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Role of Viral Diagnostics in Quarantine for Plant Genetic Resources and Preparedness

V Celia Chalam*, DB Parakh and AK Maurya
Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India

International exchange of plant genetic resources (PGR) and trade play an important role in the long-distance dissemination of destructive viruses/strains. The ICAR-NBPGR has been empowered for quarantine processing of imported PGR including transgenics meant for research purposes. Adopting a workable strategy of post-entry quarantine growing/inspection followed by use of combination of detection techniques viz., electron microscopy, ELISA and RT-PCR, 45 viruses of great economic and quarantine importance were intercepted in imported PGR in the last three decades (1985-2016). The interceptions include 19 viruses not yet reported from India viz., *Barley stripe mosaic virus*, *Bean mild mosaic virus, Bean pod mottle virus*, *Broad bean mottle virus, Broad bean stain virus, Broad bean true mosaic virus*, *Cherry leaf roll virus*, *Cowpea mottle virus, Cowpea severe mosaic virus,* Dioscorea latent virus, *Garlic virus C*, High plains virus, *Maize chlorotic mottle virus*, *Pea enation mosaic virus*, *Peanut sunt virus*, *Pepino mosaic virus, Raspberry ringspot virus, Tomato ringspot virus* and *Wheat streak mosaic virus*. Besides, 21 viruses not known to occur on particular host(s) in India were intercepted and 23 viruses were intercepted in germplasm imported from CGIAR centres. Fourteen viruses including nine viruses not reported from India have been intercepted in transgenic germplasm. Even though some of the intercepted viruses are not known to occur in India, their potential vectors exist and also congenial conditions to multiply, disseminate and spread destructive exotic viruses/strains. India also need to establish a strong network of interconnected accredited laboratories able to quickly diagnose new viruses/strains; enhance surveillance capacity and develop early warning systems. This makes it imperative that India should put in place a "National Plant Pests Diagnostic and Certification Network" to enhance preparedness.

Key Words: Diagnostics, Plant genetic resources, Quarantine, Viruses

Introduction

The global movement of plant genetic resources (PGR), agri-horticultural produce and products has the potential of introducing new pests including viruses which may pose potential risk to the agriculture of the importing country. The unrestricted exchange of seed lots has been attributed to the worldwide distribution of many economically important viruses such as *Bean common mosaic virus*, *Soybean mosaic virus*, *Pea seed-borne mosaic virus*, *Wheat streak mosaic virus*, *Peanut mottle virus,* etc. A number of exotic plant viruses have been introduced into India along with imported planting material causing serious crop losses. These included *Banana bunchy top virus* (BBTV), *Peanut stripe virus* etc. BBTV was introduced and spread into India from Sri Lanka in 1940 (Magee, 1953). The international spread of BBTV is primarily through infected planting material (Wardlaw, 1961). In India, an annual loss of Rs.40 crore due to BBTV has been estimated in Kerala alone. These introductions highlight the fact that increased pace of international travel and trade, had exposed countries to the danger of infiltration of exotic viruses harmful to the agriculture.

The National Plant Protection Organizations are responsible for protecting their countries from the entry of new viruses and for eradicating those that have recently arrived and are still sufficiently confined for their elimination to be realistic. The strategies for biosecurity from plant viruses include stringent quarantine measures for the imported material and domestic quarantine/ use of certified virus-free seed and other planting material within the country. As per norms a workable sample of the bulk samples of seed lots/ vegetative propagules/ tissue culture-raised plants need to be inspected and tested. The detection of viruses is then carried out by the approved/ available techniques. Availability of rapid, reliable, robust, specific and sensitive methods for detection and identification of viruses determine the success of the process.

The ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), the nodal institution for management of PGR has been empowered under the

**Author for Correspondence: Email- celia.chalam@icar.gov.in; mailcelia@gmail.com*

Plant Quarantine (Regulation of Import into India) Order 2003 (hereinafter referred to as PQ Order), under the Destructive Insects and Pests Act, 1914, to undertake quarantine processing of germplasm including transgenic planting material imported for research purposes into the country by both public and private sectors. It has well-equipped laboratories and post-entry quarantine (PEQ) green house complex to undertake the task. A National Containment Facility of level-4 (CL-4) has been established to ensure that no viable biological material/ pollen/ pathogen enters or leaves the facility during quarantine processing of transgenics. At ICAR-NBPGR, adopting a workable strategy using post-entry quarantine (PEQ) growing/inspection, Electron Microscopy (EM), Enzyme-linked Immunosorbent Assay (ELISA), and Reverse-transcription Polymerase Chain Reaction (RT-PCR), a number of viruses have been intercepted in germplasm imported from many countries including Consultative Group on International Agricultural Research (CGIAR) centres (Khetarpal *et al*., 1992; Khetarpal *et al*., 1994; Khetarpal *et al*., 2001; Khetarpal *et al*., 2006; Chalam and Khetarpal, 2008; Chalam *et al*., 2005a; Chalam *et al*., 2005b; Chalam *et al*., 2007; Chalam *et al*., 2008b; Chalam *et al*., 2009b; Chalam *et al*., 2014b; Chalam *et al*., 2017; Kumar *et al*., 1991; Parakh *et al*., 1994; Parakh *et al*., 2005; Parakh *et al*., 2006; Parakh *et al*., 2008; Parakh *et al*., 2009).

The present paper discusses the interception of 45 plant viruses in quarantine processing of germplasm including transgenics imported during the last three decades (1985-2016).

Materials and Methods

Testing methods for detection of viruses in imported germplasm (seeds, *in vitro* cultures, vegetative propagative material etc.) including transgenics during quarantine as given below were employed.

