LIGGINS INSTITUTE
THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

NATIONAL RESEARCH CENTRE FOR
GROWTH AND DEVELOPMENT

LENScience Senior Biology Seminar Series

Food for a Hungry World—Optimising Plant Growth

Jacquie Bay and Karine David

Earth has a total land area of 13,056 million hectares. World population experts estimate that there are around 6 billon people on the planet. If this land resource was divided up equally, there would be around 2.1 hectares of land for every person on the Earth. However the world is not divided up evenly. New Zealand has a land mass around the same as the United Kingdom. We have just over 4 million people while in the United Kingdom there are almost 61 million people living in the same land area. Australia has a huge land mass of over 7 million square kilometres of land, but the land is not nearly as usable as New Zealand's land. The **BIOCAPACITY** of land is a measure of biological productivity and is measured in global hectares (Gha). A global hectare is 1 hectare of biologically productive space using world-average productivity levels. New Zealand has 218 Gha per sq km compared to Australia's 29Gha per sq km (FOA, 2008).

The Food and Agriculture Organisation of the United Nations reports that 923 million people worldwide were undernourished in 2007. Just under a quarter of these people were from the Asia-Pacific region, excluding India and China. Over a quarter of the total were in India (FAO, 2008). Figure 1 shows a world map as we normally see it, based on land area. Figures 2-4 show the world according to current population distribution, 2050 population and biocapacity. A comparison of these maps paints a picture of the real need for science and agriculture to work together to find better ways of maximising plant growth in order to feed the world. *"The world has no alternative to pursuing Sustainable Crop Production Intensification to meet the growing food and feed demand, to alleviate poverty and to protect its natural resources" (Shivaji Pandey, Director of FAO's Plant Production and Protection Division, 2009).* Dr Karine David and her team at the University of Auckland are conducting research that helps to understand how plants grow and can be applied to maximising plant growth, contributing to a global solution.

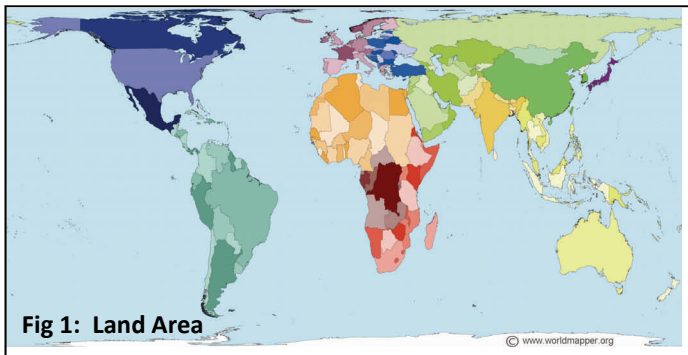


Fig 1: Land Area

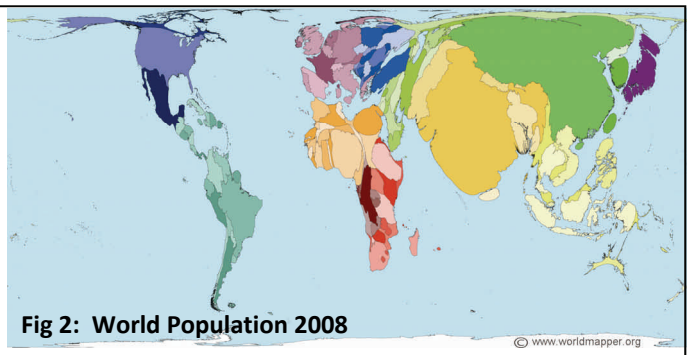


Fig 2: World Population 2008

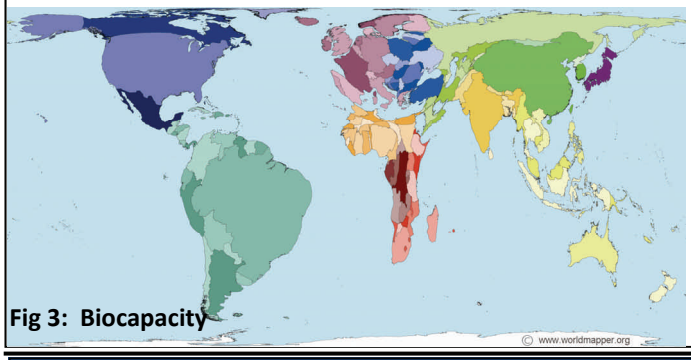


Fig 3: Biocapacity

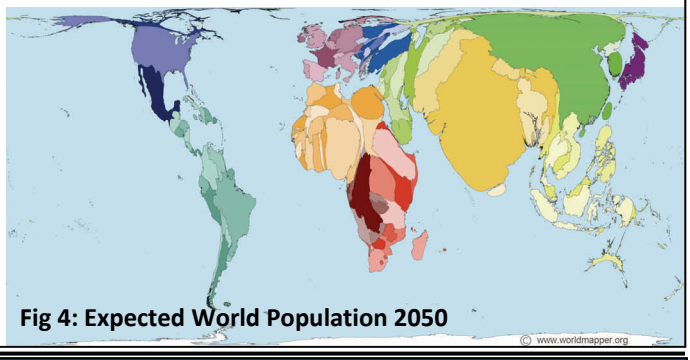


Fig 4: Expected World Population 2050

SCHOOL OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
THE UNIVERSITY OF AUCKLAND

