KEMENTERIAN SAINS, TEKNOLOGI DAN INOVASI
MINISTRY OF SCIENCE, TECHNOLOGY AND INNOVATION

RAS/5/070 DEVELOPING BIOENERGY CROPS TO OPTIMIZE MARGINAL LAND PRODUCTIVITY THROUGH MUTATION BREEDING AND RELATED TECHNIQUES (RCA)

RAS5070/9003/01
Coordination meeting to review the progress of the field trials
Hanoi, Viet Nam
3-7 July 2017

Malaysian Nuclear Agency (Nuclear Malaysia)
Ministry of Science, Technology and Innovation (MOSTI)

Project Team

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History of Kenaf in Malaysia

- Kenaf (*Hibiscus cannabinus* L.) is an annual fibre crop, native to Africa
- Main use– as raw material for paper and fibre-based industries.
- First introduced to Malaysia in 2000
- Malaysian government promoted kenaf as a new alternative crop for tobacco
- Established the National Kenaf and Tobacco Board (NKTB) in 2010
- Inclusion of kenaf in National Commodity Policy 2011-2020
- Targeted to be a new commodity in 2020
- Kenaf biomass serves as a potential material for sustainable bioenergy (bioethanol, biohydrogen) supplier in the future.


RENCANA

Potensi kenaf komoditi eksport baharu negara


Kenaf as a Bioresource for Production of Hydrogen-rich Gas

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Industri Kenaf Boleh Jadi Lubuk Emas Baru Negara

BESERI (Perlis): Kenaf adalah pokok yang ditanam untuk menghasilkan serat, yang mempunyai potensi besar dalam aplikasi komersil pelbagai industri.

Dalam hubungan ini, Lembaga Kenaf dan Tembakau Negara (LKTN) telah dipertanggungjawab untuk menerokai potensi kenaf sebagai satu komoditi baru eksport Malaysia.

Kenaf (Hibiscus Cannabinus L.) yang berasal dari Afrika, boleh tumbuh antara lima hingga enam meter dalam tempoh enam hingga lapan bulan selepas ditanam, dan ia mempunyai banyak kegunaan seperti bio komposit iaitu untuk bahan binaan, komponen bahan kereta dan perabot, pulpa dan kertas, tekstil dan sebagai bahan makanan untuk ternakan.

Kenaf biasanya akan membiak pada suhu iklim antara 10 dan 30 darjah Celsius, terutama di tanah gambut atau berpasir, dengan nilai pH 6 & 8, serta purata hujan bulanan antara 100mm dan 125 mm.

KENAF CULTIVATION IN MALAYSIA

Year	Acreage (ha)	No. of farmers
2004	0.4	1
2005	42	25
2006	112	13
2007	285	92
2008	464	167
2009	343	50
2010	1,693	409
2011	1,140	687
*2015	2,000	1,300
*2020	10,000	-

Source: Ministry of Plantation Industries and Commodities Malaysia

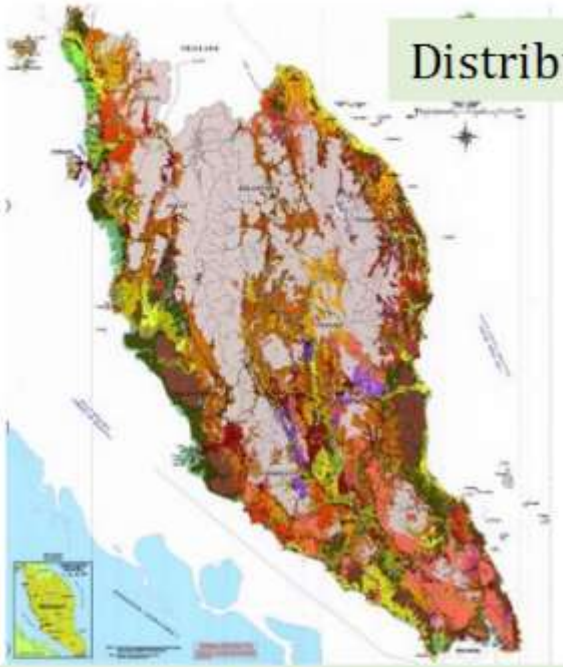
Kenaf characteristics

1. **Bast**
- outer layer fiber
- Long and strong, higher quality
- 35% of total stem weight
- For gunny sack, automative component, rope/yarn, building material etc

