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STRATEGY FOR THE PRODUCTION OF VIRUS-FREE PLANT GERMPLASM UNDER *IN-VITRO* CONSERVATION AND EXCHANGE — A BRIEF REVIEW

C.A. Kumar and K.P.S. Chandel

National Plant Tissue Culture Repository,
National Bureau of Plant Genetic Resources
New Delhi–110 012

With the increase of national and international exchange of plant germplasm, the potential risk involved in distribution of plant pathogens, especially plant viruses in previously unaffected areas has increased. Realising the need for developing an efficient system for maintaining and exchanging virus-free germplasm, work has been initiated at National Plant Tissue Culture Repository of NBPGR. The strategy for production of virus-free germplasm is discussed in this brief review.

Conservation and exchange of the plant genetic resources is of considerable importance for sustained crop improvement activities. However, the germplasm infected by any pathogen impedes the progress, eventually resulting in waste of time, resources and even the valuable germplasm. Further, exchange of infected germplasm may lead to the introduction of hazardous pathogens or races into new areas (Lange, 1985). Therefore, it is very essential that adequate measures be taken for control of the pathogens associated with germplasm particularly with the accessions maintained in *in-vitro* repositories, gene banks and plant introduction stations.

National Plant Tissue Culture Repository (NPTCR), NBPGR, New Delhi, since its inception in 1986, has continued to conserve the germplasm diversity in several economically important vegetatively propagated plant species employing *in- vitro*

techniques. For facilitating its future utilization in breeding programmes and national and international exchange, elimination of virus and virus-like agents assumes special significance. Considering the importance of the diseases caused by these pathogens in the crops under *in-vitro* conservation programme currently being studied at NPTCR and in view of the necessary quarantine guidelines for exchange of germplasm, a programme on production of virus-free germplasm for conservation and exchange was initiated at NPTCR in 1990. Information on virus and virus-like diseases associated with the crops under the programme and procedure for the production of virus-free germplasm is highlighted in this presentation.

Virus diseases and their prevention

Pathogens such as fungi, bacteria, nematodes, mycoplasma like organisms, viruses, viroids etc. are the common causal agents of plant diseases. The level of success in controlling diseases depends entirely upon the types of etiological agents involved in evoking the disease and the exact mechanism by which the pathogens invade the host system. Effective chemotherapeutical control measures are available for the diseases caused by fungi, bacteria and nematodes, whereas attempts to control virus diseases in plants by using antiviral compounds have met with only limited success, because of close association of virus multiplication and host metabolism (Kartha, 1986; Long and Cassells, 1986). The only way of virus disease control is, therefore, prevention of infection.

Among all the preventive measures, removal or avoidance of source of infection is recognised as the most effective method of control. Usually the infected propagules serve as initial source of virus inoculum. Therefore, the virus diseases can be effectively controlled; if the plants are raised from the virus free planting materials. In crops, where the plants are propagated through seed, virus-free plants may be generated by using the seed harvested from the healthy plants of the population (Lange, 1985). But in the case of vegetatively propagated crops, systemically infected plants carry the viruses from one generation to another and usually remain infected in their entire life span. Thus, elimination of inoculum from such plants is the only way of virus disease control in vegetatively propagated plants (Henshaw and O'Hara, 1984).

Over the last two decades, *in-vitro* culture of plant tissue, especially meristem-tip culture or a combination of thermotherapy and meristem-tip culture has established its credibility as an efficient method for elimination of viruses from the infected plants and the method has been successfully used in more than 35 plant species (Quak, 1977; Bhojwani and Razdan, 1983). This technique is now employed as a routine procedure in most of the genetic resources centres engaged in the conservation and distribution of virus-free germplasm, particularly for clonally propagated materials (Kartha, 1986; Love *et al.*, 1987; IBPGR, 1988).

Virus and virus-like diseases associated with plant species handled at NPTCR

A number of germplasm lines belonging to tuberous crops — *Ipomoea batatas* (sweet potato), *Dioscorea* species (yams); bulbous crops — *Allium sativum* (garlic) and related wild and semi wild *Allium* species; fruit crops — *Citrus* spp., *Musa* spp. (banana); spice crops — *Zingiber officinale* (ginger), *Curcuma* spp. (turmeric), *Piper* spp.; medicinal and aromatic plants and endangered plant species — *Coleus forskholii* (Coleus), *Rauvolfia serpantina* (sarpagandha), *Sassurea lappa* (kuth), *Tylophora indica* (antamul) are being conserved in *in-vitro* form at NPTCR.

