

Mapping Metals in Seeds

Summary

Seeds store high concentrations of metal in preparation for germination. Students will map the location of the metals within an *Arabidopsis thaliana* seed using information provided by experiments utilizing synchrotron X-ray fluorescence techniques. Students will speculate (based on information provided) on the relationship between the location of elements in seeds and the function of the elements.

Pan-Canadian Objectives

Science Grade	Knowledge	Science, technology, society and the environment	Skills	ATTITUDES
10-12	313-5, 314-1, 318-1, 319-1	115-2, 116-1, 116-6, 116-7, 117-all, 118-all	212-1, 212-4, 212-5, 213-5, 214-6, 214-11, 214-12	437, 439, 440
7-9	304-3, 306-3, 307-16	109-all, 112-all, 113-all	208-2, 210-1, 210-2, 210-3, 210-11, 210-12, 211-2	422, 428, 430, 433

Special thanks to Dr. Lester Young, Agriculture and Agri-Food Research Canada, Dr. Colleen Christensen, CLS Life Sciences Scientist, and Isobel Johnston, CLS Outreach Assistant

Background

What metals are present in seeds?

Using different X-ray absorption techniques, researchers can use synchrotron to determine:

- 1) the element present in a sample (in this case a seed of the *Arabidopsis thaliana* plant),
- 2) the relative concentration of that element within the sample, and
- 3) the location of that element (by keeping track of what is recorded as the synchrotron light tracks across the sample).

Figure 1: Lester Young AAFRC

Enzymes within tissues of the seed have specific functions and also have metallic co-factors (a substance that acts with another substance to bring about certain effects). These metals are known as ‘micronutrients’. They are essential for the good health of the plant and are needed only in very small quantities. The list includes iron, manganese, molybdenum, copper, boron, zinc, and chloride. Learning more about the location of these elements and how they interact within the plant will help researchers understand how to keep the plant healthy. Micronutrients support and enhance plant health. Keeping

individual plants healthy will lead to a healthy crop, which is important for farm economy.

Students will use the synchrotron data to map the location of the elements; compare that map to a diagram of the parts of the seed; review information regarding the purpose and function of those elements; then speculate on the function of the different regions of the seed during the germination and growth process. A lot of functional information is not available yet since this a new area of active research. An intention of this lesson is to motivate students to possibly take up research or help them to understand the need for research to continue.

Activity

1. Review the parts of a seed using the *Arabidopsis thaliana* as an example. Compare the diagram to the photo. Student sheet one

Metal	Concentration (ppm)
Na	160
Mg	4 600
K	2 150
Ca	6 000
Mn	70
Fe	300
Ni	2.5
Cu	14
Zn	45

2. Discuss the process of germination. Germination starts when a seed imbibes water. As seeds imbibe water, they expand and enzymes and food supplies become hydrated. Hydrated enzymes become active and the seed increases its metabolic activities to produce energy for the growth process. In addition, the water causes turgor pressure to increase in the cells and they are able to enlarge. The first part of the seedling to emerge from the seed coat is the root (also called the radical). Eventually the shoot will also expand and emerge from the seed. Once a seedling emerges into the light, the plant turns green and begins to produce leaves. (A description of germination and a movie demonstrating the germination of *Arabidopsis thaliana* seeds can be found at this url: <http://sunflower.bio.indiana.edu/~rhangart/plantmotion/earlygrowth/germination/arabidopsis/atgermination.html>).

A good description with photos and diagrams for wheat can be found at http://www.wheatbp.net/cgi-bin/grain2.pl?topic=Germination&sub_topic=Diagrams%20and%20pictures&GS=1&sub=1.

3. Provide students with background information regarding the role that these metals play in the growth of the seed. Students sheets 2, 3 and/or 4 can be used for this purpose.

4. Provide students with data acquired using hard X-Ray Synchrotron techniques. Student sheets 5 and 6 map the location of manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn) within an *Arabidopsis thaliana* seed at either 0 or 60 degrees.

Using tracing paper (tissue paper works well) students can create a map of the location of each of the elements.

