Guidelines for a common certification scheme in the Mediterranean

M. Digiaro, A.M. D’Onghia, A. Myrta, V. Savino and G. P. Martelli

The impossibility to adequately controlling severe plant virus diseases by any type of curative treatment, including the chemical applications, has induced many countries and international organizations to undertake and promote research and sanitary improvement programs for plant species of major economic relevance.

The situation is further worsened by implementation of clonal selection and intensive trade the plant propagating material which extends and internationalises problems that would be otherwise confined in limited areas.

The inefficiency of the traditional control methods requires new approaches to the control of these diseases, involving preventive rather than curative strategies.

Among the possible measures, the utilization of "healthy" material seems nowadays the one that can ensure good results. It is based on a very simple principle: preventing the problem at the source using non infected material since the establishment of the orchards, thus reducing the inoculum from which the infection could further propagate through vectors. This measure seems even more efficient if it is considered that many virus diseases, notably in woody crops, find in humans the most efficient

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or even unique vector, through plant propagation and marketing.

The sanitary improvement of plant species is a task usually assigned to scientific institutions that, although rigorous in the application of the principles and methods to achieve the pre-established results, are often deficient in the means and organization to transfer results to farmers.

It results that in many countries vast collections of virus-free "primary sources" are available but are not within the reach of farmers and remain unused.

It seems necessary to target clonal and sanitary selection to certification requirements, establishing and automatically connecting these two steps.

What is needed is a law on certification globally addressing the problem of production, conservation and utilization of certified material, establishing the procedures for obtention of clones, identifying the concerned institutions, establishing the modes of transfer of primary sources to conservation for pre-multiplication, etc.

The two steps, involving the obtention of primary sources and certification, must be closely related to each other.

The investments necessary to obtain primary sources would be useless without a certification system able to make them profitable. Likewise a certification service without appropriate planning, resources and competent authorities for the production of primary sources would not be efficient. In the latter case, improvisation, single measures, the willingness of few would not suffice to fill the gap and to fully meet certified material demand.

Although preliminary to certification meant in a traditional sense, the obtention of primary sources, the costs of which can not be met by the private sector, should be part of a planned scenario. It should be entrusted to qualified scientific institutions working in conformity to inter-
national protocols, so as to provide absolute guarantees on the quality and homogeneity of the final product. It is not acceptable that there are materials "of equal category" obtained in different countries by different selection procedures.

To obtain primary sources it is also necessary to have all required competences (pomological, phytosanitary, enological in the case of wine grapes) working closely.

Considering the costs for obtaining a clone (field selection, diagnosis, conservation, sanitation if necessary, performance evaluation in comparative fields, homologation) and those relative to the subsequent steps of certification sensu stricto (maintenance, genetic and sanitary controls required by the protocols, etc.) the choice of the materials to improve should be well reasoned, taking into account economic, technical and social considerations. It should be reserved to the most common and locally relevant varieties.

The cost analysis and market investigations are thus an important and preliminary part of a sanitary improvement plan. Through an appropriate evaluation of these parameters it could be established whether and to what extent it would be useful to address to certification obsolete varieties, of low value and limited use.

Problems are also open in the subsequent steps of certification. Although recognizing the need for all countries to have an appropriate legislation in this matter, a common effort is crucial to harmonize the existing regulations (AA. VV., 1993). This would prevent the risk to attribute different meanings to the "certified" plant and to foster useless and protectionism-related conflicts between countries.

The first difficulty is in the label. There is now a wide range of labels, different in shape, colour and wording, whose differences and real meaning are often obscure.

A good certification scheme involves a clear, detailed and reliable label, containing given identi-
fication data (producer, lot number, Certifying Board, etc.) clearly indicating from which pathogens plants are free. General indications such as "plants free of the main pathogens", "controlled plants", "healthy plants" are only confusing. The meaning of the label "certified" for grapevines in EU countries is a typical example. In contrast with farmers' expectations and unless additional indications are provided, it does not certify the absence of virus diseases in the plant but only that the plant has been controlled for its exemption from fanleaf and leafroll. The proposal of new documents and categories of plants, (such as in EU countries the plant passport and the CAC category), introduced to favour the circulation of higher quality standard material, do not contribute towards the objective of clearness. It is difficult to make clear the substantial difference between materials covered by these types of documents even to specialists.

