An effective pest management approach in potato to combat insect pests and herbicide

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Abstract

Insect pests and weeds incur significant yield losses to potato crop worldwide. The increasing crop losses provide impetus for the development of pest management strategy that is equally effective against insect pests and weeds. In the present study, a molecular approach was used to develop transgenic potato lines (cv. Marabel) effective against Colorado potato beetle (*Leptinotarsa decemlineata* Say), potato tuber moth (*Phthorimaea operculella* Z.) and Basta[®] application. *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pTF101.1 containing *cryIAc* gene under the control of 35S and AoPR1 promoters was used to infect leaf discs and internodal explants. Phosphinothricin was used at optimal concentration (2 mg/l) for the screening of primary transformants. The standard molecular assays exhibited gene integration and expression in putative transgenics. Real-time data revealed up to ninefold high *cryIAc* transcript levels, whereas cry protein amount was estimated to 0.4 ppm in primary transformants. The analysis of first tuber progeny showed proper integration *cryIAc* and *bar* genes in subsequent progeny. The transgenic plants also showed tolerance to the application of Basta[®]. The efficacy of *cryIAc* was evaluated by allowing larvae of Colorado potato beetle (CPB) and potato tuber moth (PTM) to feed on transgenic plants. Results revealed appreciable mortality levels of different larval instars of CPB (20–100%) and PTM (50–100%). Overall, our results exhibit the potential of these transgenic lines to be used in a potato breeding program with the purpose to control insect pests and weeds.

Keywords Potato · Insect pests · Weeds · Management · Transgenics

Introduction

Recently, an estimated 37% of all crops are lost annually to pests (13% to insects, 12% to pathogens, and 12% to weeds) despite the use of pesticidal and non-chemical controls (Pimentel 2018). In potato, insect pests and diseases pose continuous threat to crop operations resulting in 40% yield losses (Beddington 2010). A wide range of insect pests damage potatoes; potato tuber moth (*Phthorimaea operculella* Z.) among lepidopterans and Colorado potato beetle (*Leptinotarsa decemlineata*, Say) among coleopterans is by far the most widespread and damaging insect pests of potato (Visser 2005; Rondon 2010).

Potato tuber moth (*P. operculella* Z.) damages crop both in the field and storage (Westedt et al. 1998), reduces the quality of the produce, and also increases the risk of pathogen infection. As it attacks both the foliage and the tuber, potato yield is reduced tremendously (Capinera 2001). The losses in storage can reach up to 100% in warmer climates (Lagnaoui et al. 2001). Potato tuber moth is an increasing agricultural problem in tropical and subtropical areas because of its ability to develop resistance to chemical insecticides.

In addition to potato tuber moth (PTM), Colorado potato beetle (CPB) is the most damaging insect pest of potato and many other members of solanaceae round the globe (Gokce et al. 2012). CPB larvae and adult feed on potato leaves thus reducing yield significantly and even kill plants. Defoliation in potato plants due to CPB results in annual yield reduction of around 30–50% or sometime no economic yield can be obtained (Zhou et al. 2012). CPB is notorious for the development of resistance to insecticides over short periods of time because of co-evolution with Solanaceous crops,

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which produce diverse secondary metabolites, thus enhancing detoxification mechanism of CPB to survive a variety of complex natural and synthetic chemicals (Kaplanoglu et al. 2017; Alyokhin et al. 2015). Therefore, there is a need of exploring new alternative methods to control the CPB infestation in the fields (Zhu et al. 2012).

The different classes of insecticides in rotation are recommended to discourage resistance development (Sharma 2013), but still, problem exists. Unfortunately, farmers in some countries spray their potato fields more than ten times during a single growing season of 4–6 months to combat these pests. Biocides pose a significant health risk to farm families and farm workers engaged in potato production (Wilson and Tegg 2012). With the emergence of higher insecticide resistant pests, even more frequent and increasingly ineffective applications of pesticides are made, raising the risk to human health and the environment (Kim et al. 2017).

