EVALUATION OF TRANSGENIC POTATO LINES RESISTANCE TO POTATO TUBER MOTH, *Phthorimaea operculella* (ZELLER)

A. MOHAMMED, E. METRY, I. M. ISMAIL, H. EL-SHISHTAWY, GH. A. GAD EL-KARIM AND T. NASR EL-DIN

Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, 9 Gamaa street Giza, Egypt

The potato, *Solanum tuberosum* L., is one of the world's most important food crops, following rice, wheat, and maize (Ross, 1986). In Egypt, the production reaches up to 3 million metric tons annually. Potato is also the leading vegetable export in Egypt, with '225,000 metric tons exported to the European market. The potato tuber moth, *Phthorimaea operculella* (Zeller), (PTM) is the most serious insect pest of potatoes worldwide. It is probably originated in South America, where the potato also originates (Goldson and Emberson, 1985). In the field, the moths lay their eggs on the potato foliage and the larvae mine the foliage and the stems. This feeding damage extends to storage, reducing potato quality and increasing the potential for pathogen infection. In India, tuber damage in storage ranges from 25 to 100% (Saxena and Rizvi, 1974) and tubers are 100% damaged in 1.5 to 2 months in Tunisia (Essamet *et al*., 1988) while in Egypt, the potato losses reach up to 100% in fields as well as in storage (Ahmed *et al*., 2013). In Sudan, 30-40% of the potatoes are stored in underground pits which could be completely destroyed by tuber moth within 2 months (Ali, 1993).

The *Bacillus thuringiensis* Crystal proteins are non-toxic to humans, animals, birds, and most beneficial insects and have been used as biological insecticides to control agricultural insect pests (Koziel *et al*., 1993). However, field applications of this biological pesticide have not always provided a cost-effective control of insect pests. 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). Jansens *et al*. (1995) was the first to develop Bt-transgenic potato lines resistant to PTM using both native and modified truncated *cryIA(b)* gene. Further potato transgenic lines expressing truncated *cryIA(b)* gene was developed and evaluated by Canedo *et al*. (1999). Field trials of transgenic lines were carried out in two Latin America countries; San Ramón (Bolivia) in 1995 and Tacna (Peru) in 1997-1998. In both trials, transgenic potato lines showed a high resistance level against PTM attack.

Three potato cultivars, 'Lemhi Russet', 'Atlantic' and 'Spunta', were transformed with five different vectors encoding *Bt-cry5* gene under different

promoters (Douches *et al*., 1998; Mohammed *et al*., 2000). The 'Spunta' transgenic tubers expressing the *Bt-cry5* under the patatin element were not successful whereas the mortality range was 25.6 and 31.1%. Two out of three Spunta lines transformed with 35S/*Bt-cry5* gene construct (G2 and G3) showed 100% mortality and other two lines with the constitutive Gelvin super promoter (S1 and S4) had the same mortality level. Furthermore, field trials on *Bt-cry5* transgenic potato lines were carried out in three different locations; one in the USA (Montcalm Research Farm at Michigan) and two in Egypt (AGERI at Giza and CIP at Kafr El-zayat), between 1997 and 2001 (Douches *et al*., 2004). None of the five *Bt-cry5*-Atlantic lines were significantly different in potato PTM damage from 'Spunta', whereas most of the *Bt-cry5*-Spunta lines were different. 'Spunta G2', 'Spunta G3', 'Spunta-S1', 'Spunta-S4' and 'Spunta 6a-3' were free of PTM infestation. The 'Spunta G2' was selected for commercialization, therefore, field trials were performed in seven locations in South Africa over five years (Douches *et al*., 2010; Visser *et al.*, 2013) and food safety and biosafety issues were also discussed (Douches *et al*., 2008).

The food sand feed safety for 'Spunta G2', 'Spunta G3' and 'Spunta 6a-3' were previously studied by Metry *et al*. (2008). The amino acids profile as well as vitamins and minerals contents of the three transgenic lines don't significantly different from Spunta. The 'Spunta G2'is extensively studied for commercialization by Douches' group, therefore, it is excluded from the field study. Herein, both 'Spunta G3' and 'Spunta 6a-3' are studied for their resistance to PTM infestation and agronomical performance in the field before proceeding towards registration for local commercial release.

MATERIALS AND METHODS

In vitro micropropagation

Three transgenic potato lines ('Spunta G2', 'Spunta G3' and 'Spunta 6a-3') resistant to PTM and non-transgenic 'Spunta' as control were micropropagated *in vitro* using nodal cutting technique as described by Roca *et al*. (1978). MS salts medium (Murashige and Skoog, 1962) was used as a basal medium supplemented with 3% sucrose, 0.4 mg/l thiamin-HCl, 2 mg/l calcium pantothenate, 1 ml of silver thioslfate solution STS (0.1 M silver nitrate and 0.1 M sodium thioslfate, with ratio of 1:4), 100 mg/l myoinsoitol and 1 mg/l gibberellic acid (GA_3).

