Clonal and sanitary selection in stone fruits

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Clonal and sanitary selection in stone fruits

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SUMMARY - Clonal and sanitary selection in stone fruits was briefly discussed. The objectives of clonal and sanitary selection were separately described. The establishment of a multidisciplinary technical team composed of pomologists and plant pathologists, was considered as an essential condition for the implementation of the selection. The different phases of selection and the conditions of conservation for the selected clones were also discussed.

Key words: stone fruits, clonal selection, sanitary selection, plant viruses

RESUME - *On discute brièvement la sélection sanitaire et clonale chez les essences fruitières à noyaux. Les objectifs de la sélection clonale et sanitaire sonts décrits séparément. La création d'une équipe technique multidisciplinaire composée par des pomologistes et des pathologistes végétaux est considerée comme une condition essentiel pour la sélection. Les différentes phases de la sélection et les conditions de conservation sont aussi passées en revue.*

Mots-clés: *essences à noyaux, sélection clonale, sélection sanitaire, virus des végétaux*

I – Introduction

For centuries, the identification of the most productive plantings had been the major concern of farmers for the establishment of new orchards. By taking the propagating material from the most vigorous and productive plants, they have unconsciously carried out a sort of ameliorating selection, which was still yielding good results in the areas where agriculture had not yet attained industrial levels.

No doubt that the spread and the establishment of new cultural techniques have enabled considerable improvements in the productive and qualitative levels of the crops thus furthering the agricultural activity in different areas. However, uncontrolled trade and the industrial production of propagating material have favoured also the spread of phytopathologic problems at the international level.

Most of the problems encountered on fruit tree species were related to two major factors:

(i) biological characteristics:

- vegetative propagation, which increased the risk of disseminating infected stocks;
- multiplication by grafting and the combination of two major components (rootstock and cultivar), increased probability of infection;
- the size and the volume of the plant influence the appreciation of the overall sanitary evaluation and need for appropriate sampling procedures;
- the lignification of the tissues induced barriers to the diffusion of pathogens and delays symptom appearance;
- the perennial status increased the potential risk of infection over time.

(ii) economical or technical method of propagation and production:

- propagation was done in nurseries through a well-defined stepwise procedure that require adequate monitoring;
- propagation in the nurseries and areas of production were usually concentrated and co-localised, from which the risk associated with the diffusion of a pathogen within the nursery or within the area of production.

So even if varietal innovations are of great interest from the economic point of view in term of adjustment to the market requirements, these need to be kept under control to give some insurance to fruit growers in term of trueness-to-type and sanitary status.

Sanitary selection, which is the oldest prevention practice for virus diseases, was still valid if soundly applied and combined with clonal selection with which it contributes to varietal improvement.

II – Objectives of sanitary selection

Sanitary selection has the scope of choosing plants free from the most detrimental viruses, to conserve and multiply the plants with their clonal descent for the longest possible time, under conditions ensuring protection from virus contamination.

The sanitary selection is at the base of the preservation and the maintenance of the potential of production in a region. It is also an efficient tool capable of improving, in a short lapse of time, the productive levels of the new plantings.

Its efficiency is directly linked with:

- the control of the nurseries and their area of production in order to avoid spreading of a disease to a production area;
- the monitoring of the given area;
- the presence of new or unsuspected pathogens, and their vectors;
- the introduction of new pathogens due to insufficient control procedures during the introduction phase (for example a problem is actually rising with *Xanthomonas campestris* pv. pruni on *Prunus* species);
- the knowledge and the possibility of applying effective detection procedures and appropriate sampling methods;
- the presence and the effectiveness of a quarantine system.

In practice, the high sanitary degradation of some fruit species and/or varieties for which it may be difficult to detect even one single healthy individual in the field exists. A good example is the 100% infection rate of cherry cv. Montagnola detected during a sanitary study carried out in Apulian orchards (Di Terlizzi *et al.*, 1998).

