



牧草およびセルロース系バイオマス
資源作物の世界におけるゲノム解
析の状況ならびに海外との国際共
同研究の取組み

北海道大学北方生物圏フィールド科学センター

山田 敏彦

講演内容

- 牧草とそのゲノム研究の最近の状況
講演者グループの成果を中心に
- バイオマス資源作物の重要性
- 米国スイッチグラスのゲノム研究
- ススキ属植物の有用性とゲノム研究の必要性
- 講演者グループの研究内容
米国DOE予算による国際共同研究など



開陽台(中標津町)

草地の各種多様な機能

- ✓ 家畜飼料としての生産機能
- ✓ 地力増進
- ✓ 土壌・水保全
- ✓ 生物相保全
- ✓ 炭素保持
- ✓ 景観保全
- ✓ レジャー・スポーツのアメニティー

牧草の種類

寒地型牧草

イネ科牧草

イタリアンライグラス, ペレニアルライグラス,
チモシー, オーチャードグラス, トールフェスク,
メドウフェスク, ケンタッキーブルーグラス

マメ科牧草

シロクローバ, アカクローバ, アルファルファ

暖地型牧草

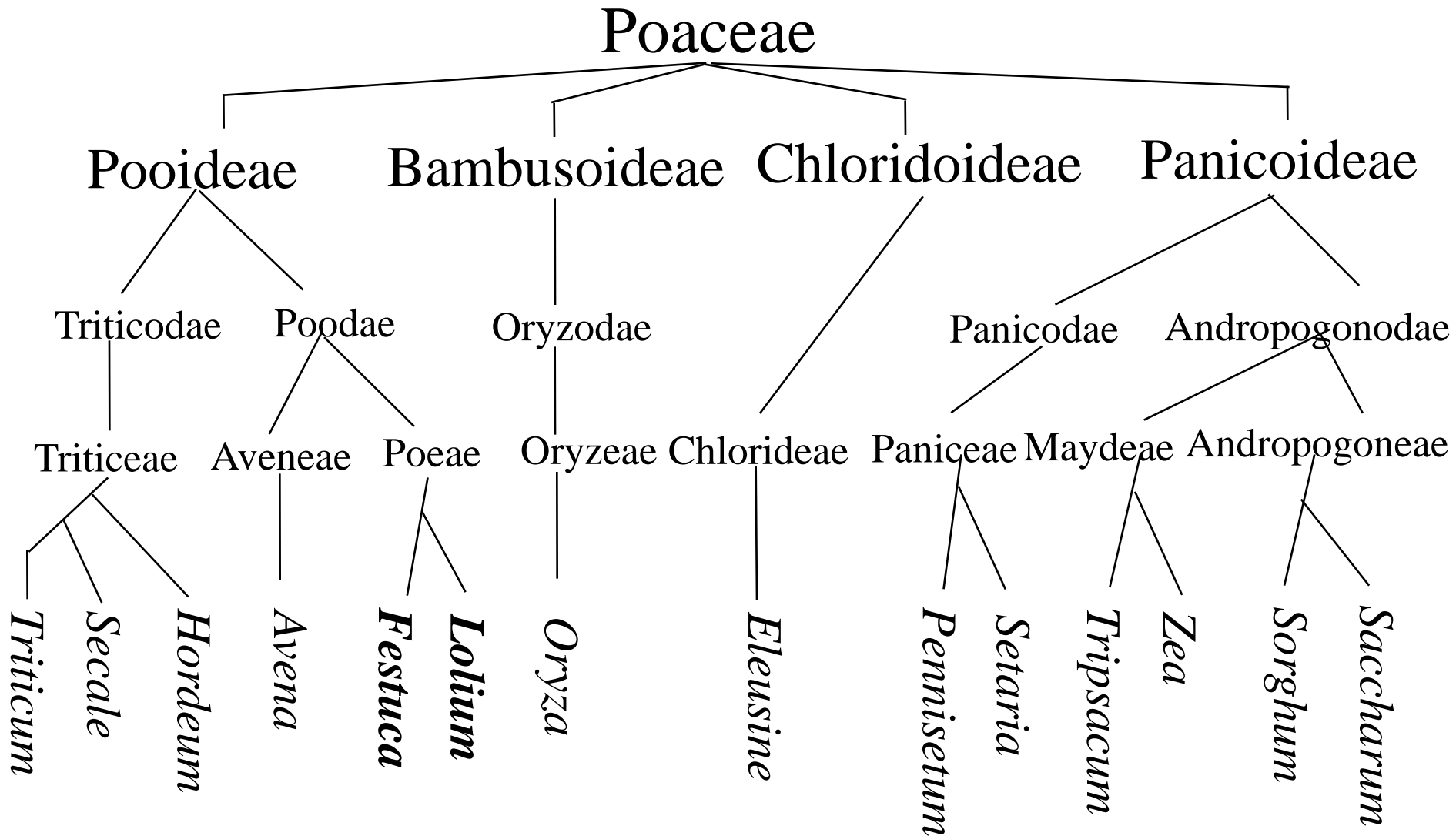
イネ科牧草

ギニアグラス, バヒアグラス, ダリスグラス, ロー
ズグラス

マメ科牧草

サイラトロなど

イネ科の分類



ペレニアルライグラス

英名 : perennial ryegrass

和名 : ホソムギ

学名 : *Lolium perenne* L.

核DNA量 :

4.16pg/2C (イネ 0.88 pg/2c)

自家不和合性(S,Z遺伝子座)

$2n=2x=14$ 同質4倍体品種あり

世界中の温暖地の草地に広く栽培され、良質な永年生牧草である。再生力は旺盛であるが、越冬性, 耐暑性, 耐干性に劣る。放牧利用に適している。また、初期生育が良好で、緑化・芝生用にも広く利用されている。



イタリアンライグラス

英名: Italian ryegrass

和名: ネズミムギ

学名: *Lolium multiflorum* Lam.

自家不和合性(S,Z遺伝子座)

$2n=2x=14$ 同質4倍体品種あり

1～越年性。初期生育が旺盛で、採草で利用される。本州以南では重要な冬作飼料作物である。



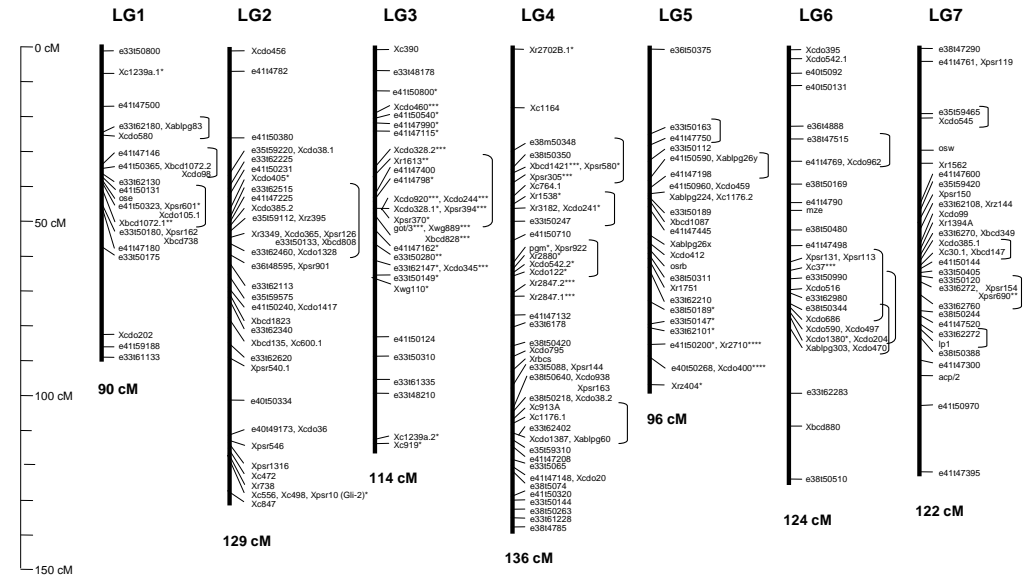
International *Lolium* Genome Initiative (ILGI)

- AV-PBC, Australia
- IGER, UK
- INRA, France
- NARCH/YPDES, Japan

P150/112

one-way pseudo-test cross population

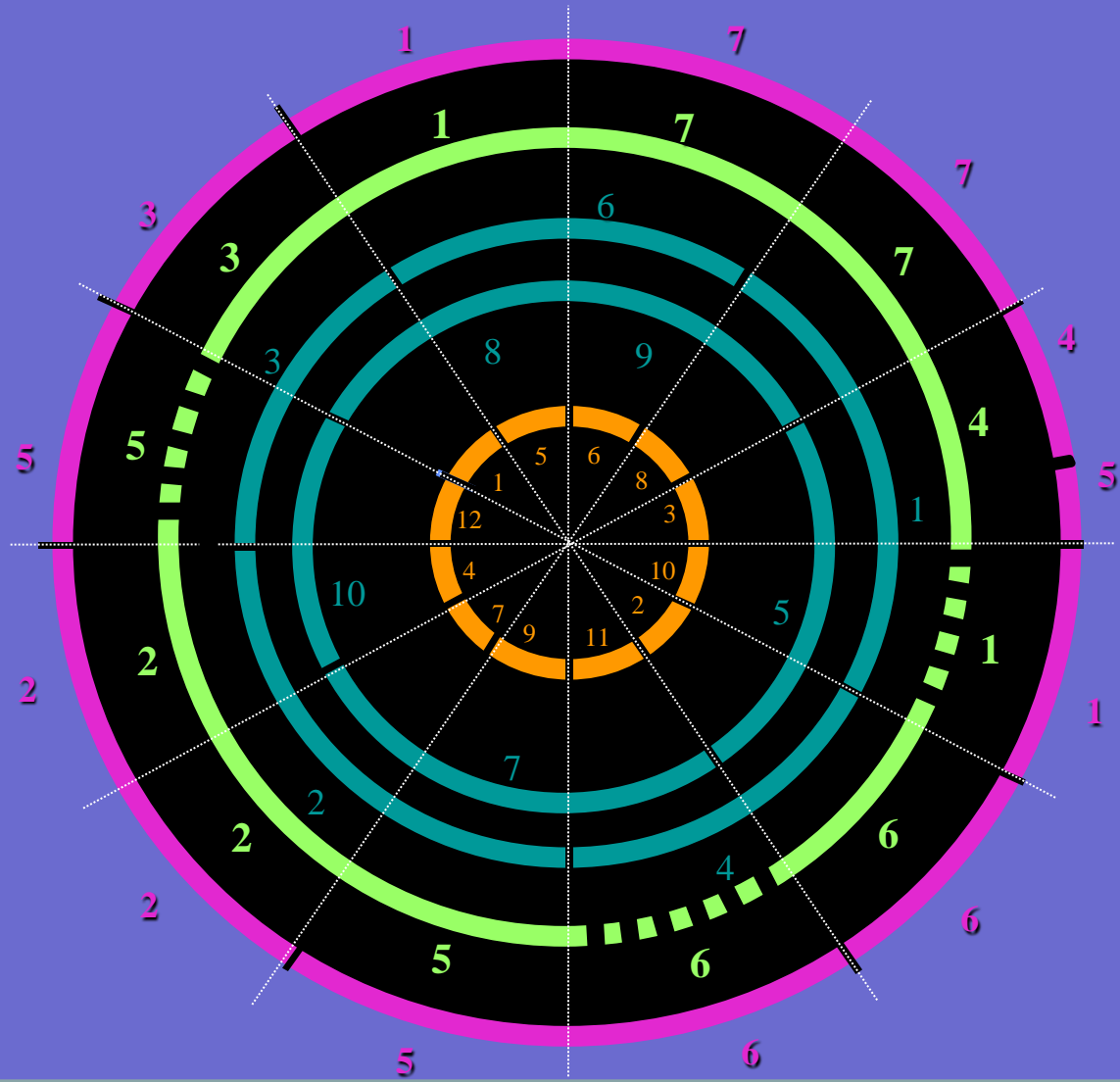
Gramineae anchor probes
(barley, oat, wheat, rice
RFLP clones)
Rice cDNAs of RGP



