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General Overview of Plant Mutation Breeding

By Soeranto

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Indonesian Research and Innovation Agency (BRIN)**

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Some Basic Definitions

- ❑ **Plant Breeding:** the science of changing the traits of plants in order to produce desired characteristics. It has been used to improve the productivity and quality in plant products for humans and animals.
- ❑ **Mutation:** the changing of structure of a gene, resulting in a variant form that may be transmitted to subsequent generations, caused by the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes.
- ❑ **Mutation Breeding:** the process of exposing seeds/plantlets to chemicals or radiation in order to generate mutants with desirable traits to be bred further or crossed them with other cultivars.

Occurrence of Mutation

- ❑ Mutation is a sudden, random and heritable change of genetic materials
- ❑ Its occurrence can be spontaneous (natural) or can be induced (using mutagenic agents)
- ❑ Induced mutation can increase mutation rates and shorten the time for enhancing genetic variation which is important in a plant breeding
- ❑ Any plant reproductive materials can be treated to the mutagenic agent in order to speed up the occurrence of mutation

About Mutation Breeding

- ❑ Use mutagenic agents (physical or chemical) for increasing **genetic variation**
- ❑ Doing selection of putative mutants starting in the M2, M3, M4 etc. until getting mutant lines/clones
- ❑ Confirmation by field/yield trials
- ❑ Combination with other related biotechnologies to fasten the breeding process
- ❑ Proposing mutant variety release
- ❑ Breeding methodology will depend on the **plant's reproductive system**

Mutation Breeding Scheme

Determining Breeding Objectives and
Preparing Breeding Materials



Mutagenesis, Radiosensitivity Study and
Generating M1 Plant Population



Generating Segregating Population of M2,
Starting Selection of Putative Mutants



Screening of
Mutants

General Breeding Objectives

- ❑ Improving yield potential
- ❑ Improving quality of products
- ❑ Improving crop adaptation
 - √ Biotic stresses (diseases, insects)
 - √ Abiotic stresses (drought, salinity, acidity etc.)
- ❑ Screenings are mostly done on the selected plants to meet the breeding objectives
- ❑ Some screening protocols are available, such as that of screening for drought tolerance in sorghum

Plant Reproductive System

- ❑ Determine plant breeding methodology
- ❑ Generative reproduction
 - Self-pollination (rice, soybean...)
 - Cross-pollination (corn, coconut...)
- ❑ Vegetative reproduction
 - Cuttings (cassava, roses...)
 - Tubers (potato, yams...)
 - Rhizomes (strawberry, imperata...)
 - Suckers (banana, bamboo...)
 - Tissue culture (any culture grown from planlets)

Plant Materials

- ❑ Plant's reproductive material (seeds, cuttings, plantlets) can be treated to the mutagenic agent
- ❑ Seeds must be dry and have a **good viability** (capable of germination under suitable condition)
- ❑ For self-pollinated crops like rice, soybean etc., original **homozygous line (variety)** must be used
- ❑ The number of seeds should be enough for studying radiosensitivity and getting the M1 seeds for further breeding program
- ❑ Prepare all the seeds in a number of paper bag according to the number of mutagenic treatments

Mutagenic Agents

- ❑ Mutagenic agent can be a chemicals (mostly from alkylating group), a physicals (radiation) or biological (like transposon)
- ❑ Mutagenic radiation (gamma rays) is the most widely used in mutation breeding program due to available gamma irradiator machine and more safe, effective and efficient in producing crop mutant varieties
- ❑ Detailed information of the globally released mutant varieties are available at the **IAEA mutant variety database**

Example of Mutagenic Chemicals

- Ethyl methane-sulphonate (EMS)
- Ethylene-imine (EI)
- Diethyl sulphonate (dES)
- Ethyl nitroso urea (NEH)
- Azide (sodium)
- Hydroxylamine (NH_2OH)
- Nitrous acid (HNO_2)
- Colchicine (can double chromosome)

