Poor nutrition can cause health problems ranging from nutrient deficiencies to obesity. Nutrient deficiencies are less common in wealthy nations where people eat a high proportion of fortified foods, which are foods with micronutrients added. Common micronutrient fortification includes adding Vitamin D to milk to promote bone health, folic acid to flour to decrease fetal neural tube anomalies, and iodine to salt for thyroid function. In countries like the United States, where much of the food purchased is processed to some degree, this is an effective solution to combat common micronutrient deficiencies.

However, adding micronutrients to mass-produced food does little to benefit populations of subsistence farmers in rural areas and developing nations. For these individuals, one way to alleviate nutrient deficiencies is through the biofortification of crops. Biofortification increases the nutritional value of crops through methods like selective breeding and genetic modification. To have the greatest impact with biofortification, it’s important to select a food “that’s a major part of the diet in any particular region that you’re targeting,” according to CSSA member Ray Glahn, a nutritional physiologist at the USDA-ARS Robert W. Holley Center for Agriculture and Health.

Many pulse crops are dietary staples, so researchers are developing biofortified varieties. Pulse crops, like lentils and beans, are highly nutritious, but there may be room for improvement, particularly when focusing on regions where pulse consumption is very high and there are limitations to the variety of foods people eat.

One micronutrient that many people do not get enough of is iron. Iron is essential, enabling our bodies to produce the oxygen-carrying hemoglobin found in red blood cells. With insufficient iron, hemoglobin levels drop, leading to the condition known as anemia. The World Health Organization estimates that 30% of the global population suffers from anemia, with many cases attributable to dietary iron deficiency.

CSSA member Thomas Warkentin, a professor at the University of Saskatchewan, points out that iron deficiency is something that is “not just in developing countries, but also probably in North America and Europe where you’ll have people who are low in iron or borderline anemic.”

Anemia in adults can lead to symptoms such as shortness of breath and fatigue. Pregnant women with anemia are at risk for having babies with a low birth weight, and children with anemia often suffer weakness and have learning disabilities. Reducing cases of anemia by increasing dietary iron could boost the productivity of the adult workforce, improve fetal and maternal outcomes, and improve the health of children.

Pulse Breeding

Increasing nutritional value through traditional breeding is no easy task. CSSA member Dr. Kirstin Bett, a professor at the University of Saskatchewan whose research focuses on the genomics and breeding of pulse crops, describes the work to develop new varieties of beans and lentils as, “playing this huge balancing act between something that’s going to perform well in the field and is going to have the right nutritional profile and the right colors.”
Performance is key for any food crop, and in beans and lentils, traits that improve performance in the field can be linked to genes that impact nutritional value and color. Canada exports pulses to other parts of the globe, but when developing biofortification of crops for subsistence farmers, researchers need to consider how a crop will perform in the region where it will ultimately be grown.

Currently, the lentils grown in North America perform poorly when planted in South Asia, and the same goes for trying to grow South Asian varieties in North America. Attempts to cross varieties from the two locations, to introduce new genetic information, have been unsuccessful. The resulting crops are poor performers and “you have to fight your way back to [local] adaptation,” Bett says.

However, new advances in genetics may give plant breeders an advantage when introducing new genes. Researchers at the University of Saskatchewan recently received funds to sequence the lentil genome. With this detailed genetic information, Bett says they are able to look at adaptations of lentils globally. This includes looking at wild relatives.

The balancing act of performance and nutrition is due to the presence of bioinhibitors, which are compounds that bind micronutrients, such as iron, so that they cannot be absorbed by the body. For some pulses, the same compounds that protect the seed in the field, like tannins and polyphenols, also act as bioinhibitors of iron when consumed by humans. So while beans and lentils contain iron, it is tied up in a form that cannot be absorbed.

Understanding the gene pathways that control seed coat color and iron bioavailability, and working with biochemists to identify the compounds involved, could lead to the development of what Bett calls “designer seed coats” for lentils and beans. “Some seed coat colors make a more robust seed at the beginning of the season because their compound will repel different microorganisms,” Bett says. And in the future, it may be possible that breeders can develop seed coats that offer protection to the plants in the field but do not interfere with absorption of iron or other micronutrients.

Iron Bioavailability of Field Peas

Biofortification research does not only focus on increasing compounds. Glahn points out that when it comes
to iron biofortification, researchers can take two approaches: One is to increase the iron content, and the other is to make the iron more bioavailable.

“If you can double the bioavailability, that’s like adding twice as much iron,” Glahn notes.

For example, the approach for increasing iron bioavailability in field peas is not focused on existing levels of iron in the seeds. Instead, researchers decrease the levels of phytate. Phytate is a form of phosphorus that binds iron and cannot be digested by monogastric animals (e.g., humans), so decreasing phytate should increase the bioavailability of iron.

