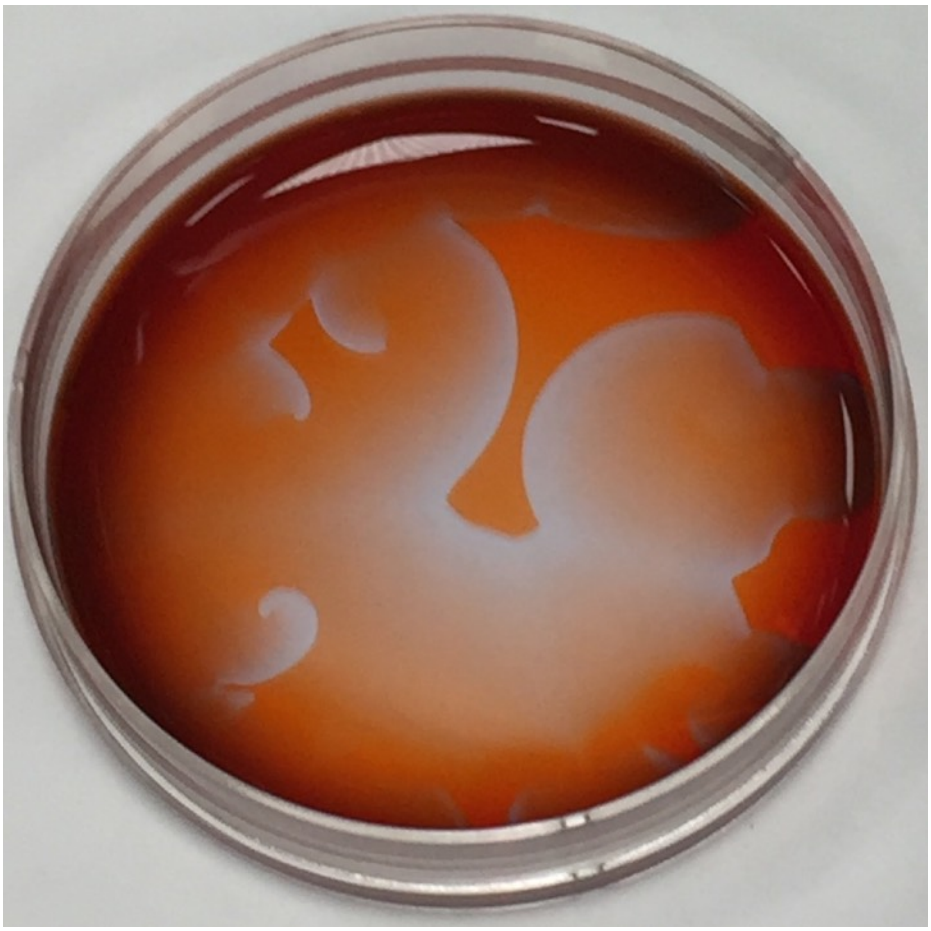
NST Part 1B Cellular and Developmental Biology Practical: Patterning

1. Chemical patterning: Belousov-Zhabotinsky reactions

The Belousov-Zhabotinsky (BZ) reaction is a chemical process that can show temporal oscillatory behavior or spatio-temporal structures as propagating waves, using a `bromate' (e.g., sodium bromate), an acid (e.g., sulfuric acid), a one electron redox catalyst/indicator (e.g., ferroin), and an organic component (e.g., malonic acid).

Boris P. Belousov discovered a form of this reaction in 1950 in Moscow. He was unable to publish his results due to a general belief that his reaction violated the 2nd Law of Thermodynamics. The original work was not published until 1981. Anatol Zhabotinsky, as a student enrolled in Moscow State University became interested in oscillating chemical reactions and heard about Belousov's discovery and attempted to meet Belousov. Due to Belousov's classified work in countering the effects of radiation and chemical weapons, Belousov was reluctant to meet with Zhabotinsky but did confirm the recipe for the BZ-Reaction to Zhabotinsky over the phone and through mail. Zhabotinsky then worked on, and refined the chemical recipes. Zhabotinsky was successful in publishing the his results in a biochemistry journal, rather than a chemistry journal. In 1968, Zhabotinsky presented his work at the *Conference on Biological and Biochemical Oscillators* in Prague, Czechoslovakia, bringing worldwide attention to the reaction.

