Updated regulation on certification of citrus propagating material in the Mediterranean region

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Updated regulation on Certification of citrus propagating material in the Mediterranean region

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Abstract. An updated regulation for the certification of citrus propagating material is presented, taking into account the EPPO standards, other European certification schemes and the current phytosanitary problems of the citrus industry in the Mediterranean region. The technical protocols for the production, conservation and use of healthy propagative material are based on the recent advances in pathogen detection and elimination in *Citrus* spp. and on the gaps in the running certification programmes. Most of the methods and procedures included in the protocols have been validated in the framework of the MNCC activity.

Keywords. Citrus – Certification – Nursery – Graft-transmissible pathogens – Trueness-to-type – Mediterranean.

Mise à jour de la réglementation sur la certification du matériel de multiplication des agrumes dans la région Méditerranéenne

Résumé. *Une mise à jour de la réglementation sur la certification du matériel de multiplication des agrumes est présentée, ceci en tenant compte des normes de l'OEPP, d'autres systèmes européens de certification ainsi que les aspects phytosanitaires des agrumes dans la région du bassin Méditerranéen. Les protocoles techniques pour la production, la conservation et l'utilisation du matériel de multiplication sain est basé sur les progrès réalisés dans la détection et l'élimination des pathogènes sur agrumes ainsi que sur les lacunes des différents programmes de certification en cours. La plupart des méthodes et procédures figurant dans ce protocole ont été validés dans le cadre des l'activités du MNCC.*

Mots-clés. *Agrumes – Certification – Pépinière – Agents pathogènes transmissibles par greffage – Authenticité – Méditerranée.*

I – Introduction

Virus and virus-like diseases are severely affecting citrus species worldwide, inducing heavy losses in many citrus growing areas. The need for detecting them and the impossibility to apply any curative treatment, including the chemical control, has led many countries and international organisations to undertake and promote research and sanitary improvement programmes.

This situation is further worsened by cloning processes and intensive trade of plant propagating material due to globalization, thus extending and internationalizing phytovirological problems that would be otherwise confined to limited areas.

As regards the Mediterranean region, citrus are infected by numerous graft-transmissible diseases, among which same, like *Spiroplasma citri*, are also spread by insect-vectors. There are no tolerant rootstocks to control the severe strains of CTV and the use of preimmunized plants is the only solution which can be envisaged when these virus strains become established. Even in this case, preimmunization can only be applied on 'healthy' nursery plants to avoid possible synergy with other CTV strains as well other virus and virus-like agents. Fortunately, 'huanglongbing' (induced by *Ca.* liberibacter spp.), which is devastating the most important citrus industries worldwide (e.g. Brazil and Florida, USA), is not present yet with its vectors in

the Mediterranean region. Preventing the entrance and dissemination of these diseases and their vectors through the use of non infected material for the establishment of citrus groves is economically and environmentally more efficient than eliminating pathogen outbreaks from which the infection can further be propagated by the vectors. Therefore the adoption of preventive rather than curative measures to control citrus graft-transmissible diseases, involving the use of 'healthy' propagating material, seems nowadays the strategy that can ensure the best results. This is particularly true if you consider that man is the main responsible in the dissemination of these diseases and, sometimes, even the only vector.

With this end in view, since 1995 CIHEAM/MAI-Bari has promoted the establishment of a dedicated Mediterranean Research Network on Certification of Citrus (MNCC), to bring together experts, Plant Protection Service officers and representatives of the private sector and make a positive contribution to the setting up of a scheme for the certification of citrus propagating material and for the monitoring of quarantine citrus pathogens. The network strategy was in line with the aims of Barcelona Declaration (1995) for the establishment in the Mediterranean region of a free trade area of agricultural products in the framework of a comprehensive Euro-Mediterranean partnership.

Citrus propagating materials "of equal category" are obtained in the various Mediterranean countries by different selection procedures and certification systems. Therefore, the harmonization of procedures, technical protocols and regulations to be applied in the Mediterranean Region has become the first priority of the network. Based on EPPO protocols and on the experience gained by several European and Mediterranean countries, during the network activity, a Mediterranean citrus certification scheme has been developed; moreover, an inventory on citrus genetic resources in the Region has also been made, aimed at providing useful information on native citrus germplasm of interest for the implementation of certification programmes at national level.

With the support of the CIHEAM/Master of Science researches, MNCC partners could investigate in each country the presence of citrus diseases with a high epidemiologic potential, such as Tristeza, transmitted by insect vectors, in order to pave the way to a national certification programme. However, certification measures must be supported by the implementation of a mandatory programme for the control of quarantine pathogens.

Based on the results obtained by the network on the pathological status of citrus in the Mediterranean, CIHEAM MAI-Bari has contributed, in close collaboration with the Mediterranean Ministries of Agriculture and research institutions, supported by cooperation aid across Europe (primarily the Italian), to the advance of certification of citrus propagating material. This programme was firstly implemented in Italy, and later extended to other Mediterranean countries (Albania, Algeria, Egypt, Lebanon, Malta, Tunisia).

II – Requirements for establishing a certification programme

CERTIFICATION of propagating material is defined as follows: *a procedure whereby candidate trees, to be used as source of material for propagation, undergo controls (trueness-to-type and sanitary) and, whenever necessary, sanitation treatments to secure varietal conformity, clonal origin of the material, a given sanitary status (freedom from a number of pathogens) as specified by regulations officially issued, or endorsed, by competent Governmental Agencies.* Based on this definition, the first step in the implementation of a certification programme in a country is drafting a dedicated law to regulate production, conservation and utilization of citrus Certified plant material.

