

Provide concise answers in the space provided after each question, or, if more space is needed, continue on the back side of the page. The potential value of each answer is 4 points unless otherwise noted in the margin.

1, Dr. Jon Stewart, who does stand-up comedy in his spare time, is a leading expert in pollen self-incompatibility. His focus has been on discovering the male factor in gametophytic self-incompatibility, and several years ago he reasoned that it must be a plasma membrane protein that would let the female self-factor (RNase) enter the pollen tube, but would not allow the entry of non-self RNase. He spent several years designing and executing experiments to prove his hypothesis.

(a) What Rule of Strong Inference was Jon breaking in his approach to discovering the male factor?

*Ans.: Rule 1: Devise alternative hypotheses to explain a phenomenon you wish to understand.*

(b) Describe an experimental approach that followed the Rules of Strong Inference to test Jon's hypothesis. In your description state the three rules of Strong Inference and point out how each rule was followed.

*Ans.: Rule 1: Devise alternative hypotheses; Rule 2: Devise a crucial experiment with alternative possible outcomes, each of which will, as nearly as possible, exclude one or more of the hypotheses. Rule 3: Carry out the experiment so as to get a credible result. Experiment: Propose alternative hypothesis that RNase gets into both compatible and incompatible pollen (Rule 1); test this hypothesis by examining whether RNase can be localized in both compatible and incompatible pollen (Rule 2), using an immunolocalization method with proper controls (Rule 3).*

2. *Arabidopsis thaliana* is self compatible.

(a) What additional genes would it need to become a self-incompatible plant?

*Ans.: Genes encoding SCR (male factor) and SRK (female factor).*

(b) Where would these genes need to be expressed for *A. thaliana* to reject self pollen?

*Ans.: SCR would have to be expressed in pollen and SRK would have to be expressed in the stigma.*

(c) Describe experimental evidence to support the answers you gave to 2(a) and (b).

*Ans.: Arabidopsis thaliana is normally self compatible, but transforming it with genes for SCR and SRK from Arabidopsis lyrata confers on the transformed plants the property of self-incompatibility.*

3. (a) In the Figure below, what is the main question being addressed, and why was it important to use **purified** SCR6 to address this question?

Stigma genotype	Purified recombinant SCR	Pollen coat protein	Pollen	Pollen tube development
$S_6S_6$	SCR <sub>6</sub>	+	$S_{13}$ , $S_2$ , or $S_{22}$	no

*Ans.: The main question being addressed is whether pretreatment of  $S_6S_6$  stigma with SCR<sub>6</sub> protein is sufficient to induce the stigma to reject non-self pollen; i.e., whether the only component needed from pollen to induce the self-incompatible reaction in a stigma is the self version of the male factor. Important to use purified SCR6 to assure this was the only pollen protein needed to induce the response.*

(b) What ultimate response is induced by the activation of SRK that affects self-incompatibility, and where does this response occur?

*Ans.: Ultimate response is stigma secretes agent that inhibits pollen germination on the stigma surface.*

(c) Indicate one intermediate step between the activation of SRK and the ultimate self-incompatible reaction, and state what would be the expected function of this step.

*Ans.: SRK phosphorylates ARK1, an E3 ubiquitin ligase. Expectation is that activated ARK1 would catalyze the ubiquitination of a protein, leading to its proteolytic destruction, and this would result in pollen rejection.*

4. (a) Senescence is a regulated stage of development, and the senescence of specific tissues can occur in young seedlings. Give an example of a tissue that senesces in young monocot seedlings, and indicate when during seedling growth it senesces.

*Ans.: The coleoptile of monocot seedlings senesces when the seedling emerges from underground into the light.*

(b) For the tissue you named in your answer to 4 (a), indicate what is the normal function of that tissue, and what is the advantage to the plant of having that tissue senesce in a young seedling.

*Ans.: The normal function of the coleoptile is to serve as a sheath to protect the coiled primary leaf of the seedling. Once the seedling emerges into the light this sheath is no longer needed for the primary leaf must uncoil and expand. The advantage of having the coleoptile senesce is that this allows its contents to be degraded and recycled to growing parts of the seedling.*

(c) All life on earth is subjected to a certain level of ionizing radiation that can cause DNA damage. Why does this fact make scientists particularly interested in lotus seeds?

*Ans.: Lotus seeds can retain their DNA relatively undamaged through over a thousand years of dormancy, so they could have some advanced mechanism for protecting their DNA.*

5. (a) In the experiments that led to the discovery of expansins, scientists used freeze-thawed tissue sections. Why did they treat the tissue in this way, and what function observed in intact plants was retained by the freeze-thawed tissue?

*Ans.: Freeze-thawing the tissue killed the cells in the tissue, but leave the cell walls in the tissue with their protein components relatively intact, thus allowing the scientist to test the role of wall proteins in wall extensibility in non-living tissue. The tissue retained the function of acid-induced increase in wall extensibility.*

(b) In the experiments that led to the discovery of expansin, scientists boiled some of the freeze-thawed tissue sections. Why did they treat the tissue in this way, and what property of the tissue was lost after this treatment?

