber at(25 ±2) ℃ and 25 μE·m^{-2}·s^{-1} light. Mortality of the potato tuber moth larvae was determined after 72 h. Percent mortality(%) was calculated as: dead larvae divided by total larvae and multiplied by 100.

For the tuber bioassay, one greenhouse-grown tuber(5~40 g) from each of the transgenic plants was placed in a Phytatray II box(Sigma, ST. Louis, MO) per replication. Holes were punched through the top for aeration. Five newly hatched larvae were placed on each tuber and kept at(23 ±2) ℃ in the dark. Four weeks later, the number of pupae, adults and larvae were counted in each box and percent mortality was calculated. The bioassay included four replications.

2　Results

2.1　Transformation and cryV constructs integrity

Four cryV constructs differing in promoters and gus gene presence or absence were created to either increase or target the expression of cryV gene(Fig.1) . The constructs were introduced into potato cv. Spunta by cocultivation of leaf discs with Agrobacterium tumefaciens. Green tumorlike callus appeared at the edge of leaf discs after twenty days culture on regeneration medium containing 50 μg·mL^{-1} kanamycin. The shoots emerged from the tumorlike callus after thirty days. On average, 10-40 shoots were produced in each callus; 38%~74% of these shoots rooted when transferred into propagation medium containing 50 g·mL^{-1} kanamycin and 200 μg·mL^{-1} Timentin. For each of the four constructs, 5-20 rooted plantlets were analyzed by PCR amplification using nptII and cryV

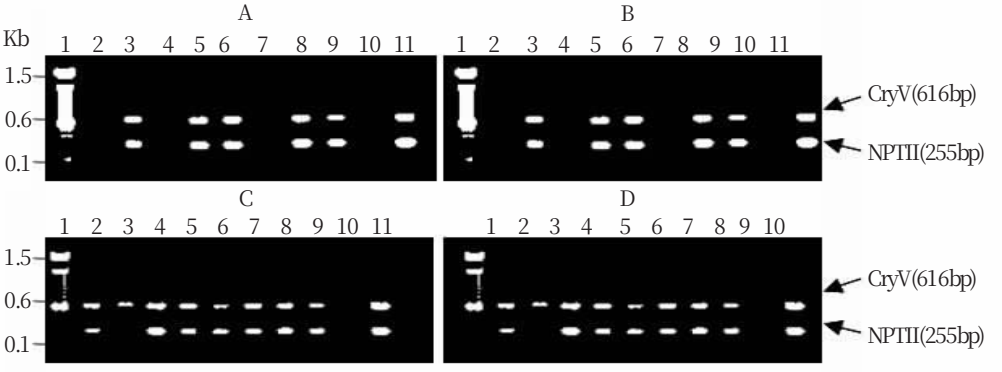

Figure 2　PCR analysis of putative transformants

(A) : Lane 1, 100 bp; ladder marker; Lane 2-9, pBICryV lines; Lane 10, ‘Spunta’ non-transgenic control; Lane 11, pBICryV plasmid. (B) : Lane 1, marker; Lane 2-9, pBIML1 lines; Lane 10, control; Lane 11, pBIML1 plasmid. (C) : Lane 1, marker; Lane 2-9, pBIML2 lines; Lane 10, control; Lane 11, pBIML2 plasmid. (D) : Lane 1, marker; Lane 2-8, pBIML5 lines; Lane 9, control; Lane 10, pBIML5 plasmid.

primer sets(Fig. 2) . All rooted plantlets containing the pBIML2 construct(patatin promoter) and pBIML5 construct(35S promoter without gus gene element) were PCR positive, whereas only 67% and 79% were PCR positive for the pBICryV construct(35S promoter with gus element) and the pBIML1 construct(super promoter with gus element) , respectively. The PCR results were further corroborated by Southern blots probed with the cryV gene.

