# Traceability of Rice and Wheat to ensure their quality using molecular and isotopic techniques

IAEA-CN-222-188

RICE & WHEAT

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# Food traceability

- To study the traceability, we have selected two food items i.e.

Wheat

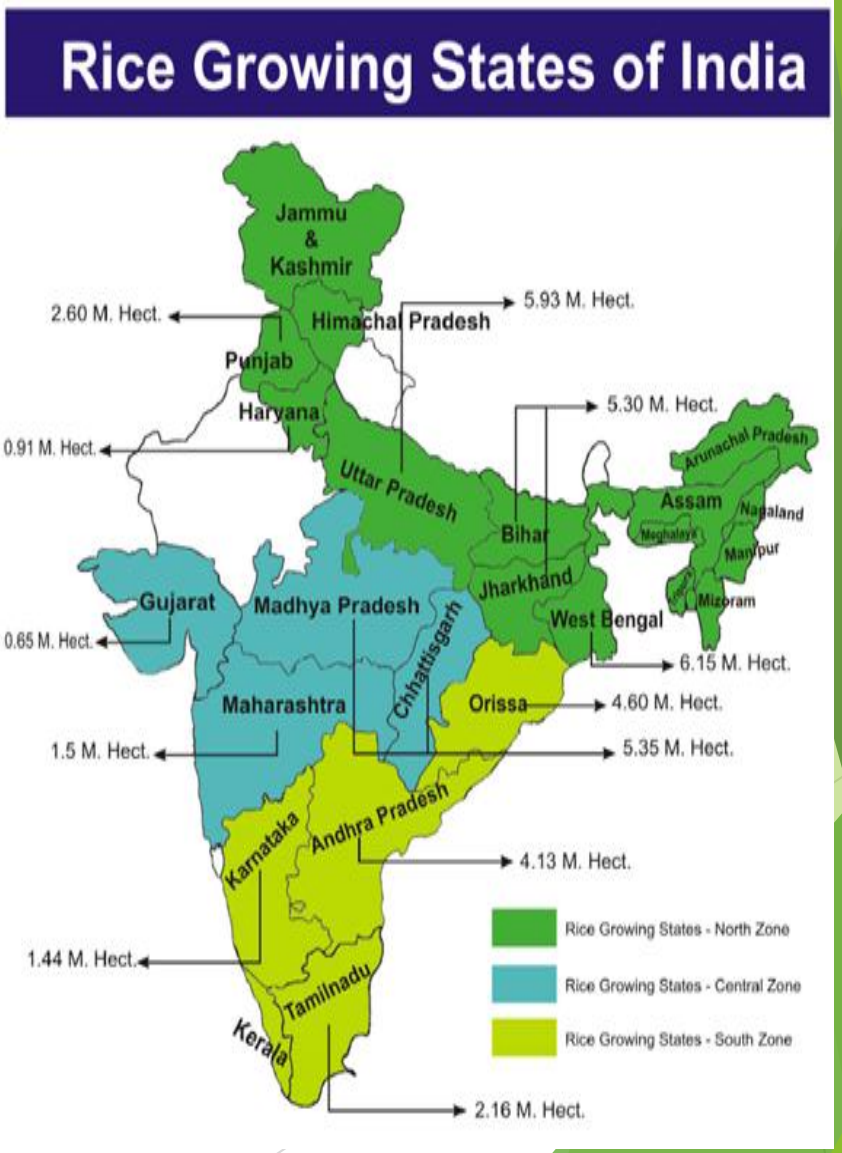
Rice

- Rice and Wheat are the staple food crops in India.

# Rice

# Rice

- Rice is one of the main grains of India. It is the staple food for the people of eastern and southern parts of India.
- India is one of the leading producers of this crop.
- Rice accounts for about 42% of total food grain production and >55% of diet in India.
- Total number of accessions range from 75,000 to 100,000 including duplicates.
- Among all rice varieties grown in India, Basmati occupies the premium position.



# Basmati Rice

- Basmati rice cultivation has remained indigenous to the Himalayan foot hills of Indian sub-continent.
- The areas of Basmati Rice production in India are in the states of Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Delhi, Uttarakhand and western Uttar Pradesh.
- Aroma and kernel elongation ratio are being highly influenced by tropical environment.

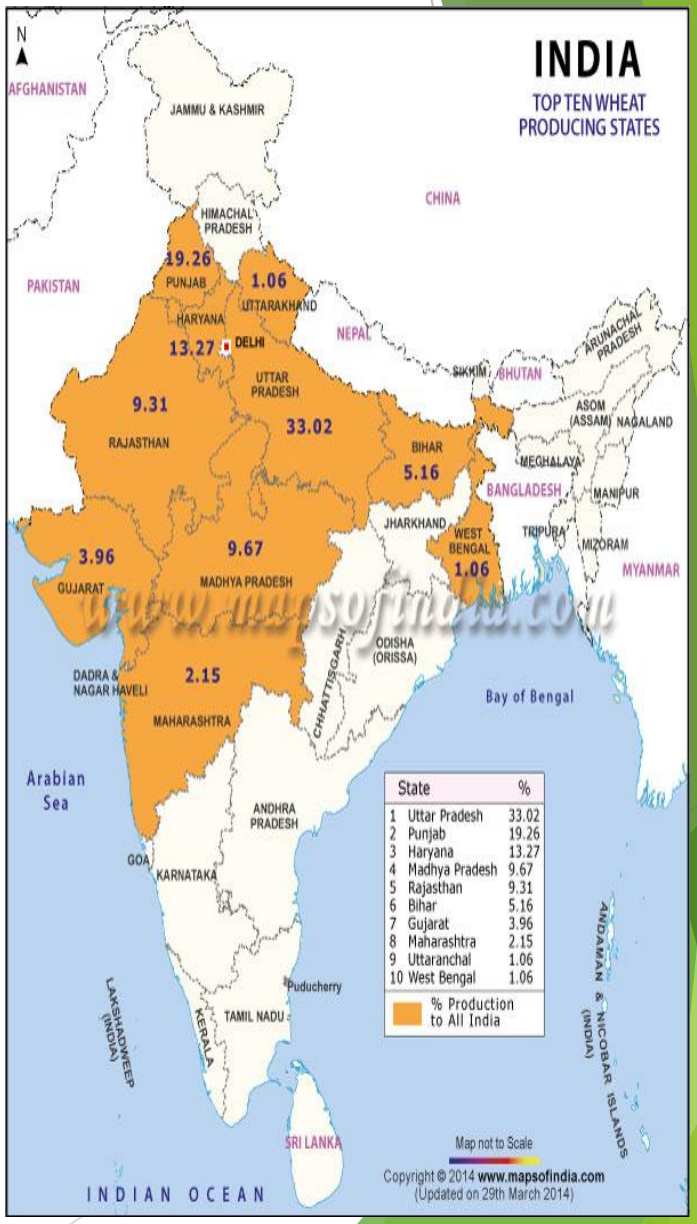
# List of rice samples collected

| S. No. | Variety of the samples | Collection Centre |
|---|---|---|
| 1. | PB1121 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 2. | Rajendra Kasturi | Rajendra Agriculture University,Pusa,Bihar |
| 3. | Uma | National Seed Corporation(NSC),Delhi |
| 4. | PB 1460 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 5. | Rajendra Marchai | Rajendra Agriculture University,Pusa,Bihar |
| 6. | PB1401 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 7. | Abhishek | National Seed Corporation(NSC),Delhi |
| 8. | PB1 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 9. | Rajendra Bhagwati | Rajendra Agriculture University,Pusa,Bihar |
| 10. | MTU 1010 | National Seed Corporation(NSC),Delhi |
| 11. | MTU 1001 | National Seed Corporation(NSC),Delhi |

# Wheat

# Wheat

- In India wheat is the main cereal crop.
- Abundant source of energy and nutrition.
- Second largest producer in the world.
- Wheat cultivated globally :
- Hexaploid bread/common wheat (*T. aestivum*) : 95%.
- Tetraploid durum wheat (*T. durum*) : 5%.
- Bread Wheat : Bread, Chapatti, Biscuits, Cakes, Pastries, etc.
- Durum Wheat : Spaghetti, Macaroni, Vermicelli, Suji, Rawa etc.
- Quality of wheat product depends on type of wheat used.



# Wheat

- Samples of Wheat were collected from different regions in India, which include Delhi, Bihar, Rajasthan, Punjab and Karnal (Haryana).
- NSC (National Seed Corporation, New Delhi) .
- IARI (Indian agriculture research institute, New Delhi).
- NPBGR(National Bureau of Plant Genetic Resources, New Delhi).
- Total 20 samples of premium wheat varieties have been collected .
- *Triticum aestivum* (bread wheat) : 15
- *Triticum durum* (durum wheat) : 5

# List of wheat samples collected

| Sl. No | Variety Of The Sample | Species Of Wheat Sample | Collection Centre |
|---|---|---|---|
| 1. | HD 2824 | *T.aestivum* | Rajendra Agriculture University(RAU),Pusa,Bihar |
| 2. | HD 2894 | *T.aestivum* | Indian Agriculture Research institute(IARI),Delhi |
| 3. | HD 3107 | *T.aestivum* | Indian Agriculture Research institute(IARI),Delhi |
| 4. | HD 2733 | *T.aestivum* | Rajendra Agriculture University,Pusa,Bihar |
| 5. | RAJ 1555 | *T.durum* | Agriculture Research Station (ARS), Rajasthan |
| 6. | HD 2967 | *T.aestivum* | Indian Agriculture Research institute(IARI),Delhi |
| 7. | HD 2643 | *T.aestivum* | Rajendra Agriculture University, Pusa, Bihar |
| 8. | HD 4725 | *T.durum* | Indian Agriculture Research institute(IARI),Delhi |
| 9. | DBW 14 | *T.aestivum* | Rajendra Agriculture University, Pusa, Bihar |
| 10. | DBW 17 | *T.aestivum* | Indian Agriculture Research institute(IARI),Delhi |
| 11. | RAJ 6560 | *T.durum* | Agriculture Research Station (ARS), Rajasthan |
| 12. | PDW 291(L) | *T.durum* | Punjab Agriculture University(PAU),Ludhiana |
| 13. | PDW 314 | *T.durum* | Indian Agriculture Research institute(IARI),Delhi |
| 14. | PBW 550 | *T.aestivum* | Punjab Agriculture University(PAU),Ludhiana |
| 15. | PBW 343 | *T.aestivum* | National Seed Corporation(NSC),Delhi |
| 16. | LOK 1 | *T.aestivum* | National Seed Corporation(NSC),Delhi |
| 17. | K 307 | *T.aestivum* | Rajendra Agriculture University, Pusa,Bihar |
| 18. | DL -788-2 | *T.aestivum* | National Seed Corporation(NSC),Delhi |
| 19. | RAJ MRI | *T.aestivum* | Agriculture Research Station(ARS), Jaipur |
| 20. | M.P WHEAT | *T.aestivum* | Local Market, Delhi |