The reference map for the p150/112 cross based on segregation data from 240 molecular markers covering 811 cM on seven linkage groups. (Genome 45: 282-295,2002)

主要なイネ科草種のゲノム比較

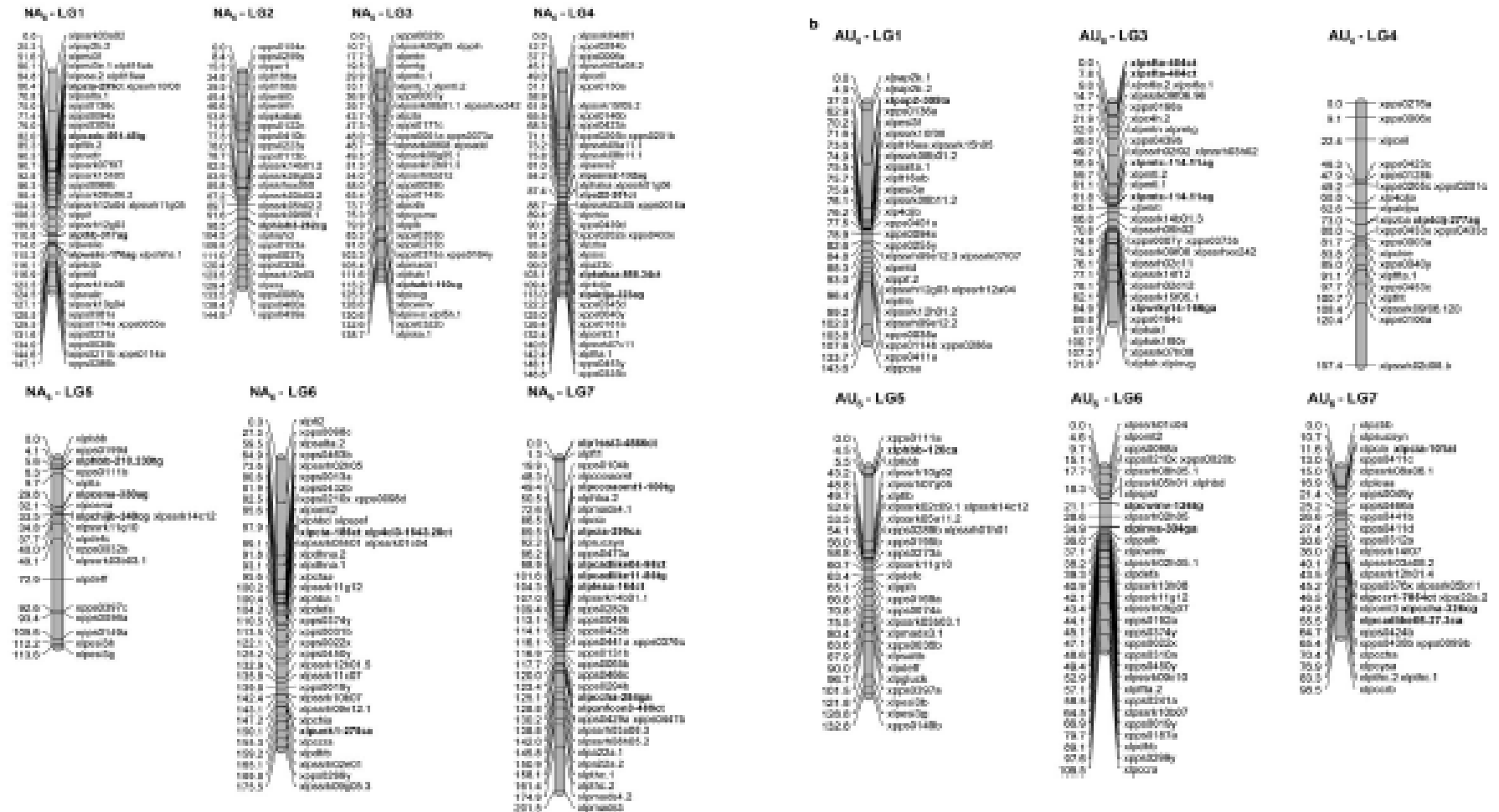
- イネ — 0.4 pg
- トウモロコシ — 2.5 pg
- ペレニアルライグラス — 2.1 pg
- コムギ — 6.0 pg



SNPマーカーによるマッピング

Cogan et al. (2006) MGG, 276, 101-112

Gene-associated SNP

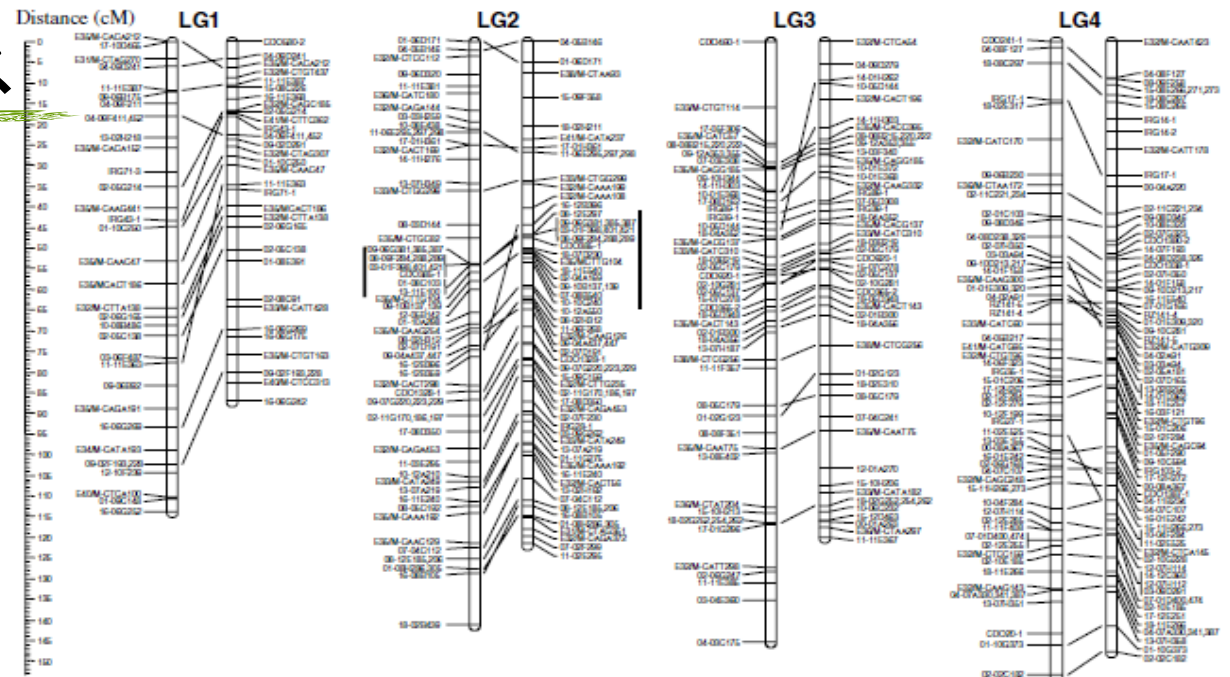


NA₆

AU₆

NA₆ x AU₆集団におけるマッピング

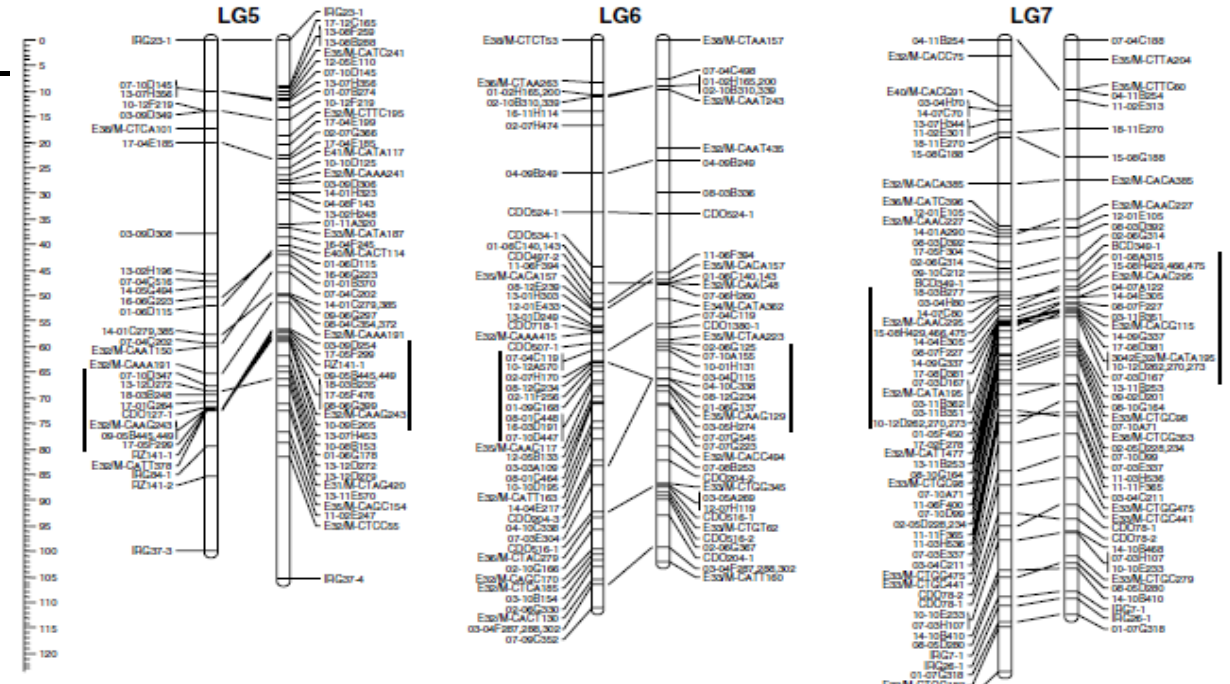
イタリアンライグラス



Hirata et al. (2006)
TAG, 113, 270-279

395個のゲノム
ライブラリー由来
SSRマーカー

218個のSSRマーカー
のマッピング



Plant and Animal Genome XX (2012)の発表から

**Susanne Barth group , Teagasc , Ireland:
Whole genome shotgun sequencing and assembly to
access the gene rich portion of the genome of *Lolium
perenne* L.**

