DETECTION OF GENETICALLY MODIFIED POTATO IN POTATO TUBERS CULTIVATED IN EGYPT

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Genetic modification (GM) was applied in modern plant breeding and has resulted in developing plants with enhanced agronomic and nutritional values. Modified crops have been manipulated through the introduction of new agronomic traits or by silencing of constituent genes involved in the susceptibility to diseases, pests or by increasing nutritional value, reducing toxins and improving desirable characteristics. Application of GM technology might have potential risks on the environment, altering of biodiversity, feasibility of the development of resistant insects and tolerant weeds. Therefore, the use of GM organisms elevates several biosafety issues. Consumers are increasingly concerned about the use of GMOs in food products, mainly in EU countries, while in North America the technology is more accepted (Deisingh and Badrie, 2005). The EU has been regulated the labelling of novel foods (Rott *et al*., 2004). James (2007) stated that the genetically modified crops were developed and commercialized in 1996. These crops were planted and commercialized in twenty-three countries with the total cultivation area reaching 114.3 million hectares. So far there has been a great increase in the surface area planted with transgenic crops.

According to the EU regulations the technique for the detection of GMO crops and foods became necessary to allow consumers to choose products and to comply with labelling regulations. Methods for detecting GMO crops are divided into three methods. The first involves nucleotide-based amplification methods, such as polymerase chain reaction. The second involves protein-based methods, such as enzyme-linked immunoassay (ELISA), and the third is

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the detection of enzymatic activities (Chiueh *et al*., 2001; Anklam *et al*., 2002).

The PCR based methods were widely applicable and could be applied for unprocessed and highly processed foods. Two types of PCR could be used for the examination; the traditional PCR, which is able to confirm the existence of GMO with the help of gel electrophoresis, and the real-time PCR that is capable not only to detect the GMO content but also to quantify it. Several PCR-based methods have been developed to detect and identify GMO in food and feed (Deisingh and Badrie, 2005; Elsanhoty *et al*., 2005 and 2006). Application of real-time PCR was applied for characterization of many crops such as corn, soybean, and GM potato *spunta* (Collonnier *et al*., 2005; Elsanhoty *et al*., 2005 and 2006; Ramadan and Elsanhoty, 2012; Elsanhoty *et al*., 2011).

Methods of detection of GM crops in raw and processed foods were reported in different countries. In Brazil, Cardarelli *et al*. (2005) analyzed food items for the presence of CaMV 35S promoter and NOS terminator. Elsanhoty *et al*. (2002 and 2013) analyzed soybean and maize samples from Egyptian and Kingdom of Saudi Arabia market. The results pointed out the presence of GM maize and soybean in the Egyptian and Saudi Arabia food market. Rott *et al*. (2004) found positive GMO soybean samples in the Canadian supermarkets using Qualitative and quantitative PCR-based methods to detect GM soybean (Roundup-ReadyTM soy) and maize (Bt176 Maximizer maize; Bt11 maize, MON 810 Yield Gard corn and T25 LibertyR Link maize) in Brazilian foods (Greiner *et al*., 2005).

Egypt relies mainly on imported crops and food (60-70%), control of these crops and food depends only on the nutritional content and the acceptable level of fungal toxins without paying attention to genetic manipulation. As a result, there are few reports on the existence of GM crops for human and / or animal consumption in Egypt. Therefore, this work was planned to detect and identify of GM potatoes tubers as seeds for cultivation in Egyptian fields during 2014-2015 by using construct-specific method for GMO detection.

MATERIALS AND METHODS

Plant material

Twenty-six potato genotypes (Adora, Flamenco, Asterix, Bartina, Cleopatra, Colomba, Desiree, Dynamica, Bildtstar, Sifra, Fortus, Liseta, Sylvana, Memphis, Mondial, Mozart, Panamera, Zin, Heros, Farida, Red scarlett, Red sun, Safari, Sophie, Spunta, Red valentine 808) have been kindly obtained from hzpc and De Nijs potato companies. These potato genotypes were grown in a farm of the Environmental Studies and Research Institute, University of Sadat City, Menofia, Egypt. These varieties were evaluated at morphological and molecular level in previous study (Khidr *et al*., 2017) and were used for DNA extraction,

detection and identification of genetic modifications depending on DNA based methods.