Adaptation and acclimatization of transgenic potato lines and control were carried out in the Bio-containment greenhouse facilities at AGERI according to Metry *et al*. (2003).

In vitro tuberization

The micropropagated plantlets were transferred to first liquid medium containing MS salt components supplemented with gibberellic acid (GA_3), 20 g/l sucrose, 500 µl benzyl adenine (BA), 10

µl naphthalene acetic acid (NAA). The plantlets were then transferred into second liquid medium consisted of MS medium supplemented with 80 g/1 sucrose, 5 ml benzyl adenine (BA), 1 mg/l, chlorocholine chloride (CCC) for 50 days in complete dark (Huiqun, 2009). The medium encourage and support the *in vitro* micro tuber formation. The microtubers were harvested and sorted out into small and large sizes and the length and width of tubers were determined and analyzed used standard error method.

Molecular analysis

PCR: Genomic DNA was extracted from the leaves of three transgenic potato lines ('Spunta G2', 'Spunta G3' and 'Spunta 6a-3') as well as non-transgenic 'Spunta' using DNAeasy plant extraction kit (Qiagen, USA) as described in the manufacturer's instructions provided with kit. A fragment of 732bp *cry5* gene was amplified using the gDNA extracted from the three transgenic lines and specific primer; Cry5s-476F; 5'-GCAGCGTGGTGAAGAGCA-3' and Cry5s-1208R; 5'-ACACGTCGCG GCTGG TGAAG-3'. The reaction conditions included 40 cycles of, denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1.5 min. An aliquot of 10 µl PCR product were analyzed on 1% agarose gel.

RT-PCR: Total RNA was extracted from the leaves of the three lines as well as non-transgenic using SV total RNA isolation system (Promega, USA) as described in the manufacturer's instructions provided with kit. The total RNA from each line was reverse transcribed using a SuperScript ™ II Reverse transcriptase (Invitrogen, USA) according to the manufacturer's instructions. The RNA template was removed from the 1st stand cDNA by 2 units of *E. coli* RNaseH (Promega) for 20 min at 37°C. The 1st strand cDNA was mixed with 2.5 units of Taq polymerase (Promega) in 1x reaction buffer, 0.5 M dNTPs, 25 mM $MgCl_2$ and 25 pmoles primers. The amplification conditions were 94°C for 2 min followed by 40 cycles of 94°C for 15 sec, 60°C for 15 sec and 72°C for 30 sec followed by 4 min at 72°C. The RT-PCR amplification was performed for using *cry5*-specific primer set mentioned is the previous section.

AFLP: Genomic DNA was prepared from the leaflets of each transgenic and control line. DNA extraction, purification and bulking were performed as described by Hussein *et al.* (2005).

The AFLP procedure was performed, with minor modifications, accord-

ing to the protocol supplied with the AFLP Analysis System I (Invitrogen, USA) manual. Approximately 400 ηg of genomic DNA of each of the four samples was digested with *Eco*RI and *Mse*I at 37°C for 2 hours. *Eco*RI and *Mse*I adaptors were ligated to the digested DNA samples to generate template DNA for amplification. Pre-amplification was carried out using 5 µl of diluted ligation products with 5-primer combinations each carrying one selective nucleotide with the following conditions, 20 cycles of 94°C for 30 sec, 56°C for 60 sec and 72°C for 60 sec. Five microliters of 50 times diluted PCR products was used for selective AFLP amplification using *Eco*RI and *Mse*I adaptor-specific primer set, each carrying three selective nucleotides. The PCR selective amplification condition was as follows: one cycle of 94°C for 30 sec, 65°C for 30 sec, and 72°C for 60 sec followed by 12 cycles of touchdown PCR in which the annealing temperature was decreased by 0.7°C every single cycle until annealing temperature goes down to 56°C. Once the annealing temperature at 56°C, another 23 cycles of pre-amplification conditions were applied to the reaction. Two µl of the reaction product was mixed with an equal volume of formamide loading buffer (98% [v/v] formamide, 10 mM EDTA, 0.005% [v/v] of each of xylene cyanol and bromophenol blue), denatured by incubating at 90°C for 5 min and quickly cooled on ice. The products were analyzed on 6% (w/v) denaturing polyacrylamide gels. The gel was run at constant power (50-55 W) until the xylene cyanol was about two-thirds down the length of the gel. The gel was silver stained using Silver Sequence DNA Staining Reagents (Promega, USA) according to the protocol described by the manufacturer.

Field trials

Two transgenic lines (‘Spunta G3’ and ‘Spunta 6a-3’) along with non-transgenic potatoes were field tested to evaluate resistance to potato tuber moth and assess their morphological characteristics. Field Trial was carried out between November, 2011 and April, 2012 at AGERI farm, Giza, Egypt.

