SEED-BORNE VIRUSES

Detection, Identification and Control

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SEED-BORNE VIRUSES:
Detection, Identification and Control

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Foreword

According to an estimate more than 90 percent of all the food crops grown in the world are propagated through seeds, and all are attacked by devastating seed-borne pathogens. The transfer of genetic stock on a global scale, either for utilization or for conservation involves possible risks of wide spread distribution of seed-borne diseases and pests. A potential hazard is the accidentally transfer of diseases and pests due to the movement of seed material, either for immediate use in breeding programmes or for evaluation and conservation at genetic resources centres. Seeds of about 200 genera of economically important crops are prone to be infected by more than three hundred viruses. The seed borne viruses that are often symptomless may pose a severe threat to local agriculture. The introduction of banana bunchy top virus (BBTV) from overseas in Pakistan had caused serious economic losses to banana plantation in the country. In order to minimize such risks, the knowledge of seed-borne viruses about their detection, identification, indexing procedures, mechanism of seed-transmission and control strategies are required to ensure that imported as well as locally produced seeds are free of seed-borne pathogens. The increase in volume of germplasm exchanged, coupled with recent advances in biotechnology has created a pressing need to have overviews of the existing knowledge about various aspects of seed borne viruses.

This publication produced by the scientists from Pakistan Agricultural Research Council, Islamabad, Pakistan (Dr. Muhammad Bashir and Dr. Zahoor Ahmad) and from Japan (Dr. Nobuo Murata) contains information on all aspects related to seed-borne viruses and has an immense value in modern agriculture. I hope that this treatise will be useful for teachers, students, plant pathologists, seed technologists, and plant quarantine specialists. My appreciation goes to the authors for their hard work and wish them success in future. I am confident that this book will prove valuable to the scientists not only in Pakistan but also throughout the region.

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Preface

There is a growing demand for improved cultivars to increase productivity on sustainable basis, essential for rapidly increasing world population particularly in developing countries. Breeding such cultivars requires collecting, conservation and utilization of plant genetic resources at national and international level. The international transfer of crop germplasm may facilitate the long distance dissemination of seed-borne viruses, some of which are of quarantine significance. Approximately 18 percent of the described plant viruses are seed-borne in one or more hosts, and it is estimated that one third of the plant viruses eventually prove to be seed-transmitted in at least one host. In order to maintain high crop productivity in modern agriculture, it is essential to minimize the risks of introducing seed-borne viruses, especially to geographical locations known to be free of such viruses.

The seed health and quarantine implications of international and adaptive testing of plant genetic resources are a major concern to international agricultural research centres and other institutions involved in such efforts. This is because germplasm collection carries with it the risk of long distance dissemination of viruses that may be harbored in or on the imported genetic material. Several instances of seed-borne viruses have recently been reported to occur in crop germplasm, e.g. soybean mosaic virus (SMV) in soybean germplasm, pea seed-borne mosaic virus (PSbMV) in pea and lentil germplasm, blackeye cowpea mosaic (BlCMV), cowpea aphid-borne mosaic (CABMV) and cucumber mosaic (CMV) viruses in cowpea germplasm, and bean common mosaic (BCMV) in *Phaseolus vulgaris* germplasm. Well documented cases tell the story of the spread of devastating viruses from one area, country or continent to another: barley stripe mosaic virus (BSMV), lettuce mosaic virus (LMV), peanut stripe virus (PStV), PSbMV, and CMV are just a few examples from an ever-increasing list of seed-transmitted viruses which have spread and have been intercepted over wide distances.

Correct identification of seed-transmitted viruses is a fundamental step towards their control. Testing techniques for virus detection should be simple, sensitive, reliable and inexpensive. It may not be possible that a single test possesses all these features, but the progress made during the last two decades made it possible for some tests, such as enzyme-linked immunosorbent assay (ELISA), immunospecific electron microscopy (ISEM), and polymerase chain reaction assay (PCR) to meet the above criteria. This publication represents the various aspects of seed-transmitted viruses

described in seven chapters. In the first Chapter, the characteristics of seed-borne viruses have been described. Various aspects on mechanism of seed transmission have been discussed in Chapter-2. This Chapter answers the questions such as, why some viruses are seed-transmitted and the others not? Why the virus is seed-transmitted at high rate in one genotype and at very low rate in other genotype of the same species? Why the virus is present in immature seed and not in mature seed? Methods of seed health testing are illustrated in Chapeter-3. The most reliable and sensitive serological techniques for the detection and identification of seed-borne viruses are presented in Chapter-4. In Chapter-5, the role of quarantine in restricting introduction of exotic plant pathogens, seed-borne viruses reported in crop germplasm in Pakistan and suggestions for improvement of quarantine implications have been illustrated. Viruses of quarantine significance are discussed in Chapter-6. Finally the last Chapter-7 illustrates the strategies for the control of seed-borne viruses. Emphasis has been given to produce virus-free seed through seed certification schemes.

The objective and aim of presenting this publication is to overview and update the current knowledge related to seed-transmitted viruses and create awareness about these devastating plant pathogens (viruses) among the plant breeders, plant pathologists, virologists, plant protectionists, seed technologists, quarantine experts, and agencies concerned with seed production systems. In addition to these, several other groups of readers may find this book useful. It is hoped that this publication will have an impact in improving quarantine measures related to seed introduction, promoting crop health and production, and safe transfer of genetic resources. The errors and omissions will be welcomed to improve the next edition.

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Islamabad
July 15, 2000

Abbreviations

ALP	Alkaline phosphatase
AVRDC	Asian Vegetable Research and Development Centre
BARI	Barani Agricultural Research Institute
cDNA	Complementary deoxyribonucleic acid
CGIAR	Consultative Group on International Agricultural Research
DAC-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
DAS-ELISA	Direct antigen coating enzyme-linked immunosorbent assay
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
FAO	Food and Agriculture Organization
GDDT	Gel double diffusion test
HRP	Horseradesh phosphatase
IARC	International Agricultural Research Centres
ICARDA	International Centre for Agricultural Research in Dry Areas
ICRISAT	International Crop Research Institute for the Semiarid Tropics
IgG	Immunoglobulin
IITA	International Institute for Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
ISEM	Immunospecific electron microscopy
JICA	Japan International Cooperation Agency
NARC	National Agricultural Research Centre
PARC	Pakistan Agricultural Research Council
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PGRI	Plant Genetic Resources Institute
PNCE	Penicinillase enzyme
PNDP	P-nitropheny diphosphate
PVP	Polyvinly pyrrophosphate
RDT	Radial diffusion test
RIA	Radio immunoassay
SCRD	Seed Certification and Registration Department
SDS	Sodium dodecyl sulphate
SSEM	Serological specific electron microscope
TAS-ELISA	Triple antibody sandwich enzyme-linked immunosorbent assay
TBIA	Tissue blot immunoassay