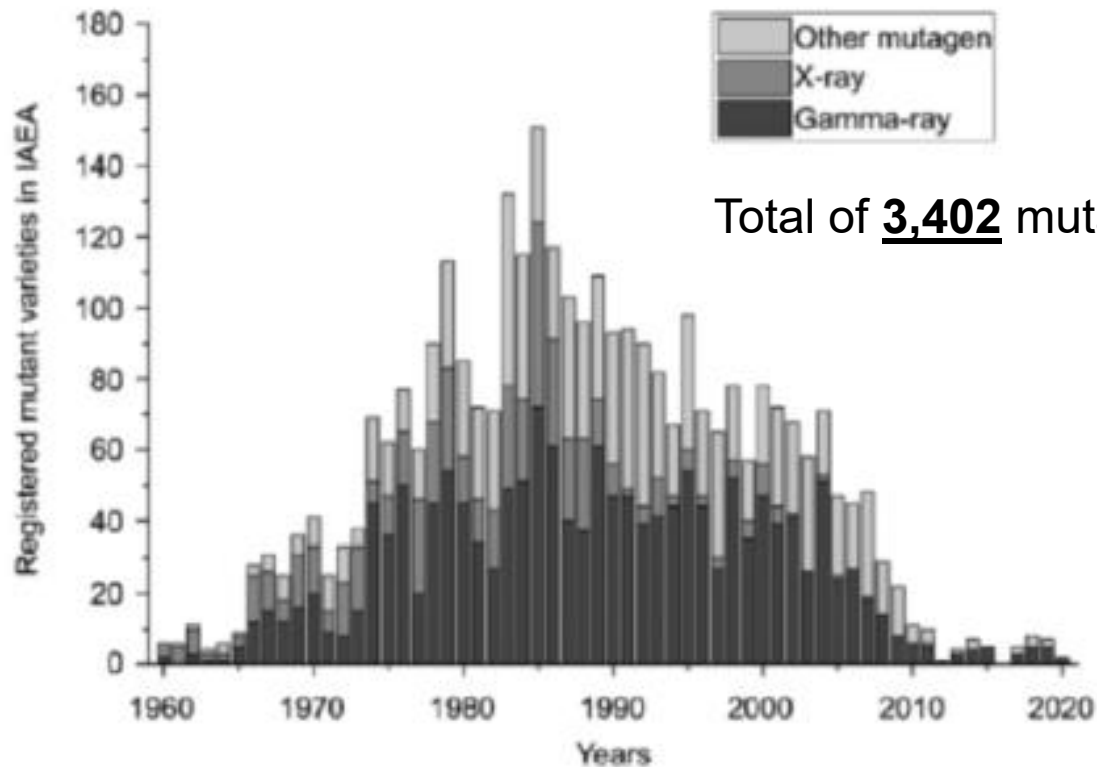
Example of Mutagenic Radiation

Types	Source	Energy	Penetration
X-rays	X-ray machine	Commonly 50-300 kV	A few mm to many cm
Gamma rays	Radioisotope and nuclear reactions	Up to several MeV	Many cm
Neutrons	Nuclear reactions or accelerators	From less than 1 to several million eV	Many cm
Beta particles	Radioactive isotopes or accelerators	Up to several MeV	Up to several mm
Alpha particles	Radioisotopes	2-9 MeV	Small fraction of a mm
Protons or deuterons	Nuclear reactors or accelerators	Up to several GeV	Up to many cm

Source: Manual on Mutation Breeding, Joint FAO/IAEA, 1977.

Global Use of Mutagenic Agent

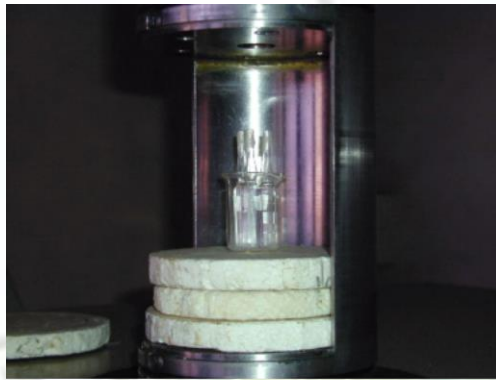
Numbers of mutant varieties registered in IAEA during 1960–2020
(based on IAEA Mutant Variety Database)



Total of **3,402** mutant varieties (2022)

Source: Ma *et al.* 2021

Gamma Irradiator located at BRIN Lebak Bulus



GC-4000A



GC-220



Panoramic Batch



Irradiator Lateks

Gamma Irradiator “Merah Putih”

Located at BRIN Serpong



Accelerator (*Under Construction*)

At BRIN Lebak Bulus



Keep in Mind (1)

- ❑ Plant's reproductive material (seeds, cuttings, plantlets) can be treated to the mutagenic agent
- ❑ Seeds must be dry and have a **good viability** (capable of germination under suitable condition)
- ❑ For self-pollinated crops like rice, soybean etc., original **homozygous line (variety)** must be used
- ❑ The number of seeds should be enough for studying radiosensitivity and getting the M1 seeds for further breeding program
- ❑ Prepare all the seeds in a number of paper bag according to the number of mutagenic treatments

Keep in Mind (2)

- ❑ Check the status of irradiator (the strength of its source) and calculate the dose rate
- ❑ Determine the irradiation dose treatments for the plant materials (in Gy unit)
- ❑ Use 0 Gy dose treatment as untreated control and high dose (e.g. 1000 Gy) as machine control
- ❑ The time of irradiation process for a given dose will depend on the dose rate
- ❑ After irradiation, the treated materials can be sown on the growth tray containing soil in the greenhouse or on the tissue culture media

Radiosensitivity Study

- ❑ Radiosensitivity is a measure of how sensitive a crop plant species is to mutagenic treatments
- ❑ Every plant species gives a specific response to mutagenic treatments, and it is usually used to estimate optimal doses for plant breeding purposes
- ❑ Optimal doses are the doses that can produce the highest possible genetic variation in the M2 population where mutant selection can be started
- ❑ Optimal doses are usually determined by accepted minimum and maximum Lethal Doses (LD) e.g. between LD-20 and LD-50 for most cereals