The experiment was three plot replicates, each with seven rows of 15 plants. Each row is 3 m long, 0.5 m space among adjacent rows, and 0.2 m plant-spacing. The transgenic lines as well as the control line were randomly distributed among the replicates; however, the tested line was planted in ranked spot only once. Plant growth was evaluated by plant height and branches and yield data was also recorded.

The field experiment was artificially challenged with PTM by seeding the plots with infested tubers. Potato tubers were infested at the lab, 25 neonate larvae were added to each tuber and allowed to grow at the room temperature. After 12 days, the infested tubers were collected and disturbed in the field. Three infested tubers were added to each row in the vicinity of plants. The experiment was PTM-challenged twice, the first challenge during the first week of March and repeat-

ed four weeks later. At harvest, tubers were evaluated for PTM damage by checking infestation symptoms of the tubers.

RESULTS AND DISCUSSION

In vitro micropropagation

The transgenic lines; 'Spunta G2', 'Spunta G3' and 'Spunta 6a-3' as well as non-transgenic 'Spunta' were successfully micropropagated *in vitro* using nodal cuttings technique. The method utilize MS basic salt medium supplemented with gibberellic acid (GA_3). Nodal cuttings were sub-cultured repeatedly on a fresh medium after 3 weeks. The appearance of *in vitro* plantlets was normal.

In vitro tuberization

The microtubers were harvested and sorted out according to size (Fig. 1). Both length and width of each size were measured and recorded (Table 1). The data was analyzed using standard error method. Results revealed that there is no significant difference of the microtuber dimensions among the three transgenic lines compared to non-transgenic potatoes. Both length and width of large and small sizes are represented in Fig. (2). The length of 'Spunta 6a-3' microtubers is smallest among the lines of both small and large size of 0.64±0.17 and 1.21± 0.26, respectively. The 'Spunta G3' shows slightly tallest small size microtubers of 0.71±0.14 while tallest large size microtubers belong to control 1.38±0.35. Other dimension parameter which is the microtuber's width showed almost the same dimension for the small size 'Spunta' control and 'Spunta G3'. Morphological characteristics of the microtubers are within the normal range of variability compared to control plants.

PCR and RT-PCR

Both PCR and RT-PCR analysis were performed on gDNA and mRNA, respectively, prepared from transgenic lines as well as 'Spunta' cultivar. All PCR and RT-PCR reactions of *cry5*-transgenic lines resulted in positive amplifications of 732 bp using *cry5*-specific primers while non-transgenic showed no PCR product (Fig. 3A & B). These results indicate the presence of active transcripts of *cry5* gene within tissues of 'Spunta G2', 'Spunta G3' and 'Spunta 6a-3' and its lack within 'Spunta'. PCR analysis was previously used to detect both *cry1A(b)* and *nptII* genes within transgenic potatoes against PTM (Hagh *et al.*, 2009) while southern bolt was selected to detect the same gene in different transgenic potatoes (Canedo *et al*., 1999). PCR analysis was also used in segregation and efficacy studies for *cry* gene in 'SpuntaG2' (Douches *et al.*, 2013).

AFLP

The banding patterns generated by AFLP analyses were examined to determine the level of polymorphism and the genetic relatedness between control and the three transgenic plants using five selective primer combinations. The amplified fragments were scored as present (1)

or absent (0). The size of AFLP fragments generated by the different primer combinations ranged from 50-500 bp (Fig. 4). A total number of 234 bands were generated from 5 AFLP primer combinations (Table 2) ranged from 43 for 8/7 to 52 for 8/8. The primer combination 4/5 revealed no polymorphic variations among the three lines and the control. On the other hand, the lowest number of polymorphic amplicons (one band) resulted from both primer combinations 8/3 and 8/8. While the highest number of polymorphic amplicons (5 bands) was observed in primer combination 8/1. The percentage of polymorphism ranged from 0% to 10.6% with a total of polymorphism 3.8%. The topology of dendrogram (Fig. 5) derived from AFLP data clustered the control and the transgenic 'Spunta G2' together (98%) while 'Spunta G3 and 'Spunta 6a-3' in another group (99%). AFLP analysis was previously used in different transgenic plants to detect genomic changes due to transformation with foreign gene. The genome similarity between non-transgenic control and six Bt-transgenic rice cultivars is more than 97% (Chandel *et al.*, 2010). They also concluded that "the transgenic plants developed by *Agrobacterium tumefaciens* infection than that of developed through particle bombardment". Similar results were also revealed by AFLP that showed limited genomic changes among three transgenic sugarcane populations produced by *Agrobacterium* infection and non transgenic sugarcane (Carmona *et al.*, 2005).