The BZ reaction, serves as a classical example of non-equilibrium thermodynamics, resulting in the establishment of a nonlinear chemical oscillator. It shows that chemical reactions do not have to be dominated by equilibrium thermodynamic behavior. These reactions are far from equilibrium and remain so for a significant length of time and evolve chaotically. An essential aspect of the BZ reaction is its so called "excitability"; under the influence of stimuli, patterns develop in what would otherwise be a perfectly quiescent medium.



Week One AM: Preparation of BZ reaction

You will be provided with aliquots of the components of the BZ reaction:

(i) Acidified sodium bromate (0.5M $NaBrO_3$, 0.5M H_2SO_4)

(ii) Acidified malonic acid (0.4M $CH_2(COOH)_2$, 0.5M H_2SO_4, plus 0.1% (w/v) SDS detergent

(iii) Solution of ferroin, a redox indicator (25mM Fe (1,10-phenanthroline)$_3$ SO4)

1. **Mix the reactants**
   Combine the reactants in a 12.5 cm diameter Petri dish. Be sure to wear gloves and use protective eye wear. Gently swirl the plate to ensure mixing of the reactants. Ferroin is a bright red colour. After mixing the solution should immediately turn a bright blue colour, followed by darkened state.
2. **Spontaneous formation of oscillatory waves**
   The solution should be left undisturbed, preferably on a white coloured surface. The solution will eventually turn again to a red colour. If the solution is held in a thin layer, points within the solution will be seen to form in blue tinted relief from the rest of the red solution. If left to develop undisturbed, these points will form travelling waves, where the reaction behaves as an excitable medium. Patterns should develop spontaneously over the course of and hour or two - even reforming if the solution is disturbed.

The BZ reaction mechanism is highly complex, with many (~50) individual reactions that fall into several categories and involve complex interplay of many inorganic and organic molecules. The oscillations in this extremely complex system arise due to the interplay of autocatalytic positive feedback and time delayed negative feedback. In the BZ reaction, the concentration of bromous acid rises sharply when it exceeds a threshold dependent upon the concentration of a second chemical species, bromide ions; the rising bromous acid concentration then causes bromide ions to be produced, which subsequently cause the bromous acid concentration to fall. Waves propagate when a high bromous acid concentration in one area spreads to adjacent areas and causes the bromous acid concentration to rise above threshold there. It is of considerable interest because of similarities to patterning, self-organisation and signal propagation in biological systems. For example, the wave behaviour of the BZ reactions is analogous to organisation of high membrane potentials on the surface of a beating heart, and many biological systems behave as excitable media.

A relevant extract from Phillip Ball's book "Shapes - Nature's Patterns: a tapestry in three parts" is provided with other online reference material. This contains a non-technical description of the processes at work in this complex set of reactions.

