

Plant Phenomics Teacher Resource



Contents

About this resource
Australian Plant Phenomics Facility2
Introduction
Phenomics speeds up plant phenotyping4
Phenomics relieves the 'genomics bottleneck'
Forward and reverse phenomics
Phenomics research
Phenomics involves cross-disciplinary research and collaboration
Research Theme 1: Increasing yield9
Research Theme 2: Adapting to climate change 13
Research Theme 3: Biofuels
Phenomics technology17
Looking inwards: studying leaves and roots with high-tech imaging systems
Looking outwards: studying plant growth in controlled conditions and in the field
Glossary
Links and resources
Phenomics sites
Plant biology sites
Molecular biology sites
Curriculum links
Unit 1: Biodiversity and the interconnectedness of life
Unit 2: Cells and multicellular organisms40
Unit 3: Heredity and continuity of life41
Unit 4: Maintaining the internal environment42
Appendix 1: Battle of the Plants
Appendix 2: Class activities with plant phenomics52

About this resource

The Plant Phenomics Teacher Resource provides background knowledge about plant phenomics research and technology for upper secondary school teachers.

The resource describes current Australian plant phenomics research, and the technology behind the research, in the form of short background 'briefing notes' with accompanying images. It also contains classroom activities you can use to bring the science of phenomics to life for your students.

We hope this resource helps you create interest among your students in this new and important field of science.

Throughout the resource, words defined in the Glossary are italicised and bolded on their first use.

The topics covered in this resource link to the Senior Secondary Australian Curriculum (Biology).

This resource has been produced by the Australian Plant Phenomics Facility.



Phone: 08 8313 0159 Email: jo-anne.pitman@adelaide.edu.au Web: www.plantphenomics.org.au © Australian Plant Phenomics Facility 2010

Contact the Australian Plant Phenomics Facility:

COPYRIGHT STATUS: © 1993–2013, the various Australian Government agencies (www.australia.gov.au) and universities.

Jo Pitman

Australian Government agencies, non-government agencies, universities and individuals have certain rights in this material.

PUBLIC ACCESS: Information and data in this document have **NOT** been placed in the public domain, but are provided for the personal non-commercial use of educators, students, scholars and the public.

Unless otherwise stated, material presented in this document may be copied for personal or research use, or published for non-commercial educational purposes, provided the source and custodians of any extracts are fully acknowledged.

DISCLAIMER OF LIABILITY: No guarantees or warranty, expressed or implied, including warranties of merchantability and fitness for a particular purpose, are made as to the currency, accuracy, quality, completeness, availability or usefulness of data, information, apparatus, product, or process disclosed, provided through this service, and no responsibility or legal liability is assumed for any damages or inconvenience arising from its use.

DISCLAIMER OF ENDORSEMENT: The views, opinions, findings, and conclusions or recommendations expressed in this document are those of the author(s) and data custodian(s) and do not necessarily reflect the views of various Australian Government agencies and universities.

Reference in this document to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favouring by any Australian Government agency and shall not be used for advertising or product endorsement purposes.

Further information contact: alyssa.weirman@csiro.au

Australian Plant Phenomics Facility

The Australian Plant Phenomics Facility has nodes in Adelaide and Canberra.

A central component of The Plant Accelerator[®] (TPA) is the first automated high-throughput phenotyping system in Australia, which remains unique in both scale and open-access policy. The system boasts a range of imaging technologies (RGB, infrared, fluorescence and hyperspectral imaging) to facilitate innovative high-quality research into plant performance in different environments. The facility's phenotyping capacity includes DroughtSpotter platforms to support precision heat and drought screenings and field phenotyping services.

The HRPPC combines expertise in plant science and engineering to develop and build (i) cutting-edge phenotyping technologies to support medium throughput phenotyping of model and potted plants in controlled environments, and (ii) novel plant phenotyping solutions to support research experiments at large scale and high throughput in the field. Field applications include ground and aerial platforms with a capacity of over 250,000 plots p.a. and the first high-resolution plant phenotyping capability for glasshouse/field environments – the Cropatron.

The APPF node at the Australian National University (ANU) has unique expertise in phenomics, bioinformatics, hardware and software development and data visualisation. This provides essential research support to APPF customers, linking phenomics data to underlying genomic variation. The node offers modern PC2 facilities and provides the only quarantine certified plant growth facilities in the ACT region. With a strong history of supporting ground-breaking plant research, including the development of open source, high throughput phenomics infrastructure and visualisation tools, the node creates open data sets for plant science researchers nationally and internationally.



Introduction

Imagine a researcher sitting at a laboratory bench, painstakingly measuring the length and width of every leaf on several hundred plants, after they have taken six months to grow to maturity and produce seed.

She collects the seed from each plant, then takes each plant out of the pot, shakes off all the soil, cuts the roots off and weighs the plant. Next, she dries the leaves and roots in an oven so that later they can be ground up in solvent to determine the chemical composition of the plant tissues.

By the time she has finished, all the plants have been destroyed to gain the measurements. Once all the data has been analysed, the few packets containing seed are kept to grow up more of the plants with the desired characteristics.

Now fast-forward a few years. The researcher again has hundreds of plants in pots to analyse. But this time, she places trays containing the pots on a conveyor belt, and turns the belt on. The plants, which are only one month old, move through a series of chambers, each one automatically taking different images and measurements.

Within a few hours, the high-tech imaging and computing systems have automatically measured the plants and obtained the necessary data. Three-dimensional images of the plant are used to calculate leaf size and plant mass. The young plants with the characteristics the researcher is looking for are selected and grown on to mature for seed; the rest are discarded.

This is not the future. It's 2013, and the plants are being analysed at the Australian Plant Phenomics Facility. Welcome to the world of high-tech, high-speed, automated plant **phenotyping**.





The high-tech automated plant analysis systems of plant phenomics have sped up traditional, time-consuming methods of plant analysis.

Phenomics speeds up plant phenotyping

Phenomics researchers study how the genetic makeup of a plant determines its phenotype – that is, how it looks and performs.

A plant's phenotype is a combination of its genetic make-up, or **genotype**, and its environment. Plants of the same genotype can have different phenotypes, depending on the conditions they are growing in.

and its environment. Plants of the same genotype can have different phenotypes, depending on the conditions they are growing in.

Analysing a plant's visible characteristics, or traits, is called phenotyping. Phenotyping can be a laborious process, taking many days, weeks or even months.

The science of phenomics speeds up phenotyping by using automated, high-tech imaging systems and computing power. Depending on the trait, phenomics techniques can cut phenotyping time down from weeks to minutes, or even seconds.

The high speed of phenomics-based plant phenotyping speeds up the process of selecting plant varieties that perform better in the field. This could mean that drought-tolerant or salt-tolerant plants, or crops that are more efficient at **photosynthesis** or can grow better under higher levels of atmospheric **carbon dioxide**, may be available in a few years, rather than decades.

Speeding up plant breeding is of crucial importance. Our planet will be expected to feed twice the population in the next 50 years, meaning that crop yields have to hugely increase, under the pressure of a changing climate, if we have any hope of feeding the world.

The faster we can speed up plant breeding, the more hope we have.



Phenomics speeds up plant phenotyping > Phenomics relieves the 'genomics bottleneck'

Phenomics relieves the 'genomics bottleneck'

The science of *genomics* – the study of genes – has produced masses of information. Phenomics can translate this information into useful applications.

Phenomics researchers look at how a plant's phenotype fits with what we know about its genes. Over the past decade, the **genomes** of many plants have been **sequenced**, giving scientists realms of information about plant genes. But, a 'bottleneck' has developed in which developments based on genomic information are unable to keep pace with the huge amount of data that genomics produces daily.

Phenomics can relieve this bottleneck by speeding up phenotyping and linking the resulting data to **gene** sequence information. In this way, gene discovery can be put to good use by the faster development of useful crop varieties.



Phenomics speeds up plant phenotyping > Forward and reverse phenomics

Forward and reverse phenomics

Forward phenomics is a way of finding the plant varieties that are the 'best of the best'. Reverse phenomics is pulling the 'best' varieties apart to discover why they are the best.

Forward phenomics speeds up plant breeding by *screening* large number of plants at the seedling stage using automated imaging technology. This makes it faster to identify interesting traits, as the plants do not have to be grown to an adult stage in the field.

Thousands of plants can be screened in pots running along a conveyor belt, and travelling through a room containing automated imaging systems such as infra-red or 3D cameras. The pots are labelled with barcodes or radio tags, so that the system can identify which pots contain plants with interesting traits. The selected plants can then be grown up to produce seed for further analysis and breeding.

In reverse phenomics, the phenotype, or desired trait – such as drought tolerance – is already known. Researchers then try to work out the mechanisms that control the trait and the gene or genes that are responsible for it.

Once the gene has been identified, plants can be screened to see if they contain that gene. Or, the gene can be bred into new varieties.



Phenomics research

Research at the Australian Plant Phenomics Facility services many projects, but three of their research themes are of great importance to the future of humankind.