Field trials

AGERI is located within populated area and away from cultivated lands; therefore the agricultural insect pest population is too low to cause noticeable damage. To overcome lack of natural infestation of PTM, the *cry5*-transgenic potato lines and the control were artificially infested twice with PTM-infected tubers in the field. Assessments of field challenge of both 'Spunta G3' and 'Spunta 6a-3' showed high resistance level against PTM attack, only 3% of the tuber yield is susceptible to larval penetration (Table 3). Results from field data showed the similar trends as those from laboratory assay, field trials and during storage experiments. Previous results showed that resistance of the transgenic lines to PTM infection reach to 100% (Mohammed *et al*., 2000, Douches *et al*., 2004; Randon *et al*., 2009; Douches *et al*., 2010).

The high mortality level of these transgenic lines is due to high expression level of the codon modified *Bt-cry5* that is regulated by the constitutive CaM35S promoter. Constitutive promoters have been deployed to drive *cry* gene expression in transgenic potatoes resistant to PTM (Canedo *et al*., 1999; Douches *et al.*, 1998, 2002 and 2013; Mohammed *et al.*, 2000). However, other inducible promoters such as light-inducible promoter, Lhca3, maize light-inducible promoter, PEPC, (Meiyalaghan *et al.*, 2006) and Green-tissue-specific, C4-PEPC-promoter (Hagh *et al.*, 2009) were also used to regulate *cry* genes for resistance to the same pest.

Because the field trials were based on planting microtubers, the yield for both transgenic and control is low compared to regular size tubers. The phenotypic variations with respect to plant height and leaf length/weight (L:W) ratio was hard to be determined. The agronomic performance is based on tubers yield of the control and the two transgenic lines. The average of tubers yield of 'Spunta G3' and 'Spunta 6a-3' showed no significant differences compared to that of 'Spunta' (Table 4). Previous agronomic trials in Michigan and Egypt showed that many of the *Bt-cry5* transgenic lines perform similarly to their non-transformed cultivar (Douches *et al*., 2004) and the same trend of results was observed for 'Spunta G2' (Douches *et al*., 2010). However, large scale agronomic trials and more parameters such as total yield/feddan, tuber size category, and specific gravity should be considered in future field trials.

The genetically enhanced potato lines undergo the mandatory pathway determined by the National Biosafety Committee which includes environmental risk assessment and food safety. This type of assessment includes; molecular characterization to determine the inserted gene and the expressed protein, nutritional composition, toxicological and histo-pathological evaluation and allergenicity tests. Moreover, the effects of genetically modified potato lines on the microbial communities in different types of soil and its impact assessment on soil ecosystem are also considered. Molecular analysis of the inserted gene and field trials are presented in the current paper while other parameters to be evaluated are in process.

SUMMARY

The potato tuber moth, *Phthorimaea operculella* (Zeller), is the major destructive insect of potato. The larvae attack foliage and tubers in the field and in storage as well. Over the past years, there was a tremendous effort to produce transgenic potato cultivar that is resistant to the larval infestation of potato tuber moth. 'Spunta' potato cultivar was previously transformed with *Bt-cry5* gene under 35S promoter construct. Three transgenic 'Spunta' lines, 'Spunta G2', 'Spunta G3' and 'Spunta 6a-3', were selected for their high resistance to PTM attack. After 14 years of storage, these three lines are subjected to *in vitro* micropropagation, tuberization and re-evaluation. Both PCR and RT-PCR confirmed the presence of *cry5* gene within 'Spunta' genome and its expression within potato plant tissues. Amplified fragment length polymorphism (AFLP) analysis revealed that the percentage of polymorphism among the three lines and non-transgenic ranged from 0% to 10.6%. Two lines 'Spunta G3' and 'Spunta 6a-3' were selected for field agronomical performances as steps for environmental safety as well as PTM challenge. Both lines are highly resistant to PTM compared to the control, only 3% of the yield shows susceptibility to larval penetration of the tubers.

REFRENCES

Ahmed, A. A. I., M. Y. Hashem, S. M. Mohamed and S. H. S. Khalil

(2013). Protection of potato crop against *Phthorimaea operculella* (Zeller) infestation using frass extract of two noctuid insect pests under laboratory and storage simulation conditions. Archives of Phytopathology and Plant Protection, 46: 2409-2419.

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, p. 297-315. *In* S. Kung and R. Wu [eds.], Transgenic plants, vol. 1. Engineering and utilization. Academic, San Diego, CA.

Carmona, E. R., A. D. Arencibia, J. Lopez, J. Simpson, D. Vargas and F. Sala (2005). Analysis of genomic variability in transgenic sugarcane plants produced by *Agrobacterium tumefaciens* infection. Plant Breeding, 124: 33-38.

Canedo, V., J. Benavides, A. Golmirzaie, F. Cisneros, M. Ghislain and A. Langnaoui (1999). Assessing Bt-transformed potatoes for potato tuber moth, *Phthorimaea operculella* (Zeller), management. CIP Program Report 1997-1989, CIP, Lima.

