



The Minister of Agriculture, Food and Forestry Policies

Having regard to the Ministerial Decree of 14 April 1997 published in Supplement no. 112 to the *Official Journal* of the Italian Republic no. 126 of 2 June 1997, transposing the Commission Directives no. 93/48/EEC of 23 June 1993, no. 93/64/EEC of 5 July 1993 and no. 93/79/EEC of 21 September 1993, on the marketing of fruit plant propagating material and fruit plants intended for fruit production;

Having regard to the Ministerial Decree of 24 July 2003 published in the *Official Journal* of the Italian Republic, general series, no. 240 (*Gazzetta Ufficiale della Repubblica italiana*, serie generale, n. 240) of 15 October 2003 organising the national (Italian) service for voluntary certification of fruit plant propagating material;

Having regard to the Legislative Decree no. 214 of 19 August 2005 published in Supplement no. 169/L to the *Official Journal* of the Italian Republic no. 248 of 24 October 2005 on the implementation of Directive 2002/29/EC on protective measures against the introduction and spread into the Community of organisms harmful to plants or plant products;

Having regard to the Ministerial Decree 4 May 2006, published in the *Official Journal* of the Italian Republic, general series, no. 168 of 21 July 2006 releasing general provisions for the production of propagating material of fruit plants and shrubs as well as for agamically-propagated herbaceous species;

Having identified the opportunity of laying down special provisions for the production of certified plant propagating material of the Strawberry ;

Having regard to the proposal about the technical protocols for the production of certified propagating material of the Strawberry approved by the National (Italian) Certification Committee (Comitato nazionale per la certificazione) in the session held on 15 and 16 June 2006, in accordance with Article 3 of the Ministerial Decree of 24 July 2003;

Having received the favourable opinion of the Phytosanitary Committee referred to in Article 52 of the Legislative Decree no. 214 of 19 August 2005, in accordance with Article 11 of the Ministerial Decree of 4 May 2006, at the meeting held on 18 July 2006;

Orders:

Article 1

Subject

1. The rules set forth in this Decree apply to certification of propagating material for the Strawberry species (*Fragaria* spp.) and its hybrids.



The Minister of Agriculture, Food and Forestry Policies

2. For the purposes of this decree, the Ministerial Decree of 4 May 2006, mentioned in the premises, will be hereinafter referred to as the "decree".

Article 2

Registration of Primary Sources

1. For the registration of Primary sources with the National (Italian) Certification Service, the plant breeder must fulfil the obligations set forth in Article 13 of the Ministerial Decree of 24 July 2003 and Article 2 of the "decree". The pomological data sheet and the phytosanitary data sheet must be prepared according to the patterns in Annex 1 of this decree.
2. For the registration of new cultivars, the pomological data sheet must comply with that provided for in UPOV or CPVO protocols.
3. New selections are allowed in the Conservation and Pre-multiplication steps, provided that they comply with the phytosanitary characteristics required and that there exists a genetic description distinguishing them from existing varieties.

Article 3

Means and Facilities

1. Means and facilities necessary to *in vivo* conservation and production of "Pre-basic" propagating material referred to in Article 4 of the "decree", must meet the requirements listed in Annex 2 of this decree.
2. Means and facilities necessary to *in vivo* conservation and production of "Basic" propagating material referred to in Article 5 of the "decree", must meet the requirements listed in Annex 3 of this decree.
3. Means and facilities necessary to *in vivo* production of "Certified" propagating material referred to in Article 6 of the "decree", must meet the requirements listed in Annex 4 of this decree.
4. Means, facilities and modes necessary to *in vitro* production of "Pre-basic" and "Basic 1" (first pre-multiplication) propagating material referred to in Article 7 of the "decree", must meet the requirements listed in Annex 5 of this decree.

Article 4

Certification of Propagating Material

1. Pursuant to Article 11 of the Ministerial Decree of 24 July 2003, for the purposes of the issuance of certification of nursery productions according to Article 12 of the Ministerial Decree of 24 July 2003 and Article 8 of the "decree", "Pre-basic", "Basic" and "Certified" propagating material which is virus-free (VF) must be free from the diseases and pathogens listed in Annex 6 of this decree.