DNA extraction

About 250 mg of tubers of each genotype was ground in liquid nitrogen using pestle and mortar to the fine powder. The genomic DNA was isolated from the ground samples using CTAB modified method according to Dellaporta *et al*. (1981).

DNA yield and quality

The concentration and purity of extracted DNA were measured at 260 nm and 280 nm using an Ultraspec 2000 spectrophotometer. The extracted DNA concentration was adjusted to 20-25 ng/µL prior to PCR using deionised water.

Oligonucleotide primers

Primers used in this study together with their target specific part of the investigated DNA are listed in Table (1). All primers were synthesized by TIB MOLBIOL, Berlin, Germany and obtained in a lyophilized state. The primers were solved to obtain a final concentration of 20 pmol/µl of each.

DNA amplification and PCR condition

PCR was carried out on Techne TC-4000 Thermal Cyclers. The PCR reaction was performed in 25 ml containing 2.5 ml PCR buffer (10x), 2 ml $MgCl_2$ solution (25 mM), 1 ml dNTPs solution (0.2 mM each of dATP, dCTP, dGTP and dTTP), 0.5 mM of each primer, 1 Unit Taq DNA polymerase, 50 ng of DNA and was completed to 25 ml with nuclease free water. Table (2) shows the PCR condition (time/temperature profiles) used for each primer pair.

Gel electrophoresis

Agarose gel preparation as well as electrophoresis was carried out using Tris-base/borate (TBE) buffer. To determine the size of the DNA fragments, DNA of known size (50, 100 bp DNA ladder, Gibco BRL, USA) together with the different amplicons were separated on 1.5% w/v agarose gel. The amplicons were made visible by ethidium bromide staining and documented using UV transillumination.

Detection of multicopy chloroplast DNA

The primer pair A1/A2 (Table1) was used as a reference control for the ability of the DNA to be amplified because its specificity to detect multicopy chloroplast DNA with a fragment of 550 bp in size (Taberlet *et al*., 1991).

Detection of genetic modifications in potato genotypes

Three primer pairs were used to detect the genetic modification in potato samples. Oligonucleotide primer pairs sequences and their targets elements are listed in Table (1). The primer pair AC2-F/ AC2-R was used to detect the *ac2* gene

which increases resistance against fungal and bacterial infections in genetically modified potato and produces a fragment size at 145 bp (Liapkova *et al*., 2001). The primer pair CaMV35S-F/CaMV35S-R was applied to detect CaMV 35S promoter sequence and the expected DNA fragment size is 105 bp (Franck *et al*., 1980). The primer Spu-35S1-F/ Spu-cryVm-R was used to detect *Cry V* gene from *Bacillus Thuringiensis* in genetically modified potato and the amplicon size was 122 bp (Elsanhoty, 2004).

RESULTS AND DISCUSSION

DNA Extraction, Concentration, and Purity

DNA was extracted from potato samples based on the complexity of their composition and technology process. The DNA samples mostly contain ingredients acting as inhibitors to the PCR reaction such as fatty acid, polysaccharides, polyphenols, and others compounds that could interfere with the isolation of the DNA or even degrade it (Holden *et al*., 2003).

Quantification of the DNA was carried out on agarose gel for all samples to detect DNA fragment corresponding to the genomic DNA. The results showed that there were no differences in the DNA obtained from any genotypes of potato samples under study and high DNA yield was obtained. Moreover, the obtained DNA was free from the grade DNA damage. The higher molecular weight of the extracted DNA is an indicator for the quality of the DNA. These factors rely on the samples itself, and the physicochemical parameters of the extraction method (Elsanhoty *et al*., 2011; Peano *et al*., 2004). The CTAB extraction method applied here led to satisfying results after PCR with DNA extracted from potato samples under investigation and hence was suited for the next steps. The quality of extracted DNA from all samples was tested by control PCR using primer pair A1/A2 (Taberlet *et al*., 1991) which amplifies part of the chloroplast genome and yields an amplicon of 550 bp in size (Fig. 1).