Visual Inspection

All the accessions (seeds, *in vitro* cultures, vegetative propagative material etc.) were examined visually. In case of seeds, shrivelled, small sized and seeds showing mottling, necrosis, split seed coat symptoms and other discolourations were discarded and incinerated.

Growing-on Test at ICAR-NBPGR, New Delhi

Apparently healthy looking seeds of legume germplasm belonging to 25,499 accessions were sown during respective *Kharif* and *Rabi* seasons in an insect-proof PEQ greenhouse in soil beds during 1985-2016. Each accession was grown in one-metre row with 15-20 plants per row. Seedlings were observed regularly after emergence till flowering. The accessions showing virus-like symptoms were further subjected to Electron microscopy (EM), Enzyme-linked immunosorbent assay (ELISA) and Reverse-transcription- polymerase chain reaction (RT-PCR), if required.

Growing-on Test of Transgenics in Containment Facility at ICAR-NBPGR, New Delhi

The transgenic germplasm belonging to 13,648 accessions were grown in containment facility. The accessions showing virus-like symptoms were further subjected to EM, ELISA and RT-PCR, if required.

Post-entry Quarantine Inspection at Indenter's Site

The germplasm other than legumes were released on an undertaking by the indenter that material will be grown in isolation under the supervision of a plant pathologist. The transgenics were released on an undertaking by the indenter that material will be grown in containment facility approved by the Department of Biotechnology, Ministry of Science and Technology, Government of India under the supervision of a plant pathologist. The accessions showing virus-like symptoms were further subjected to EM, ELISA and RT-PCR, if required.

Electron Microscopy (EM)

Two mg tissue from leaf samples showing prominent viral symptoms during growing-on test and *in vitro* cultures were taken and homogenized in 0.07M phosphate buffer, pH 6.5. Ten µl leaf extracts of each accession were deposited for one min on carbon-coated grids. The grids were then washed with 10 drops of distilled water and stained with 2% uranyl acetate. Excess of stain was removed by touching the edge of the grid with a piece of filter paper and the grids were examined under Transmission Electron Microscope (JEOL-JEM 1011).

Enzyme-linked Immunosorbent Assay (ELISA)

The leaf samples showing virus-like symptoms of different crops, representative healthy-looking samples and *in vitro* cultures were tested by adopting the classical ELISA protocol (Clark and Adams, 1977) using polyclonal antiserum against 52 viruses viz., *Alfalfa mosaic virus* (AMV), *Arabis mosaic virus* (ArMV),

Barley stripe mosaic virus (BSMV), *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean mild mosaic virus* (BMMV), *Bean pod mottle virus* (BPMV), *Bean yellow mosaic virus* (BYMV), *Broad bean stain virus* (BBSV), *Broad bean true mosaic virus* (BBTMV), *Broad bean mottle virus* (BBMV), *Broad bean wilt virus* (BBWV), *Carnation latent virus* (CLRV), *Cherry leaf roll virus* (CLRV), *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea mosaic virus* (CPMV), *Cowpea mottle virus* (CPMoV), *Cowpea severe mosaic virus* (CPSMV), *Cucumber mosaic virus* (CMV), Dioscorea latent virus (DLV), Dioscorea mosaic virus (DMV), *Garlic common latent virus* (GarCLV), *Garlic virus* C (GarV-C), *Grapevine fanleaf virus* (GFLV), High plains virus (HPV), Iris yellow spot virus (IYSV), *Leak yellow stripe virus* (LYSV), *Maize chlorotic mottle virus* (MCMV), *Maize dwarf mosaic virus* (MDMV), *Onion yellow dwarf virus* (OYDV), *Pea enation mosaic virus* (PEMV), *Pea seed-borne mosaic virus* (PSbMV), *Peanut mottle virus* (PeMoV), *Peanut stripe virus* (PStV), *Peanut stunt virus* (PSV), *Pepino mosaic virus* (PepMV), *Raspberry ringspot virus* (RRSV), *Red clover vein mosaic virus* (RCVMV), *Shallot latent virus* (SLV), *Shallot yellow stripe virus* (SYSV), *Southern bean mosaic virus* (SBMV), *Soybean mosaic virus* (SMV), *Tobacco necrosis virus* (TNV), *Tobacco rattle virus* (TRV), *Tobacco ringspot virus* (TRSV), *Tobacco streak virus* (TSV), *Tomato aspermy virus* (TAV), *Tomato black ring virus* (TBRV), Tomato mosaic virus (ToMV), *Tomato ringspot virus* (ToRSV) and *Wheat streak mosaic virus* (WSMV) (Source: Agdia Inc., USA; AC Diagnostics, USA; Adgen, UK; Bioreba, Switzerland; Biorad, USA; DSMZ Germany; Loewe Phytodiagnostica, Germany; Neogen USA; Sanofi Pasteur, France). Double Antibody Sandwich-ELISA (DAS-ELISA) was used. The leaf samples were ground in phosphate buffered saline -Tween 20, pH 7.4 + 2% polyvinyl pyrrolidone (PVP) for all except SBMV for which samples were ground in sodium sulfite + sodium azide + ovalbumin + PVP + Tween 20 buffer, pH 7.4. The extracts (1:10 w:v) were centrifuged at 3000 rpm for 3 min., and supernatant was collected. The microtiter plates of Costar (USA) were used. Immunoglobulins (IgG) to different viruses and enzyme conjugate were used at 1:100 or 1:200 dilution each as per the Company's direction. The positive and negative controls for each of the viruses used were the lyophilized leaf tissue (Source: Agdia Inc., USA; AC Diagnostics, USA; Adgen, UK; Bioreba, Switzerland; Biorad, USA; DSMZ Germany; Loewe Phytodiagnostica, Germany; Neogen USA; Sanofi Pasteur, France). The enzyme substrate used was *p*-nitro phenyl phosphate (1 mg/ml). The optical density (OD) values of samples were recorded at 405 nm using ELISA plate reader (TECAN Sunrise, Austria) at a time interval of 15 min., 30 min., 1 h, 1 h 30 min., and 2 h after incubation. A sample was considered as infected if its OD value was more than twice that of negative control (Chalam and Khetarpal, 2008; Chalam *et al*., 2005a; Chalam *et al*., 2007; Chalam *et al*., 2008b; Chalam *et al*., 2009b; Chalam *et al*., 2014b; Chalam *et al*., 2016b).