The weeds compete with potato crop in field conditions by declining yield, tuber quality, sheltering pests, enhancing diseases, and hindering agronomic practices (Zimdahl 2007). It has been estimated that weeds lead to 30–45% yield losses in most of the crops, whereas they may cause yield losses ranging between 16 and 76% in potato according to the weeds intensity competing with the crop (Tripathi et al. 1989). Control of weeds in potato is extremely harder and can be challenging to plant breeders. Weed management practices vary from non-chemical tactics to chemical control (Üremiş et al. 2009). Basta® is a non-selective herbicide used to control over 80 weed species in a wide range of crops with partially systemic mode of action that provides a higher level of crop safety than systemic alternatives like glyphosate. *Bar* gene-encoding resistance to Glufosinate, active ingredient of Basta®, has been earlier used as selectable marker for potato transformation (Padegimas et al. 2004; Figueira et al. 1994; Soto et al. 2007).

In the past decades, transgenic technology has been used to develop insect and herbicide resistant crops for reducing both yield losses and unnecessary pesticide usage (Christou et al. 2006). These transgenic plants are becoming important components of integrated pest management worldwide (Kos et al. 2009). According to the literature, use of 35S CaMV promoter has been the first choice of all researchers to express foreign protein genes constitutively in commercialized transgenic crops; however, the targeted expression of foreign gene proteins has immense significance in transgenic technology to address the concerns of unnecessary protein production, and to avoid extra metabolic burden on plants (Ahmed et al. 2017; Bakhsh et al. 2016). It has already been established that cry1 toxin shows efficacy against lepidopterans (Bravo et al. 2007); however, there are very few reports about their efficacy against CPB (Yüceer and Kayım 2012). Using transgenic approach, the present study was conducted to develop insect and herbicide resistant potato lines equally effective in controlling CPB, PTM, and showing tolerance to the application of Basta. The insecticidal gene (*cry1Ac*) used in the study was under the control of constitutive (35S) as well as wound inducible promoter (AoPR1) which activates and expresses the gene in case of any type of wound or mechanical damage localizing gene expression.

Materials and methods

Description of expression constructs

The construction of plant expression cassette has been described in Anayol et al. (2016) in detail and represented as Fig. 1. *Escherichia coli* strain JM109 cells were used for the cloning and propagation of the recombinant plasmids. For the present study, both recombinant plasmids were first confirmed using colony PCR and restriction digestion (Data

Fig. 1 Schematic representation of constructs 35SpTF101 and AoPR1pTF101 containing *cry1Ac* gene under the control of 35S CaMV and AoPR1 promoter, respectively. Phosphinothricin (PPT) was for the in vitro plant screening, whereas spectinomycin and streptomycin were used for bacterial selection

not provided) and later on electroporated to *Agrobacterium tumefaciens* strain EHA105 and confirmed by Colony PCR.

Plant material and transformation

Potato (*Solanum tuberosum* L.) cv. Marabel was used in the present study that has good agronomic characteristics and yield potential in the area, but is susceptible to insect pest damage. Prior to genetic transformation, the shoot cultures of Marabel were established from tuber sprouts and propagated in vitro using single node stem explants on basal MS medium (Murashige and Skoog 1962). The cultures were incubated in growth chamber at 25 °C, 45 µMol photons/m²/s light intensity and 60% humidity under 16/8 h of light/dark photoperiod.

The protocol for *Agrobacterium*-mediated genetic transformation was followed as described by Beaujean et al. (1998) with modifications adapted at our laboratory. Leaf discs and internodes (4–6 mm) were excised from the propagated plants in vitro and inoculated with the suspension (OD 0.6) of *Agrobacterium* strain containing targeted constructs (35SpTF101 and AoPR1pTF101) for 30 min with mid-shaking in LB liquid medium without antibiotics, followed by incubation on co-cultivation medium (MSO supplemented with 50 mM acetosyringone) for 3 days. Following co-cultivation, the explants were washed with broad spectrum antibiotic (Sulcid), dried, and cultured on regeneration selection media (MS salts supplemented with 2 mg/l of BAP, 0.2 mg/l of NAA, 1 mg/l of kinetin, and 2 mg/l of trans-zeatin). The optimized concentration of phosphinothricin (2 mg/l) was added in regeneration selection medium (RSM). The data for callus induction and number of shoots per explant was recorded. With well-developed calli, explants were transferred to shoot induction medium (MS salts supplemented with 1 mg/l of BAP, 0.1 mg/l of GA3, and PPT) for sub-culturing. Furthermore, putative transgenic plantlets with well-developed shoots and roots were shifted to pots filled with a mixture of perlite, peat, and vermiculite (1:1:1 v/v/v) for acclimatization. Transformation efficiency was calculated by dividing PCR positive plants to the total explants used.