2. **Core**
- soft inner layer (including pith)
- 65% by weight
- For absorbent, pulp and paper, biocomposite, animal bedding etc

https://www.naturalfibersinfo.org/natural-fibers/kenaf/

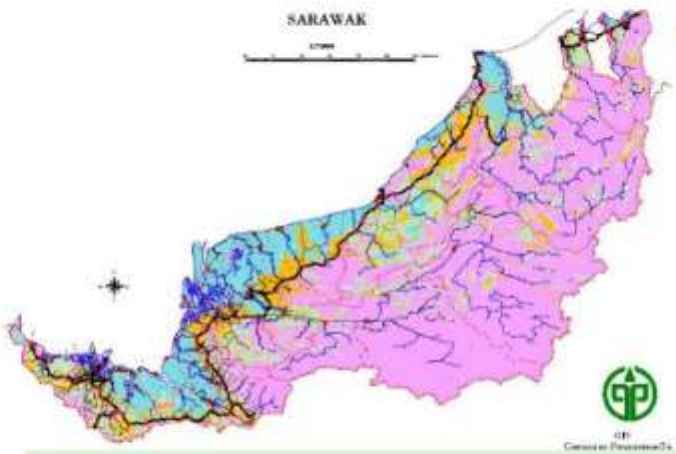
Distribution of suitable soil



Peninsular Malaysia

Physical area – 13.21 mil. ha

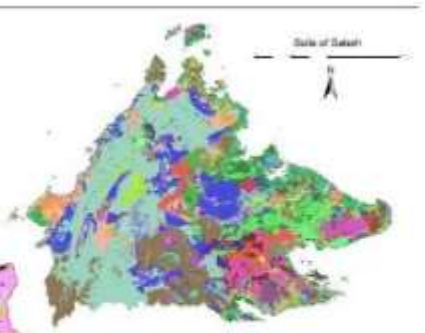
- Suitable – 53%
- Marginal and unsuitable – 47% (Peat, acid sulfate soil, sandy beach ridges, sand tailing and steepland)



Sarawak

Physical area – 12.3 mil. ha

- Suitable –28%
- Marginal and unsuitable 72 % (Steepland, swamp)



Sabah

Physical area – 7.36 mil. ha

- Suitable –30%
- Marginal and unsuitable 70 % (Swamp and steepland)

Land area distribution

- Peninsular Malaysia (39.7%)
- East Malaysia (Sarawak and Sabah) (60.3%)
- Agriculture land (33.3%)

Source: ASARI HASSAN
Department of Agriculture Malaysia
Presented at Asian Soil Partnership Consultation Workshop on Sustainable Management and Protection of Soil Resources, 12 –14 May 2015; Bangkok Thailand

Project Background

- Collaboration with National Kenaf and Tobacco Board (NKTB)
- First official meeting between Nuklear Malaysia and NKTB on 6 Sept 2015 at NKTB HQ, Kelantan
- Subsequent discussion and site visit on 17 Sept 2015 at NKTB Perlis. Both parties agreed to use Beseri dan Titi Tinggi field plots (both in Perlis) for field screenings
- Planting for 2 seasons a year

Meeting with NKTB management at Kelantan

Site visit to NKTB Perlis

Project Objectives

- To induce high rate of mutation using acute (high dose rate) and chronic (low dose rate) gamma radiation to create wider genetic variations for better selection