Reverse-transcription Polymerase Chain Reaction (RT-PCR)

RNA was extracted from samples using RNeasy ® Plant Mini Kit (Qiagen), following protocol provided by the manufacturer. Concentration (ng/µl) of isolated RNA was measured by Thermo Scientific Nanodrop Reader. Synthesis of cDNA was performed with Thermo Fischer cDNA Kit. For PCR reaction of viral cDNA, 5 µl of RT reaction was used as template. The 20 µl PCR reaction was prepared including 3 µl sterile water, 1 µl forward primer (5 µM), 1 µl reverse primer (5 µM), 5 µl cDNA template and 10 µl of 2X Go Taq master mix (Promega M7123) for each sample. PCR reactions for negative control were also prepared without adding the cDNA template. The lid of thermo cycler was kept at 99°C and pre- heating was also given. The PCR products were separated by electrophoresis of 20 µl of amplified product on 1.5% TAE agarose gel stained with ethidium bromide at 80 volts for 45 min. and image was visualized and captured by Gel documentation system (BioRad). RT-PCR was used wherever the ELISA results were ambiguous/inconclusive (Arif *et al*., 20012; Chalam and Khetarpal, 2008; Chalam *et al*., 2004; Chalam *et al*., 2008a; Chalam *et al*., 2012a;. Chalam *et al*., 2012b; Chalam *et al*., 2016b).

All plant residues and plant samples used for testing were incinerated as per quarantine procedure.

Results and Discussion

During the last three decades (1985-2016), by adopting a workable strategy such as PEQ growing in PEQ greenhouses/ containment facility and inspection, PEQ inspection at indenter's site, EM, ELISA and RT-PCR, 45 viruses of great economic and quarantine importance have been intercepted in exotic germplasm including

transgenics (Table 1). The interceptions include 19 viruses not yet reported from India viz., BSMV, BMMV, BPMV, BBMV, BBSV, BBTMV, CLRV, CPMoV, CPSMV, DLV, GarV-C, HPV, MCMV, PEMV, PepMV, PSV, RpRSV, ToRSV and WSMV. Besides, 21 viruses not known to occur on particular host(s) in India have been intercepted and these are also of quarantine significance for India. Two viruses viz., ArMV and RCVMV are present in India but with restricted distribution in India. ArMV is reported to occur only on rose in Palampur, Himachal Pradesh and RCVMV is reported only on pea from Faizabad, Uttar Pradesh. Twenty three viruses have been intercepted in germplasm imported from CGIAR centres. Fourteen viruses including nine viruses not reported from India have been intercepted in transgenic germplasm. Majority of the accessions were found infected by more than one virus (Chalam 2016; Chalam 2014; Chalam *et al*., 2005a; Chalam *et al*., 2007; Chalam *et al*., 2008b; Chalam *et al*., 2009a; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2012b; Chalam *et al*., 2012c; Chalam *et al*., 2012d; Chalam *et al*., 2012e; Chalam *et al*., 2013b; Chalam *et al*., 2013c; Chalam *et al*., 2013d; Chalam *et al*., 2014a; Chalam *et al*., 2014b; Chalam *et al*., 2014c; Chalam *et al*., 2015a; Chalam *et al*., 2015b; Chalam *et al*., 2016a; Chalam *et al*., 2016b; Chalam *et al*., 2017; Chalam and Khetarpal 2008; Khetarpal *et al*., 1992; Khetarpal *et al*., 1994; Khetarpal *et al*., 2001; Khetarpal *et al*., 2003; Kumar *et al*., 1991; Parakh *et al*., 1994; Parakh *et al*., 2005; Parakh *et al*., 2006; Parakh *et al*., 2008; Parakh *et al*., 2009; Prasada Rao *et al*., 1990; Prasada Rao *et al*., 2004; Prasada Rao *et al*., 2012; Singh *et al*., 2003).

Out of 45 viruses detected, 35 viruses either by virus species name or as genus including 26 as virus species viz., AMV, ArMV, BBSV, BBMV, BBTMV, BBWV, BSMV, BYMV, CLRV, CMV, GFLV, HPV, PEMV, PepMV, PStV, PSV, RpRSV, TAV, TBRV, TNV, TRV, TRSV, ToMV, ToRSV, TSV, WSMV and nine as genus *viz., Comovirus* (BPMV, CPMV and CPSMV), *Carmovirus* (BMMV, CPMoV) *Potyvirus* (BCMV, BCMNV, CABMV, SMV), have been mentioned in the PQ Order and require an additional declaration from exporting country that the consignment is free from one or more of these viruses on case to case basis, hence these intercepted viruses are of high quarantine significance for India.

All the plants showing virus-like symptoms were thus checked for the presence of virus and when found infected were uprooted and incinerated. A total of 226 accessions belonging to *Capsicum annuum* (47); *Glycine max* (64); *Phaseolus vulgaris* (1); *Pisum* spp. (24); *Solanum lycopersicum* (21), *Vigna catjung* (1); *V. radiata* (1); *V. subterranea* (2); *V. unguiculata* (46); *Zea mays* (19 including four accessions of transgenic *Z. mays*) were rejected due to presence of viruses of quarantine significance for India. Harvest from virus-free plants was released to the indenters.

