
Bangladesh Institute of NuclearAgriculture
BAU Campus, Mymensingh
WELCOME

# Research Plane


## Md.Saikat Hossain Bhuiyan
## Scientific Officer, Soil Science Divison

## BANGLADESH INSTITUTE OF NUCLEAR AGRICULTURE, BANGLADESH


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# Introduction

- Present condition of Soil Organic Matter

In Bangladesh, most soils have less than 1.7%, and some soils have even less than 1 % organic matter.

# What Does Organic Matter Do In Soil?

- Of all the components of soil, organic matter is probably the most important and most misunderstood.
- Organic matter serves as a reservoir of nutrients and water in the soil, aids in reducing compaction and surface crusting, and increases water infiltration into the soil.

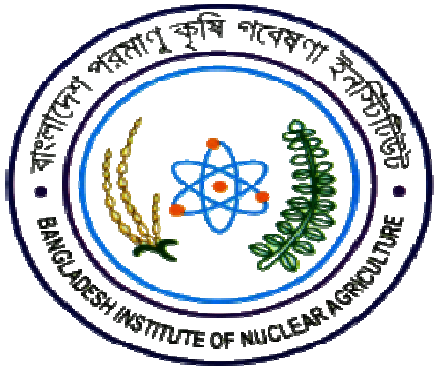
# What Does Organic Matter Do In Soil?

## Yet it's often ignored and neglected.

| Nutrients | Amount |
|---|---|
| Nitrogen | 20-30 pounds |
| Phosphorus | 4.5-6.6 pound |
| Sulfure | 2-3 pound |

**Table 1. Amount of nutrient roughly supplied by percentage of organic matter per year**

# Use of Isotope in Organic Residue Studies



# General Objective

- To determine the performance of organic matter on crop production
- To determine the amount of nutrient (nitrogen) uptake from the mixture of organic matter and soil.
- To determine the residual amount of nutrient available after 1$^{st}$ crop cultivation

# Work Plan

# Experiment :1

Name of Experiment: Organic matter preparation by using $^{15}N$ isotope as source of nitrogen

## Objective:

- To find the suitable time for Kenaf to decompose as organic matter.

# Material and Methods

Crops: Kenaf (*Hibiscus cannabinus*)

Design: CRD with three replication

# Experimental Description

**Pot preparation**: Total 27 pot use here

**Soil Requirement**: Around 07 kg soil required to fill the pot.

Only top soil are used here

**Seed rate/$m^2$** : 30 seed/$m^2$
(10seed/pot)

# Experimental Description

**Fertilizer rate: 80-100 kg N/ha, 150-200 kg P/ha and 100 kg K/ha**

**Fertilizer rate/pot: 0.7 g N, 1.4 g P and 0.7 g K (at 5.0% $N^{15}$ abundance Urea used here)**

Intercultural Operation: No need to be done

# Treatment

- **T1: Mixed all plant part (Kenaf) after 20 days of planting**
- **T2: Mixed all plant part (Kenaf) after 30 days of planting**
- **T3: Mixed all plant part (Kenaf) after 40 days of planting**

# Data collection:

- **Chemical analysis of initial pot soil,**
- **Amount of N added into soil,**
- **C:N of organic matter,**
- **Chemical analysis of soil at 20, 30 and 40 days after seed sowing**

# Experiment : 2

- **Name or the Experiment: Effects of organic matter from Kenaf residues on growth and yield of short duration grow vegetable.**

**Objective:**

- **To determine effects of nutrient uptake on vegetable plant treated with organic matter from Kenaf residues.**

# Material and Methods

## Crops: Short duration vegetable (Sawi)

## Design: Split plot with three replication

# Split plot design

Replication I

| | | |
|---|---|---|
| $D_1$ | $D_3$ | $D_2$ |
| $T_1$ | $T_2$ | $T_1$ |
| $T_2$ | $T_1$ | $T_2$ |

Replication II

| | | |
|---|---|---|
| $D_2$ | $D_3$ | $D_1$ |
| $T_1$ | $T_1$ | $T_2$ |
| $T_2$ | $T_2$ | $T_1$ |

Replication III

| | | |
|---|---|---|
| $D_3$ | $D_1$ | $D_2$ |
| $T_1$ | $T_3$ | $T_2$ |
| $T_2$ | $T_1$ | $T_1$ |

**D=Days of Cultivation**

**T=Days of Decomposition**

# Treatment

**(Days of Cultivation)**

- $D_1$= 20 days Cultivated Kenaf
- $D_2$= 30 days Cultivated Kenaf
- $D_3$= 40 days Cultivated Kenaf

**(Days of Decomposition)**

$T_1$= 20 days Decompose into soil

$T_2$= 40 days Decompose into soil

| | |
|---|---|
| $D_1T_1$ | Kenaf (20 days after sowing) and 20 days decompose |
| $D_1T_2$ | Kenaf (20 days after sowing) and 40 days decompose |
| $D_2T_1$ | Kenaf (30 days after sowing) and 20 days decompose |
| $D_2T_2$ | Kenaf (30 days after sowing) and 40 days decompose |
| $D_3T_1$ | Kenaf (40 days after sowing) and 20 days decompose |
| $D_3T_2$ | Kenaf (40 days after sowing) and 40 days decompose |

Table 2. Treatment combination of this experiment

# Experimental Description

**Pot preparation: Total 18 pot use here**

**Soil Requirement: Six kg soil/pot**

**95g/pot of total Kenaf plant material level by $N^{15}$** incorporate into soil for organic matter preparation.

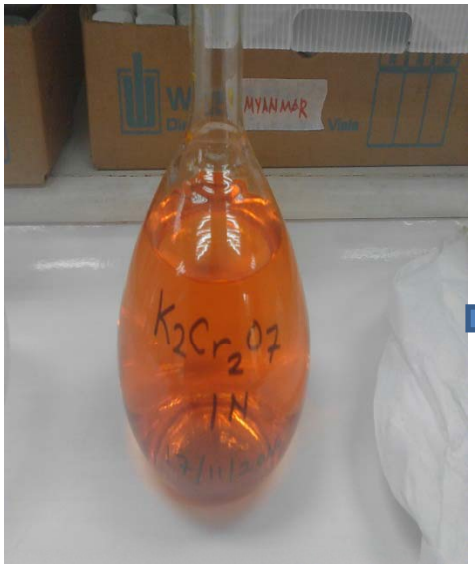
- Seed rate/pot : 2 seed/pot
- Fertilizer: Not add any chemical fertilizer
- Intercultural operation

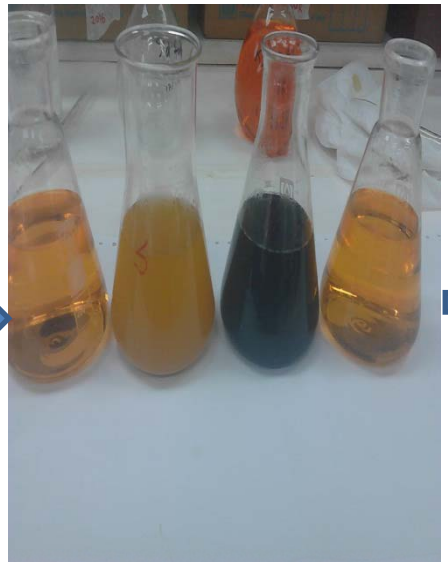
# Data collection:

- Chemical analysis of initial pot soil,
- Agronomic data on growth and yield of vegetable
- Amount of N uptake by the vegetable,
- Chemical analysis of post harvest soil

# Chemical analysis

- Total N% analysis (by Kjeldahl method)
- Total C% analysis (by walkley, A and I.A Black,1934
- Lignin content analysis (Klason lignin, using the method of the Institute of paper chemistry, Appleton)




**Reagent Preparation** **Sample Preparation** **Titration**

# Statistical analysis

- Analyses of variance were performed on all measured variables using Microsoft Excel
- Treatment effects were deemed significant at $P<0.05$, and
- Means were subsequently separated using the LSD 0.05% level.