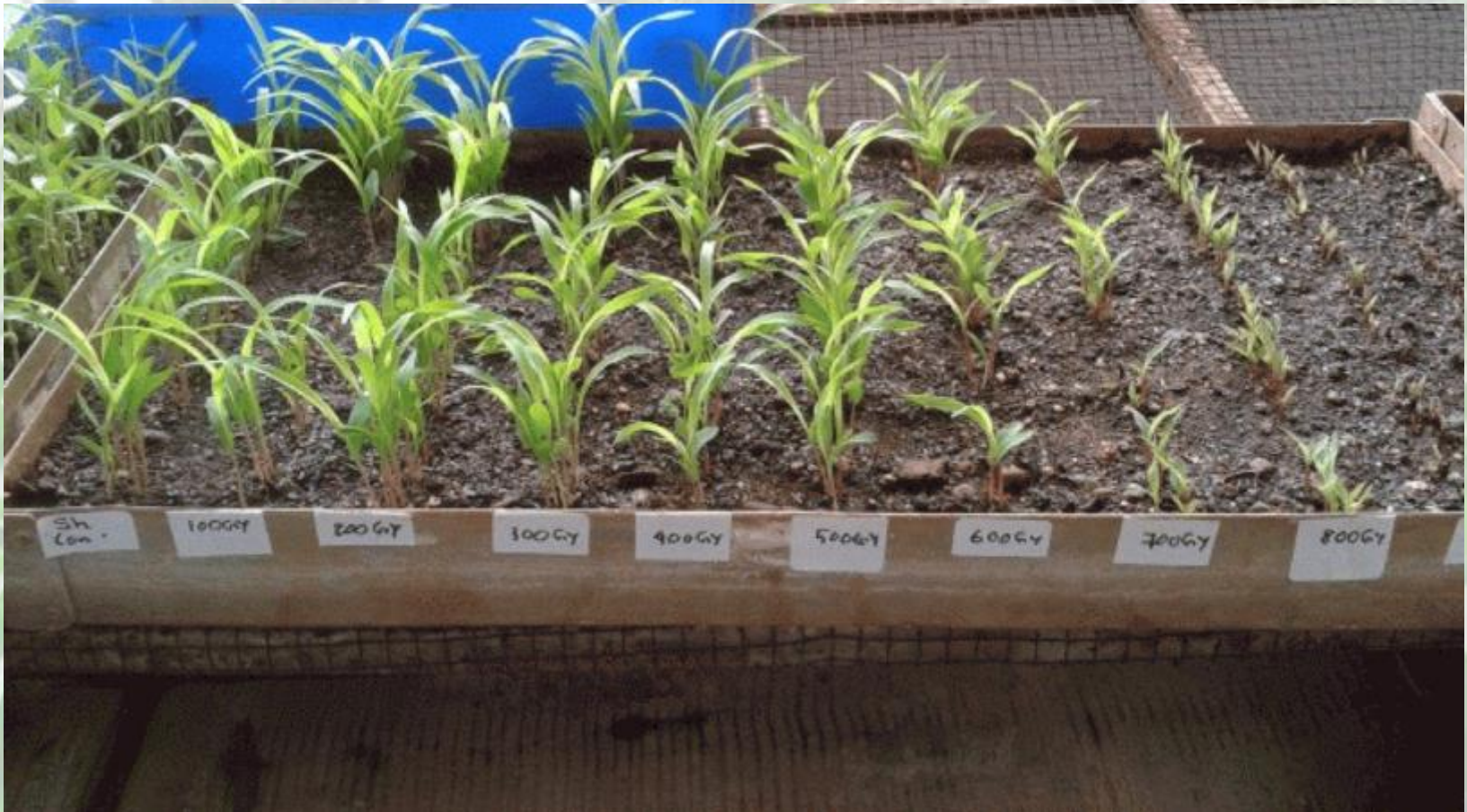
Some Terminologies

- ❑ **Lethal dose 20% (LD-20):** the mutagenic dose that can cause 20% lethality of the treated numbers of plant materials
- ❑ **Lethal dose 50% (LD-50):** the mutagenic dose that can cause 50% lethality of the treated numbers of plant materials
- ❑ **Optimal mutagenic doses:** the doses that can produce the highest possible genetic variation in the M2 population
- ❑ **Effective mutagenic doses:** the doses that can produce the highest amount of desirable mutants in a mutation breeding program

