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In Collaboration

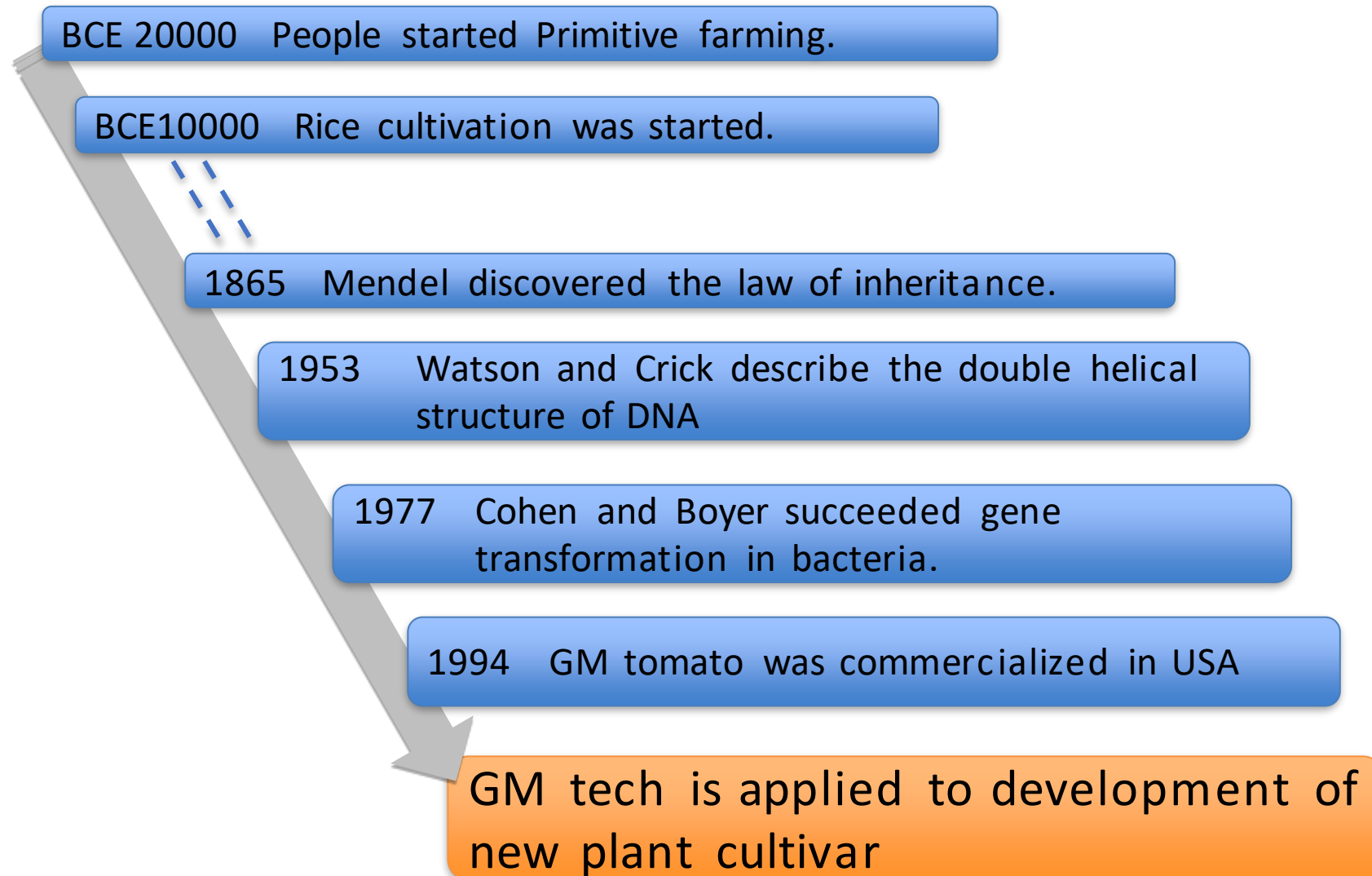
University of Miyazaki Japan 2012-2017 , 2017-2022 (**Prof. Ryo Akashi, Takahiro Gondo, PhD., Genki Ishigaki PhD.**)

Crop Mark Seed Company New Zealand 2017-2023 (**Dr. Tim Cookson**)

Badan Tenaga Atom Nasional Indonesia (**Dr. Teguh Wahyono**)

1

Timeline to apply GM tech in plant breeding



Advantages of genetic modification

- Possible to produce desired crops **without undesirables**.
- **Faster** than conventional breeding.

(Desired GM organisms can be bred in one generation.)

- Possible to introduce genes either **from distant species**, even bacteria.



6

GM crops



Flavor Saver™



Herbicide-resistant soybean



Bt corn



Blue Rose



Golden rice

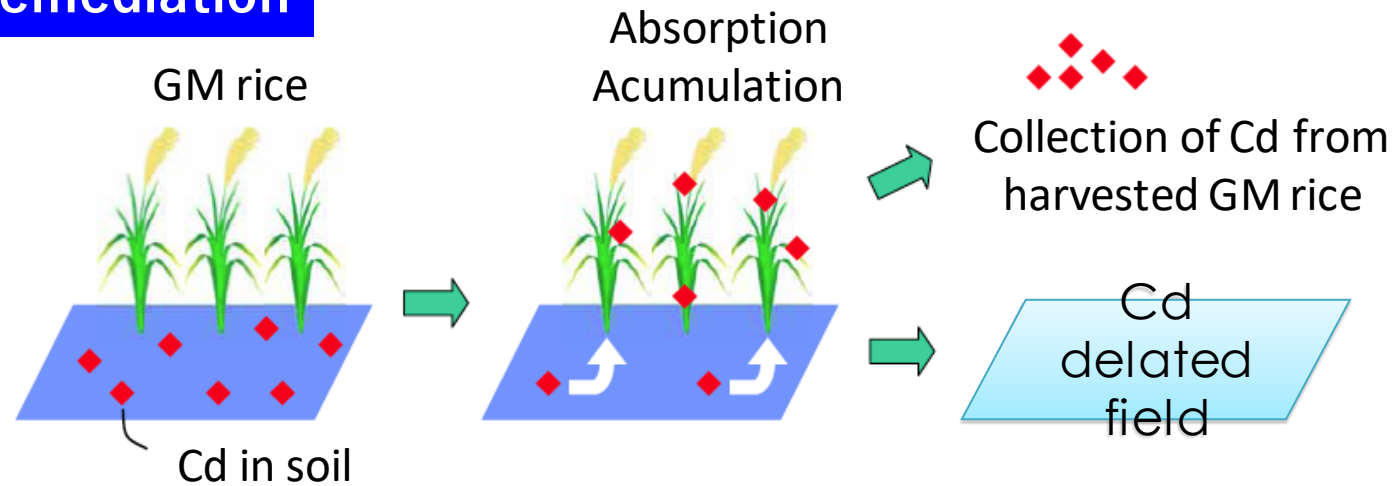


Pollen allergosis-mitigating rice

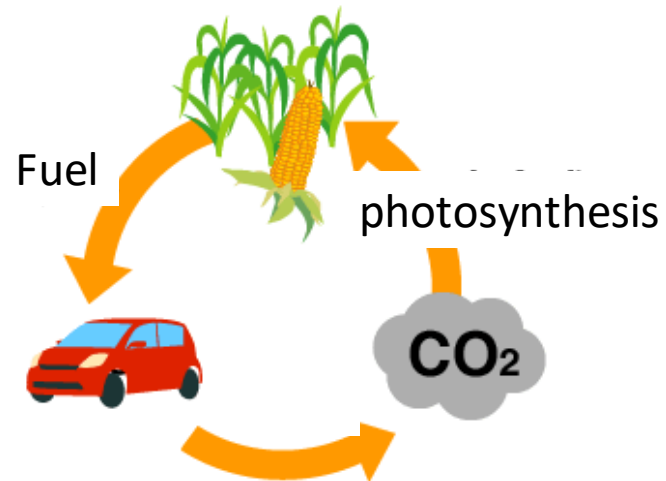
GM crops are developed not only herbicide and insect resistant crops, but also high functional crops, now.

7 New GM crops

Phytoremediation

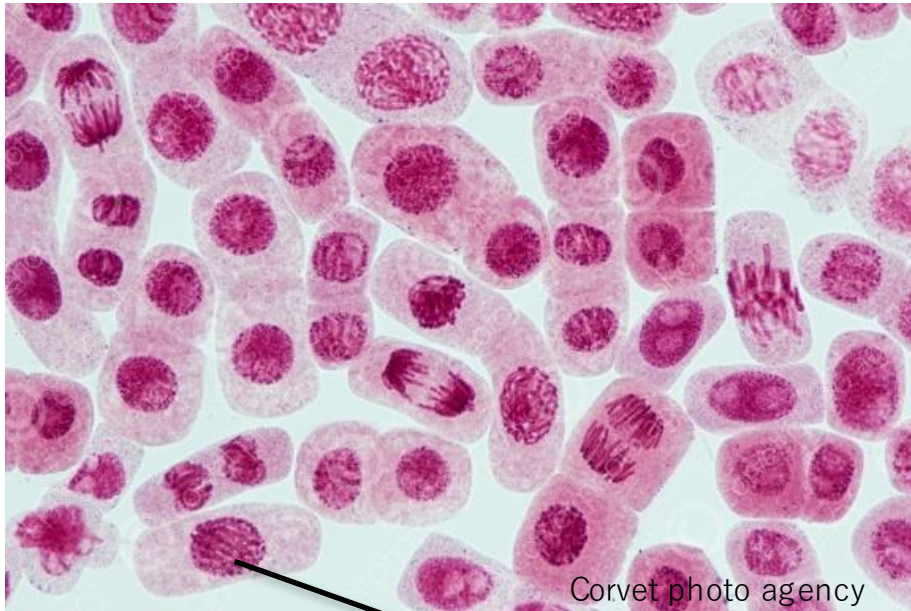


Energy



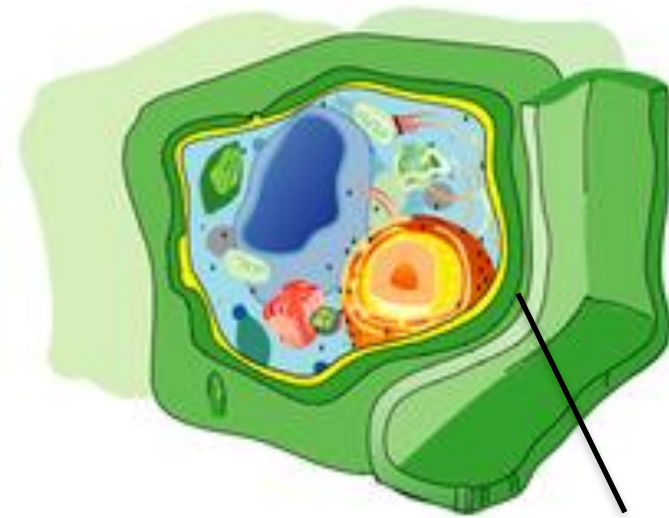
<http://manieraforista.blog67.fc2.com/blog-entry->

Plant cell and genetic transformation



Corvet photo agency

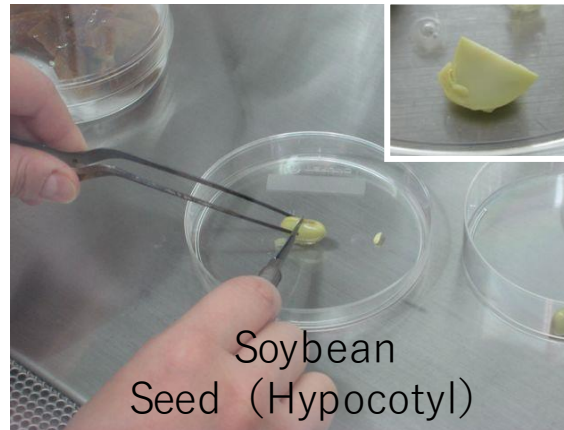
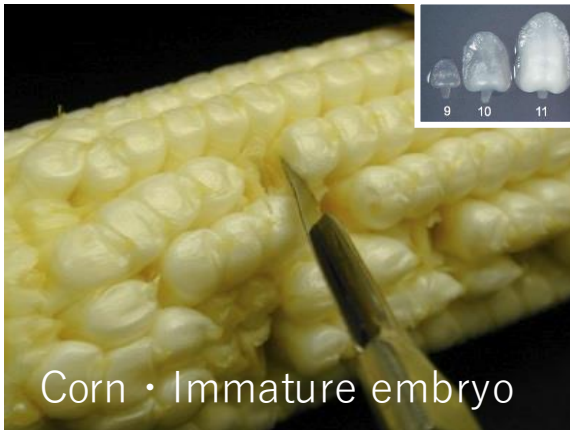
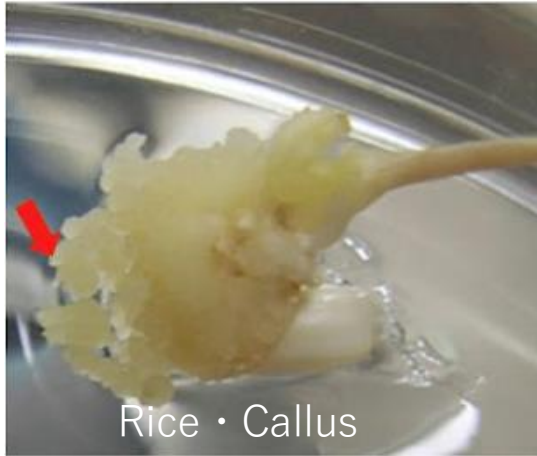
Nucleus (Chromosome)



Cell Wall

Plant transformation is difficult for cell wall.