It is then necessary to detail the protocols to be used for genetic and sanitary controls, specifying the number and type of assays, establishing the modes and sites where to keep materials during different steps of certification, harmonizing the criteria for the technical features of different structures (screen-house, for example), the distances to keep, etc.. An excellent action was taken by different European researchers to establish a commission of (true) experts with a view to define common protocols for the detection and control of infectious agents of grapevines to be adopted by member states (EUGN = European Network for the Establishment of Reference Protocols for Detection and Elimination of Infectious Agents for the Sanitary Certification of Grapevine). Similar objectives have been pursued in two additional research networks involving different Mediterranean countries (MNCC = Mediterranean Network of Citrus Certification and MNFT = Mediterranean Network of Fruit Trees) promoted by the CIHEAM/IAM-Bari for citrus and stone fruits.

The formal solution of technical and legal problems is a preliminary step towards a good certification system, although it is not enough to ensure its
success. In most cases the greatest deficiencies are observed in the implementation and in the ordinary management of the system rather than in the normative step.

Based on the previous considerations and on the past experience about certification, it seems useful to present hereby some practical suggestions and propose, in broad lines, some reference rules to those who are willing to implement certification programs of nursery production ex novo.

On a preliminary basis it is necessary to identify the existing national laws on quarantine and commercialisation of propagating material, that are to be complied with and harmonized.

The choice of the plant species to certify should be based on reasoned technical and economic considerations. The latter should take into account the actual relevance of the crop in the country, the benefits resulting from the implementation of the certification system, the costs to be incurred for establishing and maintaining the service.

As for the technical considerations, it is crucial to know the sanitary status of the crops to certify. A low incidence of phytosanitary problems could, for instance, not recommend the establishment of the certification service for some species, unless other considerations (i.e. genetic identification, commercial reasons, etc.) justify it. A typical example is olive that is subject to a high demand of certified material although it is not affected by important virus problems. On the other hand, the endemic presence of diseases of high epidemiological potential, such as Sharka of stone fruits and Tristeza of citrus, both transmitted by aphids, could make the certification action useless, unless the certification measures are supported by a preliminary and parallel eradication program.

In consideration of the expertise required, a preliminary monitoring of the local scientific institutions is necessary to support the certification service and the existing public services concerned
that should be entrusted with the management and sanitary and genetic controls required.

Lastly, since it is essential that this system is able to support itself, an active nursery sector is necessary. Nurserymen are the most important link of the whole chain and greatly influence the success of the action. This is the reason for making them aware and trained to innovate their nursery conduct following the new needs imposed by certification.

The presence of these prerogatives makes it possible the adoption and implementation of legal rules on certification.

Some of the major points involved in these rules are proposed hereinafter. They include:

I. identifying the Certifying Board, possibly within a national institution (Ministry of Agriculture, for example) responsible for the service;

II. establishing a Scientific Technical Committee (STC) for Certification including not only the representatives of the Board but also experts required in different certifications steps, ranging from the selection of primary sources to the utilization of certified material.

In particular the STC should include pathologists, pomologists, nurserymen and producers’ representatives and operates consulting body of the certifying board. STC tasks include i) working out proposals for the technical implementation protocols of each species, ii) expressing view on the action of the certification Service, iii) assessing the adequacy of the Institutions and structures to accommodate certification steps, iv) assessing the fitness of the material having access to certification;

III. establishing an autonomous Certification Service with its branch sections, responsible for
the sanitary and genetic controls included in protocols;

IV. setting up a National Catalogue of Variety, including all the selections admitted for certification. The enrolment in the register involves automatically the transfer of clones to the subsequent certification steps;

V. defining different certification steps and the types of materials produced. In accordance with the international criteria, the following steps should be included: i) obtainment of primary sources, ii) conservation for pre-multiplication (pre-base material); iii) pre-multiplication (base material); iv) multiplication (certified material); v) nursery (certifiable material);

VI. setting up increase blocks, recognized by the certifying board, possibility to utilize micropropagation to meet the particular needs of some species and favor the rapid production of base and certified materials;