Warkentin and colleagues have developed several lines of low-phytate peas, derived from a high-performing variety called CDC Bronco. They used mutagenesis, which has been successful for developing low-phytate barley and reported their methods and findings in *Crop Science* in 2012.¹ The researchers compared the two low-phytate lines to CDC Bronco and found that while the total amount of phosphate for all varieties ranged from 3.17–3.37 mg g⁻¹, the amount of P stored as phytate decreased in low lines (1.12 mg g⁻¹ and 1.01 mg g⁻¹) when compared with CDC Bronco (2.5 mg g⁻¹). The low-phytate lines also had a slightly lower seed weight (6% less than CDC Bronco) and lower yield (5.15 and 5.53 Mg ha⁻¹) compared with CDC Bronco (6.02 Mg ha⁻¹). However, Warkentin says, “we’re using those in regular breeding to regain that 15% yield penalty. Otherwise, they look and behave very similar to the progenitor variety.”

More recently, Warkentin and colleagues compared the bioavailability of iron in the low-phytate lines to normal-phytate peas. The iron bioavailability, measured as the ferritin/protein ratio, was 1.5 to 2 times greater in low-phytate lines. Their results were published in *Crop Science* in 2015.²

The authors compared bioavailability in relation to seed coat color for low- and normal-phytate peas. Darker field pea seeds often have higher levels of polyphenols, which inhibit iron absorption, and the researchers were curious how that would impact iron bioavailability for low- and normal-phytate lines.

They found that the iron concentration of whole seeds was significantly greater for the darkest seeds, but the bioavailability of iron was significantly lower in dark seeds compared with non-pigmented varieties. When the hulls were removed, the iron bioavailability increased for the dark seeds. They also looked at samples where 10% of the seed coat was added back and reported that iron bioavailability decreased to approximately the same level observed for whole seeds. This suggests that dehulling could be a way to increase iron absorption for these varieties, but the process needs to be near perfect to see the nutritional benefits.

Moving from in vitro to in vivo

The assessment of iron bioavailability in the low-phytate field peas was done using in vitro models. Glahn has developed an in vitro test that acts as an initial screening tool. “We’re growing epithelial cells, and these are human intestinal epithelial cells, and then we’re coupling that with simulated gastric and intestinal digestion,” says Glahn, describing the work as a combination of science and art. “The art is actually in realizing all the little factors that you have to control to get the system to stay consistent and responsive.”

Maintaining the consistency of cell cultures is challenging, but the lab has experienced technicians, some of whom have “been doing it for over a decade or two now.” Having this experience, well-established protocols, and consistency in results has made Glahn’s lab, “kind of the go-to center here if someone’s going to be screening staple food crops,” he says.

Working with this in vitro model, Glahn and collaborators are gaining new insight about digestion and bioavailability of micronutrients. In a study recently published in *Food and Function,* they used the model to test iron bioavailability in cotyledon cells of several bean varieties and found that about 80% of the iron in the beans is within the cotyledon cells and that cell wall is resistant to digestion in our upper intestine. As the cotyledon cells move into “the lower small intestine where there’s a significant amount of microbes you actually get breakdown of the cell walls, and thus the ability for that iron to be released,” Glahn says. “Of course, by then, you’ve bypassed the major site of iron absorption, so this is the new factor, and it could be the biggest one there is in bioavailability of iron from beans.”

When crops show high bioavailability in the in vitro model, researchers are likely to move on to animal models. “Once we narrow down an advanced line of crops or we get a high and low variety that looks great on all the cell cultures, we’ve done our breeding, then we can confirm it in chickens for very low cost relative to a human study,” Glahn says. Broiler chickens “reach their market weight in about seven weeks after they hatch, so they’re very sensitive to trace mineral deficiencies,” including iron deficiencies.

When crops finally reach trials using human subjects, researchers encounter increasing complexity. One interaction to consider, when evaluating the bioavailability of micronutrients in people, is how biofortified crops may interact with other foods. It is well documented that iron absorption increases in the presence of ascorbic acid (i.e., Vitamin C), but not everyone can drink a glass of orange juice with their meals to boost their iron intake. So researchers need to consider a person’s whole diet and other staple foods in a region because different combinations of carbohydrates and proteins can enhance or inhibit bioavailability. Glahn provides the example of beans: “If you eat [beans] with rice, you don’t get as much bioavailable iron as if you eat [beans] with a potato.”

As more varieties of pulse crops are evaluated, Glahn is seeing occurrences of high bioavailability of iron and other key micronutrients. Some even meet the current goals researchers are trying to achieve through biofortification. “Just identifying those and getting that information out there would be, could be, significant,” Glahn says.

*T. Hmielwoski, Science Editor for CSA News magazine*