Target tissues for transformation



Protocols for plant transformation (Kagakudojin publishing)

It depend on plant species for transformation.

11 Plant research by using gene transformation

1. Genetic functional analysis by using model plants



Rice



Arabidopsis



Lotus



Tomato

Analyze the function of novel genes.

2. Application of crops from model plants



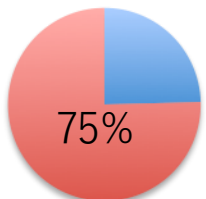
Maize
Wheat
Forage



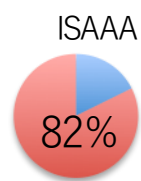
Soybean
Common bean
chick - pea

Confirm the utility of novel gene by introducing it to crops.

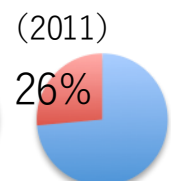
3. The estimation of safety of GM Crops



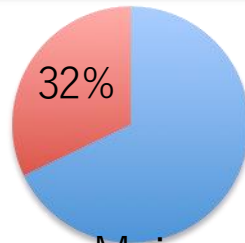
Soybean
10,000



Cotton
3,000



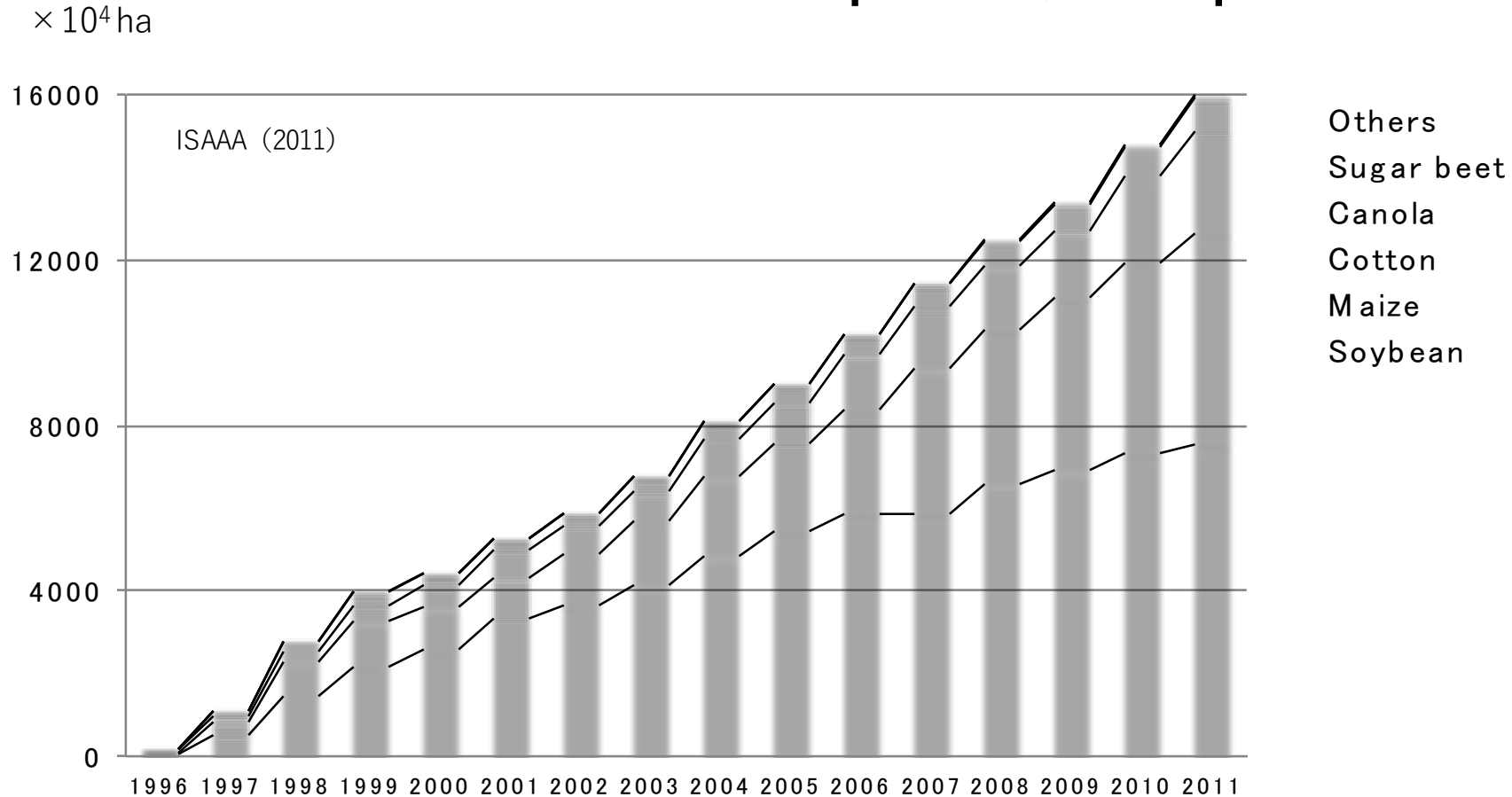
Rape
3,100



Maize
15,900 × 10⁴ ha

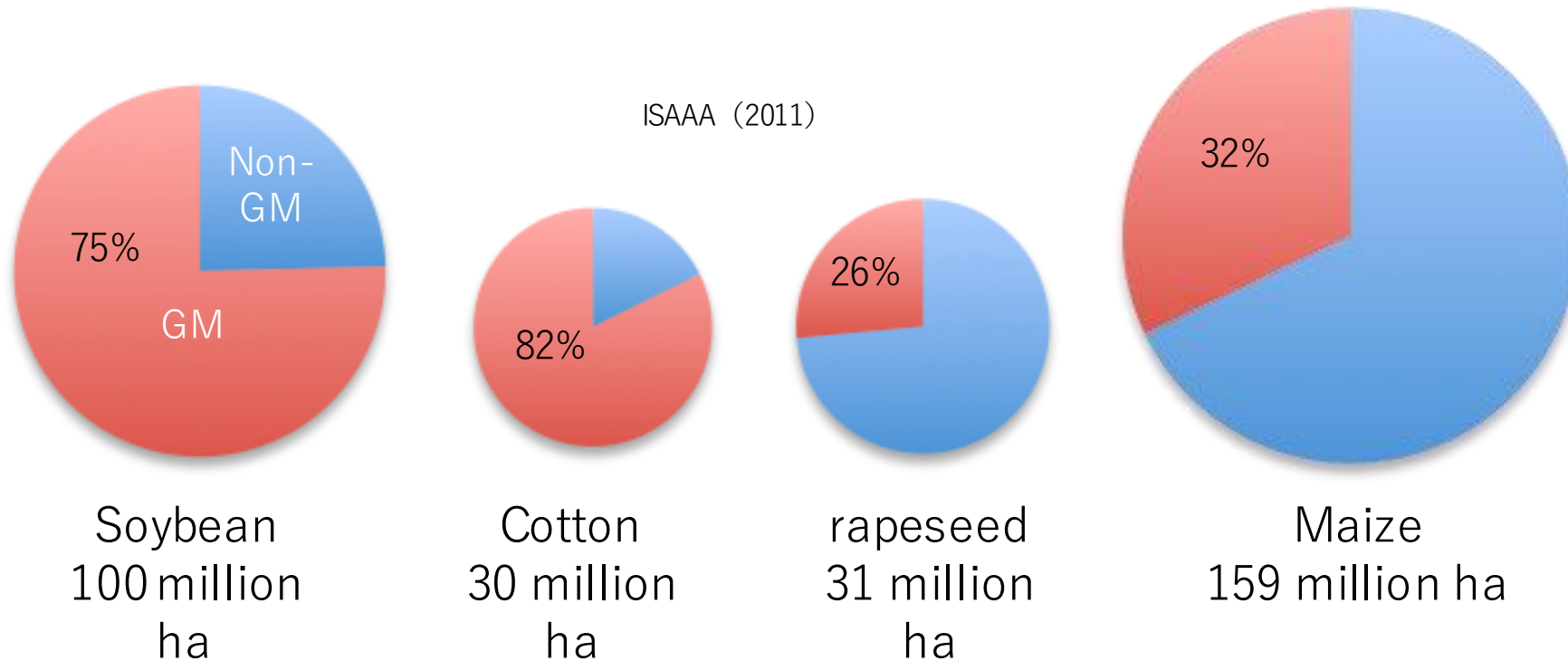
Study the biological diversity risk assessment and food safety in Japan.

Global area of GM crops (Crops)



Global area of GM crops is 160 million ha in 2011. This area is 12 % of global area of cultivation.

The area ratio of GM crops

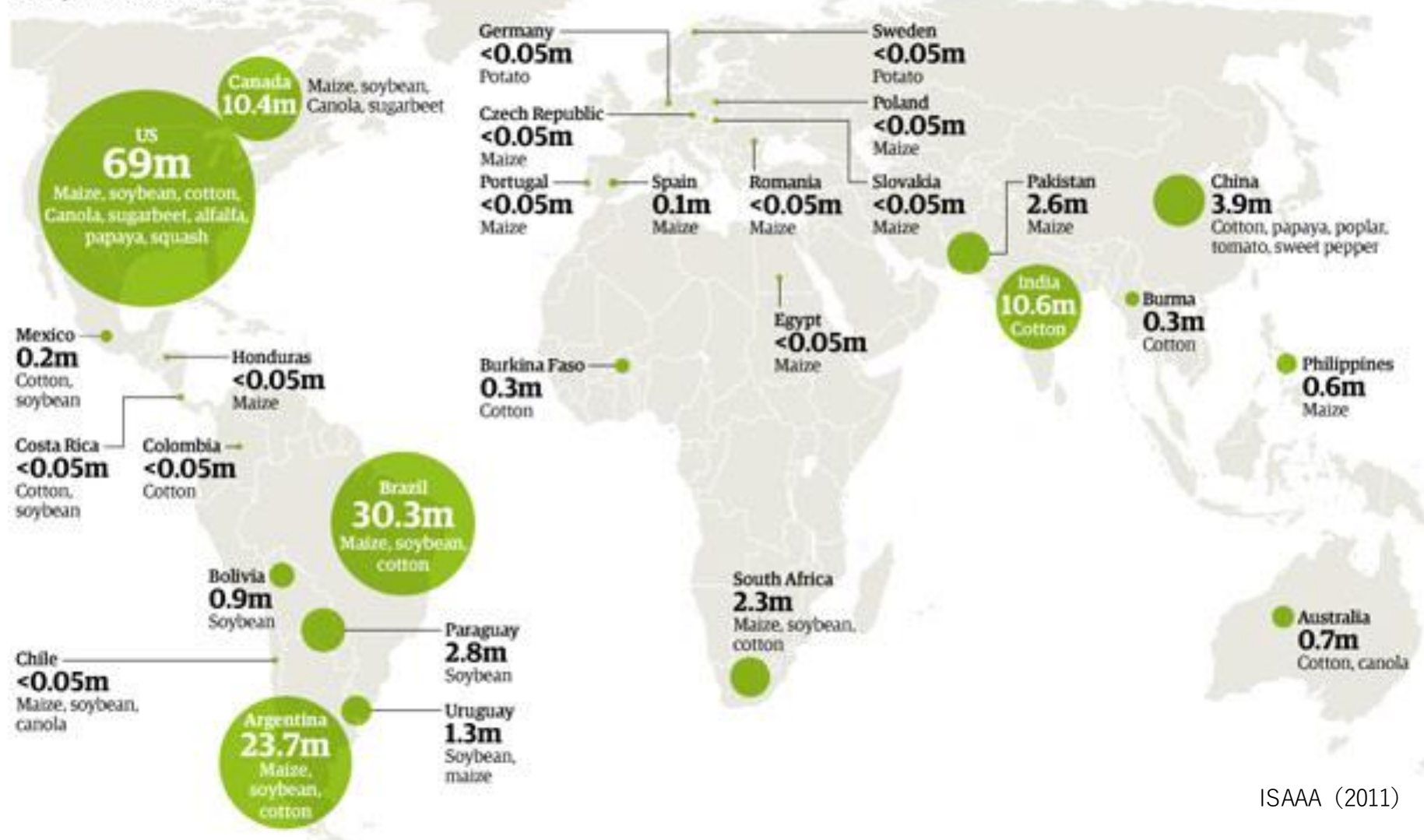


Soybean and cotton is almost GM. In 2006, the area ratio of GM crops are soybean (63%), cotton (38%), rapeseed (17%), maize (17%), the ratio increase year by year.