VII. identifying the sites where to run different steps, in accordance with the protocols of each single species;

VIII. obtaining primary sources: identification of the institutions and/or bodies in charge of the breeding of clone candidates, on the basis of the ascertained competencies;

IX. conservation for pre-multiplication: preferably unique for each species or species group, organized and run under the control of the certifying board and subject to the controls by the certification service;

X. pre-multiplication: the number of the centres is to be established according to the country's requirements, on the basis of its size and of the species relevance. For the crucial role played in these centres, they should be organized and run by public bodies, recognized
by the certifying board and subject to the controls by the certification service;

XI. multiplication: it should be managed by private citizens, either separately or in association.

General rules for organizing a certification program

1. A national Certification Service (sanitary assessment and trueness-to-type) is set up for vegetatively propagated species.

2. The Ministry of Agriculture is the certifying authority which acts on the basis of recommendations by the Scientific Technical Committee.

3. The Technical Scientific Committee is made up of:

   • n. 2 representatives of the Ministry of Agriculture, one as Secretary;
   • n. 2 plant pathologists specialized in production, conservation and use of sanitarly-improved propagating material;
   • n. 2 pomologists;
   • n. 1 representative of producers (fruit growers);
   • n. 1 representative of nurserymen.

The Committee, for specialized subjects, can resort to national and international experts.

The Committee is appointed by the Minister of Agriculture. The Committee appoints its President and will serve a period of five years;

4. Institutionally, the Certification Service works in collaboration with scientific institutions, but when is necessary (i.e. implementation and management of certain phases of activity) other professional and/or interprofessional
Guidelines for a common certification scheme in the Mediterranean organizations may be consulted upon advice of the Technical Scientific Committee.

5. A national catalogue of varieties submitted to certification is deposited with the Ministry of Agriculture. This catalogue contains the main morphological, physiological and sanitary characteristics of the primary sources (Annex-a). The registration and entry in the catalogue is authorized by the Ministry of Agriculture on the basis of the supporting documents prescribed by the protocol and upon approval of the Technical Scientific Committee. Any variety may be cancelled from the catalogue whenever one or more of the conditions required for registration are no longer met. Cancellation is subject to approval by the Technical Scientific Committee.

6. The Certification procedure includes the following steps (Annex i):

a. Obtention of primary source
It involves the pomological and sanitary selection and, if necessary, the sanitation of ecotypes of native cultivars or those grown in the country for a long period of time. These activities are carried out by the national scientific institutions. Other foreign scientific institutions may also collaborate, if needed, upon approval of the Technical Scientific Committee.

b. Conservation for premultiplication.
It consists in the conservation, under healthy conditions (Annex b), of at least two specimens of the primary source, producing propagating material of “pre-basic” category. This is done in the repository on the responsibility of the Ministry of Agriculture through scientific institutions.

c. Premultiplication.
It entails the production of propagating material of “basic category” on the responsibility of public institutions and in accordance with the guidelines drawn up for each
plant species to establish certified mother blocks. Considering the importance of the species, its distribution on the territory and the surface area of the country, more than one centre might be necessary.

d. Multiplication.
This is the production of "certified" propagating material to be distributed to the nurseries, in compliance with the protocol of each plant species. This step can be carried out by private agencies, approved by the Technical Scientific Committee.

e. Nursery.
This refers to the production, by private organizations, of rootstocks and grafted plants from certified material. The final product has to fulfil certification requirements for that species.

f. Increase.
It consists in the rapid propagation of "basic" and "certified" material in some instances, in agreement with the protocol for each plant species. The increase activity is authorized upon request by the Ministry of Agriculture and approved by the Technical Scientific Committee.

7. The Certification service is organized as follows:

- Repository: site of Conservation for premultiplication;
- Foundation Block: site of premultiplication;
- Mother Block: site of multiplication;
- Nursery: site of production of commercial certified material.

8. Propagating materials are classified in the following categories:

- Primary source – original material produced by the breeder and kept by the breeder himself or by the successors in title.
Guidelines for a common certification scheme in the Mediterranean

- **Pre-basic** (super élite) - material produced in the repository to be used for the establishment of the foundation block.

- **Basic** (élite) - material produced in the foundation block, to be used for the establishment of mother blocks.

