PLNT2530

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Unit 11

Substantial Equivalence

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Substantial equivalence: A transgenic line is said to be substantially equivalent to its non-transgenic parent if its traits, other than the transgene, are substantially equivalent.

Traits can be assessed at the level of:

- mRNA expression
- protein expression
- levels of metabolites
- phenotypes

Substantial equivalence is one of the criteria used in assessing safety of crops for agricultural use.

Mutagenesis in plant breeding

- 1930's to 1970's: Mutagenesis widely used in plant breeding to generate new mutations
- FAO: 1916 crop varieties created using mutagenesis (40% radiation)
- Two kinds: radiation and chemical mutagenesis
- Need to mutagenize many loci, and screen large numbers of progeny, to find 1 useful mutation
- Transgenics typically change only a single locus

(Oddly, the public seems to have no concern about mutation breeding.)

Batista R,Saibo N, Lourenço T, Oliveira MM (2008) Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene Proc. Natl. Acad. Sci. USA 105:3640-3645, doi: 10.1073/pnas.070788105

Hypothesis: **The genetic variation due to mutagenesis is greater than the genetic variation due to transformation**

Oryza sativa L. spp. Japonica (rice) lines:

Nipponbare
- unmutagenized, untransformed control
- ϒ-irradiated, M1 generation (“unstable”)
- *Agrobacterium*-transformed with BCBF1* gene T1 generation (“unstable”)

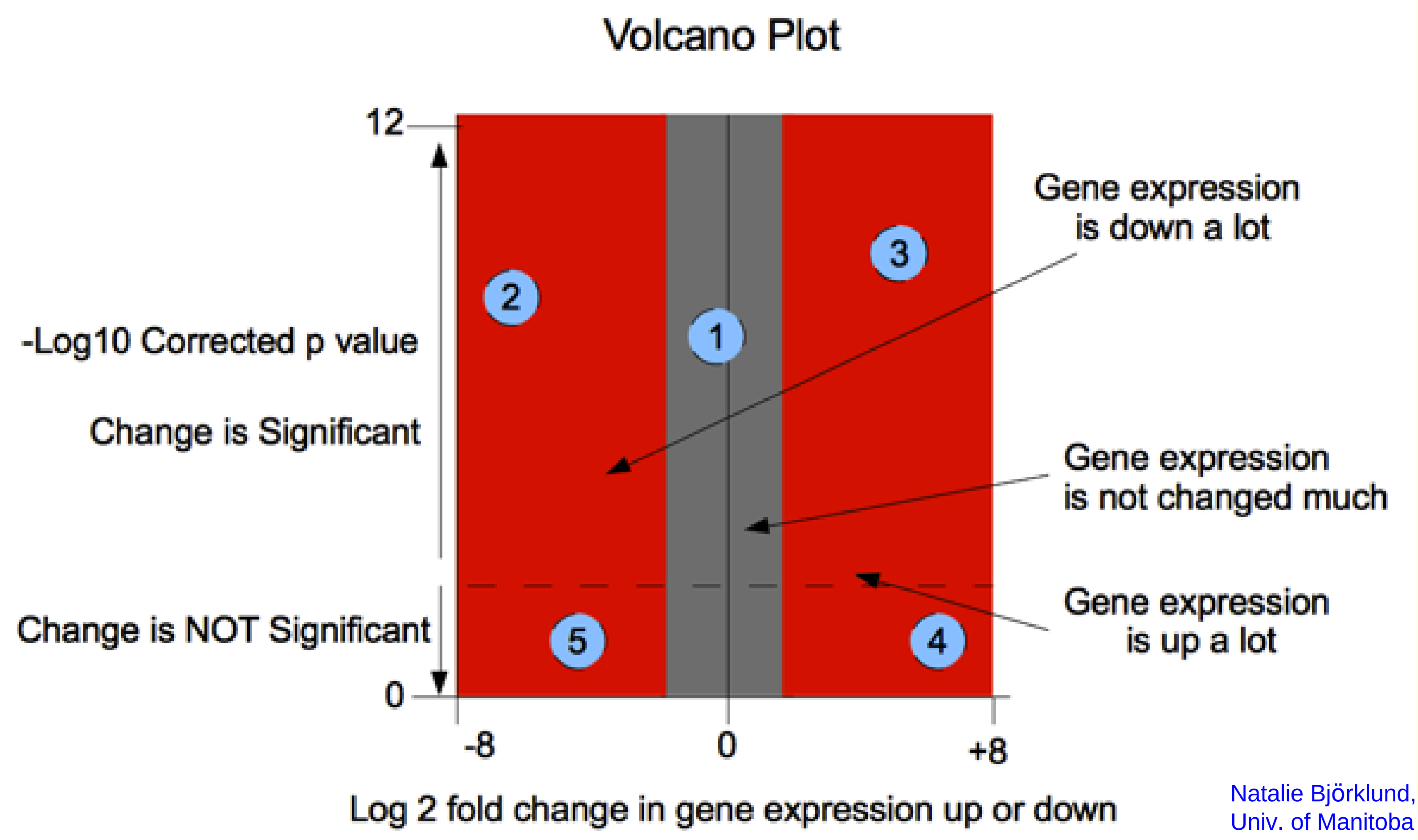
*BCBF1 - Barley C-repeat binding factor. Activated during stress; interacts with dehydration-responsive elements found in many stress-realted genes.