If monitoring is made at the right time, visual inspections may allow to quickly diagnose and discard virus-infected plants, and, but it should be kept in mind that the method is not always satisfactory. Often indexing of selected accessions is required to reveal presence of disease agents that had escaped attention, either because of confusion with other abnormalities, or because the symptoms were exceedingly mild or latent (Di Terlizzi *et al.*, 1998). In fact, certain symptoms of foliar discoloration, mottling, leaf-rolling, and deformation can be caused by agents such as: other pathogens, pests, mineral deficiencies, herbicides, pesticides, etc. Furthermore, viruses may possess hypervirulent isolates that induce distinct and readily identifiable symptoms and hypovirulent isolates whose symptoms are little perceivable. On the other hand, symptom expression may vary with the year, the climatic conditions, and the developmental stage of the host (e.g. typical

manifestations of leafroll caused by phytoplasma in apricot, peach and plum develop clear-cut symptoms in the second half of the vegetative cycle of most susceptible varieties).

Some cultivars are symptomless carriers of viruses and virus-like agents. The sole absence of typical symptoms that are usually looked for, does not permit to eliminate from selection clones that carry virus isolates that may have a depressive effect on both quality and quantity of the yield (e.g. PLMVd is latent on 20% of peach, nectarine and clingstone varieties imported from the USA) (Desvignes, 1990).

Grafting may reveal or aggravate syndromes that show little or not at all on rootstocks or scions when they stand alone. This is exemplified by certain forms of incompatibility, whose factors may not be disclosed during selection but become evident during indexing if incompatibilities arise (Delbos and Dunez, 1988; Németh, 1986).

The identification of viruses or other agents responsible for graft incompatibility has favoured the development of methods for their early detection. On the other hand, grafting can result in the co-infection by two or more viruses which are of little consequence when present singly in the rootstock and scion, but become very damaging in the grafted plant, i.e. seedlings originally infected by PNRSV (Digiaro *et al.*, 1992) and/or PDV (Boari *et al.*, 1998) combined with infected scions.

To overcome these problems, sensitive and reliable methods were developed for the detection of virus diseases, even if symptoms were not expressed at the time of selection. Currently, serological and molecular tools can be used for identification of ilarviruses, nepoviruses, trichoviruses, viroids, and phytoplasmas, but these methods must be complemented with the more traditional indexing on woody indicators.

For selection purposes, indexing remains the most appropriate detection technique. However, it has to be performed taking care that: (i) the indicator varieties are well chosen and identified; (ii) the grafting methods are selected according to the disease agent to be detected; (iii) the growing conditions are the best for the optimal symptom expression; (iv) reading and recording of symptom are made frequently and for a sufficiently extended period of time. In open field indexing, indicators are inspected several times a year for no less than 2-3 years.

Indexing may be done in a growth chamber or glasshouse under controlled conditions of temperature and light favours symptom expression, and standardised procedures (Fridlund, 1970; Boyé and Desvignes, 1996). Laboratory methods allow the identification in infected samples of viral antigens (ELISA, IEM, ISEM) and nucleic acids (molecular hybridisation with cDNA or cRNA probes, dsRNA, PCR).

Sanitary selection, despite the simplicity of its concepts, should be carried out along different steps, which are harmoniously linked. Its efficiency stems from the availability of specialised laboratories, support facilities, and skilful personnel.

III – Objectives of clonal selection

Clonal selection has the scope of identifying, evaluating and selecting clones in relation with different objectives:

- characterisation and preservation of genetic resources, the objective reminds of peculiar importance under the Rio agreements related to the conservation of genetic resources;
- identification of the best parents to be used in breeding programmes;
- identification of cultivars with the highest agronomic traits in order to improve the production.

Clonal selection deals with the identification of clones or with the characterisation of genetic variability within a population of plants. In practice for a given species, in a given area, a wide variability is observed in the agronomic performances of the plants in a field (Audergon *et al.*, 1991). These differences are related with most of the agronomic traits including blooming period, date of maturity, productivity and regularity of production, tree habit, fruit characteristics, processing adaptation, susceptibility to disease (Crossa-Raynaud and Audergon, 1991).