To this end, it is necessary to identify the existing national laws on quarantine and marketing of citrus propagating material, that are to be complied with and harmonized. As a result, the peculiarity of each country system and the compliance with the international standards, with

special reference to the Mediterranean region, shall be taken into account so as to provide absolute guarantees on the quality and homogeneity of the final product and the relative label.

Hence, the regulation shall provide for the establishment of the certifying authority, responsible for inspections and controls, and illustrate the steps from the acquisition of primary sources to the propagation of citrus plants in the nursery. Moreover, the responsible institution, the trueness-to-type and phytosanitary protocols, the facilities and controls shall be specified for each phase. In this programme all the experts involved (pomological and genetic, phytosanitary, propagation competences etc.) shall work in close cooperation. Considering the costs of clone breeding (sanitary and clonal selection, diagnosis, conservation, sanitation) and those pertaining to the subsequent steps of certification (maintenance, genetic and sanitary controls included in the protocols, etc...), the material to be used should be selected from the most widespread, long-cultivated and locally relevant varieties.

Label harmonization is fundamental for the recognition of the material throughout the whole region in order to meet Barcelona requirements for the free movement of plant materials in the Mediterranean. A good certification scheme involves the use of a clear, detailed and reliable label, reporting several identification data (producer, lot number, Certifying Body, etc...) and indicating the category '*Pathogen-tested*' or '*Pathogen-free*'. Each plant category includes a different number of pathogens from which citrus plants must be free; the list of the pathogens is clearly indicated in the regulation and must be officially harmonized across the region.

Confusion should be avoided over the «Certified» category (bleu label) and the «*Conformitas Agraria Comunitatis-CAC*» category (orange label). CAC material has the minimum requirements (clonal origin, trueness-to-type, sanitary status), which guarantees the free movement of citrus plants in EU; it is a mandatory programme, under the nursery responsibility; however CAC material is totally replacing the 'standard' material; unfortunately, controls are only visually based whereas most of citrus graft-transmissible agents can remain symptomless. As regards '*Pathogen-tested*' and '*Pathogen-free*' plant material in the framework of the certification programme, the freedom from the listed pathogens and the trueness-to-type are ascertained through objective assays, which are carried out in each step from the parent 'primary source' until the nursery plants. Moreover this programme, which is voluntary, is under the responsibility of the Certifying Body in charge for controls.

In the light of these considerations, a Mediterranean scheme for citrus certification seems to be very relevant. Moreover, since it is necessary to design a «self-propelling» and self-sustaining system, the development of an active nursery sector should be encouraged. Nurserymen are the most important players of the whole chain and the success of a certification scheme greatly depends on their support. They should be more aware of the challenges they must take up and receive training to innovate their operations in compliance with the new certification needs.

III – Conditions supporting certification legal rules

Conditions that can facilitate the adoption and implementation of legal rules on certification are the followings:

i. the identification of the Certifying Body, possibly within a national institution (Ministry of Agriculture, for example) responsible for the service;
ii. the appointment of a Scientific Technical Committee on Certification as the advisory board of the Certification, which shall include not only the representatives of the Certifying Body, but also the representatives of the professionals involved in the different certifications steps (citrus pathologists, pomologists and breeders, nurserymen and producers' representatives);
iii. the setting up of an autonomous Certification Service with its branch sections, responsible for the sanitary and genetic controls as laid down in the protocols;

iv. the creation of a National Citrus Variety Register, including all the selections admitted for certification; the enrolment in this register shall automatically lead to the transfer of the clones to the subsequent certification steps;
v. the definition of the different certification steps and types of material produced in accordance with the international criteria; the following steps should be envisaged: a) establishment of the primary sources; b) Conservation for Premultiplication (Prebasic material); c) Premultiplication (Basic material); d) Multiplication and Plant propagation (Certified material);
vi. the planting of increase blocks, recognized by the Certifying Body, to meet the specific needs of some citrus species/varieties and favour the rapid production of Basic and Certified material;
vii. the identification of the sites where the different steps shall be implemented, preferably are in areas where none of the citrus is grown or found free from vector-transmitted pathogens (i.e. CTV, *S. citri,*); therefore a Quarantine programme for the mandatory control of these pathogens is necessary in order to promptly identify and eliminate the infected sources;
viii. the establishment of the primary sources: the institutions and/or bodies which will be responsible for the breeding of clone candidates shall be selected based on their recognized competence;
ix. the Conservation for Premultiplication shall be organized and managed under the control of the Certifying Body;
x. the Premultiplication shall be organized and managed by public or public/private bodies, recognized by the Certifying Body.

IV – Regulation for the certification of citrus propagating material

This regulation is applied to the certification of plant propagating material belonging to the genera *Citrus, Poncirus, Fortunella*, as well as other genera of *Aurantioideae* and their hybrids.

The rules set forth the production of the primary source and for the organization of the certification programme (Table 1) is hereafter reported as general provisions and technical specifications.

Table 1. Organization of the Certification programme of citrus propagating material.

Steps	Plant Category	Label Color	Site	Facility	Controls
Establishment of primary sources	Primary source	Red	Scientific institutions	Screenhouse	varietal & sanitary
Conservation for premultiplication	Prebasic	White with purple band	CCP	Screenhouse	
Premultiplication	Basic	White	CP	Screenhouse	
Multiplication	Certified	Blue	SMP/RMP	Open field/ screenhouse	
			Nursery	Open field/ screenhouse	sanitary

CCP: conservation centre for premultiplication;
CP: centre for premultiplication:
SMP: scion mother plots
RMP: rootstock mother plots

1. General provisions

Art. 1 - ***Organisation of the National Certification Service (NCS)***

The regulation orders:

- the establishment of a National Service for Voluntary Certification of plant propagating material;
- the definition and implementation of Certification steps;
- the definition of categories of planting material under Certification;
- the approval of variety accessions, clones and selections which shall be subjected to certification.

