

# ゲノムワイド解析技術が拓く 計量形態学

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道具としての「形態測定学」: 量的phenotypingの活用法

## 次世代シーケンサー(NGS)が もたらしたゲノミックスの新時代



1ランで600 Gb

ヒトゲノム(約30億塩基対)  
の約200倍、  
イネゲノム(約4億塩基対)  
の約1500倍

<http://www.illumina.com>



Oxford Nanopore Technologies  
USBメモリサイズの  
シーケンサー

\$900で数10 Gb

<http://www.nanoporetech.com/>

# RRLs, RAD-seq, GBS ...

STUDY DESIGNS

## Genome-wide genetic marker discovery and genotyping using next-generation sequencing

John W. Davey\*, Paul A. Hohenlohe<sup>†</sup>, Paul D. Etter<sup>§</sup>, Jason Q. Boone<sup>||</sup>, Julian M. Catchen<sup>†</sup> and Mark L. Blaxter\*\*<sup>†</sup>

Nature Reviews | Genetics | Vol 12 | 2011 | 499

Threespine stickleback  
(*Gasterosteus aculeatus*)



100個体  
45,000SNPsをジェノタイプング



ゲノムワイド集団遺伝学

Hohenlohe et al. 2010 PLoS Genetics 6(2): e1000862

Spotted gar (*Lepisosteus oculatus*)



F1集団94個体をジェノタイプング  
65,000タグのうち15,076が多型



連鎖地図(8406マーカー)

Amores et al. 2011 Genetics 188: 799

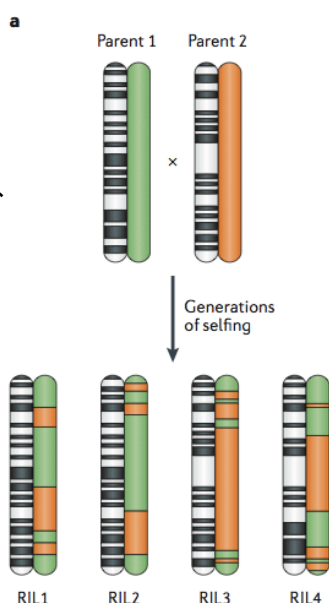
## 交配実験を行わずに遺伝子検出

(Genome-wide association study: GWAS)

従来の解析法

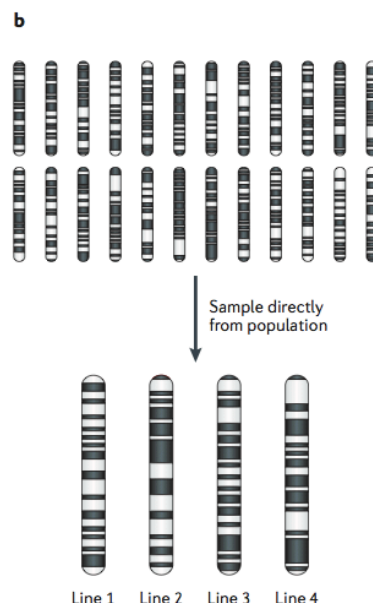
少数の親を交配し、  
その後代を解析

理由:  
親間の違い(色の違い)は分かっていても、  
詳細なDNA多型(シマシマ)まで見分けられなかった



Morrell et al. (2012) Nature Review Genetics 13:85

解像度が低い、  
材料作りに時間がかかる



GWAS

多数の個体(品種)を  
そのまま解析

理由:  
品種間に見られる  
詳細なDNA多型(シマシマ)が  
見分けられるようになった

解像度が高い、  
交配を行う必要がない

# ゲノムワイドマーカーによる選抜： ゲノミックセレクション

NATURE Vol 457 22 January 2009

NEWS

← 24頭のウシの、54,609箇所のDNA多型を、一度に解析できるチップ



**GOT A NEWS TIP?**  
Send any article ideas for Nature's News section to: [newstips@nature.com](mailto:newstips@nature.com)

## No bull: genes for better milk

On 13 January, the US Department of Agriculture (USDA) launched a service that allows dairy-cattle breeders to double their chances of selecting the best bulls to sire milk-producing cows.



about \$225, and can be done when a bull is born, thus avoiding the \$25,000–50,000 cost of raising a bull for five years to see if it sires good milk-producing offspring. “The best bulls become elite breeders.”

Using high-throughput analysis, the researchers could then compare the DNA from a young dairy bull against the chip SNPs, telling breeders which bull would be likely to sire calves that were good milk producers. The test costs

about \$225, and can be done when a bull is born, thus avoiding the \$25,000–50,000 cost of raising a bull for five years to see if it sires good milk-producing offspring. “The best bulls become elite breeders,” says Van Tassell, “The others become hamburger.”

Cost-million research project directed by Curtis Van Tassell, a geneticist at the USDA's Bovine Functional Genomics Laboratory in Beltsville, Maryland. Working with Illumina Inc. of San Diego, California, Van Tassell's team created a microarray chip containing 54,000 genetic markers called single nucleotide

a dozen traits, including milk quality and production.

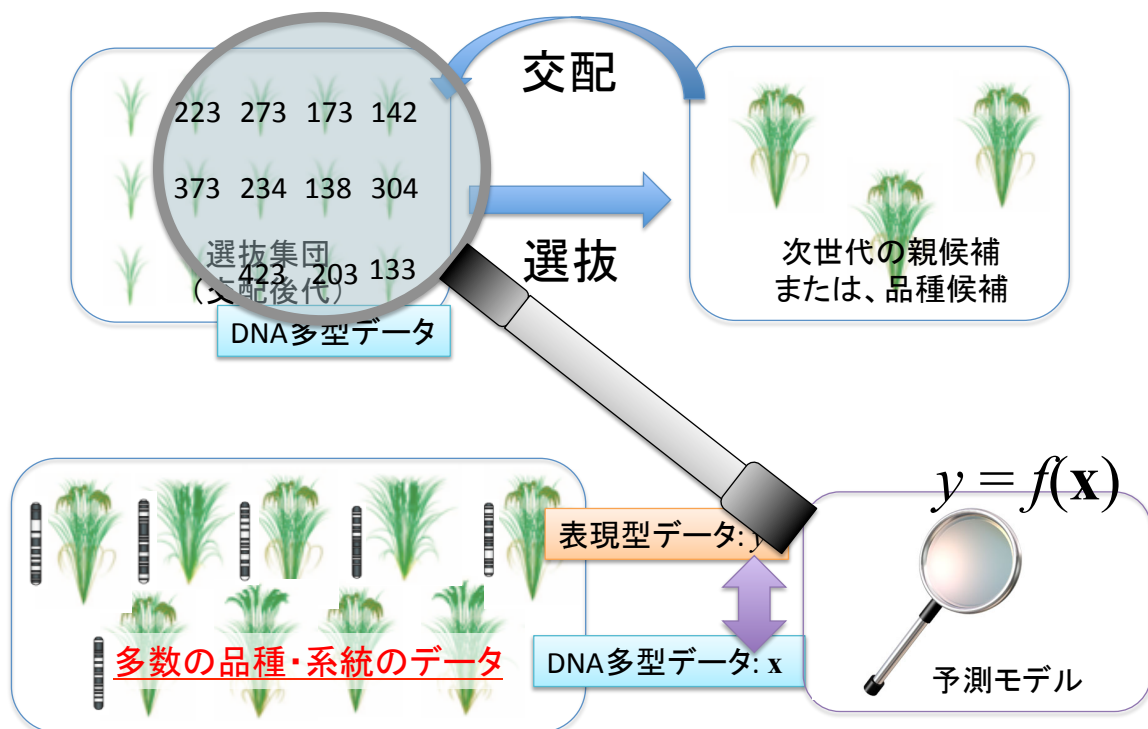
Using high-throughput analysis, researchers could then compare the DNA from a young dairy bull against the chip SNPs, telling breeders which bull would be likely to sire calves that were good milk producers. The test costs