Chandel, G., K. Datta and S. K. Datta (2010). Detection of genomic changes in transgenic *Bt* rice populations through genetic fingerprinting using amplified fragment length polymorphism (AFLP). GM Crops, 1: 327-336.

Douches, D. S., A. L. Westedt, K. Zarka, and E. J. Grafius (1998). Transformation of CryV-Bt transgene combined with natural resistance mechanisms for resistance to tuber moth in potato (*Solanum tuberosum* L.) HortScience, 33: 1053-1056.

Douches, D. S., J. J. Coombs, K. J. Felcher, M. Zuehlke, W. Pett, Z. Szendrei and J. Brink (2013). Segregation and efficacy of the *cry1Ia1* gene for control of potato tuberworm in four populations of cultivated potato. J. Econ. Entomol., 106: 1025-1028.

Douches, D. S., J. A. Brink, H. Quemada, W. Pett, M. Koch, D. Visser, K. Maredia and K. Zarka (2008). Commercialization of potato tuber moth resistant potatoes in South Africa. Tropical Agriculture - Advances in Crop Research, 10: 139-147.

Douches, D., W. Pett, D. Visser, F. Santos, J. Coombs, E. Grafius, W. Li, E. A. Metry, T. N. El-Din and M. Madkour (2004). Field and storage testing *Bt* potatoes for resistance to potato tuberworm (*Lepidoptera:*

Gelichiidae). J. Econ. Entomol., 97: 1425-1431.

Douches, D. S., W. Pett, D. Visser, J. Coombs, K. Zarka, G. Bothma, J. Brink, K. Felcher, M. Koch and H. Quemada (2010). Field and storage evaluations of 'Spunta G2' for resistance to potato tuber moth and agronomic performance. J. Amer. Soc. Hort. Sci. 135: 333-340.

Essmate, M., R. Von Arex, P. Ewell, J. Goueder, A. B. Temine and M. Cheikh (1988). Aspects techniques et economiques du problem de la teigne et du stockage des pommes de terre de saison en Tunisia. Ann. Inst. Nt. Res. Agron. Tunis, 61: 1-50.

Goldson, S. L. and R. M. Emberson (1985). The potato moth *Phthorimaea operculella* (Zeller): Its habits, damage potential and management. Special Publ., Agron. Soc., N. Z., Christchurch, New Zealand, p. 61-66.

Hagh, Z. G., H. Rahnama, J. Panahandeh, B. B. K. Rouz, K. M. A. Jafari and N. Mahna (2009). Green-tissue-specific, C4-PEPC-promoter-driven expression of Cry1Ab makes transgenic potato plants resistant to tuber moth (*Phthorimaea operculella*, Zeller). Plant Cell. Rep., 28: 1869-1879.

Huiqun, W. (2009). Effects of chlorocholine chloride on phytohormones and photosynthesis characteristics in potato (*Solanum tuberosum* L). J. Plant Growth Regul., 28: 21-27.

Hussein, Ebtissam H. A., S. S. Adawy, S. E. M. Ismail and H. A. El-Itriby (2005). Molecular characterization of some Egyption date palm germplasm using RAPD and ISSR markers. Arab J. Biotech., 8: 83-98.

Jansens, S., M. Cornlissen, R. DeClercq, 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., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright and S. V. Evola (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis.* Biotechnology, 11: 194-200.

Metry, E. A., G. M. El-Moghazy, M. S. Masoud, M. H. Youssef, T. A. Omar, T. Yehya, S. A. Mahfouz, A. M. Shokry, M. F. Emara, A. S. Hamza, T. N. El-Din and H. A. El-

Itriby (2008). Nutritional and biological evaluation of transgenic potato lines resistant to potato tuber moth. Egypt. J. Genet. Cytol., 37: 141-152.

Meiyalaghan, S., J. M. E. Jacobs, R. C. Butler, S. D. Wratten and A. J. Conner (2006). Expression of *cry1Ac9* and *cry9Aa2* genes under a potato light-inducible Lhca3 promoter in transgenic potatoes for tuber moth resistance. Euphytica, 147: 297-309.

Mohammed, A. D., S. Douches, W. Pett, E. Grafius, J. Coombs, W. Liswidowati, W. Li and M. A. Madkour (2000). Evaluation of potato tuber moth (*Lepidoptera: Gelechiidae*) resistance in tubers of *Bt-cry5* transgenic potato lines. J. Econ. Entomol., 93: 472-476.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiological Plantarium, 15: 473-497.

Roca, W. M., N. O. Espinoza, M. R. Roca and J. E. Bryan (1978). A tissue culture method for the rapid propagation of potatoes. American Potato J., 55: 691-710.

Ross, H. (1986). Potato breeding and perspectives. Verlag PareyPress, Berlin, Germany.

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

Visser, D., W. Pett and D. Douches (2013). Developing Transgenic BT-Potato in South Africa: Experiences with Field Trial Protocols. Acta Hort., 974: 51-56.