Detection of genetic modifications in potato samples

- ***Detection of ac2 gene in genetically modified potato***

Ten samples of the 26 potato genotypes were positive for the existence of the *ac2* gene (Fig. 2). The percentage of the presence of *ac2* in the examined genotypes were 38.46% in genetically modified potatoes. These results were in agreement with the results obtained by Pribylova *et al*. (2007) who used the *ac2* gene to detect the genetic modification in Potato foodstuffs because of the genetic modification '*ac2*' is not allowed in the European Union market. Furthermore, our results were in harmony with the results obtained by Song *et al*. (2017) who detect unapproved genetically modified potatoes in Korea using multiplex polymerase chain reaction and found the GM potato event E12 in one of 33 samples. They recommended that the PCR method could be effectively used to distinguish between

unauthorized GM potatoes in Korea. Moreover, these results were in concurrence with previous work carried out on the distribution of GMO in commercial foods (Elsanhoty *et al*., 2002; Cardarelli *et al*., 2005; Greiner *et al*., 2005; Margarit *et al*., 2006; Park *et al*., 2010).

- ***Detection of Cry V and** CaMV35S **in genetically modified potato in potato samples***

The DNA of the 26 genotypes was examined for the presence of genetically modified potato using primers Spu-35S1-F/Spu-cryVm-R developed by Elsanhoty (2004). Data in Fig. (3) indicated that 12 samples (46.15%) from 26 genotypes were positive for the presence of that genetic elements, the vector (pSPUD5), including a gene cassette consisting of [CaMV35S promoter - Cry5-Bt gene - NOS terminator] (Mohammed *et al*., 2000). The results were in partial agreement with those obtained by Elsanhoty *et al*. (2013) who found genetically modified plants in food samples collected from Saudi Arabia and Song *et al*. (2017) who discovered unapproved genetically modified potatoes in Korea utilizing multiplex polymerase chain reaction and provided an effective approach for identification of unauthorized GM potatoes in Korea. Similarly, the obtained results were also in concurrence with those reported by Greiner and Konietzny (2008) who analyzed 100 Brazilian foods including maize for the presence of MON 810, Bt 11, Bt 176 and T25 events. They found 11 positive samples for GM maize, 4 out of 18 maize flour and 3 out of 18 polenta samples. The majority of the positive GM maize products were not of Brazilian origin. Continuously, the presence of GM maize was screened in 32 samples of foodstuffs commercialized in Argentinean markets, eight out of 32 showed positive results (Margarit *et al*., 2006). Park *et al*. (2010) found that most of the maize in the storage products from five Korean provinces was GM, wherein about 50% of the grains were germinated.

- ***Detection of CaMV 35S promoter sequence in potatoes***

Figure (4) shows the results of potatoes samples under investigation for the detection of CaMV 35S promoter sequence derived from the cauliflower mosaic virus. As shown in the Fig. all samples under investigation were positive for the presence of CaMV 35S promoter sequence in potatoes according to the procedure used in the detection. Data indicated that 100% of the samples exhibited positive CaMV 35S promoter sequence and PCR products were observed at 105 bp. Similar results were obtained in maize and soybean by Meriç *et al*. (2014) who demonstrated that all samples were transgenic for the presence of CaMV 35S promoter as a result of PCR-based method. From these results, we could conclude that may be there were horizontal gene transfers between the genetically modified plants and non-modified plants, and or the infection of

plants by the virus in the field. This could explain why all samples under investigation were positive for the presence of CaMV 35S promoter.

Final conclusion, It could be concluded that the method of DNA isolation was suitable for the most of potato samples. DNA was successfully isolated using CTAB method. The results indicated the presence of genetically modified potatoes in the Egyptian market. On the other hand, there was no information on the label to indicate that this genotype were genetically modified potatoes to give the consumers the chance to select the suitable product or seed. From the results, it could be concluded that the Egyptian government should issue strict rules to control all of the imported raw materials and food products. Building strong regulations and reference laboratories to test for the quality control of GM crops or foods is highly recommended.