The research themes are:

- increasing plant yield
- · adapting to climate change
- · developing biofuels that don't compete with food crops.

The research within these themes covers many subject areas, but has one thing in common; it is focused on coming up with practical solutions to problems that will affect us, our children and grandchildren, well into the future.



goal for plant researchers.

Phenomics research > Phenomics involves cross-disciplinary research and collaboration

Phenomics involves cross-disciplinary research and collaboration

Phenomics researchers are not just biologists. Chemists, physicists, computer scientists, engineers, mathematicians, physiologists, microscopists, geneticists and plant breeders work together to develop new phenomics-based phenotyping methods.

Collaboration is a very big part of phenomics, as it is in all sciences. Being able to collaborate with other researchers and sharing phenomics results and techniques at meetings and conferences helps everyone find solutions to the problems threatening humanity sooner.

The Australian phenomics facility is a member of the International Plant Phenomics initiative (http://www.plantphenomics.com) and the European Plant Phenomics Network (http://www.plant-phenotyping-network.eu/eppn). These initiatives include facilities in Germany, France, Canada, Hungary, Israel, Denmark, the Czech Republic, the Netherlands and the United Kingdom. These networks bring together plant phenomics institutions and companies globally to improve collaboration.



A German phenomics researcher tests a robotic plant analysis system.

Research Theme 1: Increasing yield

The Food and Agriculture Organization (www.fao.org) predicts that the world will need to produce 70 per cent more food for the 9.1 billion people that will populate the planet by 2050.

Since the late 1960s, researchers and plant breeders have been able to increase crop yields – that is, the amount of harvestable, useful material from a plant – by an average of 2.5% a year, allowing crop production to keep pace with population growth.

But, in the last decade, yearly crop yield gains have slowed to the point of stagnation. Cereal yields, for example, are becoming limited by the capacity of plants to convert carbon dioxide from the atmosphere into the *carbohydrates* that form the planet's major food source.

Add population growth, a decline in suitable agricultural land area, competition for this land from biofuel crops, spiralling fertiliser costs, and a planet that is running out of fresh water into the mix – and the result is a looming global food crisis.

This is a problem that needs solutions as soon as possible. Plant phenomics is one part of the solution, offering the hope of speeding up plant breeding of crops that can produce more food in a changing climate.

Phenomics-based projects aimed at increasing yield include:

- 'Supercharging' photosynthesis
- Improving wheat yield
- *Brachypodium* the cereal 'lab rat'.

Annual increases in yield of maize, wheat and rice have fallen substantially in the last decade. We need to breed crops that can produce larger yields, under the influence of a changing climate.



Image: New Scientist, June 2008

'Supercharging' photosynthesis

A group of researchers that form the International C4 Rice Consortium (http://c4rice.irri.org) are trying to double rice yields by increasing its photosynthetic capacity.

Plants have two major photosynthetic mechanisms, named **C3** and **C4**. C3 plants include rice, wheat, and most other crop plants. C4 plants include maize, sorghum and sugarcane.

C4 photosynthesis is more efficient than C3, especially under higher temperatures, drought conditions, and limited nitrogen supplies. C4 maize plants, for example, produce twice the yield of C3 rice when grown with the same amount of water and nitrogen.

C4 plants have a different cellular structure compared with C3 plants, which allows them to concentrate carbon dioxide inside the leaf. If researchers can identify the genes that control this, they may be able to replace the C3 photosynthesis pathway of rice with a C4 mechanism.

The rice consortium plans to use the Australian phenomics centre to fast-track screening of rice varieties. They will use techniques such as 3D imaging and fluorescence imaging to find plants that already have some C4-like characteristics. They will then add more C4 genes to these varieties and retest them to find plants with higher yields, as well as those that use nitrogen and water most efficiently.



Rice (left) is a C3 plant, and maize (right) is a C4 plant.



In C3 plants such as rice (top), the leaf mesophyll cells (red) take up carbon dioxide and also fix carbon during photosynthesis. In C4 plants such as maize (bottom), the leaf mesophyll cells (red) pump carbon dioxide into specialised bundle-sheath cells (yellow and red), where carbon is later fixed during photosynthesis.



Improving wheat yield

Phenomics researchers are aiming to double wheat yields by searching though the world's wheat varieties to find those with high rates of photosynthesis.

One of the major limiting factors in photosynthetic performance is the inefficiency of an enzyme called *Rubisco*, which fixes carbon from the air into carbohydrates.

Some plants have better Rubiscos than others, and also use nitrogen fertiliser more efficiently.

Researchers will use the high-throughput abilities of phenomics to quickly search through thousands of wheat varieties from all over the world, looking for plants that have a better-performing Rubisco, higher rates of photosynthesis, and better ways of using its Rubisco.

Phenomics researchers based in Australia are collaborating with the International Maize and Wheat Improvement Center (www.cimmyt.org) in this research. As well as looking for wheat with improved photosynthesis, researchers are searching for varieties that will adapt best to land affected by environmental stresses, such as **nutrient** deficiency, drought and salinity.



Brachypodium – the cereal 'lab rat'

Using fast-growing 'model' plants that have had their genomes sequenced makes it easier to find genes responsible for growth and yield in food crops.

Phenomics researchers are using a small wild grass called *Brachypodium distachyon* as a 'lab rat' to speed up wheat research.

The entire genome of *Brachypodium* is known; it has many genes in common with wheat, and grows much faster. Researchers can therefore identify useful genes in *Brachypodium*, rather than searching through wheat, which takes much longer to grow and has a more complicated genome. Once the gene is known in *Brachypodium*, it is much easier to locate in wheat.

RESEARCH SPOTLIGHT

Researchers are using *Brachypodium* and phenomics techniques to study root formation and growth, to help to improve water use efficiency and yield of wheat plants. They are also searching for genes that allow plants to grow in low-nutrient conditions.

To analyse *Brachypodium* root growth and architecture, researchers float the plants in water on a clear plastic tray, and use a flatbed scanner to take high-resolution, high-contrast images. A computer program transforms the images into knowledge, by calculating root length and diameter, and analysing root branching patterns.



Image: Michelle Watt and Vincent Chochois, CSIRO

Phenomics research > Research Theme 2: Adapting to climate change

Research Theme 2: Adapting to climate change

Australian farmers have long been battling drought and increasing salinity. With climate change only predicted to make conditions tougher, urgent research into developing climate change-resistant crops is needed.

Phenomics-based projects aimed at adapting to climate change include:

- Drought-tolerant wheat
- Salt-tolerant wheat and barley.



13

Phenomics research > Research Theme 2: Adapting to climate change

Drought-tolerant wheat

The use of water by crops is a complex trait that varies at different growth stages and under different environmental conditions.

To breed wheat that can cope with dry conditions, researchers have to study how different varieties perform in the field over a whole growing season.

Combining results from phenomics technology, such as spectral reflectance to see if plants are stressed by drought conditions and infrared imaging to determine *canopy* temperature, with weather and soil data gives researchers information about how the plants cope over the whole season using phenomics field technology. This research will speed up the detection of wheat varieties that use water most efficiently, and are therefore better suited to drought conditions.

Australian researchers are collaborating with the International Center for Agricultural Research in the Dry Areas (http://www.icarda.org) and the International Centre for Maize and Wheat Improvement (http://www.cimmyt.org/) to develop drought-tolerant wheat.



In another collaboration, Australian researchers are working with the German Forschungszentrum Jülich (http://www. fz-juelich.de/portal/EN/Home/home_node. html) research centre to develop wheat with greater shoot or root growth, which will help plants cope with drier conditions.

Using time-lapse photography, researchers have studied shoot and root growth simultaneously, and have found that wheat varieties send different amounts of carbohydrate to roots and shoots at different times. For example, roots grow more in dry deep soil when the leaves receive more light.

Australian and German researchers are collaborating to develop wheat with greater shoot or root growth to help plants cope with drier conditions. Phenomics research > Research Theme 2: Adapting to climate change

Salt-tolerant wheat and barley

Two-thirds of Australian cereal crops are affected by salinity (high salt levels in the soil).

Using the Phenomobile and infrared cameras, CSIRO scientists are taking measurements of wheat and barley plants grown in saline conditions in the field, to screen for salt-tolerant varieties.

Plants growing in salinity-affected areas struggle to get enough water from the soil. They close the **stomata** in their leaves to reduce water loss, but this slows photosynthesis and reduces yield. It also heats the leaves up, which is why infrared cameras give a quick way of identifying which plants keep their stomata open more in salty conditions.

The researchers are hunting for varieties that can keep their cool in salty soil, and will study how this affects their yield.



Research Theme 3: Biofuels

Biofuels are fuels such as ethanol and biodiesel that are produced from plant matter, called 'feedstocks'.

Biofuels have often been produced using crops such as corn and soybeans. This is causing concern around the world, as such crops could not only be eaten be people instead of turned into fuel, but also compete with food crops for the best agricultural land.