Table (1): Length and width of both sized microtubers of transgenic lines and control.

Lines	Length		Width	
	Small	Large	Small	Large
‘Spunta’	0.66 ± 0.17	1.38 ± 0.35	0.42 ± 0.11	0.47 ± 0.10
‘Spunta G2’	0.69 ± 0.17	1.26 ± 0.26	0.65 ± 0.20	0.71 ± 0.19
‘Spunta G3’	0.71 ± 0.14	1.25 ± 0.28	0.42 ± 0.09	0.43 ± 0.12
‘Spunta 6a-3’	0.64 ± 0.17	1.21 ± 0.26	0.62 ± 0.14	0.64 ±0.16

Table (2): AFLP primer combinations.

Primer combinations code	Primer combinations sequences	Total	Poly	%
4/5	*Eco*R ACG/ /*Mse*I CAT	46	0	0.00
8/1	*Eco*R AGG/ /*Mse*I CAA	47	5	10.64
8/3	*Eco*R ACG/ /*Mse*I CAG	46	1	2.17
8/7	*Eco*R AGG/ /*Mse*I CTG	43	2	4.65
8/8	*Eco*R AGG/ /*Mse*I CTT	52	1	1.92
Total		234	9	3.85

Table (3): Potato tuber moth field trails.

Lines	Reps.	Total number of tubers	Infested Tubers	Mean of infes-tation ±SE	Lower/higher limits
‘Spunta’	1	354	19		
	2	214	75	4.85±0.31	4.54/5.17
	3	122	8		
‘Spunta G3’	1	157	5		
	2	188	3	0.28±0.02	0.25/0.31
	3	141	5		
‘Spunta 6a-3’	1	186	7		
	2	154	3	0.42±0.04	0.39/0.47
	3	126	4		

Table (4): Potato tuber yield from field trial.

Lines	Mean of tubers yield (grams /plant) ±SE	Lower/higher limits
‘Spunta’	44.1±0.86	43.21/44.94
‘Spunta G3’	45.9±0.76	45.18/46.71
‘Spunta 6a-3’	45.1±1.02	44.07/46.12

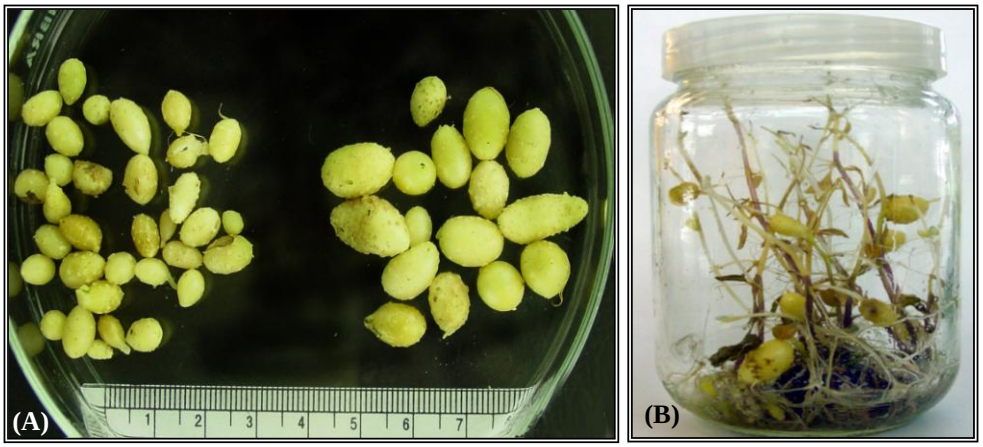


Fig. (1): (A) Microtubers are produced *in vitro* under controlled conditions and sorted out according to their size. (B) microtubers production under dark condition on second medium.