# Result and Discussion

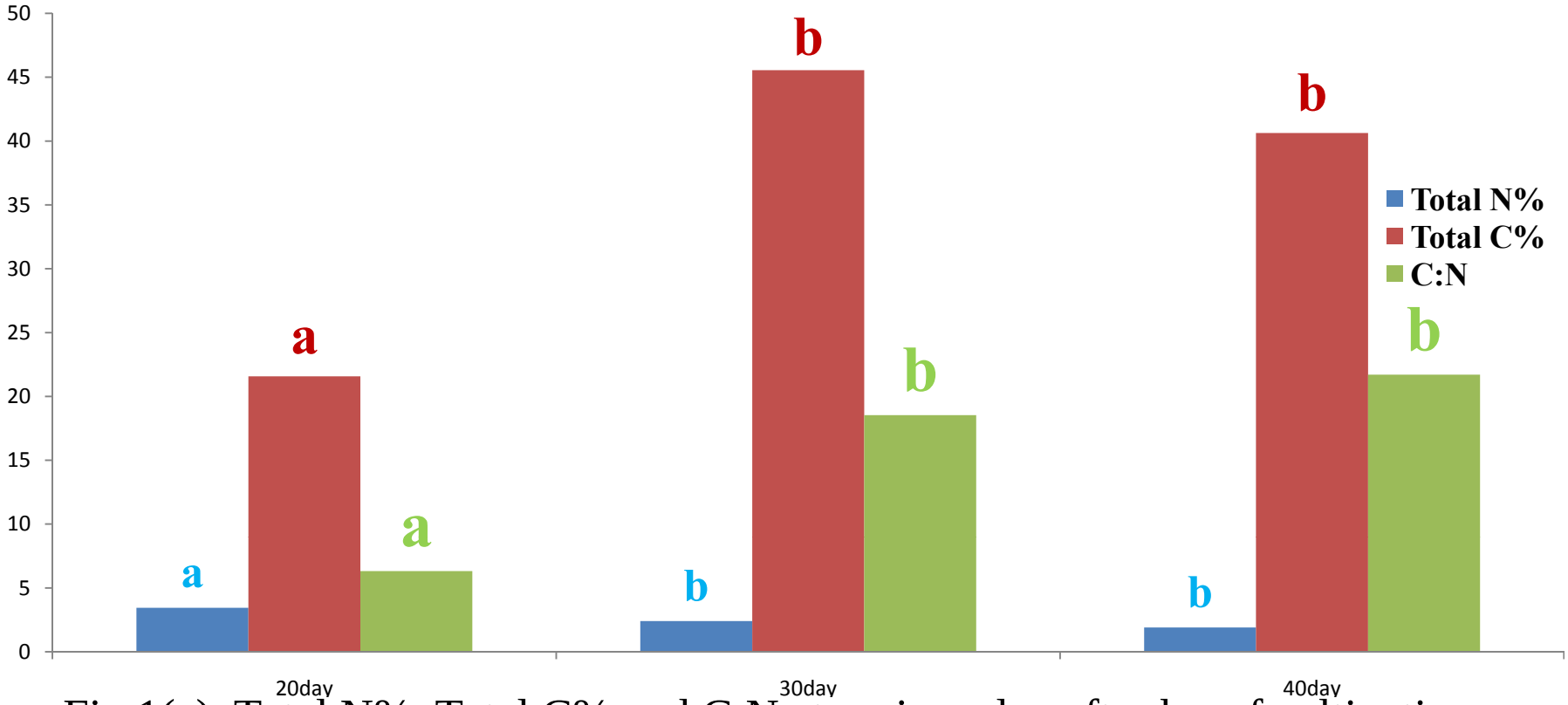



Fig 1(a). Total N%, Total C% and C:N at various day after kenaf cultivation

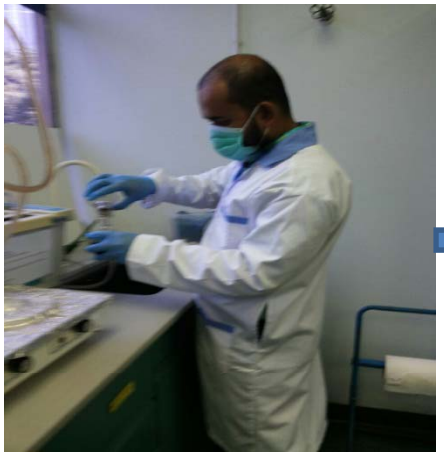

20 Days


30 Days

40 Days

Table 3(a) : Total n% and lignin% of kenaf at early vegetative stage

| Duration of cultivation | Total N% | Lignin% |
|---|---|---|
| 20 days | 3.44 | 12.35 |
| 30days | 2.42 | 14.45 |
| 40 days | 1.92 | 18.31 |



Sample preparation

Process of reflux


Drying at room temperature

# Palm et al. (2001) divides residues into quality classes based on %N, %lignin

| Class | Quality | Recommendation |
|---|---|---|
| **Class-l** | **have >2.5% N, <15% lignin** | **High quality residues** |
| **Class-II** | **with >2.5% N, >15% lignin Or with <2.5% N and <15% lignin** | **Medium quality residues** |
| **Class-III** | **with <2.5% N and >15% lignin** | **Low quality residues** |

**Considering this information kenaf plants cultivate at 40 days can be consider as low quality residues, 20 days and 30 days can be consider as high quality residue.**

# Experiment 2

# Table 3(b). Analysis of variance for significant test

| | Source of Variation | Degree of Freedom | Sum of Square | Mean sum of square | Calculated F value |
|---|---|---|---|---|---|
| Total N% | Days of Cultivation | 2 | 0.0034 | 0.0017 | 32.23** |
| | Days of Decomposition | 2 | 0.0011 | 0.0005 | 0.96 |
| | Interaction | 4 | 0.0011 | 0.0002 | 0.48 |
| Total C% | Days of Cultivation | 2 | 0.0035 | 0.0017 | 0.76 |
| | Days of Decomposition | 2 | 0.559 | 0.2796 | 33559.78** |
| | Interaction | 4 | 0.013 | 0.0032 | 391.11** |
| C:N ratio | Days of Cultivation | 2 | 964.15 | 482.075 | 56.79** |
| | Days of Decomposition | 2 | 127.65 | 63.8265 | 1.45 |
| | Interaction | 4 | 636.53 | 159.1315 | 3.61* |
| Yield | Days of Cultivation | 2 | 2718.6 | 1359.29 | 109.24** |
| | Days of Decomposition | 2 | 823.15 | 411.5755 | 8.84** |
| | Interaction | 4 | 1077.3 | 269.32 | 5.78** |