Art. 2 - ***Establishment of a National Certification Service***

The NCS for citrus plant propagating material is established at the Ministry of Agriculture; it is responsible for quality at national level and coordinates technical, administrative and scientific activities relating to Certification of plant propagating material.

Art. 3 - ***Establishment of a National Register of certified variety accessions, clones and selections***

The Register of National variety accessions, clones and selections approved by the NCS is established at the Ministry of Agriculture. The variety accessions, clones and selections are included in the National Register by a special provision.

Art. 4 - ***Registration of planting material to be Certified***

For registration of accessions, the plant breeds shall (technical specifications A):

- Maintain the primary source under appropriate facilities in order to preserve the sanitary status declared.
- File a special application to NCS.
- Deliver to the Conservation Centre for Premultiplication (CCP) the propagating material descended from the first multiplication of the primary source (source material /material of origin).

Art. 5 - ***Certification steps***

Voluntary certification of citrus plant propagating material comprises the following steps:

- Conservation for premultiplication
- Premultiplication
- Multiplication and nursery production

- **Conservation for premultiplication** (technical specifications B)

  Goals
  - Conservation under insect-proof screenhousese of «Prebasic» mother plants, deriving from primary sources registered at national level.
  - Growing under insect-proof screenhouse of «Prebasic» propagating material (seeds, cuttings, scions, buds, rootstocks, grafted plants) for the production of grafted plants to be used in premultiplication step.

  Organisation
  - Conservation takes place at the Conservation Centre for Premultiplication (CCP), registered with the NCS.

– To be registered, CCP must be provided with appropriate facilities for conservation of planting materials in healthy conditions and be supervised by a technical manager with relevant experience.
– CCP activities are coordinated by the NCS at the national level.
– The number of CCPs and their location are defined according to the needs of conservation of «Prebasic» material.
– Maps showing the exact location of existing accessions must be kept at CCP together with a farm record-book endorsed by the NCS.

Requirements

«Prebasic» material must fulfil the health and genetic requirements set forth in the technical specifications (D).

Controls and tests

Phytosanitary and genetic trueness-to-type controls and tests set forth in the technical specifications (E, F, G, H) are carried out under the supervision of the NCS and the responsibility of the CCP. Tests can be run by public laboratories or private laboratories registered with the NCS.

Certification

Certification of «Prebasic» propagating material is applied after assessment of the success of grafting for grafted plants and of rooting for self-rooted plants.

- **Premultiplication** (technical specifications B)

Goals

– Conservation under an insect-proof screenhouse of «Basic» plants, deriving from direct propagation of «Prebasic» plants or directly from the primary source.
– Growing under an insect-proof screenhouse of «Basic» propagating material (seeds, cuttings, scions, buds, rootstocks, grafted plants) for the production of grafted plants to be used in multiplication step.

Organisation

– Premultiplication takes place at the Centre for Premultiplication (CP), registered with the NCS.
– To be registered, CP must be provided with appropriate facilities for conservation of «Basic» plants in healthy conditions; moreover, they shall be supervised by a technical manager with relevant experience.
– CP activities are coordinated by the NCS at the national level.
– The number of CP and their location are defined according to the needs of Premultiplication of «Basic» material.
– Maps showing the exact location of existing accessions must be kept at CP together with a farm record-book endorsed by the NCS.

Requirements

«Basic» material must fulfil the health and genetic requirements set forth in the technical specifications (D).

Controls and tests

Phytosanitary and genetic trueness-to-type controls and tests set forth in the technical specifications (E, F, G, H) are carried out under the supervision of the NCS and the

responsibility of the CP. Tests can be run by public laboratories or private laboratories registered with the NCS.

Certification

Certification of «Basic» propagating material is applied after assessment of the success of grafting for grafted plants and of rooting for self-rooted plants.

- **Multiplication** (technical specifications C)
  Scion Mother Plant (SMP) and Rootstock Mother Plant (RMP) blocks.

Goals

- Growing of SMP and RMP in compliance with the technical specifications.
- Growing of «certified» propagating material (seeds, cuttings, scions, buds), for the production of rootstocks angrafted plants in nursery.

Organisation

- Multiplication of SMP and RMP takes place at the Centre for Multiplication (CM), registered with the NCS.
- To be registered, CM must meet the requirements referred to in the technical specification; in particular, they shall:
  - ✓ be supervised by a technical manager with relevant experience who interacts with the Certifying Body;
  - ✓ be provided with appropriate plots and facilities for management and conservation of specific production;
  - ✓ be duly equipped with mechanical means for management, conservation and transportation exclusively intended for the activities of the establishment.
- CM may include several facilities (SMP and RMP blocks, nurseries); CM facilities and means necessary for management and production of «Certified» material must meet the requirements set forth in the technical specifications.
- CM activities are coordinated by the NCS at the national level.
- Maps showing the exact location of existing plants and multiplication facilities must be kept at CM together with a farm record-book endorsed and periodically checked by the NCS.
- The number and the location of SMP and RMP blocks is defined according to the needs of propagation of «Certified» material; their size shall guarantee the annual production of a sufficient number of cuttings, scions and seeds so as to fulfil market demand.