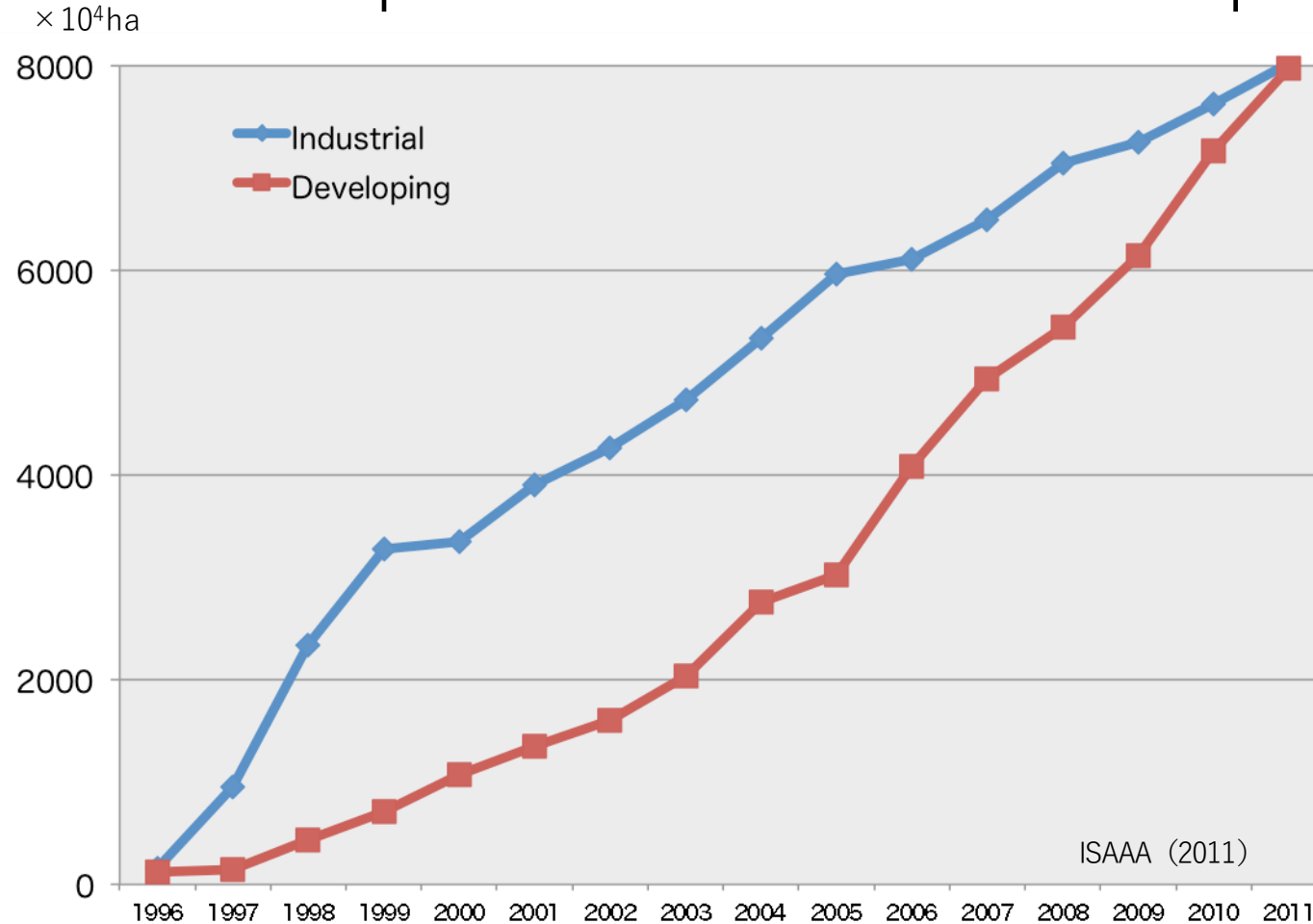
Global area of GM crops (Country)

Global status of commercial GM crops

2011, by millions of hectares

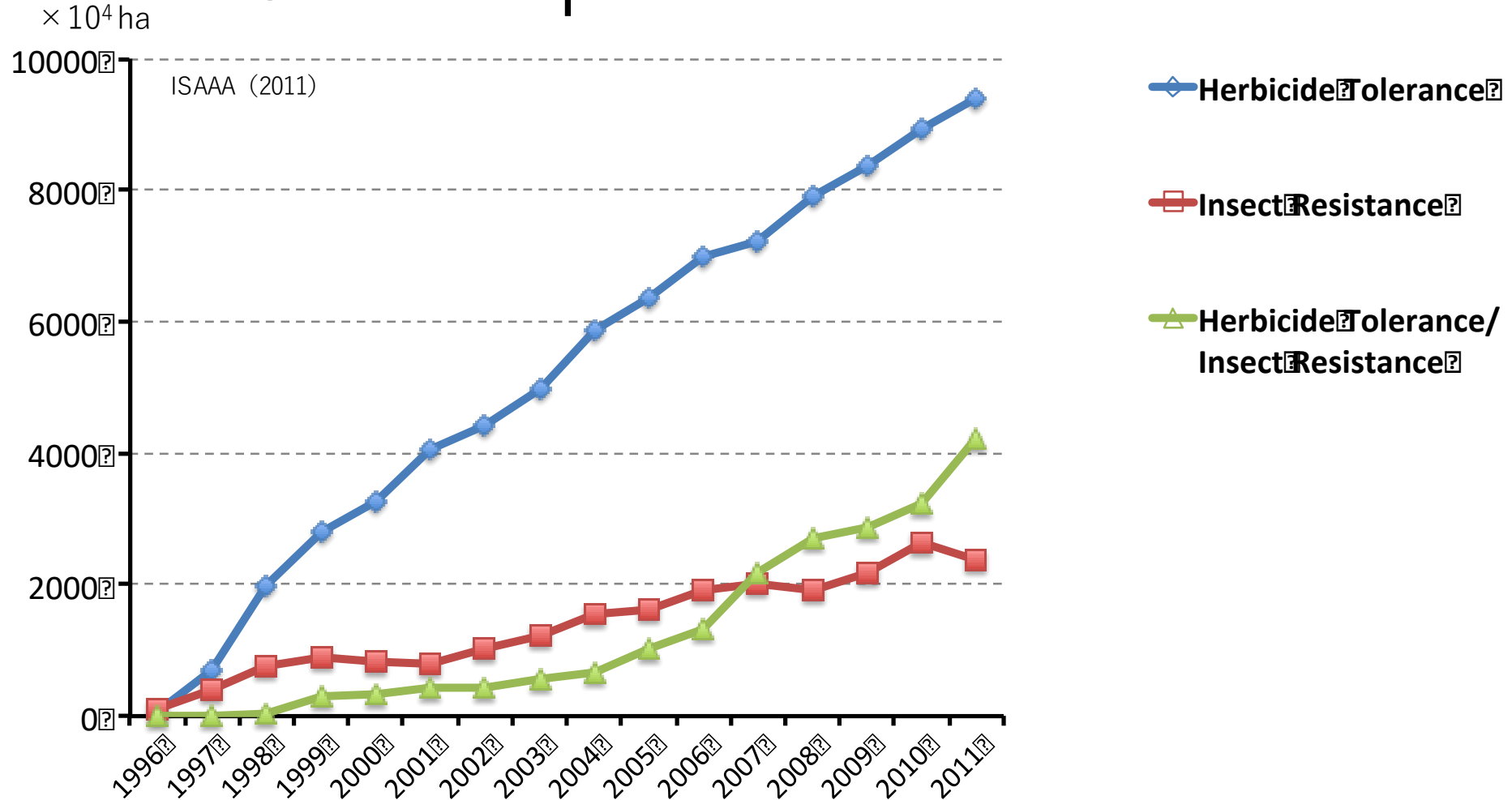


Global area of GM crops in industrial and developing country



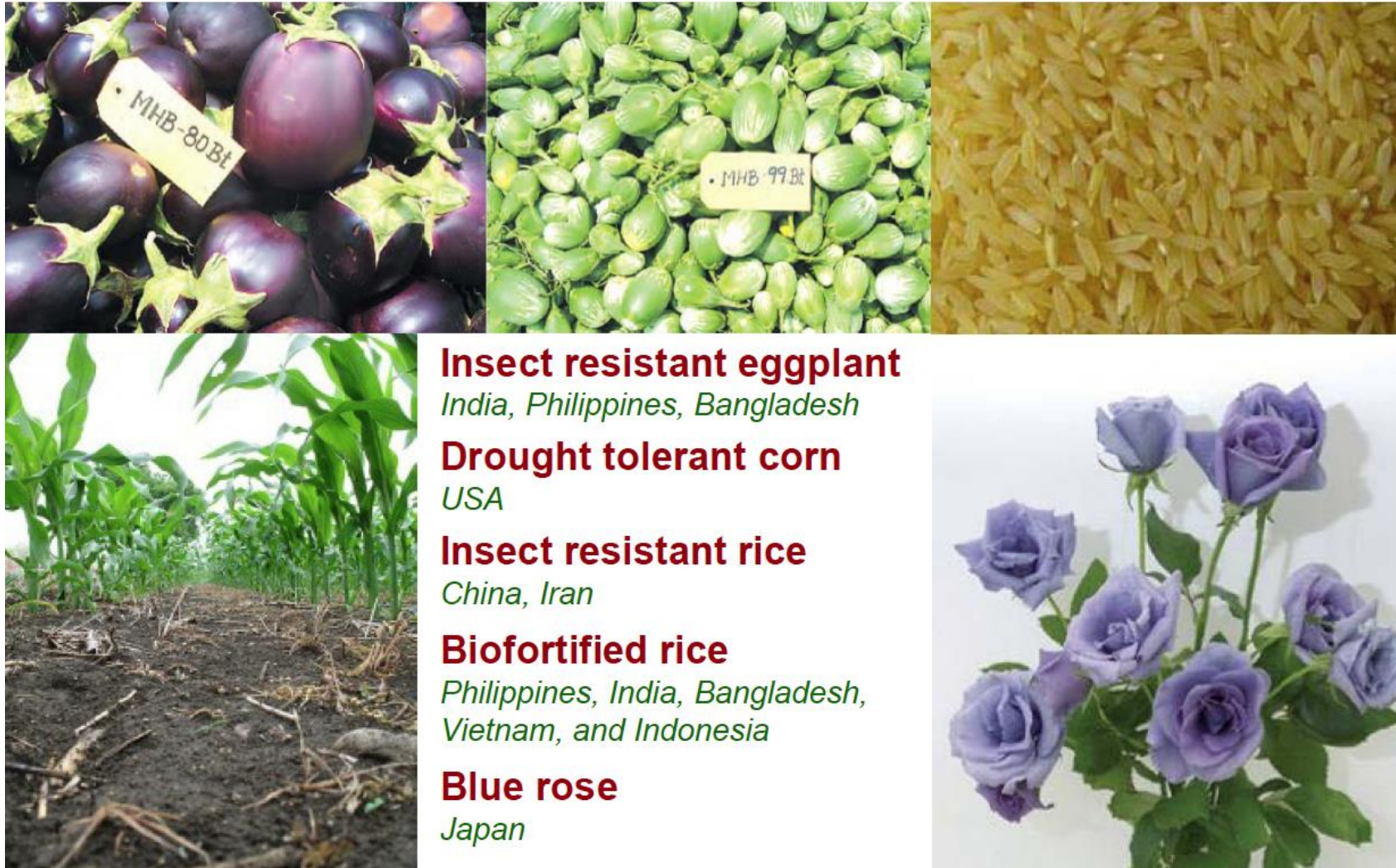
Recently, global area of GM crops of developing country is increased and became to same area in 2011.

Traits of GM crops



GM crops with herbicide tolerance and insect resistance traits increase the area in recent years.

17 GM crops nearing commercialization



Source: International Service for the Acquisition of Agri-biotech Applications (ISAAA)(2009)



Fence



Washing space



Water tank



Urgent water tank



Growing test of herbicide and insect resistance GM cotton

Application of GM Tech to Warm-season Grasses

Characteristic of temperate grass and warm season grass

	Temperate grass	Warm season grass
Origin	Temperate regions	Tropical – Subtropical regions
Optimum temperature	15 - 21°C	25°C -
Photosynthesis	C ₃ pathway	C ₄ pathway
Yield	Low - Middle	Middle - High
Quality	Middle - High	Low - Middle

Overcome the breeding barrier in warm season grass

Mode of reproduction	Limitation of cross breeding	Overcome the breeding barrier
• Cross pollination • Apomixis • Vegetative propagation	• Difficult to genetic fixation • Impossible to cross breeding	• Somaclonal variation via tissue culture • Cell fusion by using protoplast • Genetic engineering

Plant regeneration from seed-derived embryogenic callus and cell suspension cultures of bahiagrass (*Paspalum notatum*)

Ryo Akashi, Akihiko Hashimoto and Taiji Adachi

Applied Genetics and Biotechnology Division, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21 (Japan)

(Received December 21st, 1992; accepted January 12th, 1993)

We have established a high-frequency plant regeneration system via somatic embryogenesis from seed-derived callus and cell suspension cultures in 6 genotypes of bahiagrass (*Paspalum notatum*). Embryogenic callus was initiated from mature seeds on Murashige and Skoog (MS) medium supplemented with 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 3.0% sucrose and 0.3% Gellan Gum in the dark. Culture response was found to be correlated with genotype. 'Pensacola' had the best response in embryogenic callus formation, and 74% of the calli regenerated plants. Cell suspension cultures were established from embryogenic callus of 'Pensacola' in modified N₆ medium containing 1.0 mg/l 2,4-D. The suspension was composed of compact cell clusters. When smaller clusters (approx. 2.0–3.3 mm in diameter) were transferred to a solid MS medium with 3.0% sucrose but without hormones, plant regeneration was initiated at high frequency (28.6%). Morphological evidence is provided that regeneration of suspension cells occurred via embryogenesis. Regenerated plants were established to soil after culture in water at room temperature for 14 days for acclimatization.

Table II. Formation of embryogenic callus and plant regeneration in six genotypes of bahiagrass.