**Torben Asp group, Aarhus University, Denmark:
De novo genome sequencing of perennial ryegrass
(*Lolium perenne*)**

**Hongwei Cai group, Japan Grassland Agriculture and
Forage Seed Association :**

Development of SNP markers for *Lolium* species

SNP markers development using the next generation sequencing data from *L. temulentum*, *L. persicum* and Italian ryegrass

チモシー

英名 : timothy

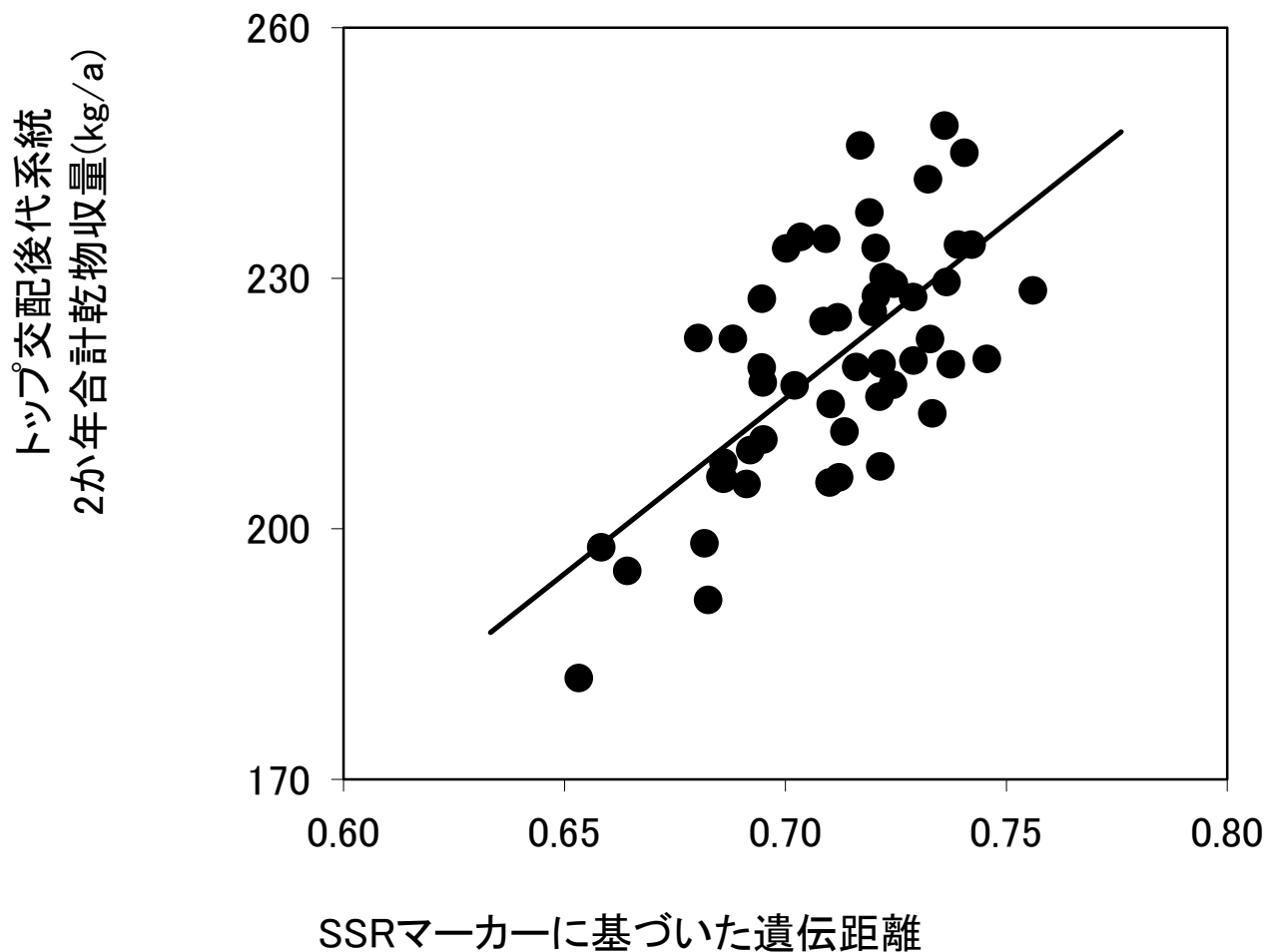
和名 : オオアワガエリ

学名 : *Phleum pratense* L.

北海道で最も広く栽培されている重要な牧草である（約7割）。再生力にはやや劣るが、越冬性はかなり優れる。牛の嗜好性が高く、主として採草用として利用されている。栽培種は六倍体である（ $2n=6x=42$ ）。