SUMMARY

This work aimed to detect the genetically modified potatoes in Egyptian market. DNA based method was used to detect genetically modified potatoes in the collected genotypes. Three primer pairs were selected to detect the genetic modification. The results indicated that 38 % of the investigated genotypes were positive for genetic modification in *ac2* gene. Furthermore, 46% (12 Genotypes) were positive for the presence of genetically modified potato that contained CaMV promoter and cry V gene. In addition to 81% from the samples under investigation were positive for the presence of 35S promoter sequence in potatoes samples. From the obtained results we recommend the Egyptian government to establish strong regulations and certified laboratories to monitor GM crops or foods in the Egyptian market.

REFERENCES

Anklam, E., F. Gadani, P. Heinze, H. Pijenburg and V. G. Eede (2002). Analytical methods for detection and determination of genetically modified organisms in agriculture crops and plant derived food products. European Food Research and Technology, 214: 3-26.

Cardarelli, P., M. R. Branquinho, R. T. B. Ferreira, F. P. da Cruz and A. G. Gemal (2005). Detection of GMO in food products in Brazil: the INCQS experience. Food Control, 16: 589-866.

Chiueh, L. C., Y. L. Chen, J. H. Yu and O. Y. C. Shih (2001). Detection of four types of genetically modified maize by polymerase chain reaction and immuno-kit methods. Journal of Food and Drug Analysis, 9: 50-57.

Collonnier, C., A. Schattner, G. Berthier, F. Boyer and G. C. Philippe (2005). Characterization and event specific-detection by quantitative

Real-Time PCR of T25 maize insert. Journal of AOAC International, 91: 143-151.

Deisingh, A. K. and N Badrie (2005). Detection approaches for genetically modified organisms in foods. Food Research International, 38: 639-649.

Dellaporta, S. L., J. Wood and J. B. Hicks (1983). A plant DNA mini preparation: version II. Plant Molecular Biology Report, 1: 19-21.

Elsanhoty, R. (2004) Quality control for foods produced by genetic engineering. PhD thesis. https://www.depositonce.tu-berlin.de/bitstream/11303/1129/1/Dokument_39.pdf.

Elsanhoty, R., A. I. Al-Turki and F. M. Ramadan. (2013). Prevalence of Genetically Modified Rice, Maize, and Soy in Saudi Food Products. Appl. Biochem. Biotechnol., 171: 883-899.

Elsanhoty, R., K. W. Boegl, J. Zagon and G. Flachowsky (2005). Development of a construct-specific, qualitative detection method for genetically modified potato spunta in raw potato and potato-derived products. Proc. of 9th Egyptian conferences of Home Economic, 19-20, September.

Elsanhoty, R., M. F. Ramadan and K. D. Jany (2011). DNA extraction methods for detecting genetically modified foods: A comparative study. Food Chemistry, 126: 1883-1889.

Elsanhoty, R., T. Shahwan and M. F. Ramadan (2006). Application of artificial neural networks to develop a classification model between genetically modified maize (Bt-176) and conventional maize by applying lipid analysis data. Journal of Food Composition and Analysis, 19: 628-636.

Elsanhoty, R., H. Broll, Grohmann L., B. Linke, A. Spiegelberg, K. W. Bögl and J. Zagon (2002). Genetically modified maize and soybean in Egyptian food market. Nahrung/food, 46: 360-363.

Franck, A., H., Guilley, G. Jonard, K. Richards and L. Hirth (1980) Nucleotide sequence of cauliflower mosaic virus DNA. Cell, 21: 285294.

Greiner, R. and U. Konietzny (2008). Presence of genetically modified maize and soy in food products sold commercially in Brazil from 2000 to 2005. Food Control, 19: 499-505.

Greiner, R., U. Konietzny and A. L. C. H. Villavicencio (2005). Qualitative and quantitative detection of genetically modified maize and

soy in processed foods sold commercially in Brazil by PCR-based methods. Food Control, 16: 753-759.

