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Original Research Article

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DETECTION OF GENETICALLY MODIFIED RICE LOCALLY AVAILABLE IN PUNJAB, PAKISTAN

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Abstract: *Rice (Oryza sativa L.) is an important cereal crop that provides food to half of the world's population. Pakistan's traditional and premium quality rice is mostly exported to Europe. Transgenic or genetically modified rice (GM RICE) has been developed in the USA, China, and other countries, like herbicide-tolerant Liberty-Link rice (LLRICE06, LLRICE62, LLRICE601) and insect-resistant Bt-63. International and national biosafety regulations allow countries to stop the import of any genetically modified organism (GMO) due to its possible safety concerns. PCR-based methods are recommended for the accurate detection of GM rice. Qualitative PCR is used to detect the presence or absence of GMOs in a rice sample. The objective of this study was to check the presence of GMOs in the rice samples collected from rice-growing areas in Punjab. Eighty-two rice samples were collected randomly from various markets/locations in rice-growing areas of Punjab for this study. Twelve bulks were prepared to represent eighty-two rice samples. The CTAB method was used to extract the DNA from rice samples to analyze it. PCR of rice bulk samples was performed using primers specific for SPS, CP, p35S, tNOS, nptII, Hpt and Bt 63 rice. Positive and negative controls were included in each PCR. Primers specific for chloroplast (CP) and Sucrose phosphate synthase (SPS) genes were used to confirm DNA quality for amplification. Then, primers specific for the cauliflower mosaic virus (CaMV) 35S promoter and the Agrobacterium nopaline synthase (NOS) terminator were used for the detection of GM rice through PCR, neomycin phosphotransferase II (nptII) and Hygromycin phosphotransferase (Hpt) were used as a selectable marker and Bt 63 rice was used in insect resistance. The gel electrophoresis of PCR was conducted using a DNA ladder and then taking of the image was through gel documentation system for visualization and analysis. According to the results, all the rice bulks representing the eighty-two rice samples were negative for p35S, tNOS, nptII, Hygro and Bt 63 rice event. These findings are encouraging and demonstrate that rice grown in kalar track of Punjab, Pakistan, is non-GMO. Such surveillance and monitoring would be largely beneficial to maintain the purity of our traditional non-GM rice and to avoid any possible mixing of GM rice seeds, especially at the time of import. All these efforts will ensure and protect our national share in the international trade of rice.*

Keywords: Rice, GMOs, Detection methods, PCR

Introduction

Rice is generally categorized into two main groups: fine long grain (Basmati) and coarse grain (non-Basmati). Basmati rice is popular in Punjab for cultivation. Basmati rice always reflects the premium quality of aromatic basmati rice cultivated in Pakistan. It accounts for 3.5 percent of agricultural value addition and 0.8 percent of GDP. The production of coarse types has recently increased (Anonymous, 2021). Pakistan produces rice to meet domestic demand and for export purposes. GM rice with tolerance to biotic and abiotic stress has been developed in some countries, including USA and China (Wang and Johnston, 2007). In recent years, rice has been a main product of foreign exchange earnings with an export of about US$ 25.7 billion

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production, import and export of GM rice are not allowed in Pakistan.

Globally, 17 million farmers cultivate genetically modified (GM) plants on an estimated 19.4 million hectares. Farmers, consumers, and the environment have all greatly benefited from the commercialization of GMOs. To improve rice productivity and plant protection, genetic modification has introduced insect resistance, herbicide tolerance, or nutrient deficiencies in rice plants. The most important trait of GM rice consists of cry genes (insect resistance) from the Bt that have been approved for general cultivation. Only three GM rice types (LLRICE 06, LLRICE 62 and LLRICE 601) have been approved for commercial cultivation in United States. Insect-resistant rice (Bt63) was identified in rice products imported from China in 2006 (Made *et al*.,2006; Grohmann and Made 2009).

Labeling is mandatory in Australia, Russia, India, China, Chile, Indonesia, Thailand, Japan, Philippines, Brazil, Saudi Arabia and Taiwan. Commercialized feed and food products contain at least 0.9 percent Korea is 3 percent and Japan is 5 percent (Bean, 2002). In the USA and Canada, GM food labelling is voluntary (Matsuoka, 2001), and European Union (EU) authorized GMO should be labeled in EU market to ensure consumer choice, whereas unauthorized GMOs are subject to a zero-tolerance (Kamle and Ali, 2013).

