Bio 328 -Spring 2005 NAME: Test #1

Please provide succinct answers in the space provided under each question. Unless otherwise noted in the margin the value of each question is 3 points.

1. (a) Why are the two kinds of self-incompatibiltiy (SI) mechanisms called “sporophytic” and “gametophytic”?

Ans.: In sporophytic SI, the male SI factor is made by the sporophyte (diploid parent), and in gametophytic SI, the male SI factor is made by the gametophyte (the haploid pollen).

(b) Contrast where the sporophytic and gametophytic SI reactions occur.

Ans.: The sporophytic SI reaction occurs on the stigma surface; the gametophytic SI reaction occurs in the transmitting tract of the style.

(c) There is evidence that ubiquitination is involved in both kinds of SI processes. Explain

Ans.: In gametophytic SI the male SI factor is itself an F-box protein, which is presumed to function as part of an E3 ligase complex that ubiquitinates proteins targeted for destruction. In sporophytic SI when the female factor (SRK) is activated by the male factor, it phosphorylates ARC1, which is a U-box E3 ligase demonstrated to promote the ubiquitination of proteins..

2. (a) What is the second Rule of Strong Inference?

Ans.: Devise a crucial experiment with alternative possible outcomes, each of which will, as nearly as possible exclude one of more of the alternative hypotheses.

(b) Describe an experiment that followed the second Rule of Strong Inference to resolve whether the simple inhibitor model to explain how the male factor of gametophytic SI functioned was true. In your answer indicate what is the simple inhibitor model, what experiment was done to test it, and why the results of that experiment supported or disproved the model.

Ans.: *The simple inhibitory model predicts that the male factor (F-box protein) binds to the female factor (RNAse) and targets it for ubiquitination and ultimate destruction by proteolysis. If this model were correct, then if you knock out the gene encoding the male factor, the pollen would not be able to ubiquitinate or destroy the RNase, with the consequence that the RNase would remove RNA from all* *pollen, and no pollen would be able to reach the egg (all pollen would be incompatible). When the experiment was actually done, the researchers found that when they knocked out the gene encoding the male factor(F-box protein), all pollen became compatible (i.e., the self-incompatibility mechanism was eliminated). The most obvious interpretation of this result is that the male factor must mediate the destruction of an inhibitor of the RNase.*

3. (a) How does ORE9 help to regulate senescence in Arabidopsis?

Ans.: ORE9 is an F-box protein that mediates the ubiquitination and destruction of protein(s) (maybe transcription factor(s)) that suppress the expression of senescence-activated genes. By promoting the destruction of this suppressor, ORE9 promotes the onset of senescence.

(b) In their publication on ORE9, why did Woo et al. consider it important to show that ORE9 interacted with ASK1?

Ans.: Woo et al. wanted to increase confidence in the conclusion that ORE9 was an F-box protein by showing that it, like all other F-box proteins, binds to a Skip-like protein (ASK1).

(c) What portion of ORE9 was critical for its interaction with ASK1? Give evidence for your answer.

Ans.: The region that contains the F-box motif is critical for ORE9 to interact with ASK1, because if this region was removed ORE9 no longer bound to ASK1 as measured by a co-precipitation assay.

4. (a) In the Figure below, which tests the role of expansin in rice walls, what is pKC1-1, and what is 2CBSU?

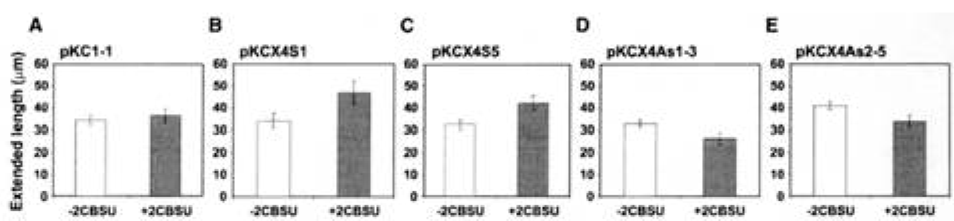

Figure 9. Measurement of Cell Wall Extensibility of Coleoptiles from Transgenic Seedlings.