# Present studies : Rice and Wheat

- Isotopic Analysis
- Physical Tests
- Biochemical Tests
- Genetic Analysis

# Isotopic analysis

- By analysis of carbon isotopes i.e. $\delta^{13}C$

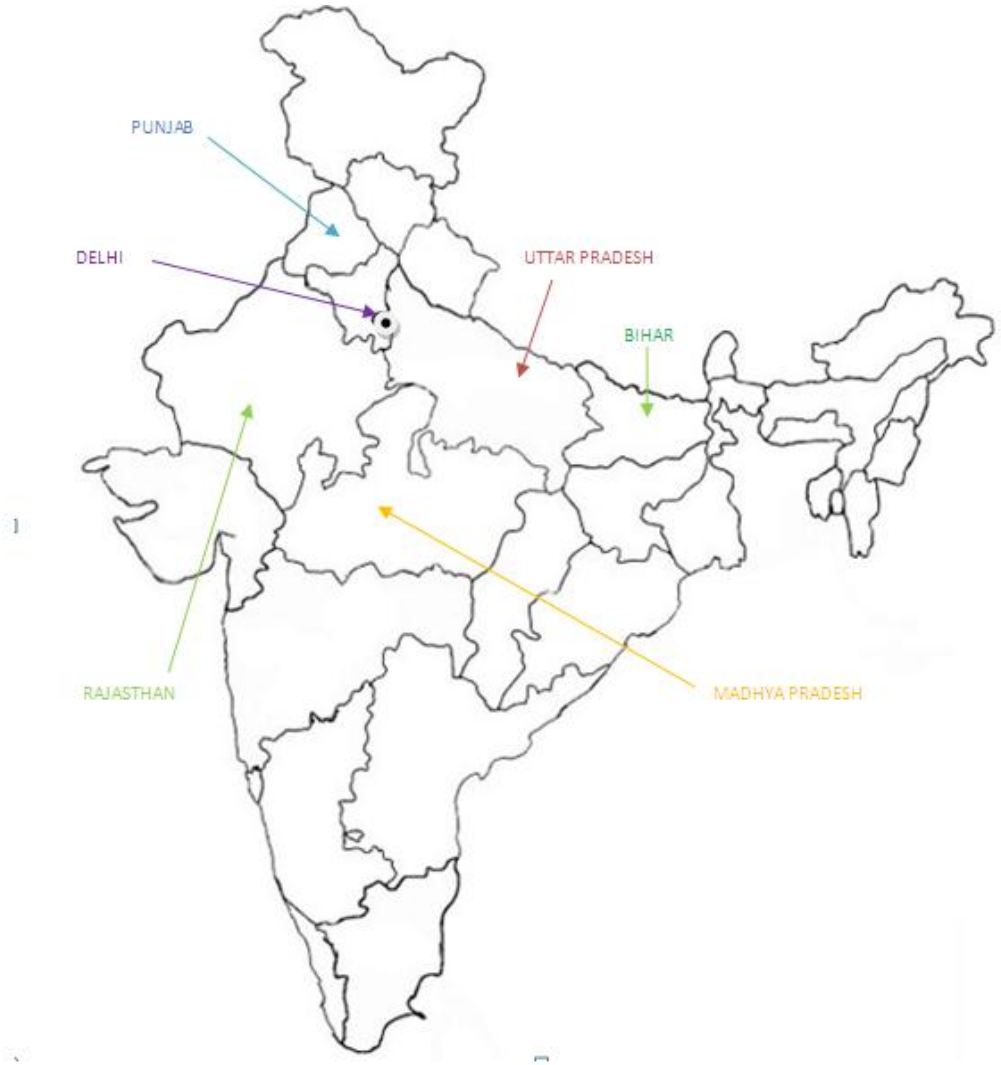
- Plant takes carbon dioxide present in the atmosphere, $\delta^{13}C$ value of plant is not the same as atmospheric $CO_2$.

**This is because of :**

1. Differential diffusion of $CO_2$, through the leaf stomata.
2. Site of assimilation and the carboxylation reaction to fix $CO_2$.

# $\delta\,^{13}C$ value for wheat

# Sample collection centre for wheat



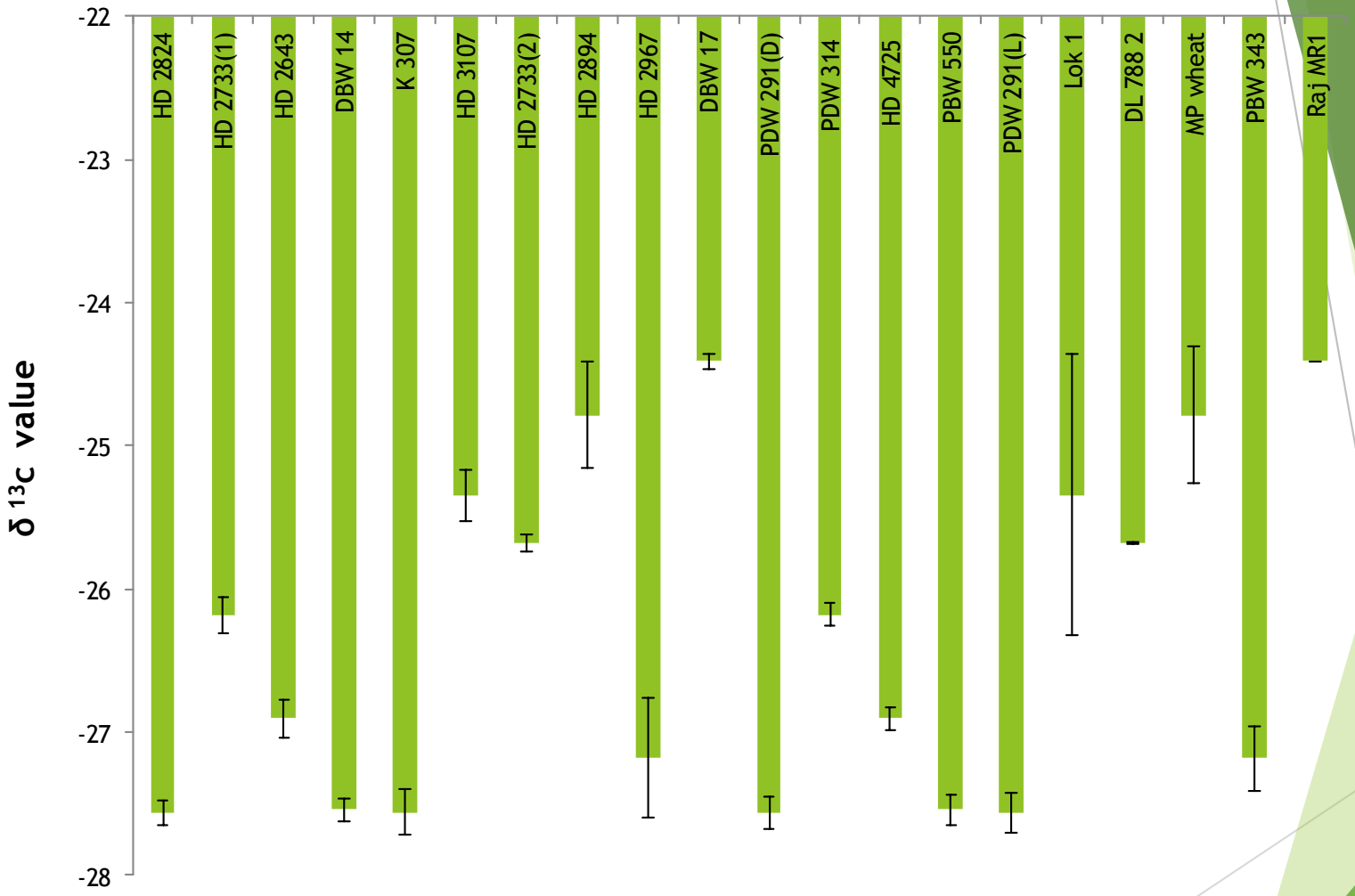
# $\delta^{13}$c value of wheat

| S. No. | Wheat Sample | (13C/12 C) $\delta^{13}C$ | Collection region |
|---|---|---|---|
| 1. | HD 2824 | -26.16 | Bihar |
| 2. | HD 2733(1) | -26.72 | Bihar |
| 3. | HD 2643 | -26.392 | Bihar |
| 4. | DBW 14 | -26.452 | Bihar |
| 5. | K 307 | -26.657 | Bihar |
| 6. | HD 3107 | -26.913 | Delhi |
| 7. | HD 2733(2) | -27.11 | Delhi |
| 8. | HD 2894 | -25.666 | Delhi |
| 9. | HD 2967 | -26.25 | Delhi |
| 10. | DBW 17 | -27.72 | Delhi |

# Cont....

| S. No. | Wheat Sample | (13C/12 C) $\delta^{13}C$ | Collection region |
|---|---|---|---|
| 11. | PDW 291(D) | -27.56 | Delhi |
| 12. | PDW 314 | -26.178 | Delhi |
| 13. | HD 4725 | -26.906 | Delhi |
| 14. | PBW 550 | -27.54 | Punjab |
| 15. | PDW 291(L) | -27.56 | Punjab |
| 16. | Lok 1 | -25.345 | Madhya Pradesh |
| 17. | DL 788 2 | -25.673 | Madhya Pradesh |
| 18. | MP wheat | -24.785 | Delhi |
| 19. | PBW 343 | -27.18 | Uttar Pradesh |
| 20. | Raj MR1 | -24.41 | Rajasthan |

# δ $^{13}$c value of wheat

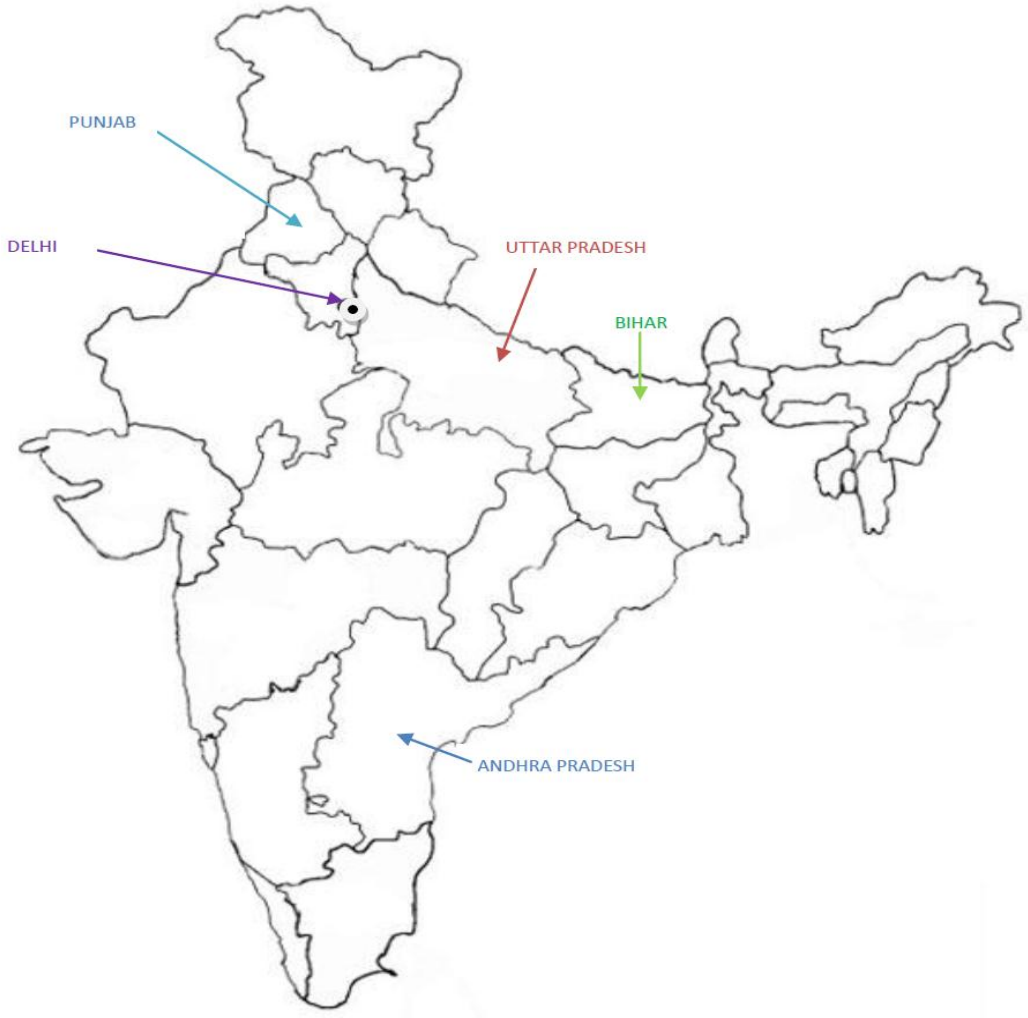


# Average of $\delta^{13}c$ value for wheat

- **Bihar :** **-26.4762**
- **Delhi :** **-26.7878**
- **Punjab :** **-27.55**
- **Madhya Pradesh :** **-25.267**
- **Uttar Pradesh :** **-27.18**
- **Rajasthan :** **-24.41**

