

Production and Distribution of Certified Propagative Material with Special Reference to Fruit Crops: The European and Mediterranean Experience

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Abstract

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Certification can be defined as a procedure whereby candidate mother plants, to be used as source of material for propagation, undergo controls to secure absence from any number of pathogens, as specified by protocols officially issued, or endorsed, by competent governmental agencies. Although certified stocks guarantee also trueness to type, their major and qualifying trait is constituted by a well-established sanitary status. The demand for certified material has grown steadily over the years, concomitantly with increased awareness that unrestricted domestic and international trade of sanitarly uncontrolled plant material has caused a tremendous and highly

threatening worldwide distribution of infectious diseases and their agents (primarily viruses, viroids, and intracellular prokaryotes). Quarantine measures, even the most effective, are not sufficient to stop the trend. However, modern technology has developed efficient tools for producing sanitarly improved stocks, detecting infectious agents and establishing effective certification schemes. The efforts made to this aim in Europe and the Mediterranean area are briefly illustrated and discussed.

Key words: certification, sanitation, Europe, North Africa, Near East.

The consensus is that if the sanitary improvement of any given crop is to be obtained, a system of preventative, protective and, often, curative measures has to be set up and implemented. Such a system may be part of what is broadly known as "integrated pest management", and comprises that complex series of interventions underlying what is currently referred to as "certification". Speaking of certification, four major questions arise:

1. What is it?

In brief, certification can be defined as a procedure whereby candidate mother plants to be used as source of material for propagation, undergo controls and, whenever necessary, treatments to secure absence from any number of pathogens, as specified by regulations officially issued, or endorsed, by competent governmental agencies.

There are two major kinds of certification:

(i) *Voluntary*. A widespread form of certification largely propitiated by the growers' increasing demand of material of known sanitary status for establishing their plantations, especially if these are long-lasting woody crops. Voluntary certification is regimented by regulations issued by a "certifying authority" (i.e. usually a branch of the country's Ministry of Agriculture, or the equivalent) but, by definition, cannot be forcefully imposed.

(ii) *Compulsory*. A type of certification enforced whenever it becomes essential to prevent the dissemination of threatening diseases liable to spread through propagative material. A few such examples are given by the mandatory certification of stone fruit trees for sharka disease recently enforced in Italy (2, 14) or by the similar scheme for citrus tristeza that has been operating for many years in Spain and, more recently, in Italy and Cyprus.

2. What does it apply to?

In principle, certification can be applied to any cultivated plant species, regardless of whether it is propagated vegetatively (cuttings, buds, tubers, bulbs, setts, offshoots, etc.) or through seeds. Thus, both vegetable and woody crops are liable to enter certification schemes, which, in fact they do.

Potato represents a primary example of an asexually propagated vegetable crop which undergoes a very strict certification. That of seed potatoes came into effect many years ago in Europe and elsewhere (5) but, in more recent times in Italy, vegetable crop nurseries began to move towards certification of both seedlings (tomato, pepper) and vegetative propagules (e.g. artichoke offshoots) (15).

However, the "classical" certification programmes, the

most widespread and the best known, are those concerning fruit trees, vines and small fruits.

There are no limitations to the kind and number of pathogens that may be considered for exclusion in a certification programme. Several species of bacteria, fungi, and nematodes are certification organisms in a number of schemes but, most certainly, graft-transmissible infectious agents like viruses, viroids and intracellular prokaryotes [mycoplasma-like organisms (now called "phytoplasmas") and fastidious bacteria] are those of major concern, and will be the object of this presentation.

3. Is there a need for it?

It is common knowledge that a progressive sanitary deterioration of vegetatively propagated crops, especially fruit trees at large (pome and stone fruits, citrus, and grapevines), has taken place over the last few decades. The primary cause of such an alarming situation resides in the increased domestic and international demand and trading of nursery products, to comply with the requirements of the expanding world agriculture, the insufficient or incomplete knowledge of the sanitary problems affecting specific crops, the presence of hosts symptomless carriers of infectious diseases, but also in the deplorable lack of appropriate sanitary control of propagating material being marketed. All this, coupled with the inefficient quarantine service of many countries (including several of the so-called developed ones), has contributed to the generalized dissemination of a number of diseases and pathogens among which the graft-transmissible ones prevail by far.