Aarhus University in Denmark, and in France and Australia. Rex Dalton

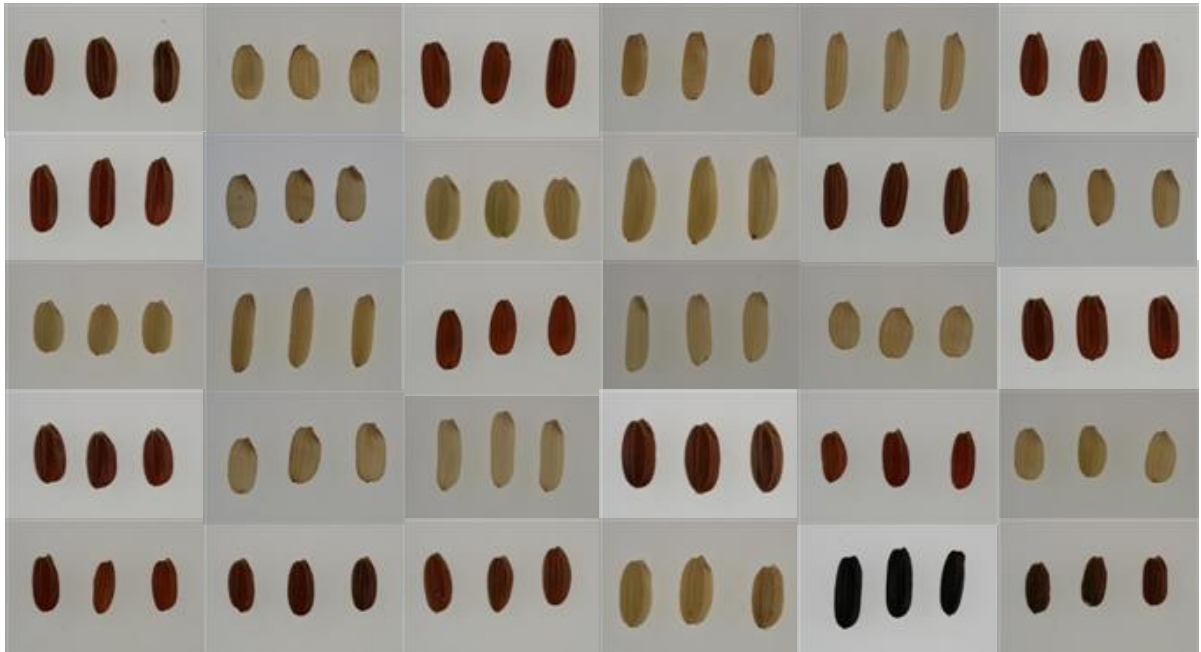
Published online 21 January 2009 | Nature 457, 369 (2009) | doi:10.1038/457369a

5

## Genomic selection (GS)



# アジア栽培イネ *Oryza sativa* の 玄米形変異

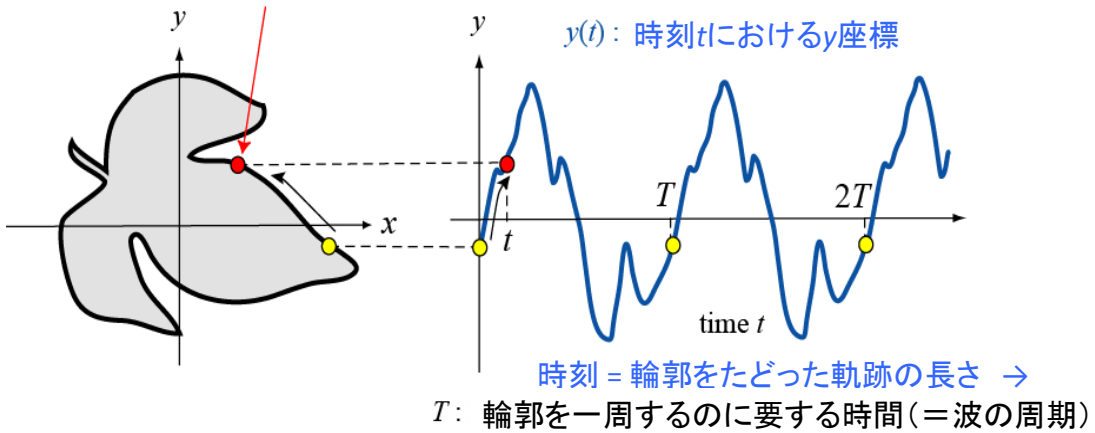


## 玄米形の解析

- 関連遺伝子の検出とその遺伝効果の推定
  - ゲノムワイドアソシエーション解析 (GWAS):  
玄米形変異に関与する**遺伝子の検出**
- 望ましい遺伝子型の効率的選抜
  - ゲノミックセレクション (GS):  
ゲノムワイドマーカーを用いて**玄米形を予測する**