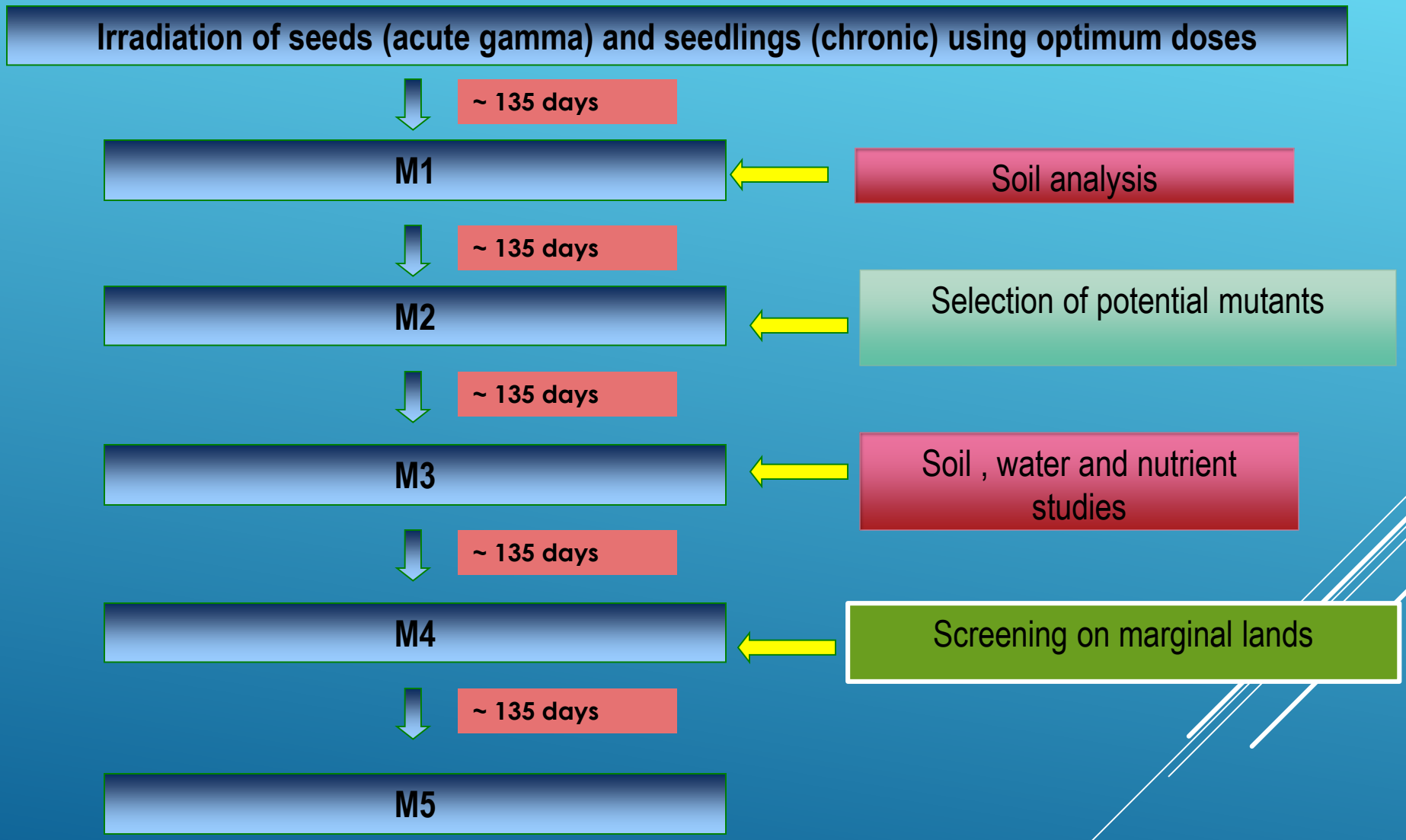
- To screen and to select potential mutant lines with high yielding and late flowering character

- To conduct nutrient and water use efficiency studies by means of isotopic techniques using N-15 and application of biofertilizer for yield improvement

NATIONAL WORKPLAN

Plant	Mutagen	Target traits	2015	2016	2017-2018
Kenaf	Gamma rays	High biomass yield Late-flowering	• Analysis of soil physical and chemical properties of Bris soil (marginal land) • Irradiation of seeds with chronic gamma radiation using 3 identified optimal doses at LD10, LD25 and LD50 and with acute gamma radiation using 3 doses at LD25, LD50 and LD75 • Planting of M1 generation at NKTB field plots on marginal land (Bris soil) in 2 locations: Pahang & Terengganu	• Selection of targeted traits such as high biomass yield and late-flowering characteristic in M2 – M3 generations	• Phenotypic selection in M4 – M5 generations • Evaluation of nutrient and water use efficiency by means of isotopic technique (N-15) and effects of nutrient package (biofertilizer + oligochitosan) on enhancement of biomass yield in M6 generation • Multi-location trials and stability test of selected potential mutant lines in M7 generation (2 cycles)

Experimental approach



Project Activities

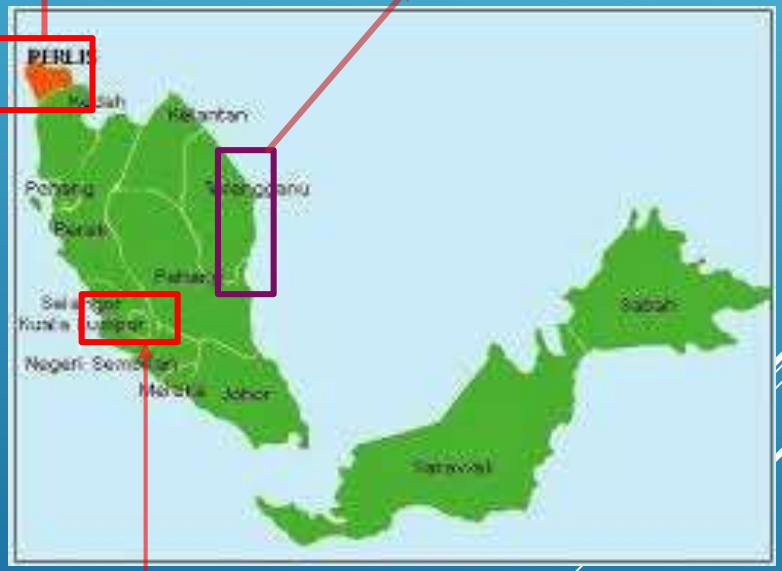
Activities	2015				2016												2017												2018							
	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
1. Irradiation of Kenaf seeds variety V36 with acute and chronic gamma irradiation for induction of mutation																																				
2. Field planting and data collection of M1 irradiated seeds at LKTN field plots: Perlis																																				
3. Harvesting of M1 and land preparation for planting of M2 generation																																				
4. Field planting of M2 plants and screening for late flowering and high yield																																				
5. Harvesting of selected M2 plants with late flowering and high yield and land preparation for planting of M3 generation																																				
6. Effects of biofertilizer and oligochitosan on yield performance of selected M3 mutant lines																																				