Genotype	No. of inoculated seeds	No. of calli formed (%)	No. of embryogenic calli formed (%)	Differentiation ^a (%)	Embryogenic ^b callus growth
Pensacola	251	87 (37.7)	35 (40.3)	74.0	+++
Nanpu	223	98 (43.9)	26 (26.5)	7.5	+++
Nanou	168	34 (20.2)	5 (14.7)	10.0	+
Common	225	43 (19.1)	14 (32.6)	0.0	++
Competider	196	59 (30.1)	24 (40.1)	27.3	+++
Tifton 54	250	121 (48.8)	15 (12.4)	20.0	++

^aFrequency of embryogenic callus, characterized by somatic embryo, green nodular spot and plantlet formation on hormone-free medium.

^b+, poor; ++, moderate; +++, strong.

Table III. The effect of hormonal pretreatment on differentiation of cell clusters of bahiagrass cv. 'Pensacola' on hormone-free medium.

NAA (mg/l)	BAP (mg/l)	No. of cell clusters treated	No. of cell clusters with green nodular spots (%)	No. of plants producing calli (%)	Total no. of regenerated plants
0.0	0.0	98	84 (85.7)	24 (28.6)	33
0.01	0.1	84	53 (63.1)	4 (7.5)	4
0.01	0.5	63	46 (73.0)	9 (19.6)	11
0.01	1.0	63	39 (61.9)	7 (17.9)	12
0.1	0.5	63	48 (76.2)	4 (8.3)	7
0.1	2.0	63	41 (65.1)	10 (24.4)	12

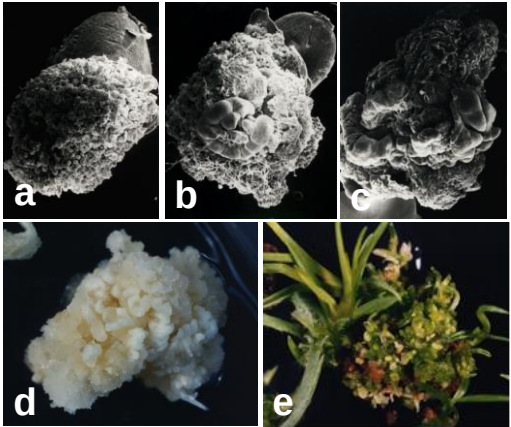


Fig. 1. Developmental stages of seed-derived embryogenic callus and plant regeneration in *Paspalum notatum* cv. 'Pensacola'. (a), (b) and (c) S.E.M. of compact callus and somatic embryo formation (*) after 10 (a), 15 (b) and 25 (c) days of culture. Bars = 1 mm. (d) Embryogenic callus. Bar = 2.5 mm. (e) Germination of somatic embryos and plant regeneration. Bar = 5 mm.

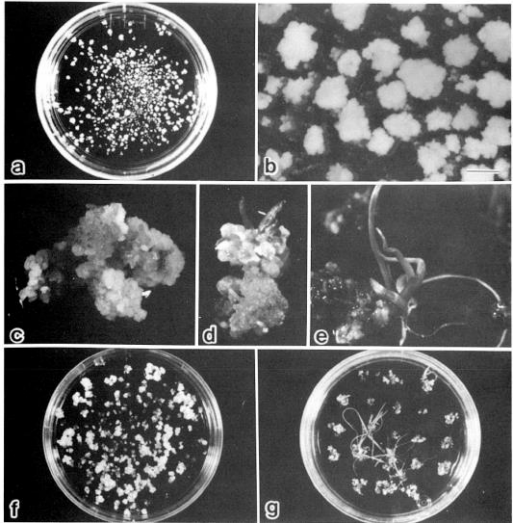


Fig. 2. Establishment of cell suspension cultures of *Paspalum notatum* cv. 'Pensacola'. (a) and (b) Typical suspension culture 3 months past initiation. Bar = 2 mm. (c) Embryogenic callus formation from suspension cells after 1 month on the solid medium. (d) and (e) Germination of somatic embryos and plant regeneration. (f) and (g) Callus formation and plant regeneration as achieved through plating method on solid medium.

Improvement of Forage Quality in Bahiagrass (*Paspalum notatum* Flüge) through Genetic Manipulation.

1. Detection of somaclonal variation using RAPD analysis in plants regenerated from suspension cultures

Synopsis

AKASHI, R. and O. KAWAMURA (1998): Improvement of forage quality in bahiagrass (*Paspalum notatum* Flüge) through genetic manipulation. 1. Detection of somaclonal variation using RAPD analysis in plants regenerated from suspension cultures. *Grassland Science* 44, 203-207.

Genetic changes in regenerated plants, caused by the tissue culture process (somaclonal variation), have become a possible means of improvement for many plant species including bahiagrass (*Paspalum notatum*). The objective of this study is to investigate genetic variation of random amplified polymorphic DNA (RAPD) markers in primary plants regenerated by an embryogenic suspension culture. RAPD markers, ten different short (decamer) oligonucleotide primers of arbitrary nucleotide sequence in combination with polymerase chain reaction (PCR) were used, did not reveal any preexisting polymorphisms in control plant seedlings. However, in three out of 28 plants regenerated from embryogenic suspension cultures variation for at least one of the RAPD markers was found. This is clear evidence of tissue culture-derived, newly-generated variation in bahiagrass.

Grassland Science 49 (5) : 456-459 (2003)

Variations in Morphological and Feeding Characteristics in Plants Regenerated from Embryogenic Suspension Cultures Derived from a Single Genotype of Bahiagrass (*Paspalum notatum* Flüge)

Synopsis

SALENG, K., M. NASE, M. NIIMI, R. AKASHI and O. KAWAMURA (2003): Variations in morphological and feeding characteristics in plants regenerated from embryogenic suspension cultures derived from a single genotype of bahiagrass (*Paspalum notatum* Flüge). *Grassland Science* 49, 456-459.

We have investigated the extent of variation in morphological and feeding characteristics in plants regenerated from six-month-old embryogenic suspension cultures originated from a single genotype of bahiagrass (*Paspalum notatum* Flüge). Twenty-eight regenerated plants were randomly selected and planted individually in pots and grown outdoors. Five control plants, which were obtained from individual seeds, were grown under the same condition. Substantial phenotypic variation was observed among regenerants, whereas the control plants were reasonably stable. Large variation was observed in the number of tillers, ranging from 10 to 84. Regenerants were compared in six morphological traits, cell wall constituents, lignin and *in vitro*-digestibility. All regenerants had higher NDF contents than the control plants although there was only a small difference in dry matter and NDF digestibility between regenerants and control plants. In this study, regenerated plants showed different morphological and feeding characteristics, and *in vitro*-digestibilities were negatively correlated with plant length (-0.56 , $p < 0.01$).

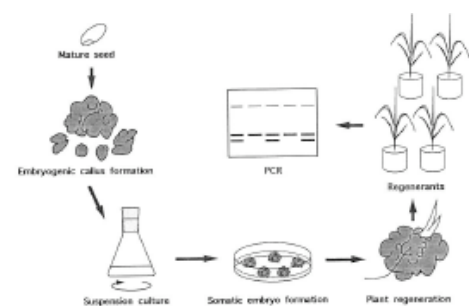


Fig. 1. Diagrammatic presentation of plant regeneration from embryogenic suspension culture of bahiagrass and screening of regenerated plants through PCR analysis.

Table 1. RAPD analysis for ten primers (OPA-01 to 10) of seedlings (control) and suspension culture-derived regenerants (R1 to 25, SCV1 and SCV2) of bahiagrass.

Plant	Number of RAPD ^{a)}									
	OPA-01	OPA-02	OPA-03	OPA-04	OPA-05	OPA-06	OPA-07	OPA-08	OPA-09	OPA-010
Control	4	2	6	8	5	5	6	8	7	8
R1-26 ^{b)}	4	2	6	8	5	5	6	8	7	8
SCV1	4	2	6	8	5	4	6	8	7	8
SCV2	4	2	6	8	5	4	6	8	7	8

^{a)} : Pattern of identical band number are also identical in position, indicating no variation.

^{b)} : Regenerants without detectable newly-generated variation.

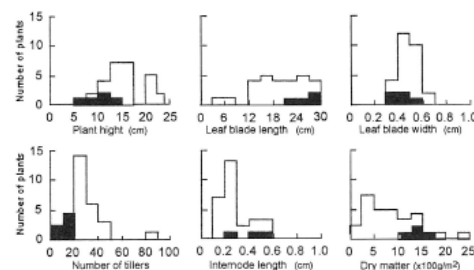


Fig. 1. Histograms of morphological characteristics and dry matter. ■ : Control plants, □ : Regenerants.

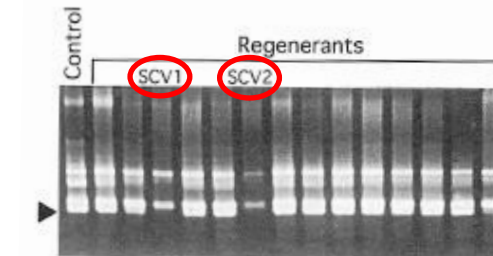


Fig. 4. RAPD pattern generated with the OPA-06 primer in plants regenerated from suspension culture. Fourteen out of 28 plants tested are shown. Two of the plants (SCV1 and 2) display OPA-6 pattern different from the control (arrowhead).

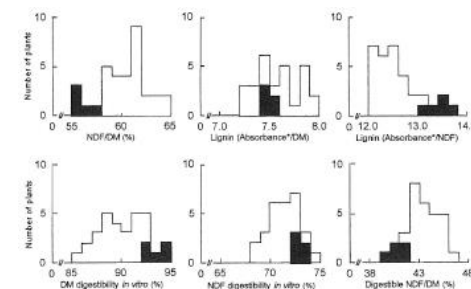


Fig. 2. Histograms of chemical composition and digestibility *in vitro*. ■ : Control plants, □ : Regenerants. * Morrison (1972).

Table 1. Correlation coefficients of morphological characteristics with digestibility and chemical composition in regenerated plants of bahiagrass.

	Plant length	Leaf blade length	Leaf blade width	Number of tiller	Internode length	DM
IVDMD	-0.56**	-0.24 NS	0.39*	0.18 NS	0.37*	-0.01 NS
IVNDFD	-0.41*	-0.10 NS	0.53**	0.40*	0.39*	-0.17 NS
NDF/DM	-0.06 NS	0.04 NS	-0.14 NS	-0.10 NS	0.03 NS	0.19 NS
Lignin/DM	-0.17 NS	-0.22 NS	-0.24 NS	-0.35 NS	0.09 NS	0.39*
Lignin/NDF	-0.11 NS	-0.14 NS	0.12 NS	0.01 NS	0.11 NS	-0.03 NS
Digestible NDF/DM	0.22 NS	0.09 NS	-0.39*	-0.28 NS	-0.27 NS	0.14 NS

IVDMD : *in vitro* dry matter digestibility ; IVNDFD : *in vitro* NDF digestibility.

NS : not significant ; * : $p < 0.05$; ** : $p < 0.01$.

Plant regeneration from suspension cultured-derived protoplasts of apomictic dallisgrass (*Paspalum dilatatum* Poir.)

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Applied Genetics and Biotechnology Division, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21 (Japan)

(Received August 13th, 1991; revision received November 25th 1991; accepted November 26th, 1991)

Protoplasts were isolated from embryogenic suspension cells of apomictic dallisgrass (*Paspalum dilatatum* Poir.). The respective suspension cultures were initiated from immature inflorescence-derived embryogenic callus. Previous to protoplast isolation, suspension cells were treated with Murashige and Skoog (MS) liquid medium without sucrose and hormones. Due to this pretreatment protoplast yield and viability were dramatically increased. A maximum protoplast yield of $4\text{--}6 \times 10^6 \cdot \text{g}^{-1}$ fresh weight was obtained. Cell division and colony formation from pretreated protoplasts were found to be best in an agarose solidified KM8p medium at a density of $5\text{--}8 \times 10^5 \cdot \text{ml}^{-1}$. The plating efficiency, based on colony formation after 2 weeks of culture, was 0.5–0.8%. Protoplast-derived colonies were transferred to a solidified MS medium containing $1.0 \text{ mg} \cdot \text{l}^{-1}$ 2,4-dichlorophenoxyacetic acid (2,4-D) for callus proliferation. The calli formed embryonic structures which gave rise to green plants in 0.2% (w/v) Gellan Gum solidified MS medium with $1.0 \text{ mg} \cdot \text{l}^{-1}$ naphthaleneacetic acid (NAA) and $0.2 \text{ mg} \cdot \text{l}^{-1}$ benzylaminopurine (BAP). The regenerated plantlets were transferred to 1/2 MS hormone-free medium for further growth and root formation. Rooted plants could be transferred to soil.

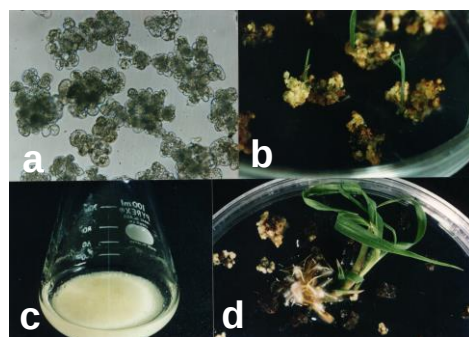


Fig. 1. Plant regeneration from cell suspension cultures of *Paspalum dilatatum* cv. 'Louisiana B-230'. (a) Large undifferentiated aggregates of cytoplasmically dense cells. Bar = 30 μm . (b) Typical suspension culture. (c,d) Plant regeneration from compact callus formed in the regeneration medium.