Researchers are trialling alternative plant species as feedstocks for biofuels; non-food plants that can grow on less productive land 'marginal' land. But, these crops will need to tolerate a wide range of environmental stresses, such as low water availability, salinity or low nutrient supplies, to be able to grow successfully in these areas.

Non-food biofuel feedstocks are called lignocellulosic **biomass** materials. This is the fibrous, woody, inedible parts of plants. Such plants include fast-growing trees, shrubs, and grasses. One species showing a lot of promise is called switchgrass (*Panicum virgatum*), which is already being used for biofuel production in the United States.



Switchgrass can grow in areas with low water and nutrient availability, and produces a high yield of biomass. However, despite its great potential, switchgrass has a very complex genome and a long *life cycle*, making its breeding very slow. Phenomics researchers are using the model plant, *Brachypodium distachyon*, to speed up this process. Because this plant is genetically close to other crops, it will be possible to translate any trait of interest to the field.

Other phenomics biofuel research aims to better understand plant structure and processes such as *cell division*, nutrient uptake and water use in non-food biofuel crops. Understanding these basic cellular processes will speed up breeding of plants that will grow most efficiently and make the most cost-effective biofuels.

Phenomics researchers are using the model plant, *Brachypodium distachyon*, to speed up the process of breeding switchgrass for biofuel production.

Phenomics technology

Phenomics technology can be used to study plants from the small scale – studying individual cells or leaves – up to the large scale of an entire ecosystem.

The information about phenomics technology in this resource has been broken up into two sections:

- · Looking inwards: studying leaves and roots with hi-tech imaging systems
- Looking outwards: studying plant growth in controlled conditions and in the field.



Looking inwards: studying leaves and roots with high-tech imaging systems

Phenomics borrows imaging techniques from medicine to allow researchers to study the inner workings of leaves, roots or whole plants.

The imaging techniques can be used to screen large numbers of plants for traits or phenotypes of interest, such as drought or salt tolerance. As plants are not destroyed, they can also be studied more than once at different stages of growth.

Phenomics imaging techniques include:

- three-dimensional (3D) imaging to study plant shape and structure
- far infrared (FIR) imaging to study leaf temperature
- fluorescence imaging to study photosynthesis and plant health
- magnetic resonance imaging (MRI) to study root growth in soil
- spectral reflectance to study plant chemical make-up.



Image: APPF

Phenomics technology > Looking inwards

Three-dimensional (3D) imaging

A series of cameras using visible light take digital photos of the top and sides of plants. The images are combined by a computer program into a 3D image of the plant.

Once a 3D image has been created, many measurements can be obtained without having to go back and look at the plant. These include:

- shoot mass
- leaf number, shape and angle
- leaf colour
- leaf health whether it is alive, dying or dead.

Plants can be analysed at any stage of growth, and can even be imaged over their entire life cycle, from germination until seed formation, and finally death.





A cotton plant prepared for 3D image analysis (left) and a 3D computer-generated reconstruction (right).

Far-infrared imaging

Far-infrared (FIR) cameras are used to study temperature. They use light in the far IR region of the spectrum (15 micrometres-1 millimetre).

Researchers can use FIR cameras to measure and calculate temperature differences either within leaves of one plant or between different plants.

Temperature differences can be used to study salinity (salt) tolerance. For example, the leaf temperature of wheat and barley plants rises as salt levels increase. Plants with leaves that can maintain lower temperatures in the presence of salt are more salt-tolerant, and should therefore grow better in saline soils. Using high-speed FIR analysis has made it possible to measure salt stress in young seedlings instead of advance plants, cutting back on the time needed for phenotyping.

FIR imaging can also be used in the field to detect plants with cool canopies. The cooler plants are the ones with superior root systems that are taking up lots of water.

FIR cameras can also measure changes in stomatal conductance, which is the speed at which water evaporates from the stomata, or pores, of leaves. Stomatal conductance can be used to calculate rates of photosynthesis.



Far-infrared images of warmer salt-treated (left and right) and cooler non-salt-treated wheat seedlings (centre).

Image: APPF

When taking far-infrared images, plants are placed in front of an acrylic screen that does not transmit infrared radiation. This gives a clearer, high-contrast background to allow more accurate analysis.



Fluorescence imaging

Fluorescence imaging is used to study plant health and photosynthesis. Fluorescence occurs when an object absorbs light of one wavelength and gives off light of a different wavelength.

Plant phenomics fluorescence imaging works by exposing the plants to red light. The researchers measure the light that is reflected back from the plants at various wavelengths between 680 and 730 **nanometres**. The reflected light is converted into false-colour signals by a computer program, allowing differences in fluorescence to be easily observed. Although only about 1% of the light falling on the plant fluoresces, this is enough to analyse water transport in the plant to measure photosynthesis and stress responses.

Chlorophyll fluorescence is affected by changes in photosynthetic performance well before any other measurable effects can be seen in plants, making it easy to see when plants are stressed, or performing well, at a very young age.





Chlorophyll fluorescence is commonly used in phenomics to see the effect of different genes or environmental conditions on the efficiency of photosynthesis. Plants are exposed to red light (left) and reflected light is converted into false-colour signals (right) to determine fluorescence levels.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is used to study plant roots. MRI uses a magnetic field and radio waves to take images of roots in the same way as it takes images of organs and soft tissues in medical applications.

The hidden nature of plant roots makes them difficult to study. Relatively little is known about how they interact with the soil environment, and how root structures form. Digging roots up destroys them, and makes it impossible to study how the root system is placed (its geometry) in the soil, or how it relates to local soil conditions.

Plants can be grown in transparent agar so that roots can be seen, and this can be done at high throughput, but this does not exactly mimic their behaviour in soil.

MRI analysis allows the 3D geometry of roots to be analysed in plants grown in tubes of soil or sand. Using MRI is not a high-throughput technique like other phenomics technology, but is useful to validate the findings of roots grown in the artificial conditions of agar pots.

> These two MRI images show the effect of temperature on root growth. Both the growth rate and formation of lateral roots are affected at the lower temperature.



Spectral reflectance

Spectral reflectance is used to determine the chemical composition of plants. Spectral reflectance is the fraction of light that is reflected by a non-transparent surface.

Depending on the part of the *electromagnetic spectrum* that is used to analyse reflectance, different matter can have different patterns of reflectance. This is called the spectral signature.

Images of plants can be taken with a 'hyperspectral' camera. This camera measures all wavelengths of light that are either reflected or absorbed by a plant, and can measure from the ultraviolet end of the spectrum (400 nm) to the near-infrared (1000 nm).

In certain regions of the spectrum, healthy, green plants have a very different spectral signature from stressed plants. But, in the visible region of the spectrum, the signatures are similar. This means that researchers can use the hyperspectral camera to tell if crop plants are stressed, for instance by saline or drought conditions, well before they can see these effects by eye.



RESEARCH SPOTLIGHT

Phenomics researchers can use spectral reflectance technology to monitor several plant properties in the field at the same time. These include the levels of chlorophyll and other pigments in leaves; and water-soluble carbohydrates and nitrogen in leaves, stems and grains. This means that researchers can determine the biochemical composition of crop plants without having to destroy the plants by harvesting.



In images taken with a normal camera, each pixel has only three bands, or colours: red, green and blue, shown on this graph. In contrast, each pixel in an image taken by the hyperspectral camera contains 340 bands that span the entire visible and near infrared spectrum.

Looking outwards: studying plant growth in controlled conditions and in the field

Phenomics researchers use a range of technology to study plant growth at different levels. They can analyse plant performance at the level of a whole plant, a small plot or large field of plants, or an entire ecosystem.

Researchers study plant varieties both under controlled conditions to analyse one variable at a time, and in the field to understand the effects of the wider environment on plant performance.

Phenomics plant growth analysis makes use of a range of technology, which operates at a range of different scales:

- remotely controlled growth cabinets
- CabScan
- TrayScan
- PlantScan
- the Cropatron
- the Phenonet
- the Phenomobile
- the Phenotower
- the Multicopter.



This infrared image of a field of crops, taken from the Phenomobile, reveals the temperature of the leaves. Phenomics technology > Looking outwards

Remotely controlled growth cabinets

Precisely controlling plant growth conditions allows all the experimental variables to stay the same except for the variable being tested.

Researchers from all over Australia and the world can change the growth conditions inside the cabinets either in person, or remotely over the internet.

The growth cabinets can control:

- · light amount and intensity
- day and night length
- temperature
- carbon dioxide content
- humidity.



Phenomics technology > Looking outwards

CabScan

CabScan is a growth cabinet with a difference. It takes images of plants 24 hours a day, seven days a week – capturing an accurate picture of growth.

The lights in the cabinets are programmed to switch on and off to create specific day and night lengths, depending on the experiment in progress.

Several cameras are attached to a robotic arm that slowly sweeps in a U-shape over trays of plants grown inside CabScan. Images are taken every 15 minutes.

- Two colour cameras produce stereo images that can be used to create 3D reconstructions of each individual plant.
- An infared camera takes images that can be overlayed onto the colour images, creating a 'heat map' of the leaves.