** 5% level of significant *1% level of significant

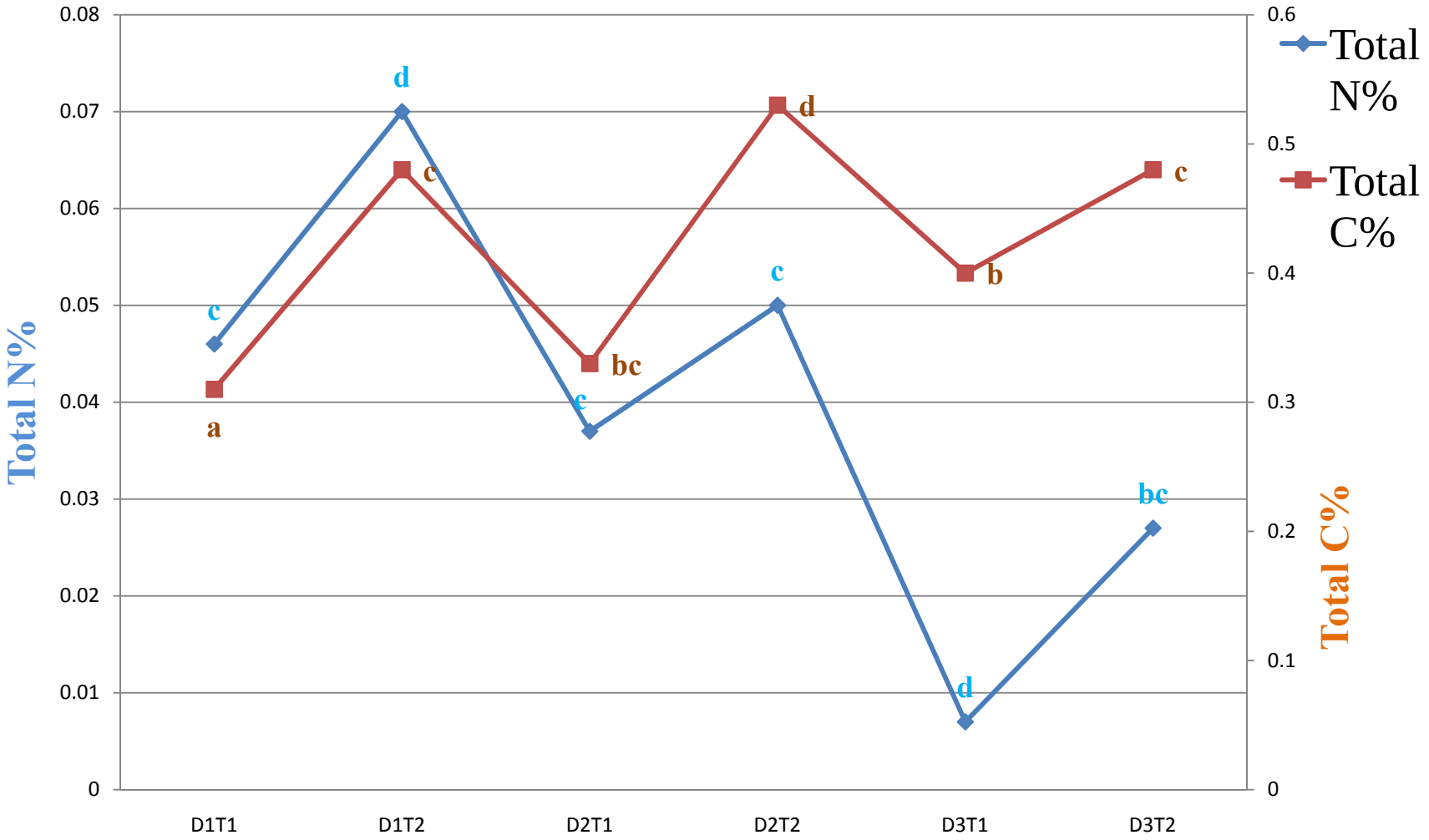



Fig 2 Total N% and C% of initial soil

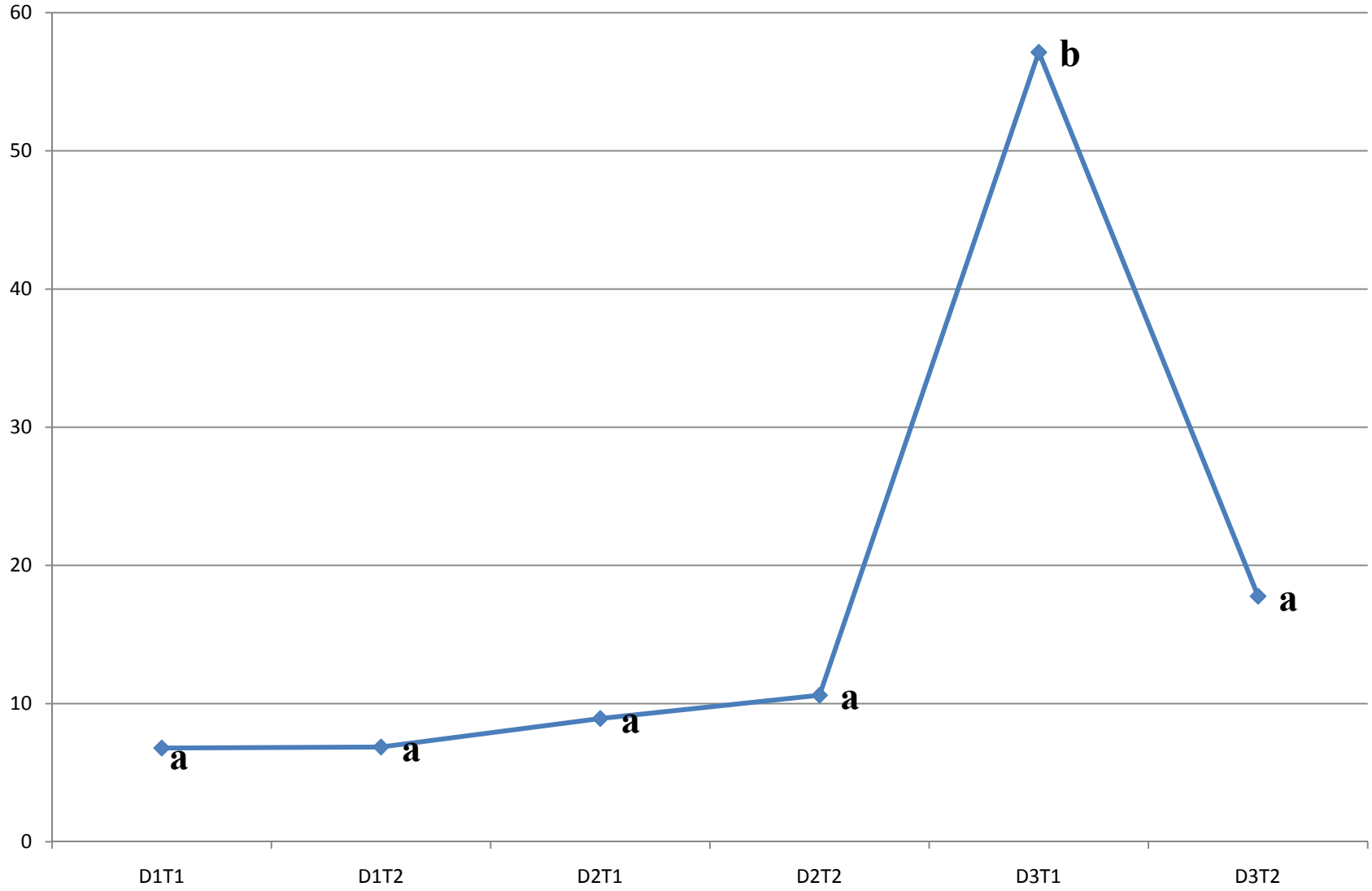
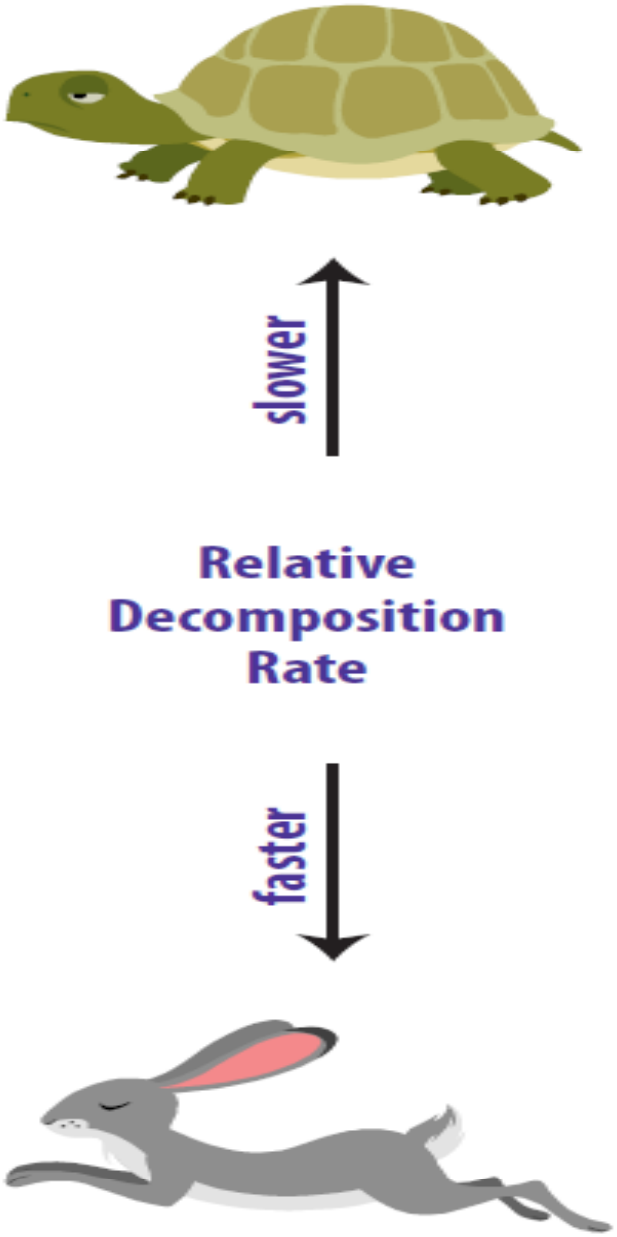



Fig 3. C:N of treated soil

| Material | C:N Ratio |
|---|---|
| rye straw | 82:1 |
| wheat straw | 80:1 |
| oat straw | 70:1 |
| corn stover | 57:1 |
| rye cover crop (anthesis) | 37:1 |
| pea straw | 29:1 |
| rye cover crop (vegetative) | 26:1 |
| mature alfalfa hay | 25:1 |
| **Ideal Microbial Diet** | **24:1** |
| rotted barnyard manure | 20:1 |
| legume hay | 17:1 |
| beef manure | 17:1 |
| young alfalfa hay | 13:1 |
| hairy vetch cover crop | 11:1 |
| soil microbes (average) | 8:1 |