An analyses of its variability highlights the implication of three major factors:

- the origin of the plant materials (genetic characteristics);
- the sanitary status (presence of viruses, viroids, or phytoplasmas);
- the environment factors (soil, climate and grafting effect).

The identification of the three factors is possible in relation with their biological characteristics (Fig. 1). The general variability can be divided within the **fluctuation** and the **variation**.

The **fluctuation** deals with the part of the variability observed in agronomic traits and due to environmental factors (*sensu lato*). It is induced by:

- the *technical practices* (irrigation, fertilisation, pruning, training system) as far as their consequences are of first importance for the economical point of view, some systems are strongly dependent on the scheme of production/commercialisation able to be used by a grower;
- the *obtention of the plants* with the impact of the rootstock expressed in term of:

(a) soil adaptation, vigour, chilling, and heat requirements with their consequences on the blooming period, interaction with the accumulation of assimilates and their consequences on the regularity of the production;

(b) resistance to disease or induced resistance to the graft cultivar; as an example apple or pear resistance to phytoplasma is able to be controlled by a resistant rootstock; a bacterial-canker (*Pseudomonas spp.*) resistance can be induced on apricot cultivar by the use of peach rootstock in place of apricot seedling or plum types;

- the climatic factors;
- the location.

As most of the agronomic traits are strongly linked with the conditions of evaluation, the proper determination of the fluctuation impact is of major importance. The only possibility to keep this part under control is to establish the plants to be compared under uniform conditions of evaluation.

How and where to set up these reference collections are a major concern. Since a major effect of the area of production is expected, care has to be taken:

- to locate the collection in a representative site of the area of production (Crossa-Raynaud and Audergon, 1991);
- to choose a rootstock adapted to the soil, to the risk associated with climatic factors (water-logging, dryness), to the biological constraints of the area of production (as for example the resistance to nematodes in areas heavily infected, or induced susceptibility to bacterial canker;
- to the cultural practices, which have to be optimised and similar to the conditions of production by the growers. This element is of primary importance since (i) fruit characteristics are strongly influenced by source/sink interactions. Thus, thinning and pruning practices must be representative of the growers' methods; (ii) it interacts with the system of commercialisation of the fruit, so that some systems which appeared really interesting in a given scheme of production/commercialisation may be wrong in another.

The **variation** integrates the part of the variability transmitted by grafting, which means the impact of the ***sanitary status*** and the one associated with ***genetics***:

- the ***sanitary status*** (virus, viroids, phytoplasmas and endogenous bacteria) strongly modifies major agronomic traits, but also not affect the viability of the plants. Fruit characteristics can be widely influenced. Furthermore attention must be paid to some additional pathogens such as bacteria or fungi. Even if these pathogens do not directly multiply in the tissue, their presence may affect the agronomic performances under

evaluation. An example can be given with *Xanthomonas campestris pv. pruni* in South-Africa where the Plant Protection Services organised the protection of the mother plants against the bacterium by multiplying bud-wood under insect-proof greenhouses as they observed a rapid degradation of the plants growth when budsticks where directly collected from already infected trees;

- it ensures that sanitary controls are followed to minimise the risk connected with the pathogens that may spread to other mother plants and disseminated through plant propagation.
- the ***genetic variability*** is at the base of the effective improvement of the production as far as it is strictly conserved and propagated by grafting. Within a defined region this variability is directly linked with:

- the presence of cultivars from different origins coming from *accessions* procedures;
- a variation in a local traditional population induced by *mutation*;
- a variation in a local traditional population induced by *seed multiplication*.

The last two types are of great importance in fruit tree where most of the traits are under polygenic control. It means that their expression depends on a large number of genes, each contributing a small effect, but having a wide effect on regulation processes.