# $\delta\,^{13}C$ VALUE for Rice

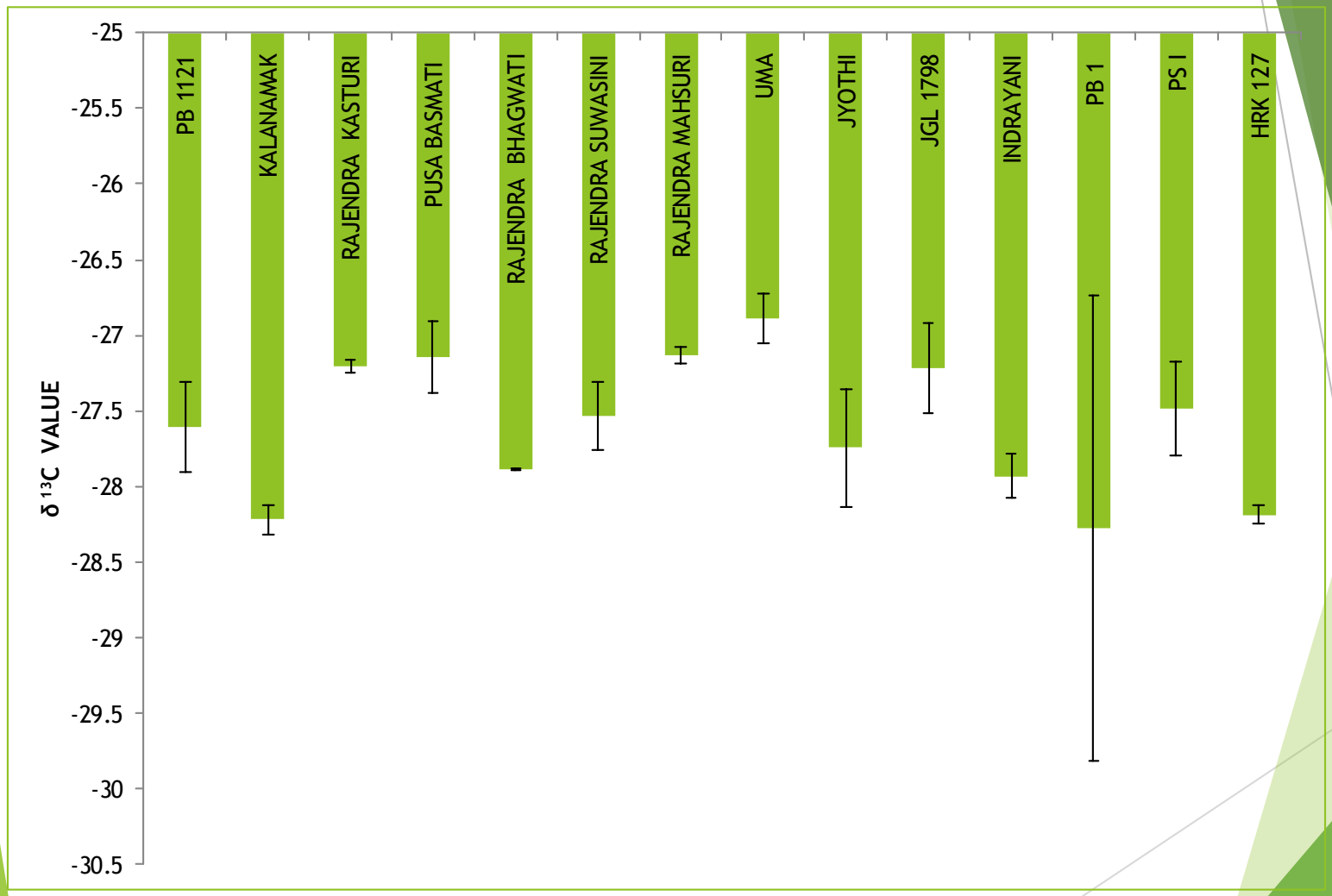
# Sample collection centre for rice



# $\delta^{13}$c value of rice

| S. No. | Rice Samples | Collection centre | (13C/12 C) $\delta^{13}C$ |
|---|---|---|---|
| 1. | PB 1121 | Uttar Pradesh | -27.607 |
| 2. | KALANAMAK | Uttar Pradesh | -28.22 |
| 3. | RAJENDRA KASTURI | Bihar | -27.206 |
| 4. | PUSA BASMATI | Bihar | -27.147 |
| 5. | RAJENDRA BHAGWATI | Bihar | -27.888 |
| 6. | RAJENDRA SUWASINI | Bihar | -27.533 |
| 7. | RAJENDRA MAHSURI | Bihar | -27.131 |
| 8. | UMA | Andhra Pradesh | -26.891 |
| 9. | JYOTHI | Andhra Pradesh | -27.744 |
| 10. | JGL 1798 | Andhra Pradesh | -27.217 |
| 11. | INDRAYANI | Andhra Pradesh | -27.932 |
| 12. | PB 1 | Delhi | -28.275 |
| 13. | PS I | Delhi | -27.486 |
| 14. | HRK 127 | Punjab | -28.185 |

# δ $^{13}$c value of rice



# Average of $\delta\ ^{13}c$ value for rice

- Uttar Pradesh: -27.913
- Bihar: -27.381
- Andhra Pradesh: -27.446
- Delhi: -27.88
- Punjab: -28.185

# Basmati rice

- Long slender
- Aromatic
- Cooked kernel elongation
- Tenderness of cooked rice

# List of rice samples collected

| S. No. | Variety of the samples | Collection Centre |
|---|---|---|
| 1. | PB1121 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 2. | Rajendra Kasturi | Rajendra Agriculture University,Pusa,Bihar |
| 3. | Uma | National Seed Corporation(NSC),Delhi |
| 4. | PB 1460 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 5. | Rajendra Marchai | Rajendra Agriculture University,Pusa,Bihar |
| 6. | PB1401 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 7. | Abhishek | National Seed Corporation(NSC),Delhi |
| 8. | PB1 (Basmati) | Indian Agriculture Research institute(IARI),Delhi |
| 9. | Rajendra Bhagwati | Rajendra Agriculture University,Pusa,Bihar |
| 10. | MTU 1010 | National Seed Corporation(NSC),Delhi |
| 11. | MTU 1001 | National Seed Corporation(NSC),Delhi |

# Genetic Diversity and relatedness : Rice

# Genetic Studies

- Genetic diversity and relatedness was studied for selected varieties using RANDOM AMPLIFIED POLYMORPHIC (RAPD) DNA .

- We examined the amplified product of primers for their size and polymorphism.

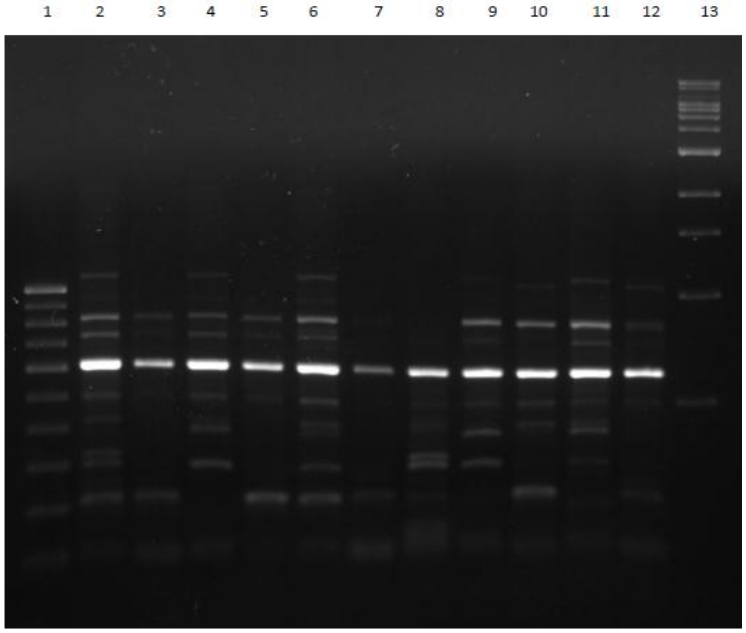
- Primers used for the study are OPO-05,OPB-11, OPB01, ,OPD-05.

# RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) primers

| S. No. | Primer Name | Sequence (5'-3') |
|---|---|---|
| 1 | OPD 5 | TGAGCGGACA |
| 2 | OPB 01 | GT T T CGCTCC |
| 3 | OPB 11 | GTAGACCCGT |
| 4 | OPO 05 | CCCAGTCACT |

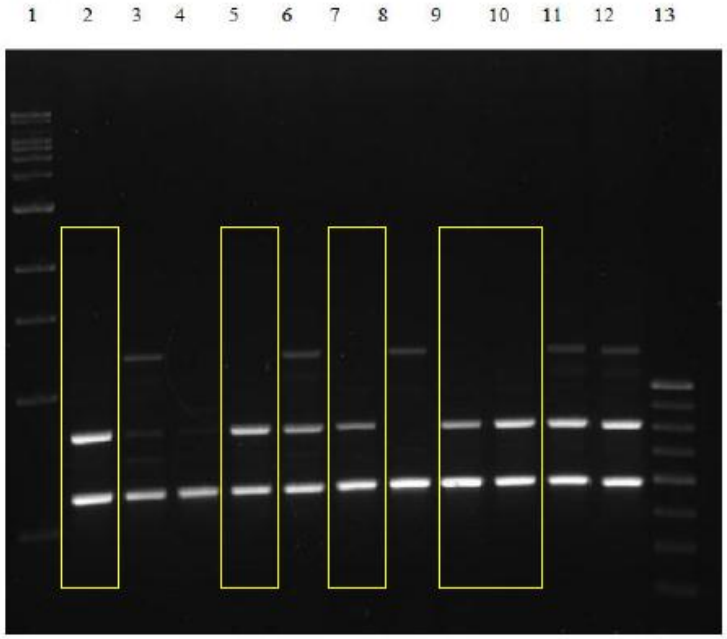
# RAPD Results

OPD-05




1-100bp ladder, 2-Rajendra Kasturi, 3-MTU1010, 4-PB1401, 5-MTU1001,6-PB1121,7-Abhishek, 8-Rajendra Marchai, 9-PB1460, 10-Rajendra Bhagwati, 11-Uma, 12-PB1, 13-supermix DNA ladder