The Minister of Agriculture, Food and Forestry Policies

Article 5

Tests and Controls

1. “Pre-basic, “Basic” and “Certified” propagating material must be subjected to phytosanitary controls and tests and genetic trueness-to-type checks as referred to in Article 5.2, (b) of the Ministerial Decree of 24 July 2003, and in Articles 4.3, 5.3 and 6.4 of the “decree”, as provided for in Annexes 7 and 8 of this decree.

Article 6

Labelling of propagating material

1. Without prejudice to what provided for in Article 9 of the “decree”, the label must comply with the characteristics listed in Annex 9 of the present decree.

Article 7

Provisional Regulations

1. Until 31 December 2011, propagating material belonging to the Strawberry species (*Fragaria* spp.) and its hybrids, even if not compliant with this decree, provided that it derives from primary sources included in the National or Regional Certification programmes and already existing at the time of entry into force of this decree, is admitted to national (Italian) certification.

This decree is sent to the Supervisory body for registration and will enter into force the day after its publication in the *Official Journal* of the Italian Republic.

Rome, 20 November 2006

The Minister: De Castro

DATA SHEETS FOR THE REGISTRATION OF THE STRAWBERRY PRIMARY SOURCE

PART I –POMOLOGICAL DATA SHEET

Country/Region **Province/Town** **Holding/Establishment**
Species: **Cultivar/Variety** **Clone:(Trade Mark,Registered Mark,Patent) Accession**

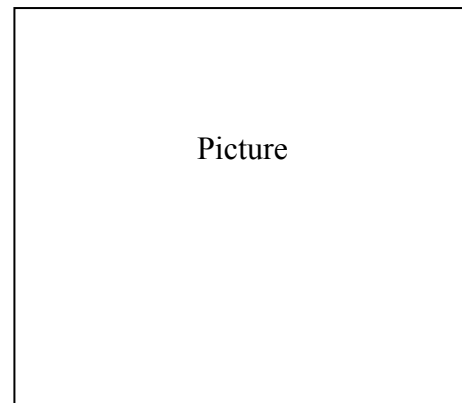
Origin of the primary source:

Cross: **Year** **made by**

Parent ♀ _____ **X** ♂ _____

Free pollination

Mutant or clonal selection: **Year:** **Identified by:**
At; **in the cultivar**



Conservation of the Primary source:

(Responsible Body)

(Location)

Belonging to GMO **YES** **NO**

Origin: _____
(in accordance with Art. 2(2) of directive 2001/18/EC of 12/03/2001

Pomological characterisation

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

Molecular characterisation:

Year _____ Laboratory _____

Molecular Markers	Number of combinations per Primer or enzyme systems	Bibliographic reference
<input type="checkbox"/> SSR		
<input type="checkbox"/> AFLP		
<input type="checkbox"/> Isozymes:		
<input type="checkbox"/> Other		

Tick if compliant

Sanitation:	Yes	No	Year/s_____
Sanitation technique used:			
Meristem tip <i>in vitro</i> culture (Establishment/Holding)		Thermotherapy	Other
Date			
			The Manager