Holden, M. J., R. Blasic, L. Bussjaeger, C. Kao, L. A. Shokere and D. C. Kendall (2003). Evaluation of extraction methodologies for Corn Kernel (*Zea mays*) DNA for detection of trace amounts of biotechnology-derived DNA. Journal of Agricultural and Food Chemistry, 51: 2468-2474.

James, C. (2007). Global Status of Commercialized Biotech/GM Crops: 2007. ISAAA Briefs No. 37. ISAAA: Ithaca, NY.

Khidr, Y. A., M. M. Arafa, S. M. M. Eldemery and R. M. Elsanhoty (2017). Detection of genetically modified potato in potato tubers cultivated in Egypt. Egypt. J. Genet. Cytol., 46: 1-17.

Liapkova, N. S., N. A. Loskutova, A. N. Maisurian, V. V. Mazin, N. P. Korableva, T. A. Platonova, E. P. Ladyzhenskaia and A. S. Evsiunina (2001). Isolation of genetically modified potato plant containing the gene of defensive peptide from Amaranthus, Prikl. Biokhim. Mikrobiol., 37: 349-354 (in Russian).

Margarit, E., M. I. Reggiardo, R. H. Vallejos and H. Permingeat R. (2006). Detection of BT transgenic maize in food stuffs. Food Research International, 39: 250-255.

Meriç, S., Ö. Çakır, N. Turgut-Kara and Ş. Arı (2014). Detection of genetically modified maize and soybean in feed samples. Genet. Mol. Res., 13: 1160-1168.

Mohammed, A. D., S. Douches, W. Pett, E. Grafius, J. Coombs, W. Liswid owati, 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.

Park, K. W., B. Lee, C. G. Kim, D. Y. Kim, J. Y. Park, E. M. Ko, S. C. Jeong, K. H. Choi, W. K. Yoon and H. M. Kim (2010). Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control, 21: 456-461.

Peano, C., C. M. Somson and L. Palmieri (2004). Qualitative and quantitative evaluation of the genomic DNA extracted from GMO and non-GMO foodstuffs with four different extraction methods. Journal of Agricultural and Food Chemistry, 52: 6962-6968.

Pribylova, R., J. Ptacek, I. Pavlik and M. Bartos (2007). Screening of

Solanum tuberosum cultivars for the “ac2” genetic modification.Veterinarni Medicina, 52: 471-474.

Ramadan, M. F., and R. M. Elsanhoty (2012). Lipid classes, fatty acids and bioactive lipids of genetically modified potato Spunta with Cry V gene. Food Chemistry, 133: 1169-1176.

Rott, M. E., T. S. Lawrence, E. M. Wall and M. J. Green (2004). Detection and quantification of Roundup Ready soy in foods by conventional and real-time polymerase chain reaction. Journal of Agricultural and Food Chemistry, 52: 5223-5232.

Song, Y. J., H. Kim and Y. H. Kim (2017). Detection of unapproved genetically modified potatoes in Korea using multiplex polymerase chain reaction. Food Control, 80: 19-22.

Taberlet, P., L. Gielly, G., Pautau and J. Bouvet (1991). Universal primer for amplification of three non coding regions of chloroplast DAN. Plant Mol. Biol., 17: 1105-1109.

Table (1): Oligonucleotide primer pairs sequence and their target element.

Primers	Sequences	Fragment length	Target element	Reference
AC2_F/ AC2_R	5’ - GTG GGA TGT GTT GCA GTC AG-3’ 5’ - CCA GCA CCA GCA AGT TTA GC-3’	145 bp	*ac2* gene which increased resistance against fungal and bacterial infections (*1*)	Liapkova *et al*. (2001)
CaMV35S-F/ CaMV35S-R	5’ - TCC ACT GAC GTA AGG GAT GAC - 3’ 5’ - CTG GTG ATT TCA GCG TGTCC - 3’	105 bp	CaMV 35S promoter sequence (promoter derived from the cauliflower mosaic virus).	Franck *et al*. (1980)
A1_F/A1_R	5’ - CGAAATCGGTAGACGCTACG - 3’ 5’ - GGGGATAGAGGGACTTGAAC - 3’	550 bp	Chloroplast gene (house keeping gene)	Taberlet *et al.* (1991)
Spu-35S1-F/ Spu-cryVm-R	5' - CTTCGCAAGACCCTTCCTC - 3' 5' - GCTGGAGAACGATTGGTGC - 3'	122 bp	CaMV promoter and *Cry V* gene from *Bacillus Thuringiensis* in genetically modified potato Spunta	El Sanhoty (2004)

Table (2): Time/temperature profiles for qualitative PCR with DNA extracted from potato tuber samples using the primer pairs.

