

DNA Fingerprinting Methodology to Distinguish Maize Open-Pollinated Varieties

Introduction

Seed relief is one intervention by which governments, donors and non-governmental organizations (NGOs) can assist smallholder farmers to recover from drought and other disasters. Large quantities of open-pollinated maize varieties (OPVs) are procured for relief seed distribution from the formal seed sector. OPVs out-yield traditional unimproved landraces, allowing increased food and seed security for farmers. In some areas, OPVs are also preferred over hybrids, because hybrid seed production requires infrastructure and expertise that is not always available. Furthermore, hybrid seeds must be purchased by farmers every year, whereas OPV seeds can be saved from year to year by the farmers. However, local seed suppliers in some years provide farmers with maize grain (instead of seed), which is often imported from abroad, resulting in poor yields due to poor adaptation to the local growing conditions.

OPVs are much more genetically variable than hybrids, but it is possible to demonstrate that a maize OPV is distinct, uniform, and stable (known as DUS testing). Measurements for DUS tests are taken from the seedling stage up to physiological maturity; therefore the minimum duration of each DUS test is normally one growing season and each test may be grown twice, over a period of two years. Field tests are carried out during the normal growing season for full expression of the measured characteristics. Data from the maize OPVs in question will be compared to data from reference samples and will be considered distinct only if at least one characteristic of each variety is clearly different in the OPV in question, compared to the reference samples. However, DUS tests are costly and time consuming because of the large number of morphological traits that need to be recorded for at least 40 plants grown in a field sown with a minimum of 3 kg of seed per OPV. Molecular markers offer a complementary method to identify and distinguish different maize samples at the DNA level in an economical and timely fashion.

Molecular marker fingerprinting

For the molecular fingerprinting of OPVs, seeds of the OPVs in question are obtained from the seed source, and 50–100 seeds are planted in the field or greenhouse. When the seedlings are about 2–4 weeks old, one leaf from at least 30 different plants are harvested. DNA is extracted from each plant, and equal amounts of the DNA from 15 plants are combined into a bulk, so that each OPV is represented by two bulks of 15 plants. The extracted DNA is checked for quality and quantity, and the required working concentration prepared for polymerase chain reaction (PCR). PCR analysis of the two bulks is performed using microsatellite markers (also called simple sequence repeats, or SSRs) that have been optimized for genotyping bulk samples. We recommend using up to 45 microsatellite markers, but fewer may be used depending on the question to be answered and the material being tested. For example, if the question is whether two different maize populations are different, both samples can initially be genotyped with only 5 markers. If a large difference in the identity or frequency of alleles of each marker is noted between the two populations in question, the analysis is finished, and the

laboratory can conclusively determine that the two populations were not the same. However, if no or very small difference is noted between populations, another 5 markers would be run; this would be repeated until a significant difference between the two populations is noted, or if no significant markers are found to be different after running 45 markers, it can be concluded that the two populations are only as different as random fluctuations caused by sampling or laboratory error would look; that they cannot be genetically distinguished from one another.

DNA fingerprinting is a specialized analysis that is run using expensive laboratory equipment, reagents and software written specifically for this purpose so it can only be done by specialists, unlike the DUS study that can be run by anyone trained in maize cultivation and careful observation.

What are the requirements for DNA fingerprinting?

1. How should you sample a seed lot?

From each seed lot, obtain a representative sample, depending on the size of the seed lot, following the sampling method outlined by the National Seed Authorities (NSA). Official samples are submitted by the National Seed Authorities (NSA), but samples will be tested that were submitted by anyone. Tests will only reflect the sample that was delivered to the laboratory. Therefore, when submitting a sample, ensure that the seeds sent are a pure and known source of the seeds in question.

2. What seed sample size is required?

A seed sample of about 250 grams (or 100–200 seeds) can be sent to the laboratory for analysis. Up to 1000 grams should be collected at the time the sample is taken from the seed lot, and the remainder of the seed can be kept under cold storage until the tests are complete. If there are doubts regarding the results of the tests, a second sample can be drawn and sent to the laboratory again for testing.

3. What information is required before sending a seed sample?

Seeds should be submitted with the following information (an example is shown below):

1. Owner-
2. Address (postal)-
3. Crop- maize
4. Cultivar/Variety-
5. Lot No. (information from the owner/sellers, so that she/he can find the same lot or bag when they get the results)
6. Size of sample- 100–200 seeds or 250 grams
7. Date sent-
8. Certified seed No/Yes
9. Tests required- DNA fingerprinting, germination

4. What documentation is required before sending a seed sample?

A. Import permit

An import permit is needed to send seed to other countries, so if the seeds originate in a country other than South Africa or Kenya, (where the tests will be run), obtain a **PLANT IMPORT PERMIT** from the country you are sending the seed to.

If you are sending seed to South Africa an import permit is obtained from:

Department Agriculture, Forestry and Fisheries,
Directorate Plant Health
Private Bag X14 Gezina 0031.
Telephone: + 27 12 319 6102
Fax: + 27 12 3196370
Website: www.daff.gov.za

The following information is required to obtain an import permit:

1. The name of the company/lab receiving seed in South Africa
2. Address of the company/lab that is going to receive the seed in South Africa
3. Place of origin of the seed lot e.g Zimbabwe
4. The purpose of sending seed e.g DNA finger printing
5. Description of plant material e.g 25 kg of seed maize

B. Phytosanitary certificate

Once the import permit has been issued from the country where the seed will be sent, obtain a phytosanitary certificate from the country where the seed comes from.

A phytosanitary certificate declares that the seed is free of pests and diseases.

In Zimbabwe a phytosanitary certificate can be obtained from:

Plant Inspector Quarantine
Ministry Of Agriculture
Department of Agricultural Regulatory Services
P.Bag 2007
Mazowe
Telephone: + 263 4 704 531-9
Email: Zpqqs@mweb.co.zw
Fax: + 263 4 700 339

5. How to send the seed samples to the lab

Once all the necessary documents have been acquired, seed samples can be sent by DHL or FedEx to the following laboratory:

Incotec Proteios SA
7 Paperbark Avenue
Ashburton 3213
Kwazulu Natal
South Africa
Tel: +27 (0)33 326 1500
Fax: +27 (0)33 326 1476
Email: Incotec@incotec.co.za
Website: www.proteios.nl

6. Costs: ZAR 120 / SSR reaction as from 1st October 2009 until further notice.

7. When to expect the results from the lab

The laboratory results will be available within 4–6 weeks. The results will be able to distinguish contaminated populations of maize OPVs from the pure sources but low levels of contamination from related sources may not be detectable.

8. For further more information contact the following websites:

<http://en.citizendium.org/wiki/Microsatellite>

<http://www.greenfacts.org/en/gmo/2-genetically-modified-crops/2-genetic-engineering.htm#3>

<http://www.abneta.org/site/pages/terminologies/mas.php>