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THE IMPACT OF GMO'S ON THE STANDARD

Transmitted by Canada

Note by the secretariat : At the last session the delegation of Canada distributed an information paper on methods and approaches to enhance varietal integrity within seed potato certification systems. The paper is being distributed at this session as an official document.

Methods and approaches to enhance varietal integrity within Seed Potato Certification

ISSUE

Seed potato inspection services normally use visual inspections to confirm varietal identity and purity. In recent years potato varieties with novel traits (PNTs) and essentially derived, morphologically similar varieties have been introduced into potato production and are challenging the way in which varietal integrity is maintained within a certification system. As cultivars from a range of national breeding programmes are increasingly moved internationally between countries, certification authorities have a new problem in confirming that the variety is what it is purported to be. Additional standardized test methods other than visual recognition may be required to confirm varietal integrity.

BACKGROUND

The identity and varietal purity of seed are essential components of a modern, efficient and effective agricultural production system. While different in practice, the objective of this recognition is to establish a valid, legal basis for the exclusive use of the variety name and to enable the breeder to gain a return on his breeding investment through royalty payments. In Canada, official recognition of new varieties has been a feature of the *Seeds Act* since 1923. Initially varieties were licensed but later were prescribed by Ministerial order. Since 1986 an administrative system of variety registration has been in place. Provisions exist for varieties to be introduced from around the world for evaluation and specialized production without registration.

In the beginning, the initial purpose of establishing a system of varietal identity was to ensure that a variety was, in fact, new and not an older, previously known variety being recycled as the latest improvement, i.e., fraud prevention. In 1928, amendments to the *Seeds Act* provided for the Minister to refuse to issue a licence in the case of a cereal variety that was "... found to possess such inferior qualities or characteristics as to impair its value for commerce." Further amendments in 1937 removed the reference to cereal grains so that all new varieties of cereals, potatoes, forage crops and turf grasses were then subject to merit evaluation.

The European Union has a very strict system in place that requires all new varieties of agricultural crops to be officially assessed for distinctness, uniformity and stability (DUS) as well as value for cultivation and use (VCU). EU legislation requires each member state to establish a catalogue of the varieties officially accepted for marketing in its territory. The EU common catalogue is a compilation of the catalogues of member states.

CURRENT SITUATION

Variety Registration:

Canada's system of variety registration and marketing (sale) is somewhere in the middle of the American and EU models. It is more official and restrictive than the US but less so than the EU. In recent years pressure has mounted in favour of the American model, arguing that market acceptance should be left to the market. Others have claimed that pre-market quality evaluations and minimum quality standards have huge downstream benefits for the agricultural handling and processing industries, as well as for producers

and ultimately consumers. Although representative samples of seed are required under the *Seeds Regulations* for all varieties submitted for registration, potatoes are the exception. Unlike true seeds, potatoes are clonally propagated and cannot be stored for an extended period of time for reference. Due to the difficulties associated with the storage and preservation of potato samples, the variety description is accompanied instead by a set of seven colour slides identifying specific characteristics of the potato plants, leaves, flowers, and tubers for variety registration.

Aside from the advantages for training staff in varietal recognition, the reference sample plays another role as a focal point for official, legal recognition of the genetic identity and purity of new varieties. But as the number and complexity of new varieties entering a system increases, further measures may be necessary to attest to the genetic identity of seed as it moves from the breeder into, and through, the seed certification system. This is also critical as seed is moved in commerce between certification systems.

Seed Certification:

The seed certification system, for small seeds, takes small quantities of breeder's seed and multiplies it under strict procedures over a series of generations to satisfy market demands. Certification is, by definition, a procedure by which a third party gives written assurance that a product, process or service conforms to specified requirements at the time of inspection. As the independent, third party seed certifying agency in Canada, the Canadian Food Inspection Agency (CFIA) requires assurances that varieties are DUS and this has generally been accomplished through the variety registration system.