Example: Radiosensitivity of Rice



Example: Radiosensitivity of Sorghum



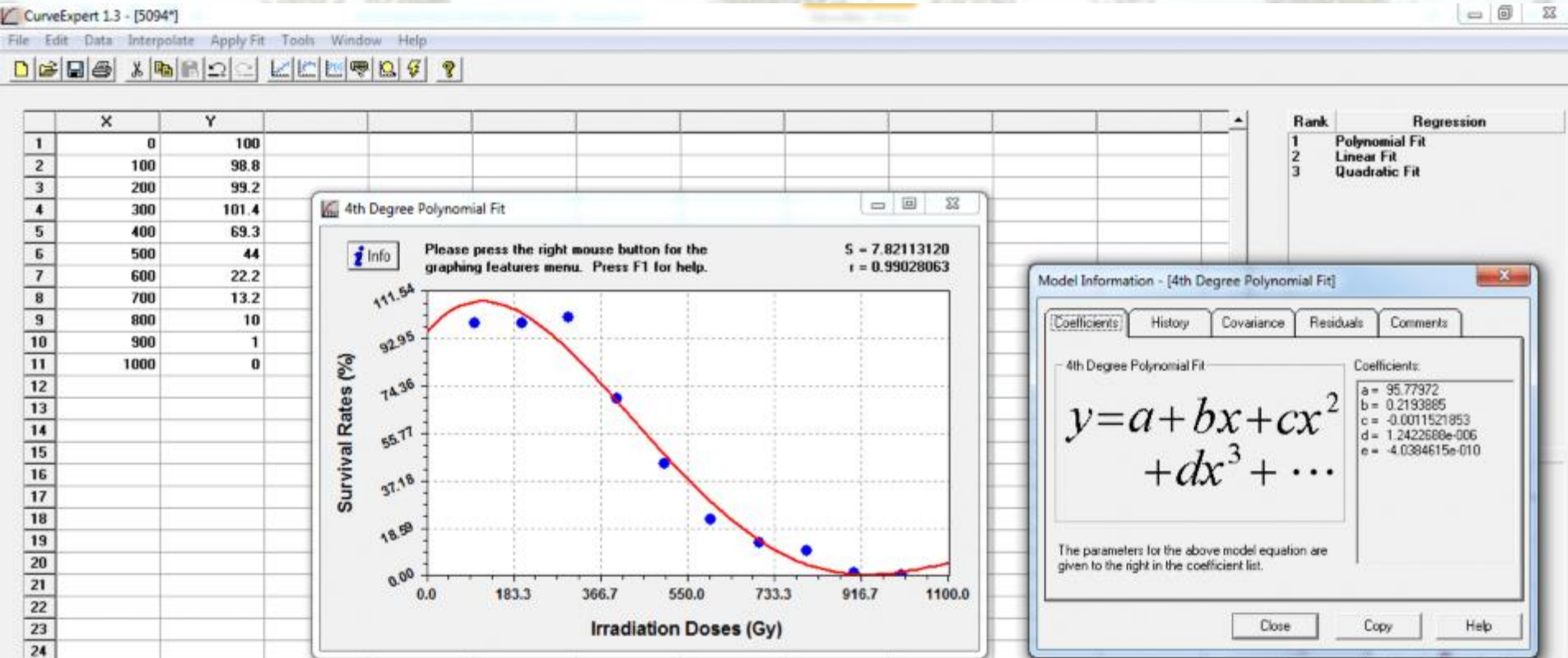
Example Data of Radiosensitivity

(Sorghum Seed, Water Content of 12%)

Radiation Doses (Gy)	Number of Irradiated Seeds	Raw Data of Survival Rates (%)	Standardized Data of Survival (%)
0	200	85,0	100
100	200	83.9	$83.9/85*100=98.8$
200	200	84.3	99.2
300	200	86.2	101.4
400	200	58.9	69.3
500	200	37.4	44.0
600	200	19.9	22.2
700	200	11.2	13.2
800	200	8.5	10.0
900	200	0.8	1.0
1000	200	0	0.0

Estimation of Optimal Irradiation Doses

(Radiosensitivity of Sorghum)



This curve gives:

- ❑ LD-20 = 343 Gy
- ❑ LD-50 = 487 Gy

Status of the M1 Plants

- ❑ Appeared plant variation in the M1 is as results of direct (physiological) effects of irradiation
- ❑ There is no genetic variation in the M1 so that we cannot do any selection in the M1
- ❑ Physiological effects in the M1 are usually measured by survival/lethal growth rates
- ❑ Lethal Doses (LD) are determined by survival/lethal rates
- ❑ We have to find the **optimal dose** i.e. the doses that can produced the highest possible genetic variation in the M2
- ❑ For most cereals, the optimal dose rates are normally in the range between LD-20 and LD-50
- ❑ The next step in mutation breeding is to generate plant population derived from the optimal doses for selection

Mutation & Plant Variation

- ❑ Mutagen causes mutations
- ❑ Mutation creates plant variation
- ❑ Plant genetic variation can be measure in the M2
- ❑ Hence, selection should be started in the M2

Variation = Variance

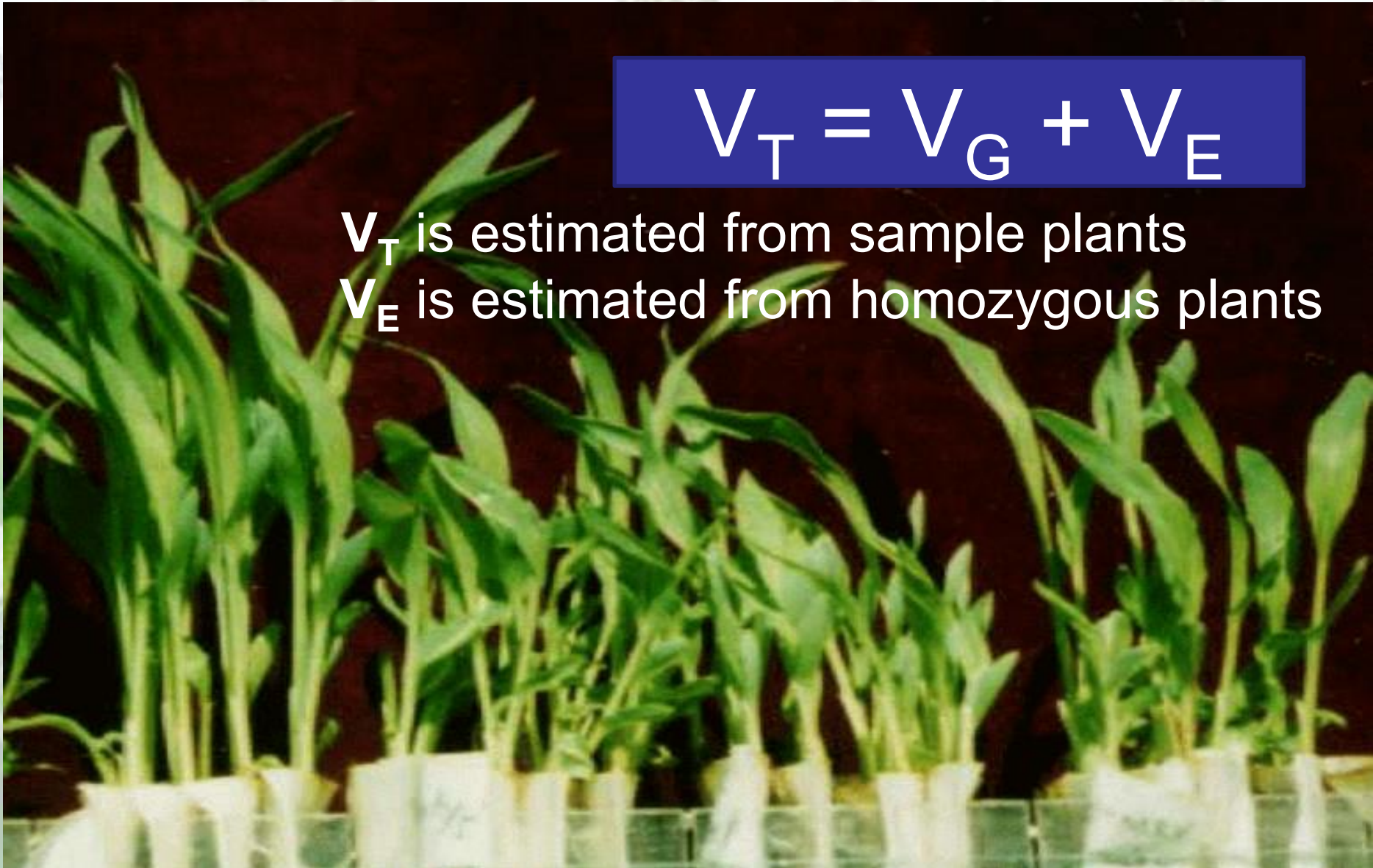
$$\begin{aligned}\text{Var}(x) &= \frac{\sum(x-\bar{x})^2}{(n-1)} \\ &= \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{(n-1)}\end{aligned}$$

