PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

Thursday, April 27, 2016 08:00 to 10:00 Frank Kennedy Brown Gym, Seats 290 - 308

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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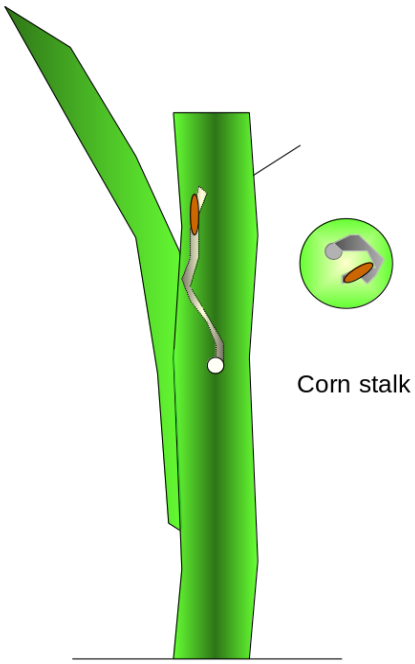
1. (10 points) The European corn-borer tunnels deeply into corn stalks and does most of its damage within the stem.

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?



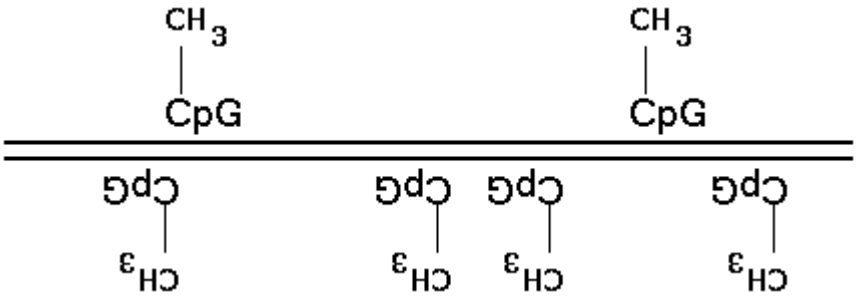
2. (10 points) In plant biotechnology, the naturally-occurring Ti-plasmid has been modified to create the binary vector system. Why is it not practical to use the unmodified Ti-plasmid as it occurs in nature, for the purposes of plant transformation.

3. (5 points) The Arctic non-browning apple represents what is arguably a new era in plant biotechnology. What is the significance of products such as the Arctic apple that distinguishes it from the previous generation of biotech crops, such as herbicide resistant soybean or canola, or Bt cotton or corn?

4. (10 points) Fill in the blanks.

Eukaryotes inactivate specific genes by methylation of ____a____ residues.

By preventing ____b____ , methyation acts as a mechanism for selectively down-regulating genes.



In embryos, methylation is often ____c____ . Subsequently during development, certain genes are methylated, and are ____d____ in the adult.

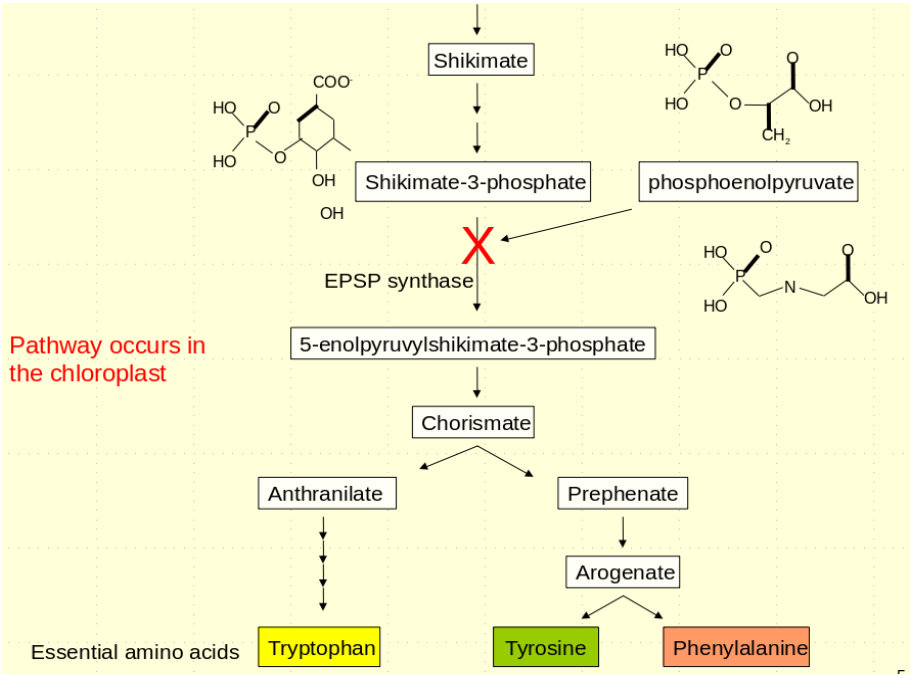
Methylation patterns are often ____e____ from one generation to the next.

