DNA FINGERPRINTING OF WHEAT STORED IN DIFFERENT CONDITIONS

Genomic DNA extraction and PCR for checking the amplifiability of wheat DNA

The genomic DNA of control wheat was extracted from 100mg of wheat flour using the commercially available Sigma GenElute Plant Genomic DNA Miniprep kit. The amount of DNA extracted was determined by measuring the absorbance at 260nm. 100-113 µg of DNA was obtained from 100 mg of wheat flour. The purity and quality of extracted DNA was evaluated by agarose gel electrophoresis. The DNA extracted from wheat flour showed an A_{260}/A_{280} ratio of 1.8 indicating the DNA was homogenous. Agarose gel electrophoresis also showed the DNA was homogenous (Figure.15).

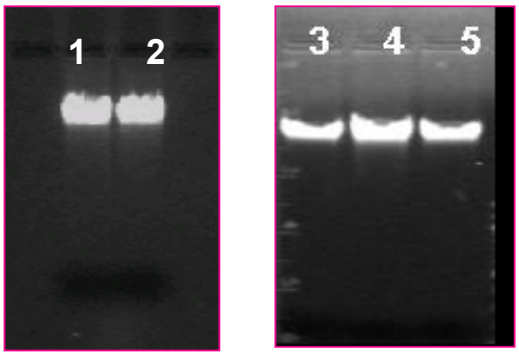

Figure 15: Agarose gel electrophoresis of DNA extracted from wheat flour.
Lane 1&2, Control wheat; Lane 3, Wheat stored in Jute, Lane 4, Wheat stored in PP and Lane 5, Wheat stored in HDPE.

Amplification of 18s rDNA gene in wheat

The quality of the extracted DNA was tested by amplification of the DNA with a eucaryote-specific PCR system (Puali et al (2000), Extraction and Amplification of DNA from 55 Foodstuffs, Mitt.Lebensum. Hyg. 91, 491-501). Positive results with the eucaryote-specific system not only show the presence of amplifiable DNA but at the same time allows a comparison of the amplifiability between samples. The DNA was

subjected to eucaryotic-specific PCR for amplification of a 137 bp fragment of the 18s rDNA gene using the primer pair TR03/TR04. The amplified fragments were electrophoresed in 2% agarose gels and visualized with ethidium bromide. Wheat flour derived DNA yielded a positive amplification signal with this system indicating the presence of amplifiable wheat DNA (Figure 16, Lanes 4&5).

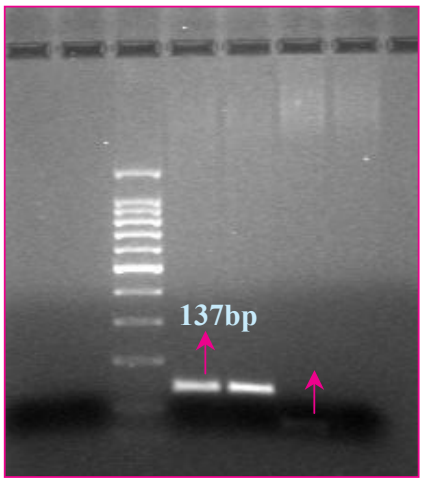

Figure 16: Amplification of the 18s rDNA gene. Lane 1, Premise Control (TRO3/TR04), Lane 2, Premise Control (Ten2/Ten3) lane 3,100 bp Ladder, Lanes 4&5 Control wheat flour, Lane 6 Control Wheat flour and lane 7, Negative Control (Maize)

PCR setup for 18srDNA amplification

Forward Primer: TRO3: 5'TCT GCC CTA TCA ACT TTC GAT GGT A'3
Reverse Primer: TRO4: 5' AAT TTG CGC GCC TGC TGC CTT CCT T'3

Reagents	Final Concentration
10x PCR buffer with $MgCl_2$	1x
dNTP solution(10mM)	200 µM
Primer TRO3	0.25µmol/L
Primer TRO4	0.25µmol/L
Taq DNA Polymerase 5U/µl	2.5U
DNA template	550ng

Temperature-time program

Initial denaturation	95°C/ 4 min: 30 sec
Denaturation	95°C/ 46 sec
Annealing	65°C/1 min: 25 sec
Extension	72°C/1 min: 25 sec
No of cycles	30
Final extension	72 °C/ 3 min: 15 sec

Amplification of 18s rDNA gene in wheat stored for 6months.

