# Introduction to mutation induction for crop improvement

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# Mutation

- A cell is said to have undergone a mutation when its genetic make up is suddenly and unexpected changed without formation and sexual diffusion of gametes.

- Heritable change in the DNA of a living cell, not caused by the common phenomena of genetic segregation or genetic recombination.

# Mutation

- A low rate of spontaneous mutation occurs continuously in all living organisms and so a small proportion of variants will always occurs, most of them are  undetectable.

- But sometimes these “variations” are detectable and beneficial and are passed on to the next generation (driving force in evolution).

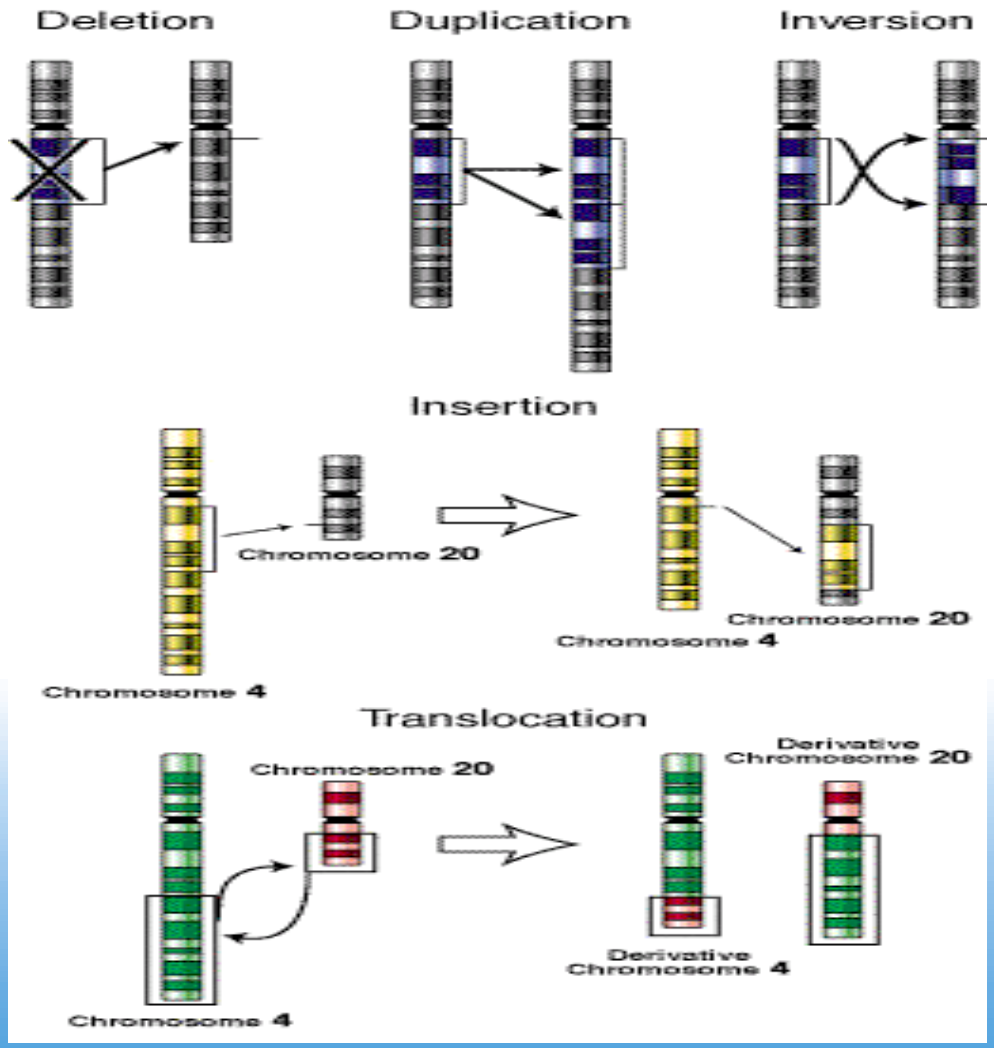
# Types of mutation

- Genome mutation
- Chromosomal mutation or structural rearrangement
- Extra-chromosomal mutation