# 楕円フーリエ記述子

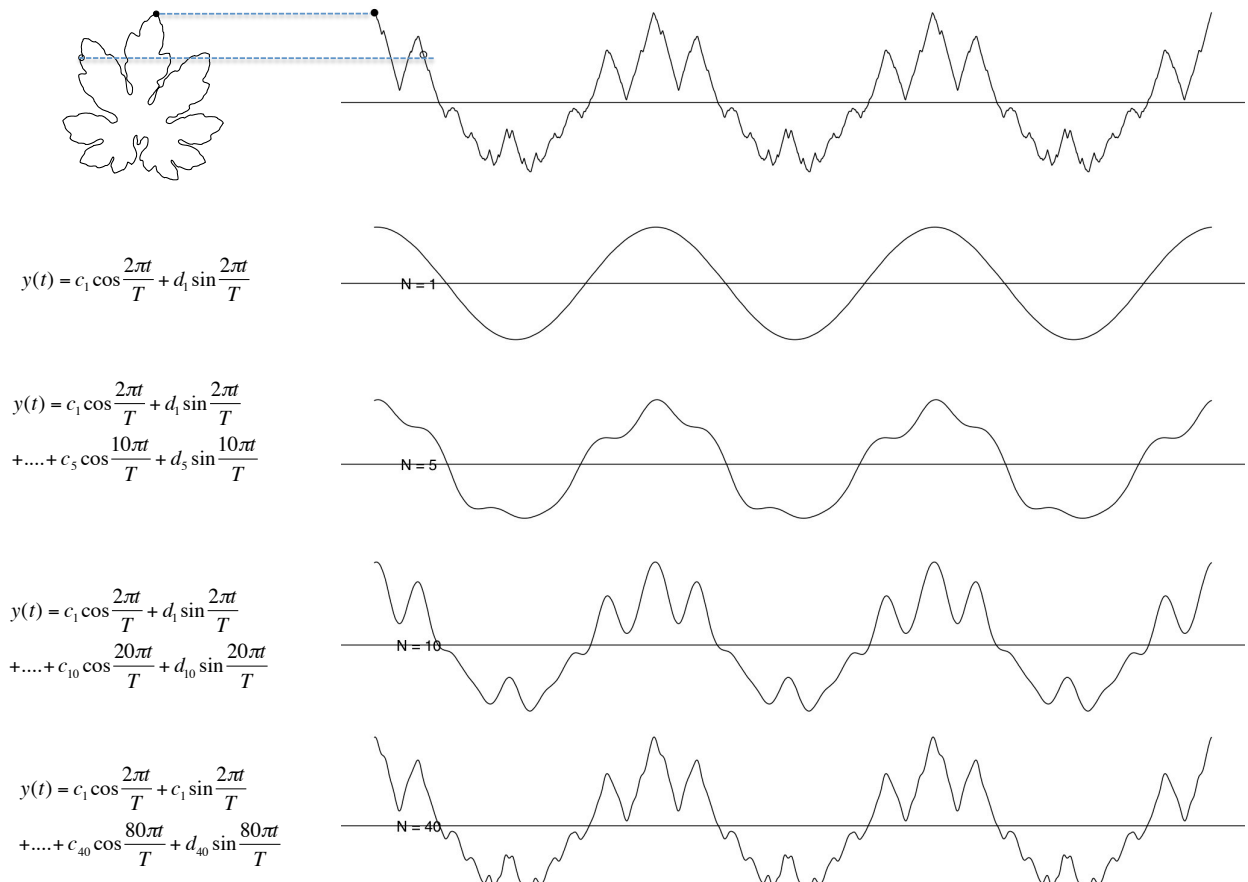
輪郭を一定速度でたどる



輪郭の形を波の形としてとらえ、波形関数のフーリエ級数展開により、形を数学的に記述する方法

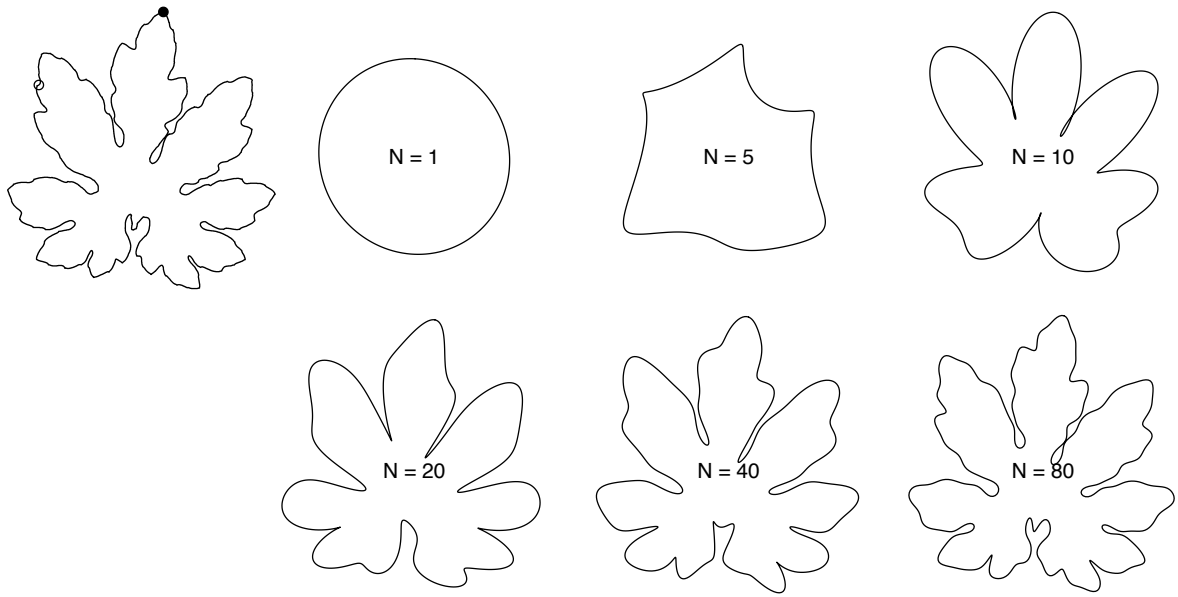
$$y(t) = \sum_{i=0}^N \left( c_n \cos \frac{2n\pi t}{T} + d_n \sin \frac{2n\pi t}{T} \right)$$