A survey of literature revealed the association of several important virus and virus-like diseases with some of the plant species under *in-vitro* conservation (Table 1). For example, *Citrus* spp. are attacked by more than 25 viruses (Garnsey, 1988). In banana, bunchy top virus disease is threatening the production in one quarter of the world's banana growing areas (Dale, 1987). Sweet potato is attacked by several viruses, out of which, sweet potato feathery mottle virus, is prevalent wherever the crop is grown (Moyer and Salazar, 1989). This virus has also been found occurring in Indian germplasm (Kumar *et al.*, 1990). Garlic mosaic virus disease is considered to be important in garlic in Taiwan, Japan, Phillippines, Germany, France (Chen and Ko, 1979; Ahmed and Benigno, 1985; Lee *et al.*, 1979; Graichen *et al.*, 1985; Messiaen *et al.*, 1981). This diestase is quite prevalent in India and has received considerable attention in recent years (Ahlawat, 1974; Ahlawat and Chakraborty, 1990; Kumar *et al.*, 1990; Khetarpal *et al.*, 1991).

Table 1 : **Important virus and virus-like diseases associated with the plant species handled at NPTCR *In-vitro* conservation and exchange**

Plant species	Disease	Reference
Allium sativum	* Garlic mosaic	Ahlawat, 1974; Chen and Ko, 1979; Kumar *et al.*, 1990.
	Garlic latent	Graichen and Leistner, 1987.
	Garlic yellow (chlorotic) streak	Xie *et al.*, 1981; Mohamed and Young, 1981.
	Onion yellow dwarf	Chen and Ko. 1979; Graichen and Leistner, 1987.
Citrus spp.	* Tristeza	Bridges, 1966; Nariani and Raychaudhuri, 1968.
	* Exocortis (viroid)	Nariani *et al.*, 1968 Duran-Vila *et al.*, 1986.
	* Greening (Procaryote)	Raychaudhuri, 1981; Garnier and Bove, 1983.
	Xyloporosis	Childs *et al.*, 1955.
	* Infectious variegation	Singh and Chakraborty, 1975; Catara, 1984.
	Stubborn (Spiroplasma)	Fawcett, 1946; Rahimian, 1983.
	* Citrus mosic	Murty and Reddy, 1975; Usugi *et al.*, 1986.
Ipomoea batatas	** Sweet potato feathery mottle	Moyer and Kennedy, 1978; Kumar *et al.*, 1990.
	Sweet potato latent	Green *et al.*, 1988.
	Sweet potato mild mottle	Hollings *et al.*, 1976.
	Sweet potato vein mosaic	Nome, 1973.
	Sweet potato yellow dwarf	Chung *et al.*, 1986.
	Sweet potato caulimo-like	Atkey and Brunt, 1987
Musa spp.	* Banana bunchy top	Vakili, 1969; Summanwar and Marathe, 1982; Dale, 1987; Wu and Su, 1990.
	* Cucumber mosaic or Infectious chlorosis	Magee, 1940; Patel and Mali, 1986; Shong *et al.*, 1990.
	Banana streak	Lockart, 1986.
	Abaca mosaic	Celino, 1956.
Tylophora indica	** Cucumber mosaic	Kumar *et al.*, 1991.

* Reported from India

** Identified in India for the first time from NPTCR laboratory.

Investigations undertaken at NPTCR during the period 1990-92 revealed the presence of sweet potato feathery mottle virus, garlic mosaic virus and cucumber mosaic virus diseases in *Ipomoea batatas, Alluim sativum* and *Tylophosra indica* germplasm collections, respectively (Kumar *et al.*, 1990, 1991a & b). Besides the above mentioned diseases, banana bunchy top and sweet potato mild mottle diseases are suspected to occur in germplasm collections based on the sympotoms observed. Above all, the presence of some other diseases in latent form in different germplasm collections can not be ruled out. This necessitates critical screening of germplasm for the freedom from viruses and salvaging of the infected material by *in-vitro* techniques.