- The part related to an *uncontrolled recombination* with a *seed multiplication* is of great importance in most of the traditional areas of cultivation as for example the apricot populations "Rouge du Roussillon" in France (Huet, 1961), "Vesuvio" in Italy or "Canino" in Morocco. The impact of this variability induced by seedling is stronger when the given species are heterozygous (e.g. apricot, almond or plum species for example). It is much more reduced, to the point of being difficult to appreciate, in homozygous species, such as peach when the given population is geographically localised such as "Missour" population in Morocco where the "Cultivar" can be reproduced by seedling.
- The part related to *mutation* is nearly "randomly" distributed and its impact is not easy to evaluate. The introduction of molecular markers will give some efficient tools to appreciate this level of variation (Baird *et al.*, 1996; Scorza, 1996).

All candidate cultivars will be objectively identified and a selection for conservation can be initiated on safer grounds.

IV - Clonal and sanitary selection organisation

The clonal and sanitary selection should be implemented with the establishment of a multidisciplinary (pomologist and plant pathologist) technical team. To keep under control the genetic factor, a clonal selection is divided into four different phases (Fig. 2).

(i) The first phase deals with the *identification of the objectives, the area of investigation and the criteria* whereby the selection has to be performed. This phase conditions the results that one wants to obtain. If the objective deals with the improvement of production, the different parties involved in the production chain must be consulted. Because the overall procedure is time consuming, it would be particularly useful to address this with the agreement of the growers, and taking into consideration the market and the consumers' requirements.

(ii) The second phase is based on a survey combining observation and selection of representative trees in the area under study. Two main objectives need consideration:

- identification of interesting cultivars;
- characterisation with defined criteria.

It consists of:

- field inspections and identification of suitable plants;
- collection of pomological and sanitary data on these plants;
- comparative processing of collected data;
- identification of primary selected clones;
- multiplication by grafting of selected clones.

(iii) The third phase is based on the sanitary evaluation of the identified cultivar (mother plant) and consists of:

- conservation of candidate clones in a repository;
- assessment of the sanitary status (indexing and laboratory tests);
- sanitation treatments (if necessary) and assessment of sanitary status.

(iv) The fourth phase is based on the constitution and the evaluation of collections. It deals with the multiplication of the accessions to address the agronomic evaluation and to realise an objective selection on the base of the quantitative traits under evaluation.

It consists of:

- planning the collection in representative sites with reference cultivars;

- collection of pomological data;
- comparative processing of collected data;
- identification of candidate clones;
- registered primary source (c/o the breeder).

It is not always possible to identify healthy plants through the sanitary selection in the field. Therefore, sanitation through heat therapy or *in vitro* culture is required. The plants obtained are retested to assess the success of sanitation from a specific virus. This control is repeated in the following years to be certain that the agent had been completely eliminated.

Generally speaking, clonal selection belongs to the classical panel of methods dealing with genetic variability management. It is one of the conventional genetic improvement procedures together with cultivar introduction or creation of new cultivars by hybridisation, mutagenesis or biotechnology as reported in Fig. 3.

It is based on the evaluation of the major agronomic traits and, as the given traits are quantitatively inherited, three major characteristics have to be considered:

- a **multitrait** approach;
- a **multilocal** evaluation;
- a **pluri-annual** observation.

Attention must be paid to the examined samples and mainly to the following factors:

- **sampling procedures** which have to be optimised; special attention has to be given to the *bias* introduced during sampling procedures and to the *number of replications* in order to keep under control the accuracy of the measurements;
- **relativization of the measurements with reference cultivars**.

By consequence, these selection procedures are leading to a progressive evaluation based in a first step on the elimination of clones either on default or redundancies up to the identification step. This is being done according to the objectives of (i) the adapted clones or (ii) the clone with the highest agronomic performances for pomologists, or (iii) the complementary clones for genetic resources management.

These studies may appear difficult to manage as they are time consuming. Some prospects exist with the introduction of the molecular markers but a lot of basic work is required to apply these methods in the characterisation and the integration of quantitative traits under a clonal selection process (Arus *et al.*, 1994; Bendiab *et al.*, 1993; Gallais, 1993; Melchinger, 1990).