Analysis of primary transformants

Transgenic plants were subjected to different molecular analysis to confirm integration and expression of introduced gene(s). For this purpose, genomic DNA was extracted using ThermoScientific GeneJET Plant Genomic DNA Purification Kit (Cat no. K0792). PCR assays were conducted using gene-specific primers to amplify internal fragments of *cryIAC* and *bar* genes from putative transgenic plants. PCR was performed in a total reaction mixture volume of 20 µl containing 1 × reaction buffer, 50 ng of DNA template, 1.5 mM MgCl₂, 1 mM of each of the dNTPs, and 0.5 µM of each primer and one unit of Taq DNA polymerase. The primers sequences, annealing temperature, and product size are given in Table 1. The plasmid DNA was used as positive control, whereas DNA isolated from untransformed plants was used as negative control. The transformants were also subjected to PCR assay with *chvA* gene to assess whether the primary transformants are contaminated with *Agrobacterium* or not. *Agrobacterium* strains LBA4404 harboring recombinant plasmid was used as positive control. The amplified DNA fragments were electrophoresed on 1.0% agarose gel and visualized by ethidium bromide staining under ultraviolet (UV) light.

qRT-PCR

PCR positive plants were subjected to real-time PCR to investigate the transcripts levels of *cryIAC* produced. For total RNA extraction, AMRESCO RiboZol™ RNA Extraction Reagent was used. cDNA was synthesized using Fermentas cDNA synthesis kit. qRT-PCR content included total Mix (2X) (Qiagen), F Primer (2 µM), R Primer (2 µM), RNase-free water. qRT-PCR temperature cycle was set up as 95 °C for 15 min, 40 cycles of 95 °C for 10 s, 58 °C for 15 s, 72 °C for 20 s and melting curve analysis was performed by incubation at 99 °C to 70 °C with a transition rate of 1.0 °C/min. For normalization, elongation factor 1-α (*eflα*) was selected as reference gene for the purpose of quantifying the expression of genes. *eflα* gene was selected based on the results of studies conducted by Nicot et al. (2005) on the identification of new

Table 1 Primer used in the study to confirm the presence of introduced gene(s) in transgenic plants

Gene	Primer sequence (5′–3′)	Product size (bp)	Annealing temp (°C)
<i>cryIAC</i>	ATGGACAACCCAAACATC TCATGTCGTTGAATTGAATA	690	58
<i>cryIAC</i> (qPCR)	ATCGGTATCAACAACCAGCA ACCTGTGGGAGAATCCTTG	55	173
<i>bar</i>	GCACCATCGTCAACCACTA ACAGCGACCACGCTCTTGAA	310	60
<i>chvA</i>	CGAAACGCTGTTTCGGCCTGTGG GTTTCAGCAGGCCCGGCATCCTGG	890	65

reference genes suitable for normalization in qRT-PCR studies. Each reaction was set up with three replicates. The threshold values of samples in target gene expression analysis were analyzed by Software of the Rotor-Gene Q (QIAGEN) RT-PCR instrument. According to qRT-PCR analysis results, the standard deviations of Ct values of the samples were calculated using Microsoft Excel program and the expression level of the genes was determined according to the $2^{-\Delta\Delta Ct}$ proportional calculation method (Livak and Schmittgen 2001).

ELISA

A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to quantify the accumulated levels of the cryIAc protein expressed in the leaves of putative transgenic plants using Envirolig kit. The leaves of primary transformants along with control were collected for protein quantification. The protocol for protein extraction and quantification was followed as per instruction provided in the kit. The OD values at 460 nm were used to calculate the amount of cryIAc protein by comparing it with the standard cryIAc protein.