Here are the figures of disaster, worldwide:

- (i) grapevines are reported to host more than 40 different viruses, about 10 virus-like diseases, 4 intracellular prokaryotes, and 6 viroids (9, 10).
- (ii) citrus is affected by at least 10 characterized viruses, over 20 virus-like diseases, 5 intracellular prokaryotes and at least 7 viroids (13, 20).
- (iii) stone fruits have more than 15 recognized viruses, more than 10 virus-like diseases, 5 intracellular prokaryotes and a couple of viroids (6).

And, thanks to the outstanding technological strides of diagnostic techniques, these figures are on the increase.

Many of these pathogens have the capacity to seriously endanger the productivity, if not the survival, of the plantations, and are to be considered as the major single cause of the decline of the fruit tree industry in certain agricultural areas.

But why fruit trees are so prone to viral infections? Rather than to their intrinsic susceptibility, this seems to be due to the way in which they are propagated and to the variety of geographical and climatic environments in which they are grown. As with all vegetatively

propagated plant species, in fruit trees there is a progressive accumulation of infectious agents that are acquired by the plants with exposure to inoculum in different places and times, and that are "clonally" perpetuated with them. This allows the survival and, sometimes, the spread of also those viruses to which individual fruit tree species are little susceptible, but that have found the way to enter the host, often quite by chance, through the fortuitous inoculation by a passing vector.

Infectious diseases are widely represented in continental and Mediterranean Europe (16), but the situation in southern Mediterranean and Near East countries is no brighter. An extensive 4-year survey (1984-97) of vineyards, citrus groves and stone fruit orchards carried out in this region under FAO patronage by J. Dunez, G.P. Martelli and A.A. Salibe, demonstrated a significant deterioration of the health of these crops due to continued propagation of infected planting material and virtual lack of provisions for its sanitary amelioration (1).

The awareness of the disastrous conditions of so many crops in such a wide area, the alarm signals launched by the scientific community and the increased demand for planting material with an acceptable health status, has compelled several countries to establish a new "clean stock" programmes, or reorganize those previously existing.

But additional efforts are needed for promoting certification schemes on a wider geographical basis. To this aim, there is no lack of supporting arguments, as exemplified by a recent excellent article by C.N. Roistacher (14) that enumerates 13 major reasons for establishing a mandatory certification programme for citrus.

4. What are the conditions needed for its implementation?

To secure the success of a certification programme, there are a number of conditions that must be satisfied before venturing into its launch, such as:

1. Existence of the problem: i.e. occurrence of phytosanitary conditions objectively calling for a public intervention;
2. Compelling request by the growers and their associations, so as to create a political consent;
3. Involvement of, and convinced participation by, the nurserymen;
4. Commitment of governmental authorities to support the programme financially, legally and logistically;
5. Adequate legislation: i.e. emanation of regulations that finalize the scheme to be enforced and regiment the production and

- marketing of certified material;
- 6. Unfailing support by scientific institutions;
- 7. Availability of technology for reliable detection of diseases and their agents;
- 8. Availability of technology for reliable elimination of diseases and pathogens (sanitation);
- 9. Appointment of the "certifying authority", i.e. a governmental service entrusted with control duties and delivery of certification labels.

Certification procedures and requirements

Certification is an interdisciplinary endeavour encompassing phytopathological (primarily virological) and pomological competences, as required by the type of analyses to which candidate certifiable stocks are submitted.

Typically, certification schemes are based upon: (i) pomological and sanitary selection in the fields, (ii) assessment of the sanitary status of selected plants and their sanitation (whenever needed), (iii) technological evaluation of the produce. The outcome of these activities is the "certifiable stock", i.e. a clone or selection true to type and possessing, as a major and qualifying trait, a well-established sanitary status.

(i) *Field selection*. It is the initial step of any programme aimed at the pomological and sanitary amelioration of a given crop. It lasts at least a couple of years, during which the candidate mother stocks (or clones, when referring to grapevines) are identified, based on the possession of superior pomological traits (vigour, productivity, bud fertility, organoleptic quality of the fruits, etc.) and, possibly, absence of visible signs of infectious diseases. Selected plants are propagated and transferred to a germplasm repository for conservation and comparison.