Part II – Testing protocols for plant health assessment

Causal agent /Disease	Acronym	Biological assays (indicators)		Serological tests ELISA		Biomecular tests	
		+	-	+	-	+	-
		Test result					
VIRUSES							
<i>(Strawberry mild yellow edge virus)</i>	SMYEV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	IC-RT-PCR RT-PCR
<i>(Arabis mosaic virus)</i>	ArMV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	IC-RT-PCR RT-PCR
<i>(Tomato black ring virus)</i>	TBRV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR IC-RT-PCR
<i>(Tomato ring spot virus)</i>	TRSV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR IC-RT-PCR
<i>(Raspberry ring spot virus)</i>	RRSV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR IC-RT-PCR
<i>(Strawberry latent ring spot virus)</i>	SLRV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR IC-RT-PCR
<i>(Strawberry mottle virus)</i>	SMV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR
<i>(Strawberry vein banding virus)</i>	SVBV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR
<i>(Strawberry crinkle virus)</i>	SCV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR
<i>(Tobacco necrosis virus)</i>	TNV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>		
<i>(Tobacco streak virus)</i>	TSV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR IC-RT-PCR
<i>(Fragaria chiloensis latent virus)</i>	FCILV	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR IC-RT-PCR
<i>(Strawberry pallidosis associated virus)</i>	SpaV	<input type="checkbox"/>	UC10 - UC11	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR
<i>(Beet pseudo yellows virus)</i>	BPYV	<input type="checkbox"/>	UC10 - UC11	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	RT-PCR
PHYTOPLASMAS							
Strawberry lethal decline (Stolbur) (XII*)	SLD					<input type="checkbox"/>	PCR
Aster yellow (I*)	AY					<input type="checkbox"/>	PCR
Multiplier disease (IV*)	MD					<input type="checkbox"/>	PCR
Strawberry green petal (I*)	SGP					<input type="checkbox"/>	PCR
Strawberry marginal chlorosis (Stolbur) (XII*)	SMC					<input type="checkbox"/>	PCR
Mexican periwinkle virescence (XIII*)	MPV					<input type="checkbox"/>	PCR
Strawberry witches' broom(I*)	WB					<input type="checkbox"/>	PCR

*Classification based on the gene coding for 16S ribosomal RNA

(Part II to be continued)

(Part II continued)

Causal agent/Disease	Acronym	Microbiological assays		Serological tests		Biomolecular tests	
		+	-	+	-	+	-
		Test result					
VIRUS-LIKE AGENTS							
Strawberry chlorotic fleck	SCF	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>			
Strawberry leaf roll	SLR	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>			
Strawberry feather leaf	SFL	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>			
Strawberry vein yellowing	SVY	<input type="checkbox"/>	UC4 - UC5	<input type="checkbox"/>			
BACTERIA							
Angular leaf spot <i>Xanthomonas fragariae</i>	X.f.	<input type="checkbox"/>	direct isolation	<input type="checkbox"/>	<input type="checkbox"/>	IFAS	<input type="checkbox"/>
					<input type="checkbox"/>	ELISA	<input type="checkbox"/>
Bacterial leaf blight <i>Xanthomonas arboricola pv fragariae</i>	X.a.	<input type="checkbox"/>	direct isolation	<input type="checkbox"/>	<input type="checkbox"/>	IFAS	<input type="checkbox"/>
FUNGI							
Antrachnose <i>Colletotrichum acutatum</i>	C.a.	<input type="checkbox"/>	direct isolation	<input type="checkbox"/>	<input type="checkbox"/>	ELISA	<input type="checkbox"/>
NEMATODES							
		Microscopic assays					
<i>Meloidogyne hapla</i>		<input type="checkbox"/>	Morpho-anatomic	<input type="checkbox"/>	identification		
<i>Pratylenchus vulnus</i>		<input type="checkbox"/>	Morpho-anatomic	<input type="checkbox"/>	identification		
<i>Aphelencooides ritzemabosi</i>		<input type="checkbox"/>	Morpho-anatomic	<input type="checkbox"/>	identification		
<i>Aphelencooides fragariae</i>		<input type="checkbox"/>	Morpho-anatomic	<input type="checkbox"/>	identification		

HEALTH STATUS: **Virus-free VF** **Virus-tested VT**

Date

The Laboratory Manager

TECHNICAL CHARACTERISTICS OF MEANS AND FACILITIES FOR *IN VIVO* PRODUCTION OF PRE-BASIC” MATERIAL

Facilities

The Conservation for Pre-multiplication (CCP) step shall be carried out in an insect-proof screenhouse, established in an area at least 100m apart from any strawberry plants.