Analysis of first tuber generation (T_1)

The tubers of positive transgenic plants were harvested and grown in green house to analyze integration and expression of introduced gene in subsequent progeny. PCR was run to amplify internal fragment of cryIAc and bar gene using protocol as mentioned above. Furthermore, the efficacy of introduced cryIAc and bar genes was evaluated by conducting leaf biotoxicity and Basta application assays, respectively.

To determine the efficacy of cryIAc under both promoters, leaf bioassays with four different larval stages of Colorado potato beetle and second and third instar larvae of potato tuber moth were conducted. Three fresh leaves along with petiole were detached from the plants; petioles were inserted into 1.5 ml Eppendorf tube containing water and wrapped with sealing film to maintain leaves fresh. The data on insect mortality were recorded on daily basis up to 72 h.

The transgenic plants were further subjected to Basta® application assay to determine the efficacy of bar gene against Glyphosate. The commercially available Basta® was applied to transgenic plants along with control using recommended dose (2000 ml/ha).

The statistical analyses were performed with Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA).

Results

Leaf discs and internodes were used as explants in transformation experiments of Marabel cultivar. The different steps of transformation are shown in Fig. 2. A total of 600

explants were excised, subjected to treatment with *Agrobacterium* strain EHA105 harboring recombinant plasmids. Calli induction from internode explants was calculated more (70%) and was compared to leaf discs (20%). Likewise, number of shoots per explants was also higher in case of internodal explants (Table 2). The regenerating shoots with a length of 2–3 cm were excised and transferred to bigger magenta boxes (107 × 94 × 96 mm). No any problem was encountered regarding rooting of regenerated transgenic shoot. The transformation efficiency from each explant was calculated by dividing PCR positive plants to the total explants used. Overall transformation was calculated as 2.33% in case of internodal explant as compared to leaf disc explant (1.66%). A total of 12 PCR positive transformants were first acclimatized in a controlled growth chamber for 2 weeks and later on transferred to greenhouse. Out of 12, six plants (3 of each promoter) were selected for further molecular analysis. The primary transformants looked similar in morphology to the control plants indicating the normal plant development and growth (Fig. 2).

To select for the positive primary transformants, PCR assays were conducted to confirm the presence of cryIAc and bar genes (Fig. 3). Results of six randomly selected plants transformed with cryIAc under 35S and AoPR1. *Agrobacterium* ability is dependent on the activation of vir genes along with chvA gene to infect the host plant. Therefore, chvA act as a promoter for the attachment of host cell to bacteria (Douglas et al. 1985). PCR assay of transformants shows product size of about 890 bp using chvA gene-specific primers if tested plants have been contaminated with *Agrobacterium* (Nain et al. 2005). Results did not reveal any amplification of such fragment indicating that transgenic plants are free of any *Agrobacterium* contamination.

Quantitative real-time PCR (qRT-PCR) was used to analyze the transcript levels of cryIAc in primary transformants. Results showed ninefold high cryIAc transcript levels in transgenic plants (Fig. 4). The plants transformed with cryIAc under the control of AoPR1 showed comparatively less transcripts as compared to plants with 35S promoter. Transgenic plants showed accumulated levels of cryIAc protein when subjected to ELISA. The expression level of cry protein varied between 0.1 and 0.4 µg/g of fresh tissue weight (Fig. 5) among different plants. Expression level of cry protein in plants with 35S promoter was slightly higher than plants with cryIAc under the control of AoPR1 promoter.

The tubers from primary transformants were harvested from green house and three tubers from each plant were sown in pots to analyze presence and expression of cryIAc and bar genes in subsequent progeny. The amplification of internal fragments in PCR assays showed transgenic plants from first tuber generation plants showing the successful transfer of introduced gene(s) in subsequent progeny (Fig. 6).

Fig. 2 Different steps of genetic transformation of Marabel cv. **a** Calli induction on optimized regeneration selection media. **b** Regenerating callus. **c** Putative shoots on selection media. **d** View of transgenic plants in green house

Table 2 Regeneration response of leaf as well as internodal explants of Marabel transformation efficiency

Explants	No. of explants	No of resist- ant calli (%)	No. of shoots/ explant	Rooting efficiency (%)	No. of PCR positive plants	Transformation efficiency (%)
Leaf discs	300	20	2.0	100	5	1.66
Internodes	300	70	4.5	100	7	2.33

Likewise, ELISA assays showed that *cryIAc* gene is being properly translated to proteins though the protein quantification varied among transgenic plants (0.02–0.05 µg/g of fresh tissue weight). The levels of cry proteins in plants transformed with *cryIAc* gene under the control of 35S promoter were relatively higher (Fig. 7).

