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CropLife International Detection Methods Project Team

Position Paper on Reference Material for Non-Grain Crops

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CropLife International recognizes the need for standardized reference materials for calibration and validation of detection methods as well as for proficiency testing of laboratories. CropLife companies seek to provide these reference materials to government agencies using a globally harmonized approach under principles that allow each company to effectively manage the distribution and use of its intellectual property.

Reference materials to be used in method calibration should be produced according to international standards and guidelines and may be certified if necessary. Reference materials are made publicly available on a single-event basis for each event in biotechnology-derived agricultural products which are commercially available. These materials are made available by the relevant CropLife company through a designated third-party source. The selection of the third-party source is based upon factors such as the following:

- Global presence,
- Operational independence,
- Dependability of supplier,
- Capacity to produce in a reasonable time, and
- Experience in working with such materials under ISO Guides.

Non-grain crops pose unique challenges for the production of reference materials. Seeds may not be available, as in the case of some vegetatively-propagated crops, or may be very small and in limited supply. The most appropriate reference material for non-grain crops may be that produced from the commodity which is offered for sale (i.e., the article of commerce) or the plant tissue from which the article of commerce is derived. For example, the most appropriate reference material for potato may be derived from potato tubers and the most appropriate material for sugarcane might be derived from the sugarcane stalk (i.e., stem). Likewise, the appropriate starting material for production of reference material might be root (e.g., carrots), leaves (e.g., spinach), or fruit (e.g., eggplant).

The fact that the article of commerce of many non-grain crops is large (in relation to its seeds), can be an advantage in the preparation of reference materials. It is often feasible to remove a small sample of tissue and test each unit for the presence or absence of a biotechnology-derived event prior to its inclusion in the bulk sample. This common feature of non-grain crops provides a unique advantage for reference material production since the purity of the aggregate sample can be ensured. In addition, many non-grain crops (e.g., potatoes, sugar cane, fruit trees) are vegetatively propagated. In vegetative propagation, a section of the mother plant (e.g., portions of potato tubers, sugarcane stalks, or grafts) is planted or grafted and the resulting plant is a genetic clone of the original plant. The risk of genetic impurity due to cross-pollination is reduced for vegetatively (i.e., clonal) propagated crops. Even in the case of articles of commerce that are seed-bearing fruit, it may be possible to remove the seed and produce reference material from the remainder of the article of commerce to eliminate any concern of adventitious presence due to seed resulting from cross-pollination. Thus, reference materials for these crops can be produced to high purity due to absence of adventitious presence within the starting material.

The article of commerce for many non-grain crops may have a high water content. In such cases, it will be prudent to reduce the water content of the article of commerce during production of reference material by using a method such as lyophilization (i.e., freeze-drying) in

order to improve the stability of the material, thus extending its lifespan. Moreover, the freezing step, which is the first step in the lyophilization process, devitalizes high moisture biological material by disrupting the cells, thus offering stewardship benefits since the ability to regenerate plants from the lyophilized material is eliminated. For larger articles of commerce, it may be necessary to cut the units into smaller pieces prior to the lyophilization process to ensure uniform freezing and thus, uniformly high-quality reference material. Cutting smaller pieces and grinding the lyophilized material into a powder provides additional stewardship benefits for seed-bearing fruits. Such processing can ensure the complete devitalization of propagatable material and presents an opportunity to remove seed from seed-bearing fruit to minimize adventitious presence as described above. The purity of the resulting reference material will still be guaranteed in such instances provided that any seeds have been removed, in cases where the possibility of cross-pollination cannot be excluded, and each article of commerce has been tested for the presence or absence of the biotechnology-derived event prior to its use to produce reference material.

As an example, certified reference materials derived from sugar beet event H7-1 (ERM-BF419) and potato event EH92-527-1 (ERM-BF421) were certified by the European Commission's Institute for Reference Materials and Measurements (IRMM) with biotechnology-derived event values of 0 g/kg for the conventional certified reference material and 1000 g/kg for the event certified reference material. Because individual articles of commerce or the tissue source thereof (i.e., sugar beet roots or potato tubers) were tested by PCR (H7-1) or colorimetric (EH92-527-1) analysis prior to reference material production, the certified values for these reference materials had no uncertainty in spite of the fact that the final certified reference material was derived from multiple individual tubers or roots. Thus, in cases where each individual article of commerce has been tested for the presence or absence of the biotechnology-derived event, the provision of the intact devitalized commodity is not necessary to ensure 100% purity of the reference material sample.

In summary, production and characterization of reference material of non-grain crops has both advantages and challenges relative to grain crops. The article of commerce should be taken into consideration to provide the most appropriate reference material for identity and purity testing. Properties such as water content must be addressed to ensure long-term stability. The larger size of the article of commerce for many non-grain crops may allow purity testing of each individual article of commerce before it is added to a composite (i.e., bulk) sample in the preparation of reference materials, therefore increasing the level of confidence in the genetic purity of the final reference material.

References

Broothaerts, W.; Bau', A.; Charoud-Got, J.; Contreras, M.; Corbisier, P.; de Vos, P.; Emteborg, H.; Meeus, N.; Oostra, A.; Teipel, K.; Trapmann, S.; and H. Emons. (2006) Certification of EH92-527-1 Fraction and Identity of Non-Modified and Genetically Modified Potato Powder, ERM®-BF421a and ERM®-BF421b. EUR - Scientific and Technical Research series. 21 pp. https://irmm.jrc.ec.europa.eu/refmat_pdf/ERM-BF421_report.pdf, accessed October 25, 2011.

Trapmann, S.; Bau', A.; Charoud-Got, J.; Conneely, P.; Corbisier, P.; de Vos, P.; Emteborg, H.; Meeus, N.; Vincent, S.; and H. Emons. (2006) Certification of H7-1 Fraction and Identity of Non-Modified and Genetically Modified Sugar Beet Powder, ERM®-BF419a and ERM®-BF419b. EUR- Scientific and Technical Research series. 19 pp. https://irmm.jrc.ec.europa.eu/refmat_pdf/ERM-BF419_report.pdf, accessed October 25, 2011.