Most of these laws are implemented using qualitative PCR technology, which allows used for quantification and detection of GMO (Fraiture *et al*.,2015). International standardization of GMO detection technologies is essential for international food and feed requirements trade. It is necessary to detect and label them to increase consumer awareness. As a result of the growing number of illegal GM rice varieties on the market, as well as moral concerns to provide consumers with informed choices, the improvement of screening procedures and monitoring programs appears on the way to be critical.

The PCR for detecting these genetic elements could be very useful in identifying unintentional mixing of GM and non-GM seeds (Shrestha *et al*., 2008). Specific primer elements such as 35S promoters, coding sequences nos terminators, CP gene, SPS gene, nptII, Hpt and Bt 63 rice can be used. However, detecting specific elements does not allow for the differentiation of various GM events involving the same specific elements (Yang *et al.*, 2005). In general, agarose gel electrophoresis distinguishes qualitative PCR products by size. So, this study mainly focused onPCR-based GMO detection of rice.

Objectives of the study:

1) The present research has been conducted to investigate the presence of GMOs in the rice samples from rice-growing areas in Punjab, Pakistan.
2) Collection of rice samples from rice growing area of Punjab.
3) Transgenic analysis for the detection of GMOs in the rice samples through polymerase chain reaction (PCR).

Materials and methods

The present research work under this study was conducted at Agricultural Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE) in collaboration with the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad (UAF) during the year 2021-22.

Sample Collection and Preparation

In 2020, 82 rice samples were randomly taken from several local markets in Punjab as shown in Table 1. Twelve bulks were prepared to represent eighty-two rice samples. Twelve bulks representing eighty-two rice samples were selected in this study in Table 2.

Table 1: Rice samples collected from kalar track of Punjab

Sample No	Bulk No.	Rice Samples	Main Cities
1	B1	Kissan Basmati 1509	Nankana
2	B1	Kainat	Nankana
3	B1	Supri	Nankana
4	B1	Kainat	Nankana
5	B1	PK-1121 Kainat	Nankana
6	B1	Kainat	Nankana
7	B1	Supri	Nankana
8	B1	Kainat	Nankana
9	B2	Kainat Sela	Nankana
10	B2	Super Narowal	Nankana
11	B1	Super Basmati	Nankana
12	B3	Super Kainat Steam	Sheikhupura

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13	B3	Kainat 1509	Sheikhupura
14	B3	Super Basmati	Sheikhupura
15	B4	Kainat 1121	Sheikhupura
16	B4	Super Basmati	Sheikhupura
17	B4	Rice 86	Sheikhupura
18	B3	Kainat	Sheikhupura
19	B3	Kainat	Sheikhupura
20	B3	Kainat 1509 steam	Sheikhupura
21	B3	Supri SKP	Sheikhupura
22	B3	Super Basmati	Sheikhupura
23	B3	Kainat	Sheikhupura
24	B6	Super Basmati	Muridke
25	B5	Super Basmati	Muridke
26	B5	Super Basmati	Muridke
27	B5	Supri 86	Muridke
28	B5	Super Basmati 515	Muridke
29	B5	Super Chenab	Muridke
30	B6	Steam Kainat	Muridke
31	B6	C-9 non-Basmati	Muridke
32	B6	86-Rice Broken Rice	Muridke
33	B6	Super Basmati	Muridke
34	B6	Super Fine	Muridke
35	B6	Supri-86	Muridke
36	B6	Super Basmati	Muridke
37	B6	Super Brown New	Muridke
38	B8	Kainat Steam Rice	Kamoki
39	B8	Super Basmati	Kamoki
40	B8	Supri-86+Steam Rice	Kamoki
41	B7	Super Basmati (Kernel Super)	Kamoki
42	B9	Super Basmati	Gujranwala
43	B9	Super Basmati	Gujranwala
44	B10	Basmati-386	Gujranwala
45	B10	Super Kernel (Basmati)	Gujranwala
46	B10	KS 1509 (Hybrid)	Gujranwala
47	B10	Supri	Gujranwala
48	B10	Kainat	Gujranwala
49	B10	Super Kernel (Basmati)	Gujranwala
50	B9	Super Basmati	Gujranwala
51	B10	Super Basmati	Gujranwala
52	B10	Super Basmati	Gujranwala
53	B10	Basmati 86	Gujranwala
54	B10	Super Kernel	Gujranwala
55	B8	Supri - Kaccha Purana	Kamoki

[Citation: Latif, A., Shafique, M.T., Bano, M., Bibi, A., Bibi, T., Gulnaz, S., Khan, R.A.R., Mallhi, A.R., Sarfraz, S., Ahsan, A., Iqbal, H.M.N., Latif, A., Nazar, M.Z.K., Asif, M. (2023). Detection of genetically modified rice locally available in Punjab, Pakistan. *Biol. Clin. Sci. Res. J.*, **2023***: 175*. doi: https://doi.org/10.54112/bcsrj.v2023i1.175]