Procedure for the production of virus-free Germplasm

The production of virus-free germplasm includes mainly three aspects i.e. routine virus indexing, selection of virus-free mother plants and elimination of viruses from the infected mother plants by meristem-tip culture combined with various treatments (Fig. 1). Routine virus indexing plays a key role in the production of virus-free plants, as only after testing the plants for specific viruses, they could be identified as virus-free or infected. Often it becomes necessary to repeat the process to ensure virus-free status of the mother plants selected from the field and in meristem-tip culture raised plants.

In order to develop routine virus indexing techniques, first of all the viruses are detected, identified and purified, and then the detection techniques are standardized. In certain cases where the virus detection kits are readily available, they may be procured and used in routine virus indexing.

The following detection techniques are generally applied in routine virus indexing :

i) Infectivity assays — These methods are based on the transmission of viruses by artificial means to the susceptible indicator or test plants and visual examination for the symptoms produced on them after a certain incubation period. Most frequently employed tests are mechanical sap inoculation and graft transmission (Noordam, 1973).

ii) Serological tests — The basic principle involved in these tests is detection of viruses in the infected plants on the basis of visible or measurable reaction between virus (antigen) and its homologous antiserum (poly or monoclonal antibodies). In

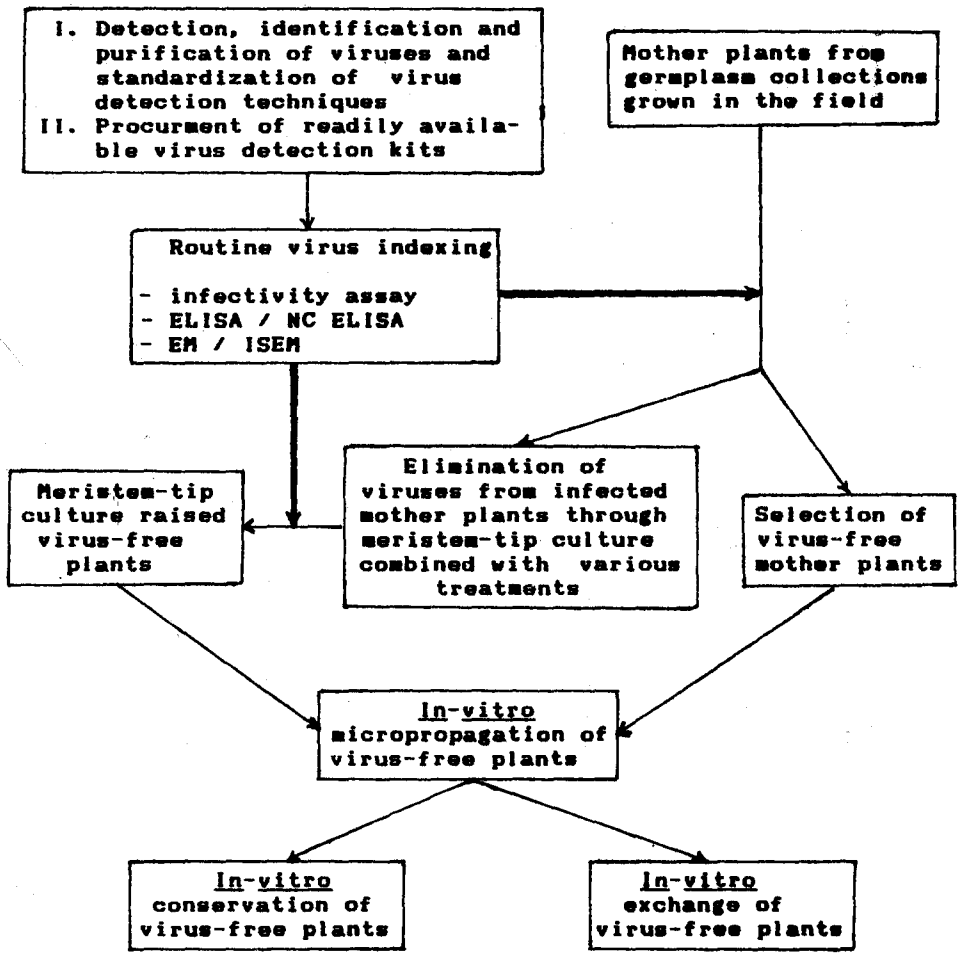

Fig. 1. : Diagramatic scheme for the production of virus-free germplasm under *in-vitro* conservation and exchange.