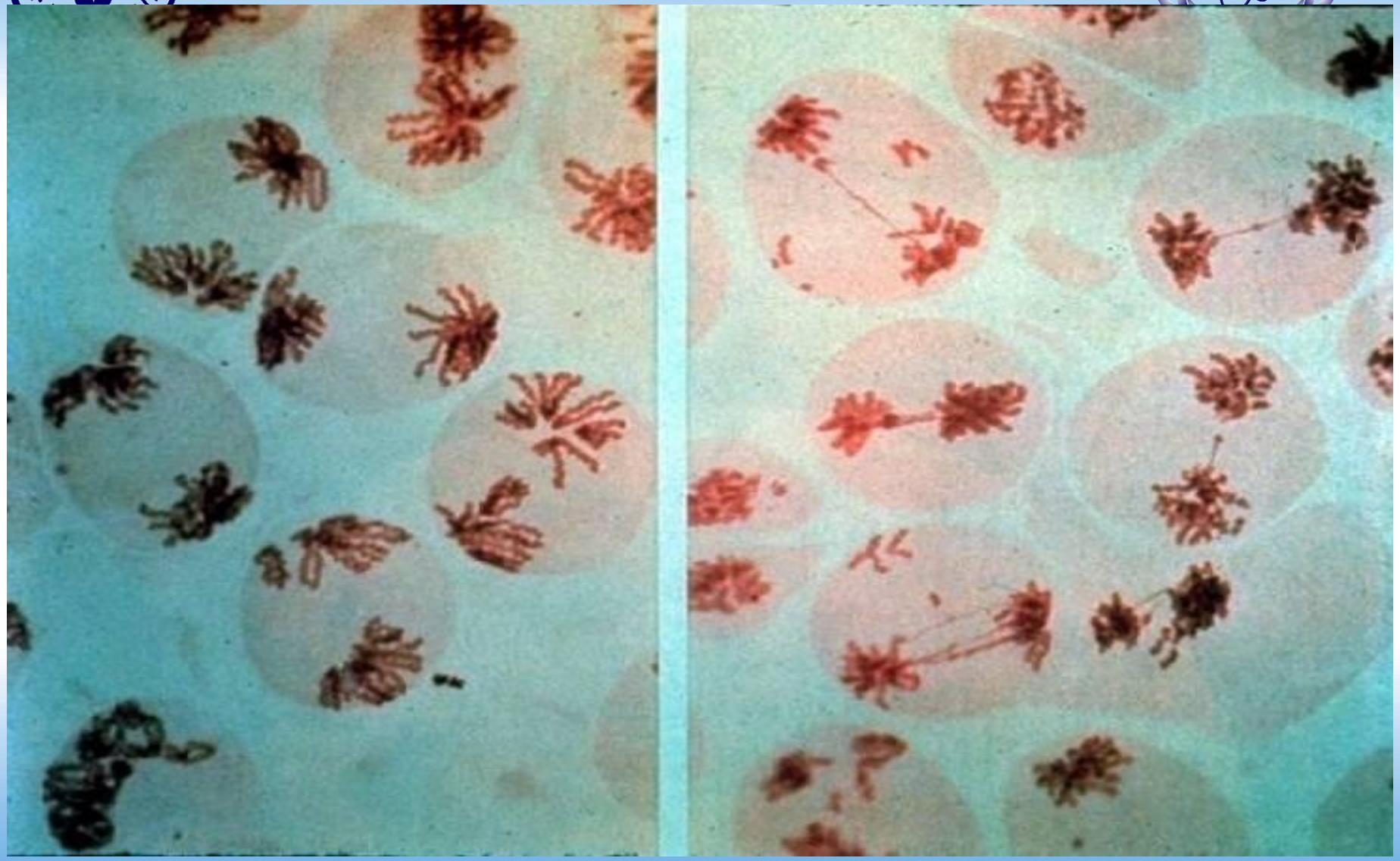
- Gene mutation

# Chromosomal mutation or structural rearrangement

- Translocation
- Insertion
- Inversion
- Duplication
- Deletion



# Mitotic abnormalities



NORMAL

IRRADIATED

# Gene mutation

| Type of gene mutation | Effect on the code (7 amino acids) |
|---|---|
| **Normal code** | THE FAT OLD CAT SAW THE DOG |
| **Addition (frame shift)** | THE EFA TOL DCA TSA WTH EDO G |
| **Deletion (frame shift)** | THE ATO LDC ATS AWT HED OG |
| **Substitution** | THE FAT OLD BAT SAW THE DOG |
| **Duplication** | THE FAT TFA TOL DCA TSA WTH EDO G |
| **Inversion** | THE TAF OLD CAT SAW THE DOG |

# Impact of mutation on protein sequence

- **Frameshift mutation:** cause by insertion or deletion
- **Nonsense mutation:** cause a premature stop codon and often result of non-functional protein product
- **Missense mutation:** single nucleotide is changed to cause substitution of a different amino acid
- **Neutral mutation**: is a mutation that occurs in amino acid codon which results in the use of different  but chemically similar , amino acid. (eg. AAA to AGA , arginine)
- **Silent mutations**: are mutations that do not result in the a change to the amino acid sequence of a protein

# Impact on function

- **Loss-of-function mutations**: Loss of function, often referred to as a null allele) and often recessive. Exceptions for  haploid organism

- **Gain-of-function mutations**: These mutations usually have dominant phenotypes

- **Dominant negative mutations** have an altered gene products that act antagonistically to the wild-type allele. These mutations usually result in an altered molecular function (often inactive) and are dominant or semi-dominant

- **Lethal mutations** lead to the death of the organisms which carry the mutations.

- A **back mutation** or **reversion** is a point mutation that restores the original sequence and hence the original phenotype.