The system can also use infrared spotlights with a short flash to take 'night vision' pictures. Researchers can then determine how darkness affects plant growth.



RESEARCH SPOTLIGHT

CabScan has been used for drought tolerance experiments. Half the plants were watered as normal, and the other half were kept dry. The CabScan images revealed that the drought-simulated plants were indistinguishable from watered plants for 48 hours, but after this time, their growth was rapidly affected.

Each tray or pot in CabScan – as well as TrayScan and PlantScan – has a barcode, similar to the codes on products in the supermarket. As the trays cycle through the cabinet, they pass a barcode scanner, which sends the information to a computer program. This allows researchers to track which plants have been scanned.





Image: CSIRO

TrayScan

The TrayScan system holds a mini-conveyor belt that cycles trays of plants through a high-tech array of cameras.

A chamber within the TrayScan cabinet holds three different camera systems.

- Colour cameras take images from the top, back and sides of the trays to allow 3D imaging of plants.
- A thermal infared camera and a chlorophyll fluorescence imaging system take images from overhead.

RESEARCH SPOTLIGHT

TrayScan is being used by United States' researchers to screen large populations of *Brachypodium*. They hope to find plants that accumulate high yields of biomass in their shoots.

TrayScan can fit 15 trays, each holding up to 20 pots, and takes just 10 minutes to image a full cabinet's worth of plants. The system is kept in a room with a controlled environment, so that researchers can set the temperature and light levels.



Phenomics technology > Looking outwards

PlantScan

PlantScan uses digital imaging to allow non-invasive analyses of plant morphology and function.

A conveyor belt can hold 30 plants at once, which travel through the imaging chamber on trays. A mechanical system accurately positions single plants while hundreds of images are taken, with each plant scan taking only one minute.

The system uses three high-resolution cameras, two infrared cameras and two lasers that pulse light off the plant's surface. Once the scan is complete, the raw data is sent to a supercomputer at the Australian National University. It merges the information from the images to give an accurate model of the plant.

Using the 3D models, researchers can determine measurements such as leaf thickness, plant height, stem length and plant surface area – all without having to physically measure a thing, or even look at their plants. The infrared images can also be overlayed on the colour images, creating a heat map of the leaves that can be used to study temperature differences.



RESEARCH SPOTLIGHT

PlantScan is being used to understand how plant function and architecture are related to photosynthetic efficiency and light harvesting. For example, researchers are currently seeing how rice leaves orientate towards the sun for maximum photosynthetic efficiency.



Cropatron (mobile and static)

The Cropatrons provide a canopy-like environment in which to study the impact of climate change on crops.

They allow plants to be grown in the field, but under a controlled environment. Researchers can then measure the effects of environmental conditions, such as carbon dioxide levels, water availability, light levels and daylength, and temperature and humidity.

The 'mobile' Cropatron is a system of see-through polythene tunnels that are big enough to drive a tractor through – about 32 metres long and 16 meters wide. Crops are planted inside the tunnels, and an imaging crane runs along rails at the top. The crane contains all the imaging systems of the Phenomobile.

The 'static' Cropatron is effectively a tall glasshouse that houses plants grown in one-metre-cubed tubs. The tubs create a mini-canopy to simulate field conditions. The crane used in the mobile Cropatron can also fit inside the glasshouse to measure aspects of the environment and take images.



RESEARCH SPOTLIGHT

Researchers are currently growing rice crops in the Cropatron as part of an International Rice Research Institute (http://www.irri.org/) project. Forty lines of rice are being grown and analysed to quantify their photosynthetic and growth properties, in both the Cropatron and in controlled growth cabinets. The phenotypes of each line will then be mapped back to the rice genome, allowing researchers to precisely determine the effects of field conditions on growth.



a climate-controlled, field-like environment. Phenomics technology > Looking outwards

Phenonet

The Phenonet is a network of data loggers that collect information from a field of experimental crops and send it back to researchers at the laboratory.

This saves daily visits to field sites, which is especially useful if the site is in a remote area.

The data loggers, which were developed by CSIRO (http://www.csiro.au), are linked to sensors that monitor the physical and environmental conditions affecting plant performance, such as air temperature, soil moisture and temperature and crop canopy temperature.

The sensors include a far infrared thermometer to measure canopy temperature, a chlorophyll fluorescence sensor to measure photosynthetic activity, a weather station, soil moisture sensors set at different depths, and a camera to monitor crop development and flowering.

The sensors take measurements every 10 seconds, and average the measurements over a 5-minute period. The data is transmitted 24 hours a day to a base station, which is connected to the 3G network. The base station then sends all the information to a 'virtual laboratory' server at the Plant Phenomics Centre in Canberra.

The recorded field data helps researchers to select new plant varieties suited to difficult growing conditions. The Phenonet's continuous analysis of plant growth and performance can be linked to both climate conditions in the field and the plant's genetic make-up, helping to improve and speed up breeding.

The Phenonet's modules are

across Australia.



Phenomobile

The Phenomobile is a buggy that moves through a field of plants, taking measurements as it goes.

As a researcher drives the Phenomobile travels through the field site at 3–5 km per hour, it collects measurements from the plot directly beneath it.

The equipment carried by the Phenomobile includes:

- · digital cameras to estimate leaf greenness and ground cover
- · far infrared cameras to monitor canopy temperature
- a stereo-imaging system of two digital cameras combined with three lasers to create
 3D reconstructions of plots, which are used to measure leaf area, the volume (*biomass*) of plants, plant height and plant density
- a laser that shines red light onto the plants, which is used in combination with spectral reflectance sensors to determine the crop's chemical composition.



difference – researchers drive the Phenomobile through a crop site. Phenomics technology > Looking outwards

Phenotower

The Phenotower is a cherry-picker that researchers can use to take infrared and colour images of a field plot from 16 metres above the canopy.

The data is used to compare canopy temperature, leaf greenness and groundcover between different plant lines at the same time.



researchers to take images of many plants at once.

Multicopter

The Multicopter can take infrared and colour images of a field from just a few centimetres above the ground to a height of up to 100 metres.

Looking a lot like a mini-helicopter, the Multicopter is less than one metre across, and has six propellers. It can be remotely controlled, and can also be programmed to follow an automatic flight path. It can quickly image an entire field of crop plants, helping researchers take measurements of many plants at the same time.

Packing a lot of punch into a little space, the Multicopter will be equipped with a computer, a GPS, and colour and infrared cameras. The infrared and colour images can be used to identify the relative differences in canopy temperature. This indicates plant water use, which is an important trait to understand.

The Multicopter is currently in a testing phase with a normal camera, with plans for further development.



RESEARCH SPOTLIGHT

The Plant Phenomics Centre is also trialling 'airborne thermal imaging' by mounting cameras into the fuselage of a light aeroplane. The plane can fly over a field of crops in a few minutes, giving researchers an almost-instant map of hundreds of plots.

Such large-scale thermal imaging can help remove experimental bias. For instance, a good-performing plant line might only be performing well because it's in a good spot. The images quickly reveal which areas of soil in the field are cooler, and which are warmer. Researchers will then know to test the good-performing line in a different spot.



The multi-rotor Unmanned Aerial Vehicle (UAV) is equipped with a high-resolution camera. The UAV can take off and land vertically and follow a flight plan using GPS.

Glossary

Biomass

The biological material that makes up living, or recently living, organisms such as plants.

C3 photosynthesis

A photosynthetic pathway found in most plants, in which the first product is a 3-carbon molecule.

C4 photosynthesis

A photosynthetic pathway found in many tropical plants and grasses, in which the first product is a 4-carbon molecule.

Canopy

The above-ground part of a plant crop.

Carbohydrates

Compounds containing carbon, oxygen and hydrogen, such as sugars and starch.

Carbon dioxide

A gas in the atmosphere taken up by plants during the process of photosynthesis. Carbon dioxide is a greenhouse gas.

Cell

The basic unit of all organisms; a small membrane-bound compartment containing structures called organelles.

Cell division

A parent cell dividing into two or more daughter cells.

Chlorophyll

A pigment found in the chloroplasts of all green plants, which converts light energy from the Sun into a chemical form to begin the process of photosynthesis.

Electromagnetic spectrum

The range of all possible wavelengths of electromagnetic radiation, or waves of energy. Includes visible wavelengths of light, and invisible wavelengths such as radio waves, microwaves, ultraviolet and infrared radiation.

Fluorescence

The absorbance of light of one wavelength and emission of light of a different wavelength.

Gene

A sequence of DNA that either encodes a protein or has a regulatory function.

Genome

All of an organism's genetic information.

Genomics

The study of an organism's genome.

Genotype

The genetic makeup of an organism. An organism's genotype interacts with the environment to affect its physical appearance, or phenotype.

Life cycle

The length of time a seed takes to germinate, grow, flower, and set its own seed.

Morphology

The structural features of an organism.

Nanometre

One millionth of a millimetre.