Bengal
- unmutagenized, untransformed control
- *Agrobacterium*-transformed with ScFv (immunoglobulin; no expected effect), T3 generation (“stable”)

Estrella A
- unmutagenized, untransformed control
- ϒ-irradiated, semi-dwarf phenotype, self-pollinated for 10 generations (“stable”)

RNA from whole 12-day old seedlings
Microarray: 51,279 probes ie. distinct transcripts

Volcano plots compare differential expression, between two conditions, for thousands of genes

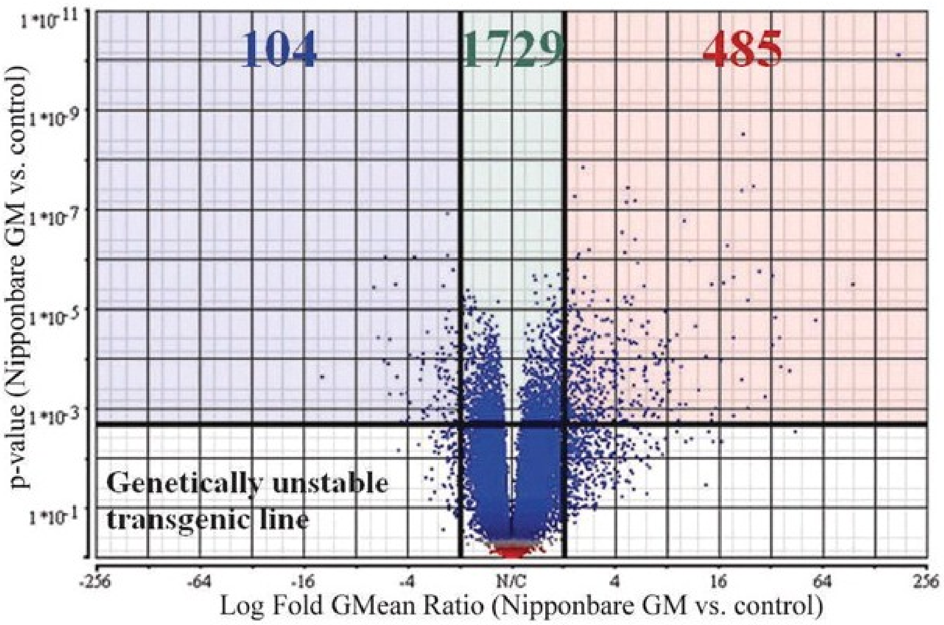


Natalie Björklund, Univ. of Manitoba

p-value: A measure of statistical significance. p is the probability that the observed change gene expression of a gene is just due to random variance in expression between genes, when many genes are observed.

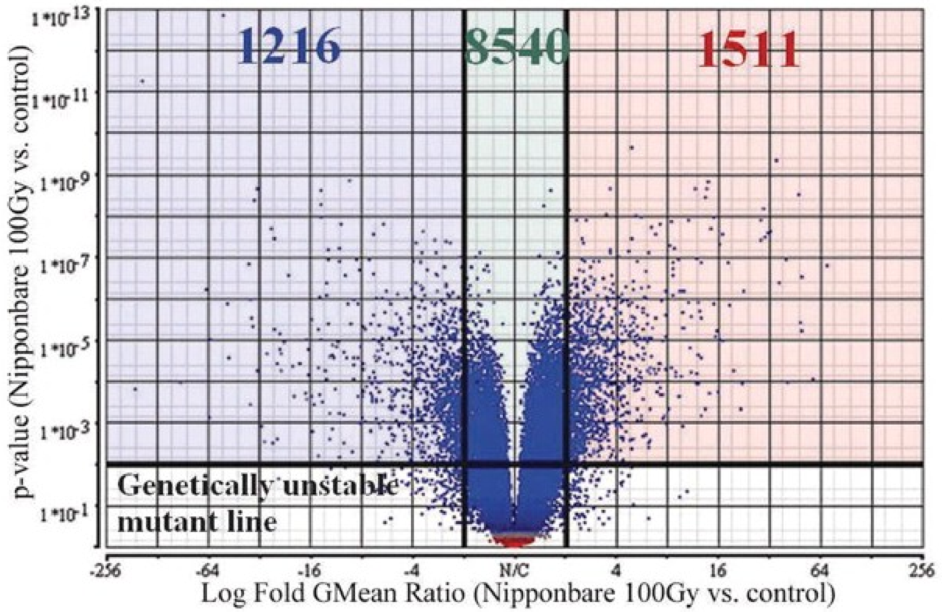
Comparison of gene expression in early “unstable” generations