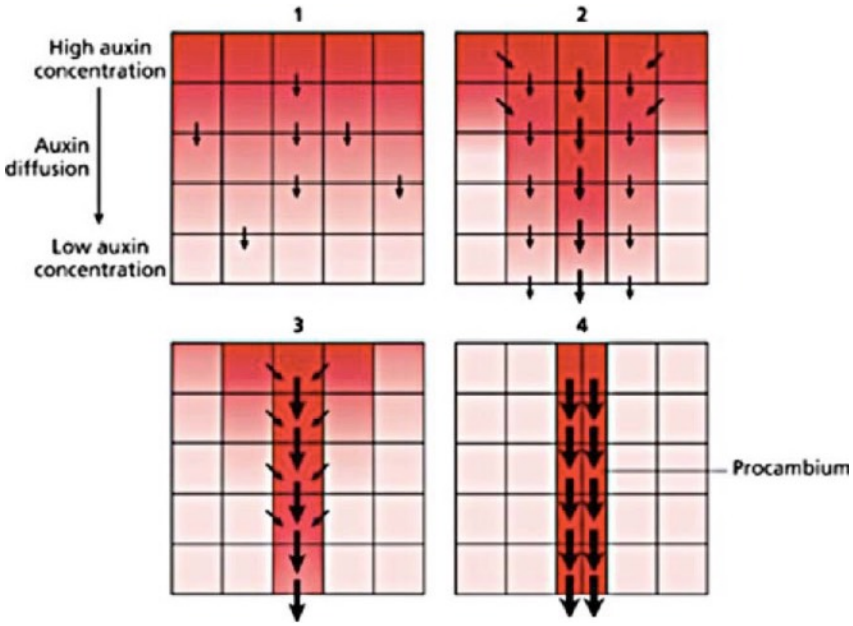
2. Cellular Patterning: Xylogenesis

The differentiation of plant cells into xylem vessels or tracheids with lignified walls is triggered by auxins. Auxins will induce xylogenesis in a number of plant tissues and, as in many other hormone-mediated processes in plants, there may also be a requirement for cytokinins and/or gibberellins (Sugiyama and Konamine, Cell Differentiation Dev. 31, 77, 1990).

Explants of Jerusalem artichoke (*Helianthus tuberosus* L.) tuber are composed predominantly of storage parenchyma cells. The explants can be used as "living test-tubes", exposed to different growth factors, and assayed for effects on growth and differentiation. Exposure of the explants to auxin and cytokinin has been shown to trigger the formation of ectopic xylem vessels (Dalessandro & Roberts, Am. J. Bot. 58:378, 1971; Dallesandro, Plant Cell Physiol. 14: 1167, 1973). Under different conditions, the xylem vessels can form as scattered individuals, or as precisely connected strands similar to those found after wounding of plant vascular traces. Generally, the differentiation of xylem vessels is a precise and absolute event. In the lateral direction, adjacent cells may adopt very different fates. In contrast, cells may adopt coordinated fates longitudinally. Some forms of feedback and communication must be responsible for symmetry breaking and coordination of cell fates. (D.E. Hanke & S.J. Green. *The contribution of auxin and cytokinin to symmetry breaking in plant morphogenesis.* in Shape and Form in Plants and Fungi, 1994)

The selective traffic of auxin regulates cell polarity in plant tissues. A combination of influx and highly selective efflux transporters coordinate the flow of auxin within the plant and control cell fates and outgrowth. Further, auxin regulates the flux of its own traffic through plant tissues. Apical-basal polarity in plant embryos, outgrowth of the root and shoot meristem and initiation and maintenance of the plant vascular system are all dependent on this precise feedback-regulated traffic of auxin.

Current models for auxin based regulation of plant cell growth and differentiation rely on a "bucket brigade" style polar traffic of auxin, via specifically localised efflux carriers throughout the plant. Some form of positive feedback between efflux carrier and auxin flux results in canalisation of long-distance pathways for auxin traffic. The same mechanism also provides a route for local control of cell fates through cellular responses to auxin.



As you will discover, application of synthetic auxin to undifferentiated tissue causes the formation of xylem vessels in Jerusalem artichoke tuber explants. Cells of the xylem can be easily visualised due to the formation of highly characteristic cell wall thickenings that are formed by the deposition of secondary deposits of cellulose and lignin. Synthetic dyes like safranin O can be used to stain and mark any cells that differentiate within a tissue explant.

