PRECAUTIONARY NOTES ON AVOIDING CONTAMINATION

It is well known that plant breeding is a *numbers game*, and that screening genotypes requires a substantial effort in both the field and the laboratory. Collecting a representative sample and minimizing soil and dust contamination from harvesting or postharvest processing equipment poses a considerable analytical challenge when breeding iron- and zinc-dense crops.

Before using equipment—especially processing equipment—for sample preparation, determine what material the equipment is made of and whether it could be a source of contamination. For example, many grinding mills have stainless steel components, which generally are thought not to cause iron contamination. However, some stainless steel comes in various levels of hardness and quality and can be a source of contamination. Another example is black rubber products, commonly found in various threshing equipment, which can introduce exogenous iron and zinc.

The following general sampling protocols were developed by HarvestPlus plant breeders and their collaborators. These protocols are a *work in progress* and may be refined over time.

HANDLING HARVESTED MATERIALS

The staff who collect and handle samples must understand the importance of avoiding contamination. Common contaminant sources when sampling or handling crops in the field or the laboratory include:

- *soil or dust on hands or equipment,*
- *skin-care products (such as hand lotions) on bare hands,*
- *dirty or rusty equipment (such as sampling containers or threshing blades),*
- *lodging of crop plants (in the case of cereals) that result in the inflorescence lying in soil or water,*
- *contact or accidental mixing with other samples.*

MAINTAIN POSTHARVEST EQUIPMENT

Do not use any equipment that has rusty parts. If you are unsure whether your equipment is causing contamination, equipment should be tested. To test, prepare replicates of two to three varieties and analyze for the mineral content of the samples. A high level of iron with a correspondingly high level of aluminum or titanium may indicate contamination. For example, if a milled rice sample has a higher iron content than its corresponding brown rice sample, then the iron in the milled rice is most likely a contaminant.

Before and after using equipment, use a clean brush, a clean cloth, or compressed air to remove dust and plant material. Clean the equipment after each sample to avoid cross-contamination. When equipment is not in use, keep it covered to protect it from dust and water or moisture (to avoid rusting).

COLLECTING A REPRESENTATIVE SAMPLE

Inherent genotypic variability for iron and zinc has been observed among plants of the same genotype and even within a single plant. For example, iron content varies spatially within the inflorescence of wheat; hence the need for a representative sample that truly reflects the real iron and zinc values of the genotype.

For grains, there are two ways to collect a representative sample:

- *Pile the grains evenly on a clean acid-washed tray, flatten the pile, and spread the grains in a circle (see Figure 1). Divide the circle into four roughly equal parts. Discard two diametrically opposite quarters, and remix the remaining two parts. Repeat the quartering procedure until the amount of grains is reduced to the amount of sample required for the experiment.*
- *Use stainless steel automatic dividers (commonly called sample splitters) to randomly divide grains into two, four, or more streams, any one of which can be taken to represent the gross sample. Repeat the sample-splitting procedure until the amount is reduced to the desired sample amount.*

For roots and tubers, the method illustrated in Figure 1 also can be applied. Divide each root or tuber into four roughly equal parts, and discard two diametrically opposite quarters; combine the two remaining quarters as the representative sample. To account for variation between roots or tubers on the same plant, combine diametrically opposite quarters from several tubers or roots from the same plant.

STORING SAMPLES

At any stage of the crop-sampling process when sample material must be transported, packaged, or stored, the importance of a clear labeling and clean, contaminant-free, pest-free environment cannot be overemphasized. Seed and samples must be stored properly to avoid contamination and ensure their integrity for analysis.

First and foremost, always label the sample vessel with the name of the sampled crop, variety name, location, and date.

Whenever a sampling protocol calls for storage, the vessel—like any postharvest processing equipment—must be clean and uncontaminated. Paper bags and envelopes should be new and unused, preferably taken from a sealed package that ensures that they are free of soil, dust, and other contaminants; for most dry samples to be stored between preparation and analysis, #1 coin envelopes are the perfect size.

Plastic containers, plastic trays, and Petri dishes should be acid-washed to ensure their cleanliness. Wash the vessels with soapy (i.e. Pyroneg) water, then rinse in reverse osmosis (RO) water. Place the vessels in a 10% nitric acid bath for 24 hours, then rinse with high-purity water [preferably Milli-Q water (>18-milliohm resistivity)]. If acid washing is not possible, then wash vessels with soapy water and rinse with RO water; however, there is a slight chance of contamination in the absence of acid washing.

For long-term storage, naphthalene mothballs can be used to avoid insect infestation. In such a case, place sealed sample bags in a carton or other box, and place the mothballs around the sample bags (not inside the sample bags—not touching the crop samples).

GRINDING

Grinding often is a prerequisite to sample digestion and analysis, and a contaminant-free grinding mill must be used. The following HarvestPlus crop-sampling protocols mention using a Retsch grinding mill with Teflon chambers and zirconium balls and the IKA A10 grinder (used by many HarvestPlus collaborators). However, these mills are not the only suitable ones on the market. To obtain a list of mills that HarvestPlus has tested so far (with assessments of their performance), contact Dr. James Stangoulis.

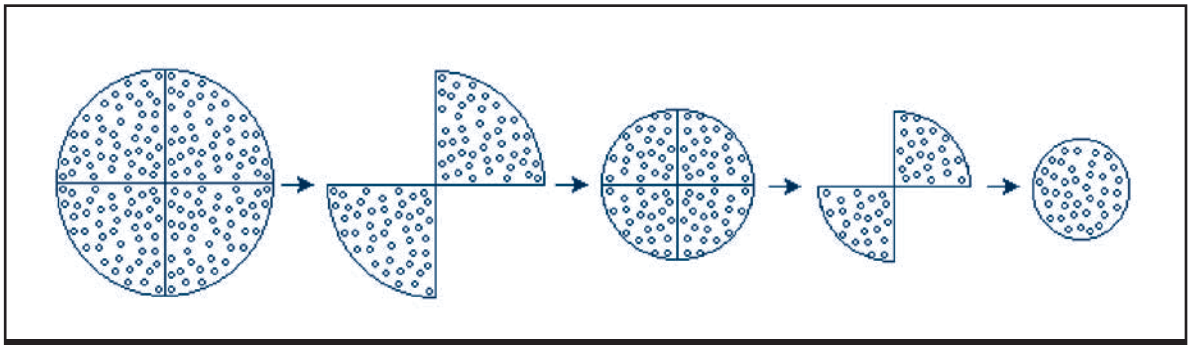

Figure 1: Collecting a representative sample from roots and tubers.

For more information, contact:

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