Ans.: pKC1-1 is an empty vector used as a control, 2CBSU is the chemical agent that turns on the transgene (coding for expansin sense message or antisense message).

(b) In the Figure above, it appears that the results in B are the same as in C, and the results in D are the same as in E. What hypothesis was being tested in these experiments and why did the authors feel the need to duplicate their results in two different experiments?

Ans.: The hypothesis is that the extensibility of walls is greater the more expansin is in them. To be confident that the phenotype of the transgenic plant is due to the kind of gene it moved into it instead of where it inserted in the genome, it is necessary to show that the same phenotype can be observed in two independently produced transgenic plants carrying the same transgene.

(c) In D and E, "As" stands for antisense. By what mechanism does an antisense construct influence expansin expression?

Ans.: An antisense message of a gene will base pair with the sense strand for that same gene, forming a double-stranded RNA, which will be destroyed by the RNAi mechanism in cells. Thus plants expressing the antisense version of a gene will typically have a suppressed level of the sense mRNA, and less message for expansin typically leads to less expansin protein.

(d) Expansins and natriuretic peptides play complementary roles in regulating plant cell growth. Explain.

Ans.: Expansins increase the extensibility of walls needed for growth, and natriuretic peptides induce a decrease in water potential in cells (increased salt uptake), thus increasing the turgor needed for growth.

5. (a) Some fungi are pathogenic and some fungi are beneficial to plants. What gene is induced in potato by beneficial fungi, and what is the benefit of this induction to the potato?

Ans.: Gene encoding a phosphate transporter, which enhances the ability of the plant cell to take up phosphate from the fungus into itself, thus improving its nutrition.

(b) What is the evidence that for the induction of the gene noted in (a), cell-cell contact between the symbiotic partners is required? Your answer should describe the experimental methodology used to address this question.

Ans.: Promoter-GUS contructs for the phosphate transporter gene demonstrate that the only potato cells expressing the phosphate transporter gene are those that are occupied by hyphae of the symbiotic fungus.

(c) Regarding the question of whether mycorrhizal fungi assist in plant mineral nutrition, BRIEFLY describe the experiment that addressed this question, the results of the experiment, and the conclusion drawn .

Ans.: This experiment tested whether mycorhizal fungi can deliver phosphate nutrients to their host roots. In this experiment, radioactively labeled phosphate was added to a dish containing mycorhizal fungi, and the fungi were allowed to grow into the soil and establish contact with the roots of the pine seedling. The fact that radioactivity was subsequently found in the pine seedling indicated that the fungi could help the seedling harvest phosphate from their surroundings.

6. (a) In what distinctly different microdomains within the chloroplast would you find PSI mainly located and PSII mainly located, and how is the domain of PSI related to its function?

Ans.: PSI would be located mainly along the stroma lamellae portion of the thylakoid membranes, and PSII would be located mainly in the grana stacks (or appressed membranes), A key product of the activity of PSI is NADPH, which is used in the stroma of the chloroplasts to help drive the carbon fixation cycle, so PSI is located close to where its product will be used.

(b) What is LHCII, what redox conditions control the microdomain occupied by the chlorophyll proteins in it, and how do modifications in the structure of the proteins in LHCII afffect which microdomain of the chloroplast they occupy?

Ans.: LHCII is light harvesting complex II, the major light-harvesting protein in plant chloroplast membranes. Under conditions in which the ratio of reduced plastoquinone to oxidized PQ is high, LCHII is phosphorylated by a protein kinase and moves to the stroma lamellae. When this ratio is low, phosphate groups are removed from LCHII by a phosphatase and it moves to the grana membranes.

7. (a) Mel Brooks, who is a farmer in his spare time, decides to build a 10 acre enclosed greenhouse to grow his Blazing variety of soybeans (a C3 crop) in Georgia under high light & high temperature conditions, expecting this will increase his yield. Based on your knowledge of the carbon fixation cycle, advise Mel on whether this is a good idea and explain why. Your advice should include the terms Rubisco, CO_2, O_2, oxygenase.