The Potato Section of CFIA, is the seed certifying agency for seed potatoes. Seed potatoes produced from Nuclear class (Pre basic -TC) can remain in the certification programme for no more than seven years. Each growing season the seed moves sequentially downward through the classification system regardless of the disease status of the lot. This "flush-through" system is designed to minimize the risk of disease build-up and allow for commercial quantities of seed to be sold for ware production. As in many countries potatoes intended for re-planting may be multiplied without official oversight, but in Canada such material cannot be sold as seed. This would be in contravention of *Part II of the Seeds Regulations* and various provincial laws which require that only certified seed potatoes be eligible for planting.

Within the certification system the standards for varietal identity and purity are outlined in the *Seeds Regulations Part II*. The standards for purity are in compliance with the UN/ECE standards. A zero per cent tolerance for varietal mixture is applied on visual inspections for Nuclear (Pre-basic TC) through Elite 4 (Field Generation 5) classes of certified seed in Canada. Stability of a variety is essential to maintain these rigid standards in production.

Plants with Novel Traits (PNTs):

The introduction of PNT varieties (including genetically modified (GM) varieties) and their lack of acceptance in certain markets, means that varietal purity and identity are more important than ever before (e.g., adventitious presence of GM traits in non-GM varieties as well as the presence of unapproved traits). The potential development of non-food products from potatoes only increases this importance. Therefore the ability to track and maintain varietal identity on farm through the seed multiplication system prior to ware production is critical to the confidence buyers have in the seed purchased.

In Canada, regulation of modified plants is product based, not process based and addresses all plants with novel traits regardless of how they were derived (conventional breeding, mutagenesis, recombinant DNA techniques etc.). Plants with novel traits are defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable, population of a cultivated species of plant in Canada and that has been intentionally selected, created or introduced into a population of that species through a specific change. By definition, transgenically modified varieties and

varieties derived through mutagenesis are considered PNTs. The PNT variety may be morphologically identical to the traditional variety from which it was derived even though they have unique genotypes.

Traceability:

The success of any seed potato certification programme is its ability to effectively identify and trace back problems that may arise within seed lots. A key in this process is the maintenance of variety and stock identity as an essential tool in investigating and eliminating a problem whether it be quality or disease related.

Most Seed Potato Certification programmes rely exclusively on visual inspection procedures to ensure that the standards for identity and purity are met. Official tags or movement records are also required by certification authorities as evidence of the derivation of seed stocks. This paper trail provides the basis of information to which a certification authority undertakes its business and is maintained by a unique system of identification for every grower and each crop produced on a farm. This identification is used as a reference on all seed tags, certificates, and shipping documentation.

Information on seed source is also recorded on the application forms for crop inspection. Documentation provides evidence that all germplasm, micro-tubers, plantlets, or mini-tubers, entering seed potato production as Nuclear class (pre basic-TC) have been produced from pathogen free material under protected environmental conditions. All varieties multiplied and maintained as Nuclear (pre basic-TC) must be physically separated and each facility must also document the location of each variety. As seed moves through production, all seed source information can be traced back through the individual crop certificates.

Labelling:

Certification authorities must be confident that the information provided on official seed tags, including variety name, category and class accurately represents those seed potatoes. Specific trait characteristics for each variety, such as disease resistance are documented as part of the variety registration process and those characteristics become synonymous with the variety name. Additional information on the variety is not required in the certification system, as the variety name, along with category and class etc.... is stated on every movement document. This is essential, especially in the case of unique varieties or varieties which possess specific characteristics or traits, including PNT's as they are developed and recognized by the varietal name. Certification systems are not obligated to highlight the merits of any one variety and leave this responsibility to the Industry. Nonetheless, official seed certifying authorities must have complete confidence that potatoes varieties which are duly certified, marketed and sold are identity preserved and meet the standards for purity. The buyer in turn must have confidence in the ability of the certification authority to verify the information presented.