Among the viruses intercepted 19 are not known to occur in India and their potential vectors exist and so also the congenial conditions for them to multiply, disseminate and spread the destructive exotic viruses/ strains. The risk of introduction of 45 viruses or their strains into India was thus eliminated. If not intercepted, some of these viruses could have been introduced into the country and caused economic yield losses. Besides, the harvest obtained from virus-free plants also ensured conservation of virus-free exotic germplasm in the National Genebank.

In order to highlight the importance of interception of viruses in exotic germplasm an example of *Bean pod mottle virus* (BPMV) is given below:

BPMV, not reported from India, was detected and intercepted in exotic germplasm of *Glycine max* from USA (Chalam *et al*., 2014b). BPMV is seed-transmitted at a rate of 0.037 to 0.1% in *G. max*. It causes yield-reductions ranging from 3 to 52.4% and seed coat discolouration. Seed coat mottling and dark pigments have often been problematic for soybean farmers and industry as it reduces consumer acceptance (Giesler *et al*., 2002). If not intercepted, BPMV could have been introduced into the country and established as favourable environmental conditions are available in India. If introduced, it would have caused an annual yield loss of Rs.2632.5 million with the incidence of 1%. The Possible yield losses were calculated based on soybean production (10.53 m t) and minimum support price (Rs. 2500/q) during 2014-15 (Anonymous, 2016).

The present findings thus highlight the importance of plant quarantine in minimizing/ eliminating the risk of introducing destructive exotic viruses along with seeds/ *in vitro* cultures of germplasm including transgenics.

V. Conclusions and Future Perspectives

Due to the exchange of PGR and liberalized trade under WTO there is an increasing possibility of introduction of exotic viruses if stringent quarantine measures are not

Table 1. Plant viruses intercepted in germplasm imported into India for research purposes during 1985-2016

S. No.	Virus intercepted	Host	Source of import	Reference
1	[cd]*Alfalfa mosaic virus* (AMV)	*Glycine max*	AVRDC (Taiwan), IITA (Nigeria), Brazil, Canada, Costa Rica, Myanmar, Sri Lanka, USA	Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		Phaseolus vulgaris[c]	CIAT (Colombia), Canada, Kenya, Nepal, Russia	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		Pisum sativum[c]	ICARDA (Syria), Russia, Spain, USA	Chalam *et al*., 2014b
		Vicia faba	ICARDA (Syria), Spain	Chalam *et al*., 2009a; Chalam *et al*., 2014b
		Vigna mungo[c]	Bhutan	Chalam *et al*., 2014b
		V. radiata[c]	IITA (Nigeria), Japan	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata[c]	CIAT (Colombia), IITA (Nigeria), Italy, USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008
2	[bcde]*Arabis mosaic virus* (ArMV)	*G. max*[ce]	Canada, Costa Rica, USA[e]	Chalam *et al*., 2014b; Chalam 2016; Chalam *et al*., 2016a
		V. unguiculata[c]	IITA (Nigeria), Italy	Chalam 2016; Chalam *et al*., 2016a
3	[acde]*Barley stripe mosaic virus* (BSMV)	*Hordeum vulgare*	USA	Chalam and Khetarpal 2008; Khetarpal *et al*., 2006
		Triticum aestivum[f]	USA[f]	Chalam 2014; Chalam 2016; Chalam *et al*., 2013b; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2015b; Chalam *et al*., 2016b
		Zea mays[f]	Philippines[f], France[f], USA[f]	Chalam 2014; Chalam 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2016a
4	[c]*Bean common mosaic virus* (BCMV)	*G. max*[c]	AVRDC (Taiwan), IITA (Nigeria), Brazil, Canada, Costa Rica, Thailand, USA	Chalam *et al*., 2014b; Chalam *et al*., 2016; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		P. vulgaris	CIAT (Colombia), CIS, Hungary, Kenya, Nepal, Russia, USA	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Khetarpal *et al*., 1994, Khetarpal *et al*., 2001
		V. radiata	AVRDC (Taiwan), Japan, USA	Chalam and Khetarpal 2008; Chalam *et al.,* 2014b; Khetarpal *et al.,* 2001
		V. subterranea[c]	Ghana	Chalam *et al*., 2014b
		V. unguiculata	AVRDC (Taiwan), IITA (Nigeria), Guyana, Italy, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a;
5	[c]*Bean common mosaic necrosis virus* (BCMNV)	*G. max*	Costa Rica	Chalam *et al*., 2016a
		P. vulgaris	CIAT (Colombia), Kenya, Russia	Chalam *et al*., 2007; Chalam *et al*., 2014b
		V. marina[c]	AVRDC (Taiwan)	Chalam *et al*., 2014b