5. (10 points) Explain why a single gene insertion at a single locus in a transgenic plant will usually give a single band on a Southern blot, when the same gene is used as a hybridization probe. Draw a diagram that illustrates your point.

6. (5 points) In nature, *Agrobacterium tumefaciens* lives within its plant host. Is this relationship symbiosis or parasitism? Explain your reasoning.

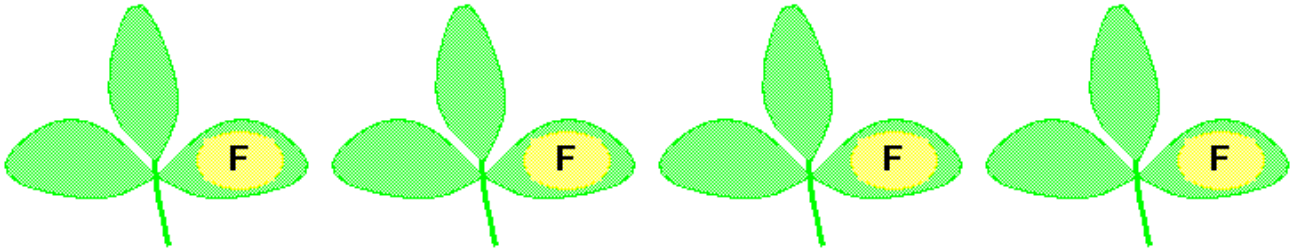
7. (5 points)

A diagram of the shikimic acid pathway is shown at right. Give the name of the chemical that inhibits this pathway at the point indicated by the X.

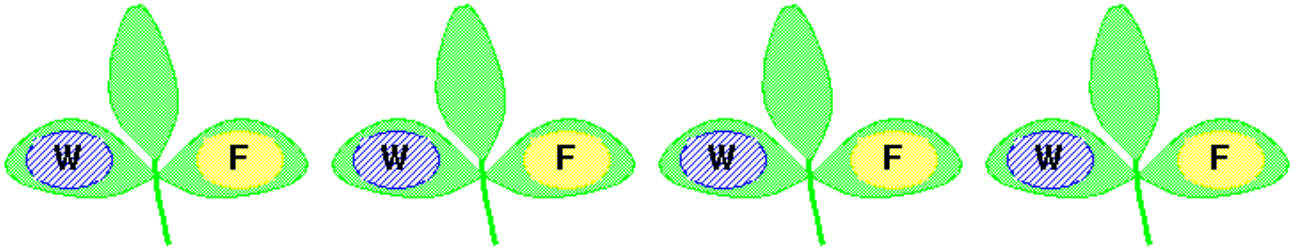


8. (15 points) Soybeans were transformed with a pea gene (P) for resistance to fungi. You wish to determine whether the P gene will confer fungal resistance in soybean. To do this, you would inoculate leaves with a fixed concentration of fungal spores suspended in sterile water (**F**) and observe whether resistance or susceptibility is seen. One leaf is inoculated, and the other left inoculated as a control. After observing the response, RNA is isolated from leaf tissue to observe changes in gene expression by RNAseq. Expression of genes is compared between fungal-treated leaves and control leaves.

a) Part of the experimental design includes inoculating 4 different plants at the same time. Why are 4 plants inoculated, as opposed to 1? What would be missed if we did the experiment only once? (You can ignore the trivial reason that 4 plants would yield more RNA.)



b) An alternative design is shown below, in which control leaves, rather than remaining uninoculated, are inoculated with sterile water (**W**). Why is this a better experimental design?



c) There are two possible ways to extract the RNA. One is to pool inoculated leaves from all 4 plants, and do a single RNA extraction. The other is to isolate RNA independently from each leaf and sequence each RNA sample separately. This is a lot of extra work. Why is this a better experimental design? In other words, once you have the four measurements for each gene, you're going to average them anyway, so why not just pool the leaves and simplify things? What information would you be throwing away if you pooled leaves?