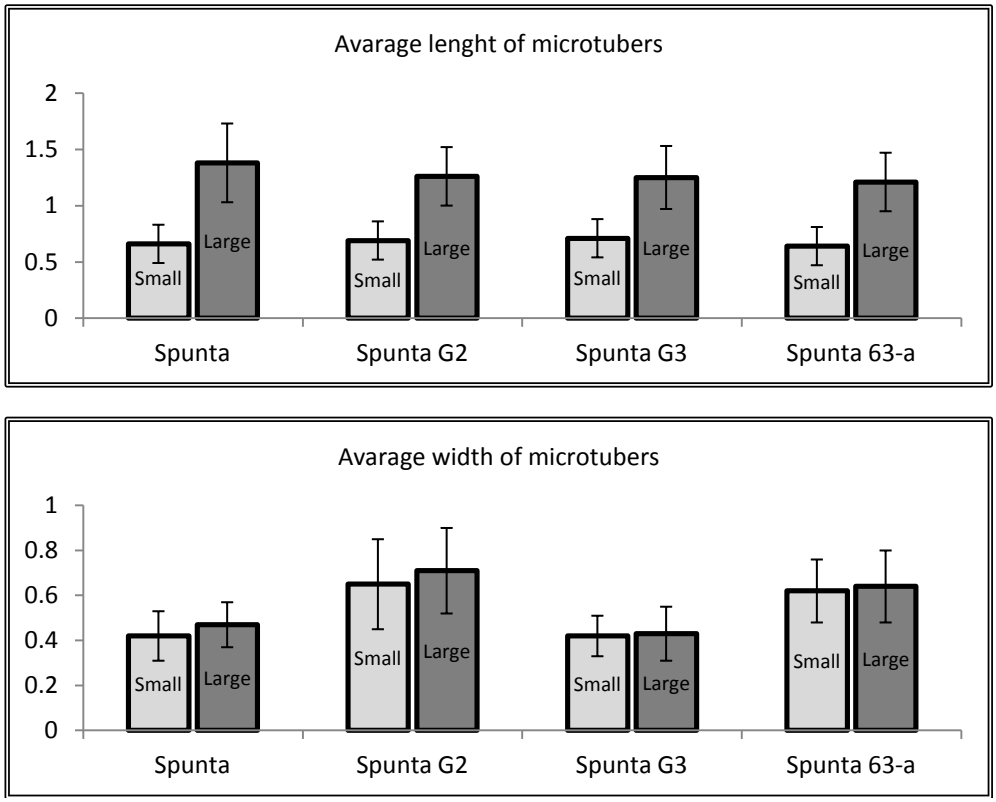


Fig. (2): Average length and width of small and large sized microtubers of the two transgenic potato lines of ‘Spunta G3’ and ‘Spunta-6a-3’ compared to the control ‘Spunta’.

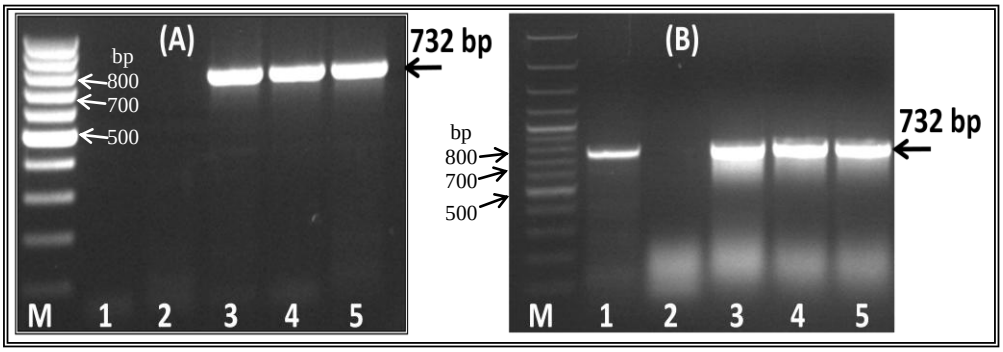


Fig. (3): PCR (A) and RT-PCR (B) analysis of three transgenic lines compared to non-transgenic potato line. The expected size of amplified products using cry5-specific primers is 732 bp. Lane M; 100 bp plus DNA ladder, lane 1(A); negative control using ddH_2O and specific primer set, lane 1(B); positive control using cry5 DNA as template, lane 2; non-transgenic 'Spunta' as a control and lanes 3 through 5 are transgenic potato lines, Spunta G2', 'Spunta G3' and 'Spunta-6a-3', respectively.