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Chapter 1

Characteristics of Seed-borne Viruses

1.1 Introduction

One of the most characteristic and interesting relationships of plant viruses to their host plants is the high degree of protection possessed by embryos of seeds against invasion by viruses that affect the mother plant. Despite this protection, a considerable number of plant viruses have been able to cross this barrier and perpetuate from one generation to the next through seeds. In general where seed transmission occurs, it is usually limited to a relatively small percentage (2-5%) of the seeds to affected plants, but in certain cases the occurrence of high percentage (up to 100%) of seed transmission is reported. (Cooper, 1976). More than 300 plant viruses are reported to be seed-borne in one or more host species and the number of such reports continue to increase (Hampton, 1983).

Collecting, conservation and utilization of plant genetic resources and their global distribution are essential components of crop improvement programmes. International exchanges of germplasm and introduction of advanced breeding material to conduct regional trials is increasing day by day. The movement of germplasm involves a risk of accidentally introduction of plant quarantine pests along with the host plant material. The pathogens that are often symptompless, such as viruses, pose a special risk. In order to minimize this risk, rigurous and effective testing (indexing) procedures are required to ensure that distributed material is free of viruses that are of quarantine concern. Viral pathogens are especially difficult to detect and identify through routine quarantine procedures, because these pathogens need specialized skills and equipment under specific laboratory conditions. Unfortunately viruses cannot be eliminated from contaminated seed lots by seed treatment or other means. Additionally in "***growing on tests***" the seed-borne viruses may not express visible symptoms to be detected.

The exotic crop material is being evaluated directly by most of the researchers under field conditions without any testing for seed-borne viruses under greenhouse or laboratory conditions. This practice provides an opportunity to the virus(s) to be introduced in a new location. Once a virus is introduced and established, it becomes difficult to eliminate or eradicate. Thus, in order to avoid the introduction of new viruses through infected seeds in the country, there is an

urgent need to establish effective quarantine measures. Precise diagnosis of seed-borne viruses is pre-requisite both for pre and post-entry quarantine purposes. Detection and identification of the causative virus or viruses is the first requirement to develop strategies for the control and better management of viral diseases. In this chapter characteristics of seed-borne viruses have been discussed.

1.2 Characteristics of Seed-borne Viruses

In nature, the viruses which are transmitted through seeds are confined to a few plant virus genera such as Cryptovirus, Hordeivirus, Ilarvirus, Nepovirus, Potyvirus, Tobamovirus, and Tobravirus. All Cryptoviruses are seed transmitted with high frequency. On the other hand most members of some genera of plant viruses such as Luteovirus, Carmovirus, Carlavirus, Dianthovirus, Fijivirus, Phytoreovirus, and Tenuivirus, seem not to be seed transmitted, while for most members of other genera, seed transmission is very rare, e.g. Rhabdovirus, Closterovirus, Carlavirus and Geminivirus. The frequency of seed-transmitted viruses in various recognized virus genera is given in Table-1. The reasons why some viruses are seed transmitted and the others not, are not fully understood. Generally, the viruses which are restricted to the phloem tissue are not seed transmitted. Nevertheless, seed-transmissibility of any individual virus is often host specific, sometimes specific to strain of the virus, and can be temperature dependent. In general the viruses that are seed transmitted have some characteristics more or less common. These characteristics are as follows:

a. Most seed transmitted viruses are readily sap transmissible to one or more host plants, indicating an ability to invade parenchyma tissue.
b. The symptoms of most of the seed transmitted viruses include mottling, local necrotic or chlorotic lesions, etch, and other abnormalities in the parenchymatous tissue.
c. Most of the viruses transmitted by aphids in non-persistent manner are seed transmissible.
d. Viruses that tend to reach high concentrations in host plants e.g. Capilloviruses, Hordeiviruses, Necroviruses, Potexviruses, Tobamoviruses, Tombusviruses and Tymoviruses are often seed-borne in some hosts.
e. Viruses transmitted by leafhoppers or plant hoppers e.g. Fijiviruses, Geminiviruses, Marfaviruses and Phytoreoviruses) or by aphids in a persistent manner e.g. Lueoviruses and Rhabdoviruses are not seed transmitted.
f. Viruses transmitted by nematode vectors (Nepoviruses) appear to be more often seed transmitted than those transmitted by other types of vectors.
g. There is no relationship between seed transmission and particle morphology or nucleic acid type.

Table-I: Frequency of seed-transmitted viruses in various recognized virus genera

Virus Group.	Number of Members	
	In Genus	Seed-Borne (%)
Alfamovirus	1	1
Bromovirus	5	1
Carlavirus	47	2
Caulimovirus	7	0
Closterovirus	15	1
Comovirus	15	6
Cucumovirus	4	4
Dianthovirus	3	0
Geminivirus	16	1
Hordeivirus	3	2
Ilarvirus	13	8
Luteovirus	29	0
Nepovirus	30	22
Potexvirus	38	4
Potyvirus	117	16
Rhabdovirus	74	1
Sobemovirus	10	2
Tobamovirus	18	7
Topovirus	1	1
Tymovirus	18	3
Viroids	15	5

Based on 4th Report of the International Committee on Taxonomy of Viruses (Matthews, 1982)

1.3 Terminology

The following terms in the text are defined according to Neergaard (1977).

Seed-transmitted: Seed-transmitted viruses are those that are transferred from one place to the other through the agency of the seed and cause infections of the plant produced by germination of such seed.

Seed-borne: Seed-borne viruses are carried in, on, or otherwise within the seed.

Infected seeds: Infected seeds are those in which the virus has penetrated into the tissues of the seed and is often in a resting stage.

Contaminated (infested) seeds: Contaminated (infested) seeds are those in which the virus is carried adhering to the external surface of the seeds.