# Induced mutation

- Is induced artificially and aims to produce variation, e.g. useful in plant breeding

- It is a complimentary tool for breeding with specific objectives.

# Physical mutagens

- **Ionizing electromagnetic irradiation** (energy range)
  - ❖ X-ray (10 – 100 keV)
  - ❖ Gamma-rays (100 – 3000 keV)
  - ❖ Cosmic rays (10 – 1000 MeV)
- **Ionizing atomic particle irradiation**
  - ❖ Alpha (~5 MeV)
  - ❖ Beta-rays (Radioisotopes) (10 – 2000 keV)
  - ❖ Accelerators (ion beam) (10 – 1000 MeV
  - ❖ Neutrons (0.001 – 10000 keV)

# Chemical mutagens

- **Alkylating agents**
  - EMS (ethyl methane sulphonate)
  - deS (diethyl sulphate)
  - NMUrea (N-methyl-N-nitrosourea)
  - Chemical agents
- **Others**
  - $NaN_3$ (sodium azide)
  - DNA precursors such as BUdR (5-bromodeoxyuridine); 2-Amino purine and other base analogues

# Mutagenesis

Mutagenesis is described as the exposure or treatment of biological materials to a mutagen that raises the frequency of mutation above the natural spontaneous rate.

# Important concepts for physical mutagenesis

The number of mutations induced depends on:

- the radiation types
- the radiation method
- the dose rate
- the applied dose

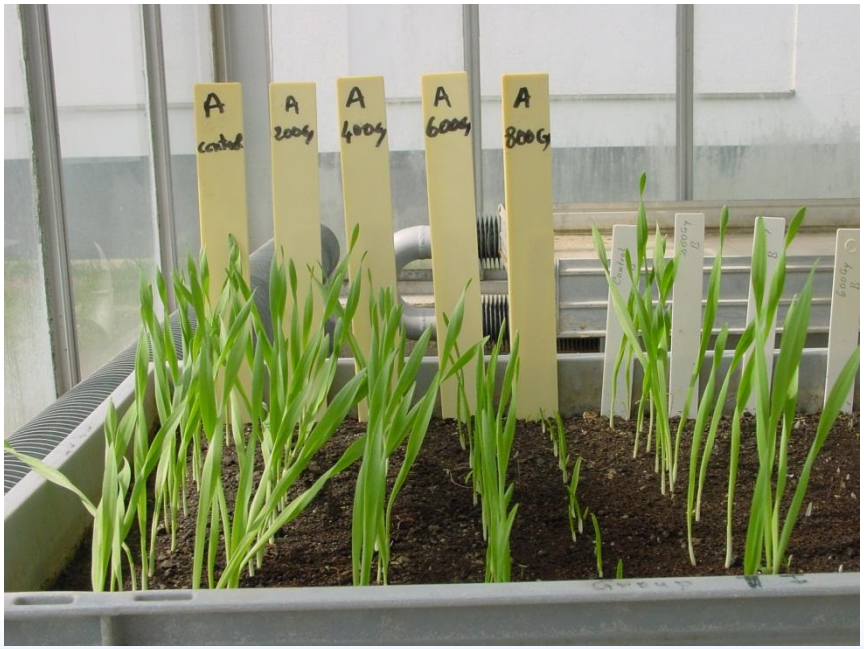
# Methods used

- Acute irradiation: applied only once, the total dose is administered at a high dose rate in a single exposure (minutes or hours)
- Chronic irradiation: the total dose is administered at a low dose rate in a single exposure but for days or weeks
- Dose fractionation or split dose irradiation: the total dose is administered interrupted by time of interval.