Activities	2015	2016	2017	2018
7. Harvesting of selected M3 lines treated with biofertilizer and oligochotosan and land preparation for planting of M4 generation				
8. Nutrient and water use efficiency of selected M4 mutant lines by means of isotopic technique/screening on marginal lands (bris)				
9. Harvesting of selected M4 mutant lines with water-use efficiency and land preparation for planting of advanced lines in M5				
10. Planting of selected high yield and late-flowering mutant lines in M5 generation using recommended biofertilizer and oligochitosan package				
11. Farmers Field Day for field demonstration of promising mutant lines and preparation of Final Report for Project Completion				

FIELD PLOT LOCATIONS (BESERI AND TITI TINGGI) IN THE STATE OF PERLIS

Existing plots

Proposed future plot – Bris coastal areas



NUCLEAR MALAYSIA

MUTATION BREEDING

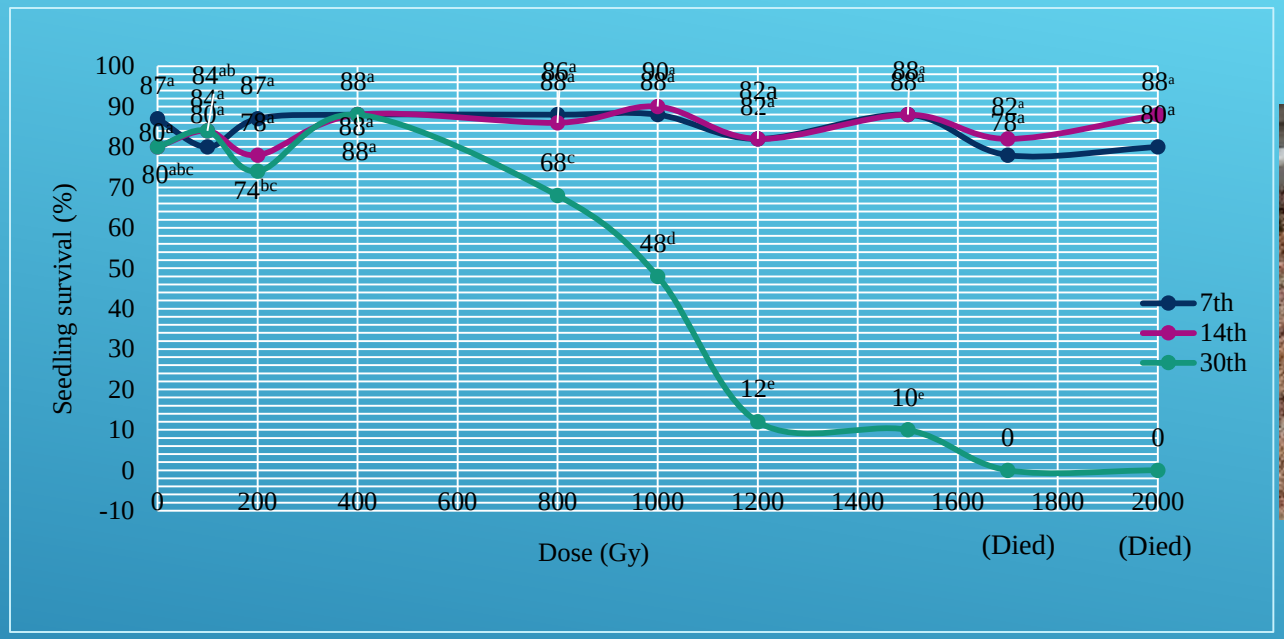
Acute Gamma Irradiation

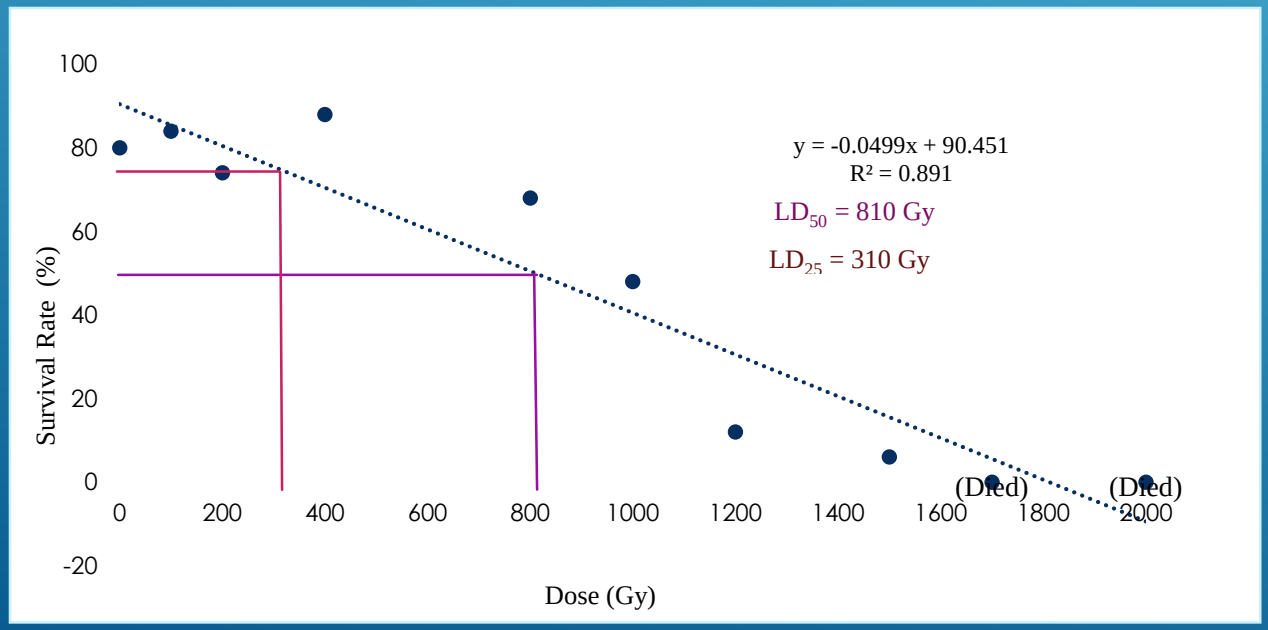
Biobeam Gamma Cell – acute gamma

Chronic gamma irradiation

Treatment	Ring Number	Dose Rate (Gy/h)
1	3	0.3
2	4	0.17
3	5	0.11
4	7	0.05
5	9	0.03
6	11	0.02
7	Control	0 Gy

Radiosensitivity test for acute gamma irradiation