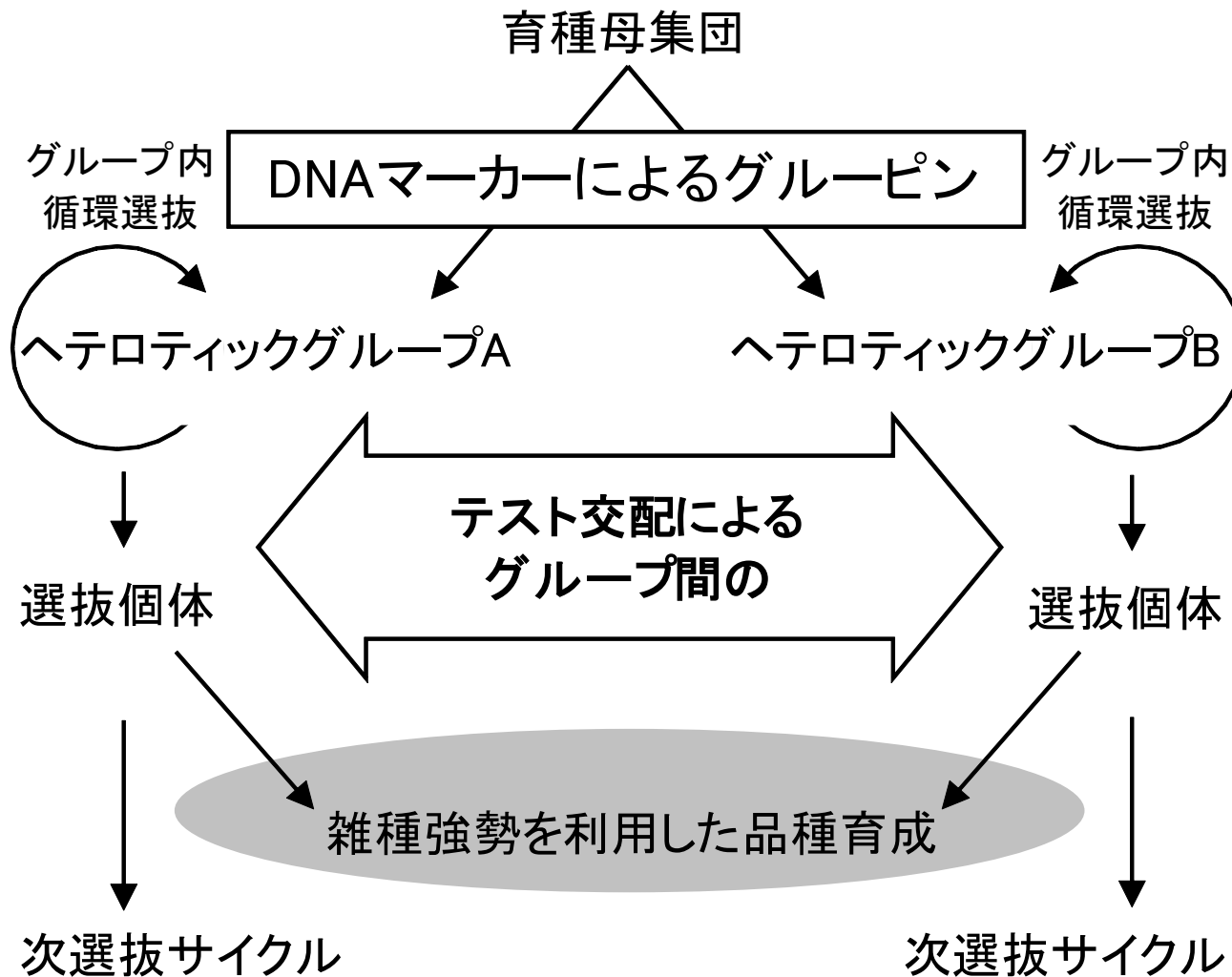


種子親-テスター間遺伝距離と トップ交配後代の収量

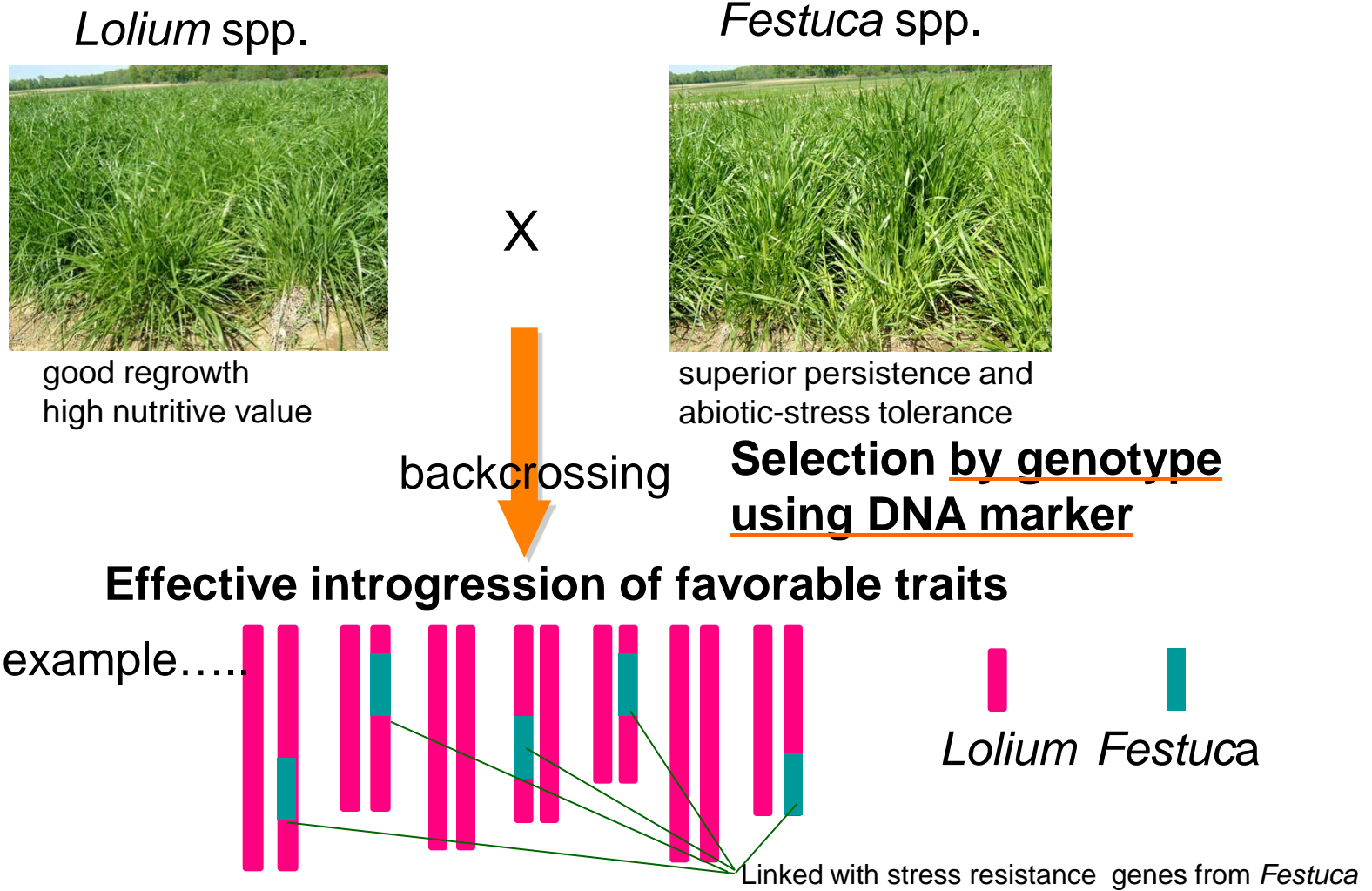


Tanaka et al., Crop Sci 51, 612-620, 2011

相反循環選抜への移行の提案



Introgression breeding using DNA-marker selection



DNA markers to distinguish between different species are required for MAS in hybrid breeding.

Species specificity of marker is important factor for hybrid breeding in which multiple genotypes are involved.

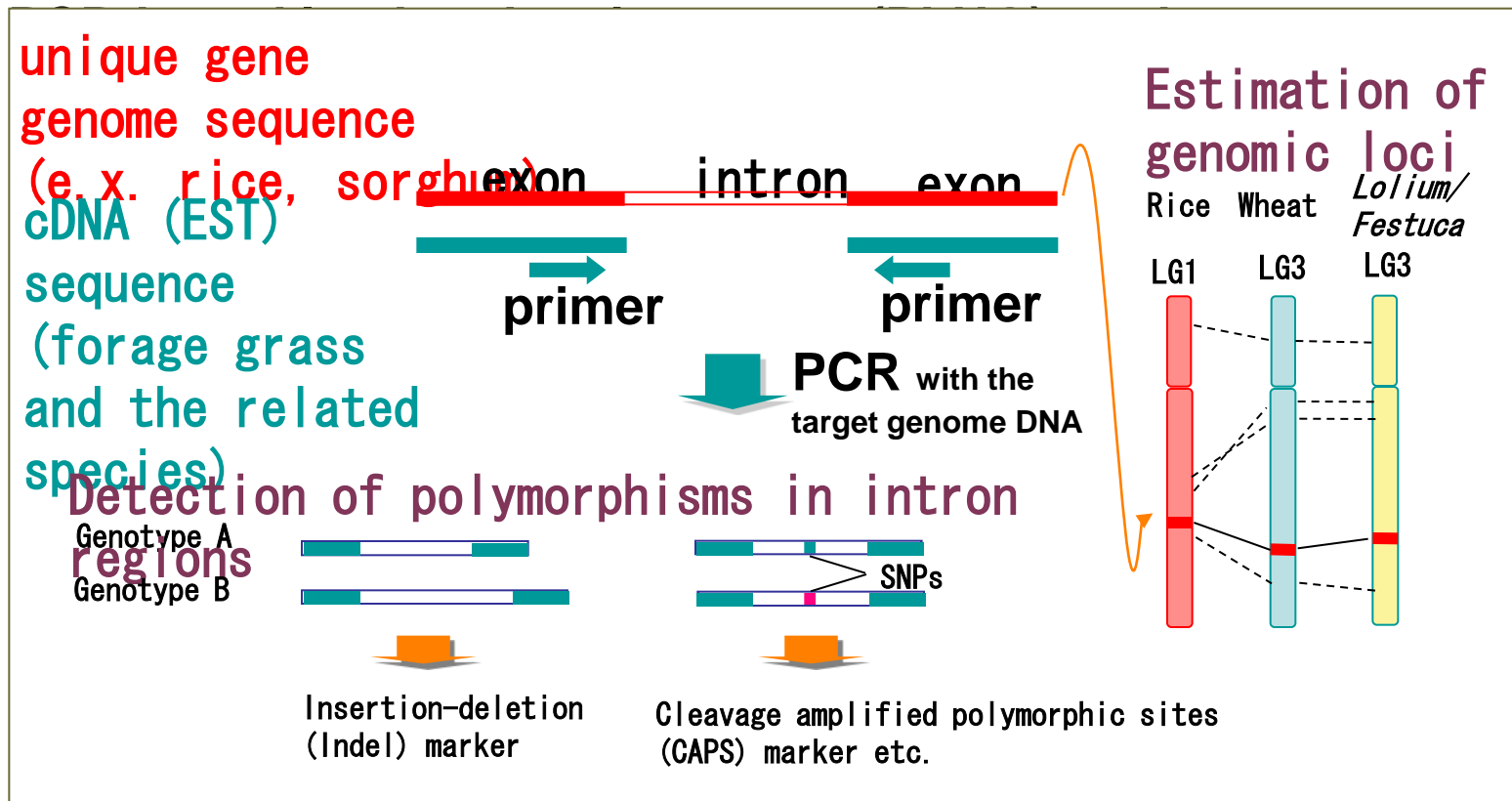
Concept of intron-flanking EST markers using comparative genomics

Intron-flanking EST marker

Wei et al. (2005) *Theoretical and Applied Genetics*

Comparative anchor-tagged sequence (CATS) marker

Fredslund et al. (2006) *BMC Genomics*

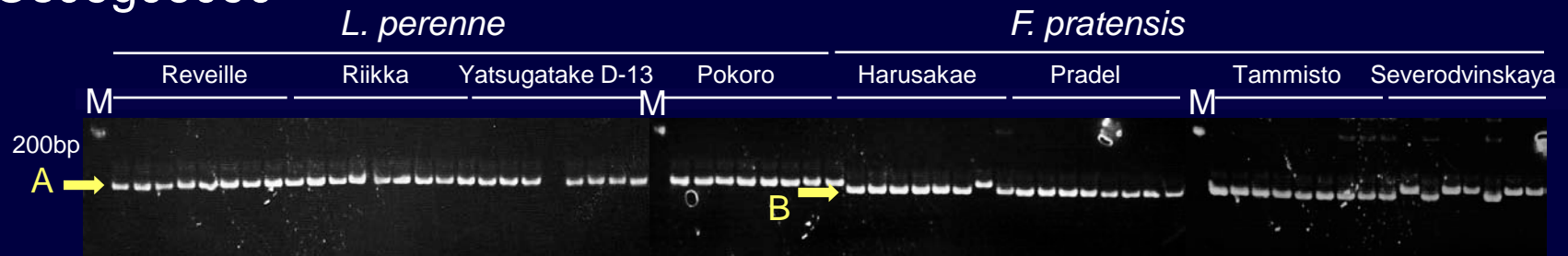


Tamura et al. (2009) TAG

Specificities of intron-flanking EST markers to *Lolium* and *Festuca* genomes

- Intron-flanking EST markers were developed by screening using each one cultivar *F. pratensis* and *L. perenne*
- Genotyping of *L. perenne* and *F. pratensis* (each 4 cultivars x 8 plants)
- Specificity index, S value was calculated
= maximum difference of fragment frequency

Os06g06090



$$\begin{aligned}
 & {}_A F_{Lp} = 0.97, {}_A F_{Fp} = 0.19, & {}_B F_{Lp} = 0, {}_B F_{Fp} = 0.81 \\
 & \text{Difference of } F_A = |0.97 - 0.19| = 0.78, & \text{Difference of } F_B = |0 - 0.81| = 0.81 \\
 & \therefore S_{Lp} = 0.78, S_{Fp} = 0.81
 \end{aligned}$$

The 2011 Eastern Japan Earthquake and Tsunami on 11th March 2011

Failure of Cooling Systems at Fukushima I Nuclear Power Plant after Earthquake and Tsunami 2011