9



$x(t)$  についても同じように波を重ね合わせる際の重み (フーリエ係数) を求める

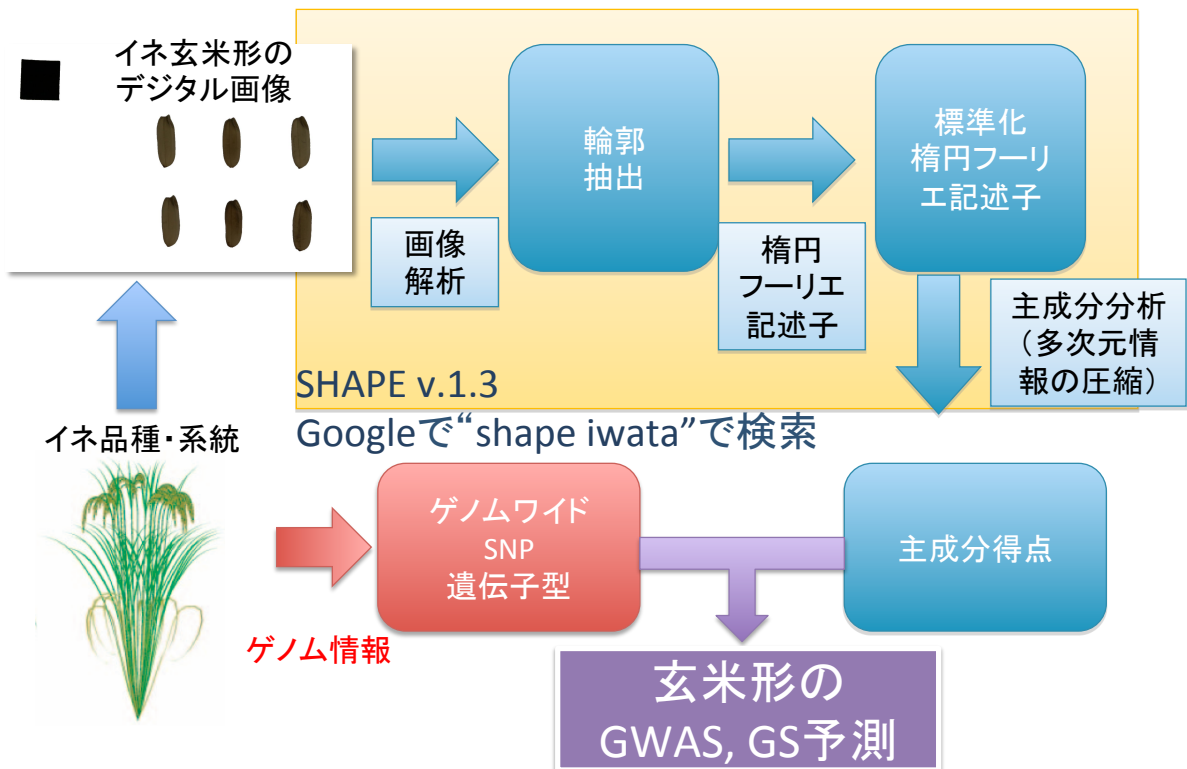
# 楕円フーリエ記述子による近似



*N*を大きくすればするほど（より周期の短い波まで足し合わせれば合わせるほど）近似はよくなっていく（ただし、あまり大きくすると不要な変動（ノイズ）を捨てる）

11

## 解析の流れ



# 解析材料

## データセット S1

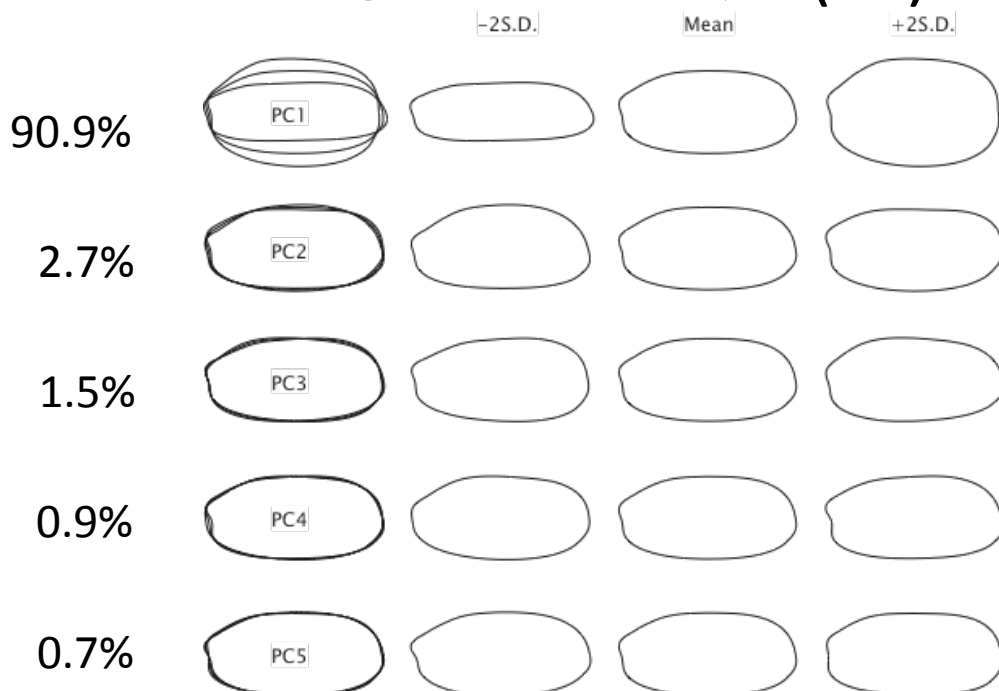
- 179 アクセッション  
(インディカ、ジャポニカの両方を含む。多くは在来品種)
- 3753 SNPs  
(fastPHASEで補完)