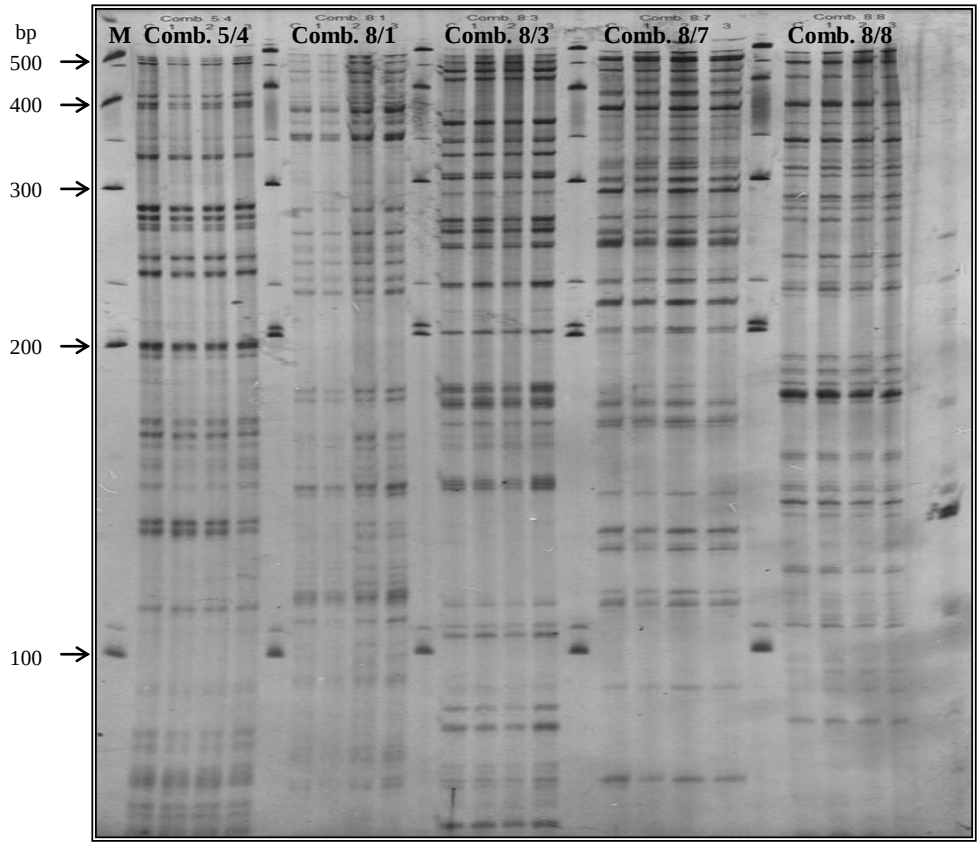


Fig. (4): AFLP profiles of the four potato samples (C: control, non-transgenic potato lines, 1, 2 and 3: transgenic potato lines (Spunta G2, Spunta G3 and spunta 6a-3)) as revealed by four primer combinations. M: 100 bp DNA ladder.

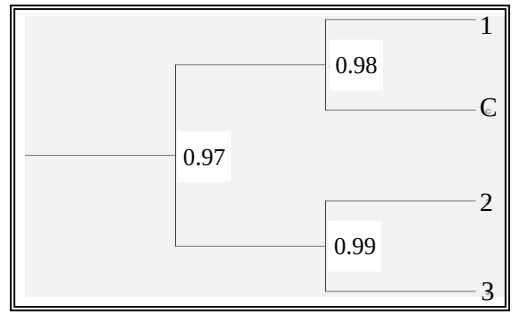
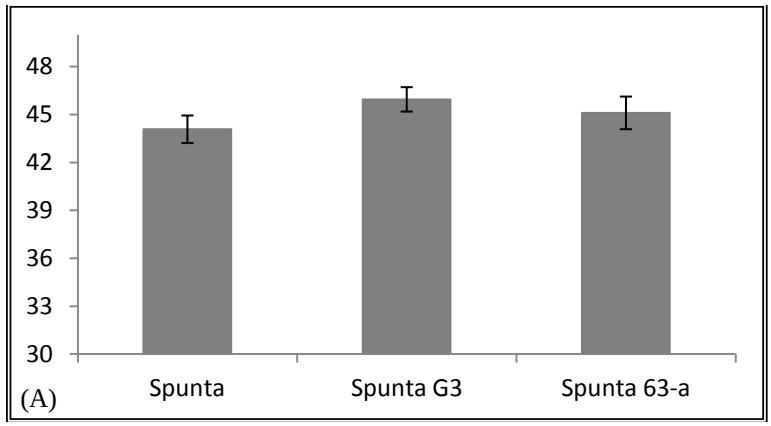


Fig. (5): Dendrogram of the four potato samples based on AFLP data; C: control (non-transgenic potato) and 1, 2 and 3 lines: three transgenic plants 'Spunta G2', 'Spunta G3' and 'Spunta 6a-3'.



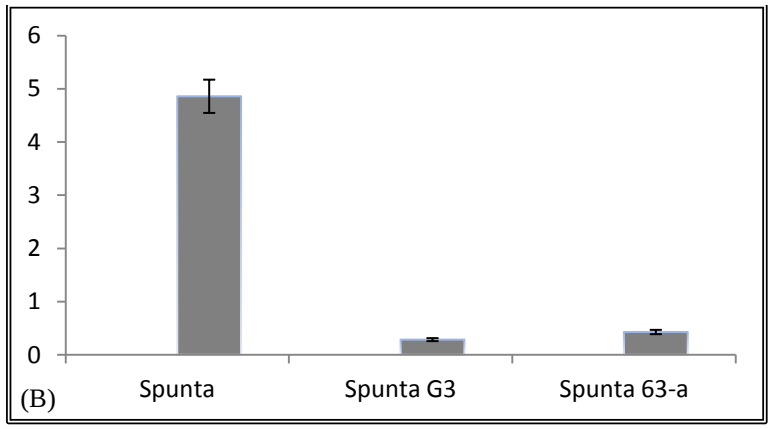


Fig. (6): Field trials of 'Spunta', 'Spunta G3' and 'Spunta 6a-3'; (A) tuber yield grass/plant for each line and (B) mean of infested tubers with potato tuber moth.