Feeding 9 Billion People

So what will it take to feed the expected 9 billion people who will inhabit the Earth by 2050? This is a complex issue that requires significant scientific knowledge about how to grow food. The issue is centred on **energy,** and **maximising plant growth** is critical. All food chains start with plants that convert light energy into chemical energy via photosynthesis. No matter what humans are eating, plants are the starting point for ensuring that there is adequate food for the world's populations. Plant growth is influenced by genetic factors, environmental factors (water, carbon dioxide, light, nutrients) and hormones within the plant. The Plant Molecular Sciences Lab at the University of Auckland has a large team of scientists who work on understanding of plant growth and collaborate with Plant and Food Research to produce healthier fruit faster with less environmental impact.

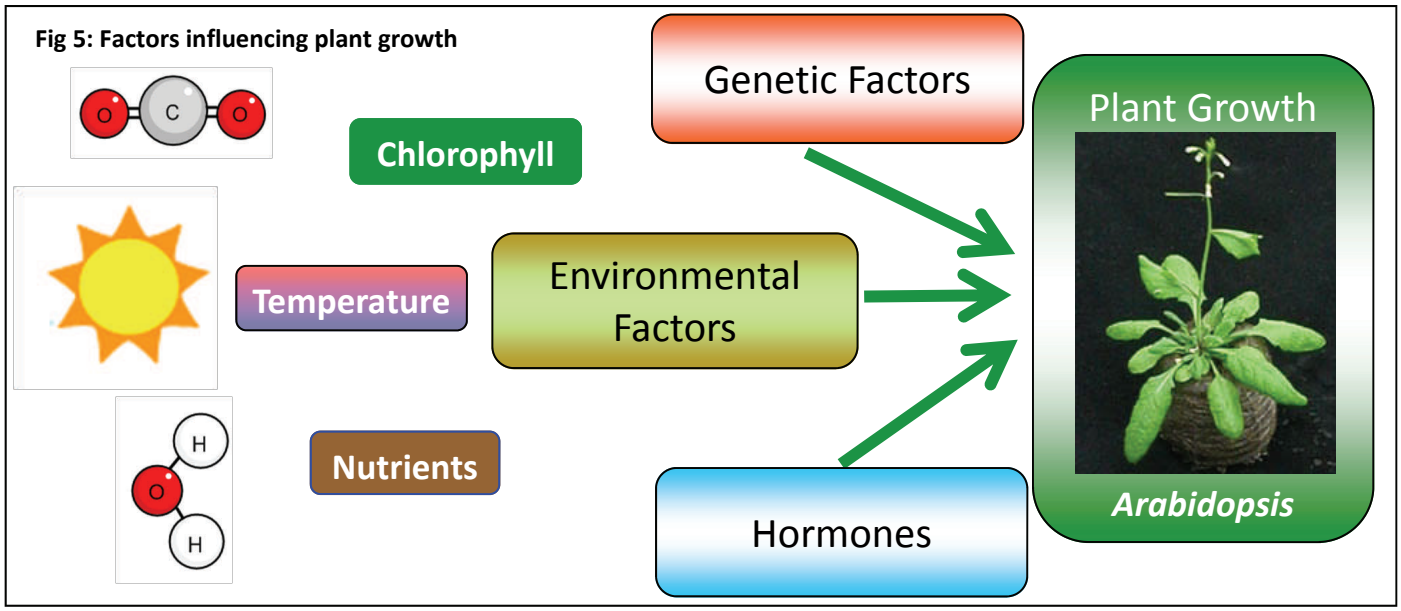


Fig 5: Factors influencing plant growth

Raging Hormones

Just like teenagers—plants have hormones. These chemical messengers can travel to different parts of the plant. Once they reach their "target" site, the hormone will act to change the way the cells behave, controlling the way in which the plant grows. This control is achieved by the turning on or off of genes that affect growth. Knowing about what plant hormones do and how they do this is vital information that can be used to enhance the way plants are grown for food production. By adding plant hormones to crops, the quality and quantity of food that is produced can be enhanced. Much of the food we eat today is grown with the help of plant hormones. Table 1 below shows the 5 major groups of plant hormones, their actions and their commercial application. Auxin is the best known of these and has a number of highly significant commercial applications.

Hormone	Action	Commercial Application
Auxins	Influence cell division, cell elongation, cell differentiation affecting apical dominance, tropisms, flowering, abscission and senescence.	Development of transgenic plants without seeds (e.g strawberry, tomato, eggplant); applied to seedless fruit to make them grow larger; encourage root formation in plant cuttings and growth in plant tissue culture; as weed killers
Gibberellins	Increases elongation growth- e.g. they cause the rapid growth of internodes in stems making the plant grow taller.	Applied to increase fruit size (e.g. Grapes) and increase the distance between fruit on a stem. Can be used to break seed dormancy—encouraging the seed to germinate.
Ethylene	Promoted ripening of fruits and abscission (falling) of leaves.	Can be used to make fruit ripen earlier than it would naturally.
Cytokinins	Regulate cell division.	Promotes the growth of lateral buds in flowering plants.
Abscisic Acid	Inhibits other hormones—promoting dormancy in plants and seeds.	No commercial applications as the cost of producing synthetic versions of this hormone are too high.