## データセット S2

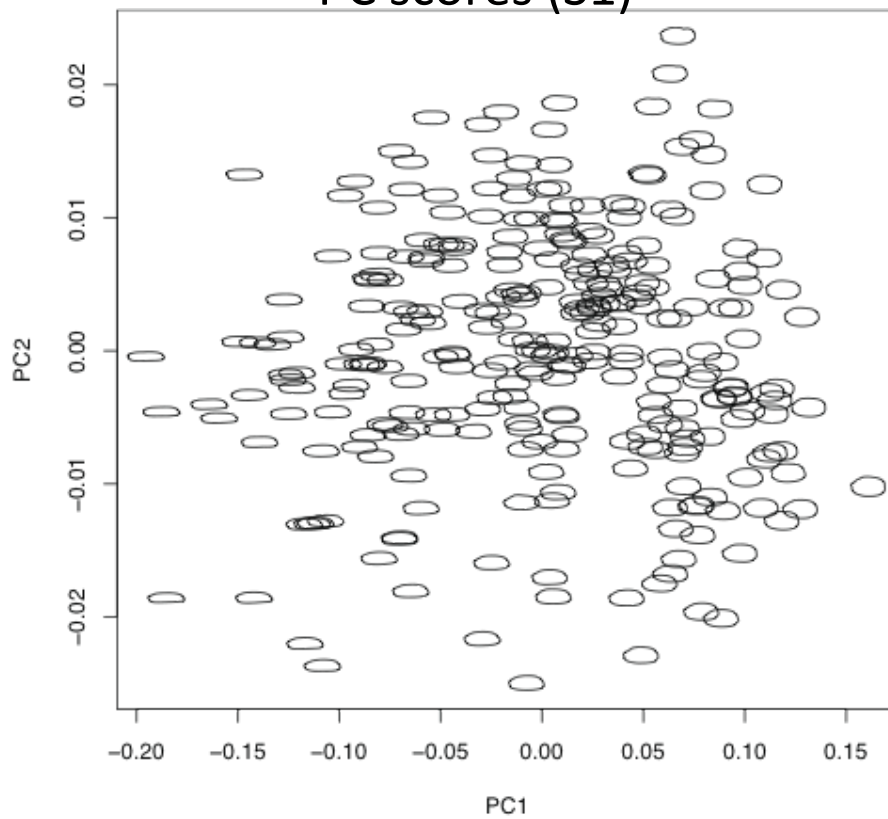
- 386 アクセッション
- 1311 SNPs

For S2, all materials were downloaded from <http://ricediversity.org/>

## イネ玄米形の主成分 (S1)

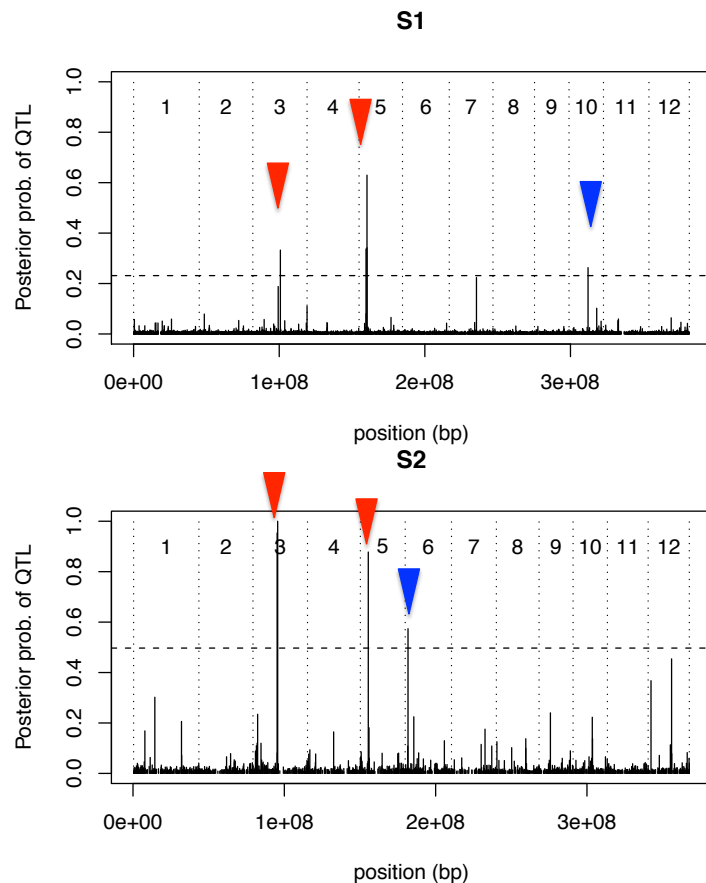


## Shape variations explained by PC scores (S1)



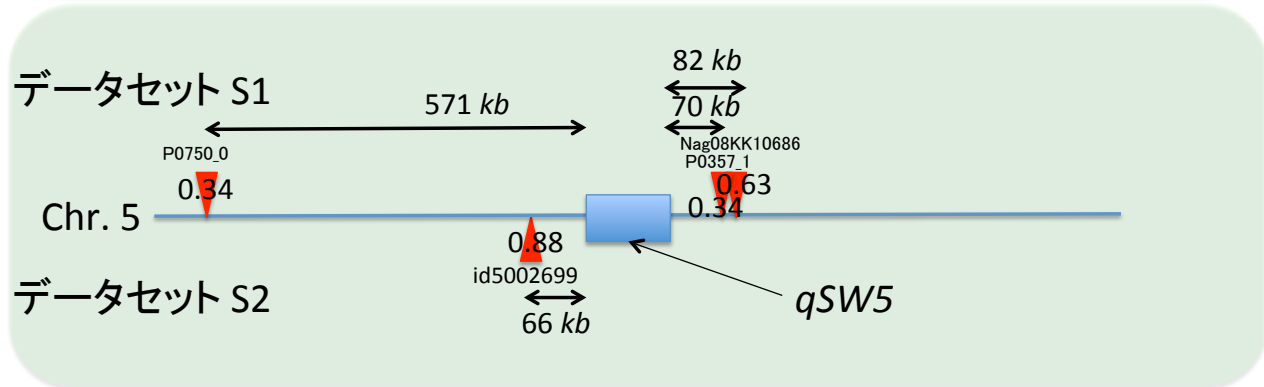
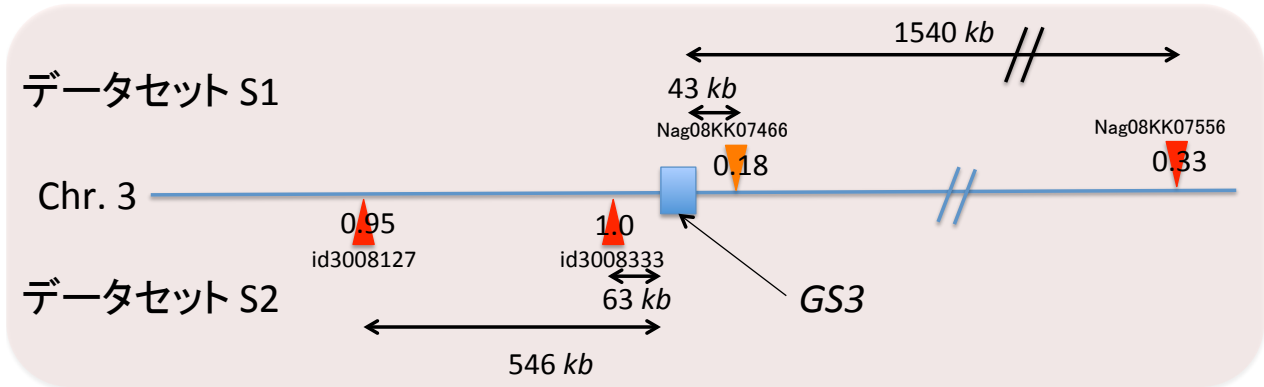
## GWAS結果