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

a) mechanism: Transformation occurs by insertion of a foreign DNA sequence into a chromosome.

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

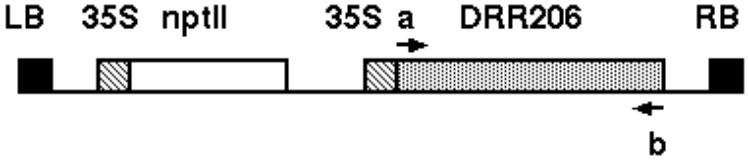
Describe how genome editing using the CRISPR-Cas9 system differs from transformation, with respect to a and b.

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. (15 points) There are many ways of verifying transformation of a plant, each with its advantages and disadvantages. The construct shown below contains two genes, under the control of the 35S promoter, between the T-DNA left and right borders. The DRR206 gene has been shown to provide some resistance to the blackleg fungus in transgenic canola. The nptII gene encodes neomycin phosphotransferase.

The following table lists a number of possible experiments, and the types of information that might be obtained. A "yes" means that the experiment is likely to provide a particular type of information. A "no" means that the experiment will not tell us that information. Some of the table has already been filled in.

Your job is to rewrite the table in your answer book, and complete it using yes/no answers. To save time, feel free to shorten or abbreviate the column and row headings, as long as you keep them in the same order.



	What the experiment tells us:			
	Presence of DRR206 gene	Copy number of DRR206 gene	Expression of DRR206	Presence of nptII gene
Experiment:				
PCR of genomic DNA using primers a and b	**yes**	**no**	**no**	**no**
Southern blot using DRR206 gene as a probe				
Northern blot using DRR206 as a probe				
assay for root growth in kanamycin				
test for resistance to blackleg		**no**		

12. (5 points) What are the characteristics we should look for in choosing a plant species for production of pharmaceuticals?

13. (10 points) The slide below comes from the lecture material on Substantial Equivalence, in which the expression of over 51,000 genes was compared in different transgenic, mutagenized and

a) What does comparison of gene expression between the three unmutagenized, untransformed rice lines tell us?

b) To compare transgenics with controls, the authors used two different genes, BCBF1 and immunoglobulin. Why did they choose these two particular genes for evaluating the effects of transformation on gene expression?

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), 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

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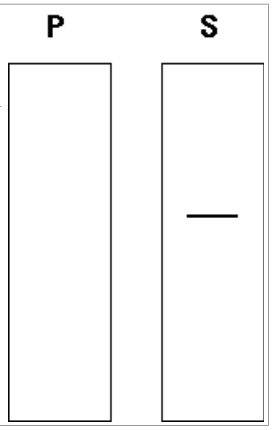
14. (10 points) The following Southern blots were done during a project to transform soybean with a gene from pea for fungal resistance (**P**). Explain the results in B and C in light of what we have discussed regarding the basic genetics of transgenes.

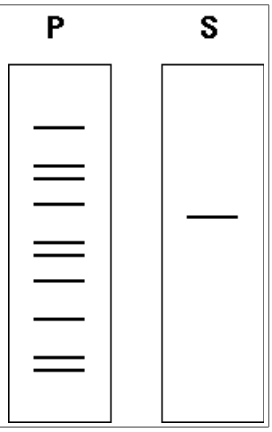
A

A Southern blot was done using DNA from the original, untransformed parent. The southern was probed with gene P, and the blot was washed and reprobed with the probe from a single copy gene from soybean, S. Not surprisingly, the Pea gene does not hybridize with soybean DNA, while a single band is seen with the soybean gene S, indicating that S is a single copy gene.



B

Soybean was transformed with gene P, and a Southern blot was done with DNA from a T_0 transformant. The transformant shows many bands for gene P. The blot was reprobed with S as a control.



C

The T_0 transformant from B is back crossed to the original parent for 3 generations. Each generation, progeny are crossed back to the original untransformed line, referred to as the recurrent parent.

The southerns are repeated using DNA from a BC3 plant, with the results shown at right.

T_0 x untransformed
|
v
BC1 x untransformed
|
v
BC2 x untransformed
|
v
BC3