Primer pair	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Final elongation
AC2_F/ AC2_R	3 min at 95°C	30 S at 95°C	30 S at 62°C	40 S at 72°C	35	5 min at 72°C
CaMV35S-F/ CaMV35S-R	2 min at 95°C	30 S at 95°C	30 S at 64°C	30 S at 72°C	35	5 min at72°C
A1_F/A1_R	5 min at 95°C	30 S at 95°C	30 S at 60°C	1 min at 72°C	35	7 min at 72°C
Spu-35S1_F/ Spu-cryVm_R	2 min at 95°C	30 S at 95°C	30 S at 60°C	40 S at 72°C	35	5 min at 72°C

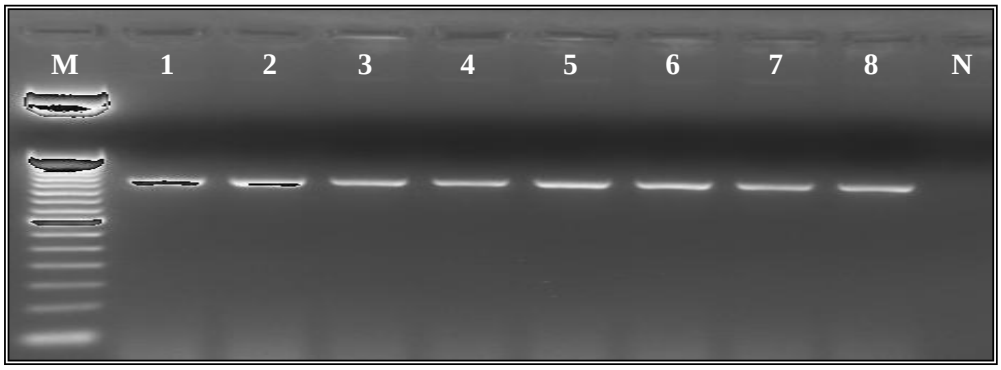

Fig. (1): Agarose gel electrophoresis shown a fragment length of 550 bp for detection of chloroplast DNA in potato DNA gene using the primer pair A1/A2. From the left: lane (M), 50 bp DNA ladder, Lanes 1-8, (Adora, Flamenco, Asterix, Bartina, Cleopatra, Colomba, Desiree, Dynamica genotypes as an example) positive for the presence of chloroplast DNA gene, Lane (N), negative control.

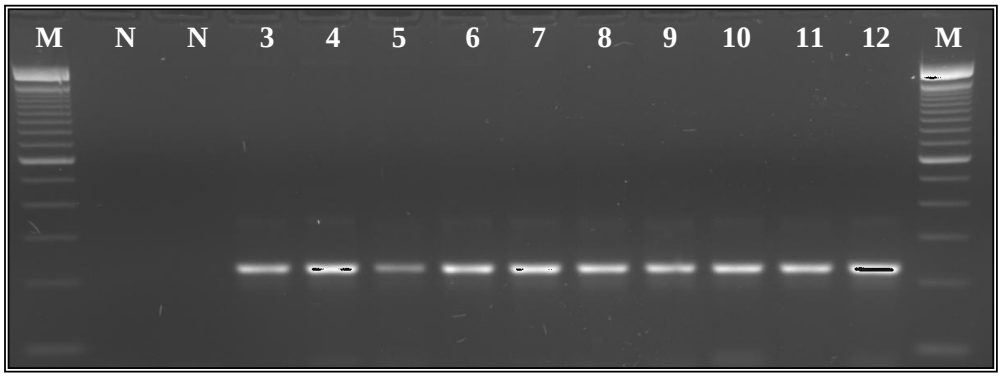

Fig. (2): Detection of the *ac2* gene using the primer pair AC2-F/ AC2-R which amplify 145 bp in length. From the left: 100 bp ladder (lanes M), PCR negative control (lanes (N), and lanes 3-12 positive samples (Flamenco, Asterix, Bartina, Bildtstar, Sifra, Fortus, Liseta, Sylvana, Memphis, Mondial) for the presence of *ac2* gene.

