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Evolution in action using the model plant *Arabidopsis*

Introduction

Variation, mutation and selection in plants: This document describes a series of experiments that demonstrates some of the components of evolution. In part A *Arabidopsis* plants are grown in the presence or absence of sodium chloride and measurements of rate of development demonstrate variation and effect of salt stress. In part B seeds can be collected from plants that flower early and late and used to generate subsequent populations which may differ in their characteristics if selection has occurred. On going selection may see divergence in the early and late populations. Part A takes about 6 weeks, part B takes as long as you like. The use of a mutagenised seed population increases the chance of interesting variation.

Part A: Plant to plant variation and the effect of (salt) NaCl

Date	Task
Week 1	• Set up Arabidopsis project
Week 2	• Prick out Arabidopsis
Week 3	• Measure Arabidopsis • Apply salt stress
Week 4	• Measure Arabidopsis • Apply salt stress
Week 5	• Measure Arabidopsis • Transplant first bolted
Week 6	• Measure Arabidopsis • Transplant last bolted
Week 7	• Harvest seed pods (siliques)
Week 8	• Collect and store seeds

At the end of this practical:

- You will have been introduced to the practicalities of running long term experiments with model plants.
- Organised tasks within a group.
- Collected and analysed data on plant growth and development.
- Quantified the effect of salt (NaCl) on Arabidopsis plants[1]

[1] This can be done with isogenic (inbred) lines or mutagenised seeds. The latter is probably needed if the experiment is to be taken through as selection in part B.

- Used Excel to test for statistical significance and to plot graphs.

General Experimental Information

Plants will be grown in the growth rooms. Arabidopsis are grown in individual trays of plug pots. These trays are placed in drip trays and watered with tap water. Salt is applied as one watering with 1.5 litres of 60 mM NaCl. Each week plant growth and development is assessed as number of rosette leaves, rosette diameter and whether or not the plant has bolted (begum to flower).

Materials

- Drip tray x 2
- Plug pot tray x 2
- Mutagenised Arabidopsis seed
- Table salt (NaCl)
- Fine paint brush
- Growth space (~ 1 m^2, ~ 20^oC, reasonable light)
- Storage tubes

Part A – the effect of salt on growth and adaptation in *Arabidopsis*

Sowing seeds – Week1[2]

1) You will be provided with two soil filled trays of plug pots standing in a drip tray in 1-2 cm of tap water
2) Moisten the surface of the soil in each plug with tap water using a spray bottle
3) Cut a square of clean dry white paper and put a gentle crease through the centre.
4) Gently tap a small number of seeds onto a piece of folded paper. Make sure that you don't put more than you need as it's not a good idea to put unused seeds back in the packet.
5) Moisten the tip of a fine paintbrush and pick a single seed from the paper and place in the centre of a plug pot. Repeat until you have three seeds in each the centre of each plug pot.
6) Spray gently with more water
7) Label the plug pots with name, date and type of seed.
8) Repeat steps 1-7 to produce a second tray of plug pots with seeds
9) Put plug pots and drip tray in the growing area, make sure your two plug pot trays are in different trays as later you will need to change the solution that you are watering one of the trays with for a salt solution.
10) Trays will be kept watered with tap water during the week[3]

[2] Timings will depend on the temperature and light in the place the plants are grown.
[3] It's probably a good idea for a member of staff to take responsibility for watering over the week. If watered on Friday plants will be quite happy over the weekend

Pricking out: Week 2

1. After one week you should observe the cotyledons protruding from the soil. Using fine tweezers carefully remove two of the seedlings to leave one.

