DEVELOPMENT

Measuring Trace Micronutrient Levels in Crops

James Stangoulis and Georgia Guild (Flinders University)

Analytical tools to determine the levels of micronutrients in crops are an important aspect of plant breeding. Various techniques have been employed in order to quickly and accurately determine the levels of metals, particularly iron (Fe) and zinc (Zn), in plant material. These techniques include inductively coupled plasma optical emission spectroscopy (ICP-OES), atomic absorption spectroscopy (AAS), colorimetric staining, and more recently X-ray fluorescence spectroscopy (XRF).

ICP-OES and AAS

The use of analytical techniques such ICP-OES and AAS have been well established and used for many years to determine metal concentrations (including micronutrients such as iron and zinc) in various samples. ICP-OES analysis is considered the "gold standard" due to the high accuracy and low limits of detection for micronutrient analysis in plant material. However, these techniques require several steps prior to analysis including grinding the grain to form flour and digestion with acid in order to extract the metals from the flour into a solution for analysis. Due to the sensitivity of these techniques (detection possible down to the µg/kg levels), it is vital to ensure the use of high purity reagents, specialized equipment, and highly trained staff to minimize potential contamination and ensure high quality analyses. The resulting solution containing the extracted metals from the flour is then analyzed with ICP-OES or AAS. This can identify which metals are present in the flour and the concentration of these metals in the original grain sample. These pieces of equipment are expensive to purchase and run, and samples will often need to be sent overseas for such analyses. This process is time consuming and expensive both in terms of analysis cost and time required for sample preparation, shipment, and analysis.

Colorimetric Approaches

Various alternatives to the above mentioned analyses have been investigated to screen for crops with high levels of iron and zinc in an attempt to increase throughput and reduce analysis cost. One such alternative is the use of colorimetric approaches for staining grain in order to determine the concentration and localization of micronutrients in the seed. This method has shown a good correlation with ICP-OES analysis; however, this process is time consuming and not feasible when screening large numbers of samples.

X-ray Fluorescence Spectroscopy

More recently, XRF has been employed by HarvestPlus as an alternative means for micronutrient analysis and has been validated with two instruments, the Oxford Instruments XSupreme 8000 and the Bruker S2 Ranger (Figures 1 and 2). Unlike the previously discussed methods, XRF is able to analyze whole grain and flour samples without the need for digestion prior to analysis. This increases the sample throughput and reduces the pre-analysis preparation, consequently reducing the potential for sample contamination and analysis cost per sample. Additionally, XRF is much cheaper to purchase and run than ICP-OES and is easy to operate without the need for highly trained analysts, specialized facilities, or additional equipment.

HarvestPlus and its partners have developed methods for the use of XRF for screening various elements (iron, zinc, selenium, and phosphorus) in whole grain samples including rice, wheat, and pearl millet, and flour samples for larger grains such as beans and maize. The levels of detection are around 5 mg/kg levels for iron and zinc, which is ideally suited for micronutrient breeding programs. XRF is less accurate than ICP analysis; however, the results from XRF analysis show a strong correlation with ICP analysis, which makes this technique ideal for screening large numbers of samples. XRF analysis can identify which samples have the highest levels of iron and zinc, and these samples can then be sent for further analysis (such as ICP-OES) for more accurate micronutrient determination. At this stage, HarvestPlus is unable to screen for aluminum or titanium, indicators of soil contamination known to affect iron levels. These elements can be analyzed with ICP, which further emphasizes the complementary nature of these two techniques in order to ensure high-quality micronutrient analysis.

Cost / Time Effectiveness

Analysis for a single sample with XRF takes between 30 seconds and 2 minutes, depending on the crop. HarvestPlus has installed 18 of these instruments in various countries, and each instrument has been able to analyze 100–200 samples per day. These instruments have effectively paid for themselves when considering the thousands of samples analyzed each year with XRF rather than more expensive, alternative analyses.

Figure 1: Oxford Instruments XSupreme 8000

Figure 2: Bruker S2 Ranger