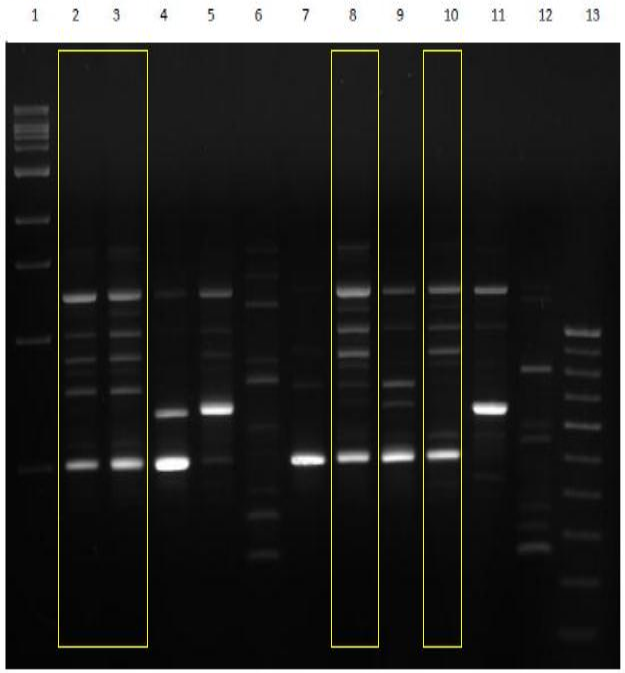
OPB-11




1-Supermix Dna ladder 2-PB1, 3- Rajendra Bhagwati, 4- Rajendra Marchai, 5- PB1401, 6- Uma, 7- PB1460, 8- Abhishek, 9- PB1121, 10- Rajendra Kasturi, 11- MTU1010, 12- MTU1001, 13-100bp ladder

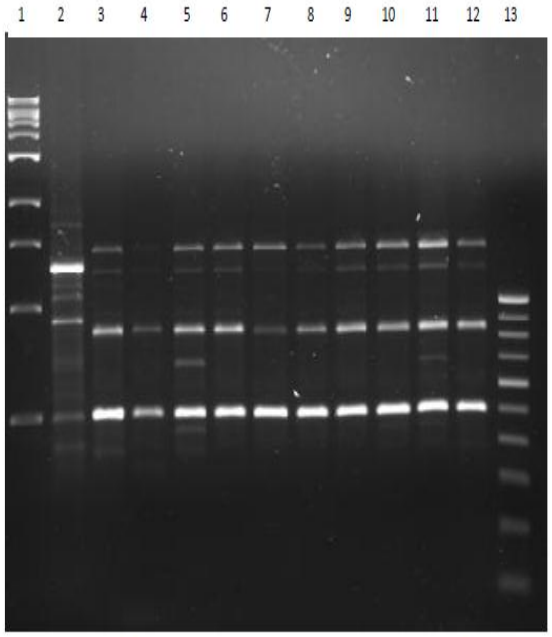
# RAPD Results

OPB-01




1-Super mix DNA ladder, 2-PB1, 3-PB1401, 4-Abhishek, 5-Uma, 6-Rajendra Kasturi,7-RajendraMarchai, 8-PB1121, 9-Rajendra Bhagwati, 10- PB1460, 11-MTU1010, 12- MTU1001, 13-100bp ladder.

OPO-05




1-Super mix DNA ladder, 2- Rajendra Marchai , 3-MTU1001,4-Abhishek,5- Rajendra Bhagwati,6-Uma-,7-MTU1010, 8- PB1121,9- PB1401,10- PB1460,11- PB1,12- Rajendra Kasturi,13-100bp ladder

# RAPD results

- Similarly **OPD-05** is also not efficient in differentiating Basmati rice but helped in study diversity among the selected Rice varieties

- Amplified product of **OPB-11** primer showed similar banding pattern among **Basmati rice** varieties and using the same primer, **Rajendra Kasturi** also showed similar banding pattern.

- **OPO-05** showed similar banding pattern among all rice varieties except **Rajendra Marchai**, thus not efficient in differentiating Basmati from non Basmati Rice.

- **OPB-01** showed similar banding pattern among **all Basmati Rice.**

# Simple Sequence Repeats

- Simple sequence repeats are a class of genetic polymorphism commonly used for mapping, linkage analysis and to trace inheritance patterns.

- They are tandem repeated sequences, where the repeating unit is 1 to 4 nucleotides long. The number of times the unit is repeated in a given SSR can be highly variable, a characteristic that makes them useful as genetic markers .

- For identification of aromatic rice lines, genotypic analysis was done using SSR markers.

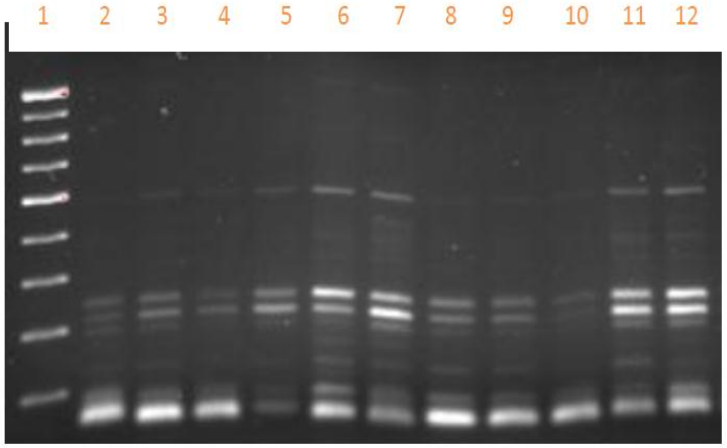
# Simple sequence repeats

## SSR markers used for grain quality traits

| Primer Name | Sequence | Annealing Temp. ($^0$C) | Chromosome Locus |
|---|---|---|---|
| RM223 | Rev. GAAGGCAAGTCTTGGCACTG<br>Fwd. GAGTGAGCTTGGGCTGAAAC | 55 | 8 |
| RM342 | Rev. ACTATGCAGTGGTGTCACCC<br>Fwd. CCATCCTCCTACTTCAATGAAG | 55 | 8 |

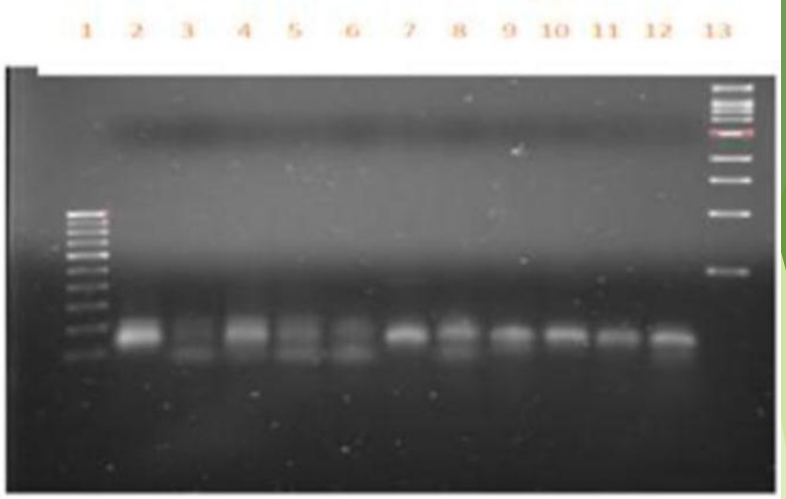
# Simple Sequence Repeats for Aroma

RM223




1-100bp ladder, 2-Uma, 3-PB1, 4- PB1121, 5- PB1460, 6- PB1401, 7-Abhishek, 8-Rajendra Kasturi, 9- Rajendra Bhagwati, 10- Rajendra Marchai, 11-MTU1010, 12 MTU-1001.

RM342




1-100bp ladder, 2-Rajendra Marchai, 3-PB1121, 4-PB1, 5-Rajendra Kasturi, 6-PB1460, 7-MTU1010, 8-PB1401, 9-MTU1001, 10-Uma, 11-Abhishek, 12-Rajendra Bhagwati, 13-Super mix DNA ladder

# Simple Sequence Repeats : Results

- RM223 has shown 3 bands in non aromatic varieties and 2 bands in aromatic varieties in between 200-300bp.

- RM342 has shown single band of 200bp in non aromatic varieties

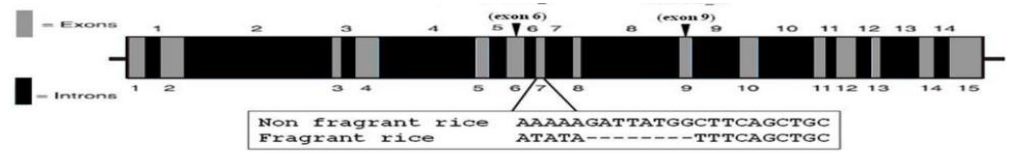
# Primer Designing

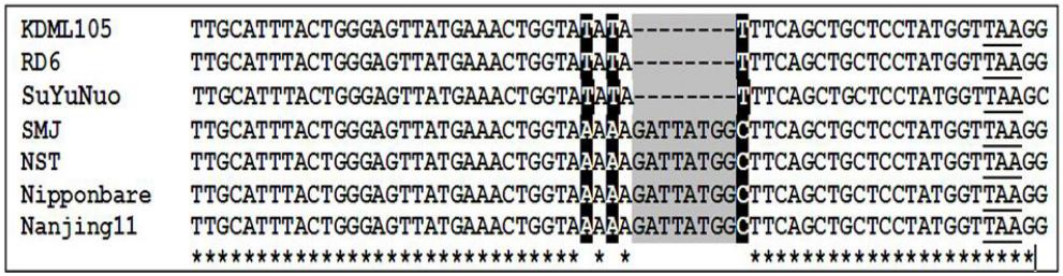
- To study genotypic differences in aromatic/non-aromatic rice, primers were designed for exon 7 of *Badh2* gene of *fgr* region on chromosome-8 encoding enzyme BADH2 (**Betaine aldehyde dehydrogenase isoform 2**).

- The *Badh2* in aromatic rice has an 8-bp deletion and 3 SNPs in exon 7. This mutation at exon 7 of *Badh2 in* aromatic rice related to 2AP accumulation (**2-Acetyl-1-pyrroline**).

# Primer designing

Two primers were designed for this *Badh2* gene that flank 8 bp deletion and 3 SNPs in order to differentiate aromatic from non aromatic rice.