Planting of M1at Beseri and Titi Tinggi (22 Oct 2015)

Liming process

Seed planting using seeder

Fertilizer application

Observation and data collection

Doses; 0, 100, 200, 300, 800, 1300

Harvesting of M1 population – Beseri Plot (18 Feb 2016)

Briefing before harvesting

Seed harvesting

Collection of seeds

Seeds were dried to 10-12% moisture content before storage and planting

Seed moisture content after harvesting

Seed moisture content after drying

Seed drying facility

Agronomic data collection on irradiated kenaf

Harvesting of M1 – Titi Tinggi (10 Mar 2016)

Titi Tinggi plot

Seed harvester

Sorting out plants for data collection

Project members

Plantings of M2 (29 Mar 2016) & M3 (13 Feb 2017)populations

Using seeder for unselected seeds

Manual planting for selected M2 promising mutant lines

Plot labelling according to dose and treatment

Harvesting and Data recording on M2 plants (5 Sept 2016)

Recorded Characters;

- Plant height
- Number of pods per plant
- Number of nodes per plant
- Internode length
- Stem girth
- Number of seeds per pod
- Seed weight
- Fiber weight (fresh/dry)

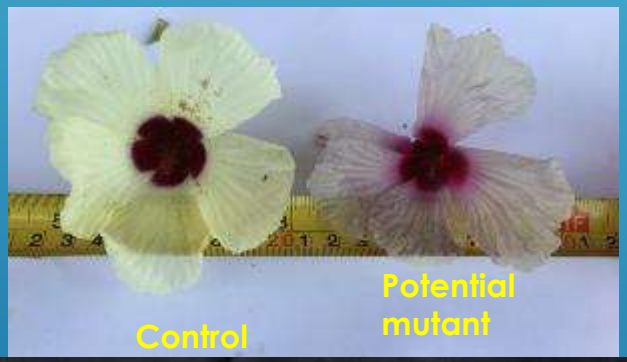
Promising mutants – higher biomass (bast fiber), different flower colour, leaf shape