To identify gene integration efficacy among the constructs, the numbers of T-DNA copies in the cryV transgenic lines were determined by Southern blot analysis Fig. 3. The number of T-DNA copies was estimated by insert intensity in a single site and different insert locations within genomic DNA. The results show the number of integrated copies varied from one to more than nine across the four types of constructs. Four to nine copies of the cryV gene were found in most of the transgenic plants containing the pBIML5 construct, with up to 5 insertion sites estimated in these lines. The copy numbers for the other constructs were lower and located only in one or two sites.

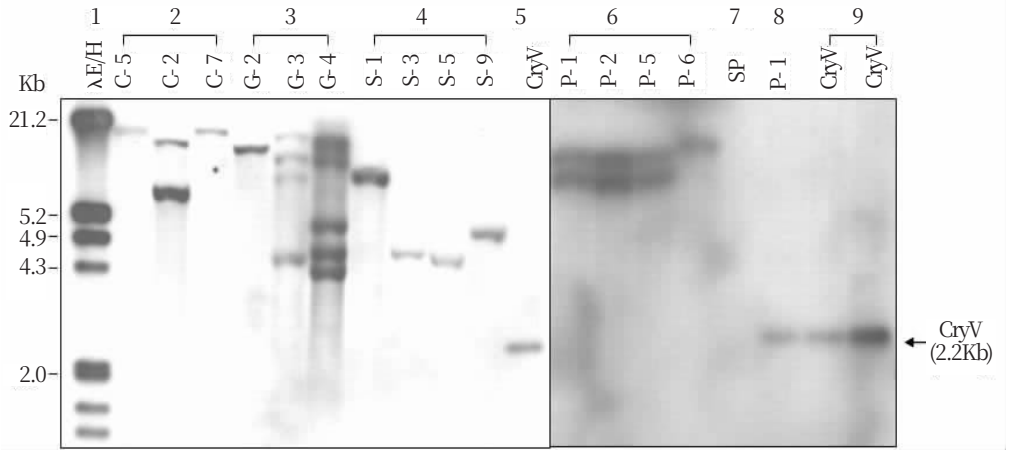

Figure 3 Southern blot analysis of genomic DNA from CryV-Bt transgenic potatoes

DNA is digested by XbaI, except of the lane marked 8 in which DNA is digested by BamHI. The probe is specific for the CryV-Bt gene. Lane 1, λDNA/EcoRI +HindIII marker; Lanes marked 2, the pBICryV lines; Lanes marked 3, the pBIML5 lines; Lanes marked 4, the pBIML1 lines; Lane marked 5, CryV-Bt DNA(6 pg) ; Lanes marked 6, the pBIML2 lines; Lane marked 7, ‘Spunta’ non-transgenic control; Lane marked 8, the P-1 line digested by BamHI; Lanes marked 9, the CryV-Bt DNA of 6 pg and 12 pg, respectively.

2.2 cryV gene transcription

cryV gene transcription in various transgenic plants was examined by northern blot analysis. Of all constructs, the pBIML5 construct(35S promoter without gus gene) displayed the highest level of cryV transcripts, especially for the line G-3(Fig. 4) . A moderate amount of transcripts was obtained from pBIML1 construct(super promoter with gus gene) , comparable to the pBICryV construct(35S promoter with gus gene) which contributed a lower level of Bt gene transcription. Transcription of the cryV gene in the pBIML2 construct(patatin promoter with gus gene) was not detected in the leaf.

2.3 Insecticidal activity in transgenic plants

The biological activity of the cryV against Phthorimaea operculella larvae was examined using a

sample of PCR-positive transgenic plants of each construct type. In the detached leaf feeding bioassay, mortality of potato tuber moth larvae on the transgenic plants was between 5% and 83% after 72 h of feeding, compared to 8% in the non-transgenic cv. Spunta. Growth of the larvae fed on transgenic plants containing pBIML1, pBIML5 and pBICryV constructs was severely restrained during the first 2-3 days of feeding(Table 1), and all larvae were dead after 5-7 days of feeding. There was very little evidence of feeding damage to the leaves of those transgenic plants. On the other hand, the leaves of non-transformed cv. Spunta were seriously damaged after 3 days of feeding, concurrent with a rapid growth of larvae. Transgenic plants containing the pBIML5 construct(without gus element) caused the highest mortality(80%~83% mortality), significantly greater than some of the Bt transgenic plants with the pBIML1 construct(13%~78% mortality) and some of the Bt transgenic plants with the pBICryV construct(50%~70% mortality). No insecticidal activity was found in the leaves of pBIML2 transgenic plants containing the patatin promoter.