- **Certified** - material produced from mother blocks to be used directly for the establishment of commercial plantings or to be used in nurseries for grafting, rooting and seedling production. Grafted plants are certified when conditions of production comply with the certification rules.

For the above categories, the sanitary status envisaged may be of two types:

- **Virus-free** material free from all viruses and virus-like agents known for the species considered.

- **Virus-tested** material free from the viruses or virus-like agents specifically mentioned in the certification protocol and listed in the labels.

9. In order to settle questions related to the management of the certification service, the Ministry of Agriculture is assisted by the Technical Scientific Committee, acting as a technical advisory board.

The Ministry of Agriculture promotes genetic and sanitary controls on the repository and foundation blocks through scientific institutions. The scientific Institutes responsible for the afore-mentioned tasks are approved by the Ministry of Agriculture upon the recommendation by the Scientific Technical Committee.

The mother blocks and nurseries according to paragraphs 6d and 6e are inspected and controlled by the certifying authority.
The "protected" and the "non-protected" cultivars of public and private breeders, in the country and abroad, may enter the certification service. For the introduction of this material in the certification service, the persons concerned, or their legal representative, shall apply to the Ministry of Agriculture, providing the documents as specified in Annex (c). Individual genotypes will be delivered to the repository for evaluation of the sanitary status and trueness-to-type and registration in a specific national catalogue.

Applications for the recognition of the multiplication centre and for obtaining the genetic and sanitary certification of nursery material produced shall be submitted to the Ministry of Agriculture following the schemes of Annex (e) and (d), herewith enclosed.

The propagating material may be certified only for the persons, or their legal representatives, authorized to perform the nursery activity in compliance with the laws in force.

Certified plants must be accompanied by official certificates issued by the Ministry of Agriculture following the scheme of Annex (f) which is an integral part of the present regulation. The official certificates are labels whose colour is “white with blue band” for "pre-basic" material, “white” for "basic" material and “blue” for "certified" material. This certification guarantees that all the operations of control have been made according the provisions of the present regulation. In particular, the certification recognizes one of the two types of sanitary status of propagative material in accordance with the conditions set in the protocols for each single species: virus-tested and virus-free.

The persons or their legal representatives submitted to the inspection as well as the Institutions, Agencies and Inspectors are
Guidelines for a common certification scheme in the Mediterranean

requested to supply the Ministry of Agriculture and the Technical Scientific Committee with all information necessary to verify the proper functioning of their activities.

15 The Multiplication Centre that intends to be recognized by the Ministry of Agriculture should fulfil the following conditions:

▪ putting in to the Ministry of Agriculture an application for the recognition of the multiplication centre (Annex e);

▪ nominating a technician as responsible for contacts with inspection and certification authorities;

▪ having appropriate organization, infrastructures and equipment for the production, conservation and transportation of the material, to be used only for the activities of the centre.

16 Micropropagation can be applied in the pre-multiplication and multiplication steps. This is subject to the specific technical regulations detailed in Annex (g) and (h).

17 The present regulation, after its publication in the Official Journal, should be included in the Official Collection of laws of the country.

Selected References


Di Terlizzi, B., Audergon, J. M., Caglayan Yildizgordu, K., Djerbi, M., Gavriel, I., Khouri, W., Myrta, A., Pallas, V., Shriri, M., Varveri,
M. Digiaro, A.M. D’Onghia, A. Myrta, V. Savino and G. P. Martelli,


Guidelines for a common certification scheme in the Mediterranean

ANNEX (a)

National catalogue of varieties

<table>
<thead>
<tr>
<th>N°</th>
<th>Cultivar</th>
<th>Primary Source</th>
<th>Breeder</th>
<th>Official Recognition</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pomoloical Sanitary</td>
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</tbody>
</table>
ANNEX (b)

Technical features of the facilities for the conservation of prebasic material

Prebasic material is to be kept in conditions of absolute isolation to avoid possible contamination.

The material will be grown in pots of adequate diameter containing sterile soil and kept in facilities (screenhouse) meeting the following requirements:

- The floor has to guarantee the isolation of the pots from the soil. Should the pots be buried, the alleys will be covered with gravel or any other inert material securing an adequate drainage.

- The roof has to be rigid, and a double net has to be used on the sides to prevent insects from entering inside.