Requirements

«Certified» propagating material must fulfil the phytosanitary and genetic requirements set forth in the technical specifications (D).

Controls

Phytosanitary and genetic trueness-to-type controls and tests on «certified» material set forth in the technical specifications (E, F, G) are carried out under the supervision of the NCS and the responsibility of the CM; tests can be run by public laboratories or private laboratories registered with the NCS.

Certification

Certification of «certified» propagating material is applied after the assessments set forth in production specifications.

Nursery

Goal

Growing of «certified» rootstocks and grafted plants for commercial production.

Organisation

- Multiplication takes place in nurseries registered with the NSC; it can be carried out only by physical persons or legal entities authorised to produce nursery material and declaring that they use the propagating material registered with NCS, in accordance to the provisions laid down in the present regulation.
- Maps showing the exact location of existing plantings must be kept in the nurseries together with a farm record-book endorsed and periodically checked by the NCS.

Requirements

«Certified» propagating material must fulfil the health and genetic requirements set forth in the technical specifications (D).

Controls

Phytosanitary and genetic trueness-to-type controls and tests on «certified» propagating material set forth in the technical specifications (E, F, G, H) are carried out under the supervision of the NCS and the responsibility of the nurseryman. At the request of the NCS, tests can be run by public laboratories or private accredited laboratories, registered with the NCS.

Certification

Certification of «certified» propagating material is applied after assessment of the success of grafting for grafted plants, of rooting for self-rooted plants.

Art. 6 - *Categories of propagating material*

Propagating material is comprised in the following categories:

- **Primary source.** Material of origin selected or produced by the plant breeder and kept by the plant breeder or parties entitled.
- **Prebasic.** Material produced from plants obtained from first propagation of the «Primary source» and maintained in the CCP in the number of 2 mother plants minimum.
- **Basic.** Material produced from plants obtained from first propagation of «Prebasic» material (or from the primary source when «Prebasic» material is not available), and maintained at the CP in a variable mother plant number (2 minimum) depending on importance of the cultivar in question;
- **Certified**.
  - ✓ *Scion and Rootstock Mother Plants.* Material produced from plants obtained from first propagation of «Basic» material, and maintained at the CM in a variable mother plant number depending on the importance of the cultivar in question.
  - ✓ *Nursery Plants.* Material (self-rooted plants, grafted plants, rootstocks) obtained from the first propagation of seed and/or scion mother plants intended for commercial use.

Art. 7 - *Sanitary status of propagating material*

For the purposes of plant certification, two categories of sanitary status are defined (technical specification D):

– *Pathogen free* – (PF). Material free from viruses viroids, virus-like, phytoplasmas and other major systemic infectious, known for the species in question at the time of enforcement of the specific regulation on certification, as indicated in the technical specifications.
– *Pathogen tested* – (PT). Material free from virus, viroids, virus-like, phytoplasmas and other main infectious agents of major economic importance, listed in the technical specifications.

If plants are grafted with material with a different sanitary status, the final category will be the lower one.

Art. 8 - *Label*

Propagating material, produced in the meaning of the present regulation, comes with the label of a different colour according to the production step.

In particular, the label colours are as follows:
- ✓ red for the «Primary source»;
- ✓ white with a purple diagonal bar for «Prebasic» material;
- ✓ white for «Basic» material;
- ✓ blue for «Certified» material.

The label shall report:
- ✓ the reference regulation of the country;
- ✓ the Certification body;
- ✓ the plant category;
- ✓ the species name (botanical name included);
- ✓ the variety, clone;
- ✓ the rootstock;
- ✓ the production year;
- ✓ the producer's code;
- ✓ the lot number;
- ✓ the quantity;
- ✓ the sanitary status.

Art. 9 - *Sanitary and trueness-to-type checks*

The holder is responsible for the different categories of propagating material, «Primary source», «Prebasic», «Basic» and «Certified» material. The competent Authority exerts control on all plant categories in accordance with the technical specifications (E, F, G, H).

Art. 10 - *Fees/Costs*

For patented accessions, the plant breeder or parties entitled shall bear the costs of conservation and production of propagating material at CCP et CPC; the nurseryman shall bear the costs for non patented accessions.

Art. 11 - *Provisions for propagating material deriving from other Certification schemes*

Propagating material deriving from Certification schemes of other countries approved by the National Certification Programme are admitted to the National Certification.

Admission procedures shall meet the following requirements:

– plant category must be «Prebasic» or «Basic», with the sanitary status *Pathogen-free*;

– during plant health assessment, conservation shall be carried out under the insect-proof screenhouse, separately from national accessions;
– sanitary checks shall be run following the procedures for the «Primary source» *Pathogen-free*, in compliance with the technical specifications A.
– sanitary and trueness-to-type checks shall take place under the supervision of the NCS and the responsibility of CCP. Tests can be run by public or private laboratories, registered with the NCS.

2. Technical specifications

A. **Documents for the registration of the «Primary source»**

✓ Declaration stating the methods used for the production of the «primary source».

✓ Morpho-pomological data sheet with pictures and/or documents.

✓ Phytosanitary data sheet regarding the plant sanitary status with respect to the diseases and pests included in Table 1.

✓ Declaration indicating the place and methods for primary source maintenance under healthy conditions and the person responsible for maintenance.

✓ Declaration stating that the «primary source» is free from quarantine pests (no included in the certification list).

✓ For accessions of patented varieties, a copy of the patent documents (application and issue) and a list of beneficiaries. For accessions of unpatented varieties, a declaration stating this condition.

