PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

09:00 am to 11:00 am Monday, April 13, 2015

Answer any combination of questions totalling to exactly 100 points. If you answer questions totalling more than 100 points, answers will be discarded at random until the points equal 100. This exam is worth 40% of the course grade. The questions available total to 120 points.

Hand in these question sheets along with your exam book. Question sheets will be shredded.

Ways to write a readable and concise answer:
i. Just answer the question. Save time by specifically addressing what is asked. Don't give irrelevant background if it doesn't contribute to the question that was asked.
ii. Avoid stream of consciousness. Plan your answer by organizing your key points, and then write a concise, coherent answer. Make your point once, clearly, rather than repeating the same thing several times with no new information.
iii. Point form, diagrams, tables, bar graphs, figures are welcome. Often they get the point across more clearly than a long paragraph.
iv. Your writing must be legible. If I can't read it, I can't give you any credit.

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1. (10 points) Of the "Omics" technologies, Metabolomics stands out as the most difficult, compared to DNA, RNA, or protein sequencing. Explain the basic concept behind metabolomics, and why its methodologies need fine tuning for different species, tissues, or developmental stages.

2. (10 points) You are planning a research project in canola (*Brassica napus*), a tetraploid species very closely-related to *B. rapa*. There is available at NCBI a high quality genomic sequence for *B. rapa*, although only 60% of the genome has so far been sequenced. Also available is the complete genome for *Arabidopsis thaliana*, for which 100% of the genomic sequence is available.

Describe the tradeoffs associated with constructing a microarray consisting of 60-mer oligonucleotides, designed from either *B. rapa* or *A. thaliana*, to measure gene expression levels in *B. napus*.

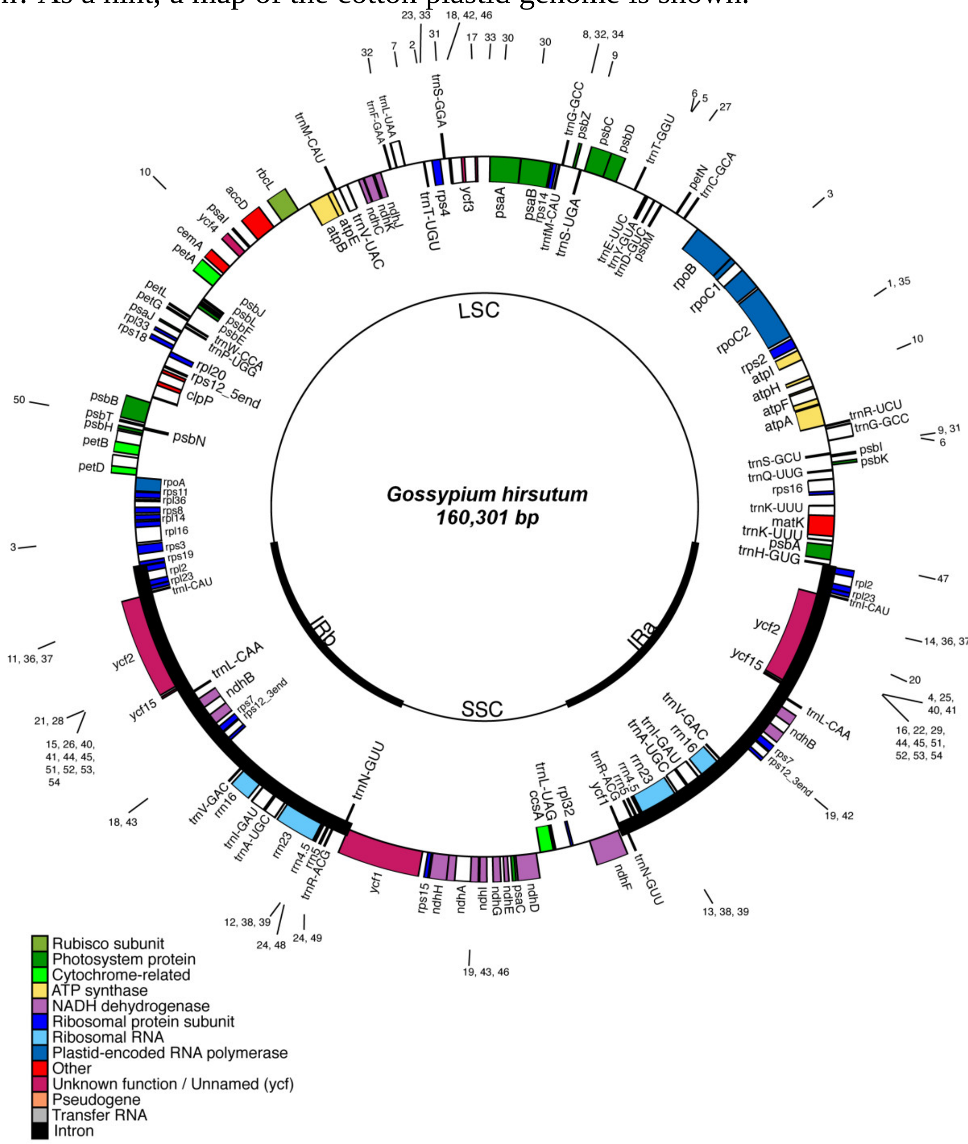
3. (10 points) *Agrobacterium* has evolved an elaborate mechanism for transforming plant cells. Describe what the function of the T-DNA in nature, and explain how this benefits *Agrobacterium*.