56	B8	386- Steamed	Kamoki
57	B8	Super Kernel (Old)	Kamoki
58	B8	Super Brown	Kamoki
59	B8	Supri Sehla	Kamoki
60	B8	Super Brown	Kamoki
61	B8	Super (New)	Kamoki
62	B8	386 (New)	Kamoki
63	B8	1121 Steamed (old)	Kamoki
64	B8	Super Kernel (New)	Kamoki
65	B8	1509 Sehla	Kamoki
66	B11	KS- Hybrid	Hafizabad
67	B11	Kainat - Steamed	Hafizabad
68	B11	Super Kernel	Hafizabad
69	B11	Nikko-386	Hafizabad
70	B11	Adhwar Supri	Hafizabad
71	B11	1509 Sehla	Hafizabad
72	B11	Tota 386	Hafizabad
73	B11	Adhwar Kainat	Hafizabad
74	B12	Super Kernel (old silky)	Hafizabad
75	B12	Supri	Hafizabad
76	B12	Kainat Super	Hafizabad
77	B12	Super Kernel (old)	Hafizabad
78	B12	Kainat Sehla (Steamed)	Hafizabad
79	B12	Kainat (old)	Hafizabad
80	B12	Kainat (new)	Hafizabad
81	B12	Kainat Sehla	Hafizabad
82	B12	Super Kernel Basmati (new)	Hafizabad

Table 2: Number of Samples in each Bulk

Sr. No.	Bulk No.	No of Samples	Main Cities
1	B1	9	Nankana
2	B2	2	Nankana
3	B3	9	Sheikhupura
4	B4	3	Sheikhupura
5	B5	5	Muridke
6	B6	9	Muridke
7	B7	1	Kamoke
8	B8	14	Kamoke
9	B9	3	Gujranwala
10	B10	10	Gujranwala
11	B11	8	Hafizbabad
12	B12	9	Hafizbabad

Genomic DNA extraction of Rice Samples

DNA from these samples was extracted by using the CTAB method (Doyle and Doyle, 1990). DNA concentration was measured with a spectrophotometer.

Quality of DNA

The DNA of rice samples was loaded on a 0.8% or 1% agarose gel for the confirmation of extracted DNA, whether it is present or not and for quality check. The quality of DNA was checked by running 5µl DNA on 0.8% agarose gel prepared in 0.5xTAE buffer. The DNA samples given smeared in the gel were rejected, and re-isolated and good-quality of DNA was achieved.

Dilution of DNA for PCR (Working dilution):

Twelve dilutions were prepared from Stock DNA samples to measure the DNA concentration for best amplification The DNA concentrations were measured through a spectrophotometer.

PCR Analysis:

PCR condition

The PCR analysis was carried out in a thermal cycler (96 valves, Bio-Rad (Thermo Fisher Scientific). 2xPCR Taq master mix (Cat No. G013, Thermo

Fisher Scientific) was used to amplify target DNA. Finally, a PCR reaction was performed in total volume of 25 µl.

PCR primers

The oligonucleotide primers synthesized from euro fins Genomics Company. Seven primers were used for this study (Table 3).

Table 3: Primers and their sequences

Sr. No	Prime Name	Target Gene / Element	Sequence (5'-3')	Product size	Source
1	Hyg-F Hyg-R	Hygromycin resistance gene (Hpt)	AGA ATC TCG TGC TTT CAG CT ACA TTG TTG GAG CCG AAA T	510 bp	GMDD*
2	35s-1 35s-2	35S Promoter (P35S)	GCTCCTACAAATGCCATCA GATAGTGGGATTGTGCGTCA	195 bp	ISO 21569
3	HA-nos118-f HA-nos118-r	Nos Terminator (tNOS)	GCATGACGTTATTTATGAGATGGG GACACCGCGCGCGATAATTTATCC	118 bp	ISO 21569
4	SPS-F SPS-R	Sucrose phosphate synthase (Rice endogenous reference gene)	ATC TGT TTA CTC GTC AAG TGT CAT CTC GCC ATG GAT TAC ATA TGG CAA GA	287 bp	GMDD*
5	Kana-F Kana-R	Kanamycin resistance gene (nptII)	ATG ACT GGG CAC AAC AGA CA CCA GAA AAG CGG CCA TTT TC	550 bp	GMDD*
6	CP-3 CP-4	Plant chloroplast	CGA AAT CGG TAG ACG CTA CG GGG GAT AGA GGG ACT TGA AC	650 bp	GMDD/ISO21569 GMDD/ISO21569
7	T51F T51R	Bt63rice construct based	gACTgCTggAgTgAT TAT CgACAg A AgCTCggTA CCT CgA CTT ATT CAg	83 bp	

Agarose gel electrophoresis:

The check was the quality and concentration of DNA on 0.8 % (w/v) agarose gel by comparing them with standard DNA.