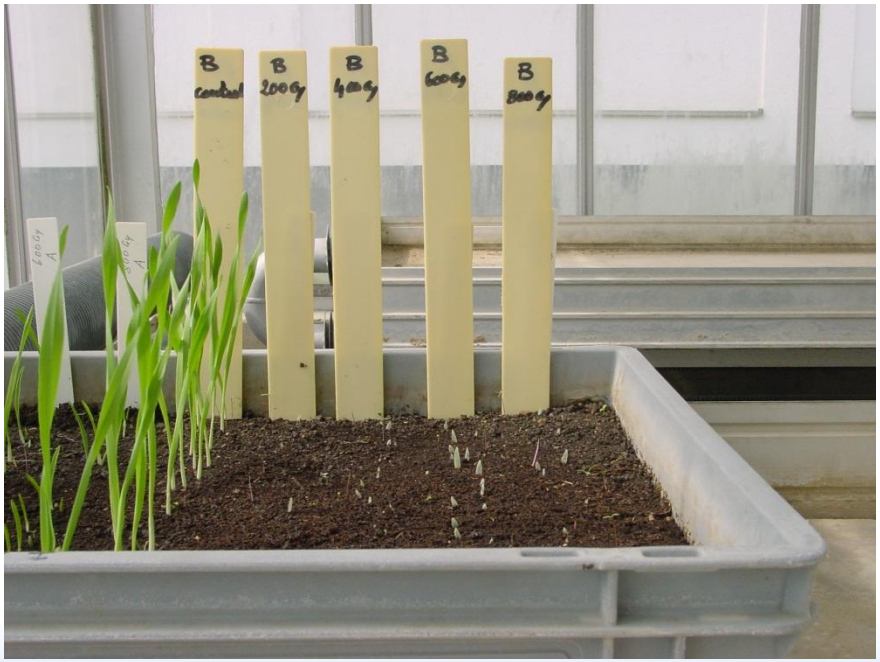
# Influencing factors

- **Environmental factors**:
  - Oxygen
  - Water content
  - Post irradiation
  - Temperature
- **Biological factors:**
  - Nuclear and interphase chromosome volume (ICV)
  - Genetic and varietal difference
  - Biochemical contents

# Moisture effect on radiation test

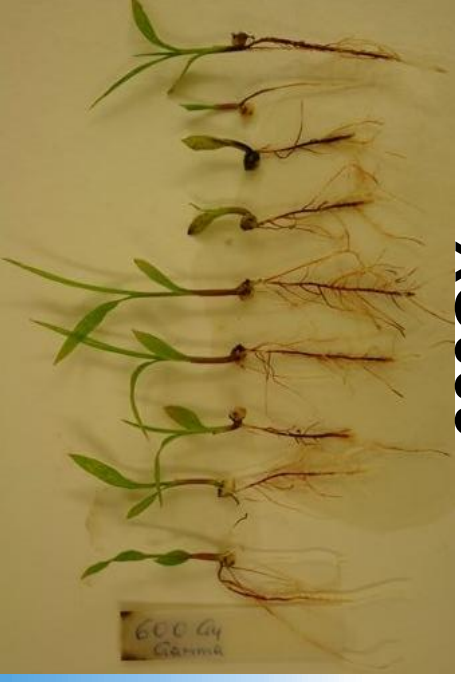



**12-15% Moisture**




**100% moisture**

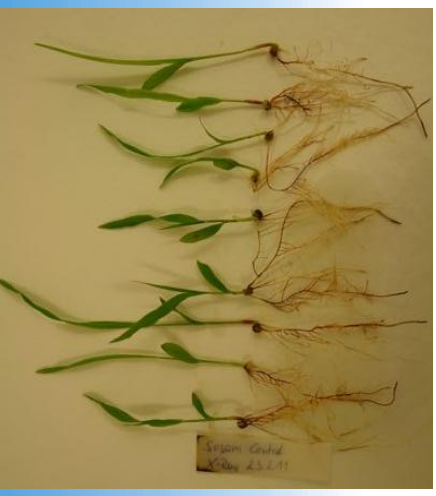
# Sandwich blot




600GY



400GY




200GY


Control

# Important concepts for chemical mutagenesis

The following parameters have to be investigated to establish procedures for experiment standardization

- Dose (concentration X time)
- pH
- Physical and chemical properties of the agents
- Interaction with the culture media
- Post treatment condition

# Influencing factors

- Pre-soaking
- Treatment duration
- Temperature during treatment
- Hydrogen ion concentration
- Dry back
- Post washing
- Storage of treated materials

# Radiation or toxicity test

- Is a relative measure that gives an indication of the quantity of recognizable effects of radiation on the irradiated objects

- $LD_{50}$ (Lethal dose): the dose at which 50% of the irradiated object die due to the treatment

# Type of materials

- Whole plants
- Seed (12.5-13% seed moisture contents)
- Meristems (*in vitro*)
- Pollen
- Cells or tissues in culture

# Efficiency of the radiation or toxicity test

Different parameters have been used to assess the efficiency mutagens

- Germination rate
- Seedling changes( height, biomass, hypocotyl,…)
- Leaf changes (chlorophyll, structure,…)
- Survival rate
- Seed set