Source: USDA Natural resources conservation service

# Fig 4. Yield response of Sawi (*Brassica juncea*) on organic matter

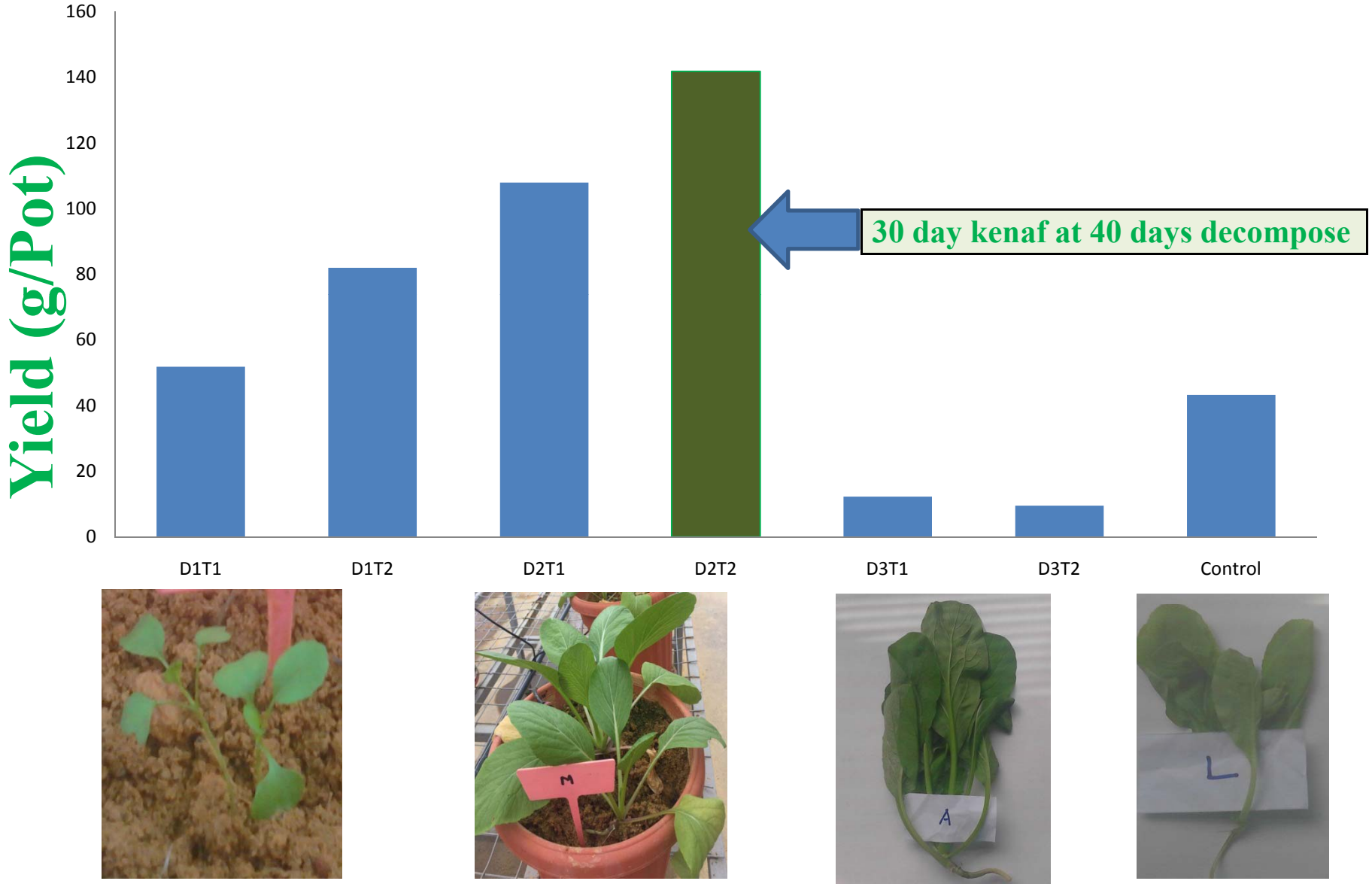
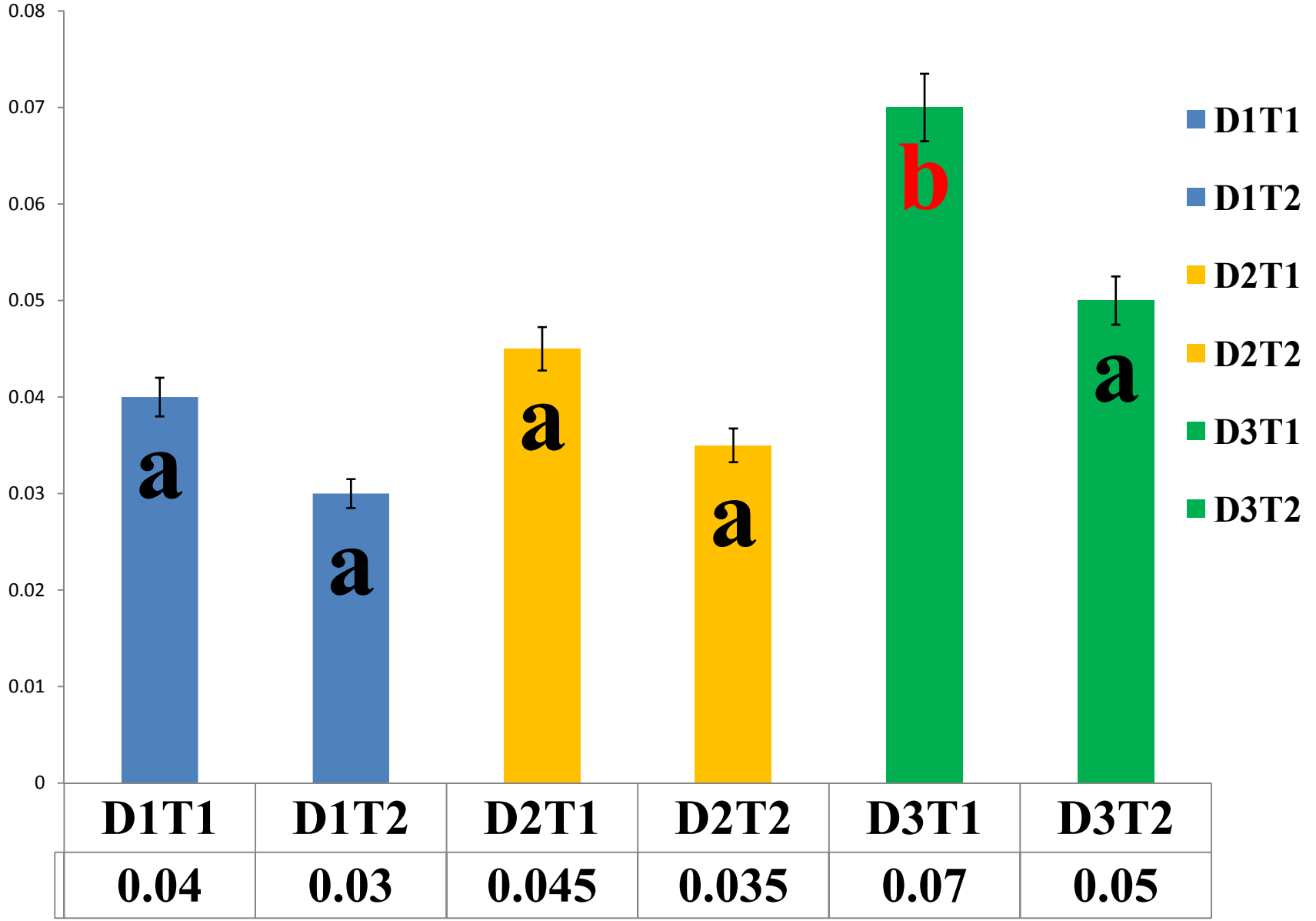


**Fig 5. Residual amount of nutrient (Total N%) available after 1st crop cultivation**




| D1T1 | D1T2 | D2T1 | D2T2 | D3T1 | D3T2 |
|---|---|---|---|---|---|
| 0.04 | 0.03 | 0.045 | 0.035 | 0.07 | 0.05 |

# Summary

**The age for Kenaf that suitable to be used as organic matter is at 30 days after sowing. Then, the appropriate time to decompose the kenaf into the soil is 40 days time. By using this time frame, we can grow vegetable plants without additional fertilizer and maintained organic farming.**

# Parte-2

## Molecular Analysis of Rice Mutant

- Heritable variation is central to the success of plant breeding.
- Molecular marker are used for **early generation testing**

- DNA Extraction
- DNA quantification
- RAPD Analysis
  i) PCR Cycle optimization
  ii) Optimization of $MgCl_2$ concentration
  iii) Primer optimization
  iv) RAPD Analysis using primer ???

