PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

Thursday, April 14, 2016 18:00 to 20:00 EITC 2, Rm. 150, Seats 1 - 14

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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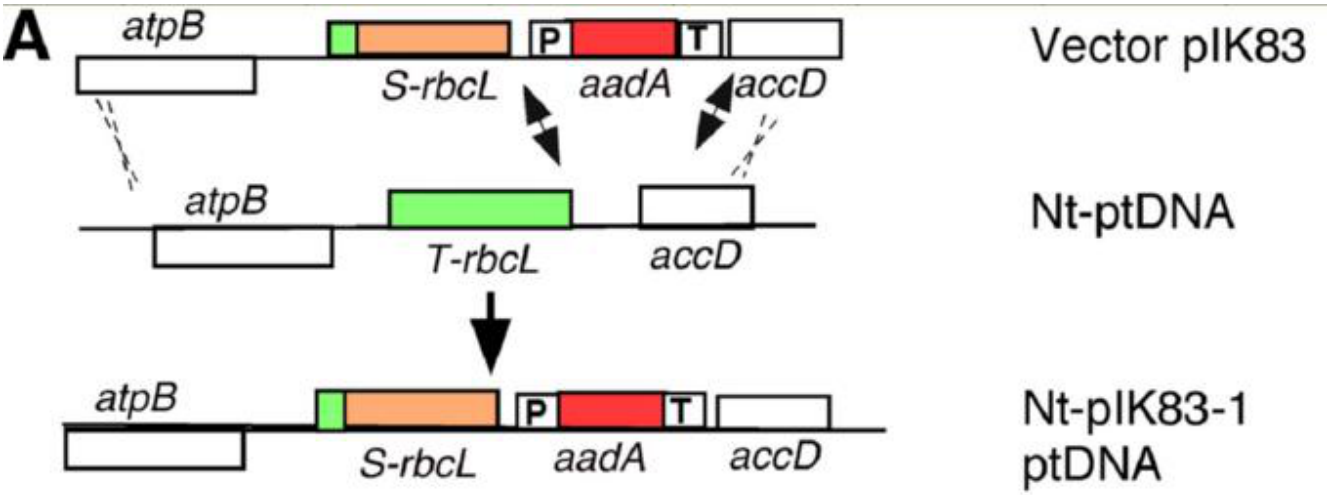
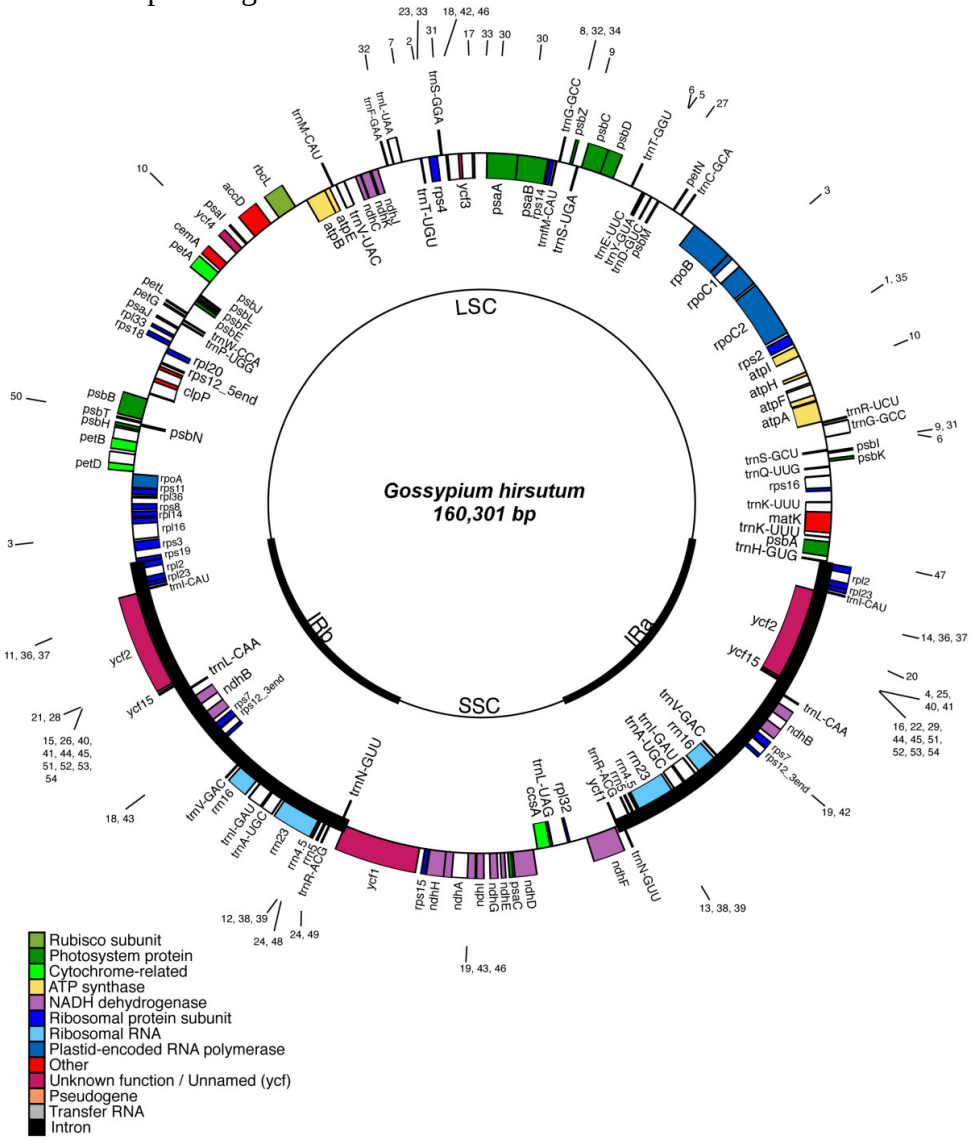
1. (10 points) In the example given in class, tobacco chloroplasts were transformed using a construct containing the following:

- **bacterial gene aadA**, which codes for spectinomycin resistance
- **atpB and accD**, from the tobacco plastid genome
- **S-rbcL** - sunflower RUBISCO (ribulose bisphosphate carboxylase/oxygenase), large subunit. This gene can easily substitute for the native tobacco copy of the gene, but has a distinct sequence that is easy to verify as being the sunflower gene.



1. Figure:
Spectinomycin resistant plants were selected on media containing spectinomycin. What aspect of the design of pIK83 ensures that no important functions of plastid genes will be disrupted? Would you expect to see transformant lines in which the construct integrated into the nuclear genome, rather than the plastid genome? Why or why not?

2. (5 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? As a hint, a map of the cotton plastid genome is shown.



3. (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.

4. (10 points) Describe <u>one</u> of the following:

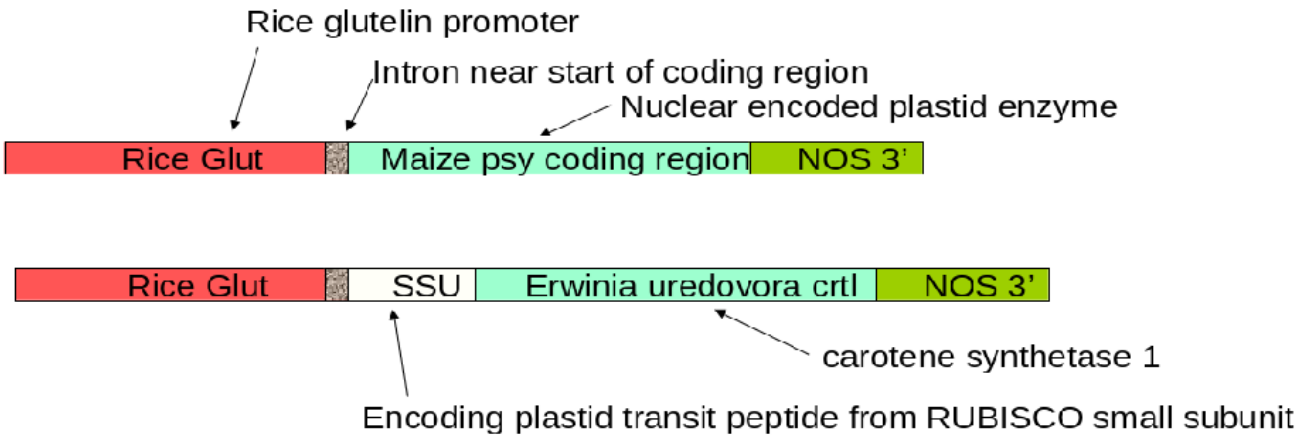
- Transcriptional Gene Silencing
- Posttranscriptional Gene Silencing