The screenhouse size shall allow the proper development of plants proportioned to the container volume; moreover, the following requirements shall be fulfilled:

- a. isolation of growing containers from the ground or the flooring by:
 - i. a suitably designed French drain, covered with fine gravel or any inert material providing for effective drainage;
 - ii. a layer of concrete or different material. In such a case containers and benches for runner rooting shall be kept on supports at least 20cm high off the ground;
- b. a French drain, all around the screenhouse, at least 80cm wide and at least 20cm deeper than the inside flooring;
- c. isolation from surface water flow through a kerb or a similar isolating structure;
- d. hard roof and walls with a double 20/10 mesh (20 wires/cm warp and 10 wires/cm weft) net and entrance with double net walls and double door;
- e. appropriate equipment for tool disinfection and disposable clothing for persons who have access to the conservation premises.

Growing

- a. The “Pre-basic” plants shall be grown individually in containers of appropriate volume;
- b. when mother plants are brought in, they shall be numbered on the spot in a progressive order and in an indelible fashion;
- c. the soil or growing medium shall be found free from the nematodes *Longidorus elongatus*, *L. macrosoma*, *Xiphinema diversicaudatum*, *Meloidogyne hapla*, *Pratylenchus vulnus*, *Aphelenchoides ritzemabosi*, *A. fragariae*, *Ditylenchus dipsaci*; freedom from the above shall be substantiated by an official document;
- d. mother plants and their descendants belonging to different accessions shall be kept separated by isolating means/facilities to maintain their genetic identity and prevent contamination;
- e. “Pre-basic” plants shall be obtained through direct propagation of the primary source by runner multiplication or micro-propagation.

Production

- a. “Pre-basic” material shall be propagated under a screenhouse in the same conditions as above;
- b. Any delivery of material by the Centre shall be at all time recorded and immediately notified (by fax and/or email) to the locally competent Regional Phytosanitary Service.

TECHNICAL CHARACTERISTICS OF MEANS AND FACILITIES FOR *IN VIVO* PRODUCTION OF “BASIC” MATERIAL

“Basic” material is produced in two steps, according to the procedure specified in Part I and Part II of the present annex.

Part I – First Pre-multiplication (PC1)

Facilities

The first Pre-multiplication (PC1) step shall be carried out in an insect-proof screenhouse which fulfils the following requirements:

- a. roof and walls with a 20/10 mesh (20 wires/cm warp and 10 wires/cm weft) net and entrance with a double door;
- b. appropriate isolation from surface water flow and the surrounding environment; moreover, protection shall be provided from rain water in winter-autumn;
- c. appropriate equipment for tool disinfection and disposable clothing for persons who have access to the conservation premises;
- d. appropriate isolation the of growing containers from the ground or the flooring by the pavement;
- e. establishment in areas at least 100m apart from any strawberry cultivation.

Production

- a. Any delivery of material by the Centre shall be at all time recorded and immediately notified (by fax and/or email) to the locally competent Regional Phytosanitary Service.
- b. The removal of “Basic1” material and the elimination of mother plants shall be preventively notified to the locally competent Regional Phytosanitary Service.

Part II –Second Pre-multiplication (PC2)

Facilities

The second Pre-multiplication (PC2) step can be carried out in plots outdoors and the following requirements shall be met:

- a. they shall be established on soils where none of the strawberry plants have been grown for 5 years;
- b. they shall be established on soils which respond to the normal agronomic and health requirements and found free from the nematodes *Longidorus elongatus*, *L. macrosoma*, *Xiphinema diversicaudatum*, *Meloidogyne hapla*, *Pratylenchus vulnus*, *Aphelenchoides ritzemabosi*, *A. fragariae*, *Ditylenchus dipsaci*; freedom from the above shall be substantiated by an official document; moreover, soils shall be disinfected by:
 - i. methyl bromide fumigation at the rate of 50g/m² or 25g/m² if suitable plastic films are used for covering;
 - ii. nematicidal soil disinfectants, applied according to the label dosage;
- c. they shall be established in areas 500m apart from any strawberry planting; such a limit can be reduced to 250m if the plots are close to nurseries where only certified material is grown, unless more stringent instructions are provided by the locally competent Regional Phytosanitary Service.

Growing

- a. “Basic2” plants are obtained from the agamic propagation of “Basic1” material;

- b. “Basic” plants can descend directly from the Conservation for Pre-multiplication step;
- c. plants shall be subdivided into lots according to the mother plant of origin.