Table 1: Plant Hormone Action and Application

The History of the Mystery

AUXINS were the first plant hormones to be discovered and yet over 100 years on, despite extensive knowledge of how important auxins are and their commercial application, ***SCIENTISTS STILL DO NOT UNDERSTAND EXACTLY HOW AUXINS WORK!***

What is known is that Auxins can be synthesised by all plants (Woodward and Bartel 2005) and have the ability to affect cell growth, cell division, and cell differentiation. They are known to have different effects in different parts of the plant. In the stem auxins allow the plant to grow towards light by elongating the cells on one side of the stem, making it bend towards the light. In the root auxins induce new root formation, either be encouraging elongation of the root or at the right concentrations, encouraging the root to grow branches. **What is NOT KNOWN is how this happens at a molecular level.**

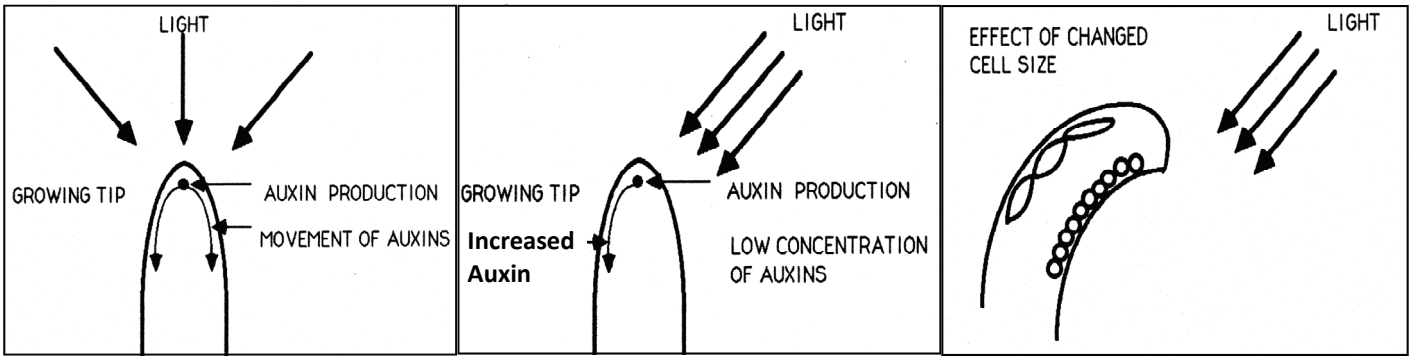


Fig 6: Auxin Action in the Shoot

When light is coming from one direction, auxin concentration increases in the side opposite the light. The cells on the opposite side to the light elongate, bending the plant in the direction of the light.

Charles Darwin

In 1880 Charles Darwin published his work "**The Movement of Plants**", in which he reported on extensive observations he had made on the ability of plants to move in response to various environmental factors. Darwin did not know what was causing this movement, but he could identify that there were consistent patterns being shown and that a substance produced in the tip was being transmitted to other parts of the plant where it was having an effect on growth.

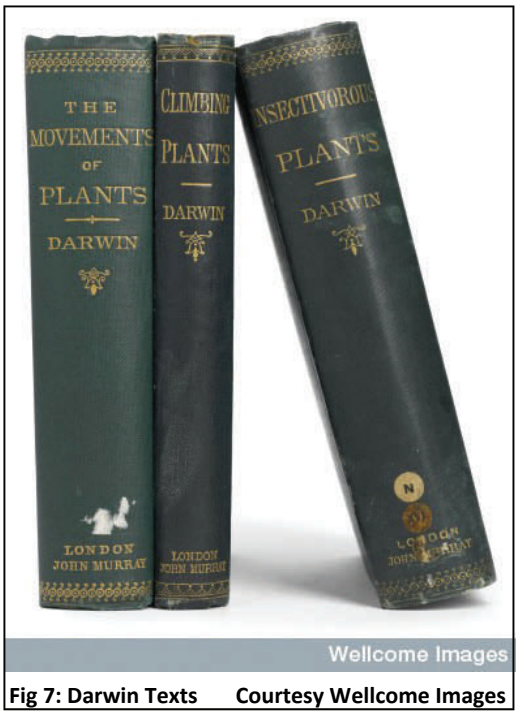


Fig 7: Darwin Texts **Courtesy Wellcome Images**

> *"In the case of the radicles of several, probably of all seedling plants, sensitiveness to gravitation is confined to the tip, which transmits an influence to the adjoining upper part, causing it to bend towards the centre of the earth." (Darwin 1880)*

Darwin also knew that the ability of the stem to bend was caused by a change in the growth patterns of the cells on each side of the stem (Fig .6) He reports on some of the known ideas around this concept, demonstrating that understanding of how auxins change cell growth has been around for over 100 years. It is clear from the quote below that in 1880 the concept that the turgidty of the cell changed to allow the cell the elongate was established.