Mutant lines	Generation	Dose (Gy)	Fresh weight (g)	Fresh Weight increase over Control variety (%)	Dry weight (g)	Increment Dry Weight over control variety (%)
M2P1	M2	200 Gy	478	143.00	124	154.36
M2P2	M2	200 Gy	298	51.65	69	41.54
M2P3	M2	200 Gy	784	298.98	44	-9.74
M2P4	M2	300 Gy	386	96.44	94	92.82
M2P5	M2	300 Gy	262	33.33	72	47.69
M2P6 leaf-palmate	M2	300 Gy	180	-8.40	45	-7.69
M2P7 (flower - yellow)	M2	300 Gy	144	-26.72	32	-34.36
M2P8	M2	300 Gy	500	154.45	97	98.97
M2P9 leaf – palmate	M2	300 Gy	172	-12.47	43	-11.79
M2P10	M2	300 Gy	306	55.73	70	43.59
M2P11	M2	300 Gy	296	50.64	77	57.95
M2P12	M2	300 Gy	258	31.30	56	14.87
M2P13	M2	300 Gy	304	54.71	75	53.85
M2P14 (flower-purplish	M2	300 Gy	190	-3.31	45	-7.69
M2P15	M2	300 Gy	206	4.83	49	0.51
M2P16	M2	300 Gy	290	47.58	65	33.33
V 36 Control	M2		196.5	-	48.75	-

Maintenance of promising mutant lines at Nuclear Malaysia's shade house

Major Outputs (Mutation Breeding)

1. Data on optimum dose for acute and chronic gamma irradiation (LD 50 ~800 and LD25 ~300 Gy for acute gamma; LD25 ~190 Gy (at ring 3 or 0.3 Gy /jam)
2. Twelve potential mutant lines (for biomass/high bast fiber) and two for flower morphology and 2 for leaf variation (palmate)
3. MSc student (UPM)
4. Publications




Palmate leaf (right)

Glasshouse screening of M2 seeds on low nutrient, ex-mining soils

- A glasshouse-scale experiment was also conducted to assess and select mutants that can grow on low nutrient soils. In this experiment, representatives of
- M2 seeds from the doses of 0, 300, 800 and 1300 Gy have been planted on troughs containing ex-mining sands (pH 8, N = 0 and P = 7.2 ppm) to pre-screen irradiated plants that can survive and grow well on this type of soil before further evaluation on marginal areas in the future.
- This experiment is still ongoing.