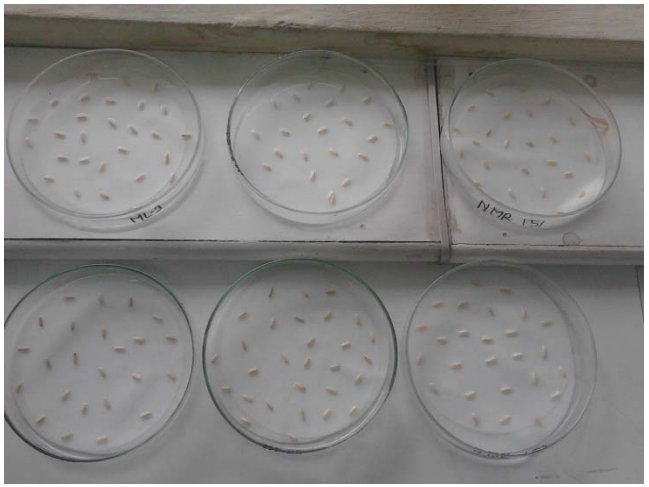
# Material

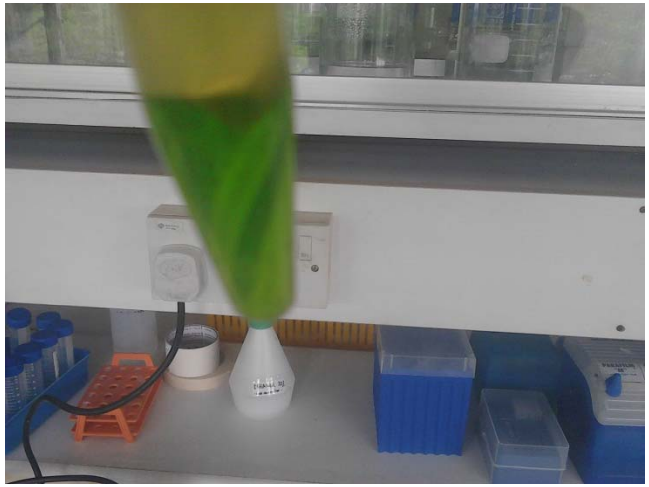
| Sample | Source of radiation |
|---|---|
| **MR219(parent)** | **--------** |
| **NMR151** | **Gamma** |
| **NMR152** | **Gamma** |
| **ML3** | **Ion beam** |
| **ML10** | **Ion beam** |
| **ML30** | **Ion beam** |
| Pongsu Seribu-2<br>**( 0Gy, 10Gy, 20Gy, 40Gy, 60Gy, 80Gy, 100Gy,120Gy)** | **Ion beam**<br>**M1 generation** |

Table 6. List of rice cultivar used in this experiment

# DNA Extraction

- Method: Modified Doyle and Doyle 1987





# DNA Quantification

- Using NanoDrop ND-1000 (Thermo Fisher Scientific,USA)

| Sample | A260/280 | Concentration (ng/µl) |
|---|---|---|
| MR219 | 2.05 | 68.55 |
| NMR151 | 2.00 | 24.1 |
| NMR152 | 2.09 | 262.35 |
| ML3 | **2.14** | 30.35 |
| ML10 | 1.97 | 52.35 |
| ML30 | 2.09 | 212.15 |

Table 7. Concentration of sample DNA

# DNA Quantification

| Sample | A260/280 | Concentration (ng/µl) |
|---|---|---|
| 0 Gy | 1.80 | 5.35 |
| 10 Gy | 1.76 | 6.75 |
| 20 Gy | 2.01 | 54.05 |
| 40 Gy | 1.97 | 160.3 |
| 60 Gy | 2.03 | 114.6 |
| 80 Gy | 2.54 | 4.85 |
| 100 Gy | 1.77 | 31.33 |
| 120 Gy | 1.54 | 4.85 |

Table 8. Concentration of sample DNA

# RAPD Reaction

| Reagent | Volume (1 reaction) | Final Concentration |
|---|---|---|
| Taq Polymerasere | 0.1 µl | 0.4 ml |
| dNTP-mix | 0.4 µl | 160 UM |
| $MgCl_2$ | 1.6 µl | 2mM |
| 10X buffer | 4.0 µl | 0.8X |
| Primer RAPD | 1 µl | 16 pmol |
| DNA template | 2 µl | 25ng |
| $ddH_2O$ | 10.9 µl | ----- |
| Total Volume | 20 µl | ------ |

**Promega, USA

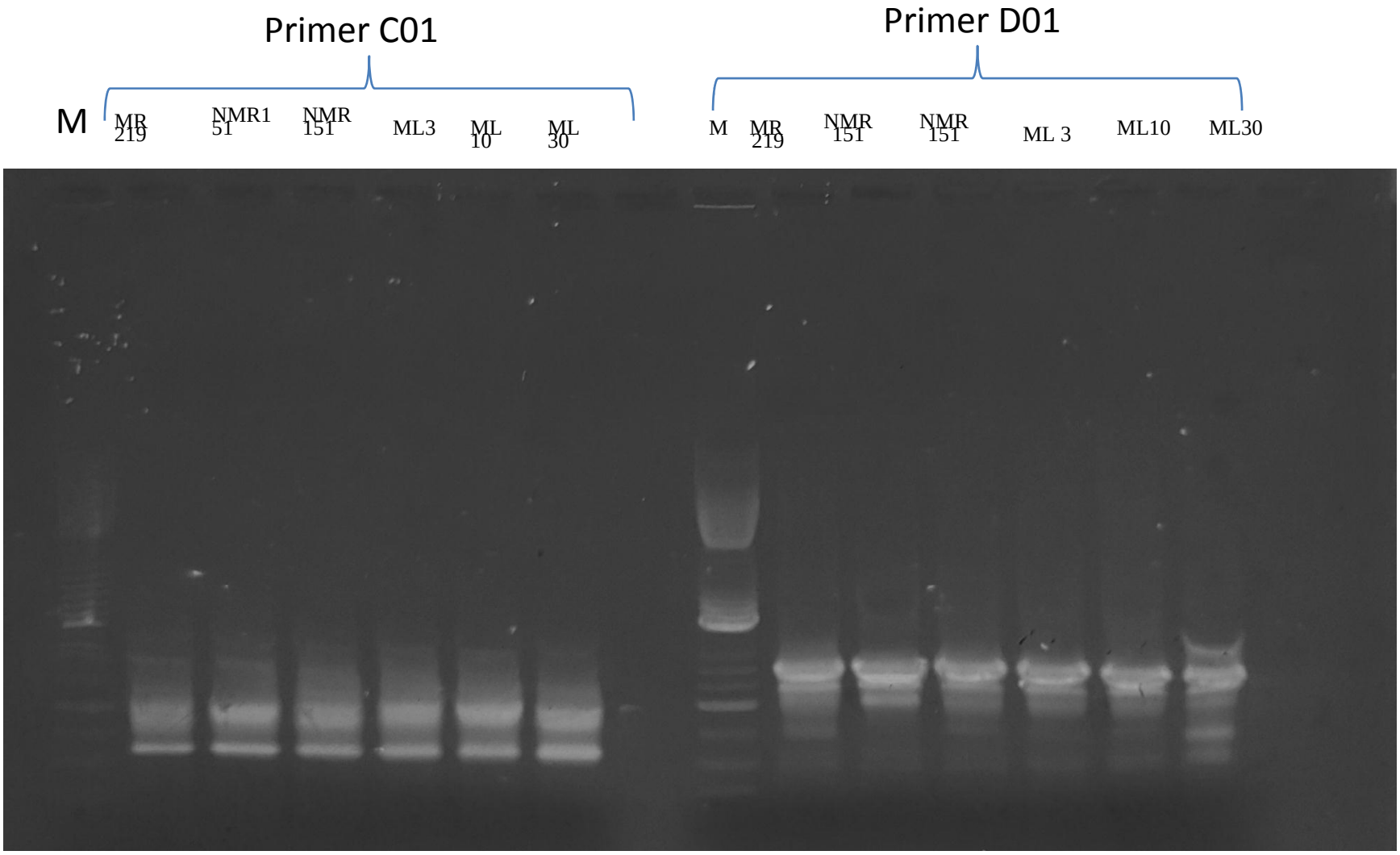
Table 9. Component of Master Mix

# RAPD PCR Program

| Steps | Temperature ($^0$C) | Time (min) | Cycle |
|---|---|---|---|
| **Initial Denaturation** | **94** | **10** | |
| **Denaturation** | **94** | **1** | |
| **Primer Annealing** | **37** | **0.30** | **40** |
| **Primer Extension** | **72** | **1** | |
| **Final Extension** | **72** | **10** | |
| **Hold** | **10** | ∞ | |