> *"Until recently the cause of all such bending movements was believed to be due to the increased growth of the side which becomes for a time convex; that this side does temporarily grow more quickly than the concave side has been well established; but De Vries has lately shown that such increased growth follows a previously increased state of turgescence on the convex side.....On the whole we may at present conclude that increased growth, first on one side and then on another, is a secondary effect, and that the increased turgescence of the cells, together with the extensibility of their walls, is the primary cause of the movement."(Darwin 1880)*

The work of a Scientific Team

Dr Karine David is group leader within the Plant Molecular Sciences Laboratory at The University of Auckland. This lab has a number of groups looking into different aspects of plant growth and offers excellent training opportunities for young scientists, some of which involve work with Plant and Food Research. Karine's team are contributing to the worldwide quest for scientific knowledge about HOW auxins work. **They are specifically trying to find out more about how the cell can tell that the auxin is there—and then how does the cell "decide" to respond?**

Members of The University of Auckland School of Biological Sciences Plant Molecular Science Laboratory.

As a hormone, auxin will have one (or more) receptor (s) which must recognise the hormone and bind to it, enabling the hormone to act in the cell (see Fig 8). In the case of auxin, that action involves turning on genes that enable plant growth. The fact that auxin has a different effect in different situations (e.g. roots vs shoots) makes understanding this very complex.

Fig 8: The action of hormones

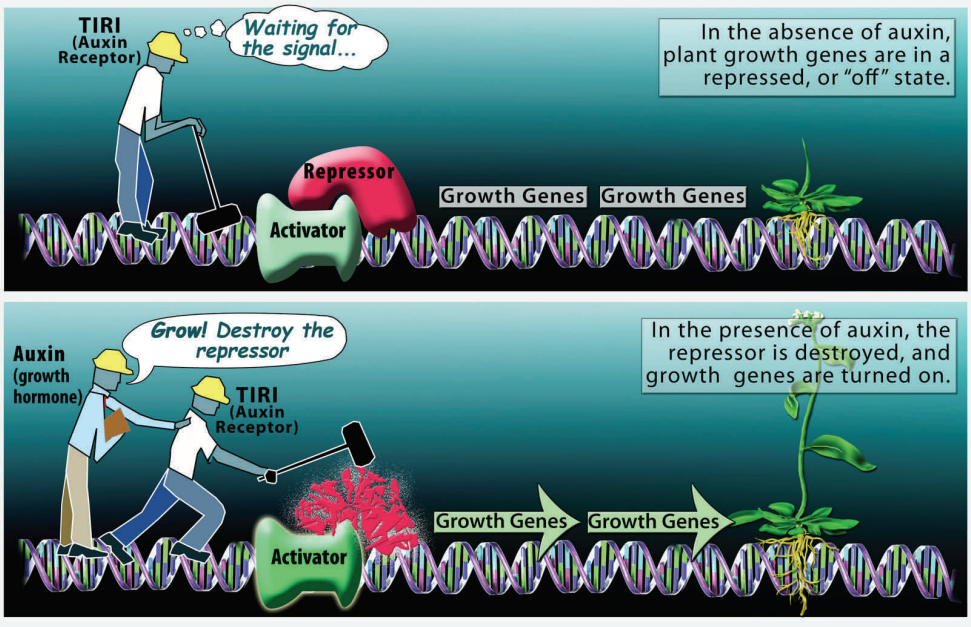
In 2005 a group of scientists from the University of Indiana (Dharmasiri et al 2005) discovered an important part of the mystery around how auxins behave. They identified a protein which was an **auxin receptor** in the cell and worked out how this receptor was making changes that caused the plant cell to grow. The protein is called ***Transport Inhibitor Response Protein 1 (TIR1).***

Photo by: David Bricker
Indiana University Bloomington biologist Mark Estelle, a member of the team who identified TIR1
Image courtesy of the University of Indiana

In order to grow, growth genes in the cell must be turned on. Normally they are not on—they are REPRESSED by a molecule that binds to the ACTIVATOR. When conditions are favourable for growth, auxin is produced and binds to the TIR1 protein. This Auxin-TIR1 complex turns on a signal to DESTROY the REPRESSOR PROTEIN. As a result, the growth genes are TURNED ON. (See Fig 9) .

Although TIR1 has been shown to be very important, evidence from Dharmasiri et al and others has shown that it is probably <u>not the only auxin receptor</u>. A protein called **AUXIN BINDING PROTEIN 1 (ABP1)** is also known to bind to auxin and is thought to be able to affect changes in cell expansion. The Auckland University group led by Karine have been investigating the link between Auxin Binding Protein 1 (ABP1) and the responses seen in the plant, **<u>trying to find out how the cell responds when auxin and ABP1 are present together.</u>**

Fig 9: Auxin control of gene expression by Transport Inhibitor Response Protein 1(TIR1) (Dharmasiri et al 2005)



When a plant is not growing, auxin is not produced, and growth genes are repressed, or turned off.

When conditions are favorable for growth, auxin is produced and binds to a molecule known as TIR1.

The auxin-TIR1 complex in turn signals for the destruction of the repressor protein that keeps growth in check, and growth promoting genes are turned on.