DATA SHEETS FOR THE REGISTRATION OF THE CITRUS PRIMARY SOURCE

Part I – Breeding information

Owner _____ **Breeding (year)**_____**executed by**_____

Original sources_____ **Sanitary selection** (period) _____ **executed by** _____

Pomological selection (period) _____**executed by**_____

Sanitation ☐ **YES** ☐ **NO** **date** _____

Sanitation method

Thermotherapy - *In vitro* shoot-tip grafting - *In vitro* somatic embryogenesis from stigma and style culture

Others _____

Part II – Morpho-pomological-genetic assays

Picture

– Morpho-pomological characterization

According to UPOV, CPVO (www.cpvo.europa.eu) or other international standards

Genus:___ **Species:**___**Cultivar:**___**Clone:**___**Genetic origin:**___**Plant characteristics:**___

External fruit characteristics - Internal fruit characteristics - Production characteristics

Behaviour towards the main physiological disorders and diseases (Facultative)

– Molecular characterization (if possible)

SSR - AFLP - RFLP - RAPD - Isoenzymes - Others

– Belonging to GMO **YES** **NO**

– Conservation of the Primary Source:

(Responsible body) (Location)

Part III – Sanitary assays

Causal agent / Disease	Acronym	Assays		
		Biological	Serological / Culture	Biomolecular
Viruses				
Tristeza *Citrus tristeza virus*	**CTV**	Mexican lime	ELISA DTBIA	RT-PCR
Leaf rugose *Citrus leaf rugose virus*	**CiLRV**	Mexican lime		
Infectious variegation *Citrus infectious variegation virus*	**CIVV**	Lemon *Eureka, Volkameriana* Etrog citron	ELISA	RT-PCR
Psorosis *Citrus psorosis virus*	**CPsV**	Sweet orange *Madam vinous, Navelina*	ELISA	RT-PCR
Satsuma dwarf *Citrus satsuma dwarf virus*	**SDV**	Lemon or Citron Dweet tangor		RT-PCR
Citrange tatterleaf *Citrus tatter leaf virus*	**CTLV**	Citrange Troyer		RT-PCR
Vein enations/woody galls *Citrus vein enation virus*	**CVEV**	Sour orange Mexican lime	*ELISA	
Leaf blotch *Citrus leaf blotch virus*	**CLBV**	Dweet tangor		RT- PCR
Viroids				
Exocortis *Citrus exocortis viroid*	**CEVd**	861-S1 Etrog citron		RT- PCR, sPAGE Hybridisation ***
Cachexia *Hop stunt viroid*	**HSVd**	*Parson's special* mandarin onto Rough lemon		RT-PCR, sPAGE, Hybridisation ***
Gummy bark** *Hop stunt viroid variant*	**HSVd**			
Other Citrus viroids	**CVds**	861-S1 Etrog citron		RT-PCR, sPAGE ***
Virus-like				
Concave gum Cristacortis Impietratura	**CG** **CCr** **Imp**	Sweet orange *Madam vinous*, *Navelina* Dweet tangor		
Citrus chlorotic disease	**CCD**	Mexican lime		
Phloem-limited prokaryots				
Huanglongbing *Candidatus* Liberibacter spp.	**HLB**	Sweet orange *Madam vinous*		PCR
Stubborn *Spiroplasma citri*	**St**	Sweet orange *Madam vinous*	Culturing	PCR

* Serologically correlated to *Barley yellow dwarf virus* (BYDV); ** Detection only by visual observations during the selection period;

*** Using inoculated Etrog citron

FUNGI	ISOLATION	BIOMOLECULAR TESTS
Foot rot *Phythophtora citrophthora*	x	PCR
Citrus root rot *Phythophtora nicotianae*	x	PCR
Mal secco *Phoma tracheiphila*	x	PCR

SANITARY STATUS: ☐ ***Pathogen-free* PF** ☐ ***Pathogen-tested* PT**

B. Means for growing and producing «Prebasic» and «Basic» material

✓ **Facilities**

The Conservation and Premultiplication steps shall be carried out in an insect-proof screenhouse in areas where citrus trees are not grown by law. If the certification facilities are already established in the citrus growing areas, these areas must be declared free from Quarantine pathogens (e.g. *Citrus tristeza virus* – CTV) and from other harmful organisms (e.g. *Spiroplasma citri,* the agent of the stubborn disease) by the Plant Protection Service.

✓ **Growing and production**

«Prebasic» and «Basic» material (scion, rootstock and grafted plant) must be obtained under the same conditions as here after specified:

- the screenhouse shall be separated by a surrounding zone at least 4m wide, kept free from any vegetation;
- the plant material shall be grown in brand new containers of appropriate volume;
- the soil or growing media shall be sterilised to remove soil pathogens;
- containers and flats for rooting and acclimatation and other sowing operations shall be kept on supports at least 20 cm high off the ground;
- flats for rooting, acclimatation and seedling beds shall preventively be disinfected with a 10% of sodium hypochlorite commercial bleach for at least 20/30 minutes;
- «Basic» mother plants shall not be maintained in pots for more than 20 years since their establishment in the insect-proof screenhouse unless otherwise provided by the NCS;
- plants with a different health status (PF and PT) may be grown under the same screenhouse provided that they are isolated by a double net;
- the planting layout shall be reported on a specific map constantly kept updated;
- records shall be kept of all operations in a special Farm Book at the CCP and the CP;
- implements shall be at all times disinfected with a 10% of sodium hypochlorite commercial bleach between cuttings;
- all diseased and doubtful plants or all plants showing any apparent abnormality shall be incinerated before the competent Authority;
- any delivery of «Prebasic» and «Basic» material shall be at all times recorded and immediately notified to the NCS.