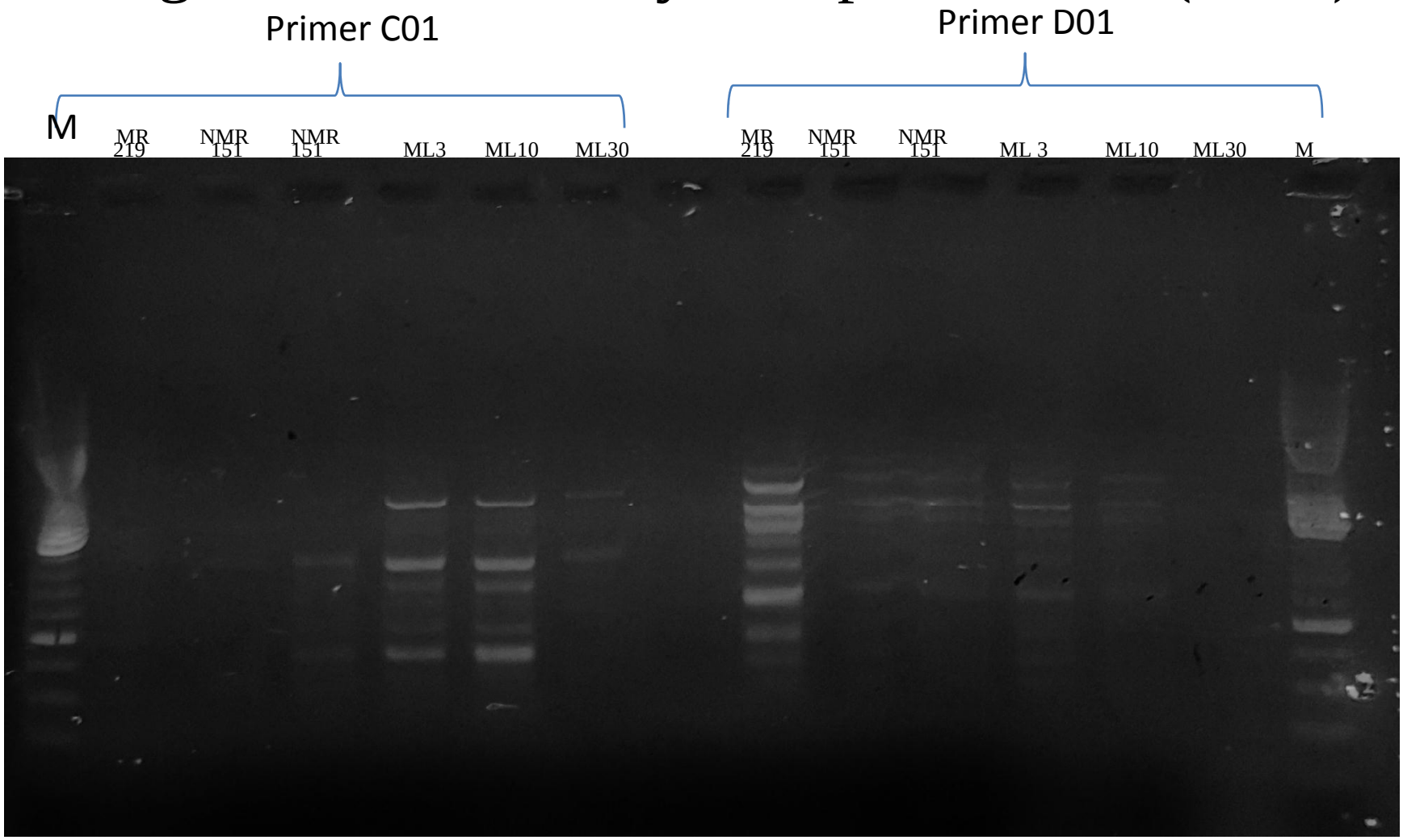
Table 10. Steps of PCR cycle

# Fig 6. RAPD PCR Cycle Optimization (50 Cycle)




* 100bp DNA step ladder (Promega)

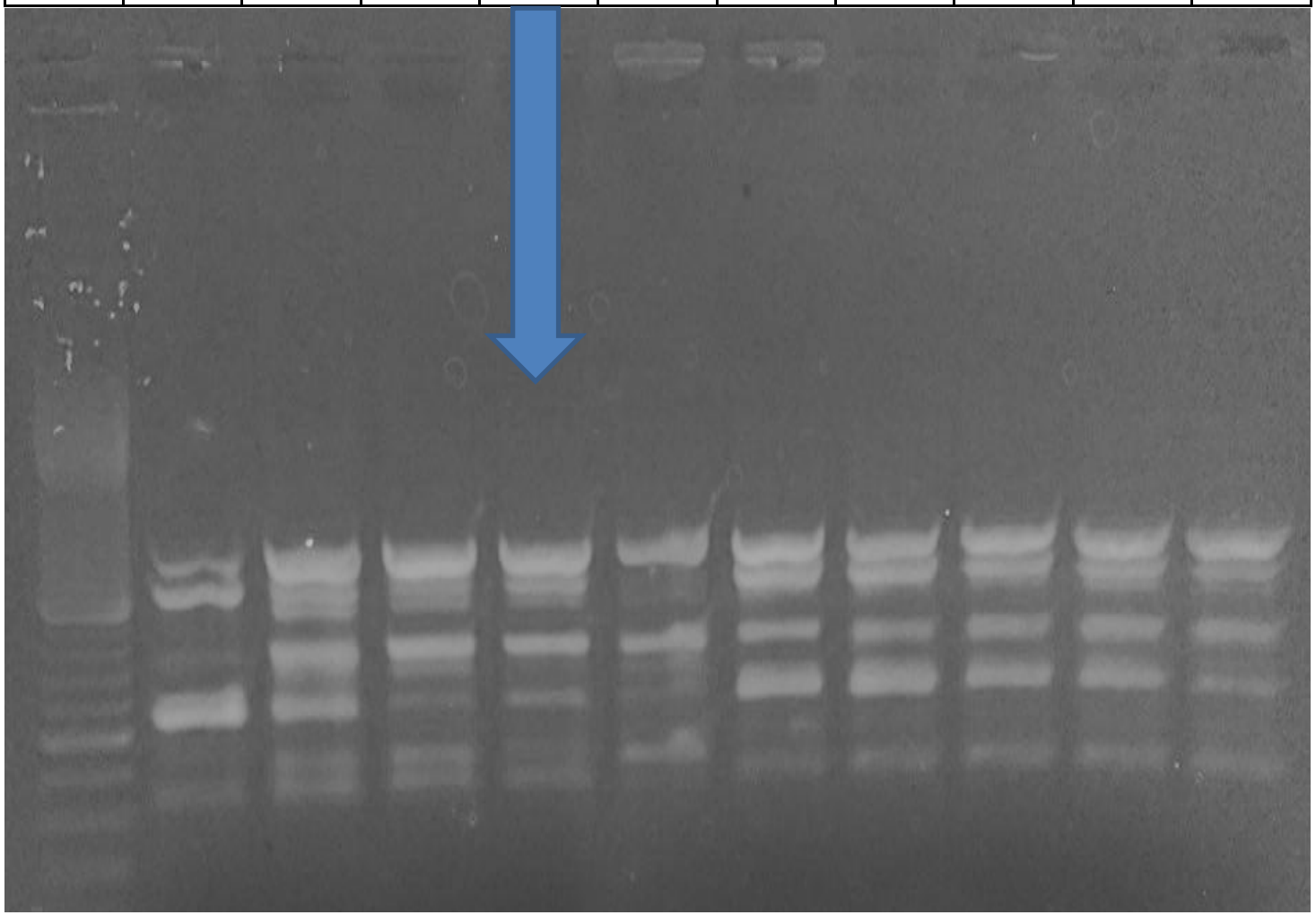
# Fig 7.RAPD PCR Cycle Optimization (30 Cycle)




* 100bp DNA step ladder (Promega)

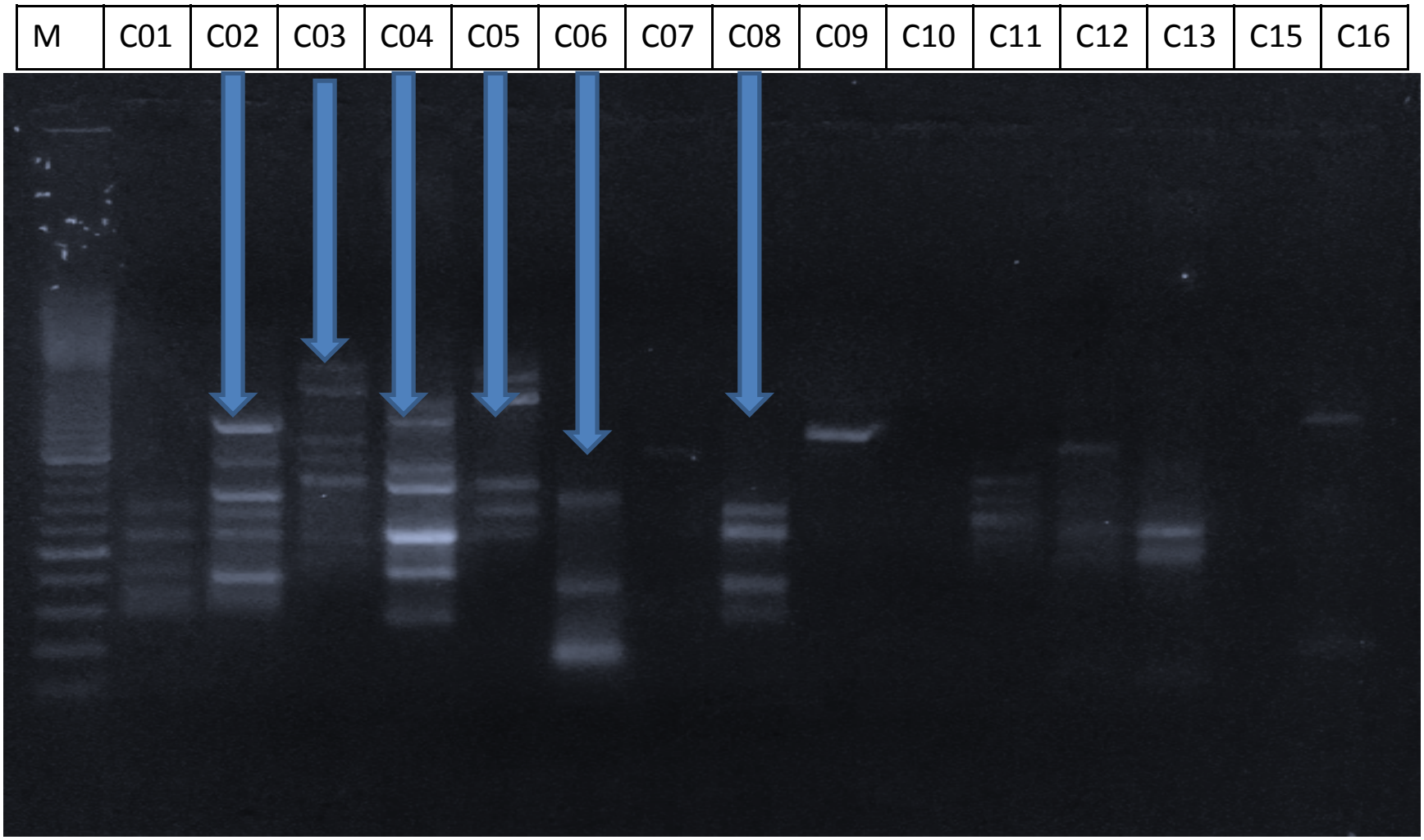
# Fig 8. Optimization of $MgCl_2$ Concentration