V - Conservation of selected clones

From the pomological point of view, the conservation of the clones and their control at each step of the certification procedure require a second set of criteria. This is because it would be difficult to evaluate selected materials for its agronomic performances during the multiplication phase.

In practice, after the identification of the candidate clone (or ecotype) a more simple, easy, repeatable, and reliable traits may be chosen to characterise the genotypes within a certification procedure.

This phase denoted "**characterisation**" is based on the simple identification of cultivars by markers under a **multitrait approach** in **one site** (location) and **on one year**. The related markers have to be stable according to the location, cultural practices, and year of observation in order to be recognised by all technicians involved in different locations, or to give the opportunity to compare different set of data.

The markers in use belong to the UPOV or IPGRI descriptors (Guerriero and Watkins, 1984). Most of them are qualitative or quantitative, standardised by reference standards, or standardised by transformations of variables (for example leaf length and leaf width are quantitative measurements strongly linked with the conditions of cultivation, while their ratio is stable for a given genotype).

Other markers under investigation that may be used in a future are molecular protein (isozyme) or DNA markers (Baird *et al.*, 1996, Takeda *et al.*, 1998). These are not currently applied because: (i) even if they can distinguish cultivars on their molecular pattern, in some cases differences may be not reliable from the agronomic point of view or (ii) different clones may be not distinguished (it seems to be particularly accurate with apple peach mutants). However, there is still great interest in term of genetic population analysis.

Selected ecotypes (candidate clones) must be kept in a collection plot where they can be inspected from the pomological and sanitary point of view. The collection plot must fulfil the conditions listed in Annex 1.

The candidate clone is a primary source with a known sanitary status, which may be registered in a registry kept by the Ministry of Agriculture or by authorised institutes. The «primary sources» are to be maintained in the screenhouse whose building characteristics (Annex 2), protect plants from natural infections.

VI - Conclusions

The clonal selection is a powerful tool focused on the identification and preservation of plant materials according to the requirements of the parties involved or interested in the (i) genetic

resources preservation, (ii) improvement of the production by selection of the best clone, and (iii) identification and screening of a population for a given character to identify the best parents for breeding programs. In practice, it integrated two complementary phases (i) agronomic performance evaluation, (ii) morphological and molecular characterisation (Fig. 4).

Sanitary selection is a tool for the control and exclusion of virus diseases. As such, it may be conducted independently from the clonal selection. However, selection is best regarded as a multidisciplinary activity, which involves plant pathologists and pomologists. If selection result in the recovery and enhancement of local germplasm of some species like peach and cherry, it also creates ideal conditions for breeding programmes developing new varieties, all based on reliable sanitary conditions.

The obtention of a «primary source» must be guaranteed by rigorous sanitary controls and using techniques such as ELISA and PCR assays.

By joining both clonal and sanitary selection approaches, the system offers guaranties at different complementary levels:

- breeders collections and genetic resources can be characterised under the best conditions (Badenes *et al.*, 1996, 1998, Takeda *et al.*, 1998);
- Plant Protection Services maintain and control the overall survey of the plant material;
- Certification Service is responsible for issuing certificates concerning the trueness-to-type and the sanitary status of a given genotype in relation with the multiplication in the nurseries (Staub *et al.*, 1996);
- Growers, the end users will optimise long term conditions of production and maximise the quality of the production.

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Annex 1-Technical characteristics to establish a collection plot of stone fruit candidate clones (cultivars and rootstocks)

- the planting should be established in a soil meeting the agricultural requirements and which has not hosted fruit tree species for at least five years;
- the soil should be free from nematode vectors;
- the field should be located 100 meters away from any fruit orchard;
- the plots should have a borderline of at least three meters, constantly tilled and kept free from any other of vegetation;
- the plants should be grafted into virus-free rootstocks;
- each plant should be grown at least in three replications;
- it should be kept free from pests and diseases;
- it should be isolated from the flow of surface water, the irrigation water must be free from pathogens or their vectors.