Courtesy: National Science Foundation. www.nsf.govt
Nicolle Rager Fuller, National Science Foundation

Research question:

- **How does the cell respond to the presence of the hormone auxin on the receptor site Auxin Binding Protein 1 (ABP1)?**
- **What is the role of Auxin Binding Protein 1 (ABP1) in the cell cycle?**

Literature Review

Science is an international, interconnected community. Research starts with a **literature review** in which the science team find out as much as possible about what is already known that may help them answer the question and design their research method. Key facts that were reported scientific literature showed that:

- ABP1 is an auxin receptor that has been known about for over 30 years (Hertel et al 1972)
- Over-expression of the ABP1 gene (producing more ABP1 than normal) in the tobacco plant allows cells that are normally not responsive to auxins to expand when they are exposed to auxins. (Jones et al 1998)
- Mutant plants that make no ABP1 show defective cell elongation, fail to organise the basic plant body plan and die early in development. (Napier et al 2002)
- Auxin acts as a "permissive" or starting signal for the cell cycle but little is known about the molecular mechanism that controls this start signal (den Boer and Murray 2000; Stals and Inze, 2001)

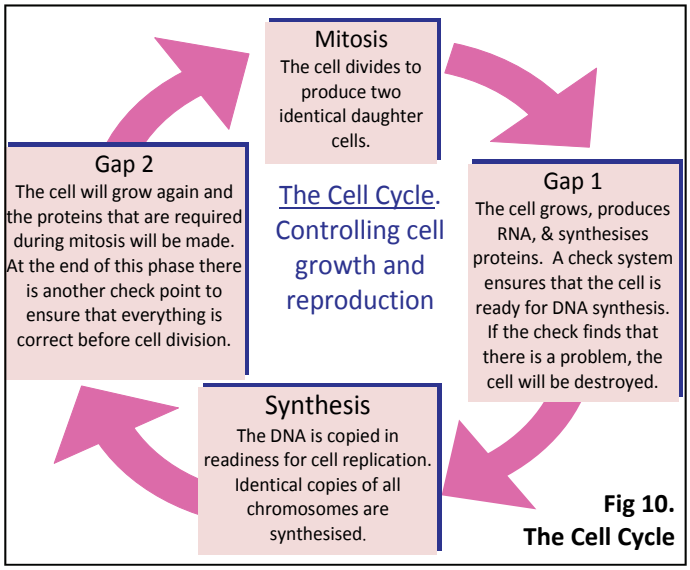


Fig 10. The Cell Cycle

You will recall from LENScience Seminar 1 *(Breast Cancer and Biotechnology)* that the cell cycle while controlling growth and reproduction in cells, is itself controlled by two families of molecules, Cyclin Dependent Kinases (CDKs) and Cyclins. Faults during the cell cycle such as inadequate growth and mistakes in the replication of the chromosomes will be picked up by these control agents and either the cell will be repaired, or the cell will self destruct (apoptosis).

The literature also shows that auxins may act directly or indirectly to regulate members of the Cyclin-dependent Kinase family of substances (John et la 1993).

The task of the research group was to find out what role ABP1 had in the cell cycle and how the plant "sensed" and responded to auxins via ABP1.

Research Method:

There are a range of methods that scientists can use to find out how a protein behaves. They include:

- OVER EXPRESSION—this means there will be *more* ABP1 in the cell than normal
- DOWN REGULATION—this means there will be *less* ABP1 in the cell than normal
- FINDING A MUTANT—a mutant that is not producing ABP1 would mean there is *no* ABP1 in the cell.

By comparing the phenotype of normal plants with the phenotype of the plants where ABP1 is either over or under expressed, scientists can build a picture of what the protein ABP1 is responsible for in the plant.

Mutant—not an option

With Auxin Binding Protein 1 (ABP1), a mutant that did not make this protein was not an option. Why? Because if you had no ABP1, the plant would not develop. In fact cell division does happen in plants that lack ABP1 due to a mutation, but they show defective cell elongation, fail to organise the basic plant body plan, and will die early in development (Callis 2005) This means that the mutant was not useful to fully explain the role of ABP1 in the cell cycle. However it did tell scientists that the auxin pathway that regulates cell division was still working in the ABP1 mutant and that ABP1 was necessary for cell elongation and early growth. But it did not explain how.

Chosen Method—Creating plants that could be down regulated for ABP1

The method decided on was to partially *DOWN REGULATE* the ABP1 protein so that there was *less* ABP1 than normal, but there was *some* being produced. This was achieved both in cell tissue culture and whole plants using tobacco plants and *Arabidopsis* (a small fast growing plant that is idea for research, see Fig 5). The mechanism used to *down regulate ABP1* was to create a **transgenic plant** that had a gene added that would repress the gene that normally produces ABP1. Exposure of these plants to ethanol vapour was used to turn on this gene, stopping ABP1 production. The tissue culture cells were grown in a broth containing auxin. The plants were grown in growth chambers where all environmental factors could be controlled. Once this was achieved, it was possible to compare the phenotype of the normal cells or plants with the down regulated cells or plants and therefore find out more about the function of the ABP1.