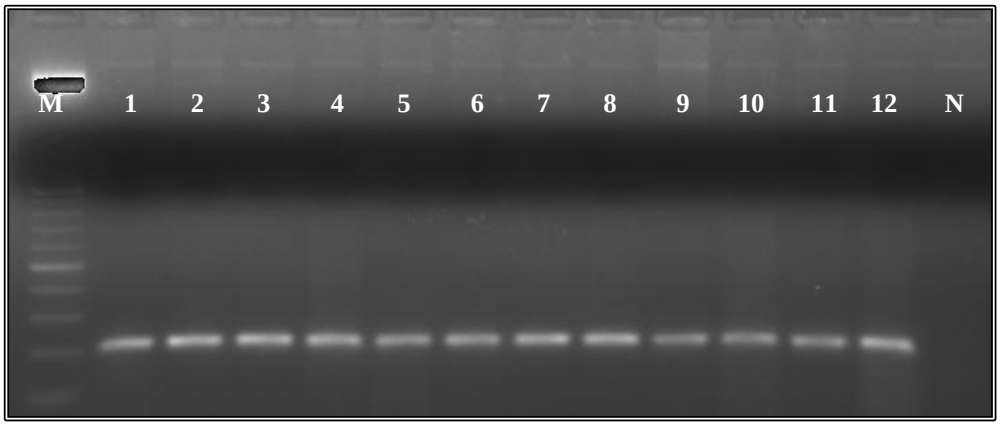

Fig. (3): Detection of the Cry V and CaMV35S genes using the primer pair Spu-35S1-F/ Spu-cryVm-R shown a fragment length of 122 bp. From the left: lane M, 50 bp, (lanes 1 -12), positive samples (Adora, Flamenco, Cleopatra, Colomba, Dynamica, Mozart, Panamera, Zin, Heros, Farida, Red Scarlett, Red valentine 808 and (lane N), negative control.

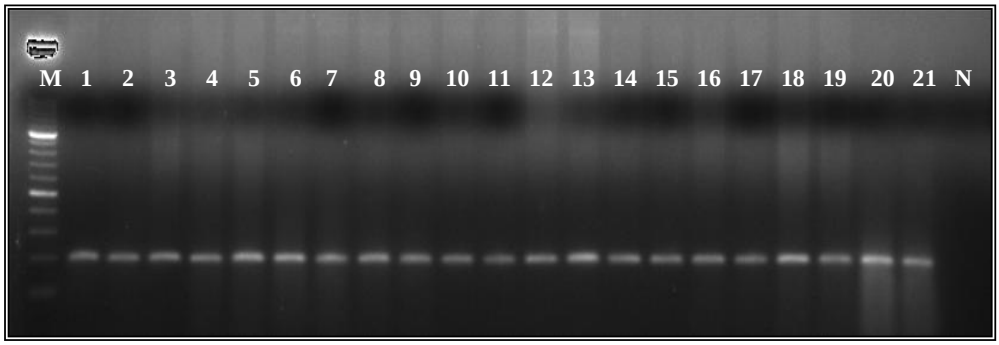

Fig. (4): Detection of 105 bp on the CaMV35S gene using of the primer pair CaMV35S-F/and CaMV35S-R. From the left: lane M, 50 bp, (lanes 1 -21), positive samples (Adora, Flamenco, Asterix, Bartina, Cleopatra, Colomba, Dynamica, Bildtstar, Sifra, Fortus, Liseta, Sylvana, Memphis, Mondial, Mozart, Panamera, Zin, Heros, Farida, Red Scarlett, Red valentine 808, (lane N), negative control.