Production

- a. Any delivery of material by the Centre shall be at all time recorded and immediately notified (by fax and/or email) to the locally competent Regional Phytosanitary Service;
- b. the removal of “Basic2” material and the elimination of mother plants shall take place under the responsibility of the Pre-multiplication Centre manager and shall be preventively notified to the locally competent Regional Phytosanitary Service.

TECHNICAL CHARACTERISTICS OF MEANS AND FACILITIES FOR *IN VIVO*
PRODUCTION OF “CERTIFIED” MATERIAL

Part I - Plants in open field

The nursery propagation shall be carried out in open field, in plots which fulfil the following requirements:

- a. they shall be established on soils which respond to the normal agronomic and health requirements and found free from the nematodes *Longidorus elongatus*, *L. macrosoma*, *Xiphinema diversicaudatum*, *Meloidogyne hapla*, *Pratylenchus vulnus*, *Aphelenchoides ritzemabosi*, *A. fragariae*, *Ditylenchus dipsaci*; freedom from the above shall be substantiated by an official document; moreover, they shall be established on soils where none of the strawberry plants have been grown for at least 4 years; this time limit can be reduced to 2 years provided that appropriate disinfection is performed by:
 - i. methyl bromide fumigation at the rate of 50g/m² or 25g/m² if suitable plastic films are used for covering;
 - ii. nematicidal soil disinfectants, applied according to the label dosage;
- b. they shall be established in areas 250m apart from any strawberry cultivation;
- c. the plots shall be homogeneous, clearly identifiable and separated from any “CAC” nursery material by a surrounding zone, at least 5m wide; the locally competent Regional Phytosanitary Service can otherwise reduce this limit provided that protective barriers are present (e.g. ditches, furrows, canals, roads, headlands etc.);
- d. in the plot the rows shall be complete and distinct per plant variety; different varieties or clones can be grown in the same row provided that they are separated by a double inter-space not lower than 2m, kept free from any vegetation;
- e. rows of different varieties shall be separated by a double inter-space kept free from any vegetation.

Furthermore it is allowed to certify, for one single cycle, the daughter plants which need one more growing cycle (Waiting Bed) provided that plant growth takes place in the conditions specified for the multiplication step in the present protocol. In this case, the number of plants shall be notified to the Regional Phytosanitary Service when they are established.

Part II- Plants grown in containers

Plants grown in containers, descended from runners collected in certified nurseries, can be certified provided that the following requirements are met:

- a. growing containers shall be isolated from the ground by a layer of fine gravel, concrete or any inert material;
- b. the growing medium shall contain peat-moss or any inert material and shall be found free from the nematodes *Longidorus elongatus*, *L. macrosoma*, *Xiphinema diversicaudatum*, *Meloidogyne hapla*, *Pratylenchus vulnus*, *Aphelenchoides ritzemabosi*, *A. fragariae*, *Ditylenchus dipsaci*; freedom from the above shall be substantiated by an official document;
- c. the area intended for growing strawberry plants shall be separated by a surrounding zone 0.5m wide, kept free from weeds;
- d. plants shall be subdivided in homogeneous and easily identifiable lots;
- f. the plots intended for growing plants in containers shall be separated from plots where “CAC” nursery material is grown by a surrounding zone, at least 5m wide; the locally competent Regional Phytosanitary Service can otherwise reduce this limit provided that protective barriers are present (e.g. ditches, furrows, canals, roads, headlands etc.);

- e. plants grown in containers shall be separated from fruiting strawberry plantings by a distance of at least 100m;
- f. the soil shall be isolated from surface water flow.