# Efficacy of the radiation or toxicity test

This assessment is done on the M2 (M3, M4,…) materials , parameters used

- Fertility
- Phenotypic mutants rate

# Examples of radiation test

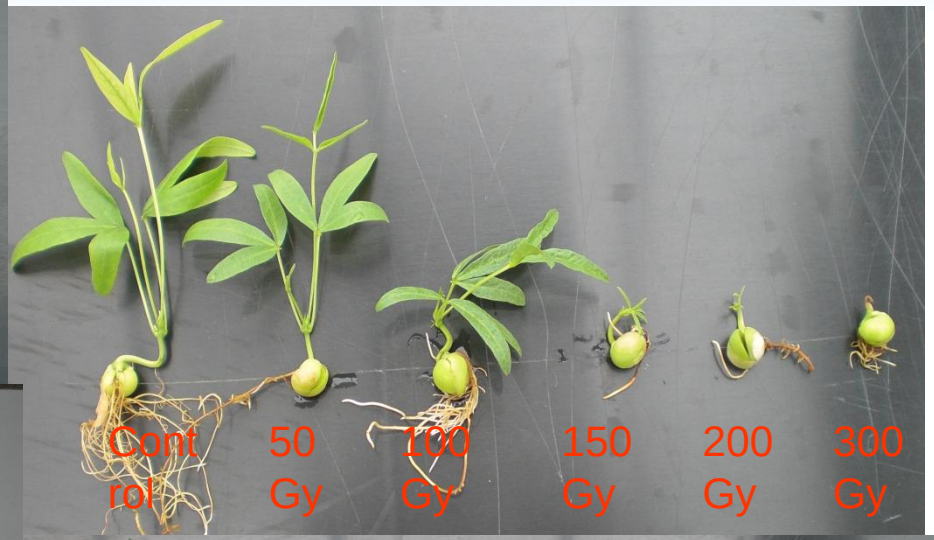

# Examples of radiation test

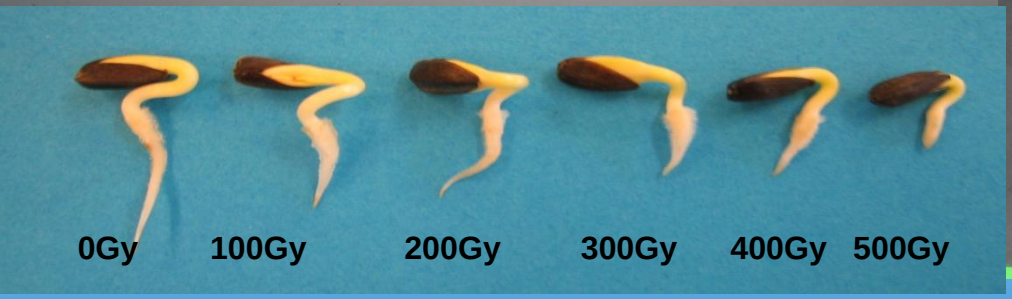

Radio resistant



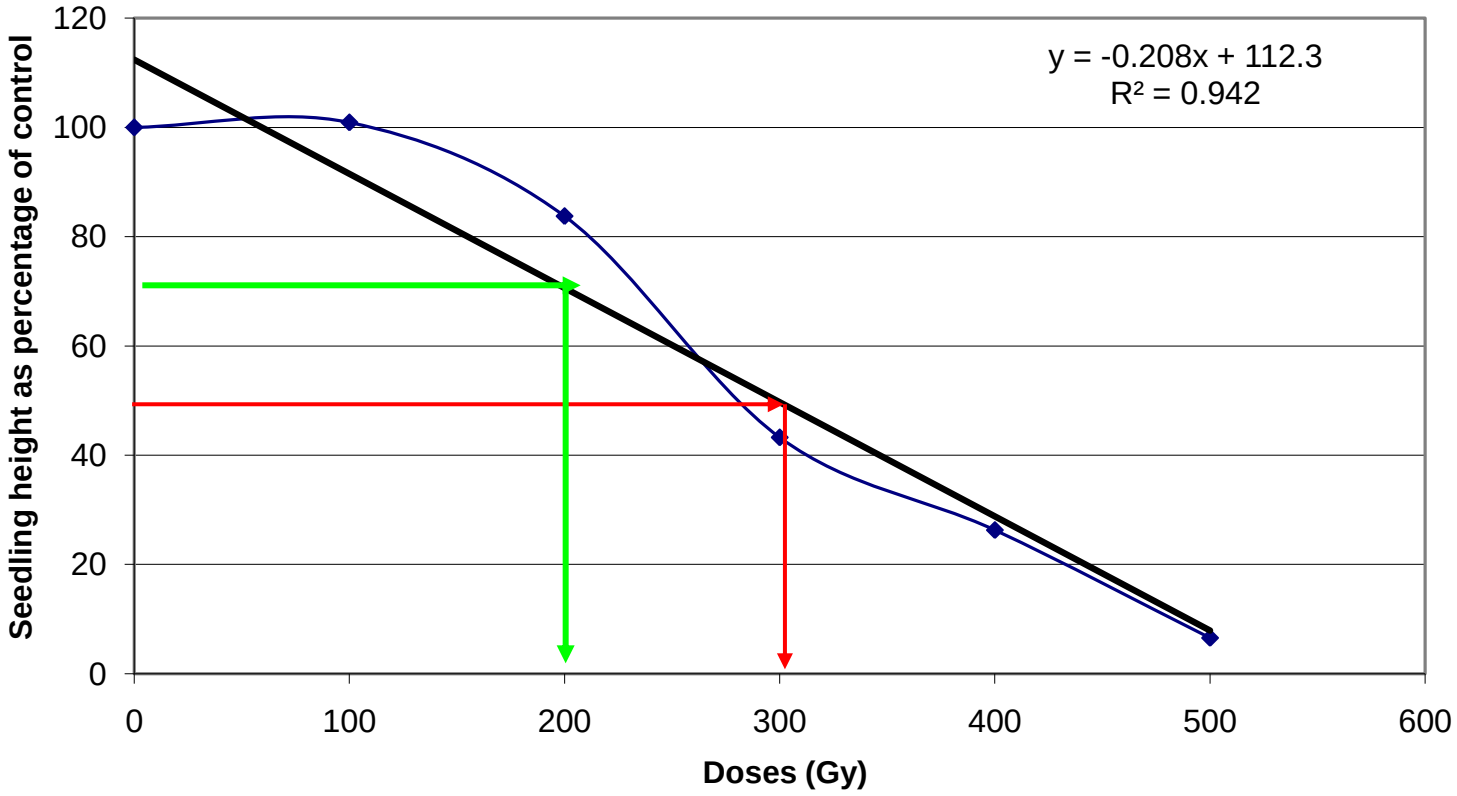
Radio sensitive

# Examples of radiation test

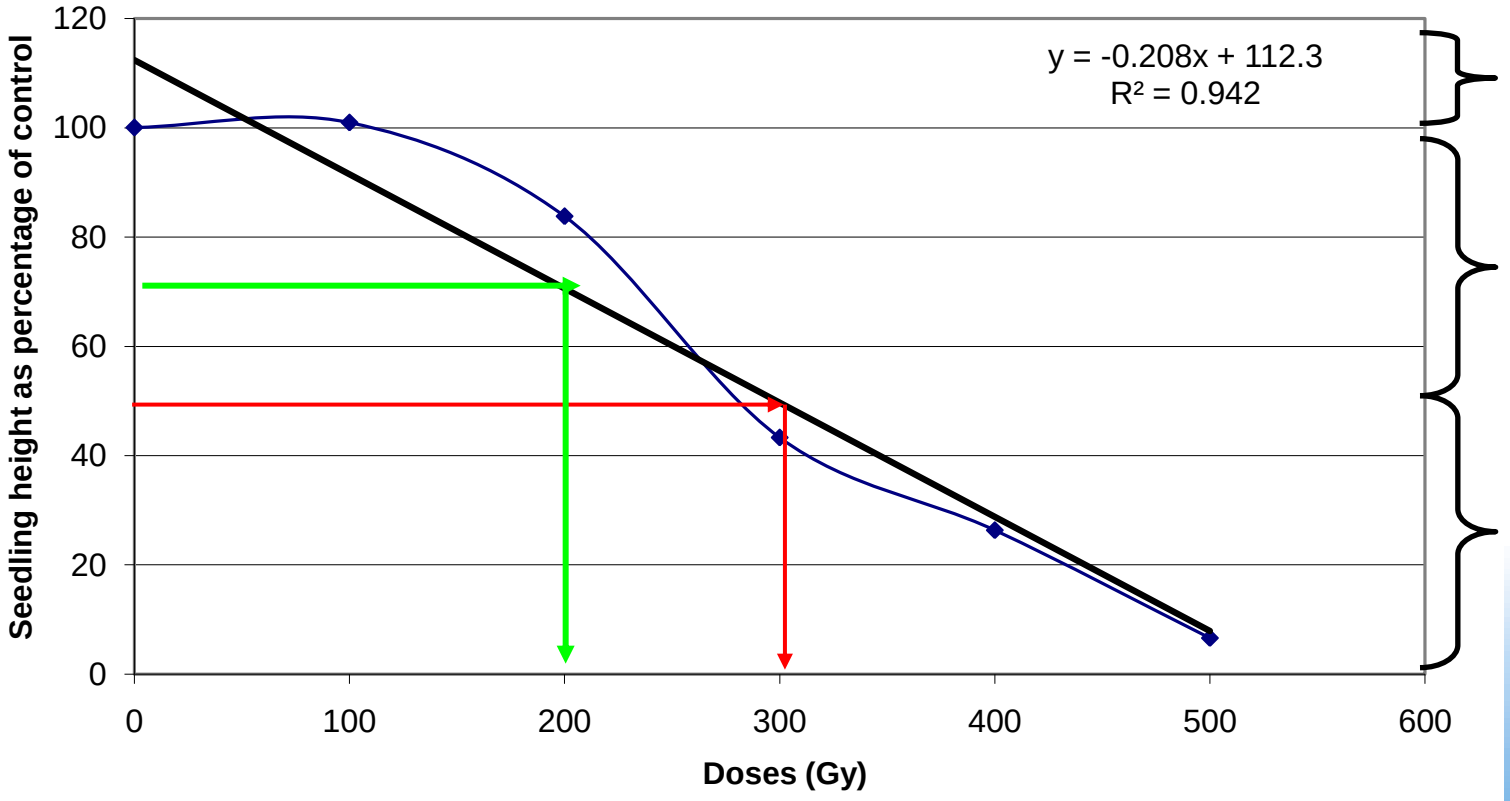



# Percentage height reduction of rice seedlings from seeds treated with Gamma irradiation



# Assessment of dosage




**Stimulation**

**Induction Mutation**

**Genes studies**

# Estimation of the optimal doses for mutation breeding - rice

| VARIETIES | Equation | LD50 (Gy) | Mutation induction LD 30 | applied Dose(Gy) |
|---|---|---|---|---|
| SNDR | y = -0.2089x + 112.38 | 298.61 | 202.87 | 250 |
| Brza | y = -0.2067x + 113.77 | 308.51 | 211.76 | 250 |
| JNK | y = -0.2305x + 102.24 | 226.64 | 139.86 | 200 |
| GHI | y = -0.2234x + 116.73 | 298.7 | 209.18 | 250 |
| Mrll | y = -0.2473x + 110.1 | 243.02 | 162.15 | 200 |
| DAM | y = -0.1978x + 111.58 | 311.32 | 210.21 | 250 |
| RNGL | y = -0.2234x + 113.06 | 282.27 | 192.78 | 250 |
| AGSZT | y = -0.2079x + 95.175 | 217.29 | 121.09 | 200 |
| MRL1-j | y = -0.1962x + 104.46 | 277.57 | 175.64 | 200 |
| KCS | y = -0.1845x + 115.44 | 354.69 | 246.29 | 250 |