Nutrient

A chemical that an organism needs to live and grow, which must be taken in from its environment.

Phenomics

The study of how an organism's phenotype fits with the information we know about its genotype.

Phenotype

The visible characteristics or traits of an organism. An organism's phenotype is the result of interactions between its genotype and the environment.

Photosynthesis

A chemical reaction in which plants use light energy from the sun to convert carbon dioxide and water into sugars and oxygen.

Rubisco

A photosynthetic enzyme that fixes carbon from the air into carbohydrates.

Screening

Testing a population for alterations in gene activity that alter an organism's phenotype.

Sequenced, sequencing (genes)

Determined or determining the order of bases that make up DNA.

Stomata

Tiny pores in plant leaves that take up gases from the atmosphere and lose water through the process of transpiration.

Stress

The negative effects that conditions such as drought, salinity or nutrient deficiency have on plant growth and yield.

Trait

An observable characteristic of an organism, such as its shape, size, biochemical properties or behaviour.

Yield

The amount of harvestable, useful material from a plant.

Links and resources

Some useful sites, including videos and animations that you can use in class, are listed here.

Phenomics sites

Australian Centre for Plant Functional Genomics: http://www.acpfg.com.au/

Australian Phenomics Facility: http://www.apf.edu.au/

Australian Plant Phenomics Facility: http://www.plantphenomics.org.au/

Australian Plant Phenomics Facility on YouTube: http://www.youtube.com/watch?v=2q3E7kFUWY8

CSIRO page on The Australian Plant Phenomics Facility: http://www.csiro.au/places/Phenomics-Facility.html

International Plant Phenomics Initiative: http://www.plantphenomics.com/

Maize Phenomics at the University of Guelph: http://www.plant.uoguelph.ca/research/homepages/elee/research/phenomics.html

NOVA: Biology meets industry – genomics, proteomics, phenomics: http://www.science.org.au/nova/078/078key.htm

Jülich Plant Phenotyping Centre, Forschungszentrum Jülich, Germany: http://www.fz-juelich.de/ibg/ibg-2/EN/organisation/JPPC/JPPC_node.html

Scanalyzer 3D plant phenotyping: http://www.lemnatec.com/product/scanalyzer-3d-plant-phenotyping

University of South Australia Phenomics and Bioinformatics Research Centre: http://www.unisa.edu.au/research/phenomics-and-bioinformatics-research-centre/

Plant biology sites

The ARC Centre for Excellence in Plant Energy Biology teacher resources: http://www.plantenergy.uwa.edu.au/education/teacher-resources.shtml

NOVA: More food, cleaner food – gene technology and plants: http://www.science.org.au/nova/009/009key.htm

BioEd Online Mendelian genetics (PowerPoint presentation and video): http://www.bioedonline.org/presentations/mendelian-genetics/f.htm

Illuminating photosynthesis (interactive animation): http://www.teachersdomain.org/resource/tdc02.sci.life.stru.methusweb/

Photosynthesis (video): http://www.teachersdomain.org/resource/tdc02.sci.life.stru.photosynth/

Plants in motion (time-lapse video): http://www.teachersdomain.org/resource/lsps07.sci.life.reg.plantmovies/

Life cycle of a seed plant (interactive animation): http://www.teachersdomain.org/resource/lsps07.sci.life.stru.seedplant/

The reproductive role of flowers (video): http://www.teachersdomain.org/resource/oer08.sci.life.stru.flowers/

From seed to flower (time-lapse video): http://www.teachersdomain.org/resource/tdc02.sci.life.colt.plantsgrow/

Ethanol biofuel (video): http://www.teachersdomain.org/resource/biot09.sci.engin.systems.ethanol/

Model organisms (interactive animation): http://www.teachersdomain.org/resource/hew06.sci.life.gen.modelorg/

Molecular biology sites

DNA libraries (interactive animation): http://www.teachersdomain.org/resource/biot09.sci.life.gen.dnalibraries/

DNA extraction (interactive animation): http://www.teachersdomain.org/resource/biot09.biotech.tools.extraction/

DNA workshop: Protein synthesis (interactive animation): http://www.teachersdomain.org/resource/tdc02.sci.life.gen.dnaworkshop/

Curriculum links

A curriculum map is provided to help you align the resource's content to the relevant sections of the Senior Secondary Australian Curriculum (Biology).

- Unit 1: Biodiversity and the interconnectedness of life
- Unit 2: Cells and multicellular organisms
- Unit 3: Heredity and continuity of life
- Unit 4: Maintaining the internal environment

Unit 1: Biodiversity and the interconnectedness of life

Science Inquiry Skills (Biology Unit 1)	Science as a Human Endeavour (Units 1 and 2)	Science Understanding
Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL001)	Science is a global enterprise that relies on clear communication, international conventions, peer review and reproducibility (ACSBL008)	The biotic components of an ecosystem transfer and transform energy originating primarily from the sun to produce biomass, and interact with abiotic components to facilitate biogeochemical cycling, including carbon and nitrogen cycling; these interactions can be represented using food webs, biomass pyramids, water and nutrient cycles (ACSBL022)
Design investigations, including the procedure/s to be followed, the materials required, and the type and amount of primary and/or secondary data to be collected (ACSBL002)	Development of complex models and/or theories often requires a wide range of evidence from multiple individuals and across disciplines (ACSBL009)	
Represent data in meaningful and useful ways; organise and analyse data to identify trends, patterns and relationships; qualitatively describe sources of measurement error, and uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions (ACSBL004)	Advances in science understanding in one field can influence other areas of science, technology and engineering (ACSBL010)	
Interpret a range of scientific and media texts, and evaluate processes, claims and conclusions by considering the quality of available evidence; and use reasoning to construct scientific arguments (ACSBL005)	The use of scientific knowledge is influenced by social, economic, cultural and ethical considerations (ACSBL011)	
	The use of scientific knowledge may have beneficial and/or harmful and/or unintended consequences (ACSBL012)	
	Scientific knowledge can enable scientists to offer valid explanations and make reliable predictions (ACSBL013)	
	Scientific knowledge can be used to develop and evaluate projected economic, social and environmental impacts and to design action for sustainability (ACSBL014)	

Unit 2: Cells and multicellular organisms

Science Inquiry Skills (Biology Unit 2)	Science as a Human Endeavour (Units 1 and 2)	Science Understanding
Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL030)	Science is a global enterprise that relies on clear communication, international conventions, peer review and reproducibility (ACSBL037)	Cells require inputs of suitable forms of energy, including light energy or chemical energy in complex molecules, and matter, including gases, simple nutrients, ions, and removal of wastes, to survive (ACSBL044)
Design investigations, including the procedure/s to be followed, the materials required, and the type and amount of primary and/or secondary data to be collected; conduct risk assessments; and consider research ethics, including animal ethics (ACSBL031)	Development of complex models and/or theories often requires a wide range of evidence from multiple individuals and across disciplines (ACSBL038)	In eukaryotic cells, specialised organelles facilitate biochemical processes of photosynthesis, cellular respiration, the synthesis of complex molecules (including carbohydrates, proteins, lipids and other biomacromolecules), and the removal of cellular products and wastes (ACSBL049)
Conduct investigations, including microscopy techniques, real or virtual dissections and chemical analysis, safely, competently and methodically for the collection of valid and reliable data (ACSBL032)	Advances in science understanding in one field can influence other areas of science, technology and engineering (ACSBL039)	Biochemical processes in the cell are controlled by the nature and arrangement of internal membranes, the presence of specific enzymes, and environmental factors(ACSBL050)
Represent data in meaningful and useful ways; organise and analyse data to identify trends, patterns and relationships; qualitatively describe sources of measurement error, and uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions (ACSBL033)	The use of scientific knowledge is influenced by social, economic, cultural and ethical considerations (ACSBL040)	Enzymes have specific functions, which can be affected by factors including temperature, pH, the presence of inhibitors, and the concentrations of reactants and products(ACSBL051)
	The use of scientific knowledge may have beneficial and/or harmful and/or unintended consequences (ACSBL041)	Photosynthesis is a biochemical process that in plant cells occurs in the chloroplast and that uses light energy to synthesise organic compounds; the overall process can be represented as a balanced chemical equation (ACSBL052)
	Scientific knowledge can enable scientists to offer valid explanations and make reliable predictions (ACSBL042)	The specialised structure and function of tissues, organs and systems can be related to cell differentiation and cell specialisation (ACSBL055)
	Scientific knowledge can be used to develop and evaluate projected economic, social and environmental impacts and to design action for sustainability (ACSBL043)	In plants, gases are exchanged via stomata and the plant surface; their movement within the plant by diffusion does not involve the plant transport system (ACSBL059)
		In plants, transport of water and mineral nutrients from the roots occurs via xylem involving root pressure, transpiration and cohesion of water molecules; transport of the products of photosynthesis and some mineral nutrients occurs by translocation in the phoem (ACSBI 060)