Ans.: Probably not a good idea to grow a C3 crop under hight light & high temp. conditions. High light favors more rapid oxygen release (from H_2O splitting in PSII); high temperature favors closing of stomates, which can lead to CO_2 depletion in leaf air spaces; high O_2 + low CO_2 favors photorespiration, in which Rubisco will function primarily as an oxygenase rather than as a carboxylase, and there will be no net fixation of carbon.

(b) Mel was also considering rotating his soybean crop with his secret code 1234 crop (a C4 variety) in his greenhouse. Using the terms malate, bundle sheath cells, and outer mesophyll cells, advise Mel on the relative productivity he could expect from this crop under the greenhouse conditions, and why.

Ans.: High light + high temp conditions of greenhouse would favor growth of C4 plants. These plants should be more productive than C3 plants because they avoid photorespiration by a mechanism in which the enzyme PEP carboxylase fixes CO_2 into a 4 carbon compound that is rapidly coverted to malate. The malate is transported to bundle sheath cells where it is decarboxylated, releasing CO_2 that is used by Rubisco to fix carbon via the C3 cycle. The PEP carboxylase and generation of malate occur in outer mesophyll cells. This pathway avoids photorespiration because PEP carboxylase is not an oxygenase and can continue to fix CO_2 even when its concentration is very low.

(c) The efficient storage of carbon in seeds is crucial to plant fitness, yet the conversion of carbohydrate to oil in seeds results in the loss of one-third of the carbon as CO_2. A recent discovery reveals a mechanism by which green seeds can reclaim some of this loss. What is that mechanism?

Ans.: The mechanism is the capture of the released CO_2 by a seed-localized Rubisco that fixes carbon by a non-Calvin-cycle pathway.

8. (a) Describe two anatomical features that <u>typically</u> distinguish the companion cells in plants that use the apoplastic mode of loading their phloem from those cells in symplastic loaders.

Ans.: Typically (though not always) apoplastic transporters have no symplastic connections with the surrounding mesophyll cells and typically they have many wall invaginations increasing the surface area of cell membrane for apoplastic transport processes.

(b) What two kinds of transport proteins would you expect to find concentrated around the periphery of the companion cells of plants that load their phloem using apoplastic transport. Give reasons for your answer.

Ans.: Proton pumping ATPases to set up the proton gradient needed for sugar uptake, and a proton-sucrose co-transporter that couples the uptake of protons to the uptake of sugar.

(c) What is stachyose, where would you expect to find it in plants, and what role does it play in symplastic transport?

Ans.: Stachyose is a high molecular weight polymer generated in companion cells by a metabolic pathway that starts with sucrose as the initial substrate. The conversion of sucrose to stachyose allows the companion cells to serve as a sink for sucrose, taking it up symplastically from surrounding cells. Because the plasmodesmata leading from the companion cells to the sieve element have larger openings *than those leading from the companion cells to the mesophyll cells, the stachyose preferentially diffuses into the sieve elements, and this accounts for the symplastic loading of the sieve elements.*

9. (a) Water potential differences ($\Delta\psi$) play a role both in moving water through the xylem and in controlling the exit of water from leaves. Explain are those roles, and for the process that controls the exit of water from leaves, what regulates the $\Delta\psi$ in the cells that control this process?

Ans.: Water always moves into cells or spaces with a lower water potential, and the movement of water from soil to the top of plants is driven by water potential gradients, with the highest water potential in the soil and the lowest in the air spaces in leaves and stems. The exit of water from plants is controlled by pores, called stomata, whose opening is dependent on the water status of the guard cells surrounding each pore. The uptake of salt into the guard cells lowers their water potential and induces them to take up water, causing them to swell, which opens the stomata. The exit of salt from guard cells reverses the process and closes stomata.

(b) In considering the maximum height a tree can grow, a recent paper by Koch et al. identified a key limiting factor as a major control on height. What was that factor, and why does it become limiting at greater heights.

Ans.: A key limiting factor is the availability of water at the top of trees. As the water column gets higher, the tension on it becomes greater, eventually resulting in embolisms that break the water continuity.