Gel documentation:

Visualize the amplification products by placing gel on a transilluminator and take photographs using UV transilluminator (Bio RAD, USA).

PCR Analysis:

PCR of rice samples was performed using primers like SPS, CP, p35S, tNOS, nptII, Hygro, Bt 63 rice. Each PCR was used specifically for +ve and –ve controls. The gel electrophoresis of PCR was done with a DNA ladder and then photographed gel with a proper documentation system.

RESULTS

Genomic DNA Extraction of Rice:

The study was conducted for GMOs detection in rice and transgenic analysis. For this purpose, DNA was extracted using CTAB method from 12 bulks representing 82 rice samples collected from rice growing areas of Punjab, Pakistan. The quality of DNA was checked by 0.8% agarose gel. Then, DNA was of good quality and it's sufficient in Figure 1.

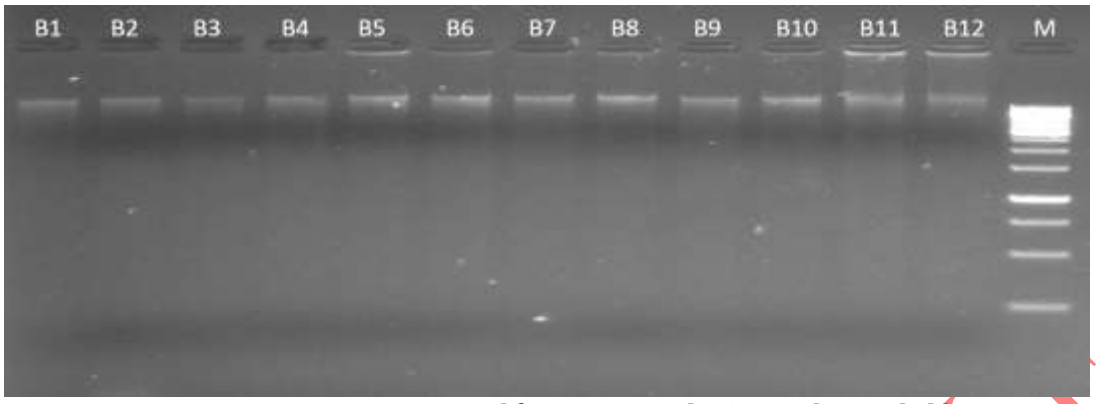

Figure 1: Genomic DNA extracted from rice samples (M=1 Kb DNA ladder)

PCR Analysis

Chloroplast (CP) specific PCR

The CP gene was amplified to confirm DNA quality from rice samples. Twelve bulk representing eighty-two rice samples were amplified, and rice specific for all DNA rice genome shows and negative samples were not amplified. Its mean CP was positive for all eighty-two rice samples shown. CP was used specifically for +ve (known rice DNA) and –ve control (PCR water), and a 650 bp band was visualized on a 2% agarose gel (as shown in Fig 2).