(Srivong et al.,1998)

# Primer designing

Oryza sativa Indica Group cultivar Nanjin

Betaine aldehyde dehydrogenase (Badh2) gene,

CTTCCTTCAGGTGTGCTAAACATAGTGACTGGATTAGGTTCTGAAGCCGGTGCTCCTTTGTCATCACACCCTGGT
GTAGACAAGGTACAGCTATTCCTCCTGTAATCATGTATACCCCATCAATGGAAATGATATTCCTCTCAATACATGG
TTTATGTTTTCTGTTAGGTTGCATTTACTGGGAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGCTGCTCCTA
TGGTTAAGGTTTGTTTCCAAATTTCTGTGGATATTTTTTGTTCTCTTTCTACTAACTCTCTATTATCAATTCTCAAT
GTTGTCCTTTTCTTTTAACTCCTTTACTTTTTAGAATTGTGATCAAGACACTTTGAGCATCATTCTAGTAGCCAGT
TCTATCCTGTTTCTTACCTTTTTATGGTTCGTCTTTTCTTGACAGCCTGTTTCACTGGAACTTGGTGGAAAAAGTC
CTATAGTGGTGTTTGATGATGTTGATGTTGAAAAAGGTACATGCCACTTGCTATGATTAACTAATTCTGAAGTGC
AGGACTTTGTAAAGCACTTAACTGAGCTGGATGCTAGACCCCCAAAAGCCCTTTTTGGTGTCTTGGGCTTGTTGC
AGAAATACTGGTCCCAGACGAGCAG.

# Primers

Two primers were designed using Primer 3 software for differentiating aromatic and non aromatic rice

1.Forward-CACACCCTGGTGTAGACAAGG

Reverse-GGGTCTAGCATCCAGCTCAG

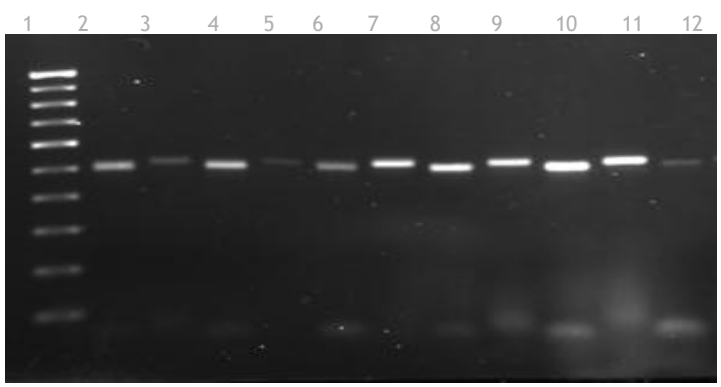
**PRODUCT SIZE: 507**

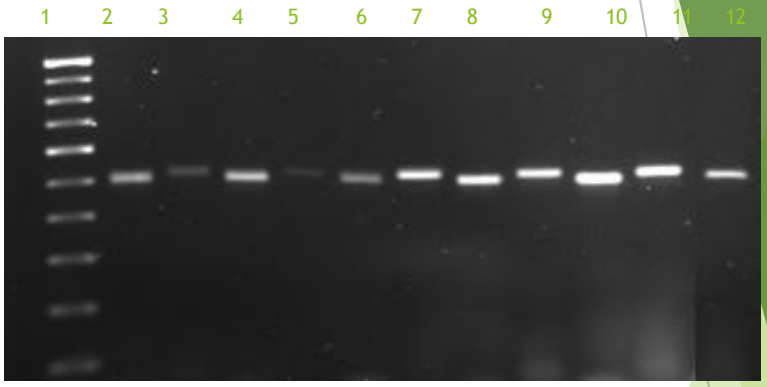
2.Forward-CGGTGCTCCTTTGTCATCAC

Reverse-GGTCTAGCATCCAGCTCAGT

**PRODUCT SIZE: 524**

# Amplified Product of Primer 1 & 2

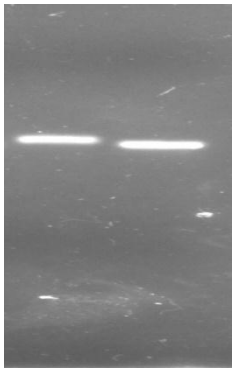


# Results

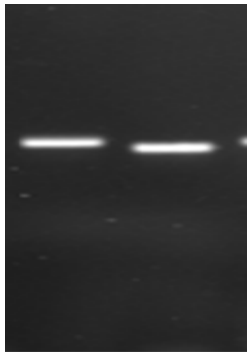
These are the two samples of each primer which were sent for sequencing to confirm the result.

Primer1



1-Uma, 2-Pb11211

Primer2



1-MTU1010, 2-PB1121

# Genetic Diversity and relatedness : Wheat

# Genetic Diversity Studies


Isolation of genomic DNA by Ctab method

DNA Purification by Phenol-chloroform method

DNA quantification by NanoDrop instrument


RAPD

SCAR

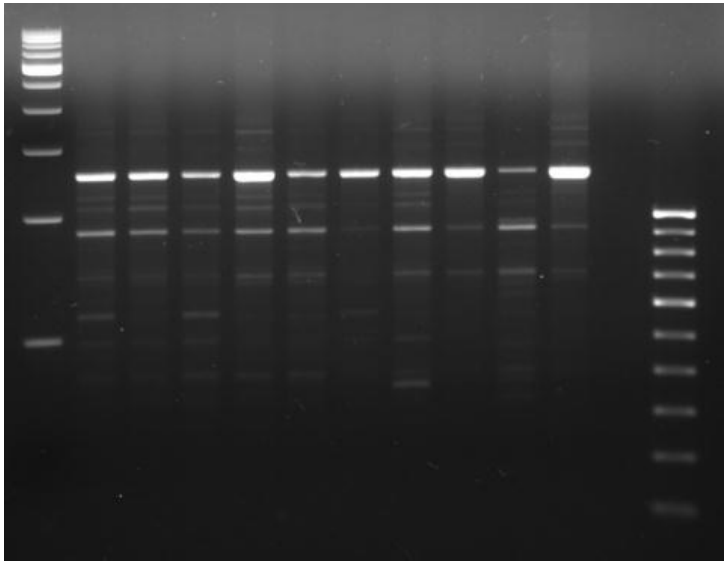
# RANDOM AMPLIFIED POLYMORPHIC DNA

- Operon primers were used

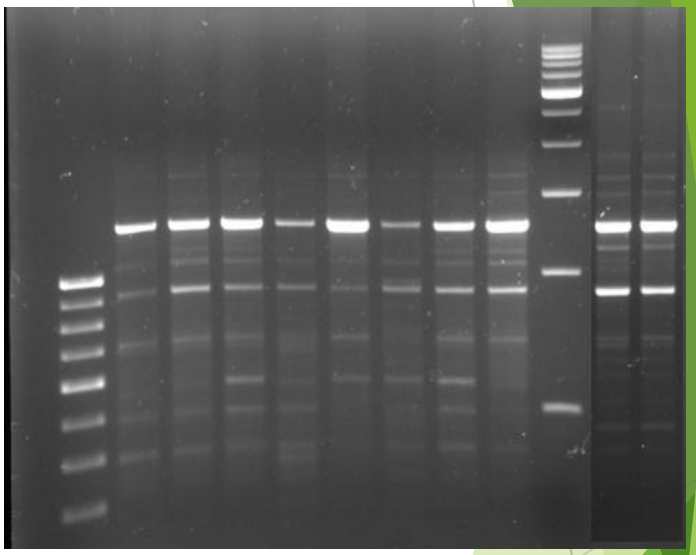
| Sl. No. | Primer name |
|---|---|
| 1 | OPA 5 |
| 2 | OPB 1 |
| 3 | OPF 17 |
| 4 | OPP 10 |

# RANDOM AMPLIFIED POLYMORPHIC DNA

OPA 5



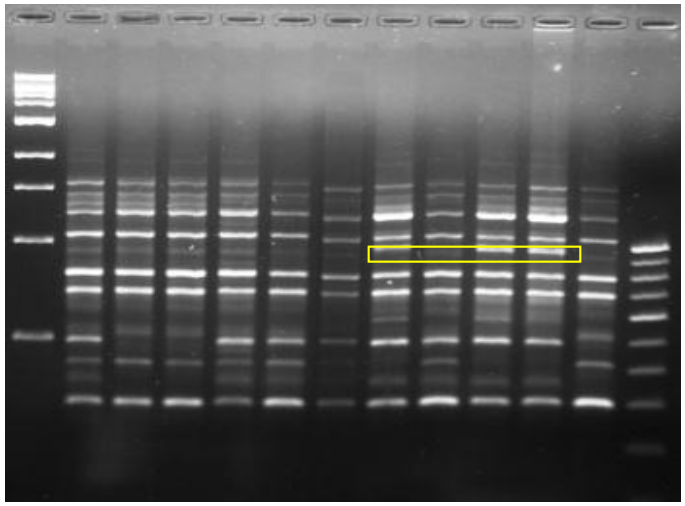
RAPD of genomic DNA of wheat samples by OPA 5: Lane 1- Supermix DNA ladder, lane 13-100bp ladder, lane 2-12 - HD 2967, DBW 14, HD 2643, Lok 1, DBW 17, HD 2894, HD 2824, HD 4725, PDW 291, PDW 314, Control respectively.



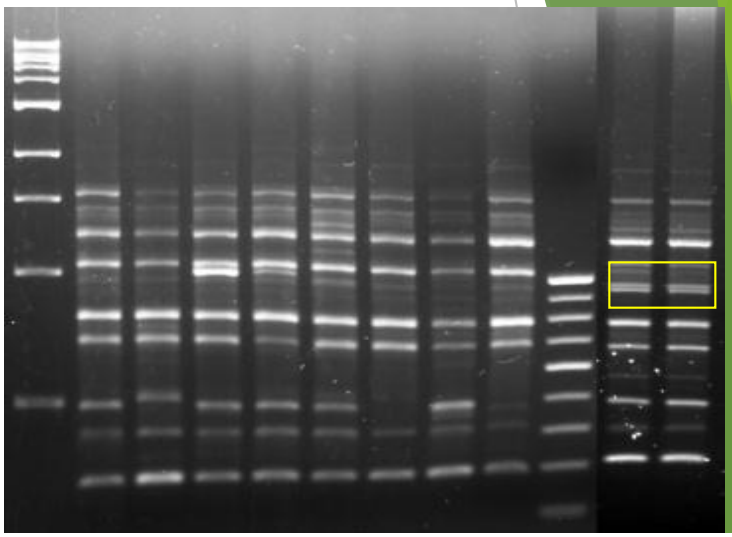
RAPD of genomic DNA of wheat samples by OPA 5: Lane 1- Supermix DNA ladder, lane 10-100bp ladder, lane 2-12 – HD 3107, HD 2733, K 307, PBW 550, Raj MR1, Market, DL 7882 PBW 343, Raj 1555, Raj 6560 respectively.

# RAPD

## OPB 1



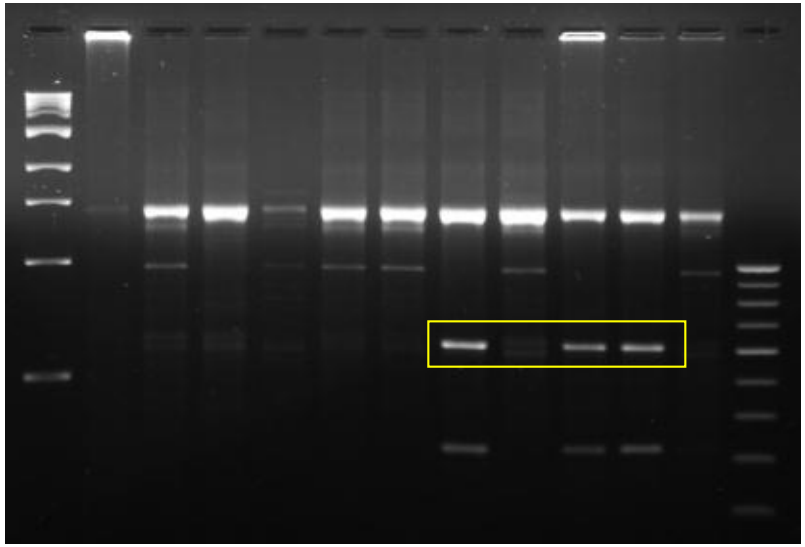
RAPD of genomic DNA of wheat samples by OPB 1: Lane 1- Supermix DNA ladder, lane 13-100bp ladder, lane 2-12 – HD 2967, HD 2377, HD2733, HD 2824, HD 2894, HD 3107, HD4725, HD 2643, PDW 291, PDW 314, PBW 343 respectively.