T_1 transgenic vs. control



104 + 485 = 589 genes differentially expressed

M_1 mutagenized vs. control



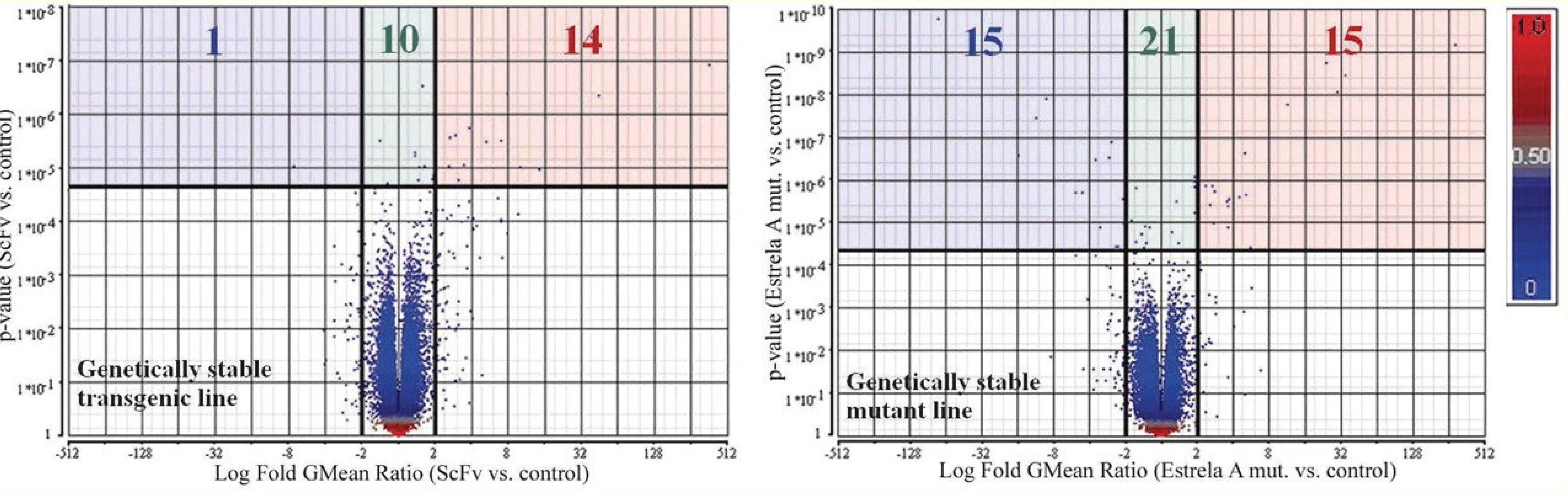
1216 + 1511 = 2727 genes differentially expressed

A note on experimental variation: In biology, each time you repeat an experiment, the measurements you make will vary. This may be due to differences in technique between replicates, or due to the lack or reproducibility of the biological system itself. **The more variability there is in expression of a gene from one replicate to the next, the greater the difference between treatments needs to be before we can say that a difference is statistically significant**. These experiments were done with only 2 replicates.