1.4 Frequency of Seed Transmission

Fortunately majority of the plant viruses are not seed transmitted in most host plant species, and those which usually infect majority of seeds are transmitted at a very low rate except in few cases. Frequency of seed transmission greatly depends on virus, virus strain, host species, host cultivar and other factors, which influence seed transmission. Extent of seed transmission varies with particular host-virus combination ranging from 0 to 100%. Different viruses have different rates of seed transmission in different host genotypes. For example, Inouye (1967) reported 30% seed transmission of pea seed-borne mosaic virus (PSbMV) in pea cultivars Oranda, 10% in Sanjunichi-Kunsusaga and over 20% in New Season and Perfected Wales. Seed transmission rate of PSbMV has been reported as high as 100% in some pea introductions of USA (Hampton, 1983; Cooper, 1976). An example of very low rate of seed transmission is sugarcane mosaic virus (SCMV) in corn: 0.1 to 0.4% (Shepherd and Holdeman, 1965). The rate of seed transmission of each seed-borne virus reported in different parts of the world in one or more hosts is presented in Appendix-1.

1.5 Factors Affecting Seed Transmission

Many factors such as temperature, host plant species or cultivar, virus or virus strain, time of infection and location of seed on the seed-bearing plant may affect the rate of seed transmission.

1.5.1 Temperature

The effect of temperature on seed transmission is well documented. For example cherry leaf roll virus (CLRV) was transmitted to 100% and 0% of seed from *Nicotiana rustica* plants grown at 20°C and 30°C respectively (Copper, 1976), whereas 95% and 55% seed transmission of southern bean mosaic virus (SBMV) was obtained in plants grown at 16 to 20°C and 28-30°C respectively (Crowley, 1959). Hanada and Harrison (1977) found that the effect of temperature on seed transmission was different not only between Nepoviruses but also between strains of the same virus. The optimal temperature for seed transmission of soybean mosaic virus (SMV) and growth of soybean plants was 20°C, whereas virus concentration was highest at 25°C (Tu, 1992). In this case the temperature may effect the rate of virus inactivation during seed maturation, as the SMV seed transmission depends on virus inactivation (Irwin and Goodman, 1981).

1.5.2 Virus-host Adaptation

Successful virus-host combination facilitates survival of the both. Symptom expression depends on the virus strain/host cultivar combinations. The isolates that

induce mild symptoms (better adapted) are more successfully seed-transmitted than those isolates which induce severe symptoms (Ghanekar and Schwenk, 1974).

1.5.3 Time of Infection

Time of infection also influence the rate of seed transmission. If early infection occurs, there are more chances of infection of ovules before fertilization than infection after flowering or fertilization. Tobacco ring spot virus (TRSV) in soybean may be transmitted through 100% of the seed if the plants are infected before flowering, but transmission is low (10-15%) if infection occurs after flowering (Crowley, 1959). Seed transmission thus appears to require that embryo be infected at early stage of development.

1.5.4 Virus Strains

Different strains of the same virus differ in rates of seed transmission in the same host species. Different ability to be seed transmitted of barley stripe mosaic virus (BSMV) strain has been observed in various cultivars of barley and wheat (McKinney and Greeley, 1965). In this study seed transmission of the different strains of BSMV varied from 0% to 53%.

1.5.5 Disease Symptoms

Generally seed-borne viruses induce mild symptoms in their hosts, however some virus–host interactions cause severe symptoms that may limit transmission through seeds. Severe virus infection may affect on flower development and result decreased seed set and restrict seed transmission e.g. Bean common mosaic virus (BCMV) causes malformation of the inflorescence to leaf-like structures (i.e. phylloidy) in *Phaseolus mungo* resulting reduced seed set (Agarwal et. al., 1976). Symptoms in seedling developing from infected seed range from very mild to severe, depending on the virus strains, host cultivar, and environmental conditions.

1.6 Longevity of Viruses in Seeds

The seed infection rate of the viruses that are carried in the pulp debris, or in the seed-coat, perisperm, and endosperm, usually declines with the passage of time. For example, infection with tobacco mosaic virus (TMV) in tomato seeds declined rapidly after one year of storage, although it was detected after 3 years (Alexander, 1960). Similarly, cucumber green mottle mosaic (CGMoV) virus infection of cucumber seeds decreased rapidly during the first 7 months of storage (Van Koot and van Dorst, 1959). Most viruses that infect embryo, and are truly seed-borne, may persist in seeds for a longer time or as long as the seeds remain viable. An interesting example is of bean common mosaic virus (BCMV), which was isolated

from bean seedlings when grown from infected seeds after 30 years of storage (Pierce and Hungerford, 1929). Wilson and Dean (1964) reported that flour of infected bean seeds was a good source of inoculum even after storage of 31 months at –20° C.

1.7 Genetics of Seed-transmission

Host and virus interaction is an important factor, that controls the capacity and extent of seed transmissibility of a virus in a host. Carroll et. al. (1979) studied inheritance of resistance of BSMV in barley lines resistant to seed transmission, they concluded that resistance is controlled by a single recessive gene in barley genotypes. Medina and Grogan (1961) reported monogenic dominance or recessiveness of BCMV in bean host.

1.8 Symptoms Expressed by Seed-borne Viruses

One of the most characteristic features associated with seed transmission is the ability of embryos, infected in the very early stage of development, to survive, grow and mature into plants that are usually productive and often produce normal plants. Moreover, the degree to which young seedlings show symptoms varies with different viruses and different host plants. In some cases the symptoms are quite visible at very early stage of growth. Symptoms of bean common mosaic virus (BCMV), blackeye cowpea mosaic virus (BlCMV) and cowpea aphid-borne mosaic virus (CABMV) usually appear on the first primary leaves when the virus is transmitted through seed **(Fig. 1.1).**

Symptoms production is influenced by temperature, at which seedlings are grown and also by light intensity. Some viruses respond to show symptoms at high temperature, whereas the others need low temperature to express symptoms e.g. barley seeds infected with barley stripe mosaic virus (BSMV) show better symptoms at 75° F to 100° F, whereas seedlings from tobacco seeds infected with tobacco ring spot virus (TRSV) do not show symptoms unless grown at low temperature. In general the seedlings from virus infected seeds show symptoms that are similar to those shown by naturally infected mother plant.