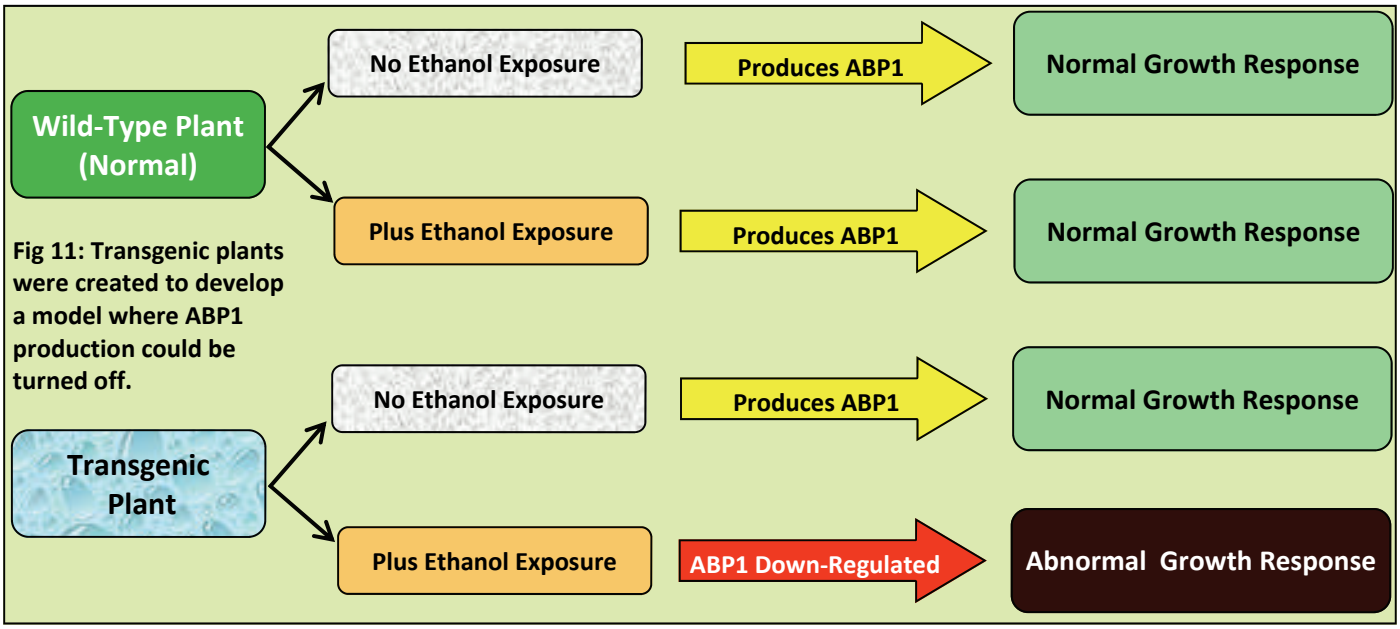


Fig 11: Transgenic plants were created to develop a model where ABP1 production could be turned off.

Data Gathering:

The growth of the plants and the cells was monitored and observations were made using:

- **Light microscopy** for histological observations

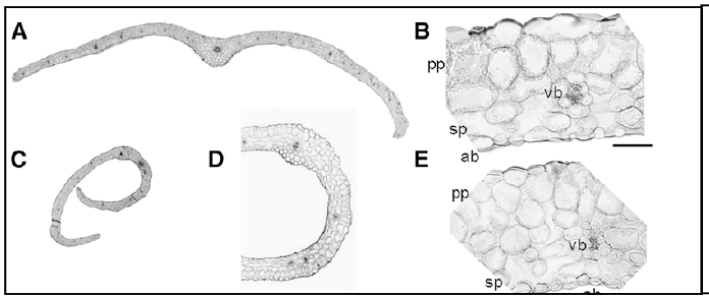


Fig 12: Observations made using light microscopy

A: Cross section of a wild-type leaf
B: Detail of the cross section in A
C: Cross section of a leaf from a plant where ABP1 has been down regulated
D: Detail of the cross section C
E: Detail of the cross section C

- **Scanning electron microscopy** to enable measurement of cells

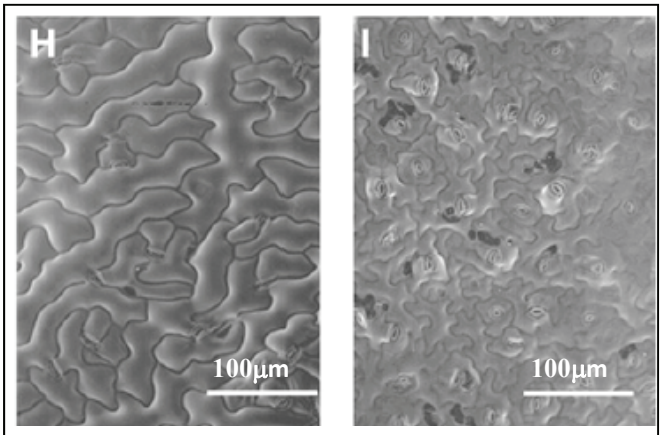


Fig 13: Observations made using SEM

H: SEM Leaf Surface Wild Type Plants

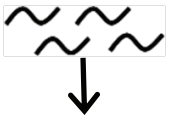
I: SEM Leaf Surface APB1 Inactivated Plants

- **Quantitative Real Time Polymerase Chain Reaction** (qRT-PCR) was used to amplify and analyse target DNA molecules to identify whether genes are being expressed or not during the cell cycle.

Fig 14: Using PCR Technologies

PCR—the polymerase chain reaction, amplifies DNA and is used in multiple ways. In gene expression studies PCR is used to amplify specific fragments of a gene to find out whether that gene is being expressed in the tissue being studied.