The same Bt transgenic lines tested in the leaf bioassays were used for the tuber bioassay. After four weeks of feeding, adults emerged from the cv. Spunta non-transgenic tubers and from most of the tubers of the pBIML2 lines. In contrast, five lines containing pBIML5, pBIML1 or pBIcyV constructs possessed 100% mortality, respectively, and no feeding damage was observed as the newly hatched larvae were dead within the days of the bioassay. The living insects on the lines with less than 100% mortality(e.g. C-5 and G-4) exhibited a delayed development. The lines which displayed a high mortality to potato tuber moth in the leaf bioassay were also highly lethal to potato tuber moth in the tuber bioassay (Table 1). The level

Table 1　Insecticidal activity in transgenic plants

Constructs	Transgenic lines	Tuber bioassay % mortality		Leaf bioassay % mortality (72h)		Mining
pBICryV	C-2	100	c	70	bc	N
	C-5	81	bc	50	b	N
pBIML1	S-1	100	c	73	bc	N
	S-2	13	a	13	a	Y
	S-4	100	c	78	c	N
pBIML2	P-2	24	a	5	a	Y
	P-5	34	a	13	a	Y
	P-6	27	a	5	a	Y
pBIML5	G-2	100	c	83	c	N
	G-3	100	c	80	c	N
	G-4	69	b	80	c	N
Spunta		25	a	8	a	Y

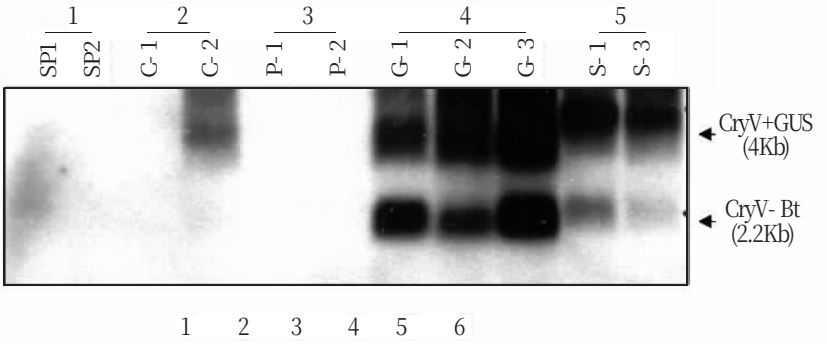

Figure 4　Northern blot analysis of Cry V Bt gene transcripts in leaf

Lanes numbered 1 are ‘Spunta’non-transgenic plants; lanes numbered 2 are pBICryV lines; lanes numbered 3 are pBIML2 lines; lanes numbered 4 are pBIML5 lines; lanes numbered 5 are pBIML1 lines.

of tuber damage control paralleled the mortality of larvae(data not shown) .

3 Discussion

Four cryV constructs were created, transformed into potato and evaluated by leaf and tuber bioassays with the potato tuber moth. The results show that the greatest level of potato tuber moth control was achieved with the transgenic lines containing the pBIML5 construct. Although most larvae did not survive more than 5 days on the leaves of the transgenic lines carrying the pBIML5, pBIML1 and pBICryV, the early death of larvae was most pronounced in the transgenic plants with pBIML5 construct(80%~83% mortality) . This effect may be critical to minimize the foliar damage of the potato tuber moth and to reduce potato tuber moth population levels in the field. This experiment also indicates that the deletion of the gus gene from cryV construct leads to an distinct increase of cryV gene expression.