| M | 0.4 | 0.8 | 1.2 | 1.6 | 2 | 2.4 | 2.8 | 3.2 | 3.6 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|



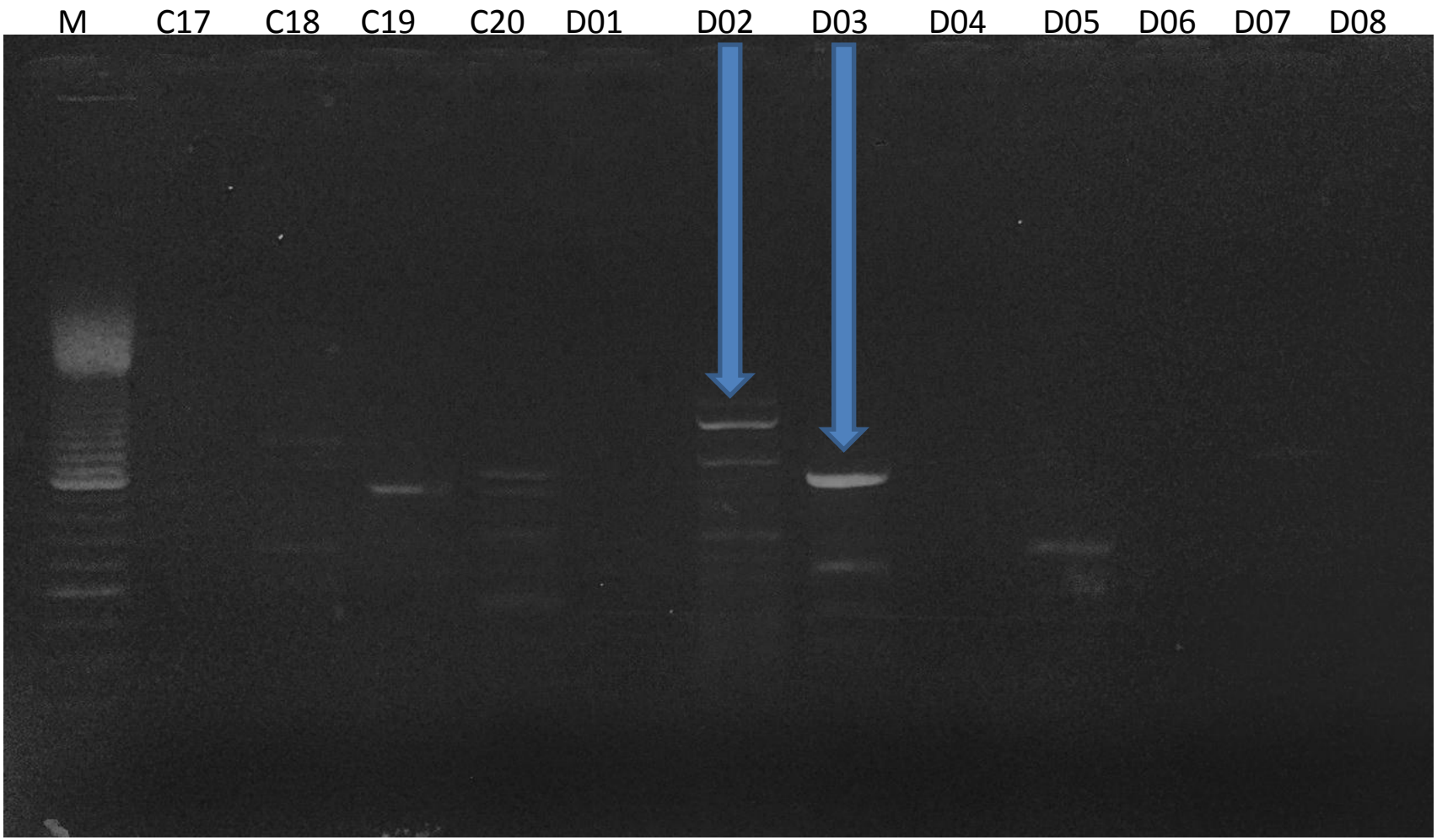
* 100bp DNA step ladder (Promega)

# Fig 9. RAPD Primer Optimization for Rice




* 100bp DNA step ladder (Promega)

# Fig 10. RAPD Primer Optimization for Rice

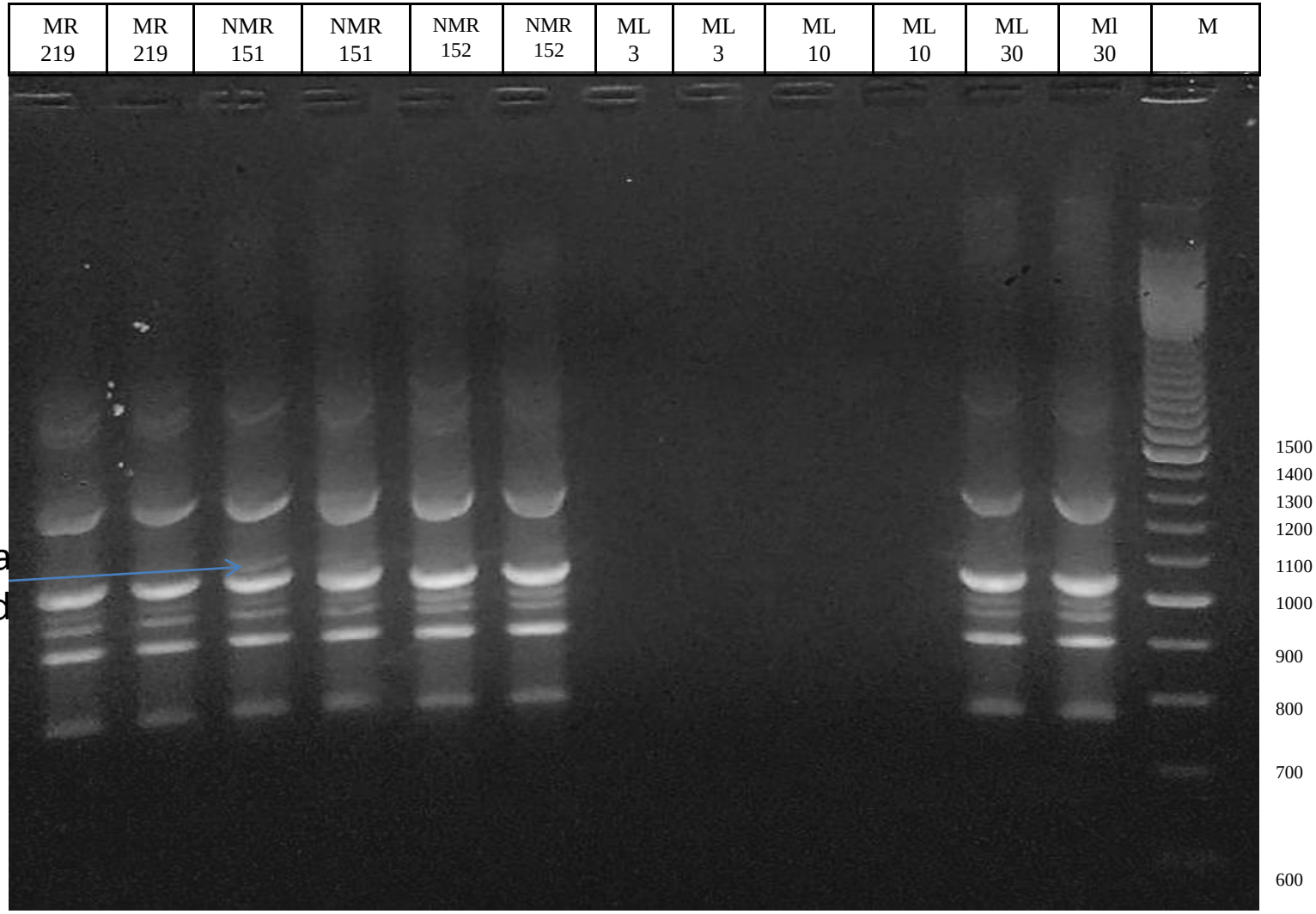



* 100bp DNA step ladder (Promega)