RNA is extracted from the cells



Reverse Transcriptase reaction produces cDNA from the RNA

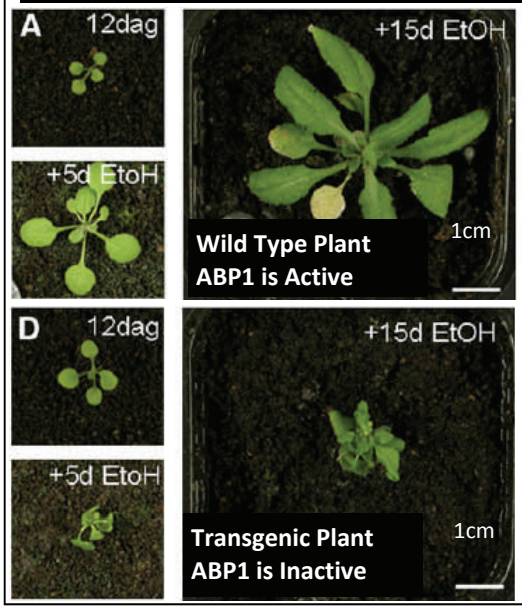
The Polymerase Chain Reaction (PCR) using specific primers produces multiple copies of the target DNA

The target DNA is analysed using Gel Electrophoresis. A variation on PCR called REAL TIME PCR uses fluorescent labels and provides a quantitative analysis of the PCR product.

Findings:

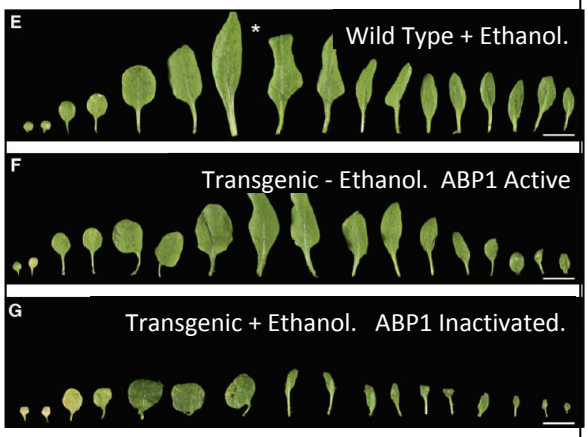
1. When ABP1 is Down-Regulated (Inactivated), Plant Growth is Reduced.

Fig 15: Comparisons of leaf growth with and without ABP1



A: Wild Type Plants
12dag = 12 days after germination. This is when the plants were exposed to ethanol
+5d EtHO = 5 days after exposure to ethanol
+15d EtOH = 15 days after exposure to ethanol

D: Transgenic Plants that when exposed to ethanol will not produce ABP1
12dag = 12 days after germination. This is when the plants were exposed to ethanol (ABP1 is turned off)
+5d EtHO = 5 days after exposure to ethanol
+15d EtOH = 15 days after exposure to ethanol



Turning Genes On and Off
Comparison of F and G shows that the down-regulation of the ABP1 gene is controlled by exposure to ethanol. This is called conditional down-regulation. Bar = 1cm

Table 2: Surface Cell Area of leaves in plants with and without ABP1 Down-Regulation

	Adaxial Leaf Surface	
Genotype	Wild Type	ABP1AS
Leaf number	5	5
Mean cell surface area (μm^2)	1810	527
SD	98	34
n	202	327
Calculated number of cells · mm^{-2}	552	1897

Cell Cycle Control

The cell cycle is controlled by molecules called **Cyclins**. The scientists knew that **CyclinD6** controls the check point between GAP 1 and SYNTHESIS in the cell cycle and **CyclinB1** controls the check point between GAP 2 and MITOSIS.
The scientists wanted to find out whether the genes, CyclinD6 and CyclinB1 were in turn controlled by ABP1.
To answer this question they used Real-Time PCR to measure the amount of the CyclinD6 or CyclinB1 gene that was being expressed in plants where ABP1 was gradually being down-regulated. RT-PCR is used to show whether target genes (CyclinD6 and CyclinB1) are being expressed in plants.
Fig 15 shows that the more ABP1 was down-regulated, the less CyclinD1 gene was expressed. This told the scientists that ABP1 was needed for CyclinD1 to do its job in controlling the cell cycle check point. They found similar result for CyclinB1. This information confirmed that if ABP1 was not present, ClyclinB1 and CyclinD6 were not made. This means that ABP1 is controlling the cell cycle by controlling Cyclin production.