The improvement in the Bt expression by deleting the gus reporter gene from the cryV construct may be due to a number of factors. The low potato tuber moth mortality on some transgenic lines may be partially due to the interaction of the gus and cryV genes. Dale et al.[22] and Belknap et al.[23] reported that the gus gene had a negative effect upon the physiology of agronomic traits in transgenic potato. The deletion of gus element eliminates the possibility of its negative action on cryV gene expression. In addition, the opportunity for T-DNA integration into genomic DNA may be increased by shortening the T-DNA length. The present results show that the copy numbers of cryV gene in the pBIML5 transgenic plants were higher than in transgenic plants containing other constructs and higher than the copy numbers reported for some maize, rice, tobacco and potato transformants as well[10, 23, 24]. The higher copy number, consequently, may contribute to a higher accumulation of mRNA transcripts of cryV gene(Fig. 4) . High copy number is not acceptable for seed propagated crop, however, the potato is routinely propagated asexually. Finally, the cryV gene may produce more sufficient transcripts and the mRNA might be more stable by limiting the DNA template only to the cryV element.

Bt expression in leaves may reduce tuber damage because newly hatched larvae typically feed on the foliage before dropping to the ground and feed on the tuber. As an alternate host resistance management strategy, it might be advisable to deploy Bt protein only in the tuber. According to this principle, we introduced a tuber-specific promoter(class-I patatin element) into the cryV construct(pBIML2) . In our experiments, the transcription signal was not detected in leaves as expected, but only a faint transcriptional signal was observed by northern analysis of the tuber RNA derived from some pBIML2 transgenic lines (data not shown) . This was consistent with the tuber bioassay result where larval growth was moderately inhibited in transgenic lines such as P-5 (data not shown) . These results suggest that the use of the patatin promoter may not be successful for targeting high level of Bt gene expression in tuber.

The low tuber Bt expression in pBIML2 lines containing the patatin promoter may be attributed to the following reasons. First, transgenic expression can be influenced by the chromosomal integration site (position effect) . Position effect may explain the lack of correlation between the expression levels and copy numbers of cryV gene in some transgenic lines, and may also explain the poor insecticidal activity in pBIML1 line 2(S-2)(Tables 1) . If the position effect is the major factor, then we should be able to select Bt lines with higher levels of gene expression from a larger population of transformants. The Bt toxin transcript of tuber may also be unstable, probably due to inefficient posttranscriptional processing or rapid turnover of mRNA. We have found a lower level of steady-state transcripts in tubers than in leaves(data not shown) , and the Bt RNA was degraded during tuber storage to a low molecular weight form. This process, together with the insufficient functioning of patatin promoter, might interfere with tuber cryV

expression. In addition, the gus gene fused with the cryV gene in this construct may limit the cryV expression as discussed earlier, and the Bt expression level may be increased by deleting the gus gene from the patatin construct. We have recently engineered a patatin construct without the gus element and will conduct transformations with this new construct to further study the effect of the patatin promoter.

Ni et al.[14] reported that a super promoter, mannopine synthase promoter with its activator and triple octopine synthase activators, is 156-fold stronger than the 35S promoter. In our experiment, the super promoter supported higher expression of the cryV gene than CaMV 35S(Table 1) , which was also verified by the existence of some lines suffering little or no damage in the leaf bioassay compared to the lines containing pBICryV construct. These results suggest that the expression of cryV protein can be increased using a high expression promoter. Furthermore, we need to compare the super promoter-CryV construct without the gus gene to pBIML1 and pBIML5 constructs. Such comparisons will help us to determine if the gus gene has a negative impact on cryV expression regulated by the super promoter and will give us a better understanding of the potential of the super promoter.