- 第1主成分で、2データセット間に共通のアソシエーションが検出された

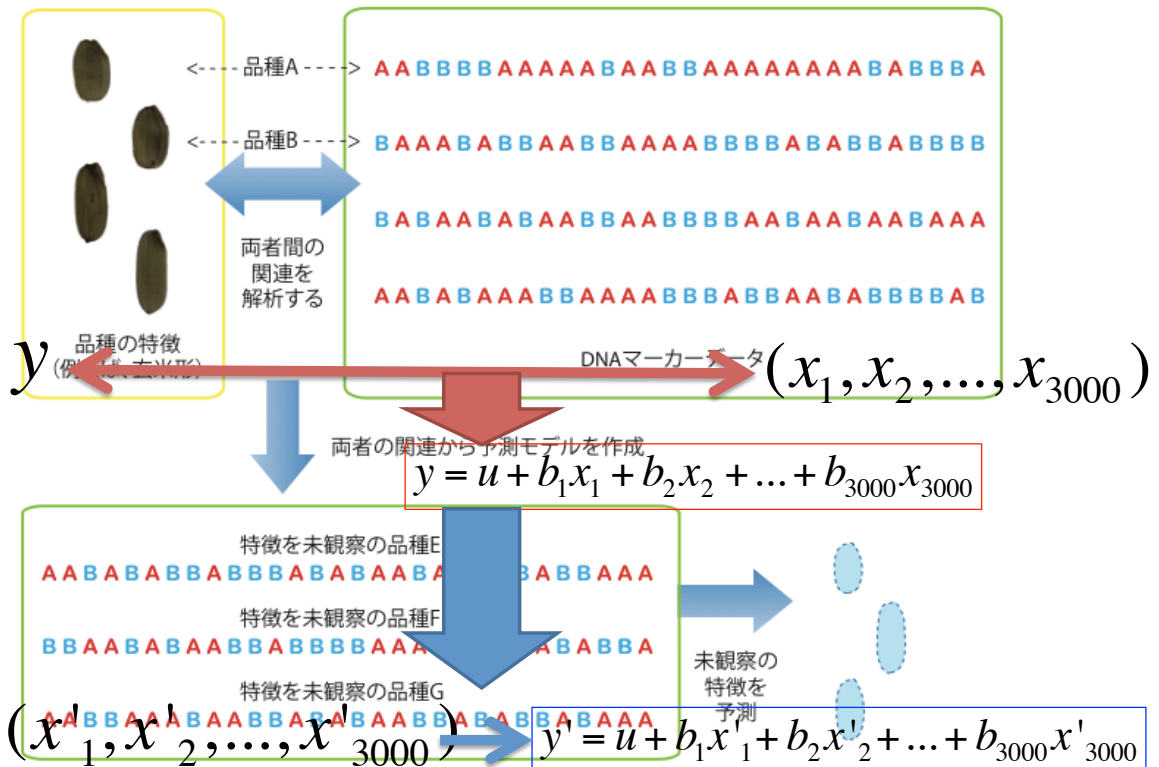




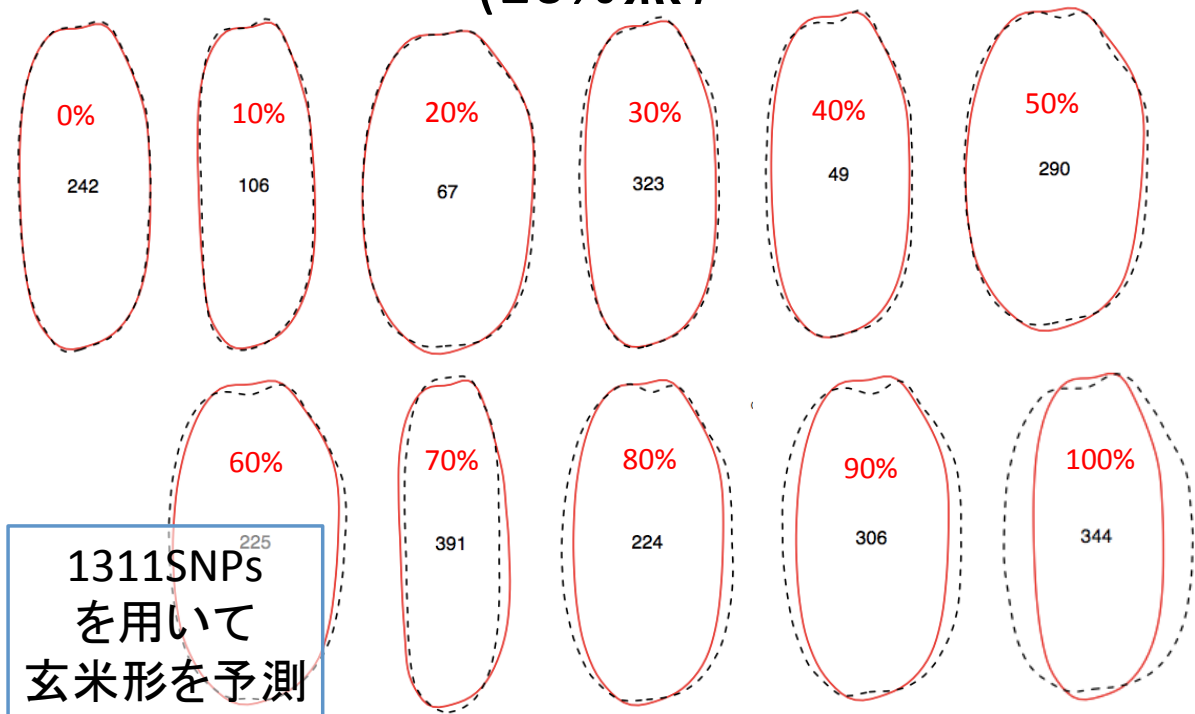
# 第3、第5染色体に検出されたアソシエーション



## ゲノミックセレクション (GS)

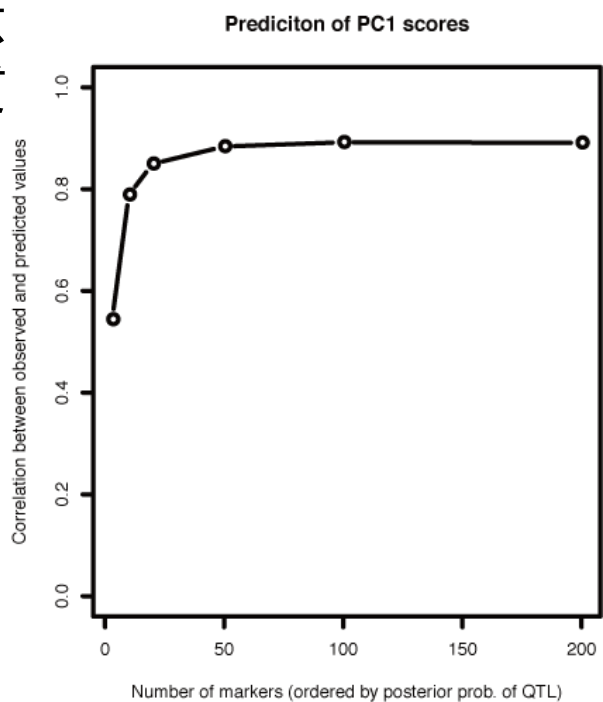


# 玄米形のゲノム予測 (S2) (10%点)



アソシエーションが有意でないマーカーも、形質の予測に必要である

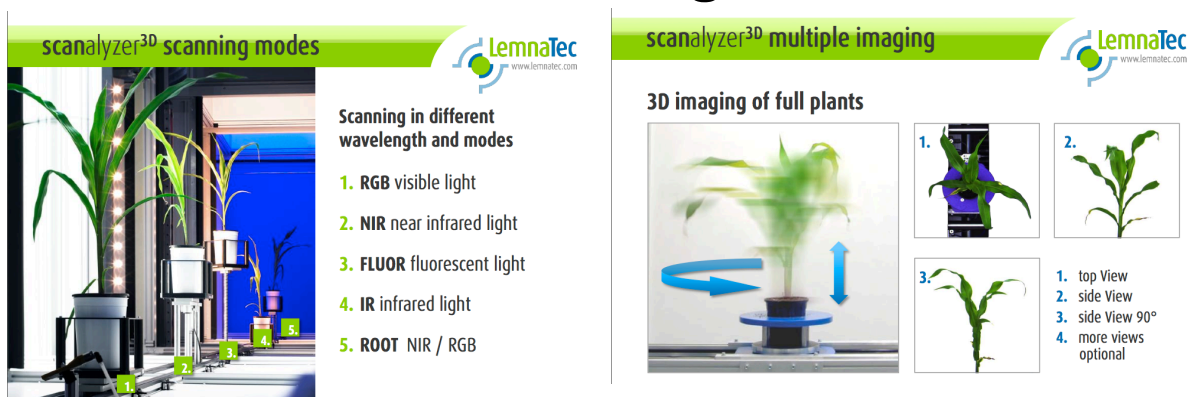
- 効果の大きなQTLだけでは、玄米形変異を十分正確に説明できない
- 多数のQTLの効果を同時に考慮した予測が不可欠である



# 生物形態のGWASとGSの展望

- ゲノム情報が安価に大量に得られるようになり、形態研究の幅が広がりつつある