The efficacy of *bar* gene in transgenic plants was assayed by the application of Basta®. The results revealed the typical symptoms of Basta application on transgenics as well as control plants (Fig. 8). The control plants started showing clear symptoms of chlorosis after the third days of Basta application, dried and finally died, whereas transgenic plants showed tolerance to herbicide treatment and leaves,

recovered later on. It is noteworthy here that transgenic plants having *cryIAc* with AoPR1 could not show tolerance to Basta application (Fig. 9).

Leaf bioassay with first instar CPB larvae showed 100% mortality of CPB in both transgenic plants expressing *cryIAc* gene under the control of 35S and AoPR1 promoters (Table 3). The second instar larvae when fed to transgenic plants, 20–80% mortality of larvae was recorded in plants expressing *cryIAc* gene under control of 35S promoter, whereas 10–60% mortality of larvae was recorded in plants with AoPR1. 35S-P2 transgenic plants exhibited 80% mortality of larvae after 72 h of experiment, while AoPR1-P3 plants showed 60% mortality. Up

Fig. 3 PCR assays of primary Marabel transformants. **a** Results showed the amplification of internal fragment (690 bp) of *cryIAC* gene. Lane M: 1 Kb plus ladder (Thermoscientific), Lanes 1–6: putative transgenic plants Marabel, Lane N: negative control, Lane P: positive control pTF101 plasmid, **b** PCR assay showed the amplification of internal fragment of *bar* gene, Lane M: 1 Kb plus ladder (Thermoscientific), Lanes 1–6: primary transformants of Marabel, Lane N: negative control, Lane P: positive control, **c** PCR results showed no amplification of *ChvA* gene in primary transformants indicating no any agrobacterium contamination. Lane M: 1 kb plus DNA ladder, Lane N: negative control, Lane P: agrobacterium chromosomal DNA as positive control, Lanes 1–6: primary transformants of Marabel

to 65% mortality of the third instar of CPB was recorded in transgenic plants with 35S, while only 10% mortality was recorded in AoPR1 plants. 35S-P1 transgenic plants showed 65% mortality of the third instar larvae after 72 h of feeding. Leaf biotoxicty assays with the fourth instar larvae of CPB showed less mortality in case of both types of transgenic plants (Table 3). Leaf biotoxicty assays with the first and second instar larvae of PTM showed more mortality rates 50–100% in both types of plants (Fig. 10; Table 4); transgenic plants with 35S showing more mortality levels of PTM.

Fig. 4 Analysis of *cryIAC* transcripts in primary transformants of Marabel by real-time PCR. Data represents means and standard errors of three replications. EF1 α gene was used as internal control to normalize data and gene expression has been indicated as a fold-increase relative

Fig. 5 Putative transgenic plants of Marabel (T0) were subjected to enzyme-linked immunosorbent assay (ELISA) to estimate amount of *cryIAC* protein produced. Amount of toxin varied among different transgenic plants, though providing protection against targeted insect pest

Discussion

The transgenic technology has resulted in improved farm yield, since the commercialization of transgenic crops that express gene(s) of economic traits like insect pests and herbicides (James 2016). Transgenic potatoes expressing *cry3A* against CPB were introduced in market in mid 90 s in USA; however, low sales, buyer focus and constant debate about GMOs resulted in withdrawal of product from the market (Cooper et al. 2004). However, current market trends may again lead to use of such cultivars to tackle the increasing pest pressure and other crop related concerns. Though not commercialized yet, there are some reports for incorporation of *bar* gene to potato genome (Figueira et al. 1994; Soto et al. 2007).