Renewable energy



Solar energy



Wind power



Geothermal energy

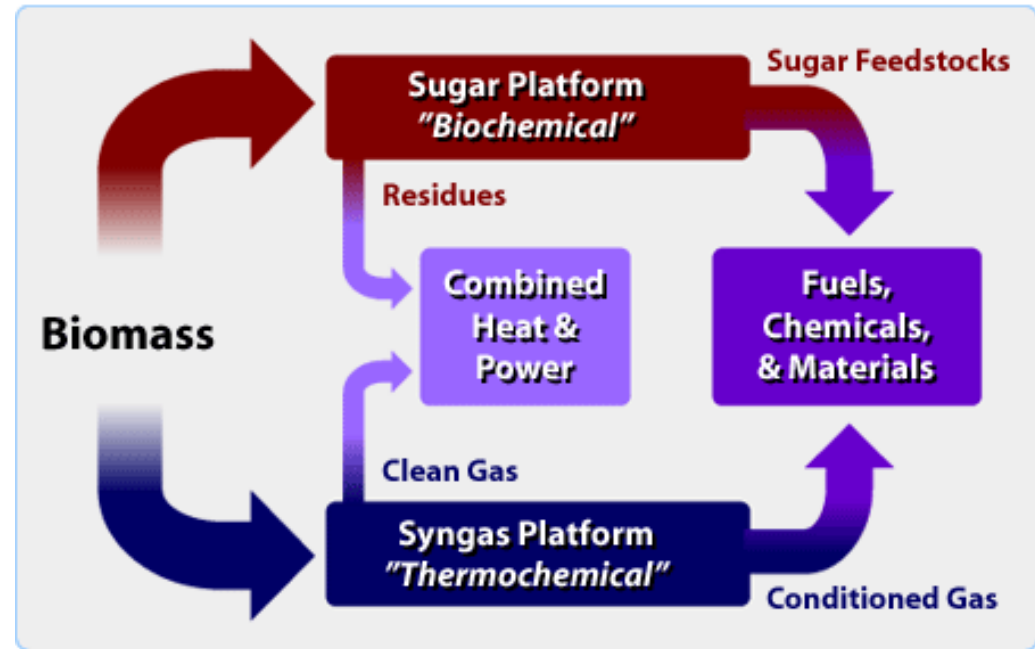


Biomass energy

Biorefinery

Feedstock production

Biorefinery Concept



Liquid transportation fuel



value-added chemicals

リグノセルロース系バイオマスの変換



コーンストーバー

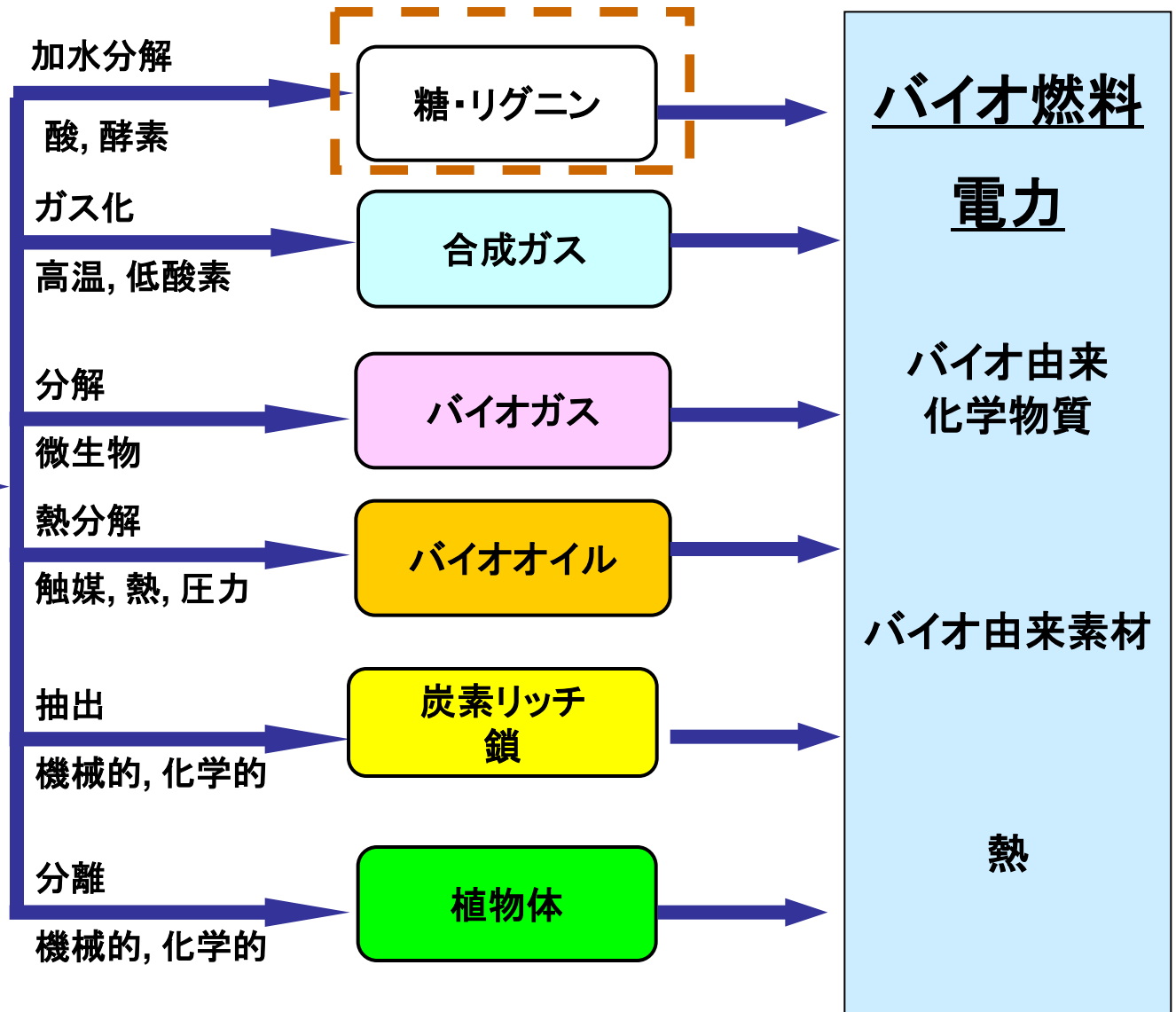


フィードストックの生産、収集、調整

スイッチグラス



雑種ポプラ



Why switchgrass as a main biofuel crop in USA?



**Native grass with high root and shoot yield compared to other grasses in USA.
High yield is achieved with low fertilizer inputs.
Great potential to improve yield through breeding, biotechnology and management.**

スイッチグラスの分布

C₄植物、2つのエコタイプが存在する

Lowlands:

バイオマス大
草丈高

湿潤、温暖な冬の
地域に分布

四倍体

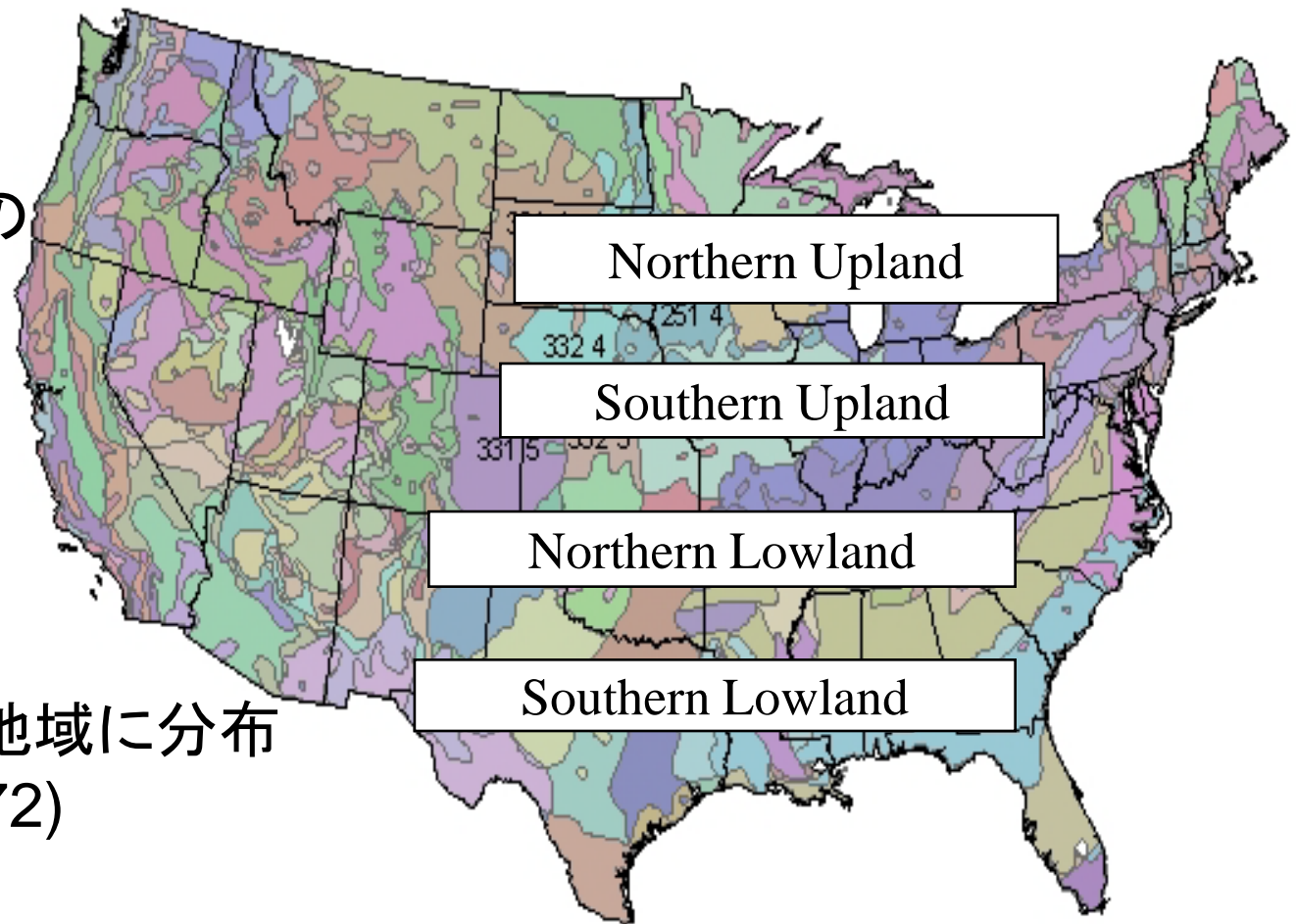
(2n=4x=36)

Uplands:

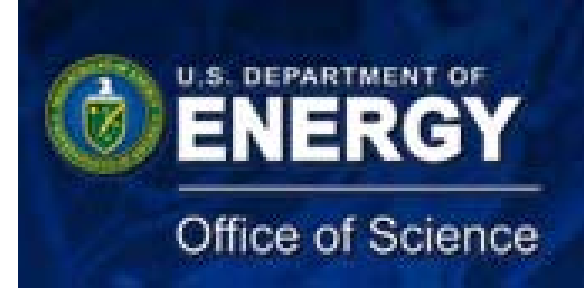
バイオマス小
草丈低

乾燥、寒い冬の地域に分布

八倍体(2n=4x=72)



Chloroplast *trnL* (UAA) intron DNA でエコタイプの分類可能 (Missaoui et al. 2006)



<http://genomicscience.energy.gov/pubs/switchgrassreport.pdf>
Switchgrass Research Group: Progress Report
January 2012

- "Association Mapping of Cell Wall Synthesis Regulatory Genes and Cell Wall Quality in Switchgrass"
- "Linkage Analysis Appropriate for Comparative Genome Analysis and Trait Selection in Switchgrass"
- "Developing Association Mapping in Polyploid Perennial Biofuel Grasses"
- "Translational Genomics for the Improvement of Switchgrass"
- "The Hunt for Green Every April: Factors Affecting Fitness in Switchgrass"
- "The Role of Small RNA in Biomass Deposition and Perenniality in Andropogoneae Feedstocks"

資源作物のゲノム研究の 対象植物



Foxtail millet



*Brachypodium
distachyon*



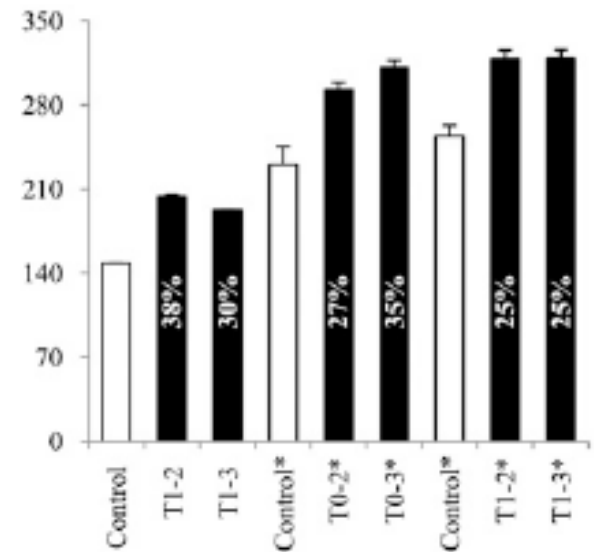
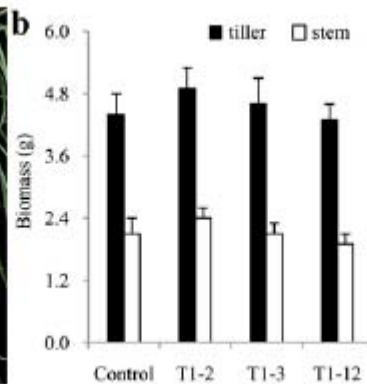
Sweet
Sorghum

Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass

Chunxiang Fu^{a,1}, Jonathan R. Mielenz^{b,c,1}, Xirong Xiao^{a,c}, Yaxin Ge^a, Choo Y. Hamilton^{b,c}, Miguel Rodriguez, Jr.^{b,c}, Fang Chen^{c,d}, Marcus Foston^{c,e}, Arthur Ragauskas^{c,e}, Joseph Bouton^a, Richard A. Dixon^{c,d,2}, and Zeng-Yu Wang^{a,c,2}

^aForage Improvement Division and ^dPlant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401; ^bBiosciences Division and ^eBioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, 37831; and ^cSchool of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332

PNAS 108:3803-3808 (2011)



Plant and Animal Genome XX (2012)の発表から

Insights into Switchgrass Genome Structure and Organization

A total of 330,297 high quality BAC-end sequences (BES) were generated, accounting for 263.2 Mbp (16.4%) of the switchgrass genome. Analysis of the BES identified 279,099 known repetitive elements, >50,000 SSRs and 2,528 novel repeat elements, named switchgrass repetitive elements (SREs). A total of 48,000 clones from each library were organized into pools and superpools (~7X coverage) and established an efficient qPCR-based screening system. 300 BACs carrying cell wall and defense response-related genes were selected and 176 are sequenced to full-length providing complete genomic sequences of rice orthologs of 259 kinases, 118 glycosyltransferases, 84 glycoside hydrolases and 13 ethylene response factors (ERFs). Comparative mapping of coding regions from 100 full-length BAC sequences and 330K BES revealed high levels of synteny with the grass genomes sorghum, rice, maize and *Brachypodium*.

Genetic Improvement of Switchgrass Feedstock for Biofuel Production

Transgenic switchgrass plant biomass showed a 30% reduction in lignin content and 57% more efficient in fermentable sugar release for biofuel production.

Genomics to Feed a Switchgrass Breeding Program

The switchgrass breeding program operated by USDA-ARS includes (1) development of single nucleotide polymorphic (SNP) markers within candidate genes that are associated with endogenous genetic variation for lignin and fermentability, (2) development of SNP markers within genes for flowering time and developmental traits for the purpose of creating reproductively compatible upland and lowland populations, and (3) development of robust and repeatable SNP marker systems and breeding methods that can be used to implement genomic selection (GS).

Characterization of the Genetic Diversity of Switchgrass Using Genotyping by Sequencing

About 1000 individuals were genotyped using GBS. Using a creative SNP calling method designed for species without reference genomes, more than one million high density SNP markers were generated.



ミスカンサス
(*Miscanthus* spp.)



Stef Reid,
Runner

Providing advanced biofuels for London 2012.



At BP, we're dedicated to fuelling the success of London 2012. We're supporting British athletes like Stef Reid and also working to provide some of the official vehicles with advanced, lower carbon fuels made from energy grasses. Find out more at bp.com/2012



London 2012. Fuelling the Future.

Crop	Average Productivity (MT ha⁻¹ year⁻¹)	Ethanol yield (liter ha⁻¹)	Seasonal water requirements (cm year⁻¹)	Tolerance to drought	Nitrogen Requirements (kg ha⁻¹ year⁻¹)
Corn		3,800 (total)	50-80	low	90-120
Grain	7	2,900			
Stover	3	900			
Sugarcane	80 (wet)	9,950 (total)	150-250	moderate	0-100
Sugar	11	6,900			
Bagasse	10	3,000			
<i>Miscanthus</i>	15-40	4,600-12,400	75-120	low	0-15
Poplar	5-11	1,500-3,400	70-105	moderate	0-50
<i>Agave</i> ssp.	10-34	3,000-10,500	30-80	high	0-12

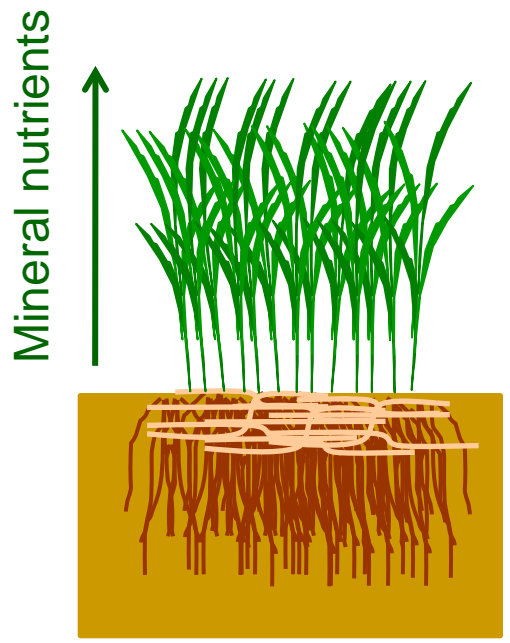
Somerville et al. (2010) Science 329: 790-792

Advantage of Perennial Grasses for Biomass Production

- ✓ A low demand for nutrient inputs
- ✓ Higher yields on relatively poor quality land
- ✓ Longer persistency
- ✓ Increase in soil carbon content
- ✓ Effect on stability and cover value for wildlife

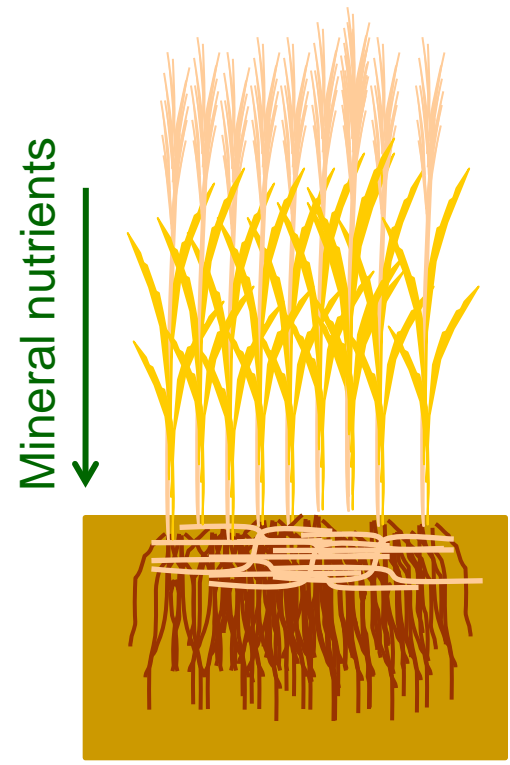
Nitrogen Use Efficiency Theory for Perennials

Spring and Summer



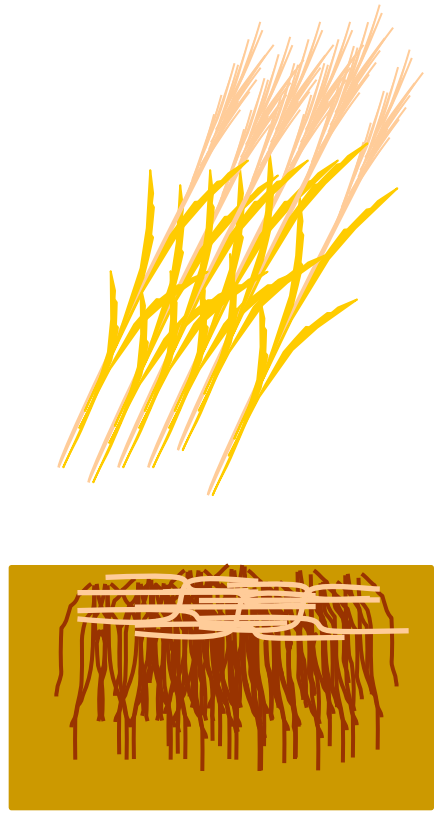
Translocation from rhizomes to growing shoot

Fall



Translocation to rhizomes as shoot senescences

Winter



Dry shoots harvested, nutrients stay in rhizomes

C accumulation rate into soil in Aso, Kumamoto, Japan



Miscanthus sinensis grassland

503 kg C ha⁻¹

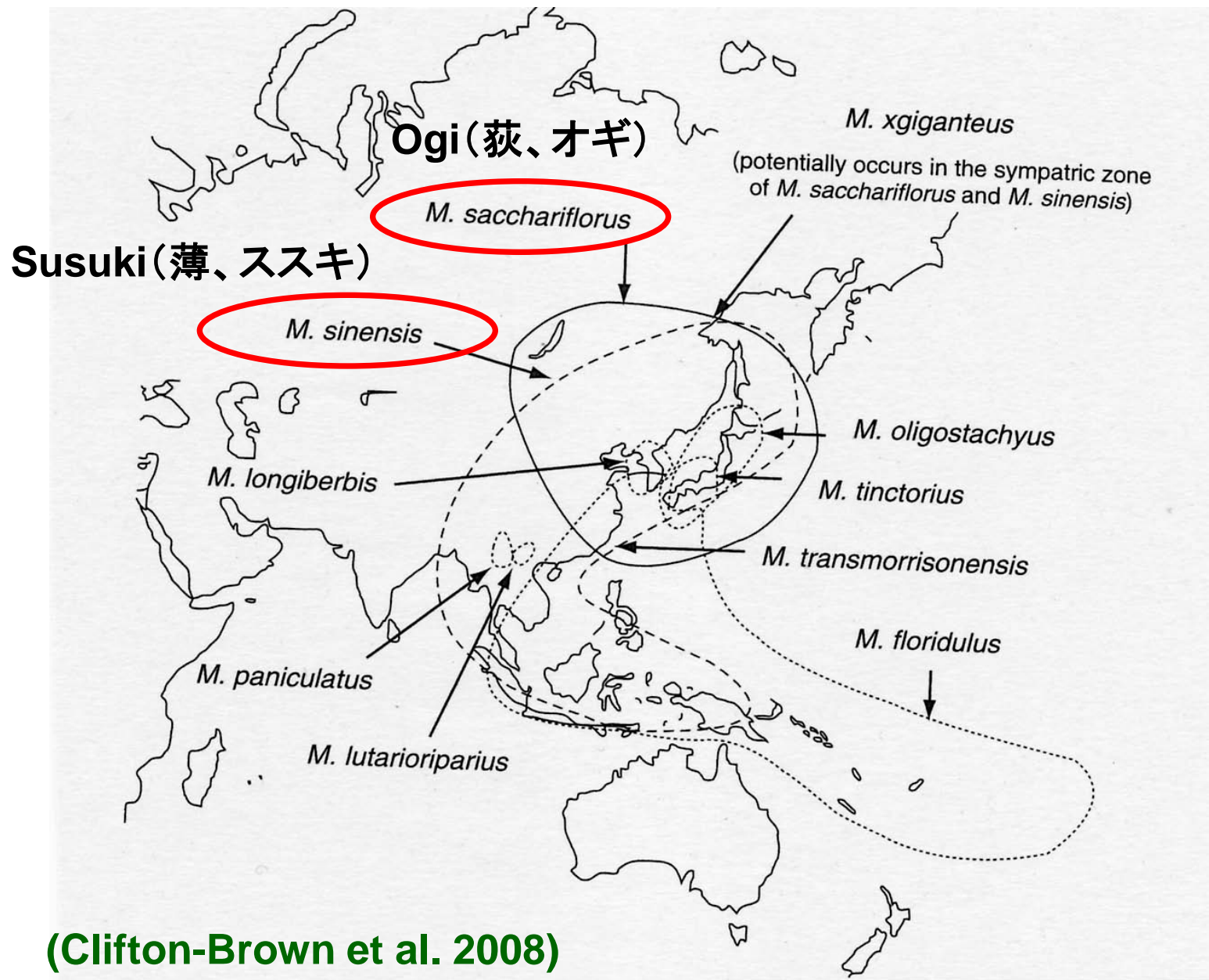
1.8 times higher in grassland than in forest



Cryptomeria japonica forest plantation

284kg C ha⁻¹ yr⁻¹

Geographical Distribution of the *Miscanthus* spp.