- 例えば、形態の定量的評価値とGWASを組み合わせることで、形態を支配する遺伝子やその遺伝システムを明らかにできる
- 例えば、ゲノムワイドマーカーだけから観察していない(できない)形態を予測することもできる
- 一方、形態情報はゲノム情報に比べるとハイスループット計測が大変である  
(phenomicsはomics分野のボトルネック)

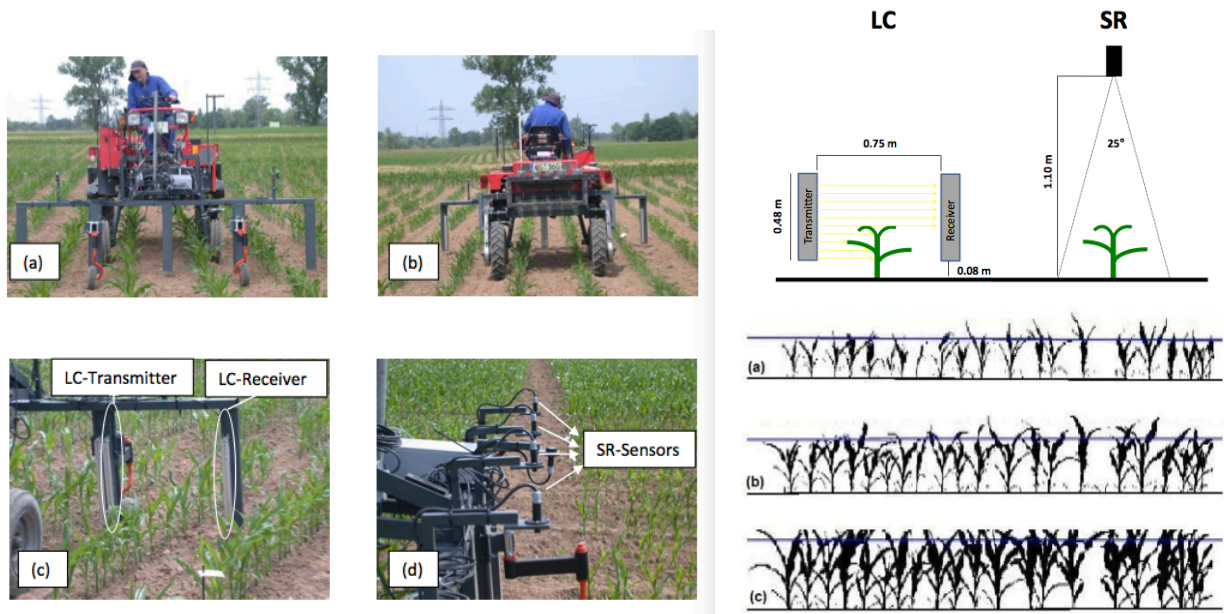
## Phenomics in the green house



[http://www.lemnatec.com/sites/default/files/application-sheets/2011/09/23/LemnaTec\\_Presentation\\_Varna\\_CMBPS.pdf](http://www.lemnatec.com/sites/default/files/application-sheets/2011/09/23/LemnaTec_Presentation_Varna_CMBPS.pdf)