1.9 Economic Importance

In the past a few viruses were known to be seed transmitted, therefore, little importance was given to these viruses. The rapid increase in the number of seed

Figure 1.1: Symptoms expressed in cowpea seedlings emerged from virus-infected seeds under growing-on test (a) BICMV and (b) CABMV.

transmitted viruses has caused a great concern of introduction of new viruses in different geographical regions of the world, therefore seed-borne viruses are given more importance than others. Even a low rate of virus transmission through seed can be disastrous to the crop and yield losses may be as high as 100% if the insect vectors in the field play an efficient role in spreading the disease. The economic implications of infection from seed depend on crop sensitivity, virus virulence, and of course, the chance of spread from infected seedlings. The other reasons for their economic importance are as follows:

1. Seeds remain viable for a longer period than the vegetative propagules, and this prolongs the potential transmission period and thus the possibility of pathogen dissemination.
2. Seed transmission provides a very effective means of introducing a virus into a crop at an early stage, providing initial foci of primary infection throughout the field.
3. In some cases, where seed transmission may be of little or no importance on the immediate crop it may still have importance in the transport and establishment of viruses over long distances.
4. In cases of very high percentages of seed transmissions, these viruses would be expected to persist several generations, even in the absence of a vector, which might add to their chance of becoming established in any area where a vector exists.
5. The viruses transmitted through seed facilitates interregional and international dissemination of viruses of exotic virus strains.
6. In certain cases these viruses also affect the seed quality and germination. Under greenhouse conditions there was a reduction of 31 to 35% in germination and reduction of 45 to 68% in yield of seeds in alfalfa infected by alfalfa mosaic virus (AMV) (Hemmati and McLean, 1977).
7. Although obviously any virus that is seed transmitted has a potential for being transported long distance in seed, nematode transmitted viruses would appear to have greater potential for this type of dissemination. Because nematode transmitted viruses attack a wide range of plant species with large percentage of diseased seeds.

1.10 Epidemiological Significance

Seed transmission of viruses is of great epidemiologically importance because it helps the viruses to cross over and perpetuate during unfavourable environmental conditions. Seed transmission may be the single most source of virus carryover from one season to another and for initiation of spread in the following season for some crops. The viruses which are disseminated through seeds have several advantages

for spread and survival. The viruses that have either no alternate hosts or sedentary types of vectors such as nematodes are of particular importance being the only mode of survival through seed. In these viruses although the transmission frequency is very low, but they play an important role in providing primary source of inoculum to initiate disease in a particular field.

One of the most obvious functions of seed transmission is the survival of viruses during periods unfavorable for growth of the host or of activity of a natural vector. The virus remains active for a longer period in seeds and increases chances for subsequent spread to new host plants. Seed-borne inoculum provides optimum distribution of disease within the crop.

Seed-borne viruses also infect perennial plants, retain virus for dispersal by animal vector. Seed infected with seed-borne viruses are usually dispersed at random during planting. The spread of virus by aphids from multiple foci of infected seedlings can be rapid and efficient. In Iran spread of bean yellow mosaic virus (BYMV) within field trials of faba bean took place from 0.2% seed-borne inoculum and led to 58% in a trial under field conditions (Kaiser, 1973). Seed transmission is responsible for the establishment of viruses in isolated geographical areas. Birds and other animals which ingest infected seeds probably contribute also to the spread of seed-borne viruses.

Chapter 2 Mechanism of Seed Transmission

2.1 Introduction

Although some viruses have the ability to be seed transmitted, but a large number of viruses lack this characteristic of seed transmission. It is not fully understood why some viruses are seed transmitted while the others are not? Viruses that are restricted to the phloem tissue cannot enter the seed, because the phloem of the mother plant and the embryo are not connected directly. With other viruses the possible explanation is that they cannot replicate and/or are degraded in meristematic tissues as the seed develops and matures. In order to understand the mechanism of seed transmission, the following three major problems related with transmission of viruses through seed require explanation:

1. Why developing embryos are not infected even by seed-transmitted viruses except in the early stages of their development?
2. Why such a great variation in percentage of seed transmission occurs among the seed transmitted viruses?
3. How all the viruses not seed-transmitted are excluded from both the gametes and young developing embryos?

The lack of plasmodesmatal connections between the developing embryo and adjacent cells may explain the inability of so many viruses to infect this tissue (Bennett, 1969; Carroll, 1972). No plasmodesmata were found in the outer wall of field bean embryos at about the twelve-celled stage (Vorra-Urai, 1974). It is not known how the virus moves into the embryo, but the contact point between the testa and the suspensor was suggested as a likely route of entry (Wang and Maule, 1993). Some seed transmitted viruses can infect the embryos of their host either by way of the pollen (and presumably by way of the egg cells also) or, to some extent, by infecting the young embryo in the early stages of its development (Crowley, 1959). In some cases the virus is inactivated during seed maturation process due to inability of virions to infect megaspore mother cell. Erratic distribution of viruses in meristemmatic cells may explain the differences in the rates of seed transmission for different host-virus combinations. Unless the viruses survive the processes associated with seed formation, maturation and storage, they are not likely to be seed-transmitted. Inactivation of viral infectivity in seed coats during maturation has

been reported for cowpea chlorotic mottle virus (CCMoV) (Gay, 1969) and southern bean mosaic virus (SBMV) (McDonald and Hamilton, 1972). There is evident that CCMoV is not seed-transmitted in cowpea because of inactivation during seed maturation process. Infections of CCMoV was recovered from the sepals, petals, stamens and pistil, but not from the seed coats of the seeds ranging from immature to mature, but not dry. No infective virus was found in the endosperm or embryo of seeds at any stage of seed maturation (Gay, 1969).

2.2 Mechanism of Seed Transmission

The phloem limited viruses, that are transmitted in a persistent manner by insect vectors are not seed-transmitted. They cannot get into the embryo of the seed. The same is true in case of mycoplasmas which, in plants, behave like phloem-limited viruses. The viruses are transmitted through seed in two ways. One is by small group of viruses such as tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), and cucumber green mottle (CGMoV) viruses. Although these viruses occur in high concentration and are very stable, but cannot enter the embryo. They infect the integuments and nucellar tissues, which later develops into the seed coat. These viruses remain ineffective after the maturation of seed. The phenomenon is still not clear, why such stable viruses cannot get into the embryo? Such viruses can be removed by heat and chemicals. They pass through the seed coat or fruit pulp into the seedlings when handled at transplanting.

The viruses, which include a large group of seed-borne viruses, and infect plants systemically, can enter the ovule via vascular bundle. Whether they are able to pass via seed to the offspring depends on a number of factors (Bos, 1989b). The viruses that enter the embryo can only do so if infection takes place before the egg cell is fertilized or before pollination. Because after pollination and fertilization, the contact between embryo and mother plant through plasmodesmata is disrupted. Such viruses can also enter the embryo on a virus-free mother plant via pollen. Once the embryo is infected, the viruses remain there as long as the seeds remain viable during storage in the gene banks. The possibility and extent of seed transmission differs according to virus and strains, and depend highly on the host species and cultivars, temperature my also play a great role.