Place the tracing paper over the Ni image. Mark where 10 is on both the x and y axes. Trace the outside of the seed. Draw a line to circle blue areas. Choose a colour or some

other way to differentiate between elements (such as slanted lines, dots, wavy lines etc) and fill the area in with your choice.

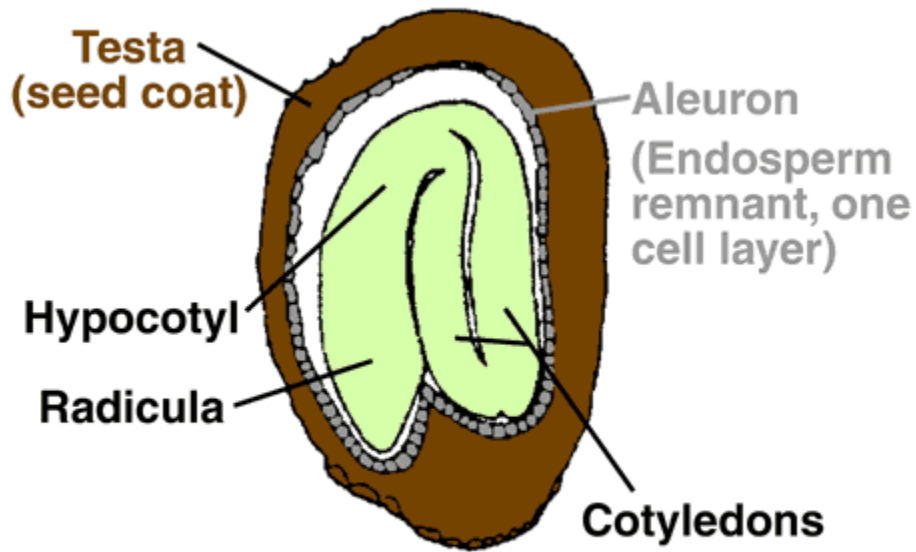
Move your drawing of the seed onto the Fe image. Line the marks on the 10 for both of the axes. Draw a line to mark the darkest areas (blue if there is any). Choose a method to designate the area and fill it in.

Repeat with Mn, Cu and Zn.

5. Compare the metal maps with the diagram of a seed and discuss the possible functions of the sections of the seed and the metals present in those areas. Student sheet 4 would provide good background for this process. The location of the Mn corresponds with the location of the embryo and the radical with a lower concentration along the groove.

6. If you would like to achieve a 3D effect, place the 0 and 60 degree diagrams of the same element side by side and use them as a stereogram. Students hold the diagrams close to their eyes (approximately a foot away) and stare at the center of the pages between the two diagrams allowing their eyes to unfocus on the diagrams. Adjust the focussing spot and the distance between the paper and their eyes until they feel the two images pull together and form a 3D image in the center of the paper. More tips on creating 3D images in this way can be found at <http://www.waynesthisandthat.com/3dimages.htm>

Student Sheet 1 – A diagram of an *Arabidopsis thaliana* seed



Arabidopsis thaliana

A photograph of an *Arabidopsis thaliana* seed



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Student sheet 3 – Micronutrients in Crop Production, adapted from Agriculture and Food SK http://www.agr.gov.sk.ca/DOCS/crops/integrated_pest_management/soil_fertility_fertilizers/micronutrients.asp

Micronutrients in Crop Production

Crops require 16 essential nutrients to grow normally. Carbon (C), hydrogen (H), and oxygen (O) derived from the air comprise greater than 90 per cent of the fresh plant tissue. Macronutrients, needed in large amounts, derived from the soil are nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg).

The remaining essential nutrients, derived from the soil, are referred to as micronutrients, because they are needed in small amounts. They are boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). Micronutrients are also important for plant growth as plants require a proper balance of all the essential nutrients for normal growth and optimum yield.

Copper (Cu): Copper is involved in several enzyme systems, cell wall formation, and electron transport and oxidation reactions. Copper is not readily transferred from older to younger leaves. In cereals, older leaves remain green and healthy with the newer leaves yellowing, wilting, and pigtailing of the leaf tips. Excessive tillering, aborted heads, delayed maturity, prolonged flowering period and poor grain filling are also symptoms. These symptoms appear in irregular patches within fields. These patches have a ‘drought-like’ appearance. Copper deficiency is often associated with increased incidence of root rot, stem and head melanosis (purpling, appears as brown patches in the field at maturity) and possibly may increase the incidence of ergot.