This report demonstrates the feasibility of increasing Bt expression in transgenic potato by modifying a flanking element and by utilizing active expression vectors. Our present bioassay results were derived from laboratory and greenhouse. Beneficial factors present in the field such as parasites, predators and adverse environmental conditions for the insect will contribute to insect control but are not factors in the greenhouse. Therefore, a stringent field test of these transgenic plants with potato tuber moth infestation will be conducted during the following years in subtropical regions (i.e. Egypt). Moreover, to support the strategy of integrated pest management, it may be necessary to develop transgenic potatoes with several cry genes that bind at different receptor sites within the insects to inhibit the formation of resistant insect populations.

4　Acknowledgments

This publication was made through partial support provided by the Key Laboratory of Soybean Biology in Chinese Ministry of Education. The research was also supported by the Michigan Agriculture Experiment Station.

References

[1] Raman K V, Palacios M. Screening potato for resistance to potato tuberworm[J]. J Econ Entomol, 1982, 75: 47-48.

[2] Lambert B, Peferoen M. Insecticidal promise of Bacillus thuringiensis[J]. BioScience, 1992, 42: 112-122.

[3] Barton K A, Whiteley H R, Yang N. Bacillus thuringiensis δ-Endotoxin expressed in transgenic Nicotiana tabacum provides resistance to Lepidopteran insects[J]. Plant Physiol, 1987, 85: 1103-1109.

[4] Fischhoff D A, Bowndish K S, Perlak F J, et al. Insect tolerant transgenic tomato plants[J]. Biotechnology, 1987, 5: 807-813.

[5] Vaeck M, Reynaerts A, Hofte H, et al. Transgenic plants protected from insect attack[J]. Nature, 1987, 328: 33-37.

[6] Perlak F J, McPherson S, Fuchs R L, et al. Expression and analysis of the insect control protein from Bacillus thuringiensis var. tenebrionis[G]//Abstracts of the First International Symposium on the Molecular Biology of the Potato, Bar Harbor, Maine. 1989: 51.

[7] Fujimoto H, Itoh K, Yamamoto M, et al. Insect resistant rice generated by introduction of a modified-δ-Endotoxin gene of Bacillus thuringiensis[J]. Biotechnology, 1993, 11: 1151-1155.

[8] Perlak F J, Deaton R W, Armstrong T A, et al. Insect resistant cotton plants[J]. Biotechnology, 1990, 8: 939-943.

[9] Koziel N G, Beland G L, Bowman C. Field performance of Elite transgenic maize plants expressing an insecticidal protein derived from Bacillus thuringiensis[J]. Biotechnology, 1993, 11: 194-200.

[10] Wunn J, Kloti A, Burkhardt P K, et al. Transgenic indica rice breeding line IR58 expressing a synthetic cryIA(b) gene from Bacillus thuringiensis provides effective insect pest control[J]. Biotechnology, 1996, 14: 171-176.

[11] Peferoen M, Jansens S, Reynaerts A, et al. Potato plants with engineered resistance against insect attack[M]// Vayda M E,. Park W C. Molecular and cellular biology of the potato. Wallingford: CAB International, 1990: 193-204.

[12] Odell J T, Nagy F, Chua Nam-Hai. Identification of DNA sequences required for ability of the cauliflower mosaic virus 35S promoter[J]. Nature, 1985, 313: 810-812.

[13] Westedt A L, Douches D S, Pett W L, et al. Evaluation of natural and engineered resistance mechanism in Solanum tuberosum L. for resistance to Phthorimaea operculella Zeller[J]. J Econ Ent, 1998,