Table I. The effect of nurse culture on colony formation of *Paspalum dilatatum* cv. 'Louisiana B-230' protoplasts.

	Cell colonies formed per 10^5 cultured protoplasts ^b	
	Total	Colony formation rate (%)
Control	148	0.03
Nurse culture ^a	3398	0.68

Table II. The effect of donor pretreatment on colony formation of *Paspalum dilatatum* cv. 'Louisiana B-230' protoplasts^a.

Pretreatment (h)	Cell colonies formed per 10^5 cultured protoplasts ^b	
	Total	Colony formation rate (%)
Control	364	0.07
12	3398	0.68

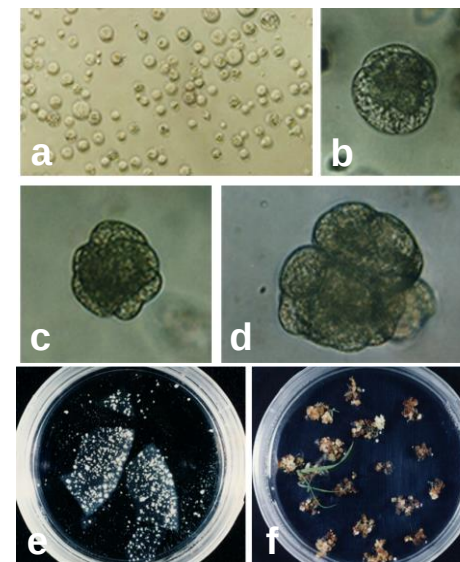


Fig. 3. Cell colony formation and plant regeneration from suspension protoplasts of *Paspalum dilatatum* cv. 'Louisiana B-230'. (a) Freshly isolated protoplasts from suspension cells after pre-treatment in PTM solution. (b–d) Cell division and cell colony formation from protoplasts after 5, 7 and 10 days of culture, respectively. (e) Colonies formed from pre-treated protoplasts after 20 days of culture. (f) Shoot and root formation from protoplast-derived callus.

Table III. The effect of protoplast culture media on protoplast-derived cell colony formation in *Paspalum dilatatum* cv. 'Louisiana B-230'^a.

Protoplast media ^b (Basal medium)	Protoplast density	No. of total colonies	Colony formation rate (%) ^c
PC 1 (MS)	3.0×10^5	16	0.005
PC 2 (MS)	3.0×10^5	47	0.016
PC 3 (MS + KM8p sugars)	5.0×10^5	56	0.011
PC 4 (MS + KM8p sugars)	5.0×10^5	92	0.018
PC 5 (KM8p)	5.0×10^5	1274	0.256
PC 6 (KM8p)	5.0×10^5	3398	0.68

Effect of BAP and Cupric Sulfate on Embryogenic Callus Formation and Plant Regeneration in Bahiagrass (*Paspalum notatum* Flügge).

Grassland Science 50, 55-58 (2004)

Synopsis

Takahiro Gondo, Ryo Akashi and Osamu Kawamura (2004) : Effect of BAP and Cupric Sulfate on Embryogenic Callus Formation and Plant Regeneration in Bahiagrass (*Paspalum notatum* Flügge). Grassland Science 50, 55–58.

We have established an efficient and stable *in vitro* culturing system for bahiagrass to proliferate highly regenerative embryogenic callus on modified medium increasing the level of cupric sulfate (CuSO_4). Highly regenerative embryogenic callus was initiated from shoot tips of germinated seeds and embryogenic callus was maintained on MS medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.1 mg/l 6-benzylaminopurine (BAP), 50 μM CuSO_4 at 31°C under the dim light condition. This modified culture could produce polyembryogenic callus which formed multiple pro-embryos over the surface of callus and gave rise to regenerability. Also, they can be maintained for more than a year without a marked loss in regenerability and appearance of albino plants during long term of culture.

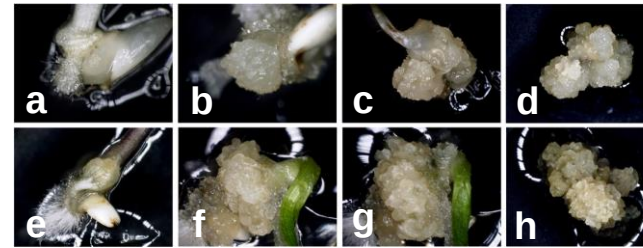


Fig. 1. Formation of embryogenic callus from mature seed of bahiagrass on different culture conditions. a-d) Developmental stages of embryogenic callus formation after 3 (a), 7 (b), 14 (c) and 28 (d) days of culture on MS medium containing 2 mg/l 2,4-D at 27°C in the dark condition. e-h) Developmental stages of embryogenic callus formation after 3 (e), 7 (f), 14 (g) and 28 (h) days of culture on MS medium containing 2 mg/l 2,4-D, 0.1 mg/l BAP and 50 μM CuSO_4 at 31°C in the dim light condition. SC, scutellum; ST, shoot tip; *, cluster of proembryos.

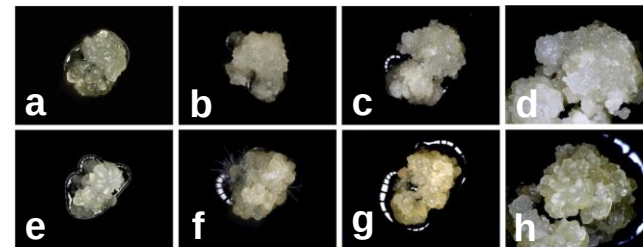


Fig. 2. Developmental stages of embryogenic callus in different sub-culture medium. a-d) Embryogenic callus cultured after 0 (a), 3 (b), 7 (c) and 21 (d) days on MS medium containing 2 mg/l 2,4-D at 27°C in the dark condition. e-h) Embryogenic callus cultured after 0 (e), 3 (f), 7 (g) and 21 (h) days on MS medium containing 2 mg/l 2,4-D, 0.1 mg/l BAP and 50 μM CuSO_4 at 31°C in the dim light condition. PE, proembryo; CPE, cluster of proembryo.

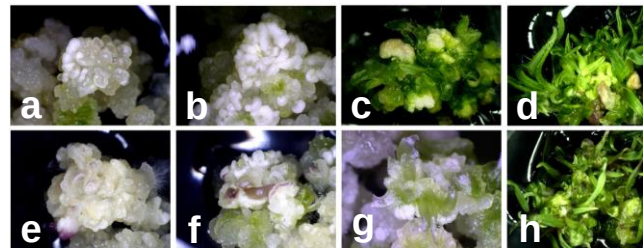


Fig. 3. Plant regeneration from embryogenic callus on different media. a-d) Somatic embryo formation (a, b) and shoot germination (c, d) from embryogenic callus on MS medium containing 2 mg/l 2,4-D at 27°C in the dark condition. e-h) Shoot germination with scutellum formation (e, f) and elongation of germinated shoot (g, h) from polyembryogenic callus on MS medium containing 2 mg/l 2,4-D, 0.1 mg/l BAP and 50 μM CuSO_4 at 31°C in the dim light condition. SE, somatic embryo; PE, proembryo; SC, scutellum; Cl, coleoptile.

Table 1. Plant regeneration from calli of bahiagrass on different regeneration media

MS-medium	Medium supplements			Number of inoculated calli ^b	Number of regenerating calli (%)	Total number of regenerated shoots	Number of shoots/regenerating callus
	2,4-D (mg/L)	BAP (mg/L)	CuSO_4 (μM)				
MS-D	2	0	0.1	63	23 (36.5)	40	1.7
MS-DC	2	0	50	63	49 (77.8)	285	5.8
MS-DB	2	0.1	0.1	63	19 (30.2)	50	2.6
MS-DBCL ^a	2	0.1	50	63	50 (79.4)	304	6.1
MS-DBCH ^a	2	0.1	50	63	54 (85.4)	425	7.9

^aCulture on MS-DBC medium at 27°C (DBCL) or 31°C (DBCH).

^bTwo-month-old microcalli derived from mature seeds on MS-D medium were pre-cultured for 21 days (see Materials and methods) and transferred to regeneration medium.

Assays of the production of harmful substances by genetically modified oilseed rape (*Brassica napus* L.) plants in accordance with regulations for evaluating the impact on biodiversity in Japan

Yoko Asanuma · Tomoe Jinkawa · Hidenori Tanaka · Takahiro Gondo · Norihiro Zaita · Ryo Akashi

Abstract Environmental risk assessment of transgenic crops is implemented under the Cartagena Protocol domestic law in accordance with guidelines for implementing the assessment established by the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the Ministry of Environment (MOE) in Japan. Environmental risk assessments of transgenic crops are implemented based on the concept of ‘substantial equivalence’ to conventional crops. A unique requirement in Japan to monitor the production of harmful substances, or allelochemicals, is unparalleled in other countries. The potential for allelochemicals to be secreted from the roots of transgenic crops to affect other plants or soil microflora or for substances in the plant body to affect other plants after dying out must be evaluated. We evaluated the allelopathic potential of seven transgenic oilseed rape (*Brassica napus* L.) lines that express glufosinate tolerance in terms of substantial equivalence to conventional oilseed rape lines, and established evaluation methods. Our results indicate no potential production of allelochemicals for any of the seven transgenic oilseed rape lines compared with conventional oilseed rape lines.

Table 1 Plant materials used in this study

Line	Control	Introduced gene	Antibiotic gene	Traits
T45	AC-Excel	<i>pat</i>	-	Glufosinate tolerance
Topas19/2	Topas	<i>pat</i>	<i>npII</i>	Glufosinate tolerance
MS1	Drakkar	<i>bar, bamase</i>	<i>neo</i>	Glufosinate tolerance and male sterility
RF1	Drakkar	<i>bar, barstar</i>	<i>neo</i>	Glufosinate tolerance and fertility restoration
RF2	Drakkar	<i>bar, barstar</i>	<i>neo</i>	Glufosinate tolerance and fertility restoration
MS1RF1	Drakkar	<i>bar, bamase, barstar</i>	<i>neo</i>	Glufosinate tolerance
MS1RF2	Drakkar	<i>bar, bamase, barstar</i>	<i>neo</i>	Glufosinate tolerance

Table 2 Evaluation of germination rate and growth of radish on soil after harvest

Line	Germination rate ^{a, b} (%)	Plant height ^c (cm)	Root length ^c (cm)	Fresh weight ^b (g)	Dry weight ^b (g)
T45	95.5 ± 3.7 ns ^d	5.44 ± 0.88 ns	8.10 ± 1.96 ns	2.86 ± 0.34 ns	0.22 ± 0.02 ns
AC-Excel	93.5 ± 5.3	5.37 ± 1.00	8.43 ± 1.69	2.76 ± 0.35	0.21 ± 0.02
Topas19/2	99.5 ± 1.6 ns	5.31 ± 0.91 ns	13.17 ± 2.21 ns	3.01 ± 0.24 ns	0.42 ± 0.02 ns
Topas	98.0 ± 3.5	5.11 ± 0.84	12.58 ± 2.58	2.95 ± 0.17	0.40 ± 0.02
MS1	93.0 ± 5.4 ns	6.42 ± 1.11 ns	7.82 ± 2.28 ns	3.09 ± 0.31 ns	0.24 ± 0.03 ns
RF1	93.5 ± 4.1 ns	6.66 ± 1.22 ns	8.46 ± 2.88 ns	2.87 ± 0.34 ns	0.23 ± 0.02 ns
RF2	94.5 ± 6.4 ns	6.14 ± 0.98 ns	8.31 ± 1.72 ns	2.88 ± 0.28 ns	0.27 ± 0.02 ns
MS1RF1	95.0 ± 6.2 ns	6.74 ± 1.14 ns	7.64 ± 3.47 ns	2.85 ± 0.30 ns	0.24 ± 0.03 ns
MS1RF2	93.5 ± 3.4 ns	6.38 ± 1.14 ns	8.33 ± 1.42 ns	2.95 ± 0.58 ns	0.24 ± 0.03 ns
Drakkar	95.0 ± 4.1	6.38 ± 1.05	8.01 ± 1.91	2.81 ± 0.30	0.24 ± 0.03