4. (15 points) What is the basis of resistance to Bt toxin that arises in populations of insect pests? Explain the principle behind the use of refugia with crops expressing the Bt protein. What does the planting of refugia hope to accomplish, and how does this work?

5. (10 points) Describe one of the following:

- Transcriptional Gene Silencing
- Posttranscriptional Gene Silencing

6. (10 points) When transforming genes into the nuclear genome, genes can insert anywhere at random. Most of the time, this doesn't knock out genes. What is different about chloroplast genomes, such that the likelihood of knocking out an important gene is greater when doing plastid transformation? What alternative approach must be used in designing constructs for plastid transformation? As a hint, a map of the cotton plastid genome is shown.



7. (10 points) What are the difficulties associated with genetic engineering to eliminate allergenicity from peanut? What experimental strategy was used to overcome these difficulties, and how well did they work?

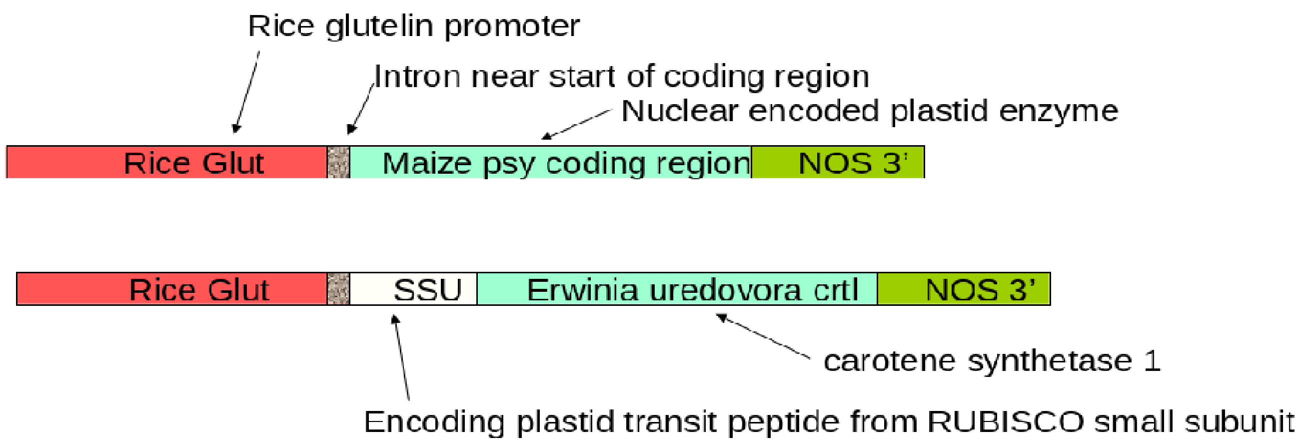
8. (5 points) List at least 2 advantages of transformation using biolistics (the “Gene Gun”) over *Agrobacterium* transformation.

9. (5 points) Define "substantial equivalence", in the context of genetically modified crops.