Unit 3: Heredity and continuity of life

Science Inquiry Skills (Biology Unit 3)	Science as a Human Endeavour (Units 3 and 4)	Science Understanding
Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL061)	ICT and other technologies have dramatically increased the size, accuracy and geographic and temporal scope of data sets with which scientists work (ACSBL068)	Continuity of life requires the replication of genetic material and its transfer to the next generation through processes including binary fission, mitosis, meiosis and fertilisation (ACSBL075)
Design investigations, including the procedure/s to be followed, the materials required, and the type and amount of primary and/or secondary data to be collected; conduct risk assessments; and consider research ethics, including animal ethics (ACSBL062)	International collaboration is often required when investing in large-scale science projects or addressing issues for the Asia-Pacific region (ACSBL073)	Proteins, including enzymes, are essential to cell structure and functioning (ACSBL080)
Represent data in meaningful and useful ways, including the use of mean, median, range and probability; organise and analyse data to identify trends, patterns and relationships; discuss the ways in which measurement error, instrumental accuracy, the nature of the procedure and the sample size may influence uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions (ACSBL064)	Scientific knowledge can be used to develop and evaluate projected economic, social and environmental impacts and to design action for sustainability (ACSBL074)	The phenotypic expression of genes depends on factors controlling transcription and translation during protein synthesis, the products of other genes, and the environment(ACSBL081)
Interpret a range of scientific and media texts, and evaluate models, processes, claims and conclusions by considering the quality of available evidence, including interpreting confidence intervals in secondary data; and use reasoning to construct scientific arguments (ACSBL065)		Mutations in genes and chromosomes can result from errors in DNA replication or cell division, or from damage by physical or chemical factors in the environment(ACSBL082)
		Differential gene expression controls cell differentiation for tissue formation, as well as the structural changes that occur during growth (ACSBL083)
		Variations in the genotype of offspring arise as a result of the processes of meiosis and fertilisation, as well as a result of mutations (ACSBL084)
		DNA sequencing enables mapping of species genomes; DNA profiling identifies the unique genetic makeup of individuals (ACSBL086)
		Mutation is the ultimate source of genetic variation as it introduces new alleles into a population (ACSBL092)

Unit 4: Maintaining the internal environment

Science Inquiry Skills (Biology Unit 4)	Science as a Human Endeavour (Units 3 and 4)	Science Understanding
Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL096)	ICT and other technologies have dramatically increased the size, accuracy and geographic and temporal scope of data sets with which scientists work (ACSBL103)	Changes in an organism's metabolic activity, in addition to structural features and changes in physiological processes and behaviour, enable the organism to maintain its internal environment within tolerance limits (ACSBL111)
Design investigations, including the procedure/s to be followed, the materials required, and the type and amount of primary and/ or secondary data to be collected; conduct risk assessments; and consider research ethics, including the rights of living organisms (ACSBL097)	International collaboration is often required when investing in large-scale science projects or addressing issues for the Asia- Pacific region (ACSBL108)	
	Scientific knowledge can be used to develop and evaluate projected economic, social and environmental impacts and to design action for sustainability (ACSBL109)	

Appendix 1

Plant growth and analysis activity

Step 1

Choose a fast-growing plant such as Brachypodium, sunflowers, marigolds, alfalfa or radish.

- Grow a number of plants to photograph at various stages: i.e. seedling stage, 2 weeks of growth and 6 weeks of growth.
- Before you start, decide which stages you will photograph: keep in mind that the bigger the plant, the greater the biomass that will be calculated, and the bigger the difference between two stages, the greater the difference in biomass will be seen.
- When you plant the seeds, place a small label on one side of the pot to ensure that you always take a photo of the same side. Make note of the size of the label: length and height in millimetres.

Step 2

Take digital photos of your plants at the desired stages. For the analysis software to assess the size of the plant, the photos must follow the protocols below:

- For consistency, ensure that all photos of all plants are taken in the same way.
- The plant should be placed on a table in front of a monotone background, such as a white or blue wall, but definitely **not** green or red.
- Take one photo of each plant, ensuring the whole plant and whole pot are in the picture, and that the label is visible (the label will be used for scale).
- Ensure that the photo is sharply focused; blurry pictures cannot be analysed by the software.
- Take note of the date the photos were taken: e.g. during Week 5 and Week 8 of the experiment.

Step 3

Download

Download the image analysis software ImageJ from http://rsbweb.nih.gov/ij/download.html. Ensure that you select the correct version for your computer. Uncompress the ZIP file under the folder where you want to install Fiji or run the installer for ImageJ.



Calibrate

1. Start the program and open your image by selecting **File** and then **Open** in the drop down menu.



2. Rotate the image by selecting **Image** in the drop down menu, then **Transform**, then **Rotate 90 Degrees Left or Right**. You can zoom in and out with the magnifier on the toolbar or the +/- keys on your keyboard.

3. Calibrate your picture by drawing a horizontal line from both sides of the label on your pot in the image using the straight line tool on the toolbar.



Define the scale

4. Define the image scale by selecting **Analyze** in the dropdown menu and then **Set Scale**. This will give you the distance in pixels.

5. Use the number of pixels in the image (222) and the size of the label noted earlier (e.g. 24 mm) to calculate the pixels per millimetre of the image. To do this, enter 24 into the **Known Distance** box and mm in the **Unit of Length** box, and click **OK**.



6. Draw a new vertical line across the label. It should be relatively close to the label's actual height. The distance appears in the ImageJ window as you draw the line. If it is not close to the actual height, repeat Step 3 under Calibration.



7. Now you can measure the height of your plant!



Calculating the area of the plant

8. To calculate the projected area, select the rectangle on the toolbar and then select your plant within the rectangle. Include as little of the pot as possible in the rectangle you select.



9. Crop the image by selecting **Image** and then **Crop** from the drop down menu. Do a colour threshold to select the plant greenness by selecting **Image**, and then **Adjust**, and then **Color Threshold**.



10. Now select a rectangle of the plant's leaves. Try to avoid including the background in your rectangle.



11. Click **Sample** in the **Threshold Color** window. The result may not be very good to begin with: you want as much of the whole plant to be red as possible.



12. Play with the slide bars until you get a nicer result and the whole plant is red, like the image below. Ensure the background is not red. Sometimes, the pot is also red; this is unavoidable.



13. Click **Select** in the **Threshold Colour** window. The red will disappear, and the plant will be selected, ready for measuring the area.



14. To measure the area of your plant, select **Analyze** and then **Measure** from the drop down menu. Right click on the fields at the top of the form and select **Set Measurements**, then tick **Area**. Your plant's area will then be given: e.g. 4514 mm².

15. Now that you have the area of your plant you can use the information to demonstrate to the class how to use excel to chart the growth of the plants.



Step 4

Analyse

Create an Excel worksheet similar to the one below, using the data you have collected from your plant growth experiment.

	Plant area (mm²), determined as described above in			
	'Calculating the area of the plant'			
Plant	2 weeks' growth	4 weeks' growth	6 weeks' growth	8 weeks' growth
Student 1	564	1568	3510	4514
Student 2	572	1286	2860	5860
Student 3	1564	6482	10598	15562
Student 4	235	568	1230	2456
Student 5	2568	7686	13840	15645
Student 6	567	1158	3510	5168
Student 7	468	1456	3548	5575
Student 8	685	1564	4682	6322
Student 9	356	1803	2684	3562
Student 10	241	1735	3085	4567
Student 11	5864	10857	17861	25864

Insert a chart in Excel, such as a line chart, to display your data. You may need to switch the row/column data to produce a chart such as the following:



Discuss with your students the trends that appear in the data. You can discuss questions such as:

- · Can you identify any periods where growth stagnated or declined?
- Can you link these periods to activities at the time of the decline?
- Did some plants outperform others, and why?

Appendix 2: Class activities with plant phenomics

1. Focus question: What is meant by phenotype?

Reference: Plant Phenomics Teacher Resource

1a. Activity for Years 6–7

Australian Curriculum References: Science K-10

• The growth and survival of living things is affected by the physical conditions of their environment.

Question: Will all seeds of the same type (species) of plant grow in the same way if environmental conditions are identical?

Equipment:

- Quick growing seeds such as radish or Brachypodium
- Soil, sand or potting mix
- Gloves
- Growing pots

Prior knowledge from Year 4 Australian Curriculum – Science

- Living things have life cycles
- Living things, including plants and animals, depend on each other and the environment to survive

Step 1: Checking prior knowledge

- What do plants need from their environment? Minerals, water, air, sunlight and a medium in which to grow.
- What are the steps in a plant's life cycle?

For flowering plants

- Seed \longrightarrow Seedling
- Seedling Mature plant

Mature plant flowers and produces seeds.

Step 2: Teacher leads with key questions

 What features of a plant can we use to describe its appearance? (Look for features such as leaf shape and size, leaf colour [light or dark green], height of plant, stem branching, arrangement of leaves etc.) The appearance of a plant can be called its phenotype.