NEXT STEPS

Although the visual inspection methods employed in Seed Potato Certification have been effective in maintaining varietal integrity, these programmes have not taken into consideration advances in science and technology that could assist in this respect. The complexity and vast number of potato varieties available make it difficult for an individual inspector to recognize a variety without extensive experience and this type of situation has led to a number of instances where varieties have been misidentified. Integrity is especially important where global trade in seed potato varieties is taking place and can be directly linked to the increased potential for the introduction of PNTs and other varieties with similar morphological characteristics in certification systems. As a result new methods should be considered for the continued management of varietal integrity in certification systems. Key considerations include the following requirements and/or recommendations.

Variety Registration:

As a precursor to certification, variety registration establishes the benchmarks for varietal identity. Although outside the scope of the UN/ECE standard it is suggested that the registration of potato varieties include the following information: (1) a reference tissue sample (may consist of two lyophilized leaf triplets), (2) seven colour slides clearly showing plant, tuber and sprout morphology, (3) A complete objective description (UPOV guidelines) identifying plant morphological characteristics and listing three definitive traits that can be used to differentiate this variety from a similar variety and (4) for transgenic PNT varieties, molecular information required to differentiate the cultivar, describing laboratory protocols and molecular data for recognition.

Handling:

Clear separation and identity preservation of varieties on farm and in laboratories are essential to maintain integrity throughout the process. On farms, growers should have field separation of all cultivars and submit maps of fields and storage locations for each lot. Each grower should be responsible for the implementation of a Quality Assurance plan identifying the steps and procedures each facility will conduct on a daily basis to meet the requirements for the production of seed potatoes. Labs should provide a Quality Assurance programme to track multiplication and maintenance systems with systematic grow outs of germplasm to verify identity/stability.

Growers may be limited to growing a maximum number of morphologically similar or identical varieties within a single farm unit. All efforts should be taken to maximize segregation during storage and handling. Handling equipment should be cleaned between cultivars such that no cross contamination occurs from field to packaging. Within each farm unit, field maps describing the planting location for each variety and storage maps should also be provided. Perhaps new seed growers should be restricted to growing only varieties which are morphologically distinguishable until they have demonstrated good farm management practices. For example, growers may be required to receive crop certificates for three consecutive years to demonstrate this ability.

Shipping:

Where the shipments are sold in bulk, a limit should also be recommended such that only morphologically distinct varieties may be shipped per consignment (tandem trailer loads) to maximize segregation of varieties. All bulk shipments shall be accompanied by bulk movement documents describing origin and destination along with the appropriate certification information. Clear, permanent separation must be maintained at all times during storage, grading and loading.

Varietal Identification:

There are several considerations that must be taken into account in attesting to varietal identification throughout the seed certification process. Simple identification methodology may include molecular analysis of DNA sequences, or protein assays either alone or in combination with one another. Several methods are available and each have positive and negative aspects to their application.

Initially, at the time of registration or entrance into certification, it is anticipated that a database of DNA fingerprints of reference samples be compiled. This serves several purposes but the main objective is to establish a baseline for the variety which can then be used to trace back or confirm varietal identity in the future, if need be. This is why reference material is needed at the entry level for registration or entrance to the system.

Testing and monitoring to ensure varietal identity throughout seed production should be required at various stages in the certification system. As production systems vary it would be simpler to assess and monitor varietal identity in parallel with adequate documentation and quality management throughout the certification

system. This could be done at the entry level and again at critical points within production to provide confidence to the buyer in the varietal identity of the seed produced.

Intensity of sampling rate and confidence obtained from the results will depend on several interactions including the specific test methodology, point of sampling and testing history. Furthermore, cost factors need to be evaluated since the processing of leaf tissue would be more cost effective than processing tuber tissue, however, the status of field progeny is best achieved through tuber evaluation. Sensitivity of testing, compositing of samples, utilization of existing post harvest or field sampling programmes need to be considered.