Fig. 6 PCR assays of first tuber generation Marabel plants. **a** Results showed the amplification of internal fragment (690 bp) of *cryIAC* gene. Lane M: 1 Kb plus ladder (Thermoscientific), Lanes 1–6: putative transgenic plants Marabel, Lane N: negative control, Lane P: positive control pTF101 plasmid, **b** PCR assay showed the amplification of internal fragment of *bar* gene, Lane M: 1 Kb plus ladder (Thermoscientific), Lanes 1–6: primary transformants of Marabel, Lane N: negative control, Lane P: positive control

Fig. 7 Estimation of *cryIAC* protein levels in first generation tuber transgenic plants of Marabel

Although there are reports of insect resistant potato, but these are either against CPB or PTM separately (reviewed in Halterman et al. 2015). In the present study, by introducing *cryIAC* (under the control of two different promoter) and *bar* gene (as plant selectable marker), we found appreciable mortality rates of CPB and PTM in leaf bioassays. Besides that, transgenic plants also showed tolerance to the application of Basta.

The dicotyledonous plants have been subjected to *Agrobacterium* mediated widely (Bakhsh et al. 2014). In present

study, EHA105 strain of *Agrobacterium* harboring recombinant plasmids was used to infect leaf disc and internodal explants of Marabel cultivar using protocol as described by Beaujean et al. (1998) with modifications adopted in our laboratory. Overall transformation efficiency in present study was recorded less (Table 2) as compared to earlier report of potato transformation (Soto et al. 2007; Veale et al. 2012; Hameed et al. 2017). The various factors, such as the type of vector, *Agrobacterium* strain, explant type, and varietal genetic background have been reported to affect the *Agrobacterium*-mediated efficiency of potato transformation (Rao et al. 2009). A total of 12 PCR positive plants belonging to 35SpTF101 and AoPRIpTF101 were transferred to the greenhouse. The transgenic plants grew well, while no abnormal phenotypes were observed. Three plants from each construct were randomly selected for further analysis.

The randomly selected PCR positive plants were analyzed further to estimate transcript levels of *cryIAC* gene (Fig. 4). 35S-P3 transgenic plant showed more than eightfold expressions as compared to others; overall expression levels were different among transgenic plants that can be attributed to different transformation events in different transgenic plants. The nucleotide sequence of the gene or the insertion point of the gene in the DNA of the transgenic variety, transgene copy number, and other related factors (Rao 2005; Bakhsh et al. 2018). Furthermore, it also led to varied *cry* protein expression among transgenic plants. The further investigation of these plants at molecular, genetic, as well as physiological levels will help in understanding the differential expression of transgenes in our future studies.

35S CaMV is widely used promoter in commercialized transgenic crops as well as for other research purposes. Being constitutive in nature, 35S promoter induces expression of foreign gene all tissues types during plant growth and development (Mi et al. 2015; Anayol et al. 2016). In the present study, *cryIAC* expression was also studied under the control of wound specific AoPRI promoter. Expression levels of *cryIAC* were recorded more under 35S promoter as compared to AoPRI that are contrary to the results earlier obtained Ahmed et al. (2017) and Anayol et al. (2016).

The transgenic plants with 35S promoter when subjected to Basta application as per recommended dose showed enhanced tolerance (Fig. 8). The control plants showed more sensitivity, starting from yellowing of young leaf tissues and finally death after seventh day of Basta application. The transgenic plants with 35S after showing typical symptoms of chlorosis and leaf injury, exhibited tolerance to herbicide treatment and recovered, whereas plant with AoPRI promoter could not be recovered. Less tolerance in AoPRI transgenic plants can be attributed to the lower expression of *bar* that needs to be further explored.

It is generally believed that *cryI* toxin shows efficacy against lepidopterans (Bravo et al. 2007); however, few