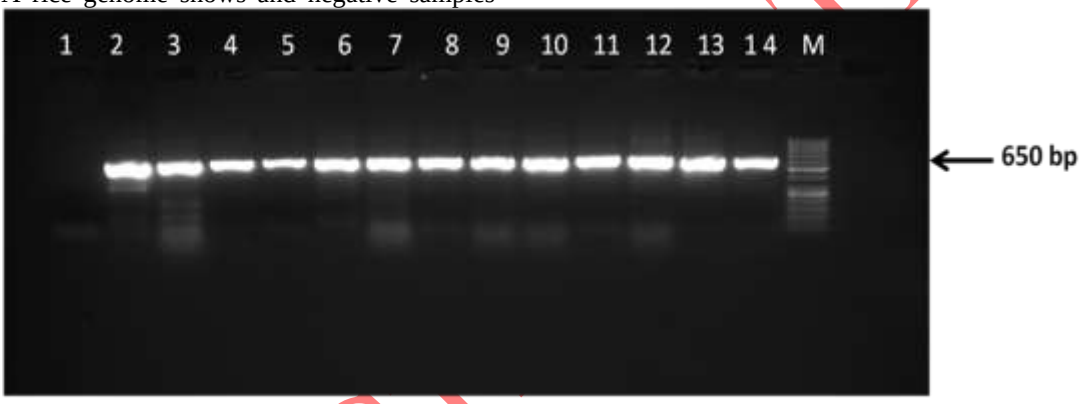

Figure 2: PCR amplification of rice samples using CP specific primer. 1)Negative control (PCR water), 2) B1, 3) B2, 4) B3, 5) B4, 6) B5, 7) B6, 8) B7, 9) B8, 10) B9,11) B10, 12) B11, 13) B12, 14) Positive control (known rice DNA), M) 50 bp DNA ladder, Primer CP specific was used forward CP-3 and reverse CP-4, Size of PCR product was used 650 bp.

Sucrose Phosphate Synthase (SPS) specific PCR

The SPS endogenous PCR was amplified to confirm the sufficient amount of DNA from rice samples. Twelve bulk representing eighty-two rice samples were amplified, and rice specific for all DNA rice genome shows and negative samples were not amplified, and so we can say that SPS was positive for all eighty-two rice samples. SPS was used specifically for +ve (known rice DNA) and –ve control (PCR water), and the 287 bp band was visualized under UV through gel electrophoresis to confirm the quality of DNA. The gel electrophoresis results were amplified for all samples shown in Fig 3.

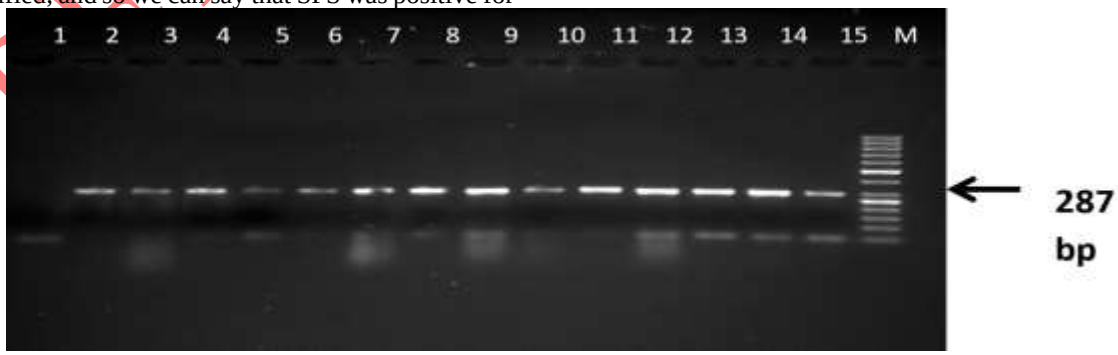

Figure 3: PCR amplification of rice samples using SPS-specific primer. 1) Negative control (PCR water), 2) B1, 3) B2, 4) B3, 5) B4, 6) B5, 7) B6, 8) B7, 9) B8, 10) B9, 11) B12, 12) B11, 13) B12, 14 and 15) Positive control (known

rice DNA), M) 50 bp DNA ladder, Primer SPS specific was used forward SPS-F and reverse SPS-R, Size of PCR product was used 287 bp.

NOS Terminator (tNOS) specific PCR

The tNOS was used to stop transcription. Twelve bulk representing eighty-two rice samples were not amplified at clear negative pointing. So we can say that tNOS was negative for all eighty-two rice samples shown. The tNOS was used specifically for +ve (Bt cotton DNA dilution) and –ve control (PCR water), and the result was analyzed through gel electrophoresis under UV illumination 118 bp band tNOS was seen (as shown in Figure 4).