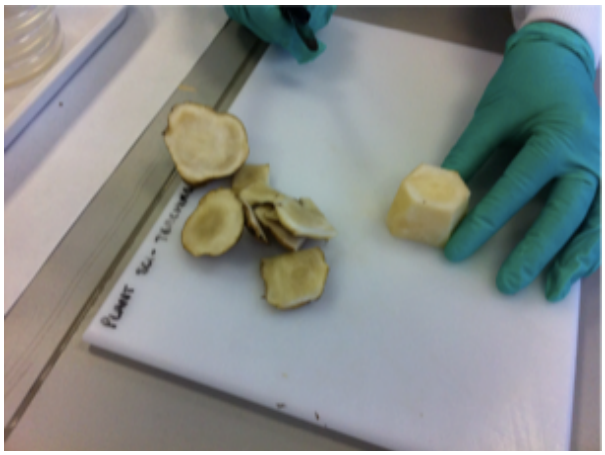
For this practical, in the first week you will produce sterile explants and grow these on different hormone-containing media. One week later, you will stain differentiated xylem elements, record patterns within the explants, and quantify the extent of differentiation under the different treatments.

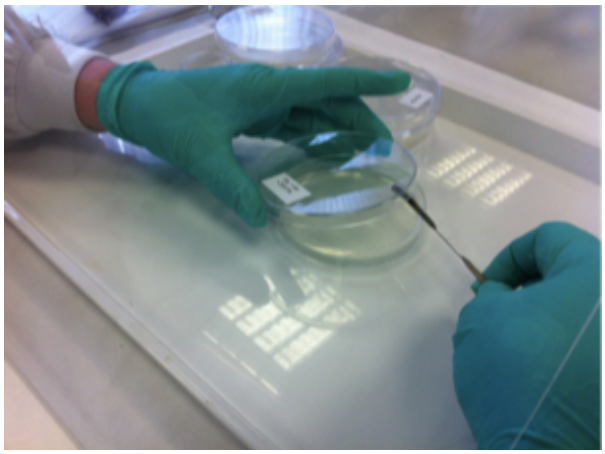
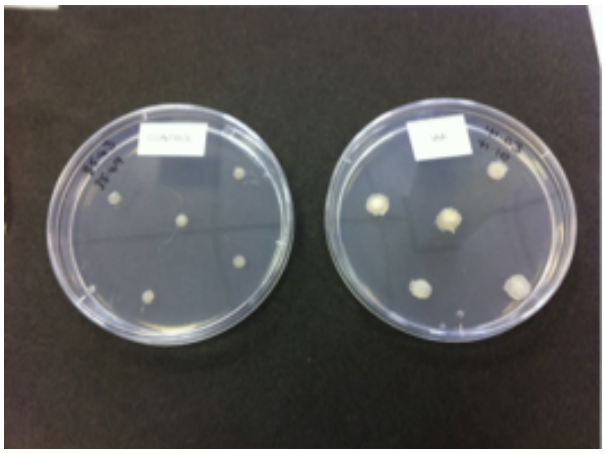
Week One PM: Preparation of explants

The aim is produce standard sized explants which are sterile, and can be cultured over the following week without suffering contamination from bacterial or fungal contamination. You are provided with sterile equipment and solutions. All equipment and materials that will come in contact with the tissue or agar should be uncovered for the shortest possible time. You are provided with ethanol and cotton wool to wipe equipment which you suspect of being contaminated.

1. **Trim the Jerusalem artichoke tube**
   On a board on the open bench, you should prepare an artichoke tuber by cutting off the cortex and epidermal tissues.
2. **Surface sterilise with sodium hypochlorite solution**
   Transfer the remaining lump of tissue to a breaker and cover with sodium hypochlorite solution for 5 minutes.
3. **Transfer under sterile "tent"**
   Transfer the beaker, tissue and sterilising solution to the perspex tent.
4. **Wash 3x with sterile water**
   Rinse and drain the tissue 3 times with an excess volume of sterile water.
5. **Trim the tuber piece with large scalpel on sterile petri dish lid**
   Cut away the top and bottom of the tissue with the large scalpel provided.
6. **Take cores of pith**
   Use the tissue borer to take cores of the central pith of the tuber. Take samples from the longitudinal centre of the tuber, avoiding vascular tissues in the outer lateral parts.
7. **Slice the tissue cores into 1mm thick disc-shaped explants**
   Cut the cores into thin (~1mm) slices with the small scalpel and deposit these in a small beaker of sterile water.
8. **Rinse twice with sterile water**
   Wash the discs with another 2 changes of sterile water in the beaker.
9. **Transfer to plates**
   N.B. weigh each plate immediately before and after addition of explants to obtain a measurement of the fresh weight of the tissue
   Transfer 5 tissue discs to each agar plate. (The plates include a control and various supplements and combinations of auxin (IAA), cytokinin (zeatin), and gibberellin (GA_3), as marked. Be sure to weigh the agar plates immediately before and after transfer of the discs, and calculate the fresh weight of added tissue.
10. **Wrap in aluminium foil, label - and incubate for 1 week at 25^{o}C in the dark**
    The agar plates should be kept in the upright orientation, stacked on top of each other,