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
6	[ae]*Bean mild mosaic virus* (BMMV)	*G. max*[e]	Canada, Colombia, USA[f]	Chalam, 2016; Chalam *et al*., 2016a; Chalam *et al*., 2017
		P. vulgaris	USA	Chalam 2016; Chalam *et al*., 2017
7	[a]*Bean pod mottle virus* (BPMV)	*G. max*	USA	Chalam *et al*., 2014b
		P. vulgaris	USA	Chalam *et al*., 2016
		V. unguiculata	IITA (Nigeria), Italy	Chalam, 2016; Chalam *et al*., 2016a
8	[de]*Bean yellow mosaic virus* (BYMV)	*G. max*[e]	IITA (Nigeria), Canada, Myanmar, USA[e]	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Parakh *et al*., 2005
		P. vulgaris	CIAT (Colombia), Nepal	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		P. sativum	Spain, USA	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria), Bulgaria, Spain	Chalam *et al.,* 2007; Chalam *et al.,* 2009a; Chalam *et al.,* 2014b; Chalam and Khetarpal 2008; Khetarpal *et al.,* 2001
9	[ad]*Broad bean stain virus (*BBSV*)*	*G. max*	AVRDC (Taiwan), Costa Rica	Chalam, 2016; Chalam *et al*., 2016a
		V. radiata	AVRDC (Taiwan)	Chalam, 2016; Chalam *et al*., 2016
		P. sativum	Spain	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria), Bulgaria	Chalam *et al*., 2007, Chalam *et al*., 2009b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Khetarpal *et al*., 2001
10	[ad]*Broad bean true mosaic virus (*BBTMV*)*	*P. vulgaris*	USA	Chalam 2016; Chalam *et al*., 2017
11	[ad]*Broad bean mottle virus (*BBMV*)*	*P. vulgaris*	AVRDC (Taiwan)	Chalam, 2016; Chalam *et al*., 2017
12	[cd]*Broad bean wilt virus* (BBWV)	*G. max*[c]	Canada, Costa Rica, USA	Chalam *et al*., 2014b; Chalam *et al*., 2016a
		P. vulgaris[c]	CIAT (Colombia)	Chalam *et al*., 2014b
		P. sativum	USA	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria)	Chalam *et al*., 2009a; Chalam *et al*., 2014b
13	[ade]*Cherry leaf roll virus* (CLRV)	*G. max*[e]	AVRDC (Taiwan), Sri Lanka, Thailand, USA[e]	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008
		P. vulgaris	CIAT (Colombia)	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria)	Chalam, 2016; Chalam *et al*., 2016a

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
14	[c]*Cowpea aphid-borne mosaic virus* (CABMV)	*G. max*[c]	AVRDC (Taiwan), IITA (Nigeria), Myanmar, Sri Lanka, Thailand, USA	Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		V. radiata[c]	AVRDC (Taiwan)	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria), Eritrea, Guyana, USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Khetarpal *et al*., 2001
15	[c]*Cowpea mild mottle virus* (CPMMV)	*G. max*	AVRDC (Taiwan), USA	Chalam *et al*., 2014b
		P. sativum[c]	Spain	Chalam *et al*., 2014b
		V. faba[c]	ICARDA, Syria	Chalam *et al*., 2014b
		V. radiata	AVRDC (Taiwan)	Chalam *et al*., 2014b
16	[c]*Cowpea mosaic virus* (CPMV)	*G. max*[c]	AVRDC (Taiwan), Canada, Costa Rica,USA	Chalam, 2016; Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a
		V. radiata[c]	AVRDC (Taiwan), USA	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria)	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata ssp. sesquipedalis	Philippines	Chalam, 2016; Chalam *et al*., 2016a
17	[a]*Cowpea mottle virus* (CPMoV)	*V. unguiculata*	Philippines	Khetarpal *et al*., 2001
		V. subterranea	Ghana	Chalam *et al*., 2014b
18	[ae]*Cowpea severe mosaic virus* (CPSMV)	*G. max*[e]	AVRDC (Taiwan), Canada, Costa Rica, USA[e]	Chalam *et al*., 2014b; Chalam *et al*., 2016a
		V. radiata	Australia	Chalam *et al*., 2014b
		V. unguiculata	Belgium	Chalam *et al*., 2014b
19	[d]*Cucumber mosaic virus* (CMV)	*Capsicum annuum*	Republic of Korea	Chalam *et al*., 2016a
		Cucumis sativus	Brazil	Chalam, 2016; Chalam *et al*., 2017
		G. max	AVRDC (Taiwan), IITA (Nigeria), Brazil, Canada, Myanmar, Sri Lanka, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		P. vulgaris	CIAT (Colombia)	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		Solanum lycopersicum	Netherlands, Republic of Korea, Thailand, USA	Chalam *et al*., 2016a
		V. faba	ICARDA (Syria)	Chalam *et al*., 2014b
		V. serratifolia	ICARDA (Syria)	Chalam *et al*., 2014b
		V. radiata	USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	IITA (Nigeria), Belgium, USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
20	[a]Dioscorea latent virus (DLV)	*Dioscorea nipponica*	USA	Chalam *et al*., 2016a
21	*Garlic common latent virus* (GarCLV)	*Allium longicuspis*	USA	Parakh *et al*., 2009
		A. sativum	USA	Parakh *et al*., 2009
22	[a]*Garlic virus C* (GarV-C)	*A. sativum*	USA	Parakh *et al*., 2009
23	[cd]*Grapevine fanleaf virus* (GFLV)	*G. max*[c]	AVRDC (Taiwan), Canada, Costa Rica, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a
		V. mungo[c]	Bhutan	Chalam *et al*., 2014b
		V. radiata[c]	AVRDC (Taiwan)	Chalam *et al*., 2016a
24	[ade]High plains virus (HPV)	*Z. mays*[e]	France[f], Thailand, USA[f]	Anonymous, 2017; Chalam, 2014; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2014a; Chalam *et al*., 2014c; Chalam *et al*., 2016a; Chalam *et al*., 2017
25	[ae]*Maize chlorotic mottle virus* (MCMV)	*Z. mays*[e]	Puerto Rico[f], Thailand, USA[f]	Anonymous, 2017; Chalam, 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2013b; Chalam *et al*., 2013c; Chalam 2014; Chalam *et al*., 2016a; Chalam *et al*., 2017
26	[ce]*Maize dwarf mosaic virus* (MDMV)	*T. aestivum*[cf]	USA[f]	Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam 2014; Chalam *et al*., 2016b; Chalam *et al*., 2017
		Z. mays[e]	Colombia, France[f], Kenya, Mexico, Philippines[f], Sudan, USA[f]	Chalam, 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2016a; Chalam *et al*., 2017; Chalam and Khetarpal, 2008; Khetarpal *et al*., 2006
27	[ad]*Pea enation mosaic virus* (PEMV)	*G. max*	Costa Rica	Chalam, 2016; Chalam *et al*., 2015a; Chalam *et al*., 2017
		P. vulgaris	Nepal	Chalam *et al*., 2014b
		P. sativum	Spain, USA	Chalam *et al*., 2014b
		V. faba	ICARDA (Syria)	Chalam *et al*., 2014b
		V. unguiculata	Italy	Chalam, 2016; Chalam *et al*., 2017
28	[c]*Pea seed-borne mosaic virus* (PSbMV)	*P. sativum*	AVRDC (Taiwan), Australia, Bulgaria, Colombia, Eritrea, Germany, Nepal, Netherlands, Russia, Spain, Syria, USA	Khetarpal *et al*., 2001; Parakh *et al*., 2006; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. faba[c]	ICARDA (Syria), Bulgaria, Spain	Kumar *et al*., 1991; Khetarpal *et al*., 2001; Chalam *et al*., 2007, Chalam *et al*., 2009a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
29	[c]*Peanut mottle virus* (PeMoV)	*Arachis hypogaea*	Indonesia, Malawi, Philippines, Uganda, USA	Prasada Rao *et al*., 2004; Khetarpal *et al*., 2006; Chalam and Khetarpal 2008; Chalam *et al*., 2017
		G. max[c]	AVRDC (Taiwan), Sri Lanka	Chalam *et al*., 2014b
		V. radiata[c]	Sri Lanka	Chalam *et al*., 2014b