Iron (Fe): Iron is a catalyst to chlorophyll formation, acts as an oxygen carrier, and aids in respiratory enzyme systems. Iron is not translocated within the plant, so deficiency symptoms first show up on the younger leaves. The classic Fe symptom is interveinal chlorosis, a pale green to yellow leaf with sharp distinction between green veins and yellow interveinal tissue.

Manganese (Mn): Manganese is a component in enzyme systems. Manganese activates several important metabolic reactions, aids in chlorophyll synthesis, accelerates germination and maturity, and increases the availability of P and Ca. Manganese is not translocated in the plant, so symptoms first appear on younger leaves. There appears to be some translocation of Mn in oat. Yellowing between the veins is the main deficiency symptom and can be confused with iron deficiency. Gray speck of oat is the most common symptom with the gray specks appearing in interveinal areas. Severe Mn deficiency in oat can cause significant loss in yield.

Zinc (Zn): Zinc is involved in enzyme systems, metabolic reactions, and is necessary for production of chlorophyll and carbohydrates. Zinc is not generally translocated within plant and is partly mobile in wheat and barley so the first symptoms appear on the younger leaves. Symptoms differ from one species to another. In wheat and barley, the older leaves may have light blotches between the veins. Younger leaves will have a normal green color and will be smaller. In flax, grayish brown spots appear on the younger leaves with shortened internodes appearing stunted.

Analysis of seed metals using the Synchrotron

Lester Young* and Martin Reaney**, AAFC-Saskatoon Research Centre

Seeds store fairly high concentrations of calcium, iron, zinc and other metals. These metals reserves are mobilised during germination as a ready supply of the enzyme cofactors necessary for rapid growth. The supply of metals in the seed allows rapid growth of the seedling until the roots are large enough to draw the metals from the soil. Many genes control the capture, transport, storage and metabolism of metals in plants, so an alteration in the concentration or location of metals in the seed may indicate a mutation in a metal handling gene.

Understanding metal physiology in plants may have implications in human and animal diets. Many people are thought to have iron or zinc deficient diets, so understanding metal storage in seeds may lead to more nutritious crops. Plants are also being studied currently for their ability to clean up heavy-metal pollution (phytoremediation). Understanding metal transport, metabolism and storage will help this area of research.

We are using synchrotron-based X-ray fluorescence spectra (XFS) to determine both the amount and location of metals in *Arabidopsis thaliana* seeds. We are using *A. thaliana* as it is closely related to canola, so genetic changes in metal handling are likely to be similar in either plant. *A. thaliana* seeds are much smaller than canola seeds and lend themselves to easier analysis in a synchrotron.

Our initial results suggest that tissues with higher levels of metabolic activity during germination have higher concentrations of manganese (Fig 1). The localisation of manganese in rapidly growing tissues is necessary as the turnover of enzyme cofactors is higher in these tissues. Manganese is also known to accumulate in mitochondria, organelles which are in higher concentrations in rapidly growing tissues. Our results provide spatial information about the distribution of metals which have been extremely difficult to obtain in the past using other techniques.

We will be looking at metal storage in different varieties of *A. thaliana* seeds plants to determine how metal accumulation varies between different populations. We will also be screening a library of *A. thaliana* seeds created at AAFC that have individual genes disabled to identify genes which affect metal storage. Identification of these genes in *A. thaliana* will enable better understanding of metal metabolism in both *Arabidopsis* and canola.

Figure 1: Insert Mn fluorescence map of a single *A. thaliana* seed. Higher concentrations of manganese are indicated by blue contour lines.