(ii) *Assessment of sanitary status*. Although many infectious agents of fruit trees (viruses, viroids and intracellular prokaryotes) have been isolated and characterized, thus can be more or less readily identified by laboratory methods, the presence of a high number of little known diseases, customarily called virus-like, makes biological testing mandatory. Biotests are represented by indexing through graft inoculation of a standardized series of woody indicators and mechanical transmissions to herbaceous host. These tests require costly infrastructures (climatized glasshouses, indexing plots) and last from a few weeks (transmission to herbaceous hosts) to several years (woody indexing). By contrast, laboratory tests are much faster (sample processing takes from a few hours to a couple of days), reliable and applicable to a thousands of samples in a season. Serology (ELISA with its countless modification and immunoelectron microscopy)

and molecular detection techniques (radioactive and cold cDNA and cRNA probes and the various PCR-based amplification systems) are the diagnostic methods of choice. Their use requires equipped laboratories, trained personnel and adequate funding due to the cost of reagents.

Methods for detection and diagnosis are described in recently published handbooks (10, 12, 13) which the readers are referred to for details.

Although most indexing and laboratory methods have been in use for many years, it became apparent that if certification schemes are to become supernational when conditions are congenial, as for instance in the case of the European Union (EU, former European Economic Community), there is the need for harmonizing and make comparable the detection procedures adopted in the different Member States. This problem is now being debated and, limitedly to the diagnosis of grapevine pathogens, is being addressed by a Study Group supported by the EU, in which the six leading viticultural countries of the Union (Portugal, Spain, France, Germany, Italy and Greece) are represented.

(iii) *Sanitation*. Because of the generalized sanitary deterioration of fruit crops in Europe, the Mediterranean and the Near East, the chances of finding field selections with a health status compatible with the requirements of most certification schemes are limited. It ensues that sanitation is, in most cases, a must.

Sanitation techniques are diversified, and can be selected according to the type of pathogen one wants to get rid of. Thus, for example:

1. most viruses are eliminated through heat therapy by exposing vegetating plants for no less than four weeks to hot air (36-38°C) and 16-18 h artificial lighting and removing buds or small shoot tips (5-10 mm in length), which are then either grafted onto healthy rootstock seedlings, or rooted under mist. Heat therapy is not applicable to viroids.
2. Viruses are also efficiently eliminated through meristem tip culture *in vitro* by excising explants comprising the meristematic dome and the first couple of leaf primordia from apical or axillary buds. This method works also with viroids, provided that the explant consists only of the meristematic dome. *In vitro* culture can be combined with heat therapy or chemotherapy for more efficient results.
3. Micrografting is the method of choice for viroid elimination, but it is efficient also for viruses.
4. Limitedly to grapevines, somatic embryogenesis may afford virus elimination.
5. Intracellular prokaryotes are readily inactivated

by placing dormant budsticks or canes in a water bath at 45°C for 3 h or at 50 °C for 45 min.

Details on sanitation techniques can be found in several reviews and books (4, 10, 13, 18, 19).

Whatever cleaning procedure is used, the material obtained (plantlets, grafted plants, rooted cuttings) must undergo indexing to establish if its healthy status is compatible with the requirements of the specific certification scheme, as established by national protocols.

In general, sanitary selection and sanitation procedures do not aim at obtaining mother stocks free from **all** known infectious agents and diseases. This would be unfeasible for a number of reasons, among which the fact that in all crops there are both infectious agents that do not cause obvious diseases, and diseases of negligible economic impact. Since the elimination of these agents and disorders regarded as "innocuous" would burden the programme and increase its cost without adequate returns, their presence is tolerated, so that mother stocks which happen to contain them are still entitled to enter a certification scheme.

To quote a practical example, in a recent proposal for the revision of grapevine certification in the EU, known and widespread diseases such as vein necrosis and vein mosaic were deliberately excluded, and no mention was made of viroids (11). Likewise, the Tunisian grapevine clean stock programme which is now being implemented, requires for the time being only freedom from grapevine fanleaf nepovirus (GFLV) and grapevine leafroll associated virus 3 (GLRaV-3).

From the above, it ensues that the loose and generalized use of the expression "*virus-free*" should be avoided because it is misleading and can be applied only to specific and well documented cases. By converse, the expression "*virus-tested*" seems more appropriate and reflects reality (for a definition of these expressions see EU Council Directive 92/34/EEC).