wrapped in aluminium foil and **labelled**. They will be incubated at 25°C for analysis next week.



Week Two: Staining of differentiated xylem cells

In addition to the analysis of the cell disks that were prepared the week before, the practical session will provide an opportunity to ask questions about revision topics and the associated lectures.

1. Inspect tissue discs and note any differences
First, retrieve your plates, and examine the explants for any obvious differences in growth.

2. Weigh the tissue discs in plastic weigh boats
Collect each set of tissue discs in a plastic weigh boat and calculate the differences in fresh weight from the previous week. Following this, the tissue will be cleared and stained as follows:

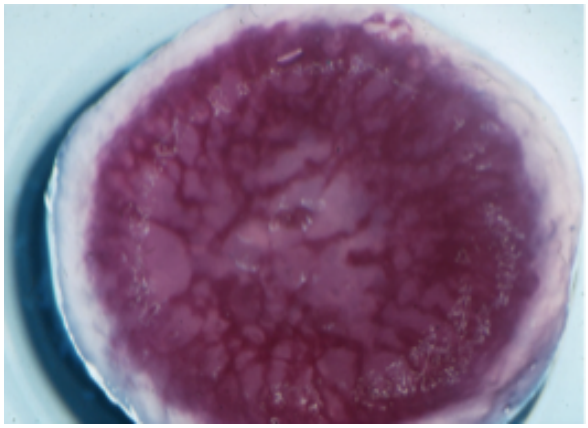
3. Incubate in 5% NaOH solution at 80C for 1 hour
Transfer the tissue discs to glass test tubes. Incubate in 5% (w/v) sodium hydroxide solution at 80°C for an hour.

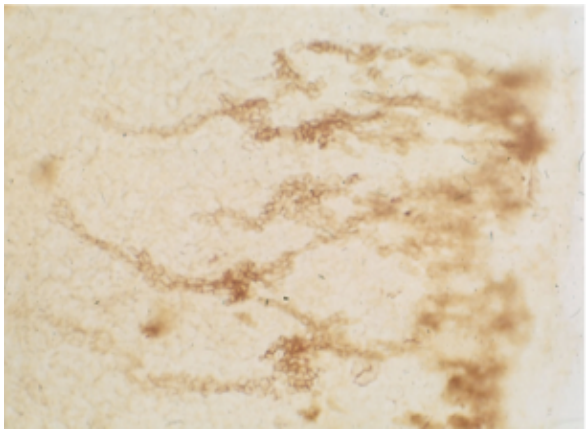
4. Wash several times with water
The tissue will become fragile after this treatment. Wash several times with water.

5. Incubate in 0.03% aqueous safranin O
Incubate in 0.03% (w/v) safranin O at 55°C for 20 minutes.

6. Destain in several changes of 1M HCl
Destain in several changes of 1M HCl over a period of an hour or so.

7. Use microscopy to inspect pattern of differentiated cells within intact discs
The patterns of xylogenesis can be seen under a dissecting microscope after mounting the tissue in a drop of glycerol on a depression slide.





Online resources including practical notes, presentation, background reading (as downloadable PDFs) and lecture materials can be found at:
http://haseloff.plantsci.cam.ac.uk
(click the "education" menu choice to navigate to the relevant section).