- What words will we use to describe the plant structures? (For example, are its leaves smooth or rough? Is the upper surface of the leaf similar to the lower surface?)
- What measurements can we use to describe a plant? (For example, what is the surface area of the leaf? What is the height of the plant? How many leaves does it have?)
- What features of a plant can we investigate to answer the question above?

Step 3: The investigation

Planning stage

- Ask your students to work in groups and decide on a plant structure that each group will measure. Once they have decided, encourage each group to frame a question, such as: *Will all the seeds* germinate to produce plants with the same leaf length?
- Work with your students to ensure that the investigation is a fair test: ensure that all conditions are kept the same and that the only factor to measure is, for example, the length of leaf. This will require determining what aspects of the germinating environment are kept identical. For example, if the seeds are to be grown in separate pots, is the soil identical? How will students make sure the light is the same? How will they make sure the water conditions are the same? Should they grow the seeds outside or in the classroom? Does temperature have an effect?
- Work with your students to develop a list of the equipment they need.

Conducting stage

- In groups, students set up the seed growing investigation.
- Each group identifies a recorder, and back-up person to assist with keeping conditions constant in each pot of seeds.
- As seeds germinate, the specific feature to be observed is measured and described.

Analysing stage

- Each group analyses its results to determine if the question of the investigation has been answered.
- The conclusions by each group are then shared with the whole class.

Further investigations

The Australian Curriculum – Science Year 6 statement provides an opportunity to expand on this simple investigation to include other variables, such as:

- Temperature germinate seeds in warm environment, in cold environment, at various temperatures
- Vary water availability
- Vary type of soil.

To demonstrate to students that environment does impact on the phenotype of a plant, they should be led to understand the importance of using the same species of seeds from the same supplier to ensure that inherited characteristics are kept constant.

1b. Activity for Years 9–10

Australian Curriculum References – Science 7–10

Biological Sciences Strand

Year 9

• Multi-cellular organisms rely on coordinated and interdependent internal systems to respond to changes to their environment

Year 10

• The transmission of heritable characteristics from one generation to the next involves DNA and genes

Science Inquiry Strand

- Formulate questions or hypotheses that can be investigated scientifically
- Plan, select and use appropriate investigation methods, including field work and laboratory experimentation, to collect reliable data; assess risk and address ethical issues associated with these methods
- Select and use appropriate equipment, including digital technologies, to systematically and accurately collect and record data
- Analyse patterns and trends in data, including describing relationships between variables and identifying inconsistencies
- Use knowledge of scientific concepts to draw conclusions that are consistent with evidence
- Evaluate conclusions, including identifying sources of uncertainty and possible alternative explanations, and describe specific ways to improve the quality of the data

Question: Which form of plant breeding would, potentially, enable some genetic diversity to be maintained in a food species?

Equipment:

- Quick growing seeds such as radish or Brachypodium
- Soil, sand or potting mix
- Gloves
- Growing pots

Extract from http://www.primalseeds.org/bioloss.htm

Since the beginning of agriculture, farmers have encouraged and developed many different traits in cultivated crops by the selective sowing of seed. Differing conditions between one village farming community and another resulted in the development of locally adapted varieties. Eventually, hundreds of thousands of distinct varieties of widely cultivated crops evolved. These varieties are known as landraces.

With the advent of modern plant breeding, much of this diversity has been lost. European landraces had disappeared by the early twentieth century and information regarding traditional varieties is very scarce.

The main reason for the loss of traditional varieties is their replacement by modern varieties. The expansion of cash crop agriculture and pasture to feed cattle has contributed to the decrease in the amount of land farmed by small farmers, who are more likely to rely upon and preserve the landraces.

This form of artificial selection for agricultural purposes has reduced the genetic diversity of modern crops.

Investigation:

1. Ask your students to form small groups. Select a single vegetable or fruit type, such as carrot or apple, and develop a list of favourable characteristics (phenotypes) for their chosen form of plant food.

2. Lead a class discussion into the steps that are taken to 'select' and grow the ideal plant food type. The terms 'phenotype' and 'genotype' can be introduced along with the concept of 'genes'.

3. At this point, distinguish between the two main forms of agricultural and horticultural plant breeding strategies:

- Selection of seed from suitable phenotypes (sexual reproduction).
- Selection of cuttings and grafting from suitable phenotypes (asexual reproduction or vegetative propagation).

4. Pose the question and lead a class discussion on the following question: which form of plant breeding would, potentially, enable some genetic diversity to be maintained in a food species?

5. At this point in the lesson sequence, students could plan, and set up, a procedure to test the genetic diversity in a seed sample from a single species. During the planning stage, outline the importance of controlling all variables except that being tested. (Use this opportunity to review the impact of environmental factors on phenotype). Ask students to brainstorm and list possible variables that could interfere with plant growth:

- Water
- Soil
- Light
- Temperature
- Wind weather if plants are to be grown outside of the classroom.

As the investigation will take some time to complete, students will also need to plan a regime to maintain the conditions where seeds are growing, as well as decide on the measurements to be taken and results to be analysed.

6. When the plants mature and seed, allow the class to pool results to assist in analysis of their data and to draw conclusions about the question in point 4 above.

Further investigations

1. If there is genetic variability within a species, how can this variability be used to identify the potentially most productive food source in a food species such as wheat? (*Refer to the Phenomics Research section of the Plant Phenomics Teacher Resource for a starting point for discussion.*) Students could research this question through secondary sources of information, or pose further ideas that could be tested in an investigation using a plant species with a rapid life cycle.

2. Investigating asexual reproduction: as a class project, students could be introduced to the broad range of strategies used to ensure genetic consistency and achievement of the desired phenotype. Some examples that could be trialled in the classroom include:

- Using eyes from potatoes
- Taking cuttings of plants such as geraniums or coleus
- Bulbs of onions
- Corms

http://plantphys.info/plants_human/vegprop/vegpropn.shtml has some illustrated examples of vegetative propagation.

3. A case study: bringing back the Wollemi pine. The use of seeds and cuttings to increase the availability of a rare species can be tracked through the story of the discovery and propagation of the Wollemi pine. The focus of the case study could be in the context of varying genetic diversity through plant propagation. Which method will increase genetic diversity more effectively? http://www.wollemipine.com/index.php is a useful website.

2. Focus question: Food for a hungry world?

Reference: *Plant Phenomics Teacher Resource – Phenomics Research section.* This provides a readily accessible description and explanation of the methods being used to identify strategies for increasing the productivity of food crops.

Activity: Year 7-8

Australian Curriculum references:

Science as a Human Endeavour

- Science understanding influences the development of practices in areas of human activity such as industry, agriculture and marine and terrestrial resource management.
- Science and technology contribute to finding solutions to a range of contemporary issues; these solutions may impact on other areas of society and involve ethical considerations.

Science Inquiry skills

• Construct and use a range of representations, including graphs, keys and models to represent and analyse patterns or relationships, including using digital technologies as appropriate

Activity

1. Internet research and percentage calculation: An excel spreadsheet on world populations from 1950 onwards can be downloaded from http://esa.un.org/unpd/wpp/Excel-Data/population.htm

a. Copy and complete the following table to show the changing world population for at least the last 20 years.

To do an accurate comparison with the graph on the following page, students could compare world population growth from 1967.

(Students with experience in table design can be challenged to design a table and mathematical calculations that will provide world population statistics to compare with the crop statistics in the table on the right.)

Year	Column A	Column B	% Increase
	Estimate World Population A	Increase	<u>Column B</u> x 100 Column A
1989			
1992			
1995			
1998			
2001			
2004			
2007			
2010			

b. To analyse this data, ask the students to select, draw up and complete a graph type to demonstrate the percentage increase in world population.

c. Increasing numbers of people in the world means that increasing amounts of food must be grown. Does the graph on the right indicate that increases in food production are keeping pace with increasing world populations?



Image: New Scientist, June 2008

2. At this point, ask students to decide on a reason why the CSIRO has focused on the yields of maize, wheat and rice. http://www.fao.org/docrep/u8480e/u8480e07.htm is a useful website to begin exploration of the following ideas.

Discussion could include:

- the meanings of the terms 'staple food' and 'cereal' to describe food crops
- places in the world where these crops grow best
- · reasons for the importance of these crops
- conditions needed for optimum growth
- the meaning of the term 'food security'.

As an assessment of student understanding, ask them to explain: 'Why is the world food situation often assessed in terms of availability of rice, maize and wheat?

3. Introduce the work of the Plant Phenomics unit at the CSIRO to outline the ways in which scientists are working to improve the yield of plants used as food (*Plant Phenomics Teacher Resource – Phenomics Research section*). To give students some idea of the importance of CSIRO's work, http://www.fao.org/giews/english/cpfs/index.htm provides information on the world food situation and crop prospects in quarterly reviews.

3. Focus: Supercharging photosynthesis

Activity: Years 10-11

Photosynthesis is a fundamental biochemical process that captures the sun's energy, transforms it and makes energy available in a form usable by the rest of the living world through food chains and food webs. Research into methods of increasing output from photosynthesis in plants will increase the availability of food resources for humans.