recent years, the serological detection methods, such as enzyme linked immunosorbent assay (ELISA) (Clark *et al*, 1988) and dot-immunobinding assay (DIBA) or nitrocellulose ELISA (NC ELISA) (Powell, 1987), which are sensitive and specific, are commonly used in routine virus indexing.

iii) Electron microscopy (EM) — Using this technique viruses can be detected in the tissues of infected materials directly by visualizing the virus particles. (Hitchborn and Hills, 1965; Horne, 1967). EM coupled with serology, known as immunosorbent electron microscopy (ISEM), is employed for distinguishing serologically unrelated virus particles in mixed infections (Milne and Lesemann, 1984).

Apart from the above mentioned techniques, several other molecular and biochemical techniques such as ELISA with monoclonal antibodies to epitopes that are highly conserved between viruses of a given taxon (Carter and ter Meulen, 1984; Halk and De Boer, 1985); isolation and analysis of dsRNA by gel electrophoresis (Kurppa and Martin, 1986); nucleic acid hybridization with cRNA or cDNA probes (Garger and Turpen, 1988; Palukaitis, 1988), and polymerase chain reaction (PCR) (Langeveld *et al.*, 1991; Lopez-Moya *et al.*, 1992) have been developed in recent years and they are all potentially very useful for routine virus indexing.

It is a well known fact that in nature, some genotypes exhibit resistance to viruses and sometimes within the population a part of the stock may remain virus-free. In such cases the plants grown in the field are tested several times for viruses and subsequently virus-free mother plants are selected for *in- vitro* micropropagation. Where the entire population of the clone is infected, the virus-free mother plants are obtained by eliminating the viruses through *in-vitro* culture techniques.

Several *in-vitro* culture methods such as meristem-tip, callus, nucellar, floral, ovule and protoplast culture can be used for obtaining virus-free plants with varying degree of success (Nadgauda and Mascarenhas, 1985; Kartha, 1986). Among all these techniques meristem-tip culture technique combined with thermotherapy has been found to be the most efficient technique for the production of virus-free plants, as the regenerated plants usually retain their genetic characteristics due to the more uniformly diploid nature of the cells (Kartha, 1986). Since, the main aim is to conserve/exchange the germplasm without disturbing its genetic

stability, only this technique is generally used to eliminate the viruses.

It is important to recognise that even after following all precautions to excise small meristem tips and subjecting them to various treatments favouring virus eradication, only a small proportion of the cultures may yield virus-free plant. Therefore, each meristem-tip culture plant has to be tested for specific viruses before using it as a virus-free mother plant (Bhojwani and Razdan, 1983; Kartha, 1986). Virus-free mother plants are then micropropagated in *in-vitro* and the healthy stocks are transferred to the medium and long-term *in-vitro* conservation. Such *in-vitro* cultures are used in national and international exchange of germplasm.

CONCLUSION

Several important virus diseases often occur in the germplasm of vegetatively propagated crop plants under field gene banks. The detection of a few hitherto unreported viruses in germplasm collections at NPTCR clearly indicates the contamination of existing collections with the viruses. Based on symptoms observed, there is a possibility of occurrence of some more viruses and their related strains in the germplasm collections. Absence of the disease symptoms does not always ensure that plants are free from viruses, as they may be present in latent form. Screening of germplasm collections against the viruses by using various sensitive biochemical and molecular techniques such as ELISA, ISEM, isolation of dsRNA, cDNA, PCR, thus become absolutely essential to ensure the virus-free status of germplasm.

In recent years *in-vitro* culture systems have assumed greater importance in the global scheme for germplasm conservation and exchange, as they have many advantages including elimination of pests and pathogens from the germplasm collections. However, *in-vitro* culture alone cannot guarantee a pathogen free, especially virus-free status of plants (Henshaw and O'Hara, 1984; IBPGR, 1988). Production of virus-free germplasm, therefore, requires a special procedure, where meristem-tip culture and virus indexing techniques are combined. The scheme is depicted by a flow diagram (Fig. 1). The approach dealt with above would eventually help to reduce the risk of viral contamination in the clonally propagated germplasm diversity being conserved and exchanged.

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