(Clifton-Brown et al. 2008)

Use of *Miscanthus* plants in Japan

- Roof materials of traditional houses
- Animal feeds
- Component of manure



Traditional house
with a thatched roof

Tourist attraction

World heritage



Shirakawa-go Village



Gokayama Village



Burning



Cutting for forage



Grazing

**Biotic pressures involved
maintenance of semi-natural
Miscanthus grassland**

Famous *Miscanthus* grassland in Japan



Aso (Kumamoto)



Soni (Nara)



Kawatabi (Miyagi)



Sugadaira (Nagano)



Miscanthus* × *giganteus

(Giant Miscanthus)

Triploid natural hybrid:

M. sinensis ×

M. sacchariflorus

Introduction to Denmark in
1935 from Japan as an
ornamental variety

Potential energy crop
since oil crisis happened
in 1970.

“Susuki” $2n=38$

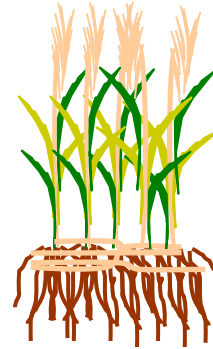
Miscanthus sinensis



Compact roots

“Ogi” $2n=4x=76$

Miscanthus sacchariflorus



Rhizomes

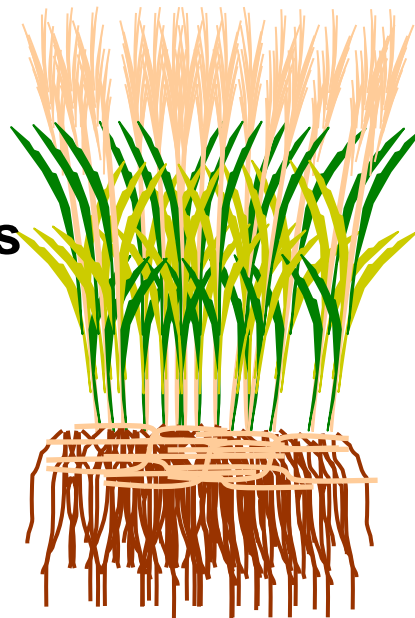
×

Natural hybrid

Triploid ($3n=57$)

M. x giganteus

Giants Miscanthus



High biomass production
(30-45 t/ha/yr)

Hybrid vigor

High density

Collection of new natural hybrids and artificial crosses will be important.

Why Use *Miscanthus*

- ✓ C4 photosynthesis
- ✓ High photosynthesis level at low temperature
- ✓ High energy ratio (output/input) 22-50
- ✓ Perenniality

Disadvantage of *M. x giganteus*

- ✓ High establishment costs of sterile triploid
- ✓ Narrow genetic background
- ✓ Less winter hardiness, especially first winter at established year

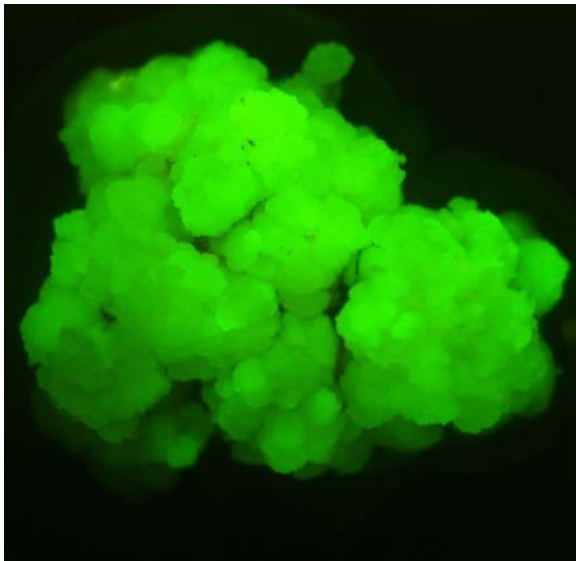
M. sacchariflorus collection in Japan



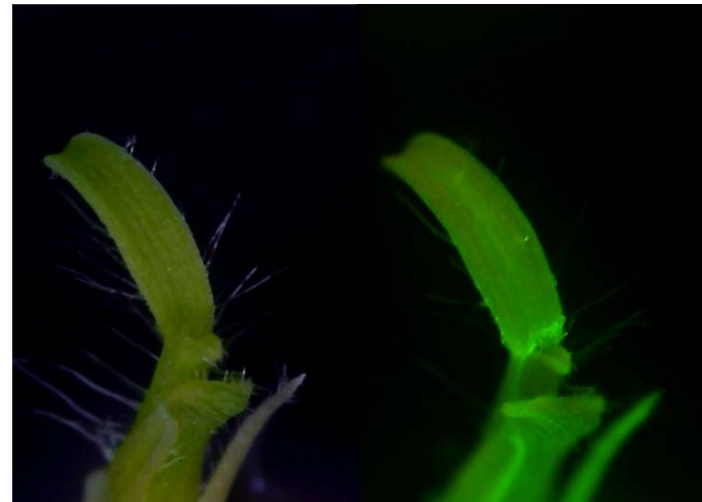


First genetically modified *Miscanthus* grass developed

Transformation system of *Miscanthus sinensis* by reporter *gfp* (green fluorescence protein) gene



GFP-illuminated genetically transformed *Miscanthus* callus

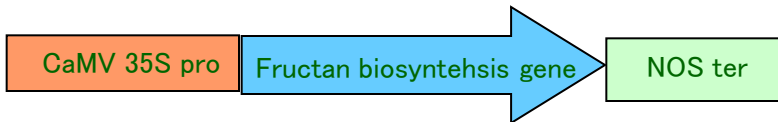


GFP-illuminated genetically transformed *Miscanthus* leaf

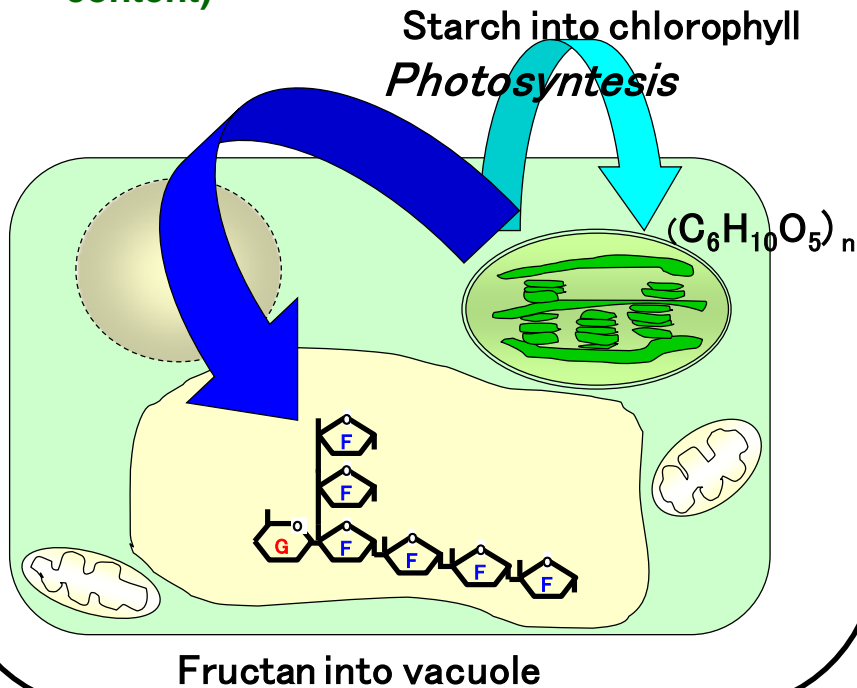
Transgenic *Miscanthus* works

Altering the content of components of cell wall by transgenic approach (carbohydrates and lignin)

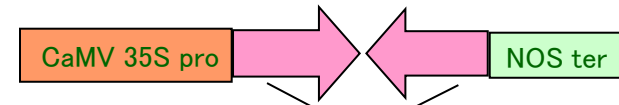
Introduction of fructan biosynthesis genes



Transgenic plants with ability to synthesis of fructan (increasing carbohydrate and reduction of lignin content)

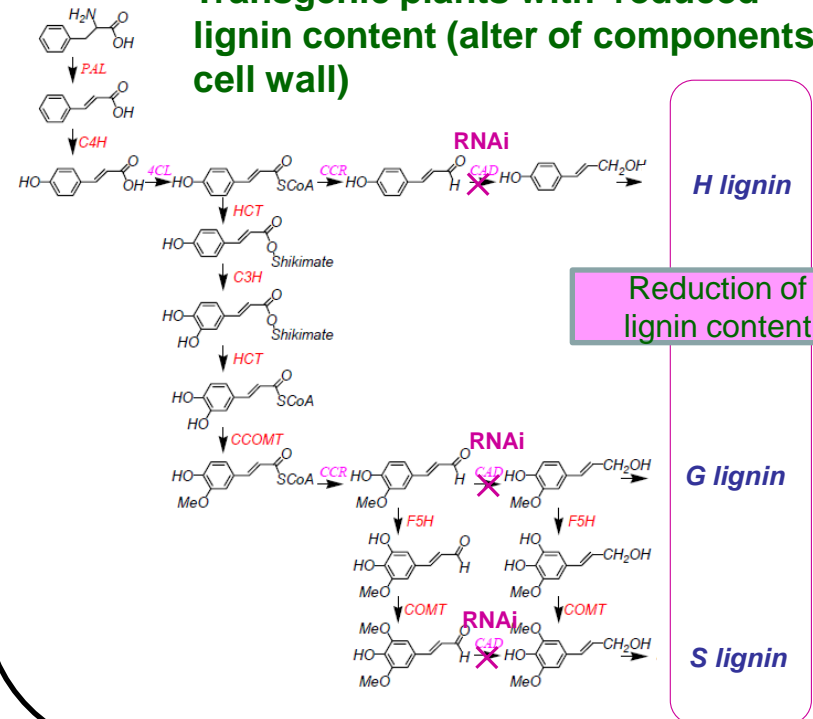


Down-regulation of Lignin genes by RNAi



Lignin biosynthesis genes (CAD, COMT) partial sequences

Transgenic plants with reduced lignin content (alter of components of cell wall)