2.2.1 Seed Anatomy in Relation to Virus Transmission

Although there is great variation in anatomical details among seeds, there are a number of generalized features significant in seed transmission of viral pathogens. The ovule (which later becomes the seed) differentiates from the ovarian placenta to which it is attached by the funiculus. **(Fig. 2.1).** The outermost layers of the ovule,

the integument, encloses the nucellus, the central meristematic tissue in which the megaspore is formed. The ovule may be loosely embedded in a fruit (e.g. a pod), or have closely adherent fruit coats (e.g. an achene or caryopsis). All of these structures are from the sporophytic mother tissues and, except for the megaspores, remain in the diploid conditions, and probably are cytopasmically connected to the mother plant. It is not surprising therefore, the virus moves into the seed and fruit coats, nucellus and sometimes the embryo sac formed from the megaspore (Crispin and Grogan, 1961). There is a great possibility of the existence of cytoplasmic connections between the plant and developing pollen grains than between the plant and developing egg cells. This is in accord with the fact of greater transmission of BCMV through pollen than through egg cells (Crispin and Grogan, 1961). If cytoplasmic connections occur between the nucellus and the embryo sac, they would permit virus entry, but one may speculate that endogenously formed egg cells lack cytoplasmic connections with nucellus, thus sealing off the egg from virus invasion. If a virus enters the embryo sac before the membrane of the egg cells is formed the pathogen could be included in it, and the embryo would be infected. The presence of virus in the embryo does not always result in seed transmission (Gold et. al., 1954).

When pollen grain germinates on the stigma, it develops a pollen tube that grows through the style into the embryo sac through the opening (micropyle) between the integuments, and releases therein two male gametes. One of these gametes unites with the egg cell and if it carries the virus, the resulting embryo may be infected. The other gamete unites with polar nuclei, and if it carries the virus, the endoplasm that is formed may be infected. Then the enlarging and differentiating zygote develops into embryo and the triploid embryo sac into endosperm. During development of seed, food may be stored in the perisperm (nucellar tissue) or in the endosperm, or translocated into embryo, especially in cotyledons.

During rapid development embryonic and endosperm tissue within the nucellus, protoplasmic bridges between these tissues (plasmodesmata) and the nucellus may be ruptured. When the seed further develops, integument and nucellus remnants form the seed coat (testa). They desiccate during maturation, and finally consist of dead tissues. Although virus-infected pollens may produce seed infection (Callahan, 1957), in some cases it may cause plant-to-plant transfer as well (Gilmer and Way, 1963).

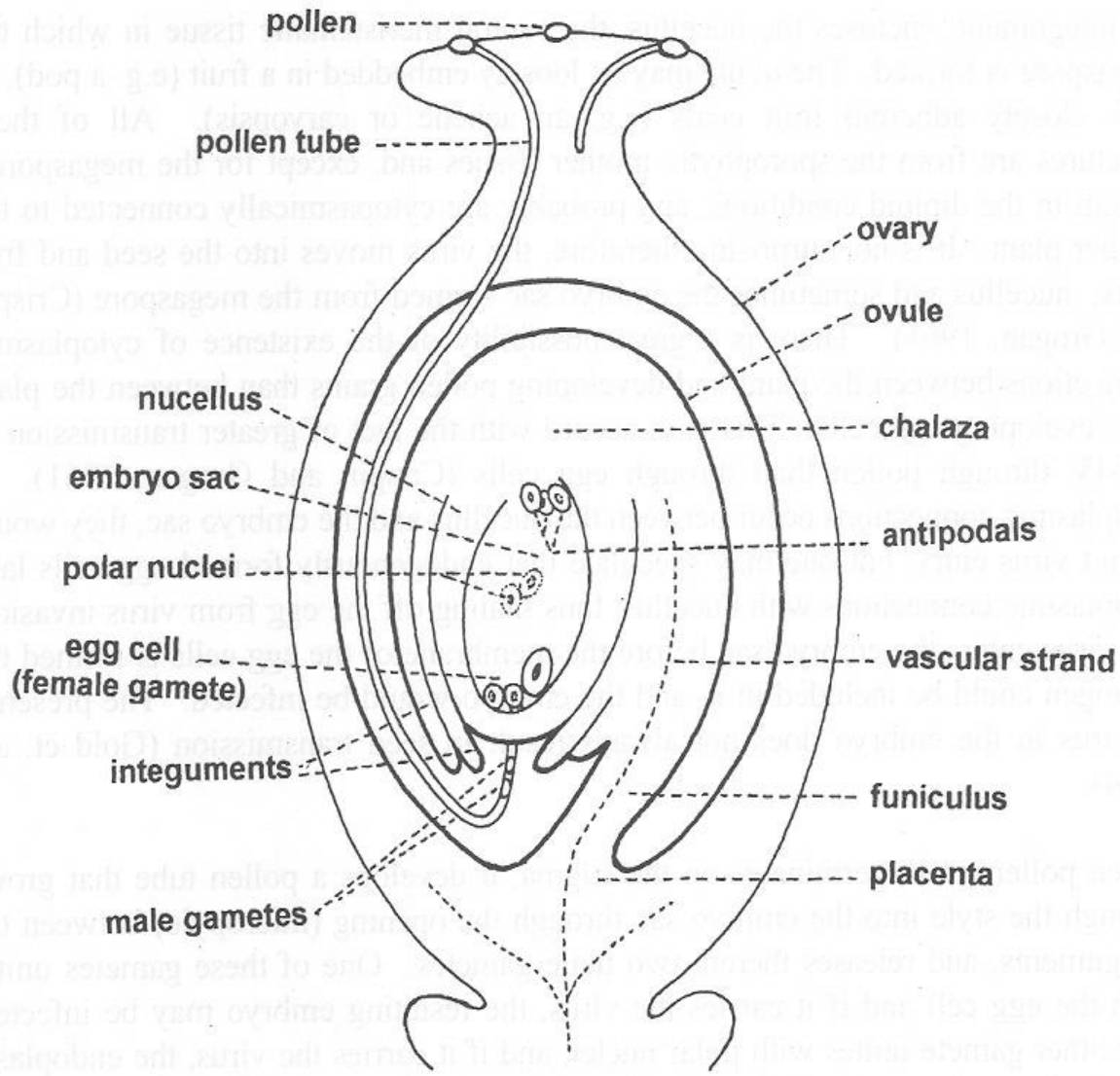


Figure. 2.1: Seed anatomy in relation to virus transmission.