Example of Plant Variation in the M2

$$V_T = V_G + V_E$$

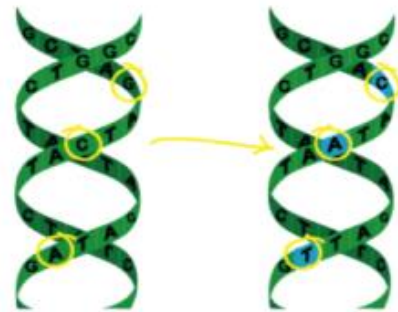
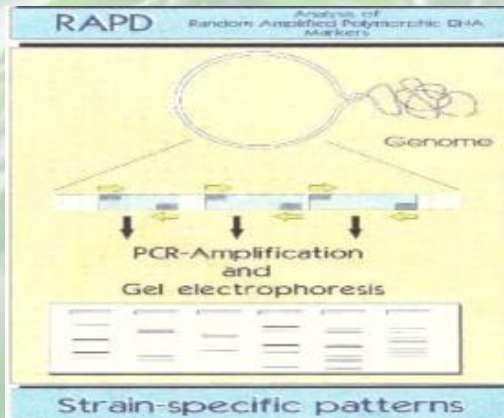
V_T is estimated from sample plants

V_E is estimated from homozygous plants

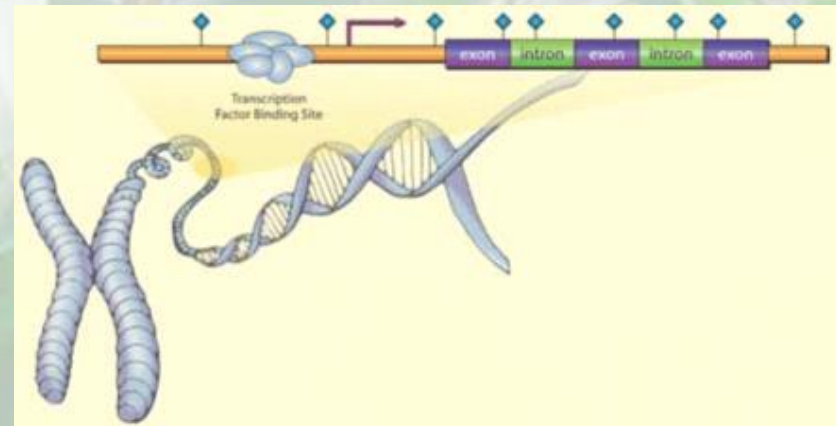


Combination with Biotechnology

- ❑ Biotechnology is used as a *tools* not a target, namely in the process of selection (*Marker Assisted Selection*)
- ❑ Or in the process of finding a new gene through *Targeting Induced Local Lesions IN Genomics* (TILLING)

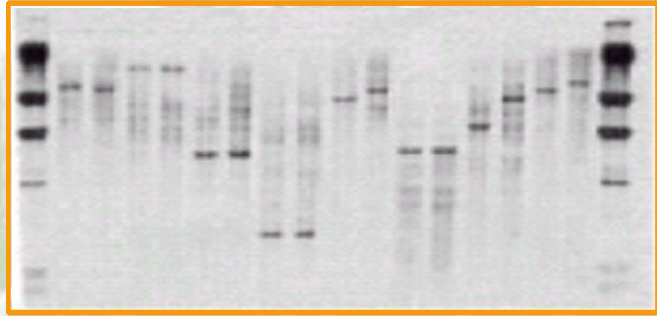


- Estimated SNPs in human genome: 10 million
- Number that have been seen twice: about two million