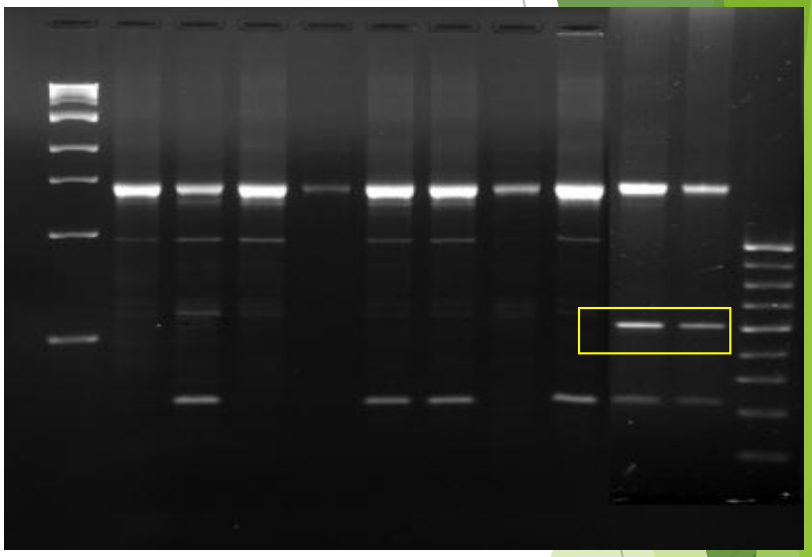
RAPD of genomic DNA of wheat samples by OPB 1: Lane 1- Supermix DNA ladder, lane 10-100bp ladder, lane 2-9 – DBW 14, DBW 17, K 307, LOK 1, DL 7882, PBW 550, Market wheat, RAJ MR1 respectively, lane 11-Raj 1555, lane 12Raj 6560.

# RAPD

## OPF 17



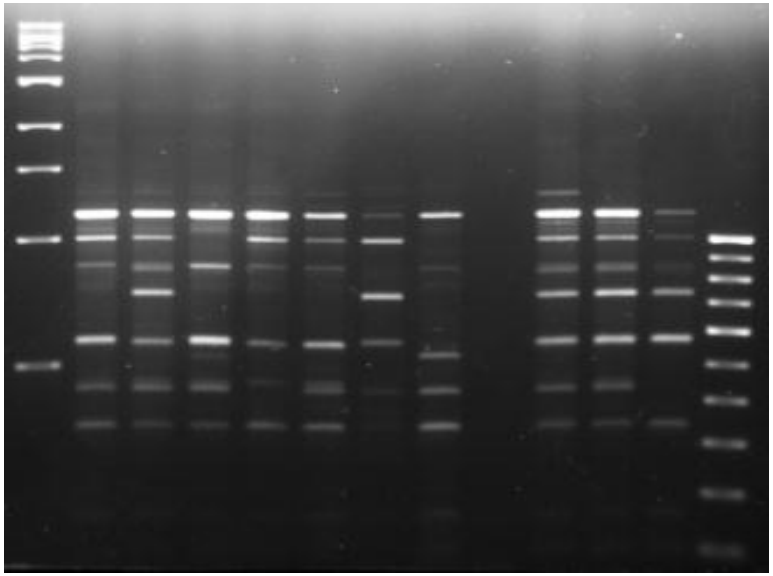
RAPD of genomic DNA of wheat samples by OPF 17: Lane 1- Supermix DNA ladder, lane 13-100bp ladder, lane 2-12 - HD 2643, HD 2733(1), HD 2824, HD 3107, HD 2894, HD 2967, HD 4725, HD 2733(2), PDW 314, PDW 291(D), PBW 343 respectively.



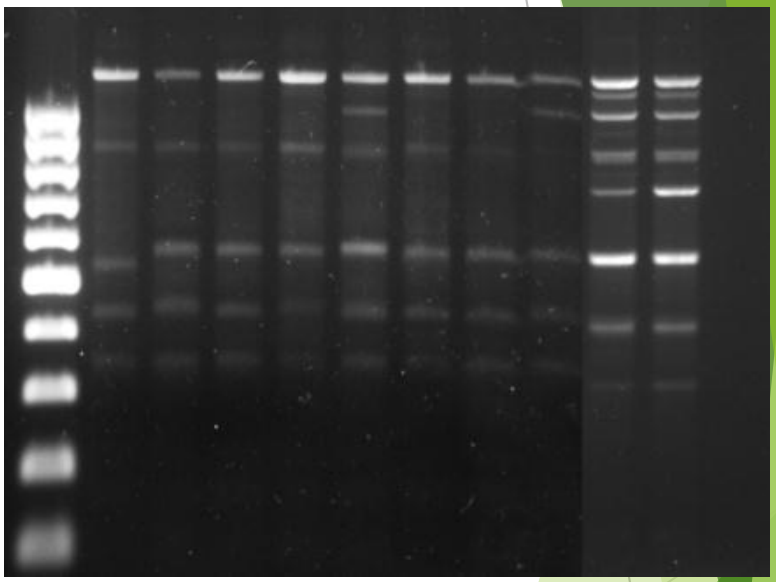
RAPD of genomic DNA of wheat samples by OPF 17: Lane 1- Supermix DNA ladder, lane 12-100bp ladder, lane 2-11 – DBW 14, DBW 17, K307, PBW 550, RAJ MR1, Lok 1, Market wheat, DL 7882 , Raj 1555, Raj 6560 respectively.

# RAPD

## OPP 10



RAPD of genomic DNA of wheat samples by OPP 10: Lane 1- Supermix DNA ladder, lane 13-100bp ladder, lane 9-control, lane2-12- DL 7882, HD 2967, HD 2824, Lok 1,HD 2643, HD 3107, PBW 343, control, PDW 291, HD 4725,PDW 314 respectively



RAPD of genomic DNA of wheat samples by OPP 10: Lane 1- 100 bp DNA ladder, lane 2-12 –HD 2733, Market, PBW 550,DBW 17, Raj MR1, K 307, HD 2894, DBW 14, Raj 1555, Raj 6560 respectively.

# Results

- OPA 5, OPP10 showed polymorphism among different varieties.
- OPF 17, OPB 1 were able to distinguish between bread wheat and durum wheat varieties.

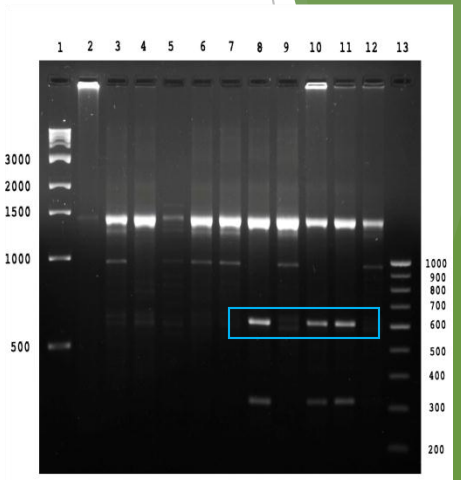
# SCAR (Sequence Characterized Amplification Region)

RAPD done by primer OPF 17 has been used

A common band of size ~600bp was commonly present in each of the durum wheat varieties (as shown in blue box)

absent in all bread wheat varieties

Basic protocol followed -

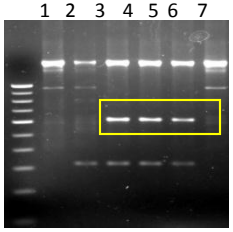



RAPD of genomic DNA of wheat samples by OPF 17: Lane 1- Supermix DNA ladder, lane 13-100bp ladder, lane 8,11,12 – durum wheat varieties, lane 2,3,4,5,6,7,10- bread wheat varieties.

**Cloning of unique band** → **Sequencing of DNA fragment** → **Designing specific primer to that sequence**

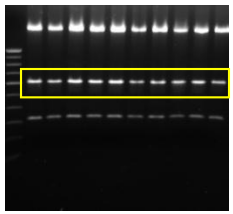
# Methodology

Before the final amplification 3 bread wheat varieties and 3 durum wheat varieties genomic DNA was again subjected to RAPD to confirm the consistency in banding pattern.



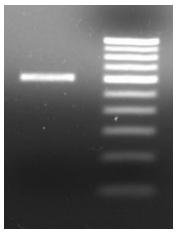

RAPD of genomic DNA of wheat samples by OPF 17: Lane 1- 100bp DNA ladder, lane 2- DL 7882, lane 3- DBW 17, lane4- PDW 291, lane 5- HD 4725, lane 6- PDW 314, lane 7- HD 2733.

Multiple RAPD amplification of genomic DNA of PDW 291(Durum) by OPF 17



Elution

Checking Precipitated product on 1% agarose gel



Cloning of DNA Sequence

Electroporation method

Positive clones

# Cloning

Transformation by Electroporation method

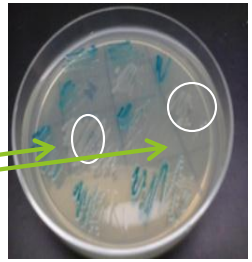
Primary screening of transformed cells

Blue colony

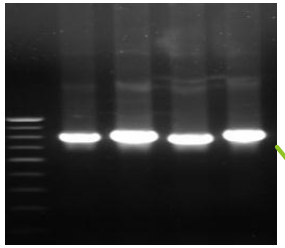
White colony

(Positive transformants)

Secondary screening



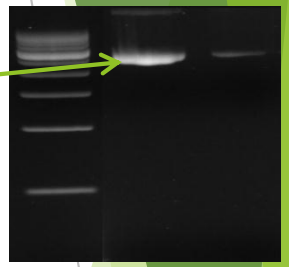
Selection of Clones having correct insert size by colony PCR(M 13 primer )



(600bp+ 211bp= 811bp)