Measurement and salt stress– Week 3

1. After 21 days since planting you should have a single small plant in the centre of each plug pot.
2. Measure the diameter of the rosette over the longest diameter.
3. Measure the number of rosette leaves.
4. Record the rosette diameter in a spread sheet for each plant in both trays.
5. Check of any plants have formed a flowering stalk (bolting) if so record the position of this plant.
6. When you have measured all the plants drain and water from each drip tray.
7. Water one tray with tap water.
8. Water the second set of plants with 750 mls of 60mM Sodium chloride (NaCl[4])

Mutants:

- The population of seeds you used has been mutagenised –that is it has been subjected to Gamma radiation which may have disrupted some of the DNA[5].
- Some of this damage will have been lethal and no adult plant will be seen, however ion some cases an altered protein may result.
- Most of the time this alteration is detrimental, but in some cases and/environments this new variation is better than that of the parent.
- This is an acceleration of the process of mutation that occurs all the time in all organisms.
- If you see a seedling with unusual features in control of salt treatments make a note of its position and sae seed form it as described for the early and late salt treated plants later.
- If this is indeed a mutant then some of the progeny will display the same phenotype.[6]

[4] The MW of NaCl is 58.43, so 1 mole (or 1000 mM) of NaCl weighs 58.43 g. To make up an l litre solution of 60 mM dissolve (58.43/1000) x 60g NaCl in 1 litre of water. For this experiment table salt and tap water would be fine.

[5] Note that these are NOY genetically modified plants in the GM sense – they are not 'dangerous' (neither are GM plants !) and you do not need a license!

[6] As Arabidopsis is an in-breeder the mutation should be apparent in subsequent generations and not be 'diluted' by wild type genes from other plants.

Measurement and Salt stress – Week 4

1. Measure the rosette diameter and number of leaves
2. Check which plants have bolted.
3. When you have measured all the plants drain and water from each drip tray.
4. Water one tray with tap water.
5. Water the second set of plants with a second 750 mls of 40mM Sodium chloride (NaCl)

Measurement and transplanting – Week 5

1. If it is still feasible record the rosette diameter and number of leaves of the control and salt treated plants. Put the data in your spreadsheet.
2. Record whether plant have formed a flowering stalk or not (bolting) Put the data in your spreadsheet.
3. Carefully remove the first two plants that bolted from the plug pot tray and re-pot them into an individual plant pot filled with compost.
4. Repeat this for the last two plants to have to have bolted, make sure these are clearly labelled.
5. You will now have two early flowering and two late flowering plants.
6. You can now dispose of the remaining plants in the plug pots.

Data analysis: the effect of salt

You can now analyse your data and compare the growth and development of the salt treated plants with those that were only exposed to water. You can put the data in an Excel spread sheet.

Mean and Standard deviation: Use the Excel spread sheet to calculate the mean and standard deviation of the data for each week.

Plotting graphs: Use Excel to plot a graph of time *vs* leaf number and time vs rosette diameter for control treatments. Using the 'add data' facility, add the second graph of change in leaf number with time for the salt treated plants. For both plots add the plus and minus standard deviations.

Part B – The effects of Selection on Arabidopsis [7]

1. Allow the early and late flowering salt treated plants to plants to grow further in their transplanted pots.
2. When the seed pods (siliques) are about 1-2 cm long stop watering and allow the plant to dry out.

[7] This additional exercise would be possible in a science club, possibly running over the two years of the six form

3. After a week or so the plants will be dry. Carefully cut off the whole flower stem at compost level and put individually into labelled paper bags.
4. Allow to dry for a week after which the seeds can be released from the siliques by gently rubbing the bag.
5. Carefully pour out the seeds into a storage tube. Label and cap the tube. Store in a dry environment at room temperature.
6. Seeds must now be stored for two weeks before they can be planted.
7. These seeds are the second generation, you will have two populations ‘early flowering’ and ‘late flowering’.
8. A second generation can now be set up using these seeds.
9. Repeat part 1, but instead of salt vs non salt plug pot trays have one tray of early flowering and one of late flowering.
10. Subject both to salt stress
11. Record the growth and development of the two different populations.
12. You can continue this selection experiment for many generations. You will have a full data set for each generation so these can be compared.
13. In particular you are applying divergent selection on flowering time (selecting early and late) when faced with salt stress.
14. Over many generations the average flowering time of the early and the late populations should diverge and salt response of the first generation to that of the first will begin to show the result of any selection that has occurred.

Useful web sites

http://arabidopsis.info/ **:** The Nottingham Arabidopsis Stock Centre (NASC) provides seed and information resources to the International Arabidopsis Genome Programme and the wider research community. From this site you can find lots of information and links about the model plant Arabidopsis.