Ideas for use of this activity

- Depending on the depth of your students' prior learning, this activity could be extended with practical investigations at each step along the way.
- The activity could be used as a guided web quest interspersed with hands-on activities.

Australian Curriculum references:

Science Understanding

Year 10

• Different types of chemical reactions are used to produce a range of products and can occur at different rates

Science as a Human Endeavour

• Advances in scientific understanding often rely on developments in technology and technological advances are often linked to scientific discoveries

Preparation/Background: (Activities 1–6 follow the Prior Knowledge section)

1. Structure of a cereal plant

- Wheat or Brachypodium seeds
- · Petri dishes, jam jar lids or similar growing surface
- Cotton wool or blotting paper

Germinate the seeds so students can identify the structures of a cereal plant. Perhaps you can grow some lawn grass or *Tradescantia* sp. to demonstrate the vegetative propagation that grasses use to spread. Including the latter would allow students to recognise a noxious weed in their gardens.

2. Reviewing the process of photosynthesis

If your students have not previously investigated the conditions necessary for photosynthesis, they should do so now. As most investigations include the test for starch as an indicator of photosynthesis, it will be important for their conceptual understanding to emphasise starch as a storage product for the glucose actually made in photosynthesis.

Some examples of activities available online include:

http://www.practicalbiology.org/areas/introductory/energy/photosynthesis/ has activities for testing the conditions necessary for photosynthesis, and uses leaves that have been exposed to different amounts of light or carbon dioxide, or that do not contain chlorophyll in every cell. Well worth a visit.

http://www.saps.org.uk/secondary/teaching-resources/284-investigating-the-behaviour-of-leaf-discs- This provides a way for students to get hands-on when investigating photosynthesis. Students punch out small discs from leaves, and float them in a syringe of sodium hydrogen carbonate solution. Once gas is evolved by photosynthesis, the leaf discs rise and fall. Students can compare the rate of photosynthesis in sun and shade plants and at different light intensities, among many other factors. Files available for download include a 2-page investigation description, as well as student and teacher notes as MS Word documents.

http://www.youtube.com/watch?v=C1_uez5WX1o is a simple little song on YouTube.

3. Movement of chemicals in and out of the plant leaf

Students at Year 10–11 level will have heard of diffusion in earlier years. However, it may be necessary to review diffusion and the concept of movement of substances from areas of high concentration to lower concentration in the context of the internal and external cellular environments in the plant leaf, as well as between the inside of the plant leaf and the external atmospheric environment. Search http://www.biology-resources.com/biology-experiments2.html under the heading of 'Transport in Plants' and 'Diffusion', to select appropriate activities that can be done either as demonstrations or hands-on activities for your students.

4. What is happening at the cellular level in plant leaves?

The purpose of this section is to confirm student understanding of the structure of plant cells, the specialisation of plant organelles and the role of chloroplasts.

If microscopes are available, guide students through the steps involved in making wet-mount slides and viewing them at the microscopic level. www.mysciencebox.org/files/cells_lab_handout.doc has instructions for making onion epidermis and Elodea slides.

5. Bringing it all together

This activity requires students to:

- · identify the sources of the raw materials of photosynthesis
- identify the products of photosynthesis
- state the importance of photosynthesis to life on Earth.

This activity could be designed as an assessment task to identify any holes in knowledge and understanding of photosynthesis and/or any misconceptions that may have developed.

6. Supercharging photosynthesis

This important step in the activity uses the information provided in the Plant Phenomics Teacher Resource to identify:

- Variations in photosynthetic adaptations in plants (C3 and C4) and the adaptive advantage of C4 plants
- Current research and its importance for future food production.

Prior knowledge:

Science education research has identified extraordinary examples of the lack of understanding of the inputs and outputs of photosynthesis. For students to grasp the importance of CSIRO's plant phenomics research into photosynthesis, it is important to identify any misconceptions on what photosynthesis means to the living world.

Students will have studied the following mandatory dot points from the Australian curriculum:

Year 7

• Interactions between organisms can be described in terms of food chains and food webs; human activity can affect these interactions.

Year 8

- Cells are the basic units of living things and have specialised structures and functions.
- Multi-cellular organisms contain systems of organs that carry out specialised functions that enable them to survive and reproduce.

Year 9

- Ecosystems consist of communities of interdependent organisms and abiotic components of the environment; matter and energy flow through these systems
- Chemical reactions, including combustion and the reactions of acids, are important in both non-living and living systems and involve energy transfer.

None of the above dot points specifically name photosynthesis as a process, and nowhere are the inputs and outputs of photosynthesis specifically mandated. With the possibility that students may not have been introduced to the concept, the activity below begins with an introduction and review of student understanding of photosynthesis.

Activities:

1. Reviewing the structure of a cereal plant



Source: http://en.wikipedia.org/wiki/File:Grass-plant-structure.png

Use the above image or similar to stimulate brainstorming about the structure of plants and the function of each part of the plant. http://www.biology4kids.com/files/plants_main.html could be used to introduce younger students or less able students to plants, their structure and function.

Ask students to fill in the table below without reference to any resources. How much do they know about plants? In using the diagram above, the role of rhizomes and stolons as vegetative reproductive organs can also be included.

Structure	Function	Chemicals exchanged with environment through this structure
Root		
Stem		
Leaf		
Flower		

Web Que	st Search Words
Stem	Leaf
Root	Bulb
Node	

2. Reviewing the process of photosynthesis

Do you believe that your students may already know about photosynthesis as a key biochemical process related to energy transformation? There is a simple flow chart available at http://www.talkabouttrees.org/ docs/09-10.pdf that might be a good starting point. The diagram below may also help students to revise their understanding of the key changes caused by photosynthesis. Ask your students to develop a word equation to describe inputs and outputs of photosynthesis.



Source: http://www.osovo.com/diagram/photosynthesisdiagrams.htm

Note that the diagram above appears to have glucose leaving the leaf and entering the air. This is, of course, not the case! Perhaps you could challenge your students by asking them to find the error in this diagram, as an example of the importance of checking secondary sources of information for accuracy?

Web Quest Search Words		
Photosynthesis	Leaf	
Energy	Starch	
Root	Carbon dioxide	
Chlorophyll		

3. Movement of chemicals in the plant leaf

The role of stomata in the diffusion of gases in and out of the leaf should be emphasised at this point in the development of the concept of plant adaptations for photosynthesis.

Traditional investigations of transpiration and diffusion can be used to model the role of stomata in the diffusion of carbon dioxide and oxygen in and out of the plant. http://www.youtube.com/watch?v=vPn5w7Xhl8Y is brief, but demonstrates the nature of diffusion through a membrane effectively.

Web Quest Search Words		
Stomate	Evaporation	
Guard cell	Membrane	
Diffusion	Atmosphere	
Oxygen		

4. What is happening at cellular level in plant leaves?

This section will require some revision of the structure of cells and the differences between plant and animal cells. It will also require students to revise the organisation of multicellular organisms into organ systems.

The plant leaf is an organ with specialised cells within that organ. Cells carrying out photosynthesis will contain chloroplasts. http://www.youtube.com/watch?v=LTglday5zak&feature=related has nice footage of chloroplast movement in Elodea cells and can be used to illustrate cytoplasmic streaming.



Source: http://en.wikipedia.org/wiki/File:Leaf_Tissue_Structure.svg

http://www.cellsalive.com/cells/cell_model.htm has an interactive model of a plant cell, which students could use to draw up and complete the following table.

Cell organelle	Diagram	Role within the cell

Web Quest Search Words	
Organelle	Leaf
Chloroplast	Nucleus
Membrane	Cell wall

5. Bringing it all together

Design and draw up a flow chart to summarise the steps in the process of photosynthesis from uptake of water in the roots and intake of carbon dioxide.



Source: http://www.caribbeanedu.com/kewl/science/science04d.asp

6. Supercharging photosynthesis

In C3 plants such as rice (top), the leaf mesophyll cells (red) take up carbon dioxide and also fix carbon during photosynthesis. In C4 plants such as maize (bottom), the leaf mesophyll cells (red) pump carbon dioxide into specialised bundle-sheath cells (yellow and red), where carbon is later fixed during photosynthesis.



Use the images and information from the *Phenomics Research* section of the *Plant Phenomics Teacher Resource* to compare and contrast the leaf structure of C3 and C4 plants.

Use http://hyperphysics.phy-astr.gsu.edu/hbase/biology/phoc.html as a starting point for student research into the following questions:

- C4 plants open their stomata at night and close them during the day, which is the reverse of other plants. Closing stomata during the day helps conserve water, but prevents CO₂ from entering the leaves. As CO₂ is necessary for photosynthesis and part of the photosynthetic reaction uses energy from sunlight, how does the C4 plant survive?
- What are the adaptive advantages of the C4 photosynthetic mechanism?
- Can the C4 photosynthetic mechanism be described as a phenotypic adaptation? Explain.
- Why is the CSIRO interested in researching C4 plants further?



www.plantphenomics.org.au