MEANS FOR *IN VITRO* PRODUCTION OF STRAWBERRY “PREBASIC” AND “BASIC”
PROPAGATING MATERIAL

- a. Apart from Conservation Centres for Pre-multiplication (CCP) and Pre-multiplication Centres (PC), *in vitro* pre-multiplication can also be performed by one or several micro-propagation laboratories registered with the Regional Phytosanitary Service; to this end the Centre and the laboratory concerned shall subscribe to a specific convention and a specific request for each accession shall be sent to the Phytosanitary Service.
- b. Acclimatation of *in vitro* material can be carried out at Conservation Centres for Pre-multiplication (CCP) and Pre-multiplication Centres (PC) as well as in one or several acclimatation facilities registered with the Regional Phytosanitary Service; to this end, the Centre and the facility shall subscribe to a specific convention.
- c. “Pre-basic” and “Basic” material shall be kept separated from propagating material of any other category by means/facilities providing for phytosanitary isolation (greenhouses, insect-proof nets, etc).
- d. The acclimatation medium shall be free from any pathogens and to this end, tested peat-moss of known origin or chemically or physically sterilised media shall be used.
- e. The initial explants for micro-propagation (*in vitro* multiplication through virus-free plant runners apices, axillary buds and meristem tips) shall be collected only from plants maintained at the Conservation Centres for Pre-multiplication (CCP).
- f. The next step can include a period of *in vitro* establishment of the material for not more than 3 months, followed by 5 subcultures at most;
- g. the material under pre-multiplication shall be renewed within 2 years since the initial explant, regardless of the number of subcultures attained.

“VIRUS-FREE” HEALTH STATUS TABLE FOR “PRE-BASIC”,
“BASIC” AND “CERTIFIED” MATERIAL:
DISEASES AND HARMFUL ORGANISMS COVERED BY THE SCHEME.

Official/ scientific name	Acronym	Health Status
		Virus-free (VF)
VIRUSES		
<i>Strawberry mild yellow edge virus</i>	SMYEV	X
<i>Arabidopsis mosaic virus</i>	ArMV	X
<i>Tomato black ring virus</i>	TBRV	X
<i>Tomato ring spot virus</i>	TRSV	X
<i>Raspberry ring spot virus</i>	RRSV	X
<i>Strawberry latent ring spot virus</i>	SLRSV	X
<i>Strawberry mottle virus</i>	SMV	X
<i>Strawberry vein banding virus</i>	SVBV	X
<i>Strawberry crinale virus</i>	SCV	X
<i>Tabacco necrosis virus</i>	TNV	X
FUNGI		
<i>Colletotrichum acutatum</i>	C.a.	X
PHYTOPLASMAS		
<i>Strawberry lethal decline (Stolbur) (XII*)</i>	SLD	X
<i>Aster yellow (I*)</i>	AY	X
<i>Strawberry green petal (I*)</i>	SGP	X
<i>Strawberry marginal chlorosis (Stolbur) (XII*)</i>	SMC	X
<i>Strawberry witches' broom (I*)</i>	WB	X
BACTERIA		
<i>Xanthomonas fragariae</i>	X.f.	X
<i>Xanthomonas arboricola</i>	X.a.	X
NEMATODES		
<i>Meloidogyne hapla</i>		X
<i>Pratylenchus vulnus</i>		X
<i>Aphelenchoides fragariae</i>		X
<i>Aphelenchoides ritzemabosi</i>		X

PHYTOSANITARY CHECKS

Part I – On “Pre-basic” material

Viruses, phytoplasmas, fungi and bacteria

Two types of checks shall be carried out:

- a. Visual inspections: every year on all plants, at the appropriate time, when symptoms are likely to be most visible;
- b. Laboratory testing: all plants under conservation for pre-multiplication (CCP) shall be tested every year and when brought into the CCP according to the procedures indicated in tables 1 of the present annex.

Part II- On “Basic” material

Two types of checks shall be carried out:

- a. Visual inspections: every year on all plants, at the appropriate time, when symptoms are likely to be most visible;
- b. Laboratory testing: according to the procedures specified below and the procedures indicated in tables 1 of the present annex:
 - i. **Viruses and phytoplasmas**: plants under pre-multiplication shall be tested every year at the rate of 2% of mother plants for each single variety in CP1 step and of 0.2% of mother plants for each single variety in CP2 step;
 - ii. **Bacteria**: in CP1 all mother plants shall be tested every year by collecting a multiple sample from 5 plants at most; in CP2, 5 plants per lot shall be tested (as specified in Annex 3, point 27 of the M.D. of 4 May 2006) by collecting a multiple sample from 50 plants (10 lots) at most;
 - iii. **Fungi**: in CP1, 30% of mother plants shall be tested every year; in CP2, 5 plants per lot shall be tested (as specified in Annex 3, point 27 of the M.D. of 4 May 2006), by collecting a multiple sample from 50 plants (10 lots) at most.

