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Section Image

Draft Seed testing protocol for Medicago sativa (Lucerne/Alfalfa)

Further information on: Genetically Modified Organisms

Introduction

1. This protocol outlines the requirements to ensure that **genetically modified (GM) *Medicago sativa* (lucerne/ alfalfa)** unapproved for import, development, field-testing or release under the Hazardous Substances and New Organisms Act (HSNO) 1996 Act

is not released into the New Zealand environment through imported seed. The protocol applies only to lucerne seed imported for sowing or sprouting. It does not apply to seeds imported for animal or bird feed, or seed which is de-vitalised in New Zealand.

2. Unapproved GM organisms are new organisms under the HSNO Act. The Act prohibits the importation, development, field-testing or release of any new organism without approval from the Environmental Risk Management Authority (ERMA). To date, no GM organisms have been approved for release in New Zealand. MAF is mandated to enforce the HSNO Act under section 28 of the Biosecurity Act 1993.
3. Importation of lucerne seed is regulated under MAF Import Health Standards

   155.02.05 – "Importation of Seed for Sowing", and BNZ.GCFP.PHR – "Importation of Grains/Seeds for Consumption, Feed or Processing – Plant Health Requirements". These standards specify the phytosanitary requirements and the overarching requirements of this protocol.
4. Importers must take appropriate measures to ensure that GM seed is not imported. This must include a testing protocol such as that described in this document, but may also include other measures such as isolation of non-GM seed from GM seed.
5. All costs associated with sampling and testing are borne by the importer. All associated MAF activities are charged on a user-pays basis.

Options for Importers of Lucerne Seed

6. Every seed lot in the consignment must be tested for the presence of unapproved GM seeds. Importers can either:

   - have the testing done upon arrival at the border or,
   - provide certification that all seed lots in the consignment have been tested individually prior to shipping.

   In order to receive biosecurity clearance, all seed lots in the consignment must have a current testing certificate[1], certifying that no GM seeds are present.

   Testing must be done in accordance with the requirements of this

protocol, by a laboratory approved to the MAF Biosecurity Standard - "Approval of Laboratories for Genetically Modified Organism Testing".

Sections 8-11 of the protocol stipulate the sampling methodology to be used. Sections 12-16 stipulate the testing methodology.

7. If requested, MAF will consider the option of area freedom from commercial GM production (on a crop:country basis). MAF will grant area freedom if the country can demonstrate that it has sufficient systems in place to provide a level of assurance equivalent to testing every seed line.
8. In addition to the above, importers of small quantities of seeds (defined as those weighing less than 0.1 kg per lot/line) for cultivar trials and/or multiplication will have three further or modified options:

    A. Test samples can be collected either by taking some seed from a number of randomly selected small packets of seed, or by taking a random selection of whole packets of seed. If GM seed is detected, the whole consignment will not be given biosecurity clearance.
    B. For cultivar testing and seed multiplication, untested seed may be imported into and grown in an appropriate quarantine facility, registered and operated according to MAF Biosecurity Authority Standard PBC-NZ-TRA-PQCON

        Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator. During growth and before pollen is produced, MAF will test leaf disc samples for GM material. If unapproved GM plants are detected then the consignment will be destroyed.
    C. For seed multiplication and re-export, untested seed may be imported into and grown in an appropriate quarantine facility, registered and operated according to MAF Biosecurity Authority Standard PBC-NZ-TRA-PQCON

        Specification for the Registration of a Plant Quarantine or Containment Facility, and Operator. The importer must sign a declaration that:

        a. the seeds have been produced under a quality assurance system to avoid contamination by GM seeds and,
        b. the seed lot is not known to contain GM seeds.

The plants will not be tested and will not receive biosecurity clearance. Once the trial is complete, all harvested seeds must be exported out of New Zealand and the remaining vegetative material destroyed.

Sampling

9. The sampling procedure is designed to collect a representative sample. It is based on a number of assumptions:
    - Individual seeds are either GM or not GM. If seeds are present which are heterozygous for the GM trait (eg. due to cross-pollination), the confidence of detection described in section 10 will be less;
    - any GM seeds present are randomly dispersed throughout the seed lot,
    - the sample will be ground and analysed as a whole, not as individual seeds, and
    - the laboratory will correctly identify the presence or absence of GM material in 99% of samples.
10. MAF requires a 95% level of confidence that the inadvertent presence of 1 GM seed in 1000 seeds (0.1%) will be detected. In order to achieve this, a sample, measured by weight, drawn from each seed line must contain at least 3200 seeds. The weight of the sample is the weight of 100 seeds multiplied by 32 and rounded up to the nearest 5 g.
11. The sample must be collected using either the standard International Seed Testing Association (ISTA) or Association of Official Seed Analysts (AOSA) methodology. The ISTA methodology is summarised in the following tables:

    For sacks (i.e. containers up to 100 kg capacity):

Containers per seed line	1-4	5-8	9-15	16-30	31-59	> 59
Number of sub-samples/container	3	2	1	15 total, taken at random	20 total, taken at random	30 total, taken at random

    For bulk bins (i.e. containers greater than 100 kg capacity):

Weight of line (kg)	100-500	501-3,000	3001-20,000	> 20,000

Number of sub-samples	5	1 per 300 kg, not less than 5	1 per 500 kg not less than 10	1 per 700 kg, not less than 40

The sub-samples must be combined to form one uniform collection. A sample of not less than 3200 seeds is then taken from this.

NOTE:1 For small packets (<1kg), thoroughly mix the contents of the packets prior to drawing a sample to ensure the contents are homogeneous.

12. Samples collected at the border, or within a transitional facility, must be done so by trained MAF staff or by organisations approved by MAF. The sample must be held under MAF supervision until it can be sent to an approved testing laboratory. The rest of the consignment must be held in a transitional facility until testing is completed.

Testing Method

13. The testing method must be based on the Polymerase Chain Reaction (PCR), designed to detect the presence or absence of GM seeds by testing for the recombinant gene sequences in GM lucerne. These are either the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, derived from the soil bacterium *Agrobacterium tumefaciens*, or the Figwort Mosaic Virus (FMV) promoter gene.

    Two types of PCR test may be used, qualitative and quantitative. While the qualitative test will definitively detect the presence or absence of GM seeds in the seed lot, the quantitative test estimates the proportion of GM seed in the lot. Both tests are acceptable, but quantitative tests must clearly report a “negative result at the limit of detection” on the certificate.

14. PCR procedures are specific to the equipment and reagents used. Procedures are expected to follow manufacturer’s recommendations and be optimised for a strong signal for each DNA target sequence. When the PCR testing procedures are being validated, GM negative controls (DNA from certified non-GM Lucerne) must have been included to ensure there was no cross-reaction with GM Lucerne DNA.
15. 15. Testing laboratories must use the best available PCR method capable of detecting GM presence in the sample at the lowest reliable limit of detection. This is currently accepted to be 0.01% GM presence (i.e. 1 in 10

000 seeds).

16. DNA is extracted from a sub-sample of the ground seed. All extraction methods must be optimised by the testing laboratory and evidence should be provided that the extracted DNA is of PCR quality. Laboratory manuals must stipulate the procedural steps for extracting, purifying and checking the quality of DNA, including the measures taken to reduce the risk of false positive results. Details of any seed cleaning procedures to remove seed dressings or soil particles that may interfere with the PCR results must be recorded.
17. Procedures must include the following quality assurance samples:
    - template DNA -free (negative) controls;
    - sample replicates;
    - sample preparation controls;
    - positive PCR controls for sample DNA extraction quality; and
    - positive controls of GM DNA from certified or validated standards, where available

Test Results and Interpretation

18. For each duplicate PCR test, results can be positive (+/+), negative (-/-), or ambiguous (+/-). Ambiguous results must be repeated by PCR. If this also gives an ambiguous result (+/-), a second DNA extract must be isolated and PCR tested. If this also yields an ambiguous result (+/-), the result must be reported as +/- (it may indicate a positive result at the limit of detection).
19. A sample that is clearly positive or negative for either the FMV promoter or EPSPS sequence is interpreted as containing or not containing GM material respectively.

    Since the EPSPS sequence is present in many ubiquitous organisms such as *Escherichia. coli*I, and FMV naturally infects some plant species, false positive/negative results can occur. Measures to improve the quality and purity of the extracted DNA such as removal of soil and extraneous plant material, will reduce the risk of false positive results.

    Where the test result is not clear, repeat testing may be required. Following this, if the result still remains unclear, MAF will make a final judgement, after consulting with ERMA New Zealand, and other sources of expert advice.

20. Seed lots which are positive for presence of GM will not be given biosecurity clearance unless approved by ERMA New Zealand under the HSNO Act.
21. Test results must clearly indicate how the testing was performed. The number of seeds ground for analysis and the weight of ground material used for individual PCR tests must be recorded.
22. Records of sampling and testing done at the border must be kept by MAF Quarantine Service staff and copies sent to the Operational Standards Team, Biosecurity New Zealand. This information is publicly available under the Official Information Act 1982 and will be reported to the ERMA New Zealand.

[1]
Means that testing has been done in the current year of importation or in one year prior. This ensures that the testing procedure continues to keep pace with changes made to this protocol.

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