Plasmid with insert

Plasmid Isolation



**Sequencing of DNA insert**

# Sequencing of desired DNA insert

- Primer M 13
- >0513_305_002_2_M13-Forward-A09.ab1

GGGACCTACTCTATAGGGCGATTGGGCCCGACGTCGCATGCTCCC GGCCGCCAT GGC GGCCGC GG GAATTCGATTAACCCG GGAAGAT GATGTCATGCTCCTTCA GGAAGATC GTCTTTGCGCA GCTGT GCAAGCTGCAC GCTACCAGCAAGCCTT GCGCTGCTATCATA GCCACAAGGTTCAT GCCCGAAGCATT GAGGAA GGT GACCTTGTACTTCG GCGCATTCAATCCGCCATG GGATCA
AACAAGTTGACGCCAAAGTGGGAGGGCCCTTATCGGGTAGCAC GAGTCACTAGACTT GGCATG GTCTGTTT GGAGACT GAAG GTGGCA GTCGC GTGCAAAACACATGGAATATC GAGCATCTTCG TAA GTTCTACCTGTAAG GCGCGATT GTCGT GCTTGGTCCCA GCAACCCCTTTTGTACAAACTTTGTCTCAAATACTTGTAACCCTTTGTACAAAGCCA GGCATAGACCAT GTGCATATGT
GAATAAATCTAAGTGTTCTACAATATTTTATGCATATTTACTTATGTACTAATGCATGATGTATTGTGGTTACATTTTGTTCGAATGAAACGACTAACATGCTGCCAAGGTCTCTACACCATTCC CTTCTCTTCTTTTCTTTTTCTAAAAGACAGAAG GATT CCTCACGTACATGTTTCCCGGGTTAATCACTA GTGAATT CGCGGCCGCCT GCAG GTCGACCATATG GGAGA GCTCCCAACGCGTT G
GATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGT GTGAAATTGTTATCC GCTCACAATTCCACACACATACGAGCCGA GCATAAGTG TA GCTGG GTGCTAT GATGAGTACCCTTAT GTGCGTCT GCCGTCAGC GCGAC GATGACGCAAAAAAA GAAAGAGTA GAGAGA GG GTATTCCGGCTTTCCA GTCGG GAAACCTGTC GTGCCA
GCTGCATTAATCGACATCGGCCAACGCGCGGGGAGAAGGC GGTTT GCGTATTG GGC GCTTCTTCCGCTTTTCCCCGCTCACTGACTCGCT GCG GCTCGGT GGTTTC GGCTTGC GG GG GAGG GG GT TATCAAGCTTCACTTCAAAGGC GG GTTATATACGG GTTATTCCACAAAATTCAGG GGTATACCCAG GGAAAGAAC GTGT GGAGCCAAAA GGGCCC GCAAG GGCCG GGAAACC GTAAA
AAGGCCCGTCTTCACTTTTTCTATAGGAGCTCACGTCTAGAGATTCCAATCAGGCTTAAGGTTCAAGTTGCGCTAAC GCGCAACG GACCTTTAAGAG GG GAT

- >0513_305_003_2_M13-Reverse-B09.ab1

GTGCCGGCATTTAGGTGACCTATAGAATACTCAAGCTATGCATCCAACGCGTTGGGA GCTCTCCCATATGGTC GACTGAG GCG GCCGCGAATTCACTA GTGATTAACCCG GGAAACATGTAC GTGAG GAATCCTTCTGTCTTTTAGAAAAA GAAAGAAGA GAAGG GAATG GTGTAGA GACCTTG GCAGCATGTTA GTCGTTTCATTCGAACAAAATGTAACCACAATACATCATGCATTA GTACA
TAAGTAAATATGCATAAAATATTGTAGAACACTTAGATTTATTCACATATGCACATGGTCTATGCCTGGCTTTGTACAAAGGGTTACAAGTATTTGAGACAAA GTTTGTACAAAAG GG GTTGCTG GGACCAAGCAC GACAATCGCGCCTTACA GGTAGAACTTAC GAAGATGCTC GATATTCCATGTGTTTT GCACGCGACT GCCACCTTCAGTCTCCAAACAGACCATGCCAA GTCTAGTGACTC G
TGCTACCCGATAAGGGCCCTCCCACTTTGGCGTCAACTTGTTTGATCCCATGGCGGATTGAATGC GCCGAA GTACAAGGTCACCTTCCTCAATGCTTC GG GCATGAACCTTGT GGCTATGATA GC AGCGCAA GGCTT GCTGGTA GCGT GCAGCTTGCACA GCTGCGCAAA GACGATCTTCCTGAA GGAGCAT GACATCATCTTCCCGG GTTAATCGAATTCCCGC GGCCGCCAT GGC GGCCG GG
AGCATGCGACGTCGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTC GTTTTACAACGTCGTGACT GG GAAAACCCTGGC GTTACCCAACTTAATCGCCTTGCAGCACATC CCCCTTTCGCCA GCTG GCGTAATAGC GAAGAG GCCCGCACC GTACGTCATATCCCAACAGTTGC GCAGCCTGAAT GGC GAATGG GACCGC GCCCTGTAGC GGCGCATTA GCGC GGCG GG
GTGGTGGTGGTTACGCGCAGCGTGACGCTACACTTGCA GCGCCTA GCGCCGCTCTTTC GCTTTCTCCCTCATTTATCGCCACGTCGCG GCTTTCCCGTCAGCTCTAAATC GG GGTCCCTTAG GTT CGATTATGCTTTACGCACCTC GACCCCAAAACCTGATAG GTGATG GTCAGCATG GGCCATC GCCTGATAGCATCTTCCCTTGAACCT GATCAGTCTTAATG GAACTTGTCCACTGAACACC
TTACCAATCTCGGCTATC

- **Multiple Sequence Alignment**
- forward sequence and reverse sequence was subjected to multiple sequence alignment by using **EMBL-EBI tool Clustal W 2**
- >aligned
- CTATAGGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCGGGAATTCGATTAACCCGGGAAGATGATGTCATGCTCCTTCAGGAAGATCGTCTTTGCGCAGCTGTGCAAGCT
GCACGCTACCAGCAAGCCTTGCGCTGCTATCATAGCCACAAGGTTCATGCCCGAAGCATTGAGGAAGGTGACCTTGTACTTCGGCGCATTCAATCCGCCATGGGATCAAACAAGTTGACGCCAAAGT
GGGAGGGCCCTTATCGGGTAGCACGAGTCACTAGACTTGGCATGGTCTGTTTGGAGACTGAAGGTGGCAGTCGCGTGCAAAACACATGGAATATCGAGCATCTTCGTAAGTTCTACCTGTAAGGCGC
GATTGTCGTGCTTGGTCCCAGCAACCCCTTTTGTACAAACTTTGTCTCAAATACTTGTAACCCTTTGTACAAAGCCAGGCATAGACCATGTGCATATGTGAATAAATCTAAGTGTTCTACAATATTT
TATGCATATTTACTTATGTACTAATGCATGATGTATTGTGGTTACATTTTGTTCGAATGAAACGACTAACATGCTGCCAAGGTCTCTACACCATTCCCTTCTCTTCTTTCTTTTTCTAAAAGACAGA
AGGATTCCTCACGTACATGTTTCCCGGGTTAATCACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCACCTAA
AT

**Primer Designing**

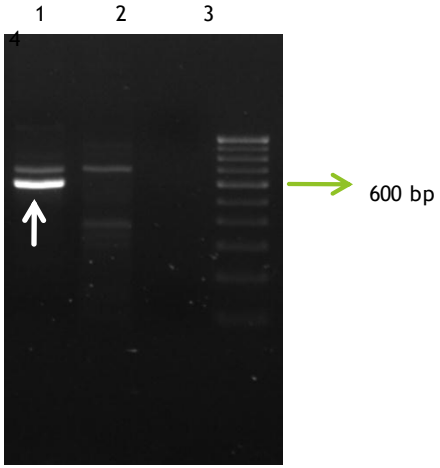
# Primer designing

Set of primers were designed with the help of **Oligocalc** tool.

AACCCGGGAAGATGATGTCATGCTCCTTCAGGAAGATCGTCTTTGCGCAGCTGTGCAAGCTGCACGCTACCAGCAAGCCTTGCGCTG
CTATCATAGCCACAAGGTTCATGCCCGAAGCATTGAGGAAGGTGACCTTGTACTTCGGCGCATTCAATCCGCCATGGGATCAAACAA
GTTGACGCCAAAGTGGGAGGGCCCTTATCGGGTAGCACGAGTCACTAGACTTGGCATGGTCTGTTTGGAGACTGAAGGTGGCAGTC
GCGTGCAAAACACATGGAATATCGAGCATCTTCGTAAGTTCTACCTGTAAGGCGCGATTGTCGTGCTTGGTCCCAGCAACCCCTTTT
GTACAAACTTTGTCTCAAATACTTGTAACCCTTTGTACAAAGCCAGGCATAGACCATGTGCATATGTGAATAAATCTAAGTGTTCTAC
AATATTTTATGCATATTTACTTATGTACTAATGCATGATGTATTGTGGTTACATTTTGTTCGAATGAAACGACTAACATGCTGCCAAG
GTCTCTACACCATTCCCTTCTCTTCTTTCTTTTTCTAAAAGACAGAAGGATTCCCACGTACATGTTTCCCGGGTTT

| Primer | | Length | Tm0C | GC % | Sequence(5'-3') |
|---|---|---|---|---|---|
| 1 | Left Primer(F)<br>Right Primer(R) | 20<br>20 | 51.8<br>51.8 | 50<br>50 | AACCCGGGAAGATGATGTCA<br>AACCCGGGAAACATGTACGT |
| 2 | Left Primer(F)<br>Right Primer(R) | 22<br>20 | 54.8<br>51.8 | 50<br>50 | AACCCGGGAAGATGATGTCATG<br>AACCCGGGAAACATGTACGT |
| 3 | Left Primer(F)<br>Right Primer(R) | 22<br>20 | 56.7<br>51.8 | 55<br>50 | ACCCGGGAAGATGATGTCATGC<br>AACCCGGGAAACATGTACGT |
| 4 | Left Primer(F)<br>Right Primer(R) | 22<br>20 | 56.7<br>51.8 | 55<br>50 | CCCGGGAAGATGATGTCATGCT<br>AACCCGGGAAACATGTACGT |

# PCR amplification of genomic DNA of PDW 291 and DBW 17 using sequence specific SCAR Primer




Lane 4-100bp marker, lane 1-3- PDW 291 , DBW 17, control.

- Specific band (~600bp) is amplified only in PDW 291 (*T. durum*) not in DBW 17*(T. aestivum*) .

# Physical-chemical Analysis of Rice

# Rice

- Eleven different varieties of Rice were selected for the study and they were collected from different regions in India, which includes Delhi, Bihar, Uttar Pradesh, Andhra Pradesh.

- Seed collection centers are:

- NSC (National Seed Corporation, Delhi).

- IARI (Indian agriculture research institute).

- NPBGR(National Bureau of Plant Genetic Resources).

# Basmati rice

- In this study in order to differentiate Basmati rice from non Basmati ,we studied three important characteristics of Basmati Rice i.e. long grain, aroma and cooked kernel elongation.

- So that we can easily differentiate this premium Basmati Rice from non-Basmati Rice.