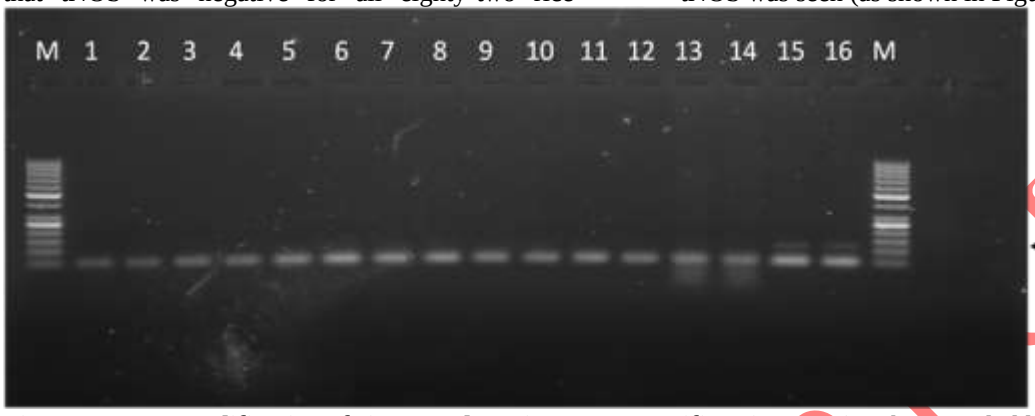

Figure 4: PCR amplification of rice samples using tNOS specific primer. M) 50 bp DNA ladder, 1 and 2) Negative control (PCR water), 3) B1, 4) B2, 5) B3, 6) B4, 7) B5, 8) B6, 9) B7, 10) B8, 11) B9, 12) B10, 13) B11, 14) B12, 15 and 16) Positive control (Bt cotton DNA), M) 50 bp DNA ladder, Primer tNOS specific was used forward HA-nos 118-F and reverse HA-nos 118-R, Size of PCR product was used 118 bp.

35S Promoter (p35S) specific PCR

The p35S was used to the expression of the gene in rice. Twelve bulk representing eighty-two rice samples were not amplified at clear negative pointing. So we can say that p35S was negative for all eighty-two rice samples shown. And p35S was used specifically for +ve (MON 531 DNA dilution) and –ve control (PCR water), and p35S was visualized through gel electrophoresis ,195 bp band p35S was seen on 2% agarose gel (as shown in Figure 5).

Figure 5: PCR amplification of rice samples using p35S specific primer. M) 50 bp DNA ladder,1 and 2) Negative control (PCR water), 3) B1, 4) B2, 5) B3, 6) B4, 7) B5, 8) B6, 9) B7, 10) B8, 11) B9, 12) B10, 13) B11, 14) B12, 15 and 16) Positive control (MON 531), M) 50 bp DNA ladder, Primer p35S specific was used forward 35s- 1 and reverse 35s-2, Size of PCR product was used 195 bp.

Hygromycin phosphotransferase (Hpt)

Hygromycin was used as a selection agent and selectable marker in rice. Twelve bulk representing eighty two rice samples were not amplified at negative clear pointing.Its mean HptII was negative for all eighty sample shown. Hygromycin was used specific for +ve (PSB 187 Plasmid 7350) dilution) and –ve control (PCR water). Through gel electrophoresis hygromycin was seen at about 510 bp band on 2% agarose gel (as shown in Figure 6).

[Citation: Latif, A., Shafique, M.T., Bano, M., Bibi, A., Bibi, T., Gulnaz, S., Khan, R.A.R., Mallhi, A.R., Sarfraz, S., Ahsan, A., Iqbal, H.M.N., Latif, A., Nazar, M.Z.K., Asif, M. (2023). Detection of genetically modified rice locally available in Punjab, Pakistan. *Biol. Clin. Sci. Res. J.*, **2023***: 175*. doi: https://doi.org/10.54112/bcsrj.v2023i1.175]

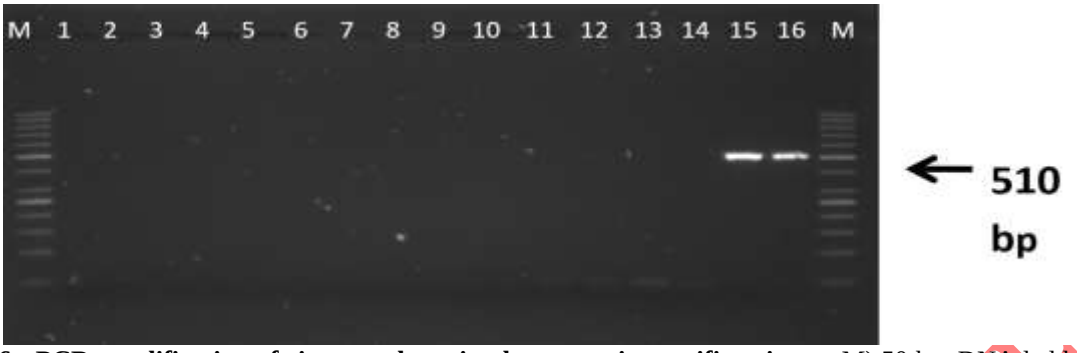

Figure 6 : PCR amplification of rice samples using hygromycin specific primer. M) 50 bp DNA ladder, 1 and 2) Negative control (PCR water), 3) B1, 4) B2, 5) B3, 6) B4, 7) B5, 8) B6, 9) B7, 10) B8, 11) B9, 12) B10, 13) B11, 14) B12, 15 and 16) Positive control (PSB 187 Plasmid 7350 dilution), M) 50 bp DNA ladder, Primer Hpt specific was used forward Hygro- F and reverse Hygro-R, Size of PCR product was used 510 bp.