| Krar | y = -0.244x + 103.99 | 221.27 | 139.3 | 200 |
| Krmn | y = -0.2013x + 107.23 | 284.3 | 184.95 | 250 |
| RsbLL | y = -0.2494x + 110.31 | 241.82 | 161.63 | 200 |
| DMK | y = -0.1992x + 112.25 | 312.5 | 212.1 | 250 |
| GB | y = -0.243x + 105.94 | 230.2 | 147.9 | 200 |

# Estimation of the optimal doses for mutation breeding - sunflower

**Table1:** **Gr50 and Gr30 values of 15 inbreds using gama rays, fast neutron irradiation and EMS solution**

| Genotypes | Gamma rays (Gy) | | Fast neutrons (Gy) | | EMS (%) | |
|---|---|---|---|---|---|---|
| | $D_{50}$ | $D_{30}$ | $D_{50}$ | $D_{30}$ | $D_{50}$ | $D_{30}$ |
| **HA-26** | 204 | 103 | 15 | 3.6 | 1.34 | 0.50 |
| **VL-A-8** | 219 | 101 | 12 | 0.6 | 1.41 | 0.55 |
| **HA-48** | 219 | 108 | 17 | 3.8 | 1.40 | 0.58 |
| **HA-19** | 118 | 5 | 9 | 0.1 | 1.55 | 0.68 |
| **OD-3369** | 170 | 35 | 11 | 0.08 | 0.69 | 0.01 |
| **V-8931-3-4-OL** | 162 | 51 | 13.5 | 1.5 | 0.82 | 0.07 |
| **HA-26-OL** | 189 | 82 | 12.5 | 1 | 1.16 | 0.43 |
| **VK-66-tph$_1$** | 335 | 219 | 20 | 9 | 1.41 | 0.53 |
| **VK-66-tph$_1$tph$_2$** | 279 | 146 | 21 | 10 | 1.54 | 0.64 |
| **VK-66-OL-tph$_2$** | 293 | 166 | 19 | 8 | 1.36 | 0.55 |
| **RUS-RF-168** | 200 | 92 | 20 | 7.3 | 1.09 | 0.30 |
| **RHA-SELEUS** | 198 | 81 | 15 | 2.6 | 1.15 | 0.39 |
| **RHA-M-72** | 191 | 94 | 13 | 1.7 | 1.46 | 0.62 |
| **CMS-ANN-15** | 235 | 145 | 13 | 0.4 | 0.94 | 0.25 |
| **RHA-S-OL-26** | 199 | 96 | 14.5 | 2 | 1.36 | 0.50 |

# Starting the mutation breeding programme

- What is known about the crop with respect to systems of reproduction?
- Which materials should be treated?
- Which method of treatment should be preferred?
- How should treated materials be handled and how large should be the population?
- What is the most efficient procedure for selection?
- Any thing known about linkage or pleiotropy?

# Starting a breeding programme

- Well defined objectives
- Is it a realistic prospect to achieve the objective (time and space)?
- The cost of the growing $M_1$ and $M_2$ generation
- The cost of harvesting and threshing the $M_1$ plants (in bulk or per plant)
- The cost of mutant selection

# Choice of mutagens

The aim is to develop methods which give the highest rate of gene mutation with the lowest chromosomal and physiological damage

# Chemical mutagens

**Advantages:**

- Point mutation predominant
- Less chromosomal damage
- High mutation rates are known in certain systems
- Possibly different mutation spectra compared with physical mutagens

**Disadvantages:**

- Penetration difficulties in multi-cellular systems.
- Difficulties in reproducibility which may be overcome by standardizing application methods
- Care has to be taken as most of the mutagens are carcinogenic (disposal)