Carbon recyclable refinery system



Inedible biomass

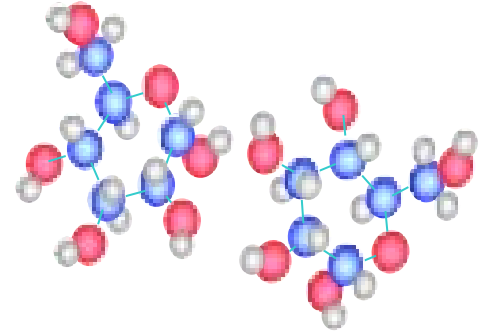
↑ biodegradation



Bioplastics

Point 1

New catalyst
→
Selective refinery



Cellulose hydrolysate
(glucose as a main carbon source for microbes)

Refinery

Carbon recycle

Point 2

High assimilation selectivity of glucose

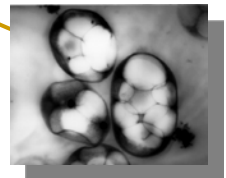
Value-added materials

manufacturing

Point 3

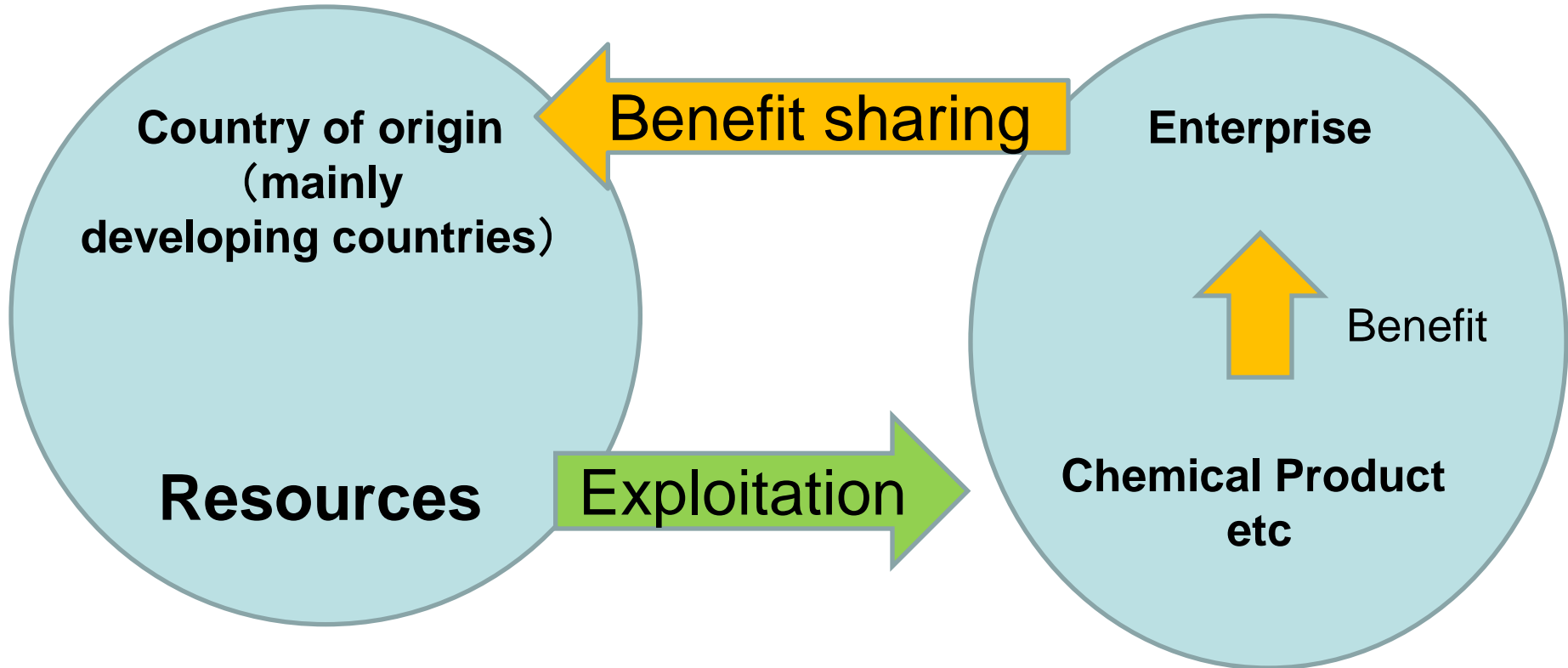


Microbial factory



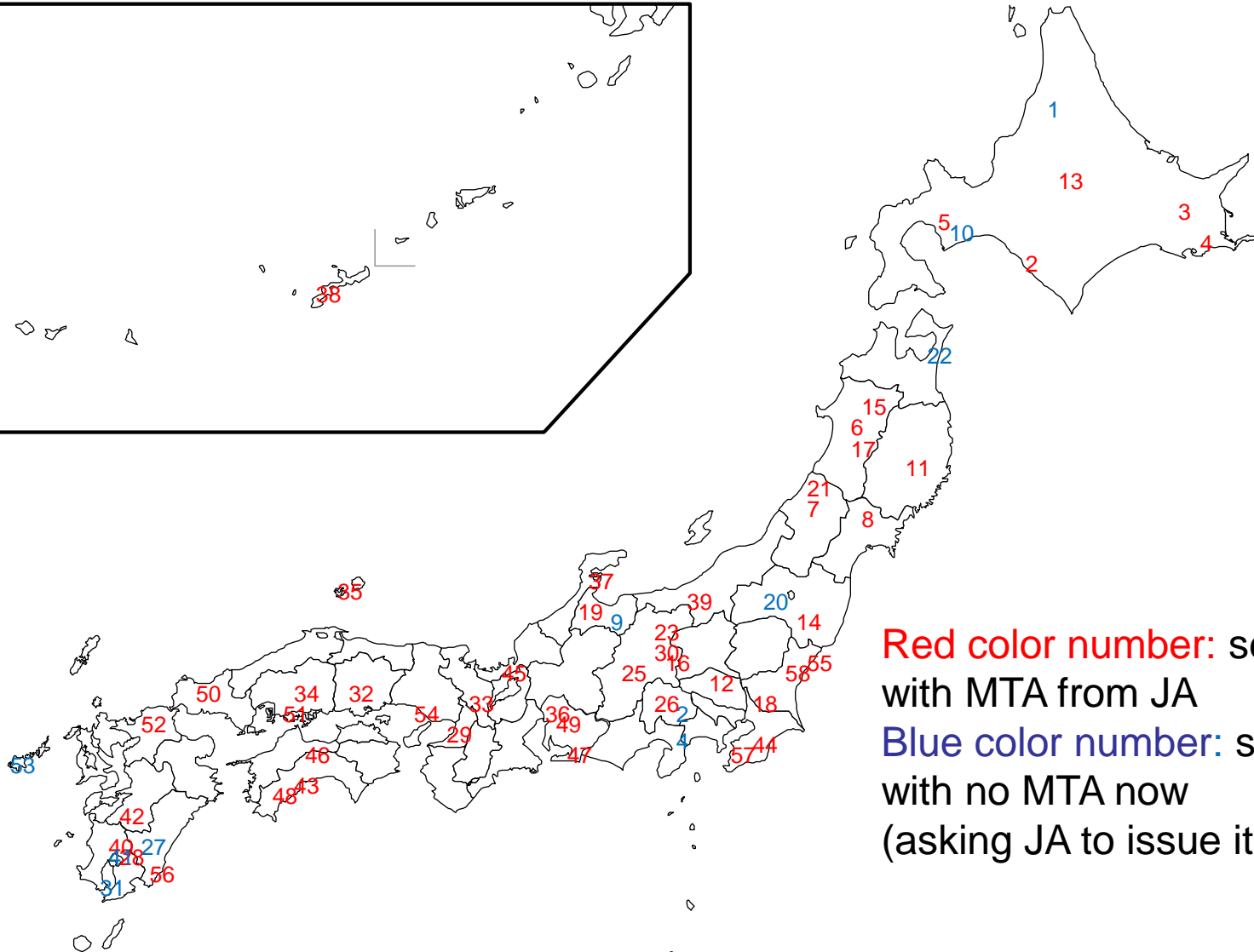
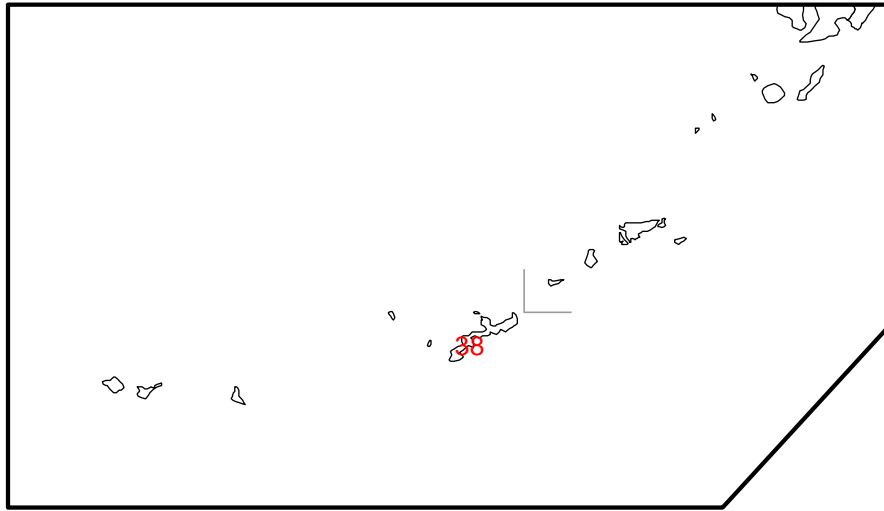
- One-pot polymerization
- under mild reaction conditions

ABS (Access and Benefit Sharing)



The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity was adopted by COP 10 meeting on 29 October 2010 in Nagoya, Japan.

Collection of *Miscanthus* seeds through Japan Agricultural Cooperatives (JAs) under the contracts of MTA by EBI grant



Red color number: seeds with MTA from JA
Blue color number: seeds with no MTA now (asking JA to issue it)

International collaboration

DOE and USDA Fund New Project for Biomass Genomics Research, 2011-2014

Quantifying Phenotypic and Genetic Diversity of *Miscanthus sinensis* as a Resource for Knowledge-Based Improvement of *M. × giganteus* (*M. sinensis* × *M. sacchariflorus*)

INVESTIGATORS: Erik J. Sacks, Joe Brummer, Megan Hall, Stephen Long, Junhua Peng, **Toshihiko Yamada**, and Chang Yeon Yu

INSTITUTIONS: University of Illinois; Colorado State University; University of California, Berkeley; Wuhan Botanical Garden; **Hokkaido University**; Kangwon National University