2.2.2 Mode of Embryo Infection by Virus

In most of the cases seed transmission is dependent on infection of the embryo at some stages of its initiation or development. Embryo infection by virus may occur in one of the following two ways:

2.2.2.1 Direct Embryo Infection

Wang and Maule (1992) reported direct invasion of the embryo in the pea cv. Vedette. In this case pollen transmission of PSbMV did not occur. However, it is not known how the virus enters into the embryo. It has been suggested that the contact point between the testa and the suspensor is the likely route of virus entry. The cell wall at the contact point is very convulated but no plasmodesmata have

been observed. They suggested an unidentified mechanism of infection that does not require plasmodesmata, thus allowing direct invasion of the embryo.

2.2.2.2 Indirect Embryo Infection

Through ultrastructural studies virus has been observed in the megaspore mother cell and egg, or in pollen mother cells and pollens (Carroll and Mayhew, 1976a ; Carroll and Mayhew, 1976b). For example, pollens of alfalfa and tobacco were observed with AMV and tobacco rattle virus (TRV) infection respectively. Both viruses are transmitted via virus-infected pollens (Frosheiser, 1974; Gaspar et. al., 1984). Several other instances of pollen transmitted viruses have been reported e.g. BSMV, BCMV, southern bean mosaic virus (SBMV) and prunus dwarf virus (PDV) are examples of pollen transmitted viruses. It seems logical that a virus that can invade male gametes should also be able to invade female gametes. The seed transmitted viruses are capable to infect ovules. In great majority of cases, ovule or embryo sac infection, by one means or other is essential for seed transmission. Nelson and Down (1933) concluded that seed transmission of BCMV depends on the ability of the virus to reach the ovule before or shortly after fertilization. Although seed transmission is dependent on infection of the embryo in the very early stage of its development by virus, some evidence indicates that this may not necessarily be true in all cases. Some viruses are able to invade the developing embryos, possibly without first having invaded ovules or embryo sac. In case of BSMV the virus was able to infect embryo even after it has reached a stage of maturity. However, such mode of infection is rare. Seed-borne TRSV was observed in megasporophote as well as in pollen of soybean (Yang and Hamilton, 1974). Seed transmission of SBMV was determined by the ability of SBMV to invade male and female reproductive meristems very clearly in their development, therefore infecting the embryo indirectly (Carroll, 1981).

2.3 Location of Virus in Seed

The viruses infecting seed are either present on the seed surface (externally seed-borne) or are present in the embryo (internally seed-borne).

2.3.1 Externally Seed-borne Viruses

Seeds from fleshy fruits (like tomato, cucumber and watermelon) systemically infected plants may have the viruses stuck to their external surfaces (confined mainly in the seed coats) and may or may not produce infected seedlings. Only very stable viruses like tobacco mosaic virus (TMV) remain viable when carried as contaminants on the seed surface. In such cases seedlings infection takes place by

mechanical contamination during handling and seedling (Broadbent, 1965). TMV remains active and viable even up to three years on tomato seeds derived from diseased plants. This type of virus transmission probably plays no significant role in spread of virus diseases.

2.3.2 Internally Seed-borne Viruses

Majority of the seed transmitted viruses are located in the endosperm/or embryo of infected seeds. Viruses present in embryos are BSMV in barley seed, SBMV in cowpea seeds, BCMV in bean seeds, CMV in cucumber seeds, peanuts tripe virus (PStV) in peanut seeds, PSbMV in pea seeds and several other viruses. Virus multiplication within the embryo is presumably reinitiated when metabolism of infected cells increases at germination (Hoch and Provvidenti, 1978). BCMV, BSMV, pea streak virus (PeSV) and possibly several other viruses indentically located are present in seed coats of immature seeds, but absent at maturity. These viruses are inactivated during seed maturation process. Only embryo-infected seeds grow into infected seedling. Viral access to the embryo is gained either directly or indirectly by infection of reproductive tissues before embryogenesis (i.e. ovule, megaspore mother cell, and pollen mother cell) or directly by invasion of the embryo during some stage of embryogenesis. The mechanisms by which these routes of virus entry are determined are not yet clear.

2.4 Inoculum Threshold of Seed-borne Viruses

New viruses are continually being discovered, and several of these are seed-borne. It would not surprise us if, with further research close to one-third of the recognized viruses were shown to be seed-borne in at least one of their hosts. The term "***inoculum threshold***" is not used to any extent by plant pathologists. "***Inoculum***" is widely used and well understood word, not only with respect to viral pathogens, but also for fungal and bacterial pathogens. The entomologist in relation to insect infestation uses "***Threshold***". When the word "***threshold***" is used, it is normally combined with a second word to form such phrases as "***damage threshold***", "***action threshold***", "***control threshold***", "***detection threshold***", - as in virus concentration necessary for detection by ELISA etc (Zadoks, 1985). These terms are used in relation to assessment of crop loss, which are sometimes difficult to define. Here we will combine the terms "***inoculum threshold***" with "***damage threshold***" to convey the concept of the maximum amount of inoculum that can be tolerated without an appreciable constraint to yield and its limitation of economic return.

Doolittle and Gilbert (1919) identified two significant aspects associated with seed-borne viruses that may act as constraints to potential yield: (a) seed transmission

provides a strategy whereby the virus can survive under conditions in which other sources of inoculum have been eliminated and (b) the seed-borne inoculum creates centres of infection from which the virus can be vectored-transmitted to nearby cultivated plants. Seed infection plays an important role in survival of inoculum, spread of inoculum, and source of inoculum in the initial stage of disease development. Some workers tried to collect data that would provide an indication of the maximum amount of seed-borne virus inoculum that could be tolerated without an appreciable loss to yield. But this aspect has been studied with only a few host-virus combinations. These few cases have several characteristics in common:

a. Level of seed transmission does not have to be high; infact, it can be exceedingly low and still be of critical importance when the few infected seedlings arising from contaminated seeds contribute the sole source of inoculum and when the virus is readily acquired and actively vectored in a crop

b. The crop must be annual, because infected seeds usually would not be the sole inoculum source in perennial crops

c. The vector is an aphid and the virus is transmitted in a non-persistent manner

d. The virus is confined to a narrow natural host range. Viruses meeting these criteria would have an " ***inoculum threshold***" of zero or close to zero.