DNA isolated from wheat stored in Jute, PP and HDPE sacks were used as template to amplify the 18s.rDNA. A positive amplification signal with this system indicating the presence of amplifiable DNA (Figure 17, Lanes 2, 3 & 4). The DNA was amplifiable in all the stored samples indicated that no detectable changes occurred during storage. The primers and PCR setup was as described above.

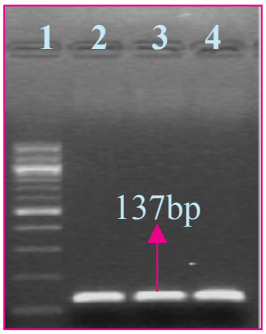

Figure 17: Amplification of the 18s rDNA gene in stored wheat. Lane 1, 100 bp Ladder, Lane 2, Wheat stored in jute bag, Lane 3, Wheat stored in PP bag and lane 4, wheat stored in HDPE bag

Amplification of Endogenous Glutenin gene in wheat

The quality of the extracted DNA was also evaluated by amplification of the DNA for the glutenin a housekeeping gene of wheat. A 67 bp fragment of the

endogenous Glutenin gene was amplified by using the primer pair Ten1/Ten2 (Terzi et al (2002) 'TaqMan PCR for Detection of Genetically Modified Durum Wheat', doi: 10.1006/jcrs.2002.0489 available online http://www.ideallibrary.com). The amplified fragments were electrophoresed in 2% agarose gels and visualized with ethidium bromide. Wheat flour derived DNA yielded a positive amplification signal of 67 bp with this system indicating the presence of amplifiable wheat DNA (Figure 2, Lane 6). Whereas maize which served as the negative control showed no PCR fragments. DNA isolated from wheat stored in jute, PP and HDPE sacks were used as template to amplify the 18s.rDNA. A positive amplification signal of 67 bp with this system indicated the presence of amplifiable DNA (Figure 18, Lanes 2, 3 & 4). The DNA was amplifiable in all the stored samples indicated that no detectable changes have occurred during storage

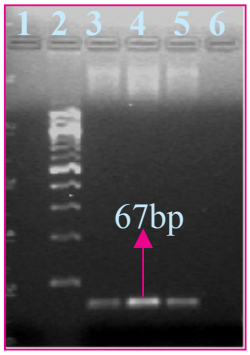

Figure 18: Amplification of the Glutenin gene. Lane 1, Premise control, Lane 2, 100 bp Ladder, Lanes 3, wheat flour stored in jute bag, Lane 4, Wheat flour stored in PP bag, Lane 5, wheat stored in HDPE bag and Lane 6, Negative control (Rice).

PCR setup for amplification of glutenin gene

Forward Primer: Ten 2: 5'GAT ATG TCG ATG CAG CGG TG'3
Reverse Primer: Ten 3: 5'ACT AGT TTG GGC GGG TCA CA'3

Reagents	Final Concentration
10x PCR buffer with $MgCl_2$	1x
dNTP solution(10mM)	200 µM
Primer Ten2	0.25µmol/L
Primer Ten3	0.25µmol/L
Taq DNA Polymerase 5U/µl	2.5U
DNA Template	550 ng

Temperature-time program

Initial denaturation	94^{o}C/ 2 min
Denaturation	94^{o}C/ 30 sec
Annealing	60^{o}C/30 sec
Extension	72^{o}C/1 min
No of cycles	35
Final extension	72 ^{o}C/ 3 min

DNA FINGERPRINTING OF PADDY AND RICE STORED IN DIFFERENT CONDITIONS

Extraction of genomic DNA from Rice and Paddy

Rice and Paddy were powdered in a hand mill and passed through a 60-mesh sieve. Genomic DNA of rice and paddy were extracted by using the commercially available Sigma GenElute Plant Genomic DNA Miniprep kit. DNA extracted was estimated by measuring the absorbance at 260nm and the purity by measuring the A_{260}/A_{280} ratio. The DNA yield was found to be in the range of 2.5-3.5µg/ 100mg of rice and paddy samples. The purity and quality of extracted DNA evaluated by agarose gel electrophoresis indicated it was it was homogenous (Figure 19a). The

DNA extracted from rice and paddy after storage in jute, PP and HDPE sacks also was homogenous and suitable for PCR amplification (Figure 19b).