Neomycin phosphotransferase II (nptII)

The nptII was widely used as a selectable marker in rice and antibiotic resistance. Twelve bulk representing eighty-two rice samples were not amplified at negative clear pointing. Its mean nptII was negative for all eighty-two samples shown. The nptII was specifically for +ve (2071+2072 dilution) and –ve control (PCR water). Visualized by gel electrophoresis of nptII was seen at about 550 bp band on 2% agarose gel. (as shown in Figure 7).

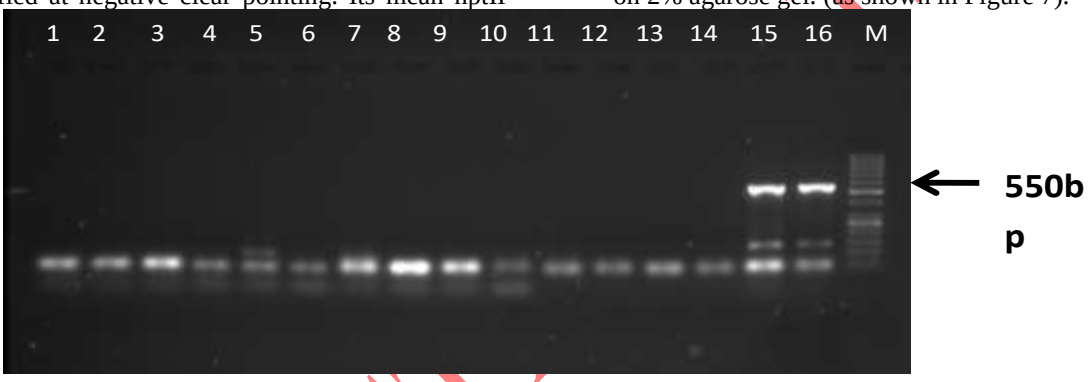

Figure 7: PCR amplification of rice samples using nptII specific primer. 1 and 2) Negative control (PCR water), 3) B1, 4) B2, 5) B3, 6) B4, 7) B5, 8) B6, 9) B7, 10) B8, 11) B9, 12) B10, 13) B11, 14) B12, 15 and 16) Positive control (2071+2072), M) 50 bp DNA ladder, Primer nptII specific was used forward Kana- F and reverse Kana-R, Size of PCR product was used 550 bp.

Bt 63 rice specific PCR

Twelve bulks represent eighty-two rice samples were not amplified at clear negative pointing. So we can say that Bt 63 rice was negative for all eighty-two samples shows Bt 63 rice was used specific for +ve (No positive control) and –ve control (PCR water) and Bt 63 rice was visualized through gel electrophoresis, no band Bt63 rice seen on 2% agarose gel (as shown in Figure 8).

Figure 8: PCR amplification of rice samples using Bt 63 Rice specific primer. M) 50 bp DNA ladder, 1 and 2) Negative control (PCR water), 3) B1, 4) B2, 5) B3, 6) B4, 7) B5, 8) B6, 9) B7, 10) B8, 11) B9, 12) B10, 13) B11, 14) B12, 15 and 16) Positive control, M) 50 bp DNA ladder, Primer Bt 63 rice specific was used forward T51F and reverse T51R, Size of PCR product was used 83 bp.

[Citation: Latif, A., Shafique, M.T., Bano, M., Bibi, A., Bibi, T., Gulnaz, S., Khan, R.A.R., Mallhi, A.R., Sarfraz, S., Ahsan, A., Iqbal, H.M.N., Latif, A., Nazar, M.Z.K., Asif, M. (2023). Detection of genetically modified rice locally available in Punjab, Pakistan. *Biol. Clin. Sci. Res. J.*, **2023***: 175*. doi: https://doi.org/10.54112/bcsrj.v2023i1.175]

PCR Analysis:

Table 4: PCR Results

0 mean no amplification, 1 mean amplification

Genetic Elements							
Samples	**SPS 287 bp**	**CP 650 bp**	**p35S 195 bp**	**tNOS 118 bp**	**Hpt 510 bp**	**nptII 550 bp**	**Bt 63 rice 83 bp**
B1	1	1	0	0	0	0	0
B2	1	1	0	0	0	0	0
B3	1	1	0	0	0	0	0
B4	1	1	0	0	0	0	0
B5	1	1	0	0	0	0	0
B6	1	1	0	0	0	0	0
B7	1	1	0	0	0	0	0
B8	1	1	0	0	0	0	0
B9	1	1	0	0	0	0	0
B10	1	1	0	0	0	0	0
B11	1	1	0	0	0	0	0
B12	1	1	0	0	0	0	0