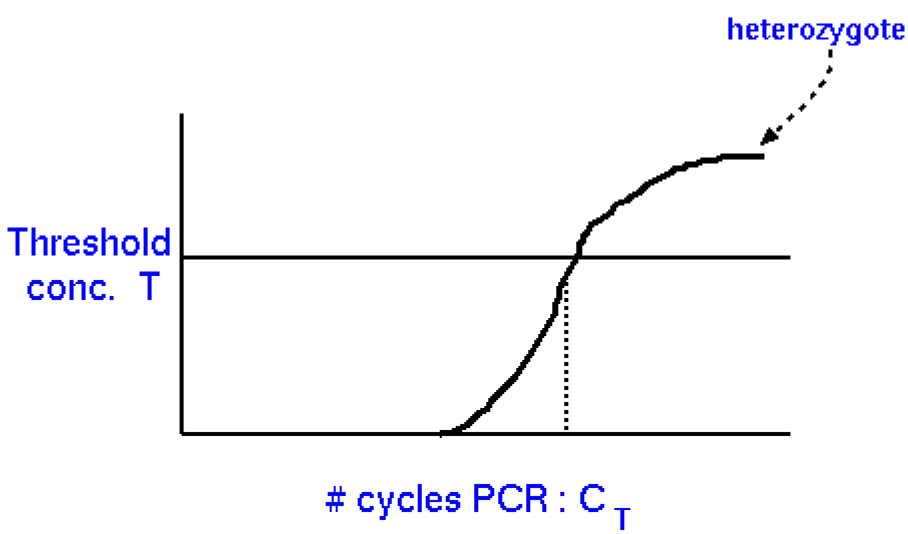
10. (10 points) The constructs used in producing Golden Rice are shown below. Answer the following questions.

a) Why was the rice Glutelin promoter used, rather than a promoter for a constitutive gene such as Ubiquitin?

b) The construct that utilizes the carotene sythase gene from the bacterium *Erwinia* had to be modified to include the transit peptide from the RUBISCO Small Subunit protein. However, the Maize phytoene synthase gene did not need to have this extra sequence added. Why not?



11. (10 points) The amplification curve for a typical qPCR reaction is shown for a gene in a plant that is heterozygous for a transformant.



Draw a similar diagram comparing the curve for the heterozygous plant with the curve for a homozygous plant.

12. (15 points) In class, we discussed the cluster diagram, showing the similarities in gene expression between transgenic lines, mutagenized lines, and non-transgenic controls, for each of three cultivars of rice (Nippponbare, Bengal and Estrella A).

Five statements, labeled A through E, are given below. Three of these statements describe one of the figures labeled I, II and III. In these figures, groupings of transgenic plants are circled, to illustrate the point being made by the corresponding statement. Two of the statements do not describe any of the figures.

Match each of the figures, I, II and III, with the statement that <u>best</u> describes that figure.

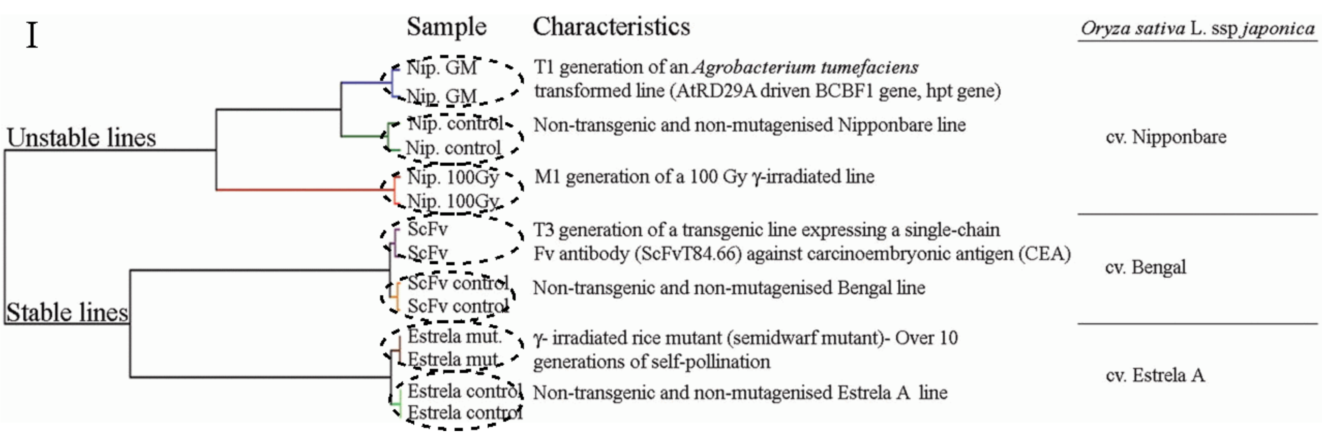
A. Variance in gene expression between rice cultivars is far greater than that caused by either mutagenesis or by transformation.