Chapter 3

Seed Health Testing

3.1 Introduction

The seed health and quarantine implications of international and adaptive testing of plant genetic resources represent a major concern to international agricultural research centres and other institutes involved in such efforts. It is evident that germplasm collections carries with it the risk of long distance dissemination of viruses that may be harbored in or on the plant or seed material that is transported. The worldwide exchange of germplasm material involves the risks of introduction and spread of a great number of viruses. Even if the viruses involved are already present, the exchanges can contribute to the accumulation of several strains of the same virus or a new virus where ecological conditions are suitable.

The presence of seed-transmitted viruses in crop germplasm needs special attention at initial stage if the disease has to be controlled at an elementary level. This is because that seed-borne virus can be introduced in contaminated seed lots to as yet uninfected countries. Sometimes plant quarantine regulations prohibit importing of plants and plant parts from specified countries because of the occurrence of pests and pathogens of quarantine significance in these countries. All countries do not have effective quarantine regulations to restrict the introduction of exotic viral pathogens through seed. In the international transfer of germplasm, viruses are generally the most difficult to detect and control, and thus constitute potential quarantine hazards. Sudden outbreaks of so-called new virus diseases may occur if the indigenous general conditions are favourable to the disease and available genotypes are susceptible. In order to avoid such situations, inspections should encompass more viruses and the detection techniques that are used could be improved. The sanitary procedures pertaining to viruses are followed to prevent introduction of new viruses through imported material.

The basis of quarantine is to delay, and hopefully to prevent the spread of pathogens from one continent, or one region to another. The quarantine laboratories should offer rapid and efficient service by employing ELISA in their routine tests. This would permit the immediate subjecting of the seed lots to screening and would avoid unnecessary delay in their release to identors. Since the detection of viruses of exotic origin (i.e. viruses not known to occur in the country) is one of the main targets of quarantine, therefore, use of ELISA is largely dependent on the availability of corresponding antiserum from foreign countries.

3.2 Germplasm Contamination by Seed-borne Viruses

Due to increased demand and utilization of crop germplasm by the breeders at national and international level, several instances of contamination of germplasm by seed-borne viruses have been documented. Lister (1978) reported contamination of world-wide-collected soybean germplasm with soybean mosaic virus (SMV). Similarly outbreak of pea seed-borne mosaic virus (PSbMV) in pea (*Pisum sativum*) germplasm was observed (Hampton and Braveman, 1979). A strain of PSbMV was also found in lentil (*Lens culinaris*) germplasm (Hampton, 1982). Bean common mosaic virus (BCMV) and cucumber mosaic virus (CMV) were detected in common bean (*Phaseolus vulgaris*) germplasm (Davis et. al.,1981; Klein et. al., 1988; Khetarpal et. al., 1994). Urdbean leaf crinkle virus (ULCV) was detected in mung bean (*Vigna radiata*) in India and in mash in Pakistan (Bashir et. al., 1991), cowpea aphid-borne mosaic virus (CABMV), blackeye cowpea mosaic virus (BlCMV), southern bean mosaic virus (SBMV), cowpea severe mosaic virus (CSMV) and CMV were detected in cowpea germplasm (Hampton et. al.,1992; Gillaspie et. al., 1995; Bashir and Hampton, 1996b). By using ELISA a few more viruses have been reported in germplasm collections and breeding lines e.g. peanut mottle virus (PMoV), peanut stripe virus (PStV) in peanut, and a strain of CMV in bean (Bharathan et. al., 1984). Bashir and Hampton (1996b) reported contamination of Plant introduction (PI) lines of cowpea with CMV, BlCMV and CABMV being maintained in Plant Introduction Centre, Griffin, Georgia, USA, while CMV and PSbMV in exotic lentil germplasm (Bashir et. al., 1995). Mehmood et. al., (1996) detected PSbMV in pea germplasm maintained in Pakistan. Recently BlCMV and CABMV have been reported from imported seeds of cowpea from Nigeria (Bashir et. al., 1999).

3.3 Introduction of Viruses Through Infected Seeds

The examples of the exotic viruses introduced through infected seeds are as follows: squash mosaic virus (SqMV) was introduced in USA by seeds from Iran (Leppik, 1964) and to New Zealand from the USA in seeds of *Cucmus melo* (Thomas, 1973). Seeds imported from Sweeden gave rise to PSbMV-infected plants in quarantine in Tasmania, Australia (Munro, 1978). Broad bean stain virus (BBSV) was detected in Australia in seedlings of *Vicia faba* raised from seeds imported from the United Kingdom (Randles and Duke, 1977). Guar symptomless virus (GSV) has been introduced to the United States from India through infected seeds of Guar (*Cyamopsis tetragonobla*) (Hansen and Laseman, 1978). Sometimes, there may be multilateral transfer of seed-borne viruses along with the exchange of germplasm.

3.4 Sampling Procedures for Seed Health Testing

The estimation of the level of seed-borne virus in a seed lot is based on direct examination of seedlings (i.e. presence of visible symptom or assay for the presence of virus or antigen) or by direct assay of seed extracts for the presence of virus or antigens. Both these methods require an appropriate sampling procedure to know the infection levels in a particular seed lot.

To decide the sample size for testing the presence of virus is complicated, particularly in viruses that are transmitted at very low rate (less than 1%). The tolerant limit could reach 1% for seed sown in regions with low vector intensities early in the season. On the other hand, the region with high vector intensities would experience major yield loss if more than one seed in 10,000 is infected. In case of lettuce mosaic virus (LMV) in lettuce, a tolerant limit of one infected seed out of 1000 has controlled this disease satisfactorily in France (Marrou and Messiaen, 1967). In California, the limit chosen was five times lower, a test giving zero infected seeds in 30,000, implies for a seed lot a 99.9% probability of having less than 0.022 infection. This limit gave excellent control of the disease (Grogan, 1980). Geng et. al., (1983) described two techniques to determine the sample size and number of tests to be carried out to have a high confidence of detecting contaminated seed lots if the disease incidence in the seed lot is above the defined tolerant limit. These two techniques are as follows:

3.4.1 Statistical Approach

While dealing with groups of seeds, there is no restriction on the number of seeds per group when there is a constant detection of one infected seed in the group. There should be chances of detecting one infected seed in 1000, while working with large groups of seeds. When this probability (assay sensitivity) is established, the test can thus be performed with reduced number of groups. For assays very practical charts help in determining the number of seeds per group and the number of groups at various levels of assay, sensitivity and tolerance limits (from 0.01 to 1%) (Maury and Khetarpal, 1989).