Test Methodology for Varietal Identification and Monitoring:

DNA fingerprints may be acquired by employing AFLP (amplified fragment length polymorphism) methodology (Vos et al. 1995) (Zabeau and Vos, 1993). This procedure is based on an initial restriction digest using two different restriction enzymes. Specific DNA adapters of known sequence are ligated to the digested genomic DNA. PCR primers specific to each adapter, plus an additional 3 bp are used to amplify fragments, selecting specifically for fragments resulting from restriction digestion by both enzymes. When the PCR product is run on a gel, dense banding patterns generally between 30bp and 100bp in size can be observed (Vrieling et al. 1997). This method may require more DNA starting material, because of the initial genomic digest, but should provide adequate variation between banding patterns for variety identification and may have more flexibility to be transferred between laboratories as specific primers are employed.

Random amplified polymorphic DNA (RAPD) is PCR based technology which uses short random primers (generally 10bp). The PCR is carried out under very low stringency to allow for maximum amplification of a variety of bands (Williams et al, 1990). In practice, this type of methodology does not produce dense banding patterns, and may be more suited to distinguishing one or two varieties compared with one or two hundred. They are also generally not reproducible in different laboratories due to variation that cannot easily be controlled.

Microsatellites may also be used for producing DNA fingerprints. Microsatellites are stretches of DNA that consist of tandem repeats of a simple sequence of nucleotides. The banding pattern resulting from PCR amplification with PCR primers flanking the repeated sequences, can then be used to identify genetic differences in populations when visualized using polyacrylamide gels (Tautz and Renz, 1984)(Tautz, 1989). This type of methodology can be used to develop cumulative information on varietal fingerprints and molecular marker databases (Ghislain et al, 1999). When compared to AFLPs, it is possible that the AFLP technology may be more easily automated for consistency between different laboratories.

Enzyme Linked Immunosorbent Assays (ELISA) methodology may be used for identification for both leaf and tuber samples. This method relies on using specific antibodies against proteins produced by individual potato varieties. Protein from sample extracts is bound to wells in the plates and hybridized with the specific labeled probes. Only the hybridized products are visualized and used for determining the presence of the specific protein. ELISAs may require more starting material (leaf/tuber) than PCR methodology but are sensitive and reliable and do not require specialized instrumentation.

Polymerase Chain Reaction (PCR) may also be used to identify potato varieties (Mullis and Faloona, 1987, and Mullis et al, 1986), DNA primers specific to the DNA sequence which is unique to a particular potato variety are required. DNA extracted from either leaves or tubers is amplified using PCR. Generally, PCR reactions are very sensitive and reliable, especially when specific primers are used, but this technique may be limited by the availability of specialized equipment and laboratory facilities as compared with the ELISAs.

When comparing protein analysis (ELISAs) with analysis at the DNA level (PCR) there are some key points to consider. PCR analysis at the DNA level may be used as an identification tool throughout the growing season on any part of the plant and for the identification of tubers in storage. Protein analysis, depending on the variety, may depend on when and where the protein is produced in the plant throughout the growing season. Depending on the promoter characteristics of the gene, some proteins may be produced throughout the entire plant at various levels, other proteins may only be produced in leaf tissue or perhaps only in the tubers.

Various test methodologies may have to be employed to identify effectively each variety within seed production. Confirmation of varietal identity may depend on variety-specific testing methods and will have to be assessed for each variety. It is important to note that preservation of varietal integrity is critical for seed certification. If at any stage of seed production the varietal integrity is in question, follow up actions will need to be taken or the seed will not be eligible for certification.

Conclusion:

Seed potato certification is efficient and effective for producing quality seed, however, new varieties are being introduced which will place additional demands on a system based on visual inspection. Additional requirements and recommendations including guidelines for planting, farm management defined in quality plans, and the implementation of modern test methodologies for the confirmation of varietal identification should all be considered. The goal is to enhance the current processes to further protect and preserve varietal integrity throughout seed production and marketing to meet the needs of the buyer and to satisfy the certification authority requirements in accordance with UN/ECE standards.