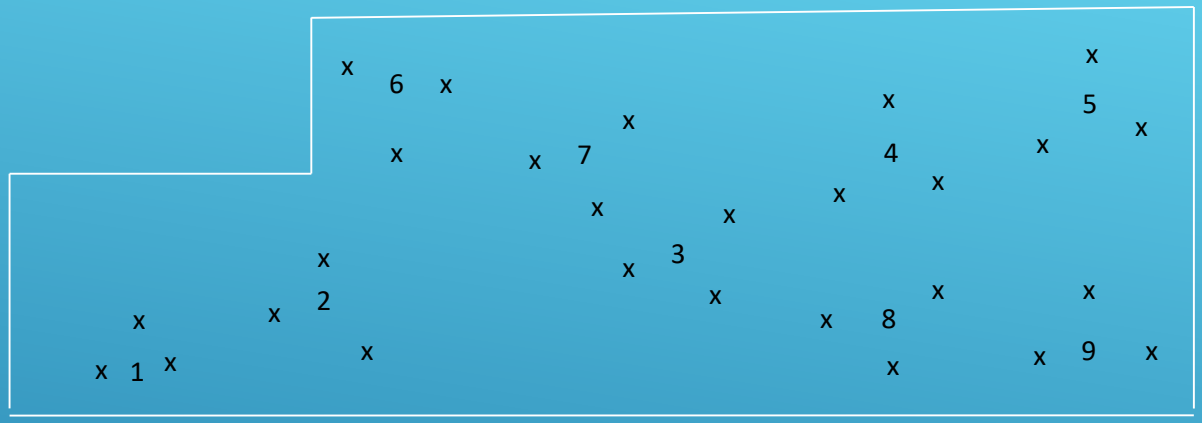
SOIL, WATER AND NUTRIENT STUDIES

Soil Analysis

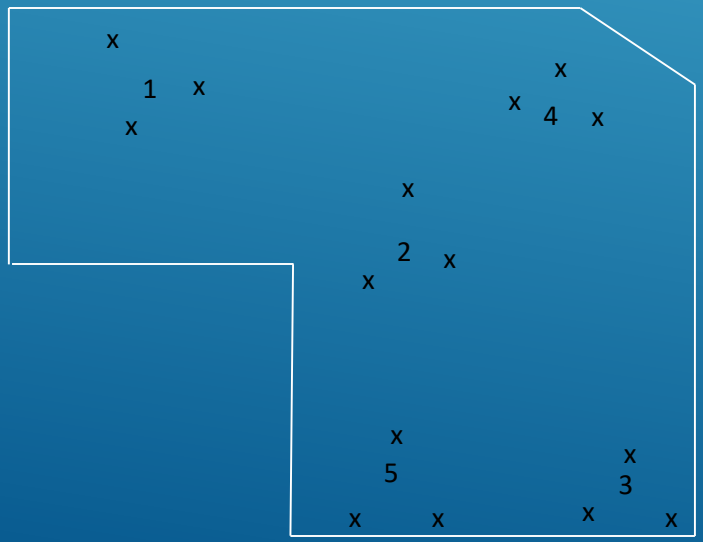
- Soil sample were taken at several points by random sampling in Beseri (1 acre) and Titi Tinggi (1 hectare) kenaf (*Hibiscus cannabinus*) experimental plot.
- At each point, about 2 soil depths were taken (10 cm and 20 cm depth) using auger at 3 random areas surrounding it
- Mixed the soil in one plastic bag.
- Moisture content also was taken at each point using soil moisture meter

* Soil types: Sandy clay loam; problem: poor drainage system

Beseri Plot



Titi Tinggi Plot



- Air dried the sample for 1 week.
- Grinding and sieving(sieve size : 0.2 cm) the soil sample
- Analysis N, P, K, and pH using soil nutrient tester model: TPY-6A.

Nutrient Efficiency Studies

Plant: Selected Kenaf (5 potential mutant lines) : S1 (M2P1), S2 (M2P4), S3 (M2P5), S4 (M2P10) and S5 (M2P11)

*Note: S4 (M2P10) showed very low germination, therefore was not counted in the analysis

Replications: 3 replicates

Location: Beseri Plot, Perlis

Treatments:

- Farmer practices (NPK Green, blue) + Oligochitosan + ^{15}N Urea 3% a.e; 20 kg N/ha **(FP)**
- Biofertilizer + Oligochotosan + ^{15}N Urea 3% a.e; 20 kg N/ha **(BO)**
- ^{15}N Urea 3% a.e; 20 kg N/ha **(C)**
- Farmer practices (NPK Green, blue) + Biofertilizer + Oligochitosan + ^{15}N Urea 3% a.e; 20 kg N/ha **(FBO)**

Treatments Application:

- NPK green was applied on 10 DAS with oligochitosan and ^{15}N (10 kg N/ha). NPK Blue on 30 DAS with oligochitosan and ^{15}N (10 kg N/ha).
- Biofertilizer (1st application) on 10 DAS with oligochitosan and ^{15}N (10 kg N/ha). Biofertilizer (2nd application) on 30 DAS with oligochitosan and ^{15}N (10 kg N/ha).
- ^{15}N (10 kg N/ha) on 10 DAS and ^{15}N (10 kg N/ha) aon30 DAS.
- NPK green on10 DAS with biofertilizer, oligochitosan and ^{15}N (10 kg N/ha). NPK Blue on30 DAS with biofertilizer, oligochitosan and ^{15}N (10 kg N/ha).

Data observation:

1. Plant height
2. Leaf colour index
3. Pod per tree
4. Length of leaf
5. Width of leaf
6. Fresh weight
7. Dry weight
8. Total N
9. ^{15}N a.e
10. Ndfa
11. Nitrogen uptake

EXPERIMENTAL DESIGN

Average Plant Height (cm) per Individual Mutant Population									Average Height (cm) per Treatment
	S1		S2		S3		S5		All mutants
Fertilizer Treatment	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	
FP	194.5	6.94	193.5	8.66	197.83	16.75	157.17	16.64	185.75
BO	181	9.20	184.5	6.41	216.33	5.34	201.33	7.16	195.80
C	184	9.98	193.5	9.92	193.5	8.63	171.0	7.44	185.50
FBO	205.67	6.97	192.67	8.52	192.67	7.88	207.67	19.48	199.67