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
30	[bcd]*Peanut stripe virus* (PStV)	*A. hypogaea*	China, Japan, Myanmar, Philippines, USA	Prasada Rao *et al*., 1990; Prasada Rao *et al*., 2004; Prasada Rao *et al*., 2012; Khetarpal *et al*., 2006; Chalam and Khetarpal 2008; Chalam *et al*., 2017
		G. max[c]	China	Prasada Rao *et al*., 2012; Chalam *et al*., 2017
31	[ad]*Peanut stunt virus* (PSV)	*G. max*	AVRDC (Taiwan), Canada, Costa Rica	Chalam, 2016; Chalam *et al*., 2016; Chalam *et al*., 2017
		P. vulgaris	USA	Chalam, 2016; Chalam *et al*., 2015a; Chalam *et al*., 2017
		V. radiata	AVRDC (Taiwan), Costa Rica	Chalam *et al*., 2016
		V. unguiculata	Italy	Chalam, 2016; Chalam *et al*., 2016a
32	[ad]*Pepino mosaic virus* (PepMV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		S. lycopersicum	Netherlands, Thailand, USA	Chalam *et al*., 2016a
33	[ade]*Raspberry ringspot virus* (RRSV)	*G. max*[e]	AVRDC (Taiwan), Costa Rica, Sri Lanka, Thailand, USA[e]	Chalam, 2016; Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam *et al*., 2017; Chalam and Khetarpal 2008
		V. unguiculata ssp. *sesquipedalis*	Philippines	Chalam, 2016; Chalam *et al*., 2016a
34	[bc]*Red clover vein mosaic virus* (RCVMV)	*P. vulgaris*[c]	USA	Chalam *et al*., 2014a ; Chalam *et al*., 2016a
35	[c]*Southern bean mosaic virus* (SBMV)	*G. max*[c]	AVRDC (Taiwan); IITA (Nigeria), Thailand, USA	Chalam *et al*., 2014b; Chalam and Khetarpal 2008; Parakh *et al*., 2005; Parakh *et al*., 2008
		P. vulgaris[c]	CIAT (Colombia)	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata	Belgium, USA	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
36	[cde]*Soybean mosaic virus* (SMV)	*G. max*[e]	AVRDC (Taiwan), IITA (Nigeria), Australia, Brazil, Canada, Costa Rica, Hungary, Nigeria, Sri Lanka, Thailand, USA[e]	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2009b; Chalam *et al*., 2016a; Chalam and Khetarpal 2008; Khetarpal *et al*., 1992; Khetarpal *et al*., 2001; Parakh *et al*., 1994; Parakh *et al*., 2005; Parakh *et al*., 2008; Singh *et al*., 2003
		P. vulgaris[c]	CIAT (Colombia), Russia	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal, 2008
		V. unguiculata	IITA (Nigeria)	Chalam, 2016; Chalam *et al*., 2016a