✓ **Increase blocks**

The production of «Basic» material (scion) from increase blocks shall fulfil the afore-mentioned requirements and be organised as follows:

- accessions under multiplication shall be distinct in easily identifiable plots;
- in the plot, the rows shall be complete and distinct per plant accession (species, cultivar and clone); if different accessions are grown in the same row, they shall be separated by a double inter-space;
- in case of grafting failure and when appropriate, top-grafting shall be made by using material of the same accession;
- well-lignified cuttings can be annually collected from each plant in the increase block but no more than five times since the grafting or the establishment date;
- the planting layout shall be reported on a specific map.

C. Means for growing mother plants and production of «Certified» material

✓ Facilities

The Multiplication step shall be carried out preferably in areas where citrus trees are not grown or in areas declared free from Quarantine pathogens (e.g. *Citrus tristeza virus* – CTV) and from other harmful organisms (e.g. *S. citri*) by the Plant Protection Service. If these conditions are not fulfilled the same Service can require the use of the insect-proof screenhouse.

✓ Growing and production

Scion Mother Plant (SMP) and Rootstock Mother Plant (RMP) blocks

Certified SMP and RMP blocks shall be established in compliance with the certification schemes and shall meet the following conitions:

- the establishments producing citrus plants must be approved by the competent Authority by:
  – submitting an official form to the competent Authority;
  – including with the applications supporting documents that testify the acquisition of the «Basic» propagating material for the block establishment;
- SMP and RMP are compulsorily registered with the competent Authority; partial or total pulling out or production cessation shall be notified to the competent Authority not later than one month;
- SMP and RMP shall be established on soils on which none of the citrus trees have been grown for at least 5 years and treated by solarisation to reduce the number of *P. nicotianae* and *P. citrophthora* propagules to an acceptable level (less than 5 propagules/ gram of soil) and eliminate nematodes;
- SMP and RMP shall be separated by a surrounding zone at least 10m wide, kept free from any vegetation;
- SMP and RMP shall be isolated from surface water flow;
- in the plot, the rows shall be complete and distinct per plant accession; if different accessions are grown in the same row, they shall be separated by a double inter-space; each plot shall be identified by a sign indicating the species, the cultivated variety (clone), the rootstock, the category, the planting date, the lot number and the number of plants;
- from each SMP not more than 1500 scions, for a maximum of 6000 buds, can be annually collected;
- missing plants shall be replaced only with propagating material of the same clone and belonging to the same category under the control of the competent Authority;
- the blocks shall be kept under continuous surveillance to control pathogens, pests and weeds;
- implements shall be at all times disinfected with a 10% of sodium hypochlorite commercial bleach between cuttings;
- SMP shall not be kept for more than 20 years since their establishment; SMP shall not be kept for more than 30 years since their establishment;
- the planting layout shall be reported on a map constantly kept updated.

Increase block

The production of «Certified» material (scion) shall fulfil the afore-mentioned requirements and be organised as follows:

- the growing medium shall be found free from *Phytophthora nicotianae* and *P. citrophthora* and from citrus nematodes;
- the growing containers shall be isolated from the ground by:

i. a layer of fine gravel or any inert material providing for effective drainage, at least 10 cm high, when mulching films are used, and at least 5 cm high when a French drain is used;
ii. a layer of concrete or different material; in such a case the containers shall be placed on supports;

- the area intended for growing plants in pots shall be separated by a surrounding zone at least 4 m wide, constantly tilled or kept free from any vegetation;
- seedlings shall be grafted at not less than 40cm from the collar;
- in case of grafting failure and when appropriate, top-grafting shall be made by using material of the same accession;
- well-lignified propagating material can be collected from each plant of the increase block non more than five times from the date of grafting, the life cycle of the increase block should not exceed 3 years**.**

Nursery

The nursery production is carried out in the seedling, grafting and rootstock beds .

The «Certified» material shall be produced in accordance with the following rules:

- to be admitted to control, the establishments producing citrus plants must be approved by the competent Authority;
- the application for admission to control can be submitted only for a production of not less than 50.000 plants; at all times the nursery declaration shall be notified to the competent Authority, including:
  i. the name and address of the declarant;
  ii. the nursery location;
  iii. a detailed map of the nursery layout reporting the lots distribution and all useful indications for the inspection;
  iv. the number and origin of the plants produced;
  v. the category to which the plants produced are likely to belong;
- to produce certified plants only soil-less culture is allowed;
- containers and flats for rooting and acclimatation and other sowing operations the flats shall preventively be disinfected with a 10% of sodium hypochlorite commercial bleach for at least 20/30 minutes;
- nurseries shall comply with the phytosanitary rules set out for plants in pots in the increase blocks as regards soil and growing media, isolation, distance of pots from the French drain and implements disinfection;
- the blocks shall be kept under continuous surveillance to control pathogens, pests and weeds;
- seedlings of species susceptible to '*Mal secco disease'* shall be covered by a 50% shading net and not less than 50m apart from other lemon groves;
- seedlings which are to be moved to the grafting bed shall exhibit at least 4 to 6 fully developed leaves so as to distinguish natural hybrids from nucellar seedlings;
- the plants shall be subdivided in homogeneous lots (per species, cultivar, clone and rootstock), made up of a maximum of 4 rows, easily identifiable and reported on a map;
- the growing containers shall be placed at a distance of at least 20cm in the row and lots shall be spaced out by at least 50 cm;
- in case of grafting failure and when appropriate, top-grafting shall be made by using material of the same accession;
- the growing cycle of the plants which are be c ertified shall not exceed three years as from the date of establishment;
- all diseased and doubtful plants or all plants showing any apparent abnormality shall be removed.

