Detection of Bt gene and seed purity in maize

Mısırda Bt geninin ve tohum saflığının tayini

Research Article

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ABSTRACT

In this study we have described the detection of Bt gene and seed purity in maize by using PCR based method. Fifty four maize varieties were used for the detection of Bt gene by using BT1 and 35S primer having size of 195bp and 301bp respectively. Of these, fifteen varieties contained Bt gene which means that these varieties can not be used for further breeding. Ninety six seeds of maize were used for purity check by bnlg 161 SSR primer. Results showed that these seeds were 95% pure with 5% impurity. So these results showed that PCR based markers were very helpful in cultivar identification which leads to the improvement in maize breeding programs.

Key Words
Bt gene, seed purity, PCR, maize

ÖZET

Sunulan bu çalışmada, mısırda Bt geninin ve tohum saflığının PCR temelli yöntemle tayini sunulmuştur. Elli dört farklı mısır türü, 1950 bazçifti ve 301 bazçifti boyutuna sahip BT1 ve 35S primerleri kullanılarak Bt geninin tayini gerçekleştirilmiştir. Örnekler arasındaki onbeş örnek Bt geni içermektedir. Bt geni içermesi daha sonraki üreme işlemlerinde kullanılamayacağı anlamına gelmektedir. Doksanaltı mısır tohumu, bnlg 161 SSR primeri ile saflık kontrolu için kullanılmıştır. Sonuçlar, bu tohumların % 5 safsızlık ile % 95 saflıkta olduğunu göstermiştir. Ayrıca, bu sonuçlar PCR temelli işaretçilerin kültür tanımlanmasında çok faydalı olduğunu ve mısır yetiştirme programlarında iyileştirmeye olanak sağlayacağını göstermektedir.

Anahtar Kelimeler
Bt geni, tohum saflığı, PCR, mısır.

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INTRODUCTION

Genetically modified organisms (GMO) are those which contains some foreign genes which express new characters improved nutritional value, resistance to virus, insect, herbicide tolerance [1,2]. In 2011, globally 160 million hectares were cultivated with soybean, maize, cotton and canola as genetically modified crops [3,4] and Bt maize covered about 11.2 million ha representing 14% of global transgenic area. Bt maize contained some proteins from *Bacillus thuringiensis* which are produced during the sporulation of the bacterium which were effective against wide variety of insects and even nematodes. Among these proteins, Cry 1Ab and Cry 1Ba genes encode that proteins which were very effective against coleopteran and lepidopteran larvae [5,6]. These genes were detected by PCR based DNA specific sequence present in all kinds of food [7,8].

In crops, species identification is problem which was done by using different criteria like morphological, biochemical and molecular methods. Identification by morphological characters was poor and time consuming process. The use of modern techniques for identification began from late 1980s and scientists used electrophoresis [9,10] and reversed-phase high performance liquid chromatography (RP-HPLC) [11, 12] was used for the detection of seed storage proteins. With the laps of time, genomic era starts and this provide a well established DNA based molecular markers for identification with high resolution power [13]. Now a day different types of molecular markers such as simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLPs) were being used for identification of clones [14], somaclonal variations [15], hybrids and breeding lines [16], cultivars [17], genetic diversity assessment [18-20] and in QTL mapping [21] of various crops like maize [22,23]. All these markers were PCR based and require very small amount of DNA which can be extracted from a single kernel.

MATERIALS AND METHODS

Extraction of Genomic DNA

One hundred and Fifty four maize genomic DNA were extracted by method as described earlier [24] and these varieties were obtained from Biotechnology Research Laboratory, Shenyang Academy of Agricultural Science, Shenyang Liaoning P. R. China, which were used for the detection of Bt gene and seed purity.

PCR for Detection of Bt gene

Detection of the Bt gene was done by using specific primers (35S and BT1) obtained from SBS gene biotech Co. China and amplification reaction was done in 10 μl reaction volume containing 5 μl PCR master mix (SBS gene biotech Co. China), 2 μl 25 μM primer, 2 μl distilled water and 1 μl DNA. The reaction was programmed (Eppendorf thermal cycler) at initial denaturation at 94°C for 5 min followed by 34 cycles of 94°C 30s, 50°C 30s, 72°C 45s and final extension at 72°C for 5 min. After PCR reaction, the amplified products were resolved on 1.8% agarose.