# Phenomics in the field



Laser curtain

Montes et al. (2011) *Field Crops Research* 121:268–273

# Phenomics in the forest

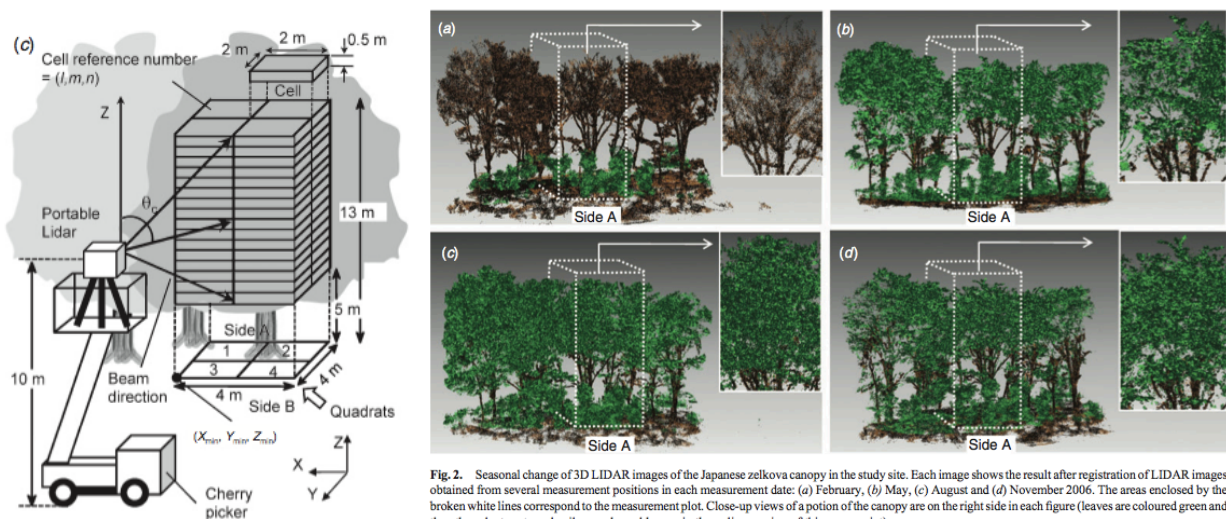


Fig. 2. Seasonal change of 3D LIDAR images of the Japanese zelkova canopy in the study site. Each image shows the result after registration of LIDAR images obtained from several measurement positions in each measurement date: (a) February, (b) May, (c) August and (d) November 2006. The areas enclosed by the broken white lines correspond to the measurement plot. Close-up views of a portion of the canopy are on the right side in each figure (leaves are coloured green and the other plant parts and soil are coloured brown in the online version of this manuscript).

Light Detection And Ranging (LIDAR) imaging

ケヤキのLAIの季節変動を計測

平均平方誤差  $0.26 \text{ m}^2 \text{ m}^{-3}$

Hosoi and Omasa (2009) *Functional Plant Biology* 36: 998–1005

# 多検体を効率よく計測

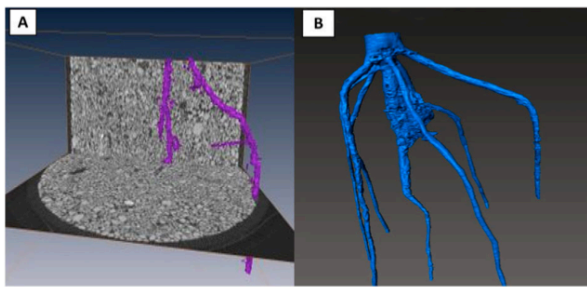


## バーコードリーダーを用いた表現型計測

トウモロコシNAM集団の計測におけるkey technologyの一つ

Kindly provided by Mr. Arturo Garcia

# 見えにくいものを見える化



Tracy et al. (2010) J Exp Bot 61:311

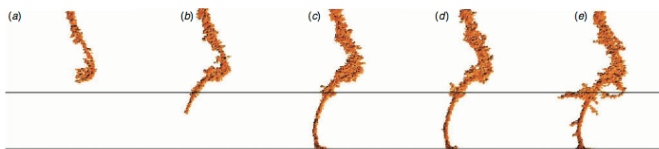
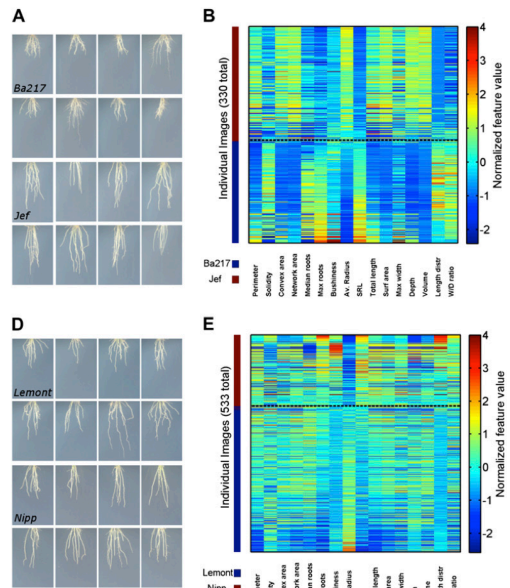


Fig. 3. Growth of a rapeseed through a soil band sandwiched between two sand layers measured with the X-ray instrument described by Gregory *et al.* (2003). The horizontal lines indicate the upper and lower boundaries of the soil band which was 7 mm deep: (a) the single root 2 days after germination; (b) after 3 days the root has penetrated into the soil layer; (c) the root then passed through the soil layer (4 days) and (d) 5 days; and (e) after 8 days, production of lateral roots had started in the soil band.



Iyer-Pascuzzi et al. (2010) Plant Physiology 152:1148

- 根の形状を解析 (NMR, X-ray CT, 透明培地など)

# 世界のPhenomics施設

The image shows two overlapping website screenshots. The top one is for the Australian Plant Phenomics Facility, featuring a navigation menu with links like 'Home', 'About', 'HRPPC', 'Plant Accelerator', 'PODD', 'IBS PDJW 2010', 'IPPS 09', 'Governance', and 'Contact'. The bottom one is for the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. It includes a search bar, a navigation menu with categories like 'Research', 'Infrastructure', and 'Events', and a main content area with news articles and a conference announcement for the 11th Gatersleben Research Conference on Chromosome Biology, Genome Evolution and Speciation.

in Gatersleben

## Phenome DBの例: PHENOPSIS DB

The screenshot shows the PHENOPSIS DB website. It features a header with logos for leperse, INRA, and AGRON OMICS. The main content area includes a navigation menu with buttons for 'PHENOPSIS DOCUMENTATION', 'DATA BROWSING AND DOWNLOAD', 'GRAPHS AND DESCRIPTIVE STATISTICS', and 'IMAGE ANALYSES AND IMAGEJ MACROS'. A central text block describes the database as an information system for Arabidopsis thaliana, providing 15 experiments, 619 genotypes, and 48,171 phenotypic data points. A 'Log On' section is visible on the right. The footer contains various icons and links like 'The PHENOPSIS movie', 'User guide', 'Suggestions or bugs?', 'Web service', and 'News'.

- PHENOPSISを用いて自動収集されたArabidopsis thalianaの画像や表現型データが、実験プロトコルや実験メタデータ（環境条件など）とともに提供されている
- 画像解析や統計解析のためのスクリプトも提供されている

# Genomicsから Phenomicsへ

## 共同研究者

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