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
37	[cd]*Tobacco necrosis virus* (TNV)	*P. vulgaris*	Nepal	Chalam *et al*., 2014b
		P. sativum[c]	Spain, USA	Chalam *et al*., 2014b
		V. faba[c]	ICARDA (Syria)	Chalam *et al*., 2014b
38	[cd]*Tobacco rattle virus* (TRV)	*P. vulgaris*[c]	CIAT (Colombia)	Chalam *et al*., 2014b
39	[d]*Tobacco ringspot virus* (TRSV)	*C. annuum*	USA	Chalam *et al*., 2016a
		G. max	AVRDC (Taiwan); IITA (Nigeria), Brazil, Myanmar	Chalam and Khetarpal 2008; Chalam *et al*., 2014b; Parakh *et al*., 2005; Parakh *et al*., 2008
40	[cde]*Tobacco streak virus* (TSV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		G. max[e]	AVRDC (Taiwan), Brazil, Sri Lanka, Thailand, USA[e]	Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		Helianthus annuus	Spain	Prasada Rao *et al*., 2012
		P. vulgaris[c]	CIAT (Colombia), Nepal	Chalam *et al*., 2014b
		P. sativum[c]	USA	Chalam *et al*., 2014b
		S. lycopersicum[c]	Netherlands, Thailand, USA	Chalam *et al*., 2016a
		V. faba[c]	ICARDA (Syria)	Chalam *et al*., 2014b
		V. serratifolia[c]	ICARDA (Syria)	Chalam *et al*., 2014b
		V. radiata	AVRDC (Taiwan)	Chalam *et al*., 2014b
		V. unguiculata[c]	CIAT (Colombia), IITA (Nigeria)	Chalam, 2016; Chalam *et al*., 2014b
41	[d]*Tomato aspermy virus* (TAV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		S. lycopersicum	Netherlands, Republic of Korea, Thailand, USA	Chalam *et al*., 2016a
		P. vulgaris	CIAT (Colombia)	Chalam *et al*., 2014b
42	[cd]*Tomato black ring virus* (TBRV)	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		G. max[c]	AVRDC (Taiwan), Brazil, Canada, Costa Rica, Sri Lanka, USA	Chalam, 2016; Chalam *et al*., 2014b; Chalam *et al*., 2016a
		P. vulgaris[c]	CIAT (Colombia), Brazil, Canada, Kenya, Russia, USA	Chalam *et al*., 2005a; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
		V. unguiculata[c]	CIAT (Colombia), IITA (Nigeria), USA	Chalam *et al*., 2008b; Chalam *et al*., 2014b; Chalam and Khetarpal 2008
43	[d]Tomato mosaic virus	*C. annuum*	Republic of Korea	Chalam *et al*., 2016a
		S. lycopersicum	Netherlands, Thailand, USA	Chalam *et al*., 2016a

Contd.

S. No.	Virus intercepted	Host	Source of import	Reference
44	[ade]*Tomato ringspot virus* (ToRSV)	*G. max*[e]	AVRDC (Taiwan), Canada, Costa Rica, Sri Lanka, Thailand, USA[e]	Chalam, 2016; Chalam *et al*., 2007; Chalam *et al*., 2014b; Chalam *et al*., 2016a; Chalam *et al*., 2017; Chalam and Khetarpal 2008
		V. radiata	AVRDC (Taiwan)	Chalam *et al*., 2017
		V. unguiculata	IITA (Nigeria), Italy	Chalam, 2016; Chalam *et al*., 2016a; Chalam *et al*., 2017
45	[adf]*Wheat streak mosaic virus*	*T. aestvum*[f]	USA[f]	Chalam, 2014; Chalam, 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam 2013b; Chalam *et al*., 2013d; Chalam *et al*., 2016a; Chalam *et al*., 2016b
		Z. mays[f]	France[f], Philippines[f], Puerto Rico[f], South Africa[f], USA[f]	Chalam 2014; Chalam 2016; Chalam *et al*., 2009b; Chalam *et al*., 2012a; Chalam *et al*., 2013b; Chalam *et al*., 2013d; Chalam *et al*., 2016a

[a]Virus not reported from India
[b]virus present in India but with restricted distribution, hence it is of quarantine significance for India
[c]Virus present in India but not recorded on the particular host on which it is intercepted, , hence it is of quarantine significance for India
[d]Virus listed as regulated pest in the Plant Quarantine (Regulation of Import into India) Order 2003
[e]Virus intercepted in transgenics also
[f]Virus intercepted in only transgenics
AVRDC = Asian Vegetable Research Development Center- The World Vegetable Center; IITA = International Institute of Tropical Agriculture; ICARDA = International Center for Agricultural Research in the Dry Areas; CIAT = Centro Internacional de Agricultura Tropical
Note: Viruses in italics are characterized and listed in the ninth report of the International Committee on Taxonomy of Viruses (ICTV); viruses not in italics are those not characterized completely but listed in the ninth report of the ICTV (Andrew *et al*., 2012).

followed. The introduced viruses could get established as virus vectors are present in the country. There is a need to strengthen the domestic quarantine system to prevent the spread of viruses with limited distribution within the country. Besides, there are tremendous opportunities to the growers for enhancing export of agricultural commodities if they meet the international quality standards by overcoming phytosanitary constraints, which may involve virus-free areas for production. Various government agencies involved in international and domestic quarantine, certification of seed and other planting material need to work in complete coordination and there is a need to create enough awareness among general public, customs officials and private stakeholders on importance of both international and domestic quarantine.

Virus detection and diagnosis are crucial for application of mitigation strategies and for exchange of PGR and safe trade. There is a need for *accredited diagnostic laboratories* at central and state level for accurate and quick detection and identification of viruses and there is a need for National Certification Programme for Seed Health in line with National Certification System for Tissue Culture-raised Plants (NCS-TCP), Department of Biotechnology, Govt. of India and need to review seed certification standards.

Establishment of a *National Repository of Diagnostics for Viral Diseases* including antisera Bank, database of primers, seeds of indicator hosts, virus reference collections (lyophilized positive controls), user-friendly diagnostics such as lateral flow strips/ dip sticks which can detect multiple viruses, multiplex RT-PCR protocols, loop mediated isothermal amplification, helicase dependent amplification protocols for detection of viruses in the field and at ports of entry, microarrays, DNA barcoding and ultimately, a cost-effective *national biosecurity chip* for diagnosis of all current threats to crop plants would be the backbone for strengthening the programme on biosecurity for plant viruses. Also, *South Asia Regional Working Group of Detection and Identification of Plant Viruses* thus need to be formed among SAARC countries for sharing knowledge and facilities.

Database on all viral diseases, including information on host range, geographical distribution, strains, etc. should be made available for its use as a ready reckoner

by the scientists, extension workers and quarantine personnel. Plant viruses of quarantine significance for India in cereals (Chalam *et al*., 2005b), grain legumes (Chalam *et al*., 2012d) and edible oilseeds (Chalam *et al*., 2013a) have been published. The Crop Protection Compendium of CAB International, UK, is an useful asset to scan for global pest data (CAB International, 2007).