- The whole construction should be isolated from surface water and from the surrounding environment.
GUIDELINES FOR A COMMON CERTIFICATION SCHEME IN THE MEDITERRANEAN

ANNEX (c)

DOCUMENTS needed to enter in the certification service

- authenticated copy of the patent (for protected varieties) with the indication of the farm patent owner authorizing its multiplication;

- data sheet for the cultivar characterization;

- documents relating to the sanitary status for the diseases reported in the protocols of the single species;

- for unprotected cultivars, a declaration stating that the cultivar is free.

The applicant is responsible for the non conformity with the declaration.
ANNEX (d)

Application to Obtain the Genetic and Sanitary Certification of Vegetatively-Propagated Plants

The undersigned nursery located at... located at.........................
Tel..............authorization n°......................... of.................

REQUESTS

the certification of the plants of the species.......................... for the following varieties ...............................................................

DECLARES

that Mr........................................ is responsible for the nursery domiciled in........................... phone........................

COMMITS ITSELF

to comply with the provisions in force;

ENCLOSES

a) Documents relating to the source of the material with indications on the number of plants and suitability of the soil as laid down in the protocols for the species.
b) Detailed plan of the nursery identifying plants of specific cultivars.

c) Receipt for the payment of ...................................................

made out to........................................for each hectare or fraction of nursery for which the certification is claimed.

ANNEX (e)

Application to Obtain the Recognition of the Centre for Multiplication

The undersigned Nursery located in...........................................................

phone....................................................authorization n°........................ of............................

CLAIMS (requests)

the recognition of the MOTHER BLOCK for the species

DECLARAS

that Mr.....is responsible for the mother block domiciled in..........................................................

........phone........................................
M. Digiaro, A.M. D’Onghia, A. Myrta, V. Savino and G. P. Martelli

COMMENTS ITSELF

to comply with the provisions in force

ENCLOSES

a) Documents relating to the source of the material with indications on the number of plants and suitability of the soil as laid down in the protocols for the species.

b) Detailed plan of the plots identifying plants of specific cultivars.

c) Receipt for the payment of ........made out to..................................................
Guidelines for a common certification scheme in the Mediterranean

ANNEX (f)

Types of Labels

1- Self-sticking label for packaged plantlets

<table>
<thead>
<tr>
<th>MINISTRY OF AGRICULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>Cv</td>
</tr>
<tr>
<td>Sanitary status</td>
</tr>
</tbody>
</table>

2 - Sealed labels for packaged plants or parts of plants or seeds

<table>
<thead>
<tr>
<th>PART OF PLANTS OR SEEDS</th>
<th>MINISTRY OF AGRICULTURE</th>
<th>PART OF PLANTS OR SEEDS</th>
<th>SEAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.</td>
<td>CV CATEGORY SANITARY STATUS</td>
<td>N.</td>
<td></td>
</tr>
</tbody>
</table>

The package shall be sealed and the label should be applied to plant in the package or a part of it (i.e. cuttings, rootlings, seeds).

3 - Sealed label for single plants

<table>
<thead>
<tr>
<th>MINISTRY OF AGRICULTURE</th>
<th>PLANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.</td>
<td>CV</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
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</tbody>
</table>

188

N. CV CATEGORY SANITARY SEAL STATUS N.
Guidelines for a common certification scheme in the Mediterranean

ANNEX (g)

General Rules

1. Explants for micropropagation (*in vitro* propagation from axillary buds), not smaller than 1 mm, shall be taken only from plants grown in the foundation block.

2. The micropropagation of chimeric clones (for example, apple spurs) is not permitted in that micropropagated plants might not correspond to the original phenotype.

3. In the procedure of propagation and rooting, the laboratories shall apply the following precautions:

   - discard any culture showing proliferation of undifferentiated tissue (callus);
   - before transfer to new medium, remove the basal part of shoots where the proliferation of undifferentiated tissue is more frequent;
   - use only shoots originated from axillary buds;
   - discard vitreous cultures and/or cultures with other morpho-physiological anomalies (fasciations).

The culture jars of the material in premultiplication or multiplication shall be kept in a well-determined site of the laboratory and individually labelled so as to be readily identified through progressively numbered tags. At the time of transplantation, the date, the progressive number of subculture of premultiplication (from 1 to 5 or from 1 to 3) or of multiplication (from 1 to 12) and the cultural step will be reported: proliferation, elongation or rooting.