✓ **Stock records keeping**

Every citrus plant producer shall at all times keep records of the amounts of plant material produced and sold, the selling date, the name of the consignee and place of destination of the plant material delivered. The account books shall be made available for inspection by the competent Authority.

✓ **Duration/validity of certification**

The certification of plant propagating material is valid for one year.

D) Sanitary conditions for *Pathogen-free* and *Pathogen-tested* «primary sources», «prebasic», «basic» and «certified» material

Causal agent	Acronym	Sanitary status	
		***Pathogen-free* (PF)**	***Pathogen-tested* (PT)**
Viruses			
Citrus tristeza virus	**CTV**	**X**	**X**
Citrus leaf rugose virus	**CiLRV**	**X**	**X**
Citrus infectious variegation virus	**CIVV**	**X**	**X**
Citrus psorosis virus	**CPsV**	**X**	**X**
Citurs satsuma dwarf virus	**SDV**	**X**	
Citrus tatter leaf virus	**CTLV**	**X**	
Citrus vein enation virus	**CVEV**	**X**	
Citrus leaf blotch virus	**CLBV**	**X**	
Viroids			
Citrus exocortis viroid	**CEVd**	**X**	**X**
Hop stunt viroid	**HSVd**	**X**	**X**
Other Citrus viroids	**CVds**	**X**	
Virus-like			
Concave gum	**CG**	**X**	**X**
Cristacortis	**CCr**	**X**	**X**
Impietratura	**Imp**	**X**	**X**
Citrus chlorotic disease	**CCD**	**X**	
Phloem-limited prokaryotes			
Spiroplasma citri	**St**	**X**	**X**
Candidatus liberibacter spp.	**HLB**	**X**	

E) Organization of controls

The «Primary source» and the «Prebasic» and «Basic» material are under the responsibility of the breeder. The 'Certified' material (scion mother plants, rootstock mother plants and nursery plants) are under the responsibility of the approved nurseryman. However the competent Authority exerts control over all plant categories (VI, VIII).

✓ **On-the-spot checks**

- **«Prebasic» and «Basic» material**

The competent Authority shall carry out periodic and regular visual inspections of this material. All plants displaying any apparent abnormality shall be submitted to laboratory testing to detect the cause and promptly adopt the relevant preventive measures. In addition, administrative checks shall be performed.

- **«Certified» mother plants**

Scion Mother Plants (SMP)

SMP shall periodically be inspected. A preliminary inspection is carried out prior to the establishment of the SMP block to check:

– the compliance with isolation conditions;
– the plant origin;
– the soil nematological and mycological analyses.

When SMP come into production, the block shall be inspected at least twice a year to assess:

– the plant sanitary status;
– the trueness-to-type;
– the production of scions.

Rootstock Mother Plants (RMP)

RMP shall periodically be inspected. A preliminary inspection is carried out to check:

– the compliance with isolation conditions;
– the plant origin;
– the soil nematological and mycological analyses.

When RMP come into production, the block shall be inspected at least once a year before harvesting to assess:

– the plant sanitary status;
– the trueness-to-type;
– the production of seeds.

- **«Certified» nursery plants**

The «Certified» nursery material shall be submitted to at least four inspections and more specifically, two inspections during the phase of rootstock growing and two inspections from grafting to removal of the grafted plants.

Rootstocks

They shall be submitted to:

– a preliminary inspection prior to the establishment of the seedling/cutting in order to check the origin of the seeds or cuttings;

– a second inspection, before trading (delivery/release/placement on the market) in order to estimate the production of rootstocks and check their sanitary status.

Grafted plants

After grafting an inspection shall be carried out in order to:

– check the rootstocks homogeneity;
– check the origin of scions;
– estimate the graft take.

One more inspection shall be carried out before trading in order to:

– check the plant sanitary and growing status;
– check the trueness-to-type;
– estimate the plants to be certified.

✓ **Laboratory testing**

All categories of propagating material shall be submitted to laboratory testing including:

a. Seeds quality control

Quality control shall include the germination rate, the species purity, the presence of insects and fungal and bacterial diseases.

b. Phytosanitary checks

The competent Authority shall randomly inspect «Prebasic», «Basic» and «Certified» material with regard to virus, viroid, virus-like agents, *S. citri*, and fungi (Table 1).

✓ **Inspection of lots**

The competent Authority can withdraw the certificate, the labels or the approval if plants do no comply with the present technical specifications.

When plants are ready for sale, the nurserymen shall inform the competent Authority within one month for the purpose of labeling.

a. Handling and packaging

– Seeds: seeds are placed in sealed packages, bearing two indelible labels, one on the outside and one on the insides;
– Rootstocks and grafted plants: they can be placed either in pots or in polyethylene bags; each plant shall bear an indelible label.

b. Stock records

Every nurseryman approved for the production and trade of citrus propagating material shall keep records of all information necessary for inspections carried out by the competent Authority, and more specifically the quantities produced and traded, the dates of sale, the name of the consignee and the destination of the plant material delivered.

✓ **Controls on trade and movement of plants**

Controls shall be carried out on plant material bearing a certification label to ascertain its origin, handling conditions, health status, freedom from pests and the presence of the certification label.

F) Phytosanitary checks

All material descended from the first multiplication of the «Primary source», or from «Prebasic» or «Basic» material supplied by any other approved certification schemes, shall be individually submitted to trueness-to-type and sanitary checks, according to the procedures reported in technical specifications A, when brought into the Conservation Centre for Premultiplication or into the other steps.