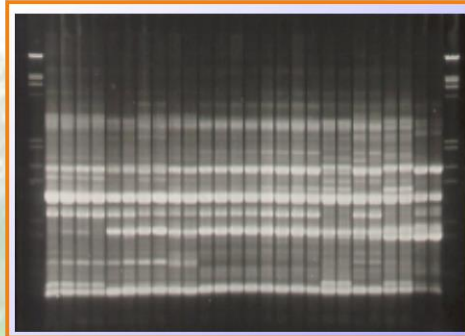


Mutant Selection Based On DNA Markers

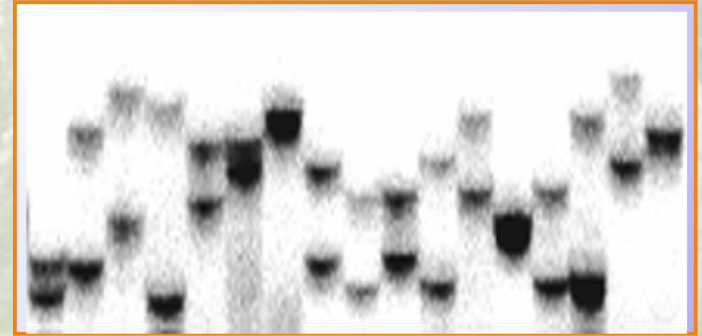
RLFP



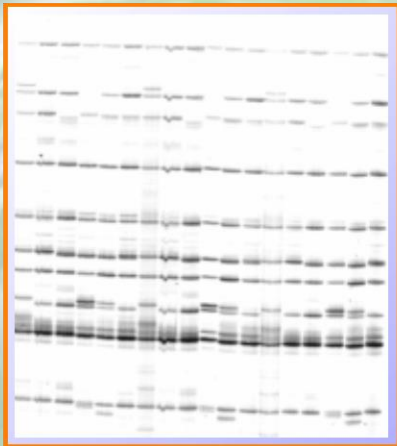
RAPD



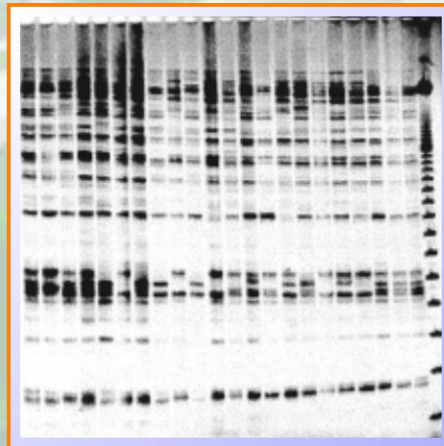
SSR



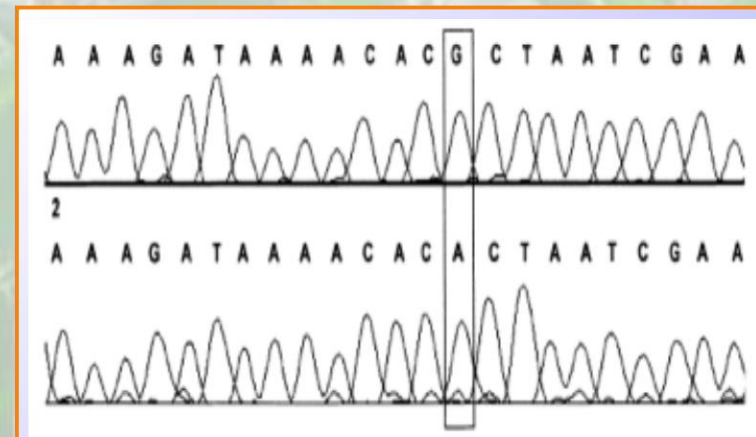
AFLP



ISSR



SNP



Facilities Needed for Mutation Breeding

- Gamma Irradiator
- Isotope Laboratory
- Plant Breeding Laboratory
 - ✓ Seed storage facilities
 - ✓ Tissue culture facilities
 - ✓ Biotechnology facilities
 - ✓ Plant screening facilities
 - ✓ Quality analysis facilities
 - ✓ Greenhouse facilities
 - ✓ Experimental fields

Plant Breeding Laboratory



Tissue Culturing Facilities



Biotechnology Laboratory



Mutant Seeds Storage Facility



Greenhouse Facility



Bird Protected Rice Mutant Nursery



Sorghum Mutant Nursery



Experimental Field



Example from Indonesia

- ❑ Rice is the main staple food for Indonesia
- ❑ Rice production, particularly in Java, is facing problem with shrinking growing areas due to land utilization for the non-agriculture purposes such as industries, roads and houses etc.
- ❑ Expanding rice field to outside Java are costly and also face problems with soil fertility, infra structures etc.
- ❑ Intensive rice cultivation (using superior varieties) becomes of important to increase rice production
- ❑ Nuclear technology has been proven to help Indonesia increase food crops production including rice, soybean, mungbean, sorghum etc.
- ❑ The first mutant variety of rice called *Atomita-1* was released in 1982

Food Crop Diversity

- ❑ Indonesia has a great potency to produce food from the available crop diversity. The tropical condition make it possible to produce carbohydrate (food stuff) all year around
- ❑ It can be from grain crops (rice, corn, sorghum etc.), tuber crops (cassava, yam, sweet potato etc.), legumes (soybean, peanut, mungbean etc.) or many kinds of horticultural crops
- ❑ Research and development have been conducted by several institutions to increase these food crops production
- ❑ In collaboration with counterparts, we have conducted some researches on increasing food crops productivity and quality by using **mutation breeding**

Potency of Dryland Farming

- ❑ In Java, most agricultural land is dominated by lowland paddy field with good irrigation system (wetland). Intensively increasing rice production can be achieved in such condition
- ❑ On the other hand, outside Java is mainly consisted of dryland agriculture ecosystem, sometime with adverse condition. We have to develop food crops that can grow and adapt well in such condition with high economic values
- ❑ **Sorghum** is a potential crop for dryland ecosystem since this crop has wide adaptability, more tolerant to adverse condition such drought, soil acidity and salinity, and has good nutritive values either for food and animal feed
- ❑ Mutation breeding of sorghum has been done successfully and some mutant varieties have been released