Preparation of *authentic reports of new viruses* and deposition of reference cultures in the National Repositories may be made mandatory. The exports may suffer due to wrong identification of viruses and reporting the same as new reports.

Need to develop *rapid response teams* at state level to deal with the sudden outbreak of viral diseases.

Develop the *web-based information portal* for management of plant viruses and regulations including database on taxonomists and dignosticians.

Revisit the national regulatory framework and and develop a mechanism for supply of virus-free seeds/ plants/ planting material within the country, be it seed distribution meant for multilocation testing under All India Coordinated Research Projects of ICAR, inland supply of germplasm by ICAR-NBPGR or seed distribution by the National/ State Seed Corporations/ private organizations. Also, need to monitor the movement of vegetatively propagated material and tissue culture-raised plants across the states. Requirement for treatment of germplasm/ commercial seed/ other material being distributed across the states to be made mandatory. Also, strengthen state certification mechanism to ensure the supply of virus-free nursery material.

Strengthen Plant Quarantine Stations dealing with bulk samples in terms of manpower, infrastructure (well equipped laboratories, treatment facilities and greenhouses) and expertise with special emphasis on advanced techniques for detection of viruses, their strains in bulk samples through regular trainings. ICAR-NBPGR has Organized Training course on "Diagnostic methods for detection and identification of pests of seed and other planting material and their management" for plant quarantine officials from DPPQS in 2011 (Gupta et al., 2011). Recently during 2015-17, ICAR-NBPGR has organized 11 Training Workshops on "Biosafety and transboundary movement of living modified organisms (LMOs)" for >300 officials of customs and plant quarantine (Bhalla *et al*., 2017). Need to organize such workshops to create *awareness on plant quarantine issues* among stakeholders such as customs officials, other airport officials, travelers, scientists and other stakeholders with diverse kind of teaching material both print and audio-visual for wider use (Chalam *et al*., 2009c).

There is an urgent need to develop a *National Plant Pests Diagnostic and Certification Network* (NPPDCN) (Fig. 1) linking the research laboratories with seed/ vegetative planting material testing laboratories and quarantine stations, which would be the backbone for strengthening the programme on biosecurity from plant pests including viruses.

The NPPDCN as proposed in Fig. 1 can be a store-house of information on pests (fungi, bacteria, viruses, nematodes, insect pests and weeds), diagnostics procedures, national data base on state-wise endemic pests, policies, and related issues. The quality of seed and other planting material will be further enhanced only if seed and other planting material health is integrated into certification procedures. We have the potential for strengthening the system but need to focus in a pragmatic manner.

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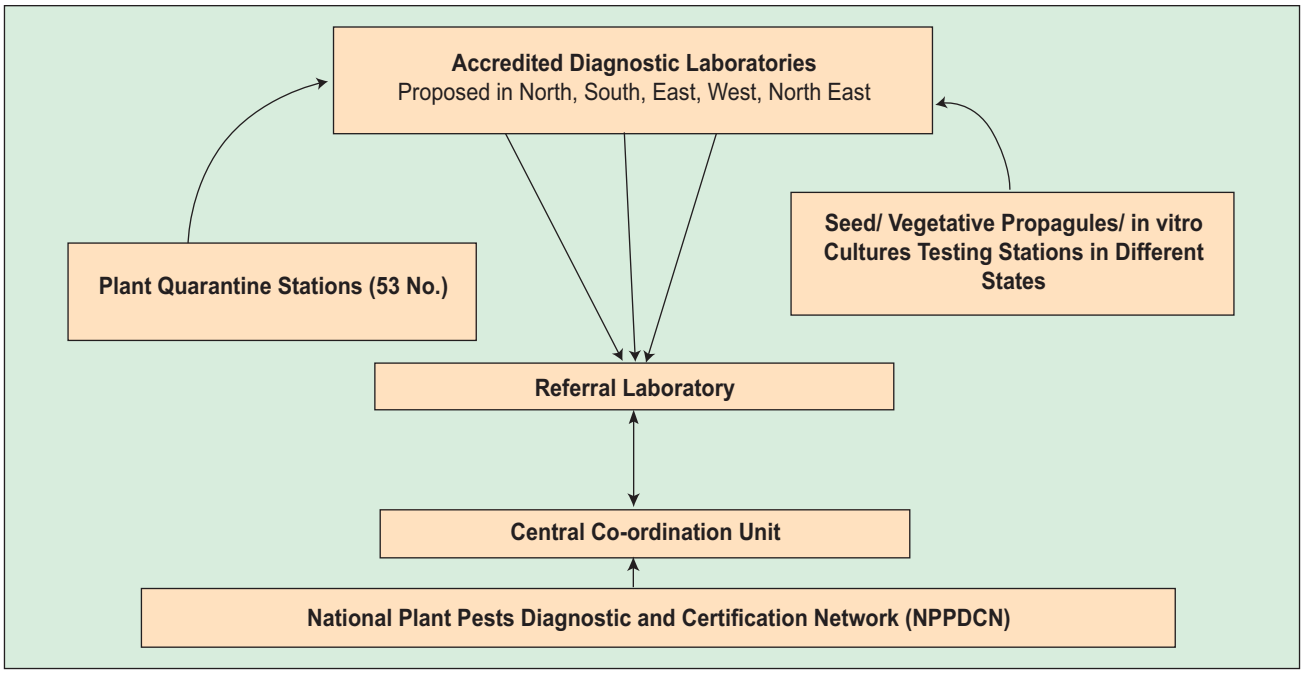

Fig. 1. Proposed Flow Chart of Networking by National Plant Pests Diagnostic and Certification Network (NPPDCN)

Source: Adapted from Chalam *et al*., 2016b

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