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AN OVERVIEW OF DNA-BASED METHODS FOR VARIETY IDENTIFICATION AT INRAN-ENSE (ITALIAN SEED CERTIFICATION AGENCY)

Document prepared by experts from Italy

1. INRAN-ENSE is the governmental seed testing agency that supervises the different aspects of the seed production in Italy, starting from the variety registration to seed certification of agricultural and horticultural species. Operating within INRAN-ENSE, the Seed Testing Laboratory located in Tavazzano, carries out laboratory tests both for official and private purposes. The laboratory is asked to verify the quality of seed lots (e.g. purity, germination) and to perform varietal tests. When these tests are addressed to the registration of new varieties, biochemical markers are used (CPVO technical protocols).

2. In the last few years some research activities were addressed to develop protocols for the use of molecular markers in different aspects of seed testing:

- Support to traditional analysis;
- Evaluation of varietal identity and purity of seed lots;
- Variety characterization.

3. Taking advantage of the flexibility of the PCR technique, different kinds of markers have been used (AFLPs, SSR) on a wide range of horticultural and agricultural species (e.g. oat, maize, soybean, rice, pumpkin, onion, bean, fennel, tomato, basil). DNA can be extracted

from different kind of tissue (leaf, seedlings, seeds) and for this reason the analysis may be conducted during the different steps of seed production and seed testing. Molecular markers can be useful in every kind of variety identity problem. Published sources are available to collect technical information about markers, sequences and level of polymorphism in the different species. Because of the wide range of application of these techniques, it is necessary to define the purpose of each analysis (e.g. genetic identity or purity), genetic characteristic of the crop (hybrid, inbred lines, self- or cross- pollinated variety) and then define the experimental plan with standard varieties, type of molecular marker and number of individuals.

4. The laboratory has experimented with some molecular techniques. Some examples are reported below.

Support to traditional analysis

5. Molecular markers can be used to handle different problems in seed testing, as morphological characteristics themselves are not always effective to solve these problems:

- Maize - *Zea mays* -Verification of a maize inbred line identity.

  Discrepancies between the official description of an inbred line and some morphological characteristics strongly influenced by the environment were detected during field controls. A comparison between the leaf tissue taken in the field and the standard seed sample was conducted by using SSR and AFLP technique.
- Rice - *Oryza sativa* - Genetic uniformity.

  During the certification controls a different level of seed pigmentation was observed in seed lots of three varieties. After a visual analysis of representative samples, the different state of expression was confirmed. Microsatellite analysis to evaluate the genetic uniformity was done.
- Maize - *Zea mays* – Identification of specific trait such as Cytoplasmic Male Sterility (CMS) by using a PCR test with specific primer. A procedure to determine the type of cytoplasm in the CSM parental line and restored hybrids is used in the framework of variety testing for registration.
- Oat - *Avena* spp. – Species identification

  The genus *Avena* includes 30 different species, both cultivated and weeds. In seed testing, identification of the different *Avena* species is often a major issue and the laboratory is asked to identify the different species. The retrieval of *Avena* seeds without typical morphology is not rare. Therefore, there is a need for new tools in seed testing to confirm the identification of *Avena* species. 33 accessions of *A. ludoviciana, A. sterilis, A. barbata, A fatua* and *A. sativa* were collected in different areas of Italy and identified by seed morphology. SSR and AFLP markers were used to characterize the samples. The information gathered allows to distinguish species and varieties and represents a new tool in seed testing.

Evaluation of varietal identity and purity of seed lots

6. The laboratory receives an increasing number of applications for variety identification and genetic purity tests on a wide range of species, in particular for horticultural species. The use of PCR-based techniques allows the detection of DNA polymorphism at random or specific loci in the genome. These tools allow variety identification and represent a step

forward, if compared with the protein based methods, because of the larger variability and the universal use (the same protocol with small modification can be applied to different species or matrix).

- Basil - *Ocimum basilicum* – Variety identification. Verification of the genetic difference among individual plantlets from a nursery with different leaf morphological characteristics and aroma by using AFLP marker.
- Tomato - *Lycopersicon esculentum* –Verification of hybrid seed lots. SSR markers have been used to describe parental lines and to evaluate the genetic quality of hybrid seed production by evaluating the presence of self-pollinated individuals in the seed lots before marketing.

Variety characterization

7. DNA-based markers provide a reliable tool for the identification of genotypes, giving a unique profile useful for the characterization of varieties. They can be also considered as a complement to the traditional morphological description, especially when a genetic description of the variety is requested.

- Characterization of local vegetables landraces – Pumpkin- *Cucurbita maxima,* Pepper – *Capsicum annuum,* Onion – *Allium cepa*.
  The heterogeneity of environmental and climatic conditions favoured the selection of a great number of vegetables landraces in Italy. Due to the reduced cultivation, many of them are facing the risk of extinction. Because of a renewed interest for the safeguard of the local tradition and biodiversity, many research projects were developed. The laboratory is involved with local groups and other research organizations in recovering of some of these old varieties with the end goal of their inclusion in the conservation varieties catalogue.
  AFLP and SSR analysis were used to evaluate the genetic difference of “Berretta da prete” (pumpkin), “Peperone di Voghera” (pepper) and “Cipolla di Breme” (onion) with respect to reference varieties to provide a molecular basis for both morphological and biochemical differences. Moreover, the combined knowledge of morphological characteristics and genetic structure of these varieties will represent an effective tool to support their recovery and maintenance.
- Hybrid rice characterization **–** In the framework of the revision of the national protocol for new rice variety listing, hybrid rice SSR profiling was proposed. The control of the hybrid formula exclusively by morphological characteristics can be difficult or even impossible. New tools are needed in order to show the relation among the parental lines and the hybrid. Microsatellite markers - because of their multiallelic nature, codominant transmission, relative abundance and extensive genome coverage - are very suitable for this purpose. Therefore, to address the need for molecular identification of rice hybrids, 12 SSRs (1/chromosome) were selected among 35 SSRs included in the Gramene database. The selection was based on the level of polymorphism in a set of 34 reference varieties. Quality scoring (presence of stutter band, reliable alleles calling) and sizing for possible multiplexing was also taken into account. A protocol with forward primer M13 tailed was considered to reduce dye labelled primer costs. As a future development, this SSR panel will be used to test all the varieties of the rice reference collection. The final purpose is to use it to characterize rice new varieties and include DNA-profiles as complementary information in the official description for the National rice catalogue.

8. In our experience, molecular markers have proved to be very flexible tools, that can concur in solving problems at different stages of seed chain production from variety registration to seedlot analysis. In the future, the development of validated panels of selected markers and reference varieties crop by crop would be desirable in order to improve their use (standardization of methods).

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