Research Counterparts

- ❑ Ministry of Agriculture
- ❑ Universities and Research Institutions
- ❑ Local Agricultural Offices
- ❑ Private Companies
- ❑ International Organizations:
 - Joint FAO/IAEA
 - FNCA
 - JSPS (Tokyo University, JAEA, IRB)
 - ICRISAT
 - APEC

Results of Mutation Breeding

- ❑ 40 rice mutant varieties
- ❑ 16 soybean mutant varieties
- ❑ 6 sorghum mutant varieties
- ❑ 4 mungbean mutant varieties
- ❑ 1 groundnut mutant variety
- ❑ 1 cotton mutant variety
- ❑ 1 tropical wheat mutant variety
- ❑ 1 banana mutant variety
- ❑ Many ornamental plant varieties

All mutant varieties were officially released by the Ministry of Agriculture of Indonesia

One of Popular Rice Mutant Varieties “Mira-1”

- High yielding (9,2 t/ha)
- Resistant to BPH
- Resistant to BLB.
- Low amylose content (19%)

**First harvest by the
President of the
Republic of
Indonesia**





**BATAN Chairman and local officials visited
“Mutant Lines” of local rice in Kerinci District**

Homogen M5 mutant line population →

Soybean Mutant Variety “Mutiara”



- High yielding (4 ton/ha)
- Big grain size (24.3 g / 100 grain)
- Tolerant to leaf spot and stem borer

Soybean Mutant Variety Developed for Tempe Industry (by Rumah Tempe)



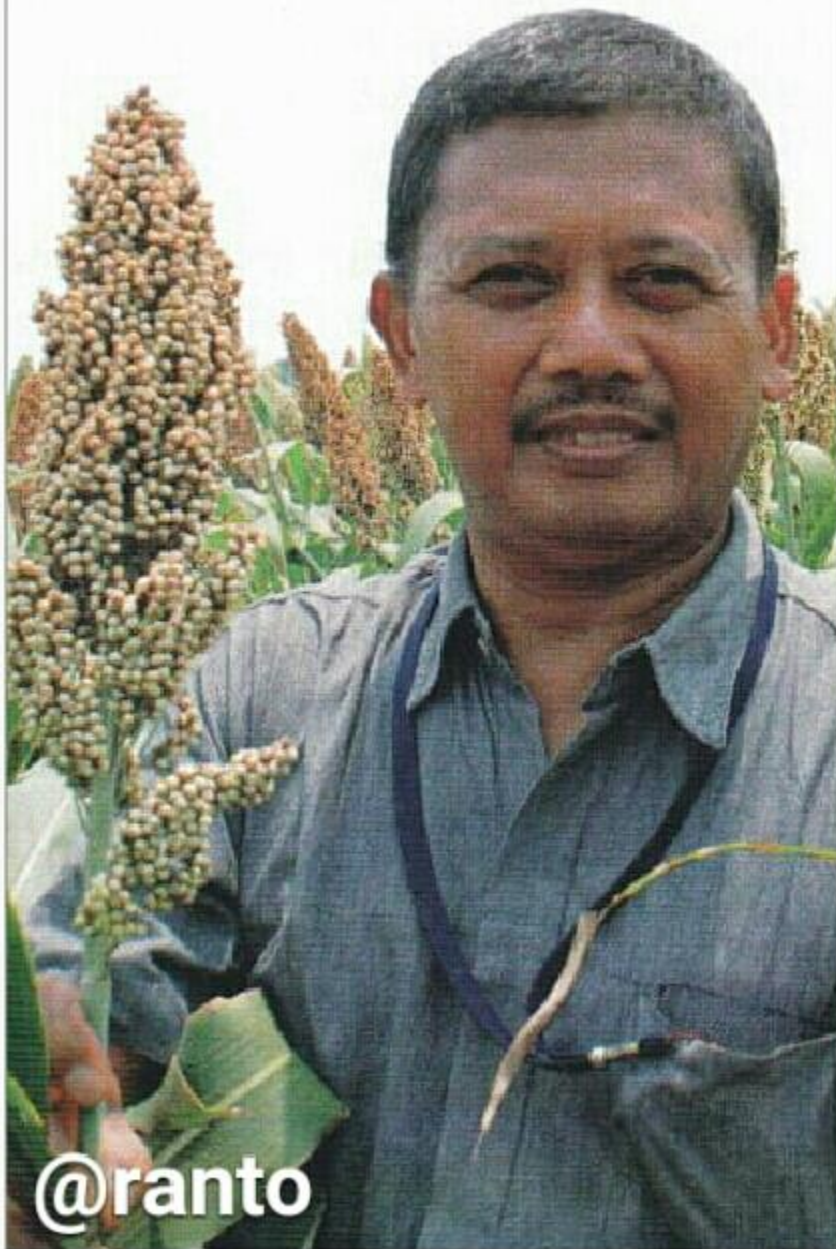
Released Sorghum Mutant Varieties

1. Pahat (grain sorghum)
2. Samurai 1 (sweet sorghum)
3. Samurai 2 (sweet sorghum)
4. Sorgamma (grain sorghum)
5. Gamma Sorbico 1 (forage sorghum)
6. Gamma Sorbico 2 (forage sorghum)

Pahat Variety (Healthy Food)



Sorghum Is Healthy Food !