PCR for Seed purity

Seed purity was checked by using specific primer (bnlg161) obtained from SBS gene biotech co and amplification reaction was done in 10 μl reaction volume containing 5 μl PCR master mix (SBS gene biotech Co. China), 2 μl 25 μM primer, 2 μl distilled water and 1 μl DNA. The reaction was programmed (Eppendorf thermal cycler) at initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C 40s, 60°C 35s, 72°C 45s and final extension at 72°C for 5 min. After PCR reaction, the amplified products were resolved on 8% PAGE followed by silver staining.

RESULTS AND DISCUSSION

In this investigation fifty four different maize varieties were used for the detection of Bt genes. Genomic DNA from these fifty four maize varieties was amplified by using specific primers. Among these fifty four varieties, fifteen contains Bt genes which are named as transgenic lines and rest of the thirty nine lines did not contain Bt gene as shown in Figure 1. These fifteen transgenic

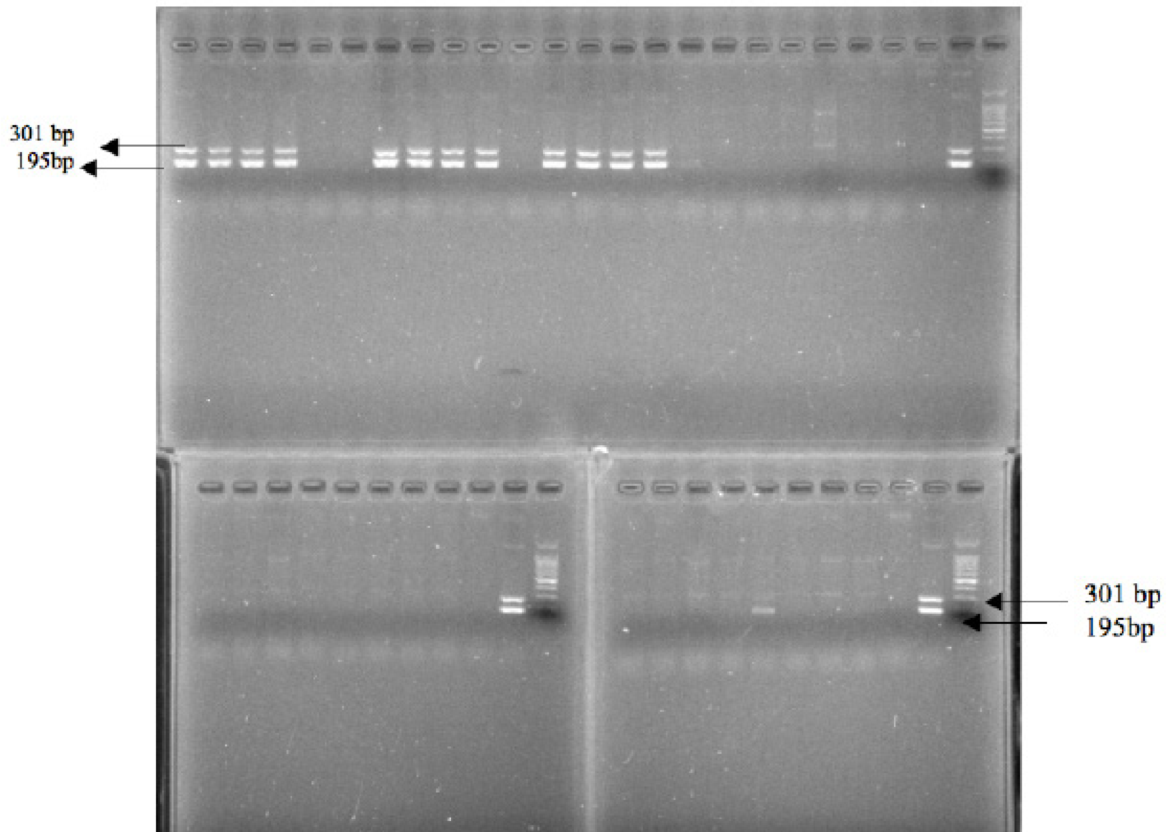

Figure 1. Detection of Bt gene in maize.

lines contained some genes from *Bacillus thuringensis* which can produce some proteins affective against insects. So these fifteen lines were insecticide resistant while the other thirty nine lines were non-insecticide resistant lines. Many studies reported the detection of Bt genes in maize and maize processed food products and all these reports used PCR based methods for detection [25-27].