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

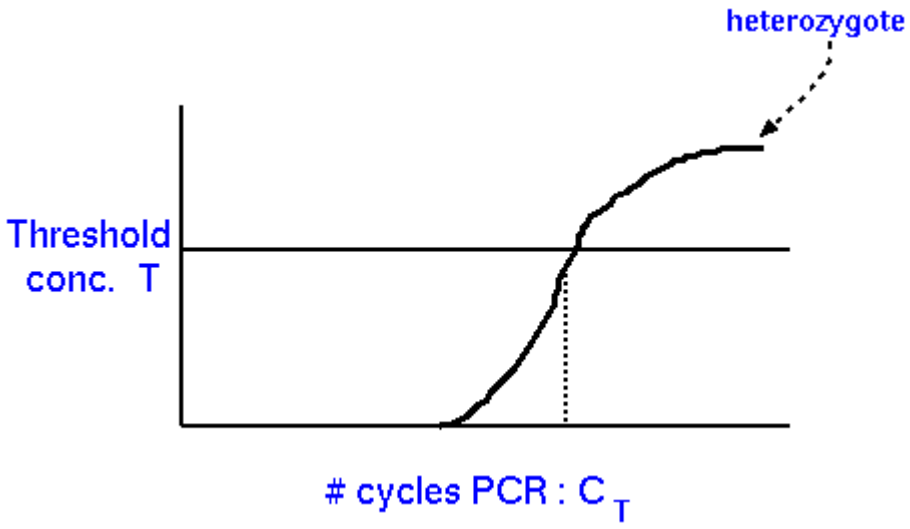
6. (10 points) The constructs used in producing Golden Rice are shown below. Answer the following questions.

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?



7. (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.

8. (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 best describes that figure.

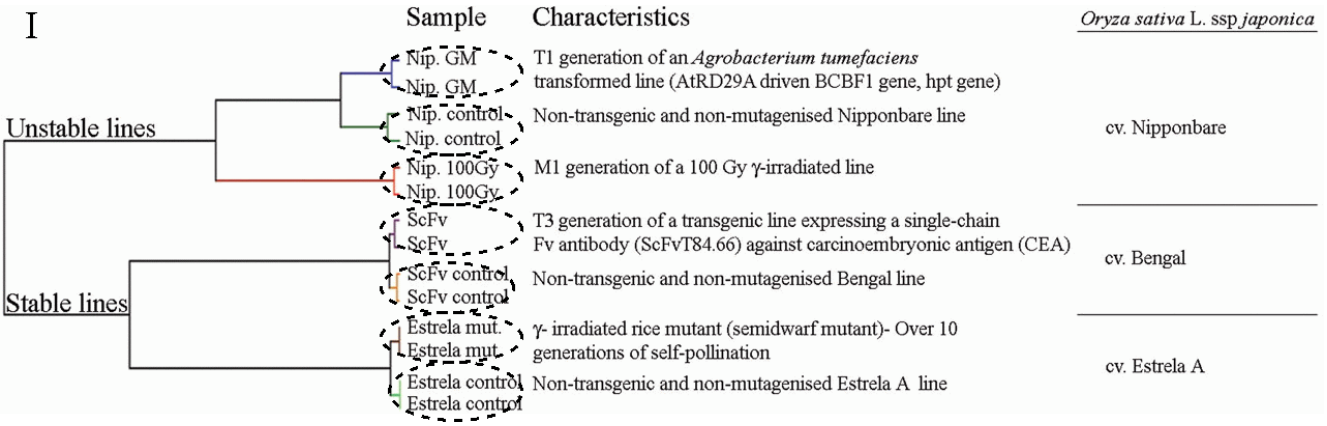
A. Variance in gene expression between rice cultivars is far greater than that caused by either mutagenesis or by transformation.

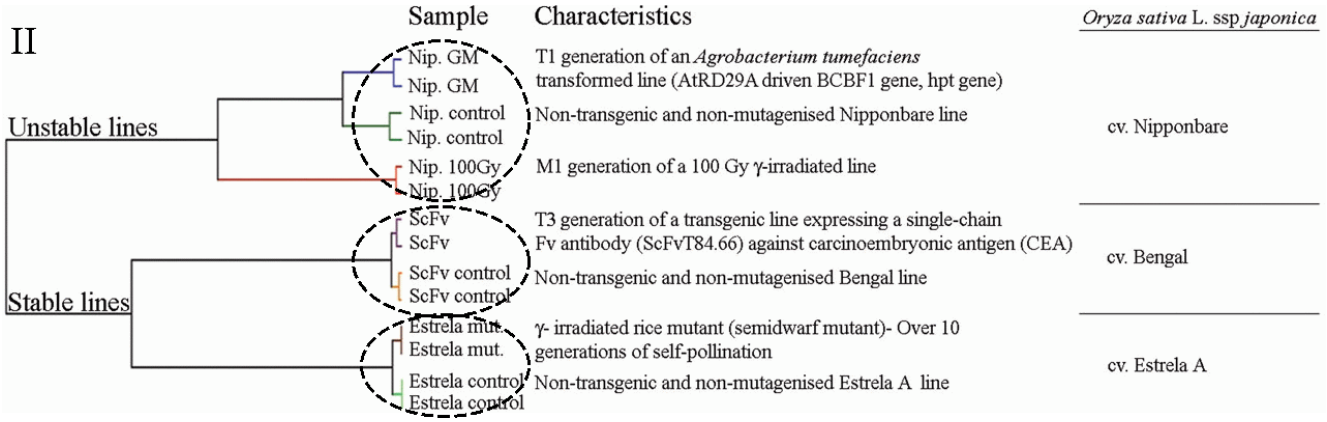
B. Both transformation and mutagenesis cause drastic changes in stress-related genes.