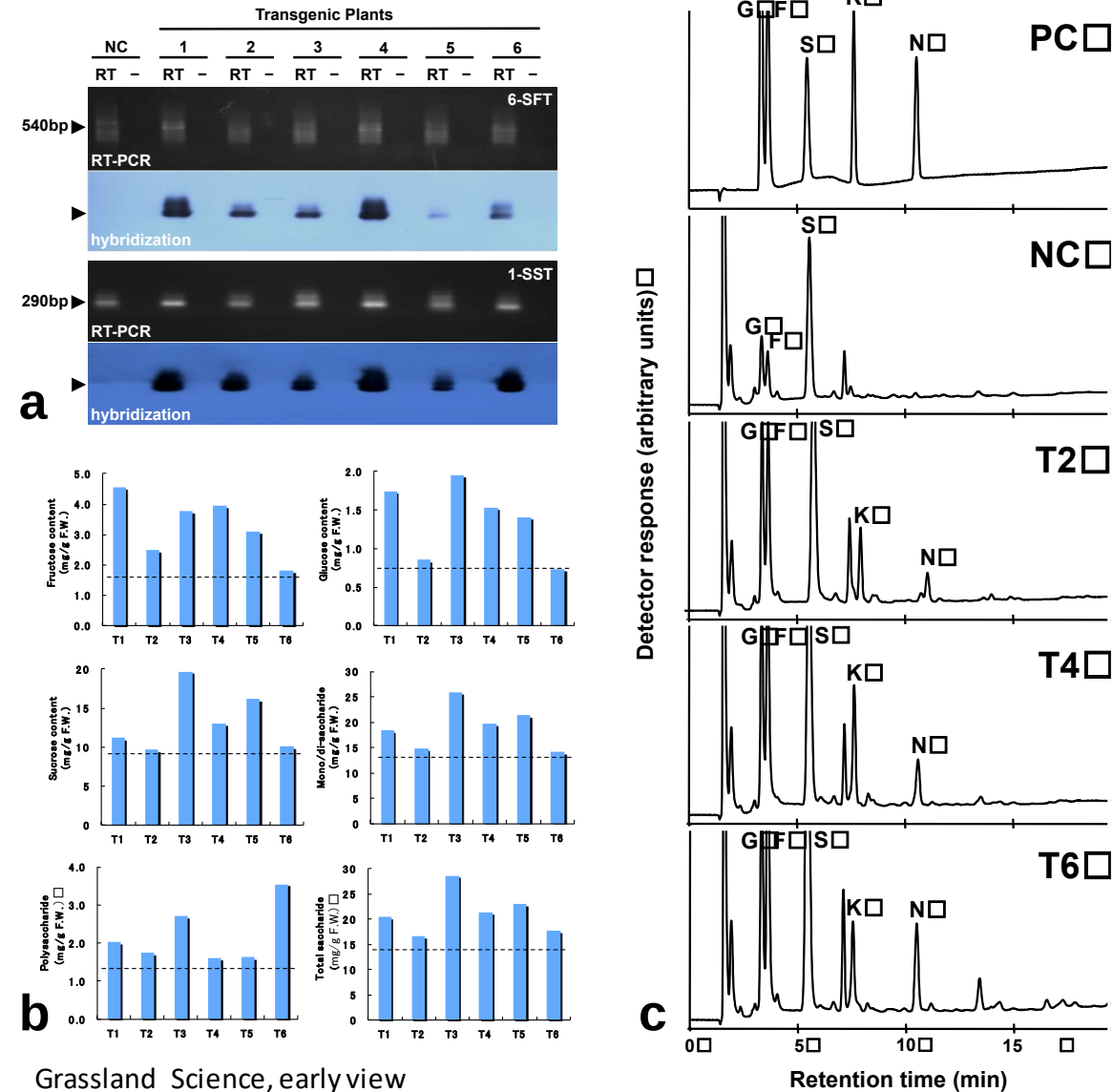
Table 3 Evaluation of germination rate and growth of radish on soil amended with dried plant materials

Line	Germination rate ^{a, b} (%)	Plant height ^c (cm)	Root length ^c (cm)	Fresh weight ^b (g)	Dry weight ^b (g)
T45	96.5 ± 4.7 ns ^d	5.69 ± 0.64 ns	11.38 ± 3.59 ns	2.03 ± 0.17 ns	0.27 ± 0.04 ns
AC-Excel	96.5 ± 4.1	5.72 ± 0.58	10.79 ± 2.42	1.98 ± 0.21	0.27 ± 0.02
Topas19/2	98.1 ± 0.0 ns	12.27 ± 1.27 ns	11.35 ± 3.04 ns	6.28 ± 0.30 ns	0.53 ± 0.03 ns
Topas	98.1 ± 0.0	12.34 ± 1.27	11.01 ± 2.22	6.30 ± 0.35	0.52 ± 0.03
MS1	96.0 ± 4.6 ns	6.32 ± 0.74 ns	7.43 ± 1.26 ns	3.60 ± 0.20 ns	0.34 ± 0.03 ns
RF1	96.0 ± 3.2 ns	6.60 ± 0.92 ns	7.39 ± 1.65 ns	3.80 ± 0.13 ns	0.36 ± 0.06 ns
RF2	97.0 ± 3.5 ns	6.57 ± 0.91 ns	7.52 ± 0.76 ns	3.71 ± 0.21 ns	0.34 ± 0.02 ns
MS1RF1	95.5 ± 4.5 ns	6.53 ± 0.85 ns	7.57 ± 1.79 ns	3.58 ± 0.30 ns	0.38 ± 0.04 ns
MS1RF2	95.5 ± 4.4 ns	6.56 ± 0.71 ns	7.57 ± 0.71 ns	3.76 ± 0.15 ns	0.37 ± 0.03 ns
Drakkar	95.0 ± 3.3	6.45 ± 0.69	7.34 ± 1.08	3.74 ± 0.23	0.35 ± 0.04

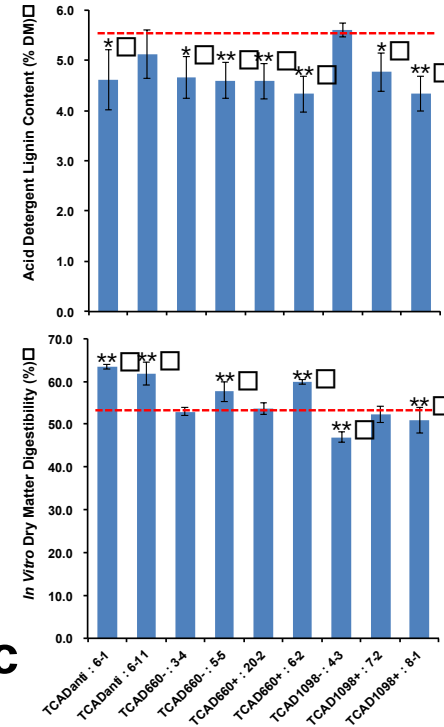
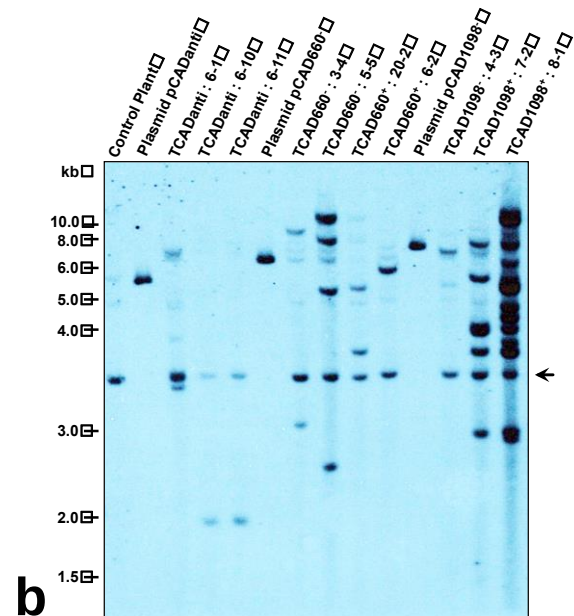
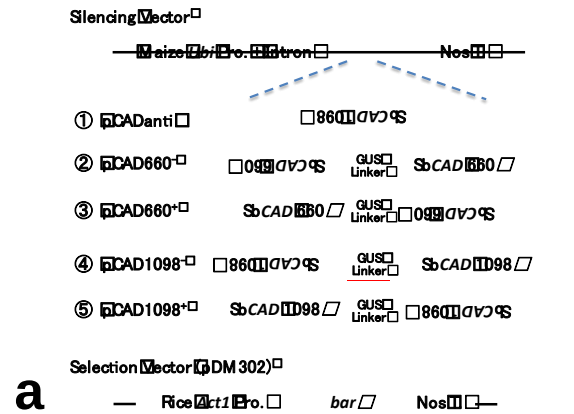
Table 4 Evaluation of values of soil microflora in soil after harvest

Line	Bacteria (×10 ⁶ CFU) ^c	Actinomyces (×10 ⁶ CFU) ^c	Filamentous fungi (×10 ³ CFU) ^c
T45 ^a	6.51 ± 1.36 ns	0.30 ± 0.63 ns	4.74 ± 1.14 ns
AC-Excel ^a	6.42 ± 1.05	0.32 ± 0.11	5.15 ± 1.09
Topas19/2 ^b	3.88 ± 0.60 ns	0.29 ± 0.06 ns	1.73 ± 0.30(×10 ⁴ CFU) ns
Topas ^b	4.38 ± 0.24	0.26 ± 0.08	2.02 ± 0.16(×10 ⁴ CFU)
MS1 ^{b, d}	4.10 ± 0.20 ns	0.38 ± 0.18 ns	5.71 ± 0.44 ns
RF1 ^b	3.66 ± 0.49 ns	0.24 ± 0.07 ns	6.04 ± 0.40 ns
MS1RF1 ^b	5.17 ± 1.01 s	0.30 ± 0.07 ns	5.25 ± 1.03 ns
MS1 ^{b, d}	3.74 ± 0.62 ns	0.12 ± 0.02 ns	6.15 ± 0.98 ns
RF2 ^b	3.83 ± 0.15 ns	0.26 ± 0.05 ns	5.24 ± 0.66 ns
MS1RF2 ^b	5.05 ± 0.13 s	0.35 ± 0.05 s	5.26 ± 0.56 ns
Drakkar ^b	3.78 ± 0.11	0.19 ± 0.08	5.15 ± 1.16

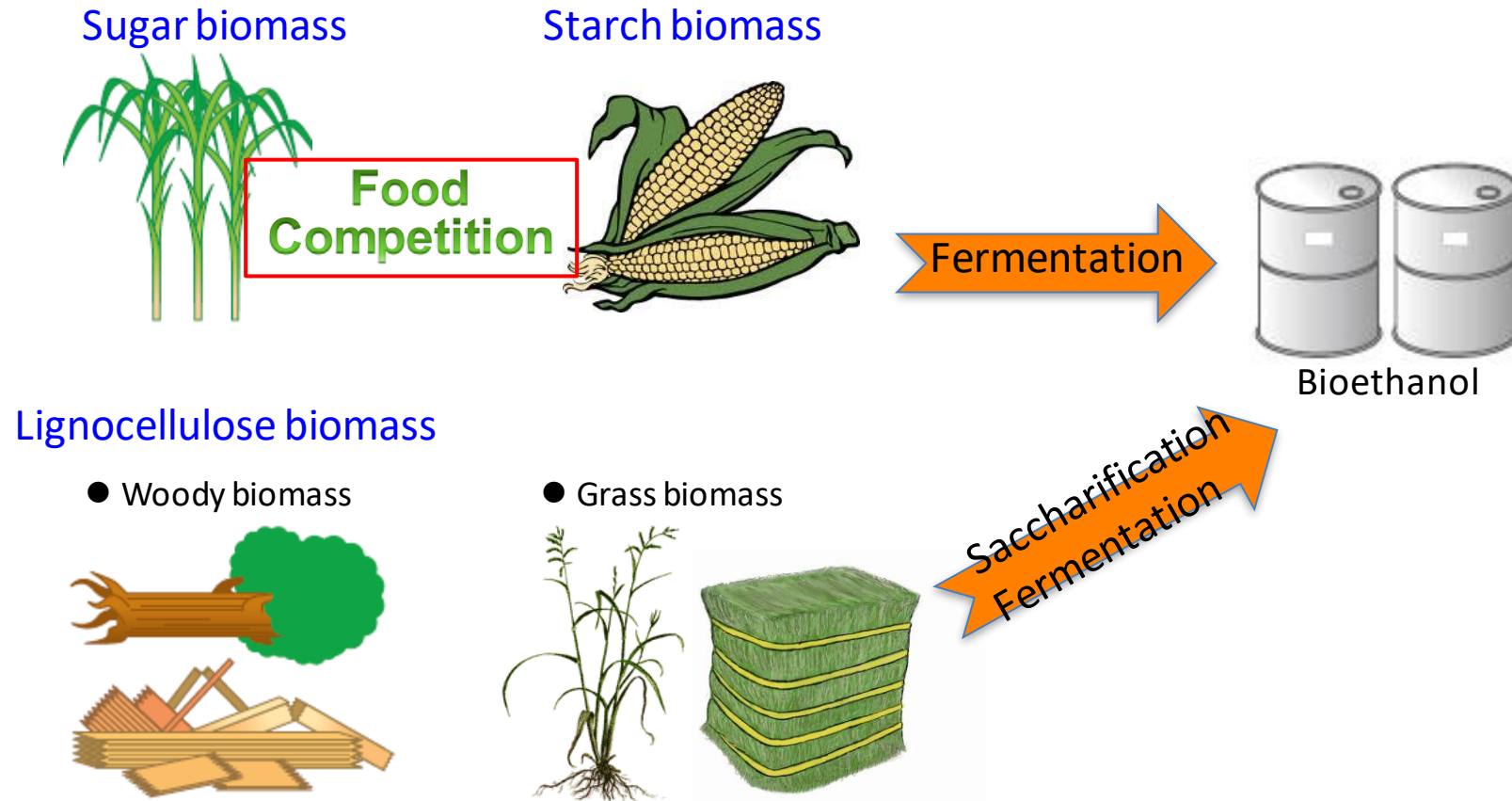
Accumulation of fructan



Decrease of lignin content and increase digestibility in transgenic plants



Bioenergy crops



- Grass biomass is
- ① high speed of growth rate.
 - ② perennial and sustainable biomass production.
 - ③ Low lignin content which inhibit saccharification.

What is napiergrass?



Presented by Prof. Ishii in Miyazaki U.