*Ans.: They boiled the tissue to denature the wall proteins. Boiled tissue lost the property of acid-induced wall extensibility, showing that wall proteins contributed importantly to this feature of walls.*

(c) Scientists used freeze-thawed, boiled tissue sections as starting material to discover expansin. Describe the experimental strategy they followed to do this.

*Ans.: Scientists used chromatography to separate wall proteins into purified fractions and then tested the ability of each fraction to restore the property of acid-induced increase in wall extensibility to the freeze-thawed and boiled tissue. The purified fraction most effective in restoring this property was expansin.*

(d) In the Rumex paper on expansin, what is the stimulus that initiates the growth response, and what is the evidence that ethylene is involved in this response?

*Ans.: The stimulus is submergence under water, and the evidence that ethylene is involved is that an inhibitor of ethylene synthesis blocks the growth response.*

6. (a) Symplastic and apoplastic pathways of mineral acquisition in roots both have a site where selection of which minerals will progress into the vascular system is made, but these sites are different. Explain.

*Ans.: Symplastic selection is made by a transporter at the plasma membrane of the root epidermal cell where the mineral first enters. Apoplastic selection is made by a transporter at the plasma membrane of the endodermal cell at the Casparian strip.*

(b) What is WAKL4, where is it located in cells, and what are two lines of evidence that it may play a role in the response of plants to high levels of zinc?

*Ans.: WAKL4 is a wall-associated protein kinase that is localized on the plasma membrane of cells. Two lines of evidence are: High levels of zinc induce increased abundance of transcripts of WAKL4 and knockout of WAKL4 reduces the zinc tolerance of plants.*

7. (a) Light excites chlorophyll molecules. In chloroplasts, what are two reactions by which the excited molecules return to their original unexcited state, and where in chloroplasts do these reactions take place?

*Ans.: Excited molecules return to their original unexcited state by resonance energy transfer, which occurs in the light harvesting complexes of PSII and PSI, and by successive electron transfers, which takes place along the thylakoid membranes in the electron transport chain.*

(b) Describe two distinct functions for plastoquinone, using the word “kinase” in one of the functions.

*Ans.: Plastoquinones are mobile electron carriers that shuttle between PSII and the Cytochrome complex. When the ratio of reduced PQ to oxidized PQ is high, this triggers the activation of a protein kinase that phosphorylates proteins in LHCII, causing them to move to PSI from PSII.*

(c) What potential roadblock does cyclic electron flow avoid and what key product of photosynthesis is not made during cyclic electron flow?

*Ans.: If electron flow from the two photosystems is faster than the available NADP molecules can be reduced to NADPH this could retard electron transport. Cyclic electron flow offers an alternative pathway to keep electron transport going. The key product not made by cyclic electron flow is NADPH.*

8. (a) What environmental condition would decrease photosynthetic efficiency in C3 plants, and why?

*Ans.: Any condition that would result in lowering the CO<sub>2</sub> concentration below the compensation point would decrease the photosynthetic efficiency of C3 plants, because this would cause RUBISCO to function as an oxygenase instead of a carboxylase, thus inhibiting the carbon fixation cycle.*

(b) The same environmental condition you note in your answer to 8(a) would not decrease photosynthetic efficiency in C4 plants. Why?

*Ans.: In C4 plants the primary carboxylase, PEP carboxylase, unlike RUBISCO, remains only a carboxylase even at high O<sub>2</sub>: CO<sub>2</sub> ratios.*

(c) In a recent Nature article, scientists related increasing global runoff of fresh water with atmospheric carbon dioxide. What appears to be the most likely explanation for this relationship?

*Ans.: Higher atmospheric CO<sub>2</sub> levels globally in recent decades would be expected to induce a relatively reduced pore size in the stomates of plants, which would result in lower transpiration rates, which would result in less uptake of rain water from soil, which would result in more fresh water runoff into streams.*

9 (a) Use the words adhesion, cohesion, xylem diameter and transpirational pull in discussing one limit to the ultimate height of trees on earth.

*Ans.: Water rises to treetops by transpirational pull of the column of water that is continuous from roots to the tops of trees. The column is supported in part by the adhesion of water to the walls of xylem cells which typically have a narrow diameter to help support the column, and by the cohesion of water molecules to one another. The transpirational pull is opposed by gravity and friction, creating tension on the column, which increases with the height of the column. Tension on the water columns of the tallest trees is close to the point of breakage, establishing this value as one limit to the ultimate height of trees on earth.*

(b) Relate stomatal movement to the transport of salts.

*Ans.: Stimuli (like light) that induce the opening of stomates, also induce the transport of salts into the guard cells, thus lowering their water potential and inducing them to take up water and swell. Stimuli that induce the closure of guard cells induce the export of salts from guard cells, thus reversing the process.*