Currently maize cultivated globally and is basic food source for human beings in many countries. It is essential to check whether it is transgenic or normal, because transgenic crops may be harmful for human consumption. Some transgenic maize such as Bt-11, MON810 and Bt-176 contain genes from *Bacillus thuringiensis* and the plant is resistant against certain lepidopteron insects such as European Corn Borer (ECB) and European Union approved these as a food or feed [28] .

To check the seed purity, DNA from ninety six seeds were extracted and amplified by specific primer. Results (Figure 2) indicated that among these ninety six seeds, five seeds belong to different parents, while rest of the ninety one seeds was same

Table 1. List of Primers used for Bt gene and seed purity.

Primer	Sequence (5′-3′)	Tm (°C)	GC (%)
35S-1	GCTCCTACAAATGCCATCA	56.0	47.4
35S-2	GATAGTGGGATTGTGCGTCA	57.3	50.0
BT1, R	CGATCAGCCTAGTAAGGTCGT	49.2	52.4
BT1, F	GGGCCCGCTGAATCCAAC	49.7	66.7
Bnlg 161 F	GCTTTCGTCATACACACACATTCA	59.3	41.7
Bnlg 161 R	ATGGAGCATGAGCTTGCATATTT	57.1	39.1

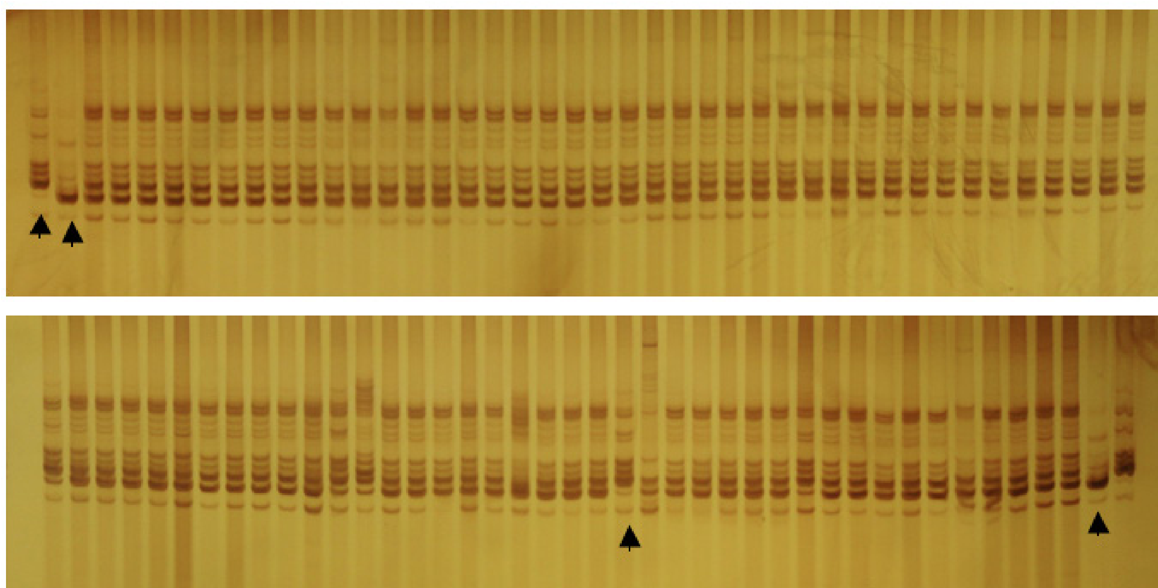

Figure 2. Purity check of maize seeds (arrows indicate the different seeds).

indicating these were pure. So the purity level in this selected panel was 95% with 5% impurity. Seed purification is very important to get the crops with better yield and if the seeds were not pure this will affects the product yield. The main cause of seed impurity was cross pollination which could be done through wind or insects. Wu et al [29] used isozymes and SSR markers for seed purity and identification, their results showed that SSR markers were reliable method for assessing the purity of the maize seeds. RAPD technique was also used for the purification of the maize hybrids [30,31]. Some researchers used both biochemical (isoenzymatic pattern) and molecular markers (microsatellite) for seed genetic purity determination revealing that microsatellite technique was fast and precise as compared to biochemical [32].

CONCLUSION

Results of this study revealed that controlling the quality of crops before breeding is very essential in yield improvement and making the pure lines. Because so many crops containing Bt genes are not declared as a safe of human and animal feed. SSR markers were widely used for the identification of crops and there are about 2300 SSR primers are available in maize database (maizegdb) which are being used for identification of variety of hybrids. So seed selling companies must check the seed purity prior to marketing with farmers.

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