@ranto

**Gluten Free
Low Glycemic
Rich Calcium
Antioxidant
High Fiber**

@nasisorgum

Mutant Variety “Sorgamma”



REPUBLIK INDONESIA
KEMENTERIAN PERTANIAN
PUSAT PERLINDUNGAN VARIETAS TANAMAN DAN PERIZINAN PERTANIAN

Tanda Daftar Varietas Tanaman

VARIETAS HASIL PEMULIAAN

NOMOR : 163/A.9/08/2025

Berdasarkan Undang-undang Nomor 29 Tahun 2000 tentang Perlindungan Varietas Tanaman; Peraturan Pemerintah Nomor 13 Tahun 2004 tentang Penamaan, Pendaftaran dan Penggunaan Varietas Asal untuk Pembuatan Varietas Turunan Esensial; Peraturan Menteri Pertanian Nomor 29 Tahun 2021 tentang Penamaan dan Pendaftaran Varietas Tanaman, bersama ini kami memberikan Tanda Daftar Varietas Hasil Pemuliaan kepada :

Nama Pemohon	: BADAN RISET DAN INOVASI NASIONAL
Alamat	: Gedung B.J. Habibie, Jl. M.H. Thamrin No. 8, Jakarta Pusat
Tanggal Penerimaan Pendaftaran	: 18 Agustus 2025
Nama Pemulia	: Sihono, Wijaya Murti Indriatama, Soeranto Human, Winda Puspitasari, Yudhistira Nugraha, Nana Supriatna, Herniwati, Anisiyah, Muhammad Iqbal, Marina Yuniawati Maryono, Tardi Suseno (Badan Riset dan Inovasi Nasional)
Kewarganegaraan Pemulia	: Indonesia
Jenis Tanaman	: Sorgum (<i>Sorghum bicolor</i> (L.) Moench)
Nama Varietas	: Sorgamma

Dengan demikian varietas tersebut telah terdaftar di Pusat Perlindungan Varietas Tanaman dan Perizinan Pertanian sesuai dengan Peraturan Perundang-undangan yang berlaku dan dicatat dalam Daftar Umum PVT, serta diumumkan dalam Berita Resmi PVT.

Jakarta, 20 Agustus 2025
Kepala Pusat,



Dr. Ir. Leli Nuryati, M.Sc.
NIP 196802251993032002

Sorgamma: Dwarf & Early Maturity

**Stay Green
and
Ratooning
Ability**



Example of Sorghum Based Food Products



WEDANG 101
HEALTHY FOOD, HEALTHY LIFE

**Cookies
Coklat
Tepung
Sorghum**



Bubur Sorgum Sehat



Newly Released Sorghum Products in Indonesia



By PT Sedana Panen Sejahtera

Prospects of Mutation Breeding

- ❑ To increase genetic variation in a mutation breeding program
- ❑ Especially for plant/crop that has difficulty in crossing or hybridization
- ❑ Breaking out *linkage genes* (e.g. rice local variety)
- ❑ Engineering new gene(s) that is not available in nature (e.g. *fusarium resistant gene* in banana)
- ❑ Drastically morphological/physiological changes in plant (e.g. sterility, flower shape and color, dwarf or bonsai plant etc.)

Safety of Mutation Products

- ❑ Mutation can occur naturally (spontaneous) or through induction (induced mutation)
- ❑ There is no difference between spontaneous and induced mutations. All are naturally safe
- ❑ In mutation, only genetic material within a plant is changed so that it can ensure the biosafety. It is completely different from that of transgenic plant where the gene transfer exists.
- ❑ Information of mutant varieties of food crop are available at the IAEA Mutant Variety Database

IAEA Collaborating Center



IAEA

International Atomic Energy Agency

Center for Isotopes and Radiation Application (CIRA)

National Nuclear Energy Agency of the Republic of Indonesia (BATAN)

IAEA Collaborating Centre

for

Plant Mutation Breeding for Climate Smart Agriculture (PMBCSA)

2017 - 2021

IAEA Collaborating Activities

- Research on mutation breeding for Climate Smart Agriculture
- Hosting IAEA meetings
- Hosting IAEA training courses (Group and Individual Trainings)
- Providing experts on mutation breeding
- Working for Asia-Pacific and Africa regions

جائزة العمل المتميز
卓越成就奖

OUTSTANDING ACHIEVEMENT AWARD
PRIX D'EXCELLENCE
ПОЧЕТНАЯ ГРАМОТА "ЗА ВЫДАЮЩИЕСЯ ДОСТИЖЕНИЯ"
PREMIO AL LOGRO NOTABLE

Plant Breeding Group, Agriculture Division
Centre for Isotopes and Radiation Application, National Nuclear Energy Agency,
INDONESIA

تقديرًا لمساهماتكم في تحقيق الأمن الغذائي والتنمية الزراعية المستدامة من خلال تربية النباتات باستخدام الطفرات

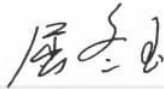
谨此表彰您利用植物诱变育种对粮食安全及可持续农业发展做出的贡献

for your contribution to food security and sustainable agricultural development through Plant Mutation Breeding

en reconnaissance de votre contribution à la sécurité alimentaire et au développement agricole durable au moyen de la sélection végétale par mutation

за вклад в обеспечение продовольственной безопасности и устойчивого развития сельского хозяйства посредством развития мутационной селекции растений

por su contribución a la seguridad alimentaria y el desarrollo agrícola sostenible mediante el fitomejoramiento por inducción de mutaciones



Mr QU Dongyu
Director-General
Food and Agriculture Organization
of the United Nations



Joint FAO/IAEA Centre
Nuclear Techniques in Food and Agriculture

September 2021



Mr Rafael Mariano GROSSI
Director-General
International Atomic Energy Agency

Hosting Many International Meetings



Hosting Many International Trainings



Providing International Experts



Working For Africa



Thank You



@Soeranto