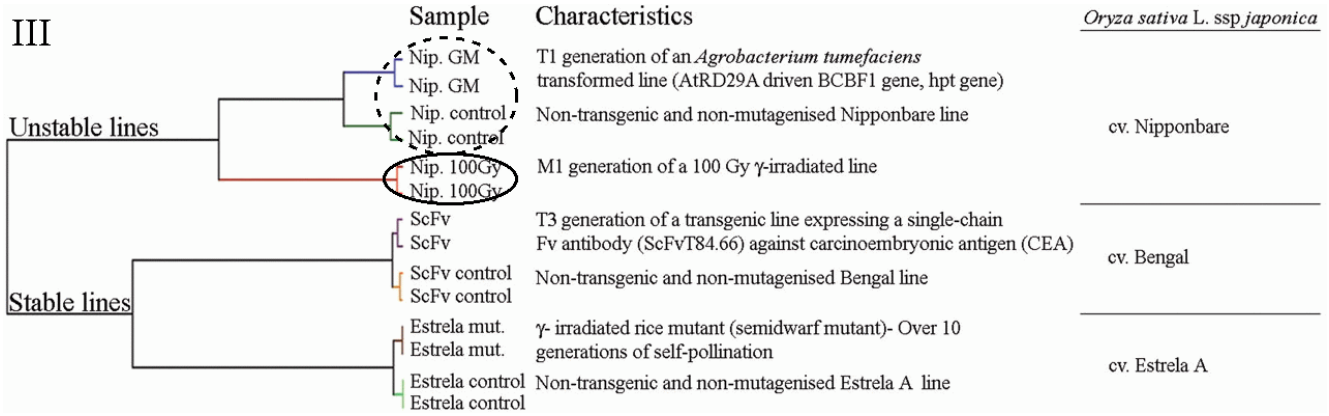
C. Observed variability of gene expression in transgenic and mutagenized plants drops off with each generation ie. gene expression stabilizes.

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.







9. (10 points) In plant transformation, the following are true:

a) <u>mechanism</u>: Transformation occurs by insertion of a foreign DNA sequence into a chromosome.

b) <u>location</u>: The site of insertion into the genome is largely random, from one transformation event to the next.

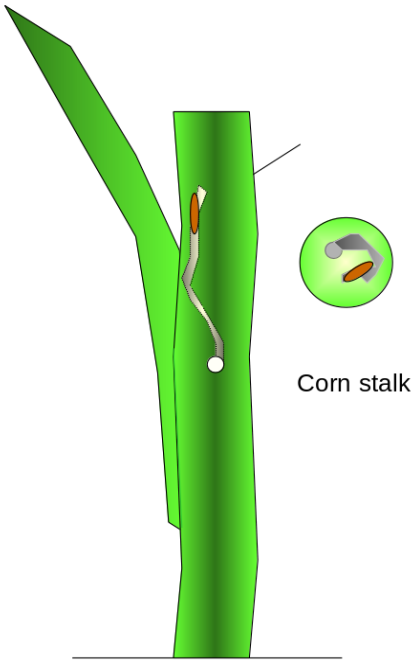
Describe how genome editing using the CRISPR-Cas9 system differs from transformation, with respect to a and b.

10. (10 points) Explain why mutations generated by the CRISPR-Cas9 system will be essentially indistinguishable from naturally-occuring mutations. Why is this important in light of regulation and labeling of genetically-modified crops.

11. (10 points) The European corn-borer tunnels deeply into corn stalks and does most of its damage within the stem.

a) Pesticides are effective against pests that eat foliage, but are not very effective against corn-borer. Explain why.

b) In what way does Bt corn solve this problem?



12. (5 points) In nature, *Agrobacterium tumefaciens* lives within its plant host. Is this relationship symbiosis or parasitism? Explain your reasoning.

13. (10 points) In plant biotechnology, the naturally-occurring Ti-plasmid has been modified to create the binary vector system. Why is it not practical to use the unmodified Ti-plasmid as it occurs in nature, for the purposes of plant transformation.