中图分类号：S532；S133　文献标识码：A　文章编号：1672-3635(2007)02-0073-05

马铃薯品种大西洋不同杂交组合后代的产量表现

张铁强，孙清华，石　瑛*，陈伊里*

（东北农业大学，黑龙江　哈尔滨　150030）

摘　要：以马铃薯品种大西洋为母本的6个杂交组合的无性一代为试验材料，对组合的各个产量性状进行评价。结果表明：综合表现较好的组合为大西洋×D2和大西洋×早大白，这两个组合产量高，单株商品薯重也较高，平均单株产量分别为621.6 g和511.0 g，平均单株商品薯重分别为426.9 g和409.2 g；尤其是大西洋×早大白组合，后代的单株结薯数量较少（4.9个），且单薯重高（107.9 g），是大薯块类型的高产组合。大西洋×克新16号组合和大西洋×F1800组合的产量中等，可结合其它性状评价其利用方向。大西洋×克新2号组合的产量较低；且单株商品薯重也较低（187.7 g），是利用价值较小的组合。大西洋×中薯4号组合的平均单株产量低（380.7 g），但单株结薯数量少（4.5个），因而单薯重较高（93.5 g），可以选择高产的单株进一步鉴定。

关键词：马铃薯；杂交组合；产量

在马铃薯杂交育种中，亲本的选择除了应考虑一般品种必须具备的性状，如高产和抗病外，还应注意选择具有当前育种目标特性的亲本，这有利于增加后代集多种优良性状于一身的无性系的数量。北美已育成的许多加工型品种就印证了这一观点，如美国利用Russet Burbank品种做亲本选育出的Russet Nuksaik，不但保留了Russet Burbank的良好外观和低还原糖的特性，还增加了抗晚疫病和卷叶病毒等特性[1]。马铃薯品种大西洋（Atlantic）是美国1976年选育的油炸薯片专用加工型品种，该品种具有产量高、食味好、油炸成品质量好等优良特性，但在中国传统的栽培条件下同时也表现出晚疫病抗性差、易退化、适应性差以及大薯空心等明显缺

收稿日期：2006-12-20

基金项目：黑龙江省科技攻关计划项目（GB01B102-07-01）

作者简介：张铁强（1980-），男，硕士研究生，从事马铃薯遗传育种研究。

*通讯作者：E-mail potato@mail.neau.edu.cn

9(2):552-556.

[14] Ni M, Cui D, Einstein J, et al. Strength and tissue specificity of chimeric promoters derived from the octopine and mannopine synthase genes [J]. The Plant Journal, 1995, 7: 661-676.

[15] Wenzler H C, Mignery G A, Fisher L M, et al. Analysis of a chimeric class-I patatin-gus gene in transgenic potato plants: High-level expression in tubers and sucrose-inducible expression in cultured leaf and stem explants [J]. Plant Mol Biol, 1989, 12: 41-50.

[16] Sambrook J, Fritsch E F, Maniatis T. Molecular cloning: A laboratory manual [M]. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.

[17] Beringer J E. R factor transfer in Rhizobium Leguminosanum [J]. J Gen Microbial, 1974, 84: 188-198.

[18] Bevan M. Binary Agrobacterium vectors for plant transformation [J]. Nucl Acids Res, 1984, 12: 8711-8721.

[19] Yadav N R, Sticklen M B. Direct and efficient plant regeneration from leaf explants of Solanum tuberosum L. cv. Bintje [J]. Plant Cell Reports, 1995, 14: 645-647.

[20] Lopes M A, Takasaki K, Bostwick D E. et al. Identification of two opaque 2 modifier loci in quality protein maize [J]. Mol Gen Genet, 1995, 247: 603-613.

[21] Saghai-Maroof M A, Soliman K M, Jorgensen R A, et al. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics [J]. Proc Natl Acad Sci USA, 1984, 81: 8014-8018.

[22] Dale P J, MacPartlan H C. Field performance of transgenic potato plants compared with controls regenerated from tuber discs and shoot cuttings [J]. Theor Appl Genet, 1992, 84: 585-591.

[23] Belknap W R, Corsini D, Pavek J J, et al. Field performance of transgenic Russet Burbank and Lemih Russet potatoes [J]. Amer Potato J, 1994, 71: 285-297.

[24] Hensgens L A M, Fomerod M W J, Rueb S, et al. Translation controls the expression level of the chimeric reporter gene [J]. Plant Mol Biol, 1992, 20: 921-938.