Fig. 8 Overview of transgenic plants along with control after 7th day of Basta application. Transgenic plants showed typical symptoms of Basta application

reports suggest that direct and/or indirect exposure via predation on primary consumers like lepidopterans may result in stunted growth in coleopteran predators (Dhillon and Sharma 2009). Our results showed significant mortality (up to 100%) of the first and second instar larvae of CPB when exposed to cry1Ac-expressing plants. Likewise, Yüceer and Kayım (2012) reported approximately 80% mortality of CPB due to cry1Ac expression. The higher mortality in the first and second instar larvae can be attributed to the lower metabolism and higher susceptibility of earlier instars (Ferro and Lyon 1991). However, the decreased mortality rates of the third and fourth instar CPB larvae were recorded. This phenomenal change in activity of Bt *cry 1Ac* can possibly due to the difference in bioactivity of digestive proteinase during different growth stages of the CPB. Our findings are comparable to the results of Michaud et al. (1995) who found that earlier instars were more prone to rice cysteine proteinase inhibitor oryzacystatin I as compared to the third and fourth larval instars. According to them, a unique digestive proteolytic system is found in growing stages of CPB

which became more active during greater instars. As activity of cry1Ac toxin is already been reported against lepidopterans (Selale et al. 2017; Bakhsh et al. 2018); therefore, higher mortality rates of PTM were recorded in our studies which are in agreement with earlier reports by Veale et al. (2012), Estrada et al. (2007) and Davidson et al. (2004).

Conclusion

We report here the development of transgenic potato lines expressing *cry1Ac* gene under the control of 35 and AoPRI promoter that showed resistance to Colorado potato beetle (*L. decemlineata* Say) and Potato tuber moth (*P. operculella* Z.) that are devastating insect pests of Potato. Besides that, transgenic lines with 35S promoter also showed appreciable tolerance to the application of Basta®. The transgenic lines can be effectively used parental material in an efficient potato breeding program.

Fig. 9 Leaf biotoxicty assays of transgenic Marabel plants fed to the first, second, third, and fourth instars of CPB larvae. Transgenic plants exhibited appreciable level of resistance against CPB larvae

Table 3 Leaf biotoxicity assays of different transgenic plants against the first, second, third, and fourth instar CPB larvae

Transgenic plants	1st instar (h)			2nd instar(h)			3rd instar(h)			4th instar (h)		
	24	48	72	24	48	72	24	48	72	24	48	72
Control	0.00b	0.00b	0.00b	0.00c	0.00c	0.00c	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
35S-P1	100.00a	100.00a	100.00a	40.00ab	40.00ab	50.00b	40.00a	65.00a	65.00a	20.00a	20.00a	20.00a
35S-P2	100.00a	100.00a	100.00a	20.00bc	60.00a	80.00ab	0.00b	20.00b	20.00b	10.00ab	10.00ab	10.00ab
35S-P3	100.00a	100.00a	100.00a	60.00a	60.00a	100.0a	0.00b	20.00b	20.00b	10.00ab	10.00ab	10.00ab
AoPR1-P1	100.00a	100.00a	100.00a	10.00bc	10.00bc	50.00b	0.00b	10.00b	10.00b	10.00ab	10.00ab	10.00ab
AoPR1-P2	100.00a	100.00a	100.00a	0.00c	20.00bc	50.00b	0.00b	10.00b	10.00b	0.00b	0.00b	0.00b
AoPR1-P3	100.00a	100.00a	100.00a	20.00ab	30.00abc	60.00b	0.00b	10.00b	10.00b	0.00b	0.00b	0.00b

Numbers with same letters within column are not significantly different from each other according to LSD test at 5% level of significance

Fig. 10 Leaf biotoxicity assays of transgenic Marabel plants fed to third instar of PTM larvae. Appreciable mortality levels of PTM were recorded**Table 4** Mortality rates of second and third instar PTM larvae when fed to transgenic plants

Transgenic plants	2nd instar larvae (h)			3rd instar larvae (h)		
	24	48	72	24	48	72
Control	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
35S-P1	0.00b	60.00b	100.00a	0.00b	50.00b	100.00a
35S-P2	0.00b	60.00b	100.00a	0.00b	50.00b	100.00a
35S-P3	0.00b	100.00a	100.00a	0.00b	100.00a	100.00a
AoPR1-P1	0.00b	0.00b	60.00b	0.00b	0.00b	50.00b
AoPR1-P2	0.00b	0.00b	60.00b	0.00b	0.00b	50.00b
AoPR1-P3	0.00b	0.00b	60.00b	0.00b	0.00b	50.00b

Numbers with same letters within column are not significantly different from each other according to LSD test at 5% level of significance

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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