Comparison of gene expression in late “stable” generations

T_3 transgenic vs. control

M_{10} mutagenized vs. control



1 + 14 = 15 genes differentially expressed

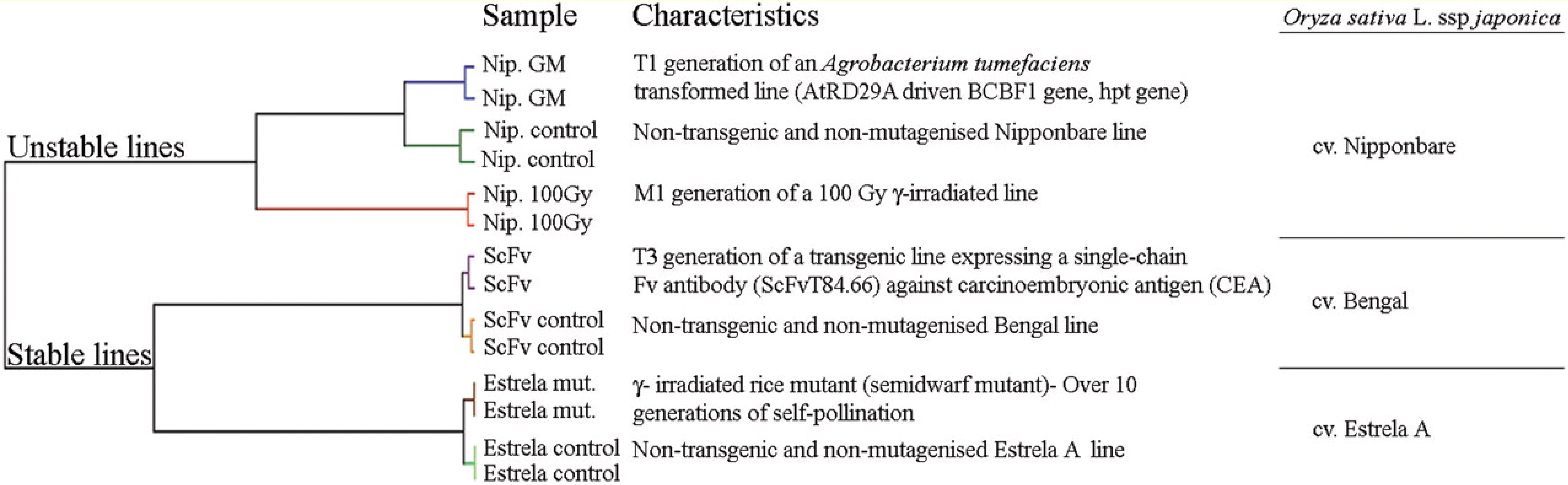
15 + 15 = 30 genes differentially expressed

Observed variability of gene expression in transgenic and mutagenized plants drops off with each generation ie. gene expression stabilizes.

Clustering of gene expression patterns

Cladogram compares:

- differences between replicates
- transgenics vs. controls
- mutagenized vs. controls
- unstable lines vs. stable lines



Sample	Characteristics	*Oryza sativa* L. ssp *japonica*
Nip. GM Nip. GM	T1 generation of an *Agrobacterium tumefaciens* transformed line (AtRD29A driven BCBF1 gene, hpt gene)	cv. Nipponbare
Nip. control Nip. control	Non-transgenic and non-mutagenised Nipponbare line	
Nip. 100Gy Nip. 100Gy	M1 generation of a 100 Gy γ-irradiated line	
ScFv ScFv	T3 generation of a transgenic line expressing a single-chain Fv antibody (ScFvT84.66) against carcinoembryonic antigen (CEA)	cv. Bengal
ScFv control ScFv control	Non-transgenic and non-mutagenised Bengal line	
Estrela mut. Estrela mut.	γ- irradiated rice mutant (semidwarf mutant)- Over 10 generations of self-pollination	cv. Estrela A
Estrela control Estrela control	Non-transgenic and non-mutagenised Estrela A line	

The farther away any two sets of gene expression data are on the tree, the more differences there are in observed levels of gene expression.
The more they cluster together, the more similarity there is in expression of genes in the microarray.

Categories of Differentially-Expressed Genes

Most of the differentially-expressed genes are stress-related genes

- Genes implicated in **stress/defense signaling or perception** eg. receptor-like kinases
- **Second messengers**, which transmit detection of a stress to the nucleus, eg reactive oxygen species (ROS), salicylic acid, ethylene, jasmonic acid
- Stress-response and systemic acquired resistance (**intercellular signalling**) eg. peroxidases, SAR, pathogenesis-related proteins
- **Protein-modification** eg. methylation, isoprenylation, lipidation, ubiquitination, scaffolds, adaptors
- Transcription factors
- Retrotransposons - mobilization of transposons is often seen in plant stress

Hypothesis: The stresses of transformation or mutagenesis bring about temporary destabilization of gene expression that is gradually lost over several generations.

Destabilization of gene expression might lead to adaptation when drastic environmental changes occur.

Conclusion: Mutagenesis causes more drastic and lasting genomic effects than transformation

- Variance in gene expression in early, unstable lines is greater in mutagenized lines than in transgenics
- Variance in gene expression decreases rapidly in transgenics
- Variance in mutagenized lines persists for many generations
- Differences in gene expression **between lines** is much greater than variance due to mutagenesis or to transformation. <u>This may be the most important observation.</u>