The transplanting operations are to be recorded daily in the stock-book with progressively numbered pages, authenticated by the Ministry of Agriculture or by Institutions delegated by the Ministry, that shall be constantly kept in the laboratory for any
control. In the said register the containers eliminated because of contamination and/or morpho-physiological anomalies of cultures as well as the containers transferred to the cold chamber, shall be recorded.

The register may contain pen-made erasures which shall allow to read what written before.
ANNEX (h)

In Vitro Premultiplication Laboratories for the Production of Mother Plants

- In vitro premultiplication is carried out by the laboratories of research institutes of the Ministry of Agriculture qualified for the species subject to multiplication or by other scientific institutions. If the number of microseedlings to be produced through in vitro culture exceeds the potentialities of the outfit, the Ministry of Agriculture may confer the production to a private interprofessional organization under the control of the qualified research institute.

- For the preparation of in vitro cultures to be delivered to commercial laboratories, premultiplication laboratories perform no more than five subcultures when required temporary cold storage is allowed. In addition a subculture for elongation and one for rooting are allowed in the production of microseedlings of rootstocks for multiplication centres.

- The shoots of the 4th subculture may be used for the production of single bud microcuttings just once.

IN VITRO PROPAGATION LABORATORIES

1. Commercial laboratories shall, within October 30 of every year, communicate by letter to the Institute responsible for premultiplication, the initial number of sterile shoots requested for each clone of woody fruit plant. Cultures in active multiplication will be delivered within the following August 31. For fruit trees and their rootstocks, it is possible to reach 12 subcultures (although alternating with just one period of cold storage between) for in vitro commercial propagation. At the end of the 12th subculture, the shoots will be transferred either at elonga-
tion or at rooting (during or at the end of this phase a period of cold storage is permitted).

2. Only during the first five subcultures, it is possible to apply the microcutting technique in order to purchase the plant material; this operation shall be recorded in the laboratory register indicating the number of initial pots and those obtained.

3. The commercial laboratories which request to in vitro multiply a certified clone will cover 100% of their production with material of this type within the third year from the beginning of this activity.

4. A specific certification will be always issued concerning the culture pots whenever the microplantlets are transferred to an acclimatising centre outside the laboratory.

5. Fruit trees and their rootstocks, in vitro produced by the authorized commercial laboratories, may be released as certified material to farmers either directly or after a growing cycle in an open-field nursery.
Guidelines for a common certification scheme in the Mediterranean

ANNEX (i)

Organization of a Certification Programme

<table>
<thead>
<tr>
<th>Steps</th>
<th>Category of Plant Propagation Material</th>
<th>Outfits</th>
<th>Requirements</th>
<th>Agencies</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanitary and clonal selection</td>
<td>Primary Sources</td>
<td>Scientific Institutions</td>
<td>Screen House</td>
<td>Scientific Institution</td>
<td>Trueness To-Type</td>
</tr>
<tr>
<td></td>
<td>Conservation Centre for Premultiplication</td>
<td>Screen House</td>
<td>Certifying Authority</td>
<td></td>
<td>Sanitary</td>
</tr>
<tr>
<td>Premultiplication</td>
<td>Basic</td>
<td>Premultiplication Centre</td>
<td>Screen House Or Open Field (Technical Protocol)</td>
<td>Public Institutions</td>
<td>Trueness To-Type</td>
</tr>
<tr>
<td>Multiplcation</td>
<td>Certified</td>
<td>Mother Block</td>
<td>Open Field (Technical Protocol)</td>
<td>Nurseryman Association And /Or Individual Nurseries</td>
<td>Trueness To-Type</td>
</tr>
<tr>
<td>Propagation</td>
<td>Certifiable</td>
<td>Nursery</td>
<td>Open Field (Technical Protocol)</td>
<td>Private Nursery</td>
<td>Only Visual</td>
</tr>
</tbody>
</table>

Colour code:
- Pre-basic ➔ white with blue band
- Basic ➔ white
M. Digiaro, A.M. D’Onghia, A. Myrta, V. Savino and G. P. Martelli

- Certified blue