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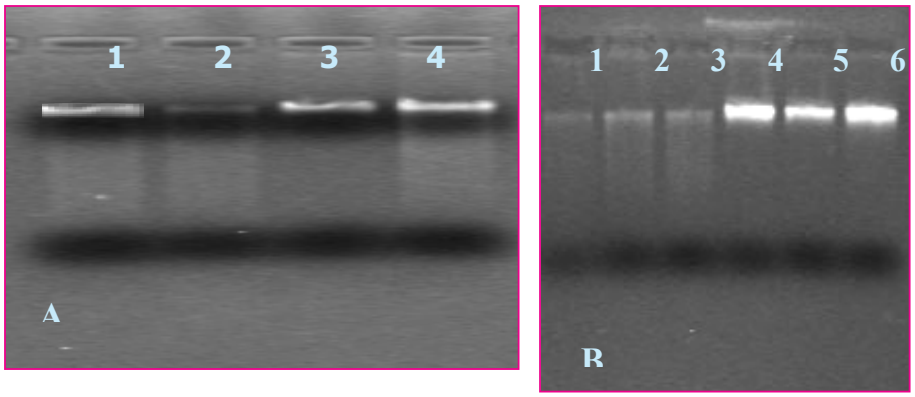

Figure 19: Agarose gel electrophoresis of DNA extracted from rice and paddy. A) Control Rice and paddy: Lanes 1&2, rice and lanes 3 & 4 paddy. B Rice and paddy after 6 months storage Lane 1-Rice stored in HDPE, Lane 2-Rice stored in PP, Lane 3-Rice stored in Jute, Lane 4 Paddy stored in HDPE Lane 5-Paddy stored in PP and Lane 6-Paddy stored in Jute

Amplification of 18s rDNA gene from Rice and Paddy samples

The quality of the extracted DNA was tested by amplification of the DNA with eucaryote-specific PCR system. Positive results with the eucaryote-specific PCR system solely show the presence of amplifiable DNA. Amplification of 18S rDNA (137 bp) gene sequence showed that the extracted DNA is of good quality for both control rice and paddy samples. A 137bp band was observed with both the rice and paddy samples of the same (Sona Masuri) variety indicating the amplifiability of extracted DNA (Figure 20). DNA isolated from rice stored in jute, PP and HDPE sacks were used as template to amplify the 18s.rDNA. A positive amplification signal of 137 bp with this system indicated the presence of amplifiable DNA (Figure 21, Lanes 2, 3 & 4). The DNA was amplifiable in all the stored samples indicated that no detectable changes occurred during storage.

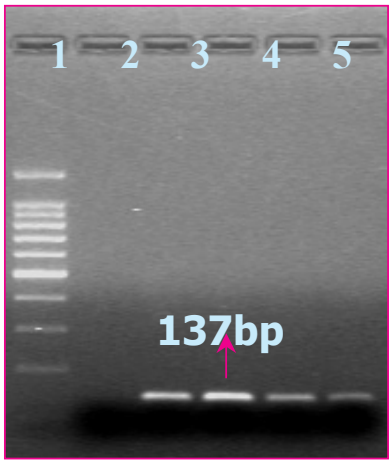

Figure 20: Amplification of 18S rDNA of rice and Paddy. Lane 1: 100bp Ladder, Lane 2 Premise control, Lane 3&4: Rice and Lanes 5&6 Paddy

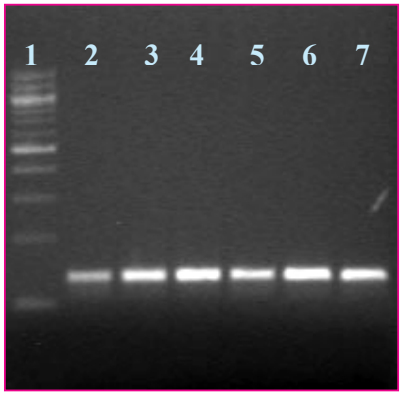

Figure 21: Amplification Of The 18s Rdna Gene. Lane 1-100bp Ladder, Lane 2-Rice Stored In Hdpe, Lane 3-Rice Stored In Pp, Lane 4-Rice Stored In Jute, Lane 5-Paddy Stored In Hdpe, Lane 6-Paddy Stored In Pp And Lane 7-Paddy Stored In Jute.

PCR setup for 18srDNA amplification of rice DNA

Forward Primer: **TRO3: 5'TCT GCC CTA TCA ACT TTC GAT GGT A'3**
Reverse Primer: **TRO4: 5' AAT TTG CGC GCC TGC TGC CTT CCT T'3**Table: 1

Reagents	Volume per Sample (µL)
10x PCR buffer with $MgCl_2$	5
dNTPs solution (10mM)	1
Primer TRO3 (4pM/µl)	5
Primer TRO4 (4pM/µl)	5
Taq DNA polymerase (5U/µl)	0.4
DNA Template	100-250ng

Temperature-time program

Initial denaturation	95°C/ 4 min 30 sec
Denaturation	95°C/ 46 sec
Annealing	65°C/1 min 25 sec
Extension	72°C/1 min 25 sec
No of cycles	30
Final extension	72 °C/ 3 min 15 sec

By looking at the genomic DNA as well as the amplified 18srDNA product (137bp) from PCR we can conclude that storing rice and paddy in different packaging materials has not affected the amplification and the DNA extracted is amplifiable.