# Physical mutagens

## Advantages:

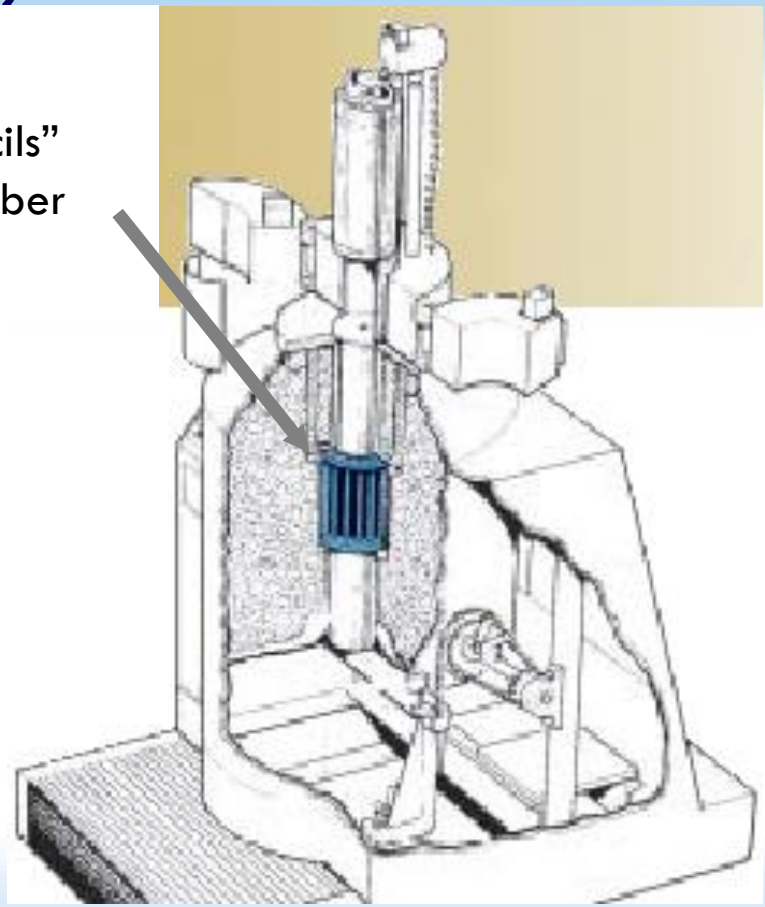
- Ionizing radiation and UV
- High penetration in multi-cellular systems except UV.

## Disadvantage:

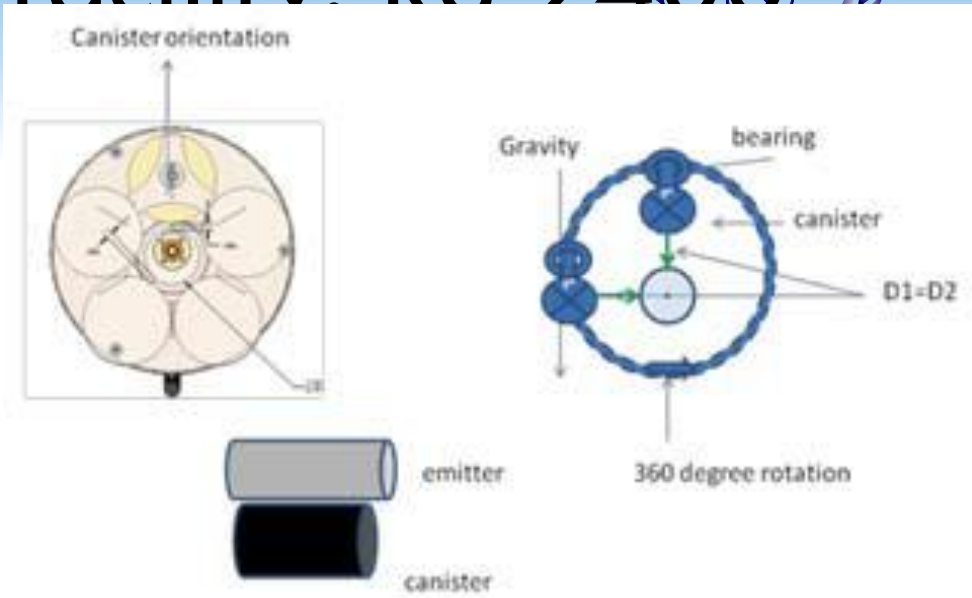
- Possibly high degree of sterility in plants regenerated from treated tissues.

# Gamma radiation facility - GC 220

Cobalt “pencils” around chamber



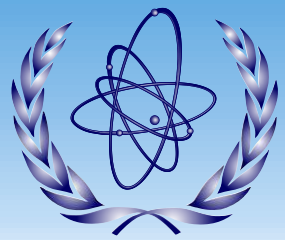
# X-ray radiation facility: RS 2400




# Some mutagenic effects observed in M1 generation

# Some mutagenic effects observed in M1 generation

# Desirable & undesirable phenotypes

# GOOD LUCK