In every step all measures shall promptly be adopted to control pests and pathogens which could pose a threat to the plant material; all operations shall be recorded in a special book.

- ✓ **«Prebasic», «Basic» and «Certified» material**

  Two types of checks shall be carried out for viruses, viroids, virus-like agents, *S. citri* and fungi:

  – Visual inspections: every year on all plants, at the appropriate time, when symptoms are likely to be most visible for each single disease; more specifically, in spring for viruses and virus-like diseases, in summer for viroid and stubborn diseases (at colour break for stubborn disease).

  – Laboratory testing: according to the procedures indicated in tables 1 and 2 of the present annex.

- ✓ **Soil and growing media in all steps**

Fungi: *P. nicotianae* and *P. citrophthora*

Mycological analysis through isolation on selective media on samples collected according to the following methods:

growing media: a sample shall be collected every $5m^{3,}$ made up of 10 sub-samples, for a total volume of at least 1 litre;

soil: before planting and, at any time, before any deep tillage, 1 sample per hectare shall be collected, made up of 10 sub-samples, for a total volume of at least 1 litre.

Nematodes: *Pratylenchus vulnus* and *Tylenchulus semipenetrans*

Nematological analysis through isolation techniques on samples collected according to the following methods:

growing media: a sample shall be collected every $5m^{3,}$ made up of 5 sub-samples, for a total volume of at least 1 litre;

soil: before planting and, at any time, before any deep tillage, 1 sample per hectare shall be collected, made up of 5 sub-samples, for a total volume of at least 1 litre.

Table 1. Procedure for the assessment of *Pathogen-free* and *Pathogen-tested* sanitary status of «Prebasic» and «Basic» Scion Mother Plants and Rootstock Mother Plants.

Organism/ Disease	ASSAYS					
	Visual inspections		Biological		Laboratory	
	Period	Frequency	Recommended indicator	Plant /year %	Sampling type and sampling time	Plant /year %
Viruses						
CTV	At growth recovery	Annual	Mexican lime	15%	Leaf peduncles, stem bark, style and stigmas / all the year round except for the coldest and the warmest months	all plants
CiLRV **CIVV**	At growth recovery	Annual	Mexican lime Lemon (*Eureka, Volkameriana*) Etrog citron	10%	Leaves in spring	25%
CPsV	At growth recovery	Annual	Sweet orange *Madame vinous, Navelina*	10%	Mature leaves in spring	25%
SDV, CLBV	At growth recovery	Annual	Dweet tangor	10%	Leaves in spring	25%
CTLV	At growth recovery	Annual	Citrange Rusk, Troyer	10%	Leaves in spring	25%
CVEV	At growth recovery	Annual	Mexican lime	10%	Leaves in spring	25%
Virus-like						
CG, Cr, Imp	At growth recovery	Annual	Dweet tangor Sweet orange *Madame vinous, Navelina*	10%	Leaves in spring	25%
CCD	At growth recovery	Annual	Mexican lime	10%	Leaves in spring	25%
Spiroplasma						
Spiroplasma citri	Late summer	Annual		10%	Leaves in late summer Fruit columella	25%
Viroids						
CEVd **HSVd** **CVds**	Late summer	Annual	Etrog citron 861-S1*	25%	Leaves in late summer	25%

**Indicator plant only for CEVd and CVds; it is used for viroid replication*

Table 2. Procedure for the assessment of *Pathogen-free* and *Pathogen-tested* sanitary status of «Certified» Scion Mother Plants (SMP) and Rootstock Mother Plants (RMP).

Organism/ Disease	ASSAYS			
	Visual inspections		Laboratory	
	Period	Frequency	Sampling type and sampling time	Tested plant/year %
CTV	At growth recovery	Annual	Leaf peduncles, stem bark, style and stigmas/all the year round except for the coldest and the warmest months	100%
CiLRV CIVV SDV, CLVB CTLV	At growth recovery	Annual	Leaves in spring	10%
CPsV	At growth recovery	Annual	Mature leaves in spring	10%
Virus-like				
CG, Cr, Imp, CCD	At growth recovery	Annual		
Spiroplasma				
Spiroplasma citri	Late summer	Annual	Leaves collected in late summer Fruit columella	20%
Viroids				
CEVd HSVd CVds	Late summer	Annual	Leaves collected in late summer	10%

G) Prerequisites for propagating material

✓ **Plant technical characteristics**

Prerequisites	Plant
Root system	Diffuse/ fasciculate
Open wounds	None

✓ **Laboratory seed testing standards**

Prerequisites	Prebasic	Basic	Certified
Germination rate	95%	90%	85%
Species purity	100%	100%	98%
Presence of living insects/rots	None	None	None

H) Trueness-to-type checks

Part 1 – «Prebasic» and «Basic» material

The trueness-to-type certification for cultivars and rootstocks is issued by the competent Authority after observing one growing and production cycle so as to assess the conformity to the phenotype at appropriate time, when the phenological characteristics are likely to be best expressed.

Later, at ripening time, a visual inspection shall be carried out every year to asses the production characteristics.

Part 2 – «Certified» Mother Plants

The trueness-to-type certification for cultivars and rootstocks is issued by the competent Authority after observing one growing and production cycle, before the collection of «certified» material. Later, at ripening time, a visual inspection shall be carried out every year to assess the production characteristics.

Part 3 – Increase Blocks and nursers material

Visual inspections shall be carried out to assess the plant growing characteristics.