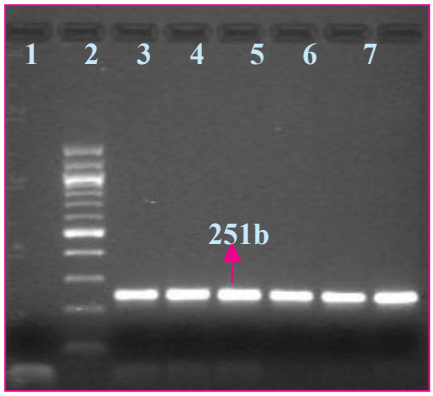

Figure 22: Amplification of the Sucrose phosphate synthase gene. Lane 1- Negative control (wheat), Lane 2-100bp ladder, Lane 3- Rice stored in HDPE, Lane 4- Rice stored in PP, Lane 5- Rice stored in Jute, Lane 6- Paddy stored in HDPE, Lane 7- Paddy stored in PP and Lane 8-Paddy stored in Jute

Amplification of Rice specific gene-Sucrose Phosphate Synthase

In order to authenticate rice and paddy samples, amplification of sucrose phosphate synthase gene the rice specific gene (endogenous reference gene) was carried out. A 251bp amplification product could be observed for rice and paddy samples stored in different packages (Figure 22). No amplification product was observed when DNA sample from wheat was used as template, which demonstrated that this system is specific for rice. The sucrose phosphate gene was amplifiable in all the stored rice and paddy samples suggest that no detectable changes have occurred during the period of study.

PCR setup for sucrose phosphate synthase gene amplification of rice DNA

PCR reaction setup

Reagents	Volume per Sample (µL)
10x PCR buffer with $MgCl_2$	5
dNTPs solution (10mM)	1
Primer SPSF (10PM/µl)	2
Primer SPSR (10PM/µl)	2
Taq DNA Polymerase (5U/µl)	0.4
DNA Template	100-250ng

Temperature programme for PCR

Initial denaturation	95°C/ 10 min
Denaturation	94°C/ 20 sec
Annealing	60°C/40 sec
Extension	72°C/1 min
No of cycles	40
Final extension	72 °C/ 3 min

EFFECT OF STORAGE ON LIPASE AND α- AMYLASE ACTIVITY OF WHEAT

Extraction: One g each of wheat flours stored in different packing material was extracted with 0.05M NaPi buffer, pH 7.4 at 4^0C. The extract was centrifuged at 10,000 × g. The supernatant was used as the source of enzyme.

Lipase Activity: Lipase activity was determined by using *p*-nitrophenyl acetate as a substrate. Lipase acts on *p*-nitrophenyl acetate and releases *p*-nitrophenol (PNP), detected at 410 nm (ε=15000 M^{-1} cm^{-1}). The activity of the enzyme was assayed using 0.01 ml of 5 mM *p*-nitrophenyl acetate in acetonitrile to 0.1M NaPi buffer, pH 7.4 containing enzyme in a total volume of 1 mL. The assay mixture was incubated for exactly 10 min at 25^0C and the absorbance was read at 410 nm. One unit of lipase activity is defined as the amount of enzyme that produces one μmole of PNP per minute at 25 ^{0}C and pH 7.5.

The lipase activity of the wheat flours is shown in Table 19. The results indicate that the lipase activity of wheat stored for six months in jute, polypropylene or HDPE sacks are similar.

Table 19. Lipase and amylase activity of wheat

Storage	Lipase Activity (Units/ g flour)	∝-Amylase activity (Units/g flour)
Jute	0.47	500.4
Polypropylene (PP)	0.48	616.3
HDPE	0.56	547.9

∝-Amylase: The ∝-amylase activity was assayed using a 2% gelatinized starch solution as substrate. The released reducing sugar was estimated using dinitrosalicylic acid. One unit of ∝-amylase activity is defined as the amount of enzyme that releases one micromole of maltose per minute at pH 5.0 and 30 °C. The amylase activity of the stored wheat is similar (Table 19). These results suggest that the packaging material has no apparent effect on the enzyme activity.