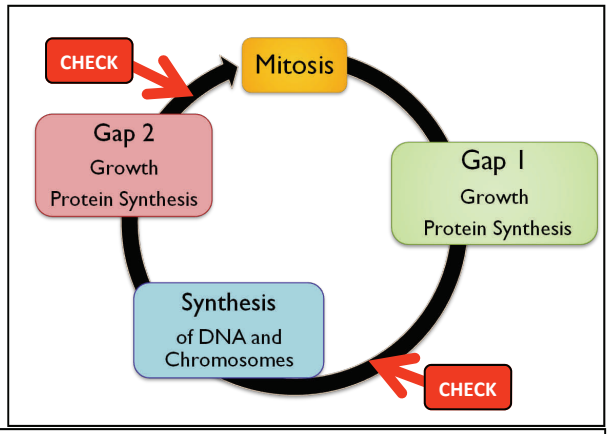
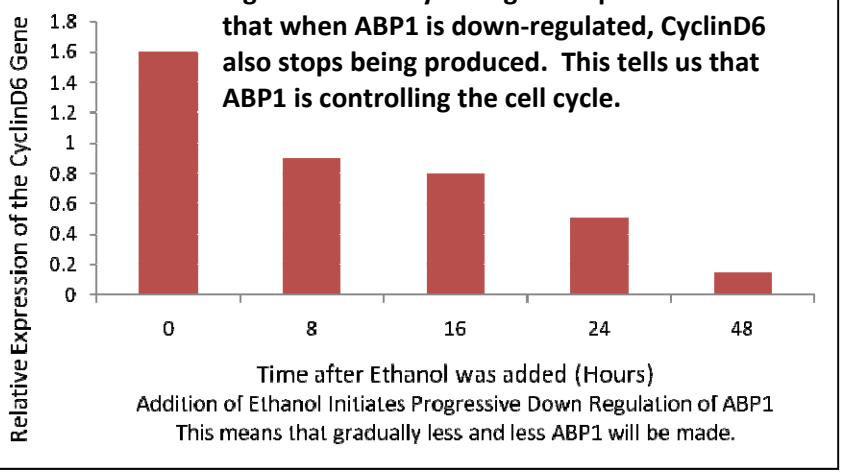


Fig 15: PCR analysis of gene expression shows that when ABP1 is down-regulated, CyclinD6 also stops being produced. This tells us that ABP1 is controlling the cell cycle.



Conclusion

The findings show that:

- when Auxin and Auxin Binding Protein 1 are present together, the cells will grow larger. **Auxin Binding Protein 1 is involved in cell expansion.**
- when Auxin Binding Protein 1 is gradually turned off, less and less CyclinD6 is produced. Given that CyclinD6 is known to control the check point in the cell cycle between Gap1 and DNA Synthesis, this tells us that Auxin Binding Protein 1 is controlling the cell cycle. The same effect was shown for CyclinB1—the molecule that controls the check point between Gap2 and Mitosis. **ABP1 is controlling the cell cycle and therefore cell division.**

Discussion

Cell division and cell expansion are two key processes for plant development. Plants develop their organs after germination. Both cell division and cell expansion are important in this process. The research has shown that ABP1 is important in both of these processes. There is however a third important component of growth - cell differentiation. This is the process during development where cells specialise.

Future Research:

There are still questions that remain unanswered which the research group know would provide useful information to help better understand how plants grow.

- They would like to understand **all** the steps between perception of auxin and the cellular response. This would involve understanding more about gene expression.
- Having found the role of ABP1 in cell expansion and differentiation, the question to ask is whether ABP1 also has a role in cell differentiation.

Direct application of knowledge:

Knowing how the plant is responding to the presence of auxins is useful in a commercial setting. Karine has a project in which she is collaborating with scientists from Plant and Food to look at control of cell expansion in apples. Cell expansion is directly related to fruit texture. Fruit texture is a complex interaction of many factors such as cell wall chemistry, cell size and shape, cell packing and cell turgor. By potentially enabling control of cell expansion, scientists could develop the ability to control phenotypic features related to fruit texture and therefore increase economic benefit.

Further Reading :

Food and Agriculture Organisation of the United Nations http://www.fao.org
FAO 2008 The State of Food Insecurity in the World
Plant Molecular Sciences, Auckland, New Zealand—Functional Genomics Texture Group
Plant and Growth Hormones General Tutorial www.biology-online.org/11/10_growth_and_plant_hormones.htm
www.worldmapper.org The World as You have Never Seen it Before
The Complete Works of Charles Darwin On-line http://darwin-online.org.uk

References:

Callis J (2005) Auxin Action *Nature* 435, 436-437
Darwin C (1880) *The power of movement in plants*. London: John Murray
den Boer, B.G.W., and Murray, J.A.H. (2000.). Triggering the cell cycle in plants. Trends Cell Biol. 10, 245–250.
Dharmasiri, N., Dharmasiri, S. & Estelle, M. (2005) The F-box protein TIR1 is an auxin receptor *Nature* 435, 441–445
Hertel R, Thomson K, Russo VEA. 1972. In-vitro auxin binding to particulate cell fractions from corn coleoptiles. Planta 107: 325-340.
John, P. C. L., Zhang, K., Dong, C., Diederich, L. and Wightman, F. (1993) Related proteins in control of cell cycle progression, the switch between division and differentiation in tissue development, and stimulation of division by auxin and cytokinin. Aust. J. Plant Physiol. 20, 503–526.
Jones, A.M. 1994. Auxin-binding proteins. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45: 393–420.
Jones, A.M., Im, K.H., Savka, M.A., Wu, M.J., Dewitt, N.G., Shillito, R., and Binns, A.N. (1998). Auxin-dependent cell expansion mediated by overexpressed auxin-binding protein 1. Science 282: 1114–1117.
Napier, R.M., David, K.M., and Perrot-Rechenmann, C. (2002). A short history of auxin-binding proteins. Plant Mol. Biol. 49: 339–348.
Stals, H., and Inzé, D. (2001.). When plant cells decide to divide. Trends Plant Sci. 6, 359–364

For further information contact Jacquie Bay, j.bay@auckland.ac.nz; Karine David k.david@auckland.ac.nz

LENScience

Bringing Schools and Scientists Together