Thus, to be effective and legally correct, certification schemes must clearly specify which pathogens candidate mother stocks must be free from, in order to be taken into the system.

There are three types of categories of propagative material produced in the course of a certification programme. These are called with different names in EU Directives and in the schemes issued by the European and Mediterranean Plant Protection Organization (EPPO):

(i) *Pre-basis* (EPPO's nuclear stock). It is the "primary source" originally obtained by the "breeder" ("obtenteur" in French, "costitutore" in Italian), i.e. the physical or juristic person who has identified (and patented) the plant selection or clone. Nuclear stocks are grown under the responsibility of the breeder, in a repository under

conditions ensuring freedom from re-infection, usually in an insect-proof greenhouse.

(ii) *Basic* (EPPO's propagation stock). Material derived from the multiplication of pre-basic stocks, propagated under conditions ensuring freedom from re-infection. Mother plants of basic category are usually grown in the field (propagation blocks) in special outfits under the direct management and control of a public Agency. Customarily basic material is delivered only to nurseries that have the necessary qualifications and is identified by a white label.

(iii) *Certified* (EPPO's certified stock). material which is produced from basic stocks by authorized nurseries under appropriate conditions. Certification is granted at this stage and labels (blue tags) are issued by the certifying authority. Then the material is delivered to the growers and leaves the control of the certification system.

There is a fourth category of propagating material called "*Standard*" (usually identified by a yellow-orange label) which is true to type but has no officially recognized health status because has never entered the certification system. Because of this, standard material represents a sanitary hazard, and is expected to disappear in due course from the EU market.

Selected stocks that have undergone certification are highly valuable; they must be grown with care and be protected as much as possible from re-infection, especially while in the multiplication and nursery phase. Thus, multiplication plots and nurseries producing certified material must be established in soils of good quality free from soil-borne virus vectors and be under superior management by highly qualified technicians. These and other requirements pertaining to safety distances and cultural practices are usually codified by official regulations.

Certification in Europe

Europe used to be, and still largely is, a composite political and social reality. No wonder then, that the intrinsic differences among countries have reflected also in their agricultural organization and politics. However, if the certification schemes operating in Western European countries are comparatively reviewed, it is possible to find many common traits, especially in the scientific background and technical approaches.

The oldest examples of certification applied to fruit trees seem to come from the United Kingdom (late 1940's in Scotland), The Netherlands and France (40 to 50 years of enforcement). In these countries, the certification is implemented by: (i) the "Plant Health Propagation Scheme (PHPS) in Britain; (ii) the General Netherland

Inspection Service for Arboricultural Produce (NAKB) in the Netherlands; (iii) the Centre Technique Interprofessionnel des Fruits et Legumes (CTIFL) for fruit trees, the Institut National de la Recherche Agronomique (INRA) and the Etablissement National pour l'Amelioration de la Viticulture (ENTAV) for grapevines in France. In this latter country, fruit trees certification became official under the authority of the Ministry of Agriculture in 1982 (7) whereas for citrus there is a clean stock programme carried out by INRA and the Centre for International Cooperation in Agricultural Research for Development (CIRAD), which may soon develop into a veritable certification scheme.

In the three above countries, the production and marketing of non-certified material is admitted, which gives a voluntary connotation to the schemes in operation, including the Dutch one on apple which guarantees trueness to type, but has three sanitary categories "virus-free", "virus-tested", and "not tested" (17).

More recently, certification schemes for different crops have been introduced in other countries:

Portugal: grapevine (1991); Denmark: fruit trees (1986); Spain: citrus (1976), fruit trees (1982), grapevine (1986); Germany: grapevine (1969), fruit trees (1976); Italy: grapevine (1969), stone fruit trees (1992), pome fruit trees (1993), walnut (1993), citrus (1993), olive (1993), Greece: grapevine (1987).

Most of the above schemes are voluntary admitting the production of standard material, with the exceptions quoted before, relative to specific highly threatening pathogens (citrus tristeza and plum pox viruses) in certain countries. In all countries the responsibility for certification rests on the Ministry of Agriculture, which can delegate certain activities to national research institutions.