# Physical analysis: rice

- They were Classified based on physical properties ; shape and size.

| S.No. | Sample name | Shape/Size | Picture |
|---|---|---|---|
| 1. | PB1121 | Long /slender |   |
| 2. | Rajendra Kasturi | Small |   |

# Physical analysis :rice

| S.No. | Sample name | Shape/Size | Picture |
|---|---|---|---|
| 3. | Uma | Medium/Bold |  |
| 4. | PB1460 | Long /Slender |  |
| 5. | Rajendra Marchai | Small/Bold |  |

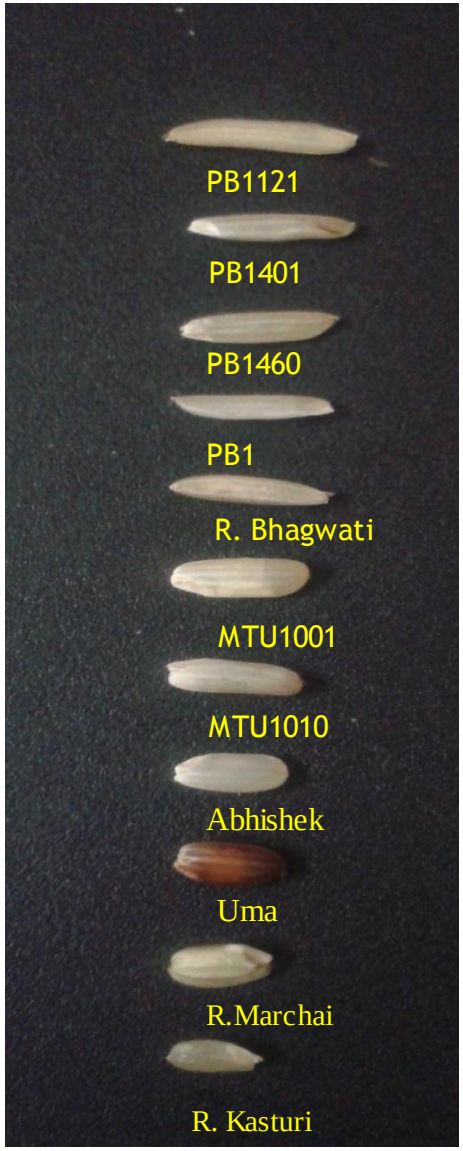
# Physical analysis : rice

| S.No. | Sample name | Shape/Size | Picture |
|---|---|---|---|
| 6. | PB1401 | Long/Slender |  |
| 7. | Abhishek | Small/Slender |  |
| 8. | PB1 | Long/Slender |  |
| 9. | Rajendra Bhagwati | Long/Slender |  |

# Physical analysis :rice

| S.No. | Sample name | Shape/Size | Picture |
|---|---|---|---|
| 10. | MTU1010 | Medium/Slender |  |
| 11. | MTU1001 | Medium/Slender |  |

# Grain length




- Grain size and shape largely determine the market acceptability of rice.
- Basmati rice are long and slender in shape.
- Their size ranges between 7 to 9 mm and their grain breadth lies between 1.5 to 2 mm

# Grain length

| S. No. | Samples | Grain length | Grain Breath |
|---|---|---|---|
| 1. | **PB1121** | **8.2** | **1.7** |
| 2. | Rajendra Bhagwati | 7.4 | 2.1 |
| 3. | Uma | 5 | 2.4 |
| 4. | **PB1460** | **7.6** | **1.6** |
| 5. | Rajendra Marchai | 4.6 | 2.9 |
| 6. | **PB1401** | **7.7** | **1.5** |
| 7. | Abhishek | 4.5 | 1.8 |
| 8. | **PB1** | **7.3** | **1.7** |
| 9. | Rajendra Kasturi | 3.8 | 1.8 |
| 10. | MTU1010 | 5.6 | 2.2 |
| 11. | MTU1001 | 5.5 | 2.1 |

Basmati rice have long/slender grain

# Introduction

- Kernel elongation after cooking is an important character of Basmati rice.

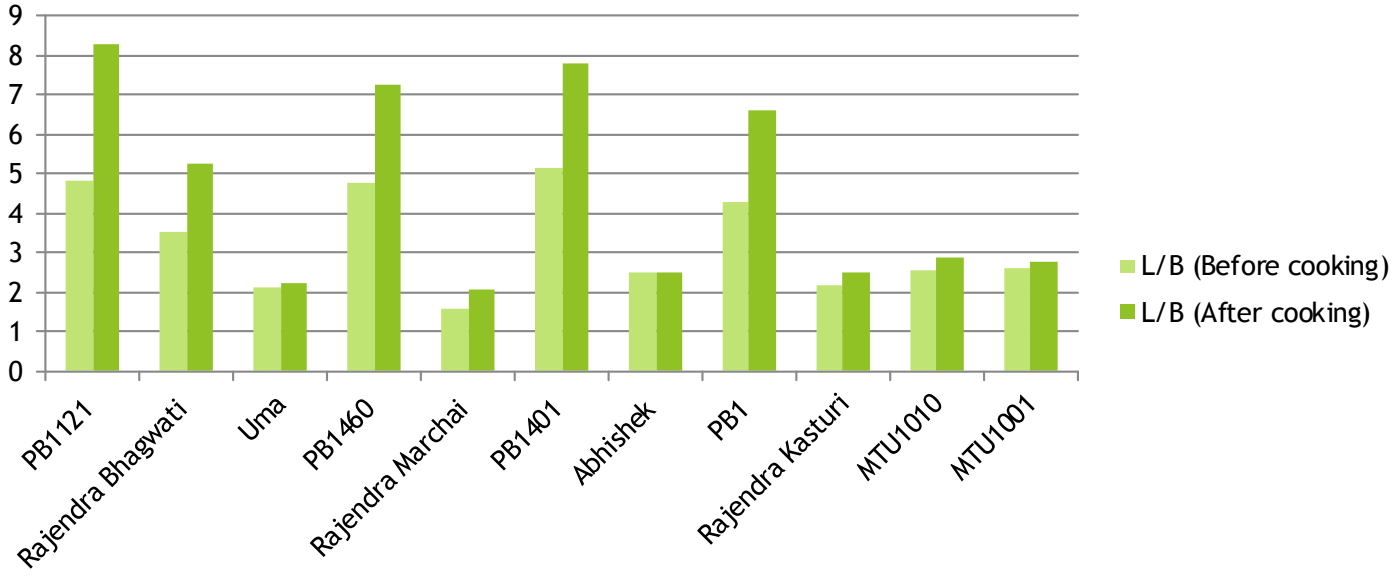
- It is a physical phenomenon which is influenced by several physicochemical and genetic factors like genotypes, anatomy, aging temperature, aging time, water uptake, amylose content, gelatinization temperature and environment.
- The trait of cooked kernel length is controlled by QTL.

# Kernel Elongation Ratio

| S.No | Samples | Before Cooking | | | After Cooking | | | |
|---|---|---|---|---|---|---|---|---|
| | | KL | KB | L/B | KL | KB | L/B | ER |
| 1. | PB1121 | 8.2 | 1.7 | 4.82 | 18.3 | 2.2 | 8.31 | 2.23 |
| 2. | Rajendra Bhagwati | 7.4 | 2.1 | 3.52 | 12.6 | 2.4 | 5.25 | 1.70 |
| 3. | Uma | 5 | 2.4 | 2.1 | 6.5 | 3 | 2.2 | 1.3 |
| 4. | PB1460 | 7.6 | 1.6 | 4.75 | 15.3 | 2.1 | 7.28 | 2.01 |
| 5. | Rajendra Marchai | 4.6 | 2.9 | 1.58 | 6.6 | 3.2 | 2.06 | 1.43 |
| 6. | PB1401 | 7.7 | 1.5 | 5.13 | 17.2 | 2.2 | 7.81 | 2.23 |
| 7. | Abhishek | 4.5 | 1.8 | 2.5 | 6.2 | 2.4 | 2.5 | 1.37 |
| 8. | PB1 | 7.3 | 1.7 | 4.29 | 14.5 | 2.2 | 6.59 | 1.98 |
| 9. | Rajendra Kasturi | 3.8 | 1.8 | 2.16 | 6.2 | 2.5 | 2.48 | 1.63 |
| 10. | MTU1010 | 5.6 | 2.2 | 2.54 | 7.8 | 2.7 | 2.88 | 1.39 |
| 11. | MTU1001 | 5.5 | 2.1 | 2.62 | 7.5 | 2.7 | 2.77 | 1.36 |

* All measurements are in mm

# Cooked Kernel Elongation




L/B ratio almost remains same for non aromatic rice before and after cooking but in case of Basmati rice it improves further .

# Aromatic and non-aromatic rice

# KOH Test Result

| S.No. | Sample Name | Aroma |
|---|---|---|
| 1. | PB1121 | + |
| 2. | Rajendra Kasturi | + |
| 3. | Uma | - |
| 4. | PB1460 | + |
| 5. | Rajendra Marchai | - |
| 6. | PB1401 | + |
| 7. | Abhishek | - |
| 8. | PB1 | + |
| 9. | Rajendra Bhagwati | + |
| 10. | MTU1010 | - |
| 11. | MTU1001 | - |

# Physical-chemical Analysis : Wheat

# Physical Analysis : wheat

| Sl. No. | Sample | Size | Shape | Kernel |
|---|---|---|---|---|
| 1 | HD 2824 | Medium | Elliptical | Medium hard |
| 2 | HD 2894 | Medium | Elliptical | Medium hard |
| 3 | HD 2733 | Medium | Elliptical | Hard |
| 4 | HD 3107 | Small | Ovate | Medium hard |
| 5 | HD 2643 | Medium | Elliptical | Medium hard |
| 6 | HD 2967 | Small | Ovate | Medium hard |
| 7 | HD 4725(D) | Long | Elliptical | Very hard |
| 8 | DBW 17 | Small | Ovate | Medium hard |
| 9 | DBW 14 | Small | Ovate | Medium hard |
| 10 | PBW 343 | Small | Ovate | Hard |
| 11 | PDW 291(D) | Very long | Oblong | Very hard |

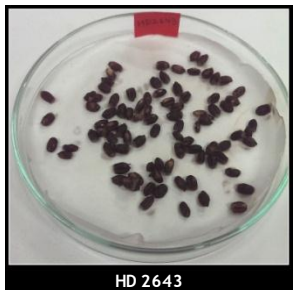
# Physical analysis : wheat

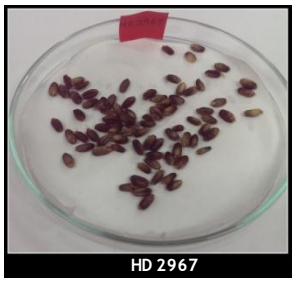
| Sl. No | Samples | Size | Shape | Kernel |
|---|---|---|---|---|
| 12 | PDW 314(D) | Long | Oblong | Very hard |
| 13 | PBW 550 | Small | Ovate | Medium hard |
| 14 | K 307 | Round | Ovate | Medium hard |
| 15 | RAJ MR 1 | Medium | Ovate | Medium hard |
| 16 | LOK 1 | Long | Oblong | Medium hard |
| 17 | DL 788-2 | Medium | Elliptical | Medium hard |
| 18 | RAJ-1555(D) | Very long | Oblong | Very hard |
| 19 | RAJ 6560(D) | Very long | Oblong | Very hard |
| 20 | M. P wheat | Medium | Elliptical | Medium hard |

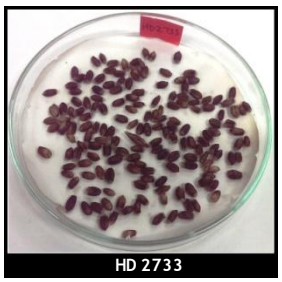
# Bio-chemical Study : wheat

- PHENOL TEST
- TYROSINASE TEST

# Phenol test





Darkened bread wheat seeds after 1% phenol treatment





Durum wheat seeds after 1% phenol treatment

# Tyrosinase test

- Tyrosinase enzyme activities.
- Disodium tyrosinate as substrate

Colour change in *T. aestivum*

Color change in *T. durum*

# Thank you