- *Pennisetum purpureum*

- Native to Africa

- Chromosome number
($2n=4x=28$)

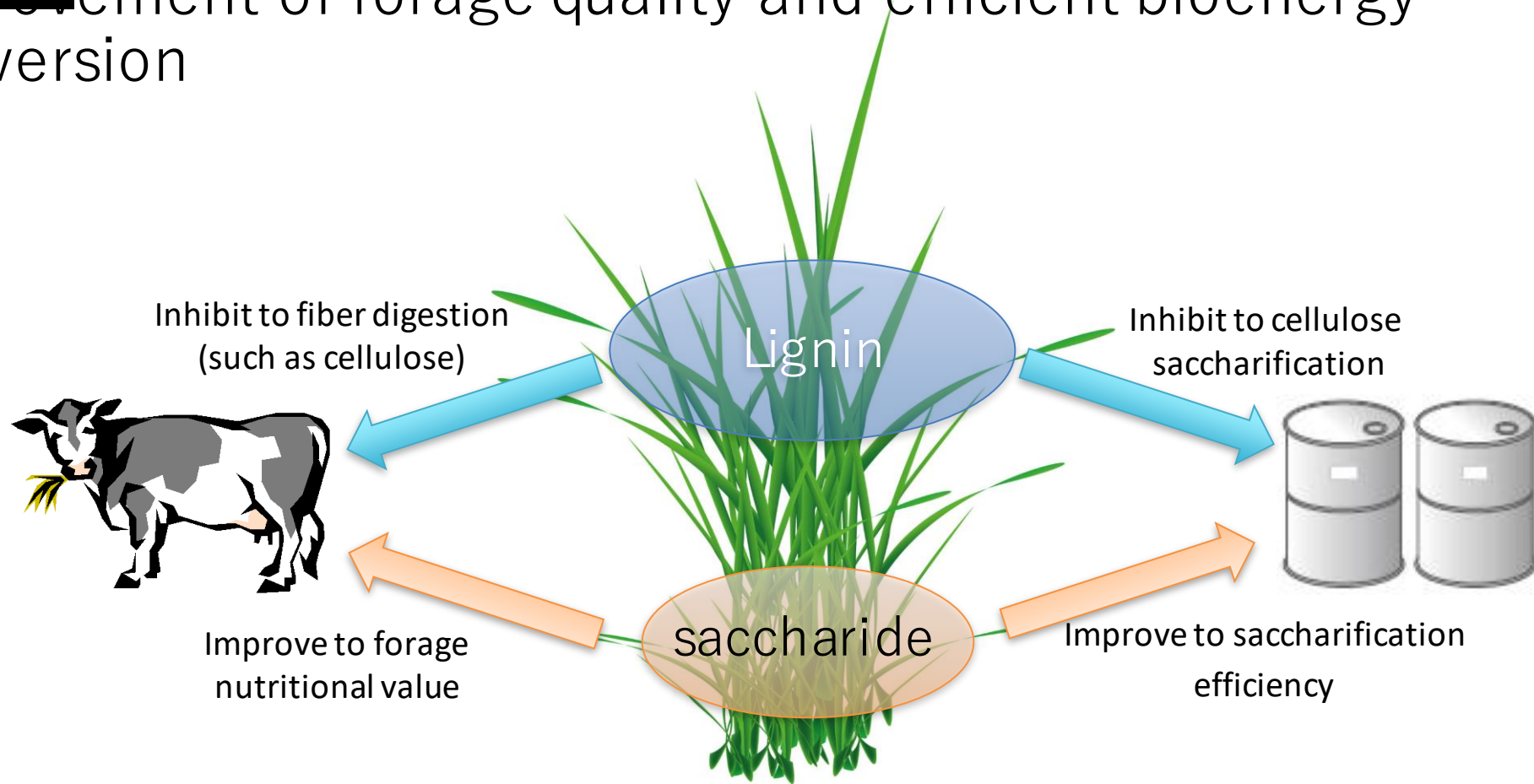
- Plant height : 2~5 m

- Perennial grass

- Production : 40~80 t/ha/y

- Vegetative propagation

Improvement of forage quality and efficient bioenergy conversion



Decrease of lignin content and increase of saccharide content is same breeding objective for improvement of forage quality and bioethanol production.

Future plan



- Fructosyltransferase genes

- CAD gene as lignin biosynthesis

Apply to
Napiergrass



We identified increase of saccharide content
decrease of lignin content in transgenic plant.

Demonstration of the utility for forage



Demonstration of the utility
for bioethanol production

Metoda Pemuliaan Mutation

Mutasi dalam pemuliaan tanaman menyebabkan perubahan pada urutan nukleotida suatu material genetik, baik DNA atau RNA yang berpengaruh terhadap struktur primer

(Chahal dan Gosal, 2003)

Perubahan pada struktur primer berdampak pada struktur sekunder, tersier, dan kuartener protein tanaman dan berpengaruh terhadap tingkah laku, morfologi dan fisiologi.

Mutagen fisika melalui radiasi merupakan jenis mutagen yang dapat digunakan dalam program pemuliaan tanaman

Sinar gamma merupakan mutagen yang paling banyak digunakan



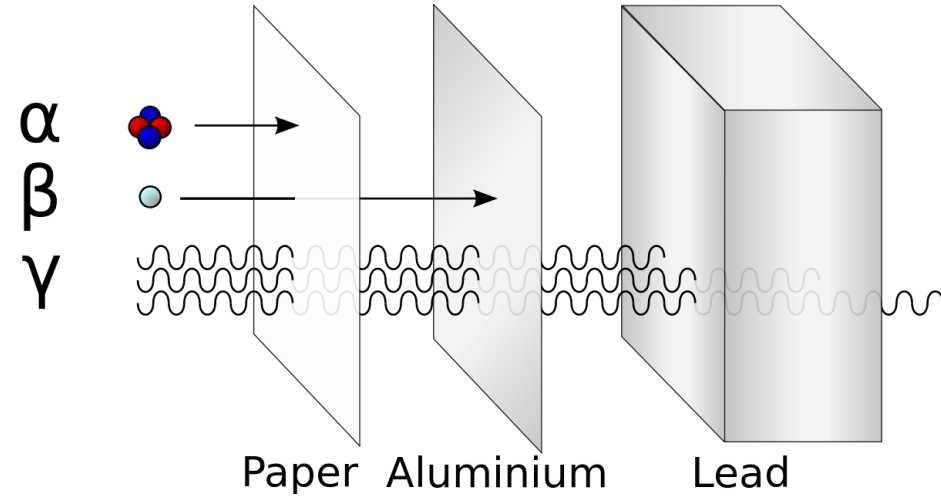
Sifat Sinar Gamma



Gammacell-220



Gamma chamber-4000A



Pemanfaatan Radiasi Gamma berdasarkan sifat-sifat yang dimiliki :

- radiasi pengion
 - daya tembus besar
 - dapat berinteraksi dengan air, DNA, kromosom & sel
-
- Sumber radiasi : ^{60}Co , ^{137}Cs
 - Instrumen : Gammacell-220 atau Gamma Chamber 4000-A

Proses Radiasi Sinar Gamma



Radiasi sinar gamma terjadinya perubahan dalam komposisi basa dan juga putusnya rantai DNA. Efek radiasi terhadap basa berperan secara langsung dalam proses mutasi gen serta menginduksi perubahan struktur kromosom yang dapat menghasilkan varietas mutan unggul.

Dosis radiasi sangat berpengaruh terhadap tingkat keberhasilan mutasi dengan satuan RAD (*radiation absorbed dose*), yaitu besarnya energi yang diserap per satuan massa atau lebih populer dengan istilah Gy (Chahal dan Gosal, 2003)..

Faktor yang Mempengaruhi Keberhasilan Radiasi

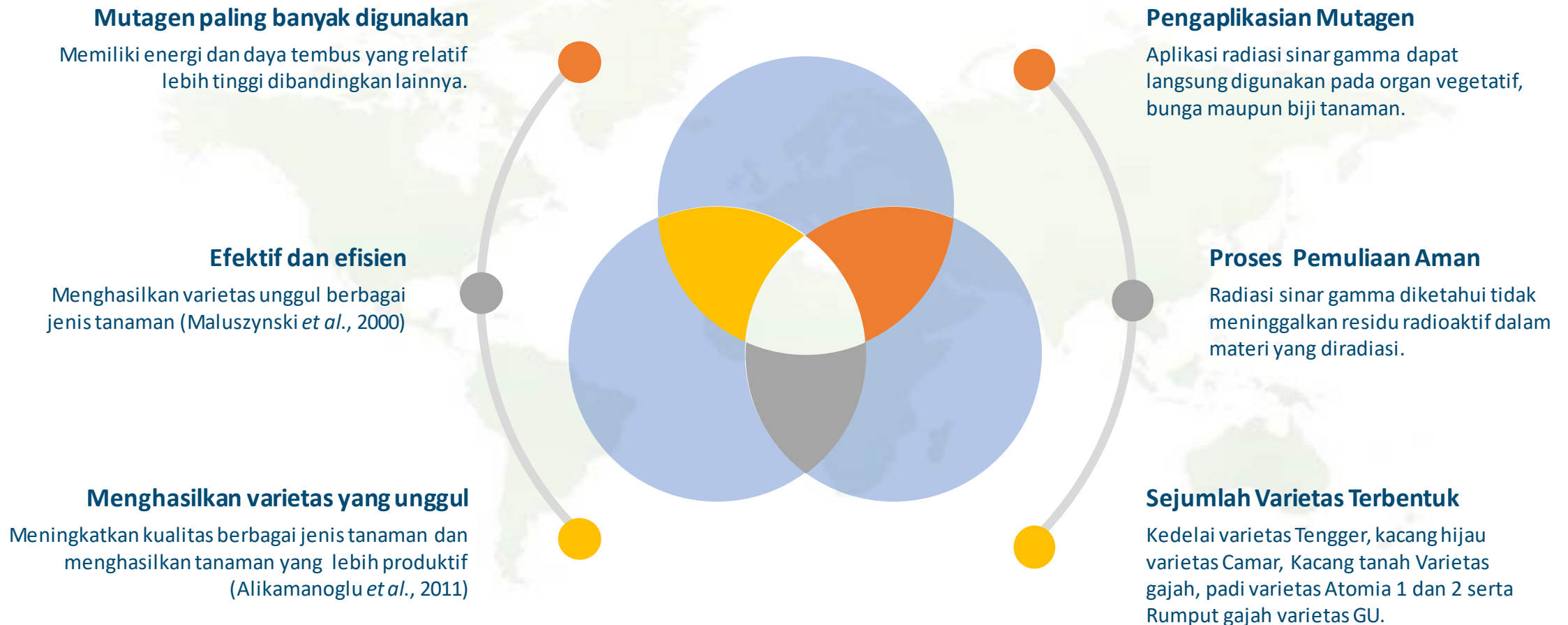
Dosis radiasi

Genotipe tanaman yang diradiasi.

Sumber eksplan



Kelebihan Radiasi Sinar Gamma



Metoda Radiasi Sinar Gamma



01

Benih Tanaman

Benih yang dipilih yang berkualitas baik dan tidak rusak dengan ciri kulit ari tidak terkelupas, tidak berlubang dan bulirnya utuh.

02

Tempat dan Label

Setiap benih dimasukkan ke tempat dan di beri label tergantung dosis radiasi yang digunakan

03

Penyinaran

Benih disinari dengan mesin iradiator Gamma Chamber, yang memiliki kekuatan sesuai dosis radiasi

04

Besar Dosis

Besar dosis radiasi merupakan fungsi dari waktu dan laju dosis yang dimiliki Gamma Chamber saat itu

Review Gamma Radiasi

Gandum
(*Triticum aestivum* L.)

100, 200, 300, 400 Gy

Dosis 100 Gy menyebabkan peningkatan jumlah anakan per tanaman.

Irfaq dan Nawab
, 2003

Triticum aestivum L.

0, 100, 200, 300, 400 Gy

Dosis 100 Gy mempengaruhi panjang dan berat akar.

Borzouei et al.,
2010

Sorgum manis

0, 100, 200, 300, 400, 500, 600, 700, 800, 900 dan 1000 Gy

Dosis 100 sampai 300 Gy mempengaruhi keragaman dari karakter tinggi tanaman, bobot biji per malai, panjang malai dan diameter batang

Surya dan Soeranto, 2009

Padi

0, 200, 300 Gy

Dosis 200 Gy berpotensi menghasilkan mutan yang lebih pendek dan panen lebih cepat.

Haris *et al.*,
2013

Padi Gogo

0, 100, 150, 200 Gy

Dosis 100 Gy mempengaruhi panjang malai dan jumlah anakan. Dosis 200 Gy mempengaruhi tinggi tanaman.

(Meliala *et al.*, 2016)

Jagung (*Zea mays* L.)

0, 100, 200, 300 Gy

Dosis 200 Gy menghasilkan tongkol paling banyak.