Table 4 shows SPS and CP genes were amplification, and p35S, tNOS, Hpt and nptII Bt 63 rice were not amplified in all eighty-two rice samples representing twelve bulks while Bt 63 rice were all negative results. Because they do not have a positive control used, they are known as non-specific primers. Based on the agarose gel electrophoresis results, except for the primers bt 63 rice and the remaining 6 primer pairs exhibited adequate consistency in amplifying the different rice samples. The PCR amplicons displayed variation in their length for the p35S, tNOS, Hpt and nptII primer.

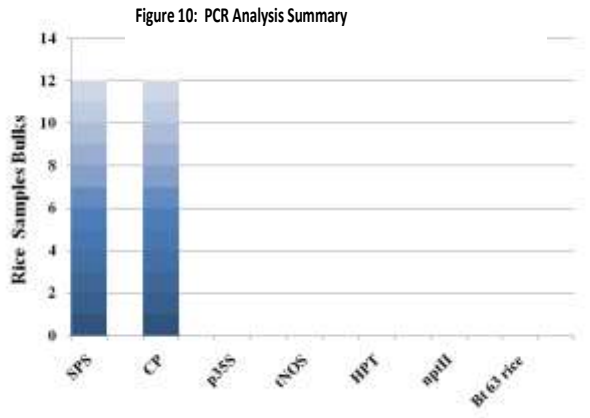

Figure 9: PCR Analysis Summary

Blue bars represent SPS and CP genes were amplified meanwhile, p35S, tNOS, HPT, nptII and Bt 63 rice were not amplified in all eighty-two rice samples (as shown in Figure 9).

Discussion

The agricultural sector is economically the most important one for Pakistan. It contributes about 19.2% to the overall GDP, and employs 38.5% of the population, either directly or indirectly. A major portion of the rice crop is affected by diseases, particularly bacterial blight, while abiotic factors like drought further reduce the net yield of rice. It has become a challenge for rice scientists to develop rice varieties tolerant to environmental stresses.

In the first part of this study, GMO rice was not present in this study conducted from the kalar track of Punjab. In the second part of this study, growing regions of Punjab do not grow rice. It is a good indicator to sustain and must be periodically because rice is used for local production and export. Rice is important for export, and farmers grow rice traditional or non-GMO used because it is good for export or benefit stakeholders or importers. Twelve bulk representing eighty-two rice samples were present non-GMO in Punjab. All eighty-two rice samples were mixed with equal-quality seed. If bulk samples were positive, then all samples were individually checked; hence bulk is important because bulking of samples are high standard practice and reduces the sample size and the labor.

In the present study, most of the DNA extracted by CTAB methods showed a high molecular weight and high purity with A260/ A280 range varies between

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1.7-1.9. This result is agreed with those of Romano and Brasileiro (2010) and Couto *et al.* (2011). A ratio higher than 2.0 generally indicates RNA contamination, while a ratio lower than 1.7 normally indicates protein contamination duringextraction process, so the good quality DNA should be ranged between 1.70- 2.00. Yields and purities of the purified genomic DNAs were measured by spectrophotometer. All samples within this range demonstrate that DNA has sufficient integrity for PCR analysis. Meanwhile, PCR water was used to prepare the sample and reduce false positive results during DNA extraction or PCR analysis. Conventional PCR is widely utilized as a qualitative assay (i.e., identifying whether the target gene is absent or present) to detect a GMO and is widely used to detect the desired gene during food processing (Song *et al*., 2011).

Conclusion

It was concluded that standard PCR could be an effective tool for screening GMO targets and that DNA isolation methods were suitable for most food products. The CTAB technique was used to isolate DNA successfully in all of the samples. The presence of GMO components was not indicated on product labels, allowing consumers to choose food products based on the safety of GM crops. To make informed decisions, labeling is essential. It may be necessary to check all imported raw materials and food products based on the results. It is recommended that strict regulations and certified laboratories be established to monitor GM foods and crops because it protects and safeguards our rice imports; breeders, farmers and traders must be vigilant to avoid mixing GM rice from foreign sources with our traditional or non-GM rice. Thus, it must be a survey**.**

Conflict of interest

The authors declared absence of conflict of interest.

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