1. *Role of the European and Mediterranean Plant Protection Organization (EPPO)*

EPPO is one of the eight Regional Plant Protection Organizations functioning under Article 8 of the International Plant Protection Convention (8) and the first, since 1991, that has engaged itself in the formulation of certification schemes for a number of different crops. These schemes are well structured and authoritative, as they result from the work of EPPO Panels for Certification composed of qualified international experts. Before being finalized, they are submitted to Ministries of Agriculture of EPPO countries for examination and approval. This, only the internationally endorsed schemes are published, makes them even more legitimate.

EPPO schemes follow pretty much those already operating in different countries, but have the distinct advantage of being up-to-date, highly informative and

consistent in format. Up to now the following have been published or are in press:

- fruit trees [OEPP/EPPO Bulletin 21, 267-277 (1991); 22,255-263 (1992); 22,265-275 (1992); 22,277-283 (1992)]
- carnation [OEPP/EPPO Bulletin 21,279-290 (1991)]
- pelargonium [OEPP/EPPO Bulletin 22,285-296 (1992)]
- lily [OEPP/EPPO Bulletin 23,215-224 (1993)]
- narcissus [OEPP/EPPO Bulletin 23,225-237 (1993)]
- grapevine [OEPP/EPPO Bulletin 24, (1994, in press)]

and those on strawberry, *Ribes* (black currant, red currant, gooseberry), hop, citrus, olive, and *Rubus* (blackberry and raspberry) are well on their way.

2. *Role of scientists*

UE scientists actively contribute to the advancement of certification programmes, not only as members of EPPO Panels, or of *ad hoc* committees appointed by national governments, but also as participants in self-appointed working groups that elaborate certification proposals of their own. For instance, one such group, made up of representatives of Portugal, Spain, France, Germany, Italy and Greece, has recently put forward and submitted to the attention of the EU Commission a proposal for updating and harmonizing grapevine certification procedures in the Community (11).

More recently, bilateral study groups from the Mediterranean Agronomic Institute of Bari (MAI-B) and the Universities of Bari, Tirana and Beirut have elaborated technical and legislative protocols for the certification of stone fruits and grapevines in Albania and Lebanon, which are now under scrutiny by governmental authorities of both countries.

3. *Role of the European Union*

The only Community certification scheme currently in operation is that activated under Council Directive 68/193/EEC on the "*Marketing of vegetatively propagated material of grapevines*". As pointed out in several instances (3, 11) this scheme is obsolete and needs a thorough revision.

Faced with this problem, and the variety of other certification programmes already implemented, or under consideration by Member Countries, the EU seems to be finally taking steps for establishing harmonized Community certifications for various crops. Enquiries are being made to gather up-to-date information, and "Standing Committees on Propagating Materials" for different crops have been appointed. The one on fruit trees is now discussing the possible establishment of a

Community certification scheme under Council Directive 92/34/EEC on the "*Marketing of fruit plant propagating material and fruit plants intended for fruit production*".

It seems, therefore that the EU may soon finalize a set of protocols for the pomological and sanitary improvement of its fruit tree industry, which will then be applied by law throughout the Community. Probably, a lot of time could be saved if EPPO schemes, which are excellent under many aspects, were adopted as such or, at least, were taken as a basis for the EU certification programme.

Certification in North Africa and the Near East

In this Region, Morocco, Algeria, Tunisia, Israel and Turkiye seem to be the most advanced in addressing certification-related issues. A fairly well established agricultural research system, the support of scientific institutions and the interest of nurseries and farmers' associations, has created a favourable environment to the establishment of clean stock programmes.

Morocco has produced certification schemes for almond (1977), citrus (1983), grapevine (1986) and olive (1987), that follow pretty much the guidelines of the European equivalents. Differences reside primarily in the admission of tolerance levels for the presence of viruses in materials of certified and, sometimes, basic category, which do not exist in the EU system (zero tolerance). A further difference rests in the fact that in the Moroccan certification scheme for olive, viruses are not taken into consideration, probably because they were not looked for in this crop.

Clean stock programmes for citrus and grapevines are being implemented in Israel, whereas Turkiye has established a similar programme for citrus, which is not yet backed up by legislative provisions, and is moving along the same lines for stone fruits and grapevines. Likewise, Algeria and Tunisia are well on the move in the framework of the UNDP-FAO Regional Project RAB/88/025.