Part III- On “Certified” material

Viruses, phytoplasmas, fungi and bacteria

Visual inspections: every year at least twice on all plants, at the appropriate time, when symptoms are likely to be most visible.

If symptoms ascribable to diseases or harmful organisms are observed on the material, laboratory tests shall be performed according to the procedure indicated in table 1 of the present annex.

Table 1: Procedure for the assessment of “Virus-free” health status of “Pre-basic”, “Basic” and “Certified” Mother Plants

Disease or harmful organism	CHECKS					
	Visual checks		Biological assays		Laboratory testing: serological or molecular	
	Frequency	Time	Recommended indicator	Sampling frequency, time and type	Frequency	Sampling time and type and Test
VIRUSES						
ArMV SMYEV TBRV TRSV RRSV SLRSV	Annual	From July to October	UC4-UC5	Annual - From July to October – Leaves	Annual	September-October – Young leaves - ELISA September – October – Young leaves RT-PCR, IC-RT-PCR
SMV SVBV SCV	Annual	From July to October	UC4-UC5	Annual - From July to October – Leaves	Annual	September – October – Young leaves RT-PCR, IC-RT-PCR
PHYTOPLASMAS						
SLD AY SGP SMC WB	Annual	From September to November		Annual – Leaves	Annual	Summer– Autumn Leaf petioles and veins– PCR
BACTERIA						
X.f. X.a	Annual	From September to November		Annual - Leaves and crowns	Annual	From September to December– Plants Direct isolation, IFAS, ELISA, PCR
FUNGI						
C.a.	Annual	From September to Novembre		Annual - Leaves, runners and crowns	Annual	From September to December– Plants Direct isolation, ELISA, PCR
NEMATODES						
<i>M. hapla</i> <i>P. vulnus</i> <i>A. fragariae</i> <i>A. ritzemabosi</i>	Annual	Growing period				

TRUENESS-TO-TYPE CHECKS

The trueness-to-type checks are based on pomological, phonological and agronomic observations with the support of molecular techniques.

Variety certification can be issued only after carrying out observations for a whole growing cycle and checking one fruit-setting on plants according to the procedure specified below:

Part I – Material under conservation (“Pre-basic”)

Visual inspections throughout the whole growing cycle, in particular at blooming.

Within the first decade of September at least two well-rooted daughter plants, produced on two runner-bearing chains shall be collected from each mother plant and they shall be individually labelled (variety, mother plant number, daughter n° 1-2).

These plants shall be immediately established outdoors and as a result, in the next Spring a sufficient amount of fruit shall be inspected to check the variety conformity.

When appropriate, checks can be intensified and shortened by growing plants in containers and moving them into a long-photoperiod (16hours/day) hot greenhouse in early January.

Part II – Material under pre-multiplication (PC1)

Visual inspections throughout the whole growing cycle, in particular at blooming.

Within the first decade of September at least two well-rooted daughter plants, produced on two runner-bearing chains shall be collected from each mother plant and they shall be individually labelled (variety, mother plant number, daughter n° 1-2).

These plants shall be immediately established outdoors and as a result, in the next Spring a sufficient amount of fruit shall be inspected to check the variety conformity.

Part III – Material under pre-multiplication (PC2)

Visual inspections repeated at least three times. Within the first decade of September at least “two” well-rooted daughter plants, produced on two runner-bearing chains shall be collected from 2% of mother plants and they shall be individually labelled (variety, mother plant number, daughter n° 1-2).

These plants shall be immediately established outdoors and as a result, in the next Spring a sufficient amount of fruit shall be inspected to check the variety conformity.

LABELLING

- Label size between 5cm x 10cm and 8cm x 16cm;
- Types per n° of plants (100 and higher amounts for multiples of 50) to place on each container.