Table 11. Sequence of Selected RAPD 10mer KITS(Eurofins Genomic)

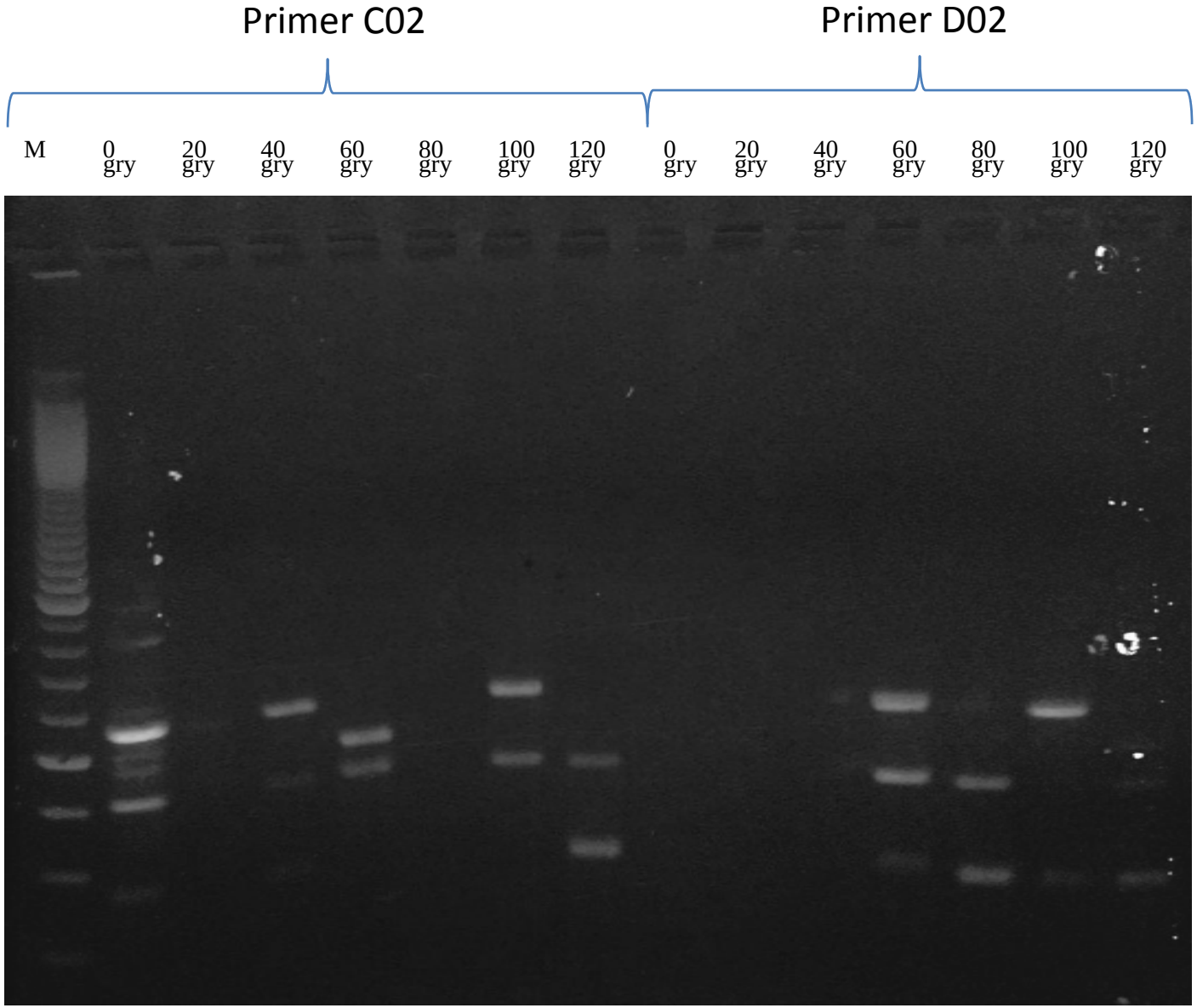
| RAPD KIT | PRIMER | SEQUENCE |
|---|---|---|
| KIT C | OPC-02 | GTGAGGCGTC |
| | OPC-03 | GGGGGTCTTT |
| | OPC-04 | CCGCATCTAC |
| | OPC-05 | GATGACCGCC |
| | OPC-06 | GAACGGACTC |
| | OPC-08 | TGGACCGGTG |
| KIT D | OPD-02 | GGACCCAACC |
| | OPD-03 | GTCGCCGTCA |

# Fig 11. RAPD profile of different rice varieties amplified with primer POC-04




* 100bp DNA step ladder (Promega)

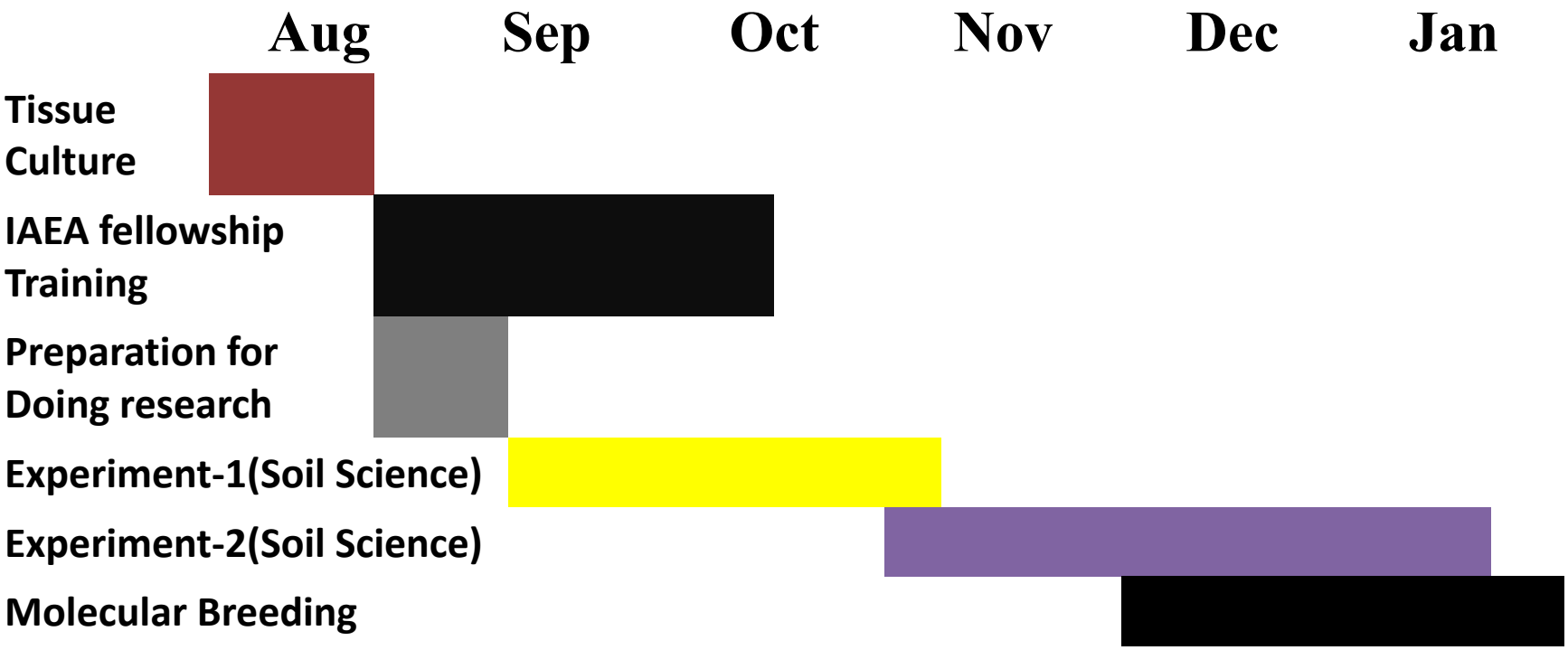
# Fig 12. RAPD Primer Optimization for Rice



# Summary of the study

- Out of 8 various RAPD primer only POC-04 able to differentiate NMR 151 to other

# Monthly View



# Learning from This Training

- Media Preparation
- Sub Culturing
- Isotopic Technique for Agricultural Research
- Total N% analysis(by Kjeldahl method)
- Total C% analysis(by walkley, A and I.A Black,1934
- Lignin content analysis(Klason lignin, using the method of the Institute of paper chemistry, Appleton)

- Preparation of Extraction Buffer, TE Buffer and others solution
- RAPD-PCR optimization
- Agarose gel preparation (1% and 1.5%)
- Gel Documentation


Thanks to All