Average Leaf Length (cm) per Individual Mutant Population									Average Leaf length (cm) per Treatment
	S1		S2		S3		S5		All mutants
Fertilizer Treatment	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	
FP	14.92	0.86	13.67	0.33	12.67	0.67	13.25	1.09	13.62
BO	13.83	0.87	14.83	0.48	13.17	0.95	13.67	0.67	13.87
C	14.08	0.71	15.0	0.58	12.83	0.48	13.0	1.06	13.73
FBO	15.42	0.55	13.5	0.67	13.83	0.65	13.92	0.80	14.17

Average Leaf Width (cm) per Individual Mutant Population									Average Leaf Width (cm) per Treatment
	S1		S2		S3		S5		All mutants
Fertilizer Treatment	Mean	Std Error	Mean	Std Error	Mean	Std Error	Mean	Std Error	
FP	10.92	0.71	10.42	0.42	9.83	0.48	9.08	0.82	10.06
BO	10.0	0.58	9.17	0.31	10.92	0.42	10.5	0.5	10.15
C	9.83	0.40	11.67	1.17	9.5	0.62	8.33	0.76	9.83
FBO	11.5	0.34	9.5	0.76	10.33	0.61	11.33	1.20	10.67

Generally, Farmer practices (NPK Green, blue) + Biofertilizer + Oligochitosan + ^{15}N Urea 3% a.e; 20 kg N/ha (FBO) was the best treatment for generating plants with higher biomass

Publication

3. Publications - 2016

Zaiton Ahmad, Faiz Ahmad, Mohd Zulmadi Sani, Mustapha Akil, Affrida Abu Hassan and Mohammad Nazri Romli (2016). Gamma-radiation induced mutagenesis for the production of new varieties of Kenaf (*Hibiscus cannabinus* L.) with high fiber and late-flowering characteristics. Transactions of Persatuan Genetik Malaysia Number 3, ms 171-174 (ISBN 978-983-2408-47-5)

Faiz Ahmad, Mohd Zulmadi Sani, Mustapha Akil, Zaiton Ahmad, Affrida Abu Hassan, Abdul Rahim Harun (2016). Effect of Gamma Irradiation on Morphological Traits in M1 Generation of Kenaf (Hibiscus cannabinus L.) V36 Variety. Seminar R&D Nuklear Malaysia , 8-10 Nov 2016, Bangi Selangor.

Mohd Zulmadi Sani, Faiz Ahmad, Mustapha Akil, Zaiton Ahmad, Affrida Abu Hasan, Abdul Rahim Harun (2016). Effect of chronic gamma irradiation on kenaf (*Hibiscus cannabinus*. L) v36 variety. Seminar R&D Nuklear Malaysia , 8-10 Nov 2016, Bangi Selangor.

Mustapha Akil, Faiz Ahmad, Mohd Zulmadi Sani, Mohamad Azizi Mohd Saliman, Zaiton Ahmad, Affrida Abu Hassan, Abdul Rahim Harun (2016). Effects of acute gamma irradiation on the growth of kenaf *(Hibiscus cannabinus* L.) V36 seedlings under controlled environment. Seminar R&D Nuklear Malaysia , 8-10 Nov 2016, Bangi Selangor.

Expected: 2017

1. Zulmadi et al. GAMMA-RADIATION MUTAGENESIS OF *Hibiscus cannabinus* L. V36 VARIETY: RADIOSENSITIVITY STUDY, PHENOTYPIC CHARACTERIZATION AND MULTIVARIATE ANALYSIS TO EXPLAIN VARIATION AMONG SELECTED M_1 PROGENIES (Jurnal Sains Nuklear Malaysia, 29(2)) – accepted for publication
2. Faiz et al. (Internation Plant Genetic Conference)
3. Mustapha et al. (Internation Plant Genetic Conference)
4. Ahmad Nazrul et al. (water and nutrient studies)

FUTURE WORK

1) **Screening of kenaf potential mutants on other marginal land types**

   Especially on the coastal bris soil in the east of Malaysia (Terengganu or Pahang State) due to their low nutrient and organic matter content.

2) Nitrogen use efficiency study

   Stable isotope 15N labelled urea 5% atom percent will be used as tracer for nitrogen uptake of kenaf mutant lines on marginal land.

3) Screening of nutrient and water use efficiency using 2 water stress treatments at 45% and 75% field capacity (carbon isotope discrimination).

4) Field demonstration (Farmer's Field Day) and dissemination of mutant seeds as well as Guideline/Protocol on Application of Biofertilizer and Oligochitosan for yield improvement of kenaf to farmers under National Kenaf and Tobacco Board.

Thank you

Terima Kasih