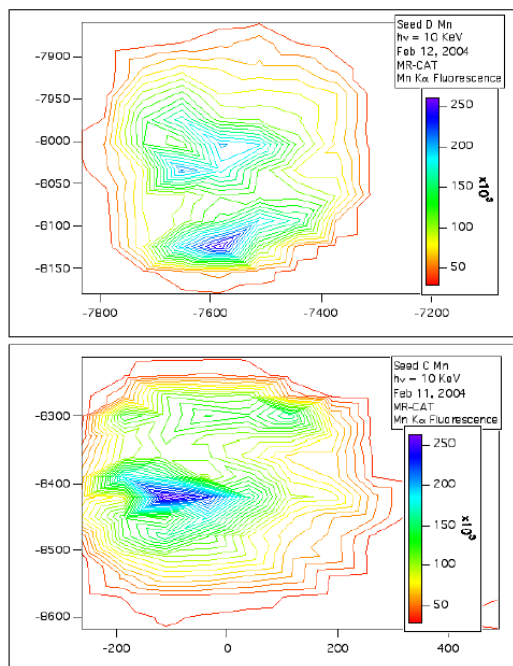


Table 1: ICP-MS determination of metal concentrations (~ppm) in 0.1 g of *Arabidopsis thaliana* seeds

Mg	Ca	Mn	Fe	Co	Ni	Cu	Zn
3580	5160	60	235	0.2	2.4	12	45

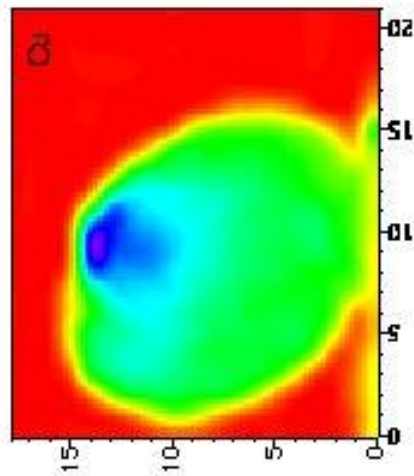
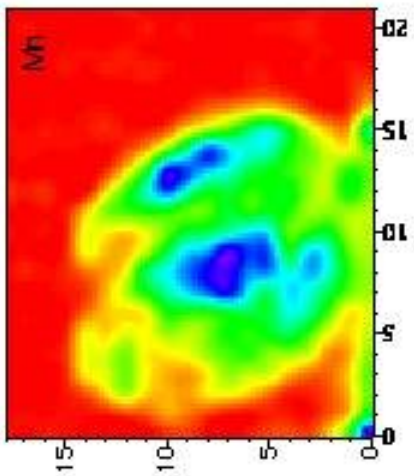
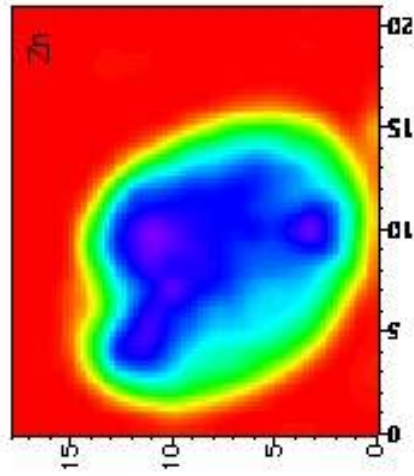
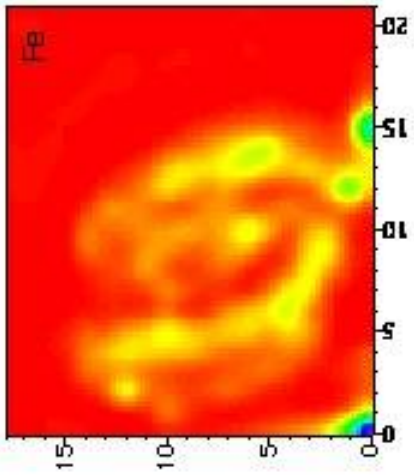
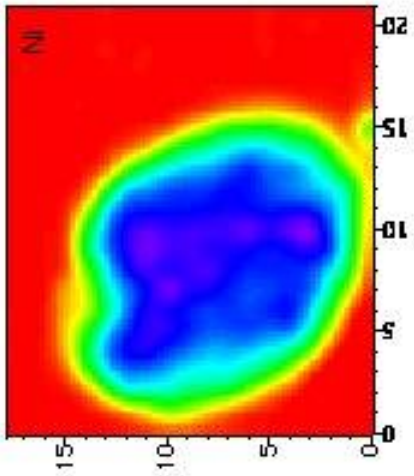
* Email: YoungLW@agr.gc.ca