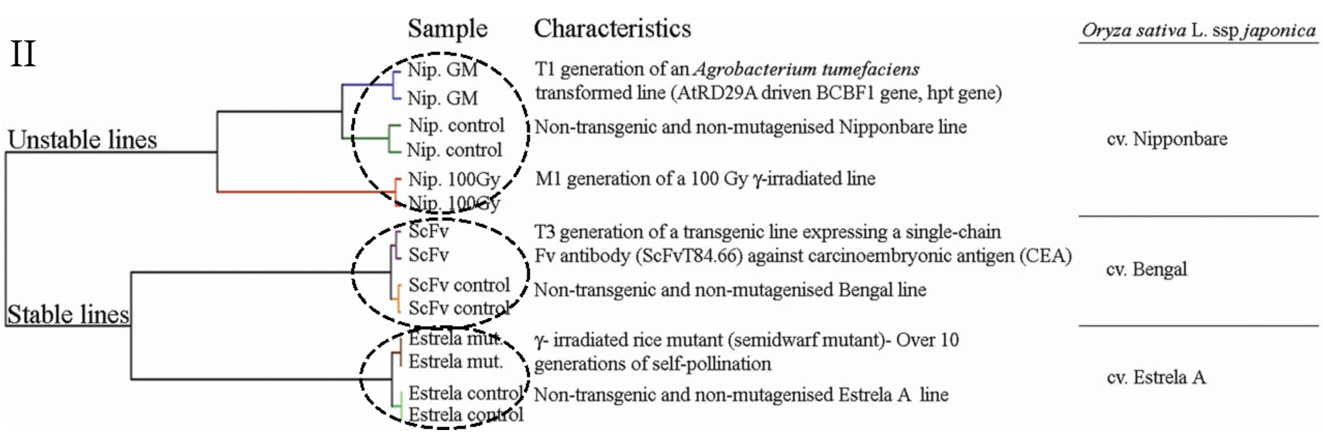
B. Both transformation and mutagenesis cause drastic changes in stress-related genes.

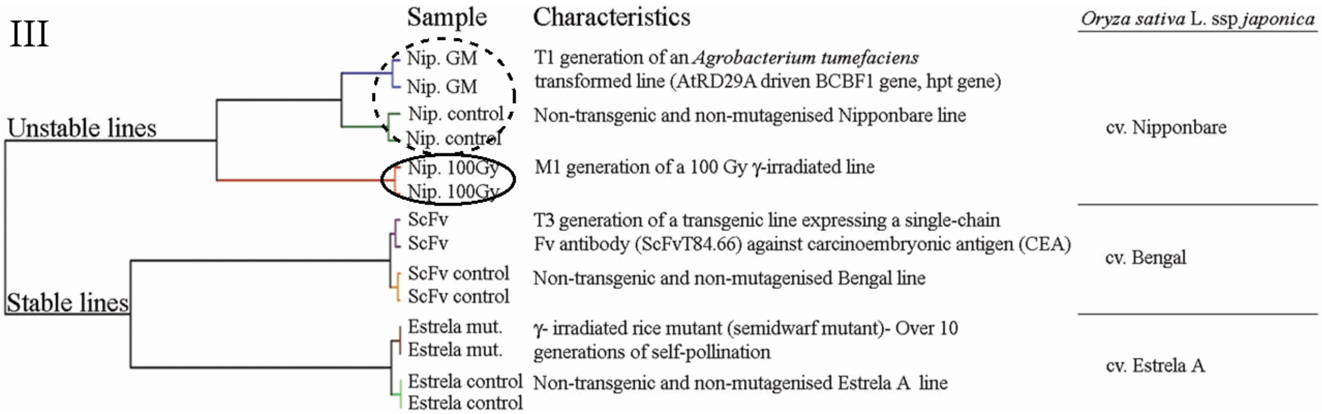
C. Observed variability of gene expression in transgenic and mutagenized plants drops off with each generation ie. gene expression stabilizes.

D. Mutagenesis causes much more drastic changes in gene expression in early generations than does transformation using *Agrobacterium*.

E. The reproducibility of the results is illustrated by the fact that replicates usually were more similar to each other than either replicate was to other treatments.






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