(Makhziah *et al.*, 2017)

Kedelai (*Glycine max*)

50, 100, 150 dan 200 Gy

Dosis 200 Gy mempengaruhi pertumbuhan dan perkembangan tanaman sehingga mempengaruhi produksi tanaman

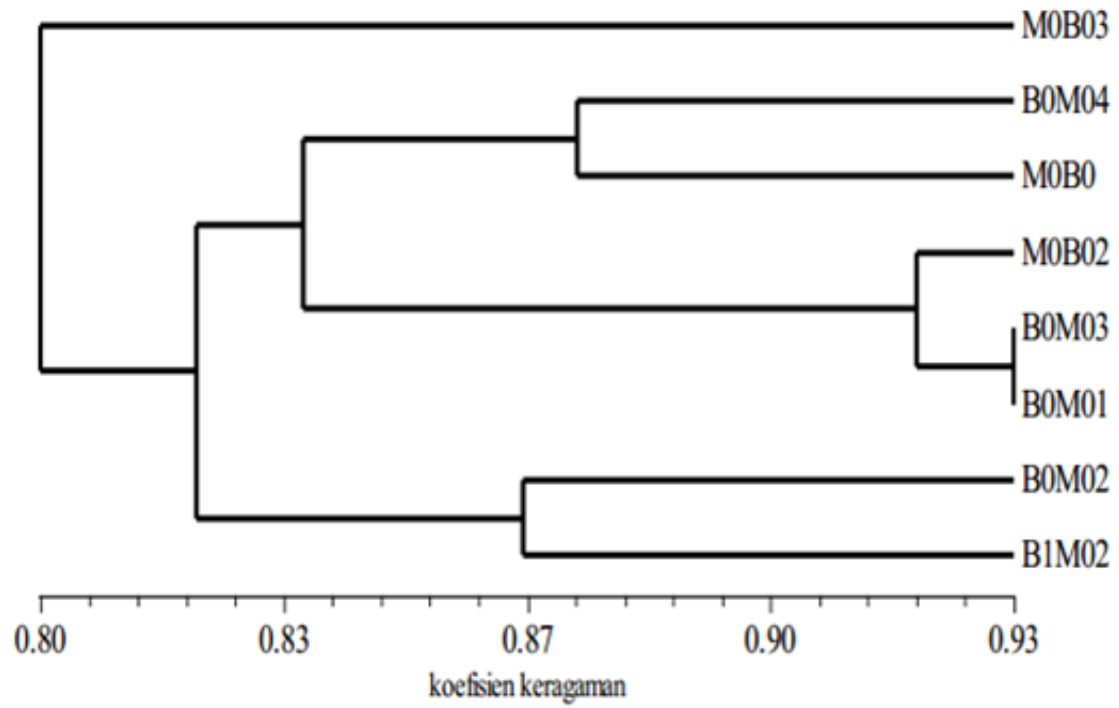
(Hanafiah *et al.*, 2010)

Padi (*Oryza sativa* L.)

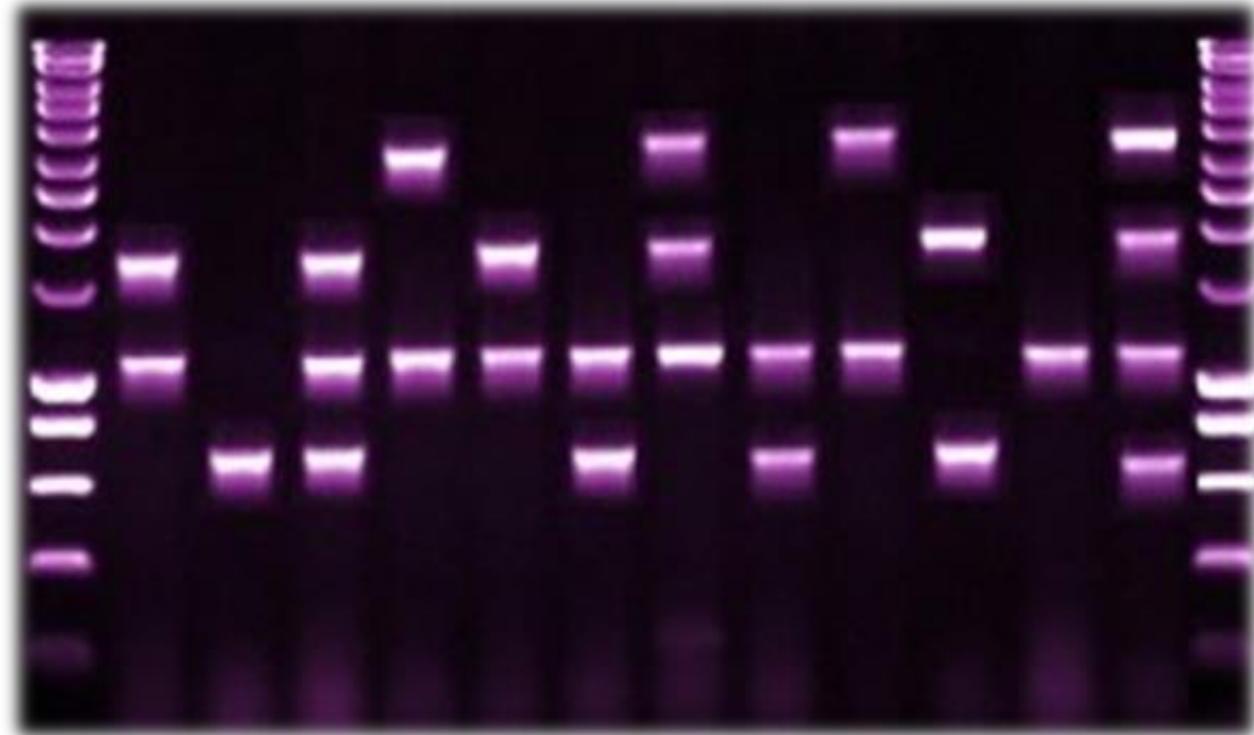
0, 150, 300, 400, 600 Gy

Dosis 150 Gy menyebabkan peningkatan jumlah daun dan jumlah anakan, produksi segar.

(Safana, 2016)



Peta filogenetik



Hasil visual dgn sinar UV

Table 2. The plant production of *Brachiaria brizantha* cv. MG5 with different doses of gamma radiation at various regrowth phases (ton/ha)

Variable	Radiation (Gy)	Regrowth phase		
		1	2	3
Dry matter production	0	1.04±0.26 ^{de}	0.69±0.11 ^{ef}	0.84±0.11 ^{ef}
	100	0.49±0.11 ^f	2.55±0.58 ^e	0.98±0.28 ^{de}
	200	0.88±0.25 ^{ef}	1.78 ±0.52 ^{bc}	0.87±0.18 ^{ef}
	300	1.11±0.09 ^{de}	1.47±0.20 ^{cd}	0.82±0.13 ^{ef}
	400	0.63±0.04 ^{ef}	2.09±0.43 ^{ab}	1.07±0.22 ^{de}
Organic matter production	0	0.91±0.23 ^{de}	0.61±0.09 ^{ef}	0.74±0.10 ^{ef}
	100	0.42±0.09 ^f	2.22±0.47 ^a	0.86±0.26 ^{def}
	200	0.75±0.22 ^{ef}	1.56±0.45 ^{bc}	0.77±0.16 ^{ef}
	300	0.95±0.07 ^{de}	1.29±0.18 ^{cd}	0.72±0.12 ^{ef}
	400	0.54±0.03 ^{ef}	1.84±0.38 ^{ab}	0.94±0.21 ^{de}

Note: Means with different superscripts differ significantly (P<0.05).

Dosis radiasi sinar gamma 100 Gy akan menghasilkan pertumbuhan dan yang berbeda pada *Brachiaria brizantha* cv. MG5. Keragaman genetik menunjukkan bahwa adanya keragaman genetik dengan ditunjukkan dua kelompok yaitu kelompok A (100, 200, 300 Gy) dan kelompok B (400 Gy).

Morfologi Tanaman Hasil Radiasi Gamma



Rumput Brachiaria (*Brachiaria brizantha*).

Pola pertumbuhan

Pertumbuhan tanaman menyebar dan setiap batang bagian terluar miring

Warna daun dan tulang daun

Warna **daun** hijau muda dengan tekstur yang terlihat nyata dengan **warna tulang daun** sedikit terlihat, engan warna sedikit kekuningan

Bentuk batang dan bulu pada tanaman

Bentuk batang kecil, dan memanjang berwarna hijau muda. **Bulu pada tanaman** ini terlihat sedikit dan rapat dengan panjang yang lebih banyak pada bagian daun

Penelitian Tanaman Pakan Radiasi Gamma

Growth and production of 2 cultivars (*Pennisetum purpureum* Schumach.) on regrowth phase

D Ananta, Z Bachruddin and N Umami

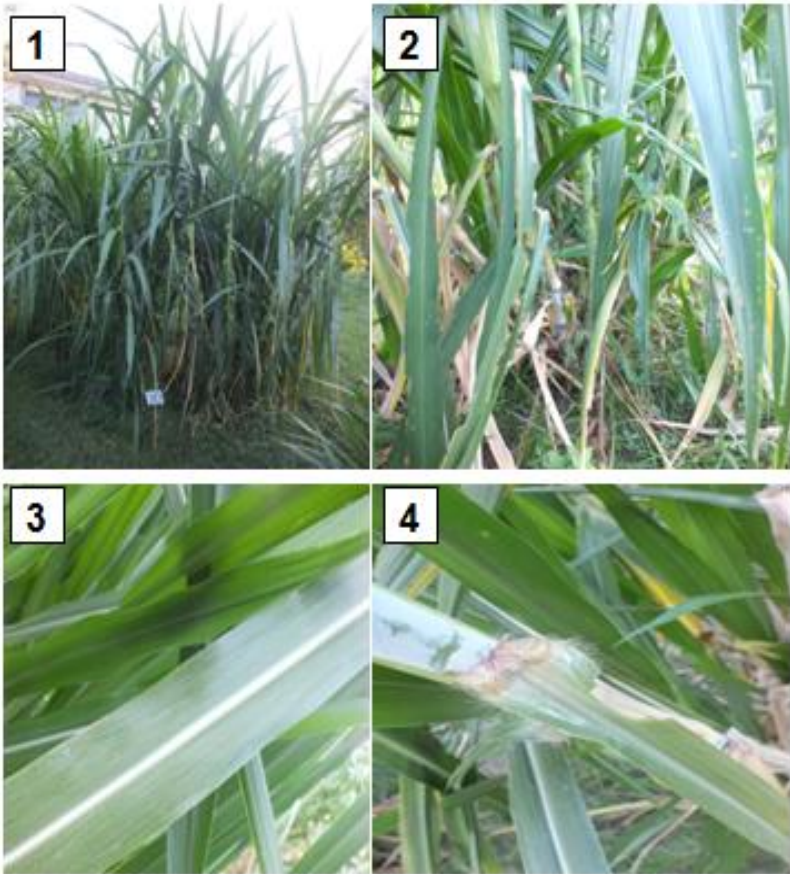
Based on the results of the study it can be concluded that napiergrass of GU-1 cultivar had a higher biomass production than local. Defoliation can increase vegetative growth and biomass production of napiergrass.

Table 3. Fresh production, dry matter production, and organic matter production of 2 napiergrass cultivars in 2 regrowth phases

Cultivars	Fresh production (t/ha)			Dry matter production (t/ha)			Organic matter production (t/ha)		
	Regrowth phase		Average	Regrowth phase		Average	Regrowth phase		Average
	1	2		1	2		1	2	
GU-1	123.53	160.05	141.79 ^a	22.24	29.46	25.85 ^a	19.73	26.19	22.96 ^a
Local	85.30	123.64	104.47 ^b	13.53	19.81	16.67 ^b	12.03	17.67	14.85 ^b
Average	104.41 ^b	141.84 ^a		17.89 ^b	24.63 ^a		15.88 ^b	21.93 ^a	

^{ab} Different superscripts indicate significant differences between cultivars and regrowth phase ($P < 0,05$)

Morfologi Tanaman Hasil Radasi Gamma



Rumput Gajah (*Pennisetum purpureum*).

Pola pertumbuhan

Tanaman tegak namun banyak terdapat batang yang tumbuh sedikit miring dan melengkung pada bagian tepi.

Warna daun dan tulang daun

Warna daun hijau tua dengan tekstur yang terlihat nyata berbulu, dengan **warna tulang daun** yang putih bersih tanpa ada tambahan warna pada bagian tepi, dan ukuran yang cukup besar.

Bentuk batang dan bulu pada tanaman

Bentuk batang besar, kuat dan keras berwarna hijau tua. **Bulu pada tanaman** ini terlihat sedikit dan rapat dengan panjang yang lebih pada bagian batang dibanding pada daun.

Perbandingan Rumput Gajah Radiasi Gamma dengan Varietas Lainnya

Tabel 3. Komposisi kimia (%) berbagai varietas rumput gajah (*Pennisetum purpureum*) .

Varietas	Serat kasar	Lemak kasar ^{ns}	Protein kasar	ADF	NDF
RG1 (Tifton)	33,27 ^a	3,49	8,90 ^{bc}	45,72 ^a	65,40 ^a
RG2 (Taiwan)	31,98 ^{abc}	2,95	7,30 ^c	44,97 ^{ab}	64,20 ^a
RG3 (Muaklek)	28,24 ^{bc}	2,88	12,35 ^a	42,50 ^c	61,71 ^{ab}
RG4 (Dwarf Indonesia)	28,00 ^c	2,93	12,58 ^a	40,37 ^{bc}	58,02 ^b
RG5 (Lokal)	32,25 ^{ab}	3,31	9,35 ^b	44,90 ^{ab}	63,99 ^a
RG6 (Gamma 1)	34,25 ^a	3,40	10,21 ^b	45,84 ^a	66,03 ^a
RG7 (Gamma 2)	33,80 ^a	3,38	9,89 ^b	45,27 ^{ab}	65,03 ^a

a,b,c : Superskrip yang berbeda pada kolom yang sama menunjukkan adanya perbedaan ($P < 0,05$).
ns : *Non Significant*

Foto Perbandingan Rumput Gajah Radiasi Gamma dengan Varietas Lokal



GU-1



GU-2



Lokal