** Email: ReaneyM@agr.gc.ca



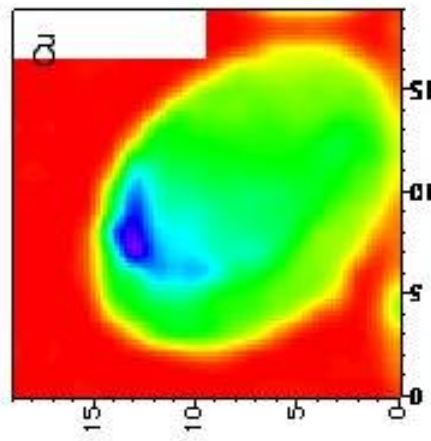
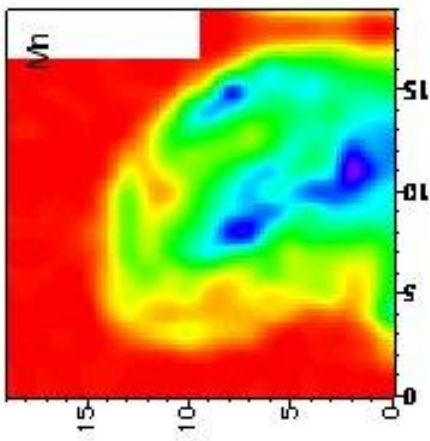
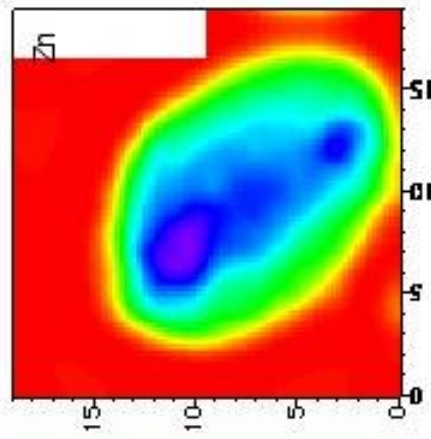
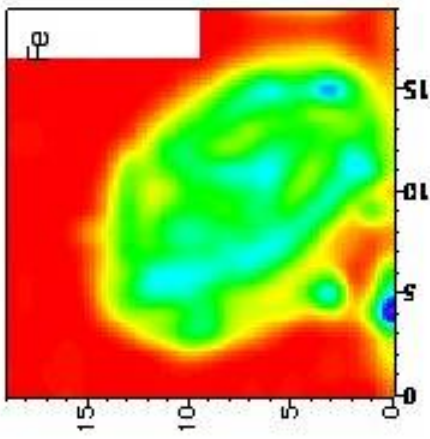
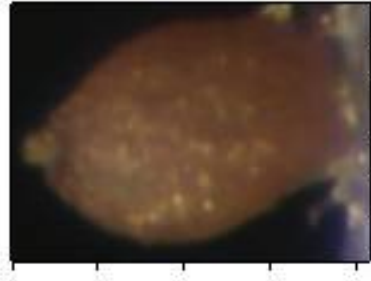
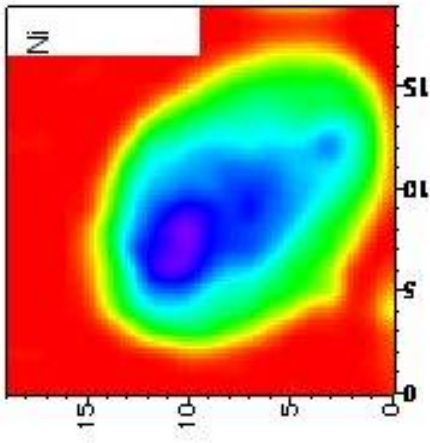
Canola Genomics Newsletter, Issue 3, July 2004

Student sheet 5 – Metal distribution in *Arabidopsis thaliana* seeds at 0 degrees
Image courtesy of Dr. L.M. Young



First At seed map, seed C

Student sheet 6 – Metal distribution in *Arabidopsis thaliana* seeds 60 degrees
Image courtesy Dr. L.M. Young



First At seed map, seed C