Annex 2 - Technical features of the facilities and means needed for the conversation of the «primary source»

- the plant material should be kept in conditions of absolute isolation to avoid contamination;
- the material should be grown in pots having adequate diameter containing sterile soil and kept in structures (screenhouse) which fulfil the following conditions;
- the floor will guarantee the isolation between pots and soil. Should the pots be buried, the alleys will be covered with gravel or any other inert materiel securing an adequate drainage;
- the roof shall be rigid, a double net used against insects as virus vectors;
- the whole structure shall be isolated from surface water and from the surrounding environment.

Genetic Variability Management

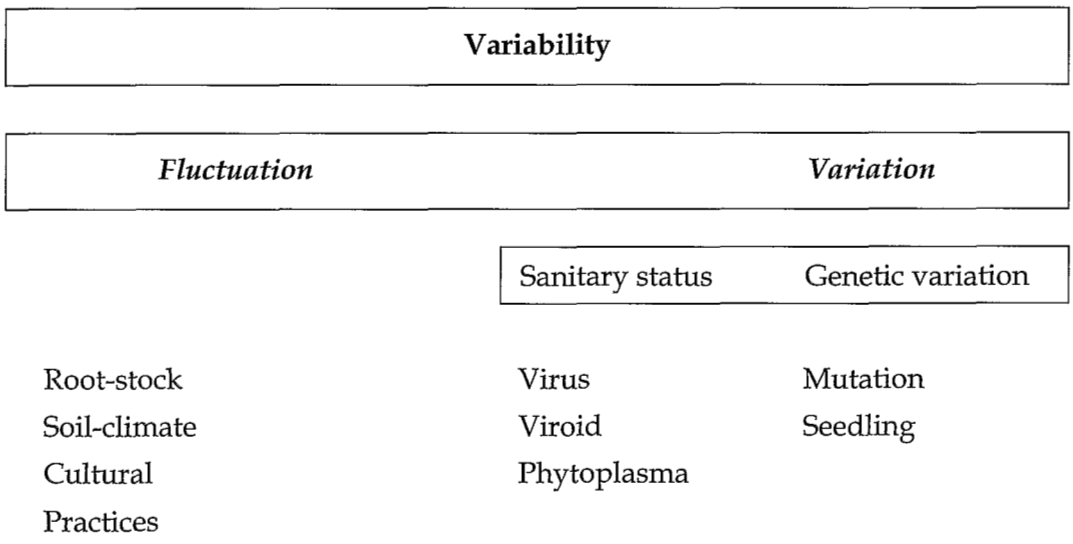

Fig. 1. Schematic representation and decomposition of the variability observed in woody plants

Objectives: genetic resources evaluation
obtention of cultivars
sanitary improvement of the plant material

Different Phases:

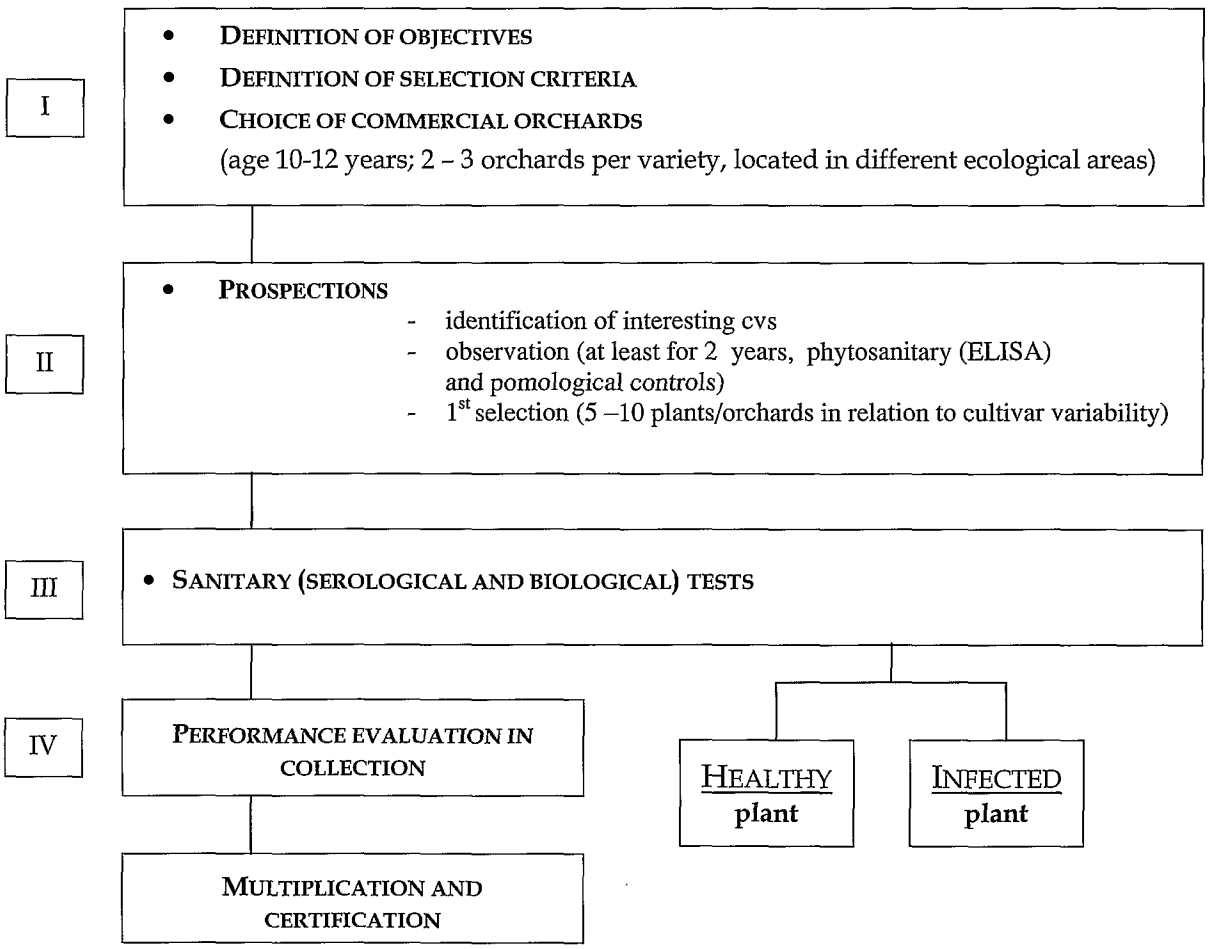

Fig. 2. Objectives and different steps in clonal and sanitary selection procedure