Role of Project RAB/88/025

The need for a regional project to improve the health status of the fruit tree industry of North Africa and the Near East, became dramatically evident during the "*FAO-EPPO Workshop on the production and handling of health (virus-tested) nuclear stocks of Mediterranean horticultural crops*", held in Bari, Italy, in October 1983. As a follow up, an inter-country cooperative project was prepared in order to reinforce scientific and applied agricultural activities in the field of crop improvement and protection. In the course of the 18th FAO Regional Conference for the Near East held in Istanbul in 1986,

when FAO was asked to promote sanitation of fruit trees in the Region and help member countries to develop their own clean stock programmes, the project was resumed and submitted to the attention of UNDP for funding. Eventually, the UNDP-FAO Regional Project RAB/88/025 on "*Control of virus diseases of fruit trees*" was approved, becoming operative in 1991.

This project embraces seven countries (Morocco, Algeria, Tunisia, Libya, Egypt, Syria, Iraq) and is structured into three networks dealing with grapevines, citrus and stone fruits, respectively. Each network has a separate coordinator and programmes of its own, but operates under the supervision and guidance of a Regional Coordinator located in Tunis. Scientific support is provided by European Institutions (MAI-B and Dipartimento di Protezione delle Piante, University of Bari, Italy; Station de Pathologie Vegetal, INRA, Bordeaux, France; Instituto Valenciano de Investigaciones Agrarias, Moncada, Spain), which assist also with training of researchers and technicians from participating countries. The major role in training is played by MAI-B, which since 1984, in collaboration with the University of Bari, offers a 9-month postgraduate international course on "Protection and sanitation of Mediterranean fruit crops" and a Master of Science degree in Plant Virology. This course had been attended by many graduate students from the countries that participate in the project, while several scientists from the same countries have spent short stays at MAI-B for intensive training in specific laboratory or field techniques.

The scopes of Project RAB/88/025 are many and can be summarized as follows:

1. Coordinate activities undertaken by participating countries in the field of crop improvement and protection;
2. Promote the establishment of structures for the production of clean stocks, their conservation and distribution;
3. Implement the exchange of information and materials among participating countries;
4. Facilitate technology transfer between institutions of developed and developing countries with special reference to diagnostic and sanitation techniques;
5. Favour the creation of specialized staff through specific training abroad;
6. Harmonize certification protocols, registration procedures and relative legislative provisions;
7. Create an information and documentation centre.

Undoubtedly, Project RAB/88/025 is ambitious and, in the end, it may not prove equally effective for all

participating countries. However, the accomplishments already achieved by the grapevine network in a very short time, demonstrate that, if the project's potentialities are wisely exploited, it will certainly contribute to the betterment of local agriculture, leaving a trace of no little consequence on the quality and competitiveness of the nursery and fruit tree industries of the region.

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الملخص

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بالتوزع العالمي للأمراض المعدية ومسبباتها (وبخاصة الفيروسات والفايرويدات والأوالي الضمن خلوية). ولم تكن إجراءات الحجر الزراعي، حتى الصارمة منها، كافية لوقف هذا الاتجاه. على أن التقني الحديثة استطاعت تطوير أدوات فاعلة لإنتاج أصول محسنة، وكشف العوامل الممرضة، وإرساء برامج تصديق فاعلة. وسيستعرض الباحث الجهود المبذولة في هذا الاتجاه في أوروبا وحوض البحر الأبيض المتوسط.

كلمات مفتاحية: التصديق، صحة النباتات، أوروبا، شمال أفريقيا، الشرق الأدنى.

يمكن تعريف التصديق على أنه عملية تخضع فيها النباتات الأمهات، التي ستستخدم كمصدر لمواد الإكثار، إلى الرقابة لضمان خلوها من عدد من الممرضات، كما ينص على ذلك البروتوكول الرسمي المستخدم، أو المعتمد من قبل هيئات حكومية كفاءة. وإضافة إلى أن الأصول المصدقة تضمن المطابقة مع النمط، فإن مواصفاتها الرئيسية والنوعية تحدد مسبقاً وبخاصة الحالة الصحية. وقد أخذ الطلب على الأصول المصدقة يتزايد تدريجياً على مر السنين، وترافق ذلك بالوعي المتزايد بأن الاتجار الإقليمي والدولي غير المحدود لمواد نباتية غير مراقبة صحياً قد أدى إلى مخاطر جدية تمثلت

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