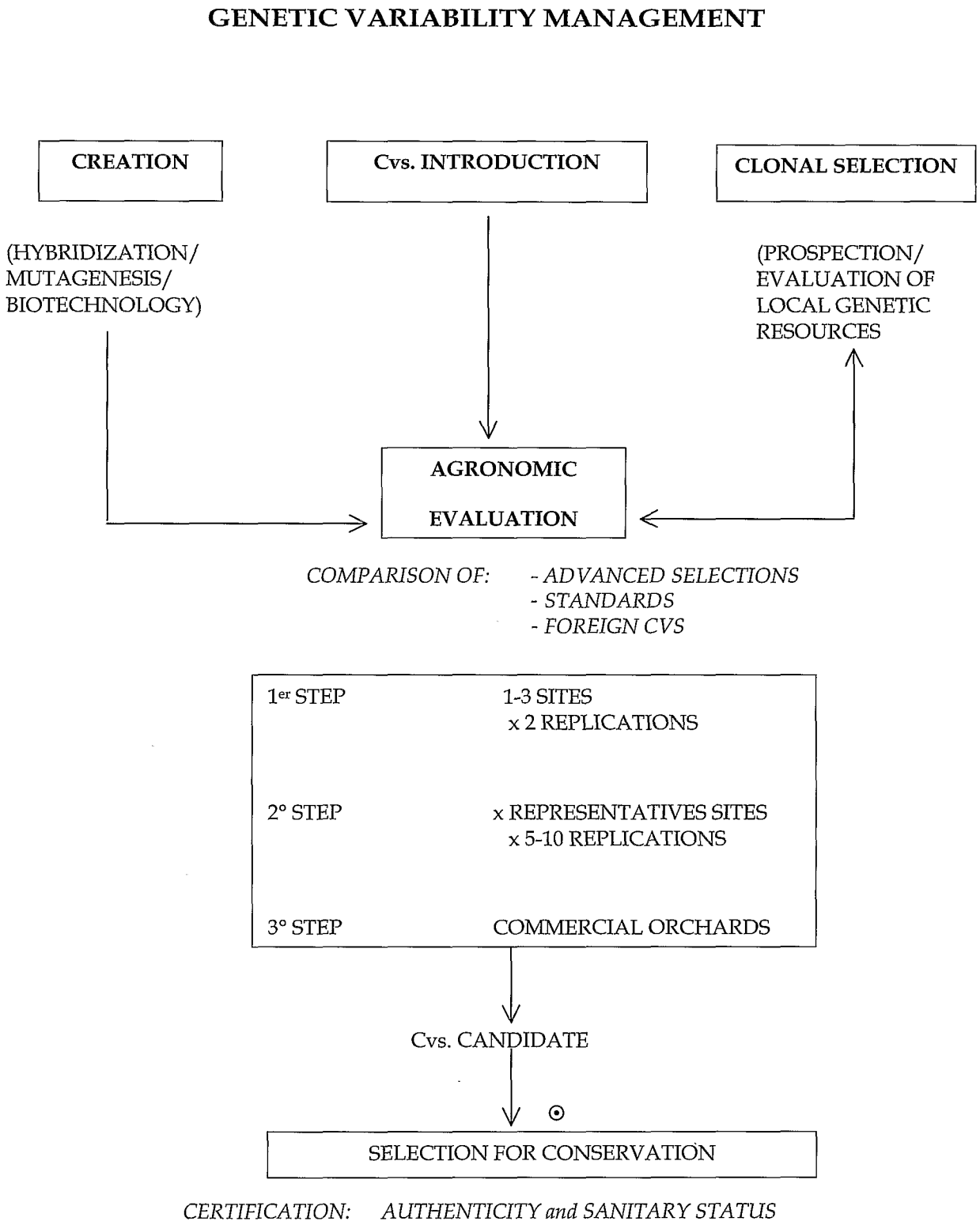

Fig. 3. Description of Fruit Tree Genetic Improvement Procedure

GENETIC IMPROVEMENT PROCEDURES

AGRONOMICAL PERFORMANCES

1. OBJECTIVES AND REQUIREMENTS

- **EVALUATION OF THE MAJOR AGRONOMICAL TRAITS**

⇨ MULTITRAIT APPROACH

⇨ ≠ SITES

⇨ ≠ YEARS

↳ **QUANTITATIVE TRAITS**

⇨ BIAS AND PRECISION
(SAMPLING / REPLICATION)

⇨ RELATIVE OBSERVATION / STANDARD

↳ **CONSEQUENCE:**

PROGRESSIVE EVALUATION

FROM ELIMINATION ON DEFAULTS

TO IDENTIFICATION OF THE BEST CLONES

CHARACTERISATION

- **IDENTIFICATION OF MARKERS**

⇨MULTITRAIT APPROACH
⇨ 1 SITE
⇨ 1 YEAR

↳ MORPHOLOGICAL MARKERS

QUALITATIVE OBSERVATION BY REFERENCE TO/STANDARDS

↳ MOLECULAR MARKERS

PROTEIN (ISOENZYMES)

DNA

Fig. 4. Comparison of selection and characterisation phases