3.4.2 Technical Approach

This approach employs the use of indirect ELISA also called antigen coating plate ELISA (ACP-ELISA), where a universal antibody conjugate is commercially available. This is an important convenience of this technique. In this case the seed extracts (antigens) are directly applied to the wells of the microtiter plate (Lommel at. al., 1982). This procedure requires highly specific polyclonal antiserum. Monoclonal antibodies can also be used in this procedure (Wang et. al, 1984).

Tests on whole seed can be performed as a first approach when the probability of

seed lots being healthy is relatively high. Seeds in a group of 5 to 10 are ground to have their extract for ELISA test. If the seeds are of large size (e.g. peanut), a portion of seed can be tested to determine the seed infection.

3.5 Testing Seeds for Virus Detection

Correct identification of seed-borne viruses is a fundamental step in any method adopted for control of a virus disease or for certification purposes. Significant progress has been made for rapid identification of viruses in leaf and seed tissues. Most of these diagnostic methods depend on the detection of virus antigens (i.e. coat protein), whereas hybridization tests permit the detection of specific viral nucleic acid by the use of isotope or non-isotope labeled complementary DNA (cDNA). Testing of progeny seedlings is the only way of determining if seed transmission occurs. However, infectivity assays or serological tests conducted directly on seeds are often used to detect seed-borne infections. Various methods that are commonly used for detecting seed transmitted viruses are discussed below:

3.5.1 Visual Examination

There are some reports on externally visible seed abnormalities associated with virus infection of seeds. For example, seed coat mottling in soybeans is associated with soybean mosaic virus (SMV) infection in the seed (Phatak, 1974; Ross, 1970) as compared with normal whitish seeds. Abnormal seeds have black or brownish-red bands or zones radiating from the long axis of the hilum. Bands are more or less symmetric on both sides of the hilum and are solid with streaks on the outer margins. Some seeds show only diffuse grayish zones. Similarly, the cracking of seed coat in pea seeds is due to PSbMV infection (Stevenson and Hagedorn, 1970). Some workers reported that seed transmission is often associated with latent infection, and is notably prevalent with nematode-transmitted viruses (Lister and Murant, 1967). In such cases, infected seeds are also normal in appearance. Consequently, seed-coat abnormality is not always indication of the virus-infection; further tests must be performed to confirm the results.

3.5.2 Growing-on Test

This test is based on virus symptoms appearance in the seedlings emerging from virus-infected seeds. The classical example of such a test adopted in routine testing is that of LMV in lettuce seeds. In this test, called "*controlled environment room test*", seedlings are grown in a controlled chamber or greenhouse and the presence of virus is determined after 2-3 weeks, when the seedlings express virus symptoms **(Fig. 3.1).** The major disadvantages of this test are the following:

Figure 3.1: Germplasm evaluation for seed-borne viruses by growing on test (a) screening of groundnut and (b) lentil germplasm.

1. It takes a long time to standardize growing-out test in order to obtain reproducible symptoms.
2. A lot of controlled room/greenhouse space is required.
3. The symptoms may be confused with deficiency symptoms in growing seedlings.
4. The possibility of having a latent infection (symptomless).

In this test the seeds to be tested are grown in pots filled with sterile soil in an insect-free greenhouse. The seedlings are observed for virus symptoms. The presence of virus is confirmed either by serological or infectivity tests.

3.5.3 Infectivity Test

In this test the presence of virus is assayed by inoculating extracts of seed or seedlings on indicator plants such as *Chenopodium amaranticolor, C. quinoa, Phaseolus vulgaris, Vigna unguiculata, Datura stramonium* etc. This method could also reveal the symptomless or latent infection of plants as in growing-on test. In case of LMV, this test was found very sensitive as even one infected seed in a sample of 700 lettuce seeds give positive reactions (Marrou et. al., 1967). Group analysis has been developed for the elimination of seed lots infected above the threshold value 0.1% and standardized as critical method for certification of lettuce seeds (Marrou and Messiaen, 1967). In group analysis, 10 samples of 700 seed each, are inoculated on young leaves of *Chenopodium quinoa* at 4-6 leaf stage. The inoculated plants are kept in the greenhouse or in growth chambers at 25°C under a photoperiod of 16 hours for 4-15 days. The plants are examined after 5-12 days for symptoms that may appear on inoculated leaves in form of chlorotic local lesions. Virus concentration is assessed based on the number of local lesions appeared. However, this test has the following limitations:

1. It needs a span of time to standardize the indicator hosts and to record the symptoms.
2. A lot of controlled environmental chamber or greenhouse space is required.
3. The test is laborious and time consuming for working with large samples for routine testing.

3.5.4 Electron Microscopy (EM)

Electron microscopy (EM) has been used to detect virus particles in pollen, pistil, ovaries and parts of seeds, such as endosperm and embryos either in

tissue homogenate or directly *in situ* in ultrathin sections. EM may not be the most sensitive test, as Phatak (1974) could not detect particles of LMV in extracts on infected lettuce seeds. He could detect particles of BSMV, BCMV, and SMV in extracts of seeds of plumules of their respective hosts. EM is not used for routine testing of seed samples. However, in conjunction with other tests, the electron microscope examination of sap (chop method) in combination with negative staining remain a very rapid and useful technique for checking infection by viruses.

Chapter 4 Serology in Virus Detection

4.1 Introduction

Serological tests are the most important assays for detection and identification of seed-borne viruses. Such tests are based on the binding capacity that individual antibody has for its own specific homologous antigen. When warm blooded animals are injected with a foreign protein (or virus) it stimulates the production of antibodies in animal blood. The antibodies thus formed are immunoglobulin (IgG) which combine with special part of virus at some specific amino acid sequence (the antigenic site) called *antigenic determinant* or *epitope*. Blood serum containing such antibodies is called "*antiserum*". Antibodies in this serum will bind with the homologous antigen to produce a precipitate and this is the basis of serological tests for virus detection. Serological tests are very specific and reliable since antibodies will only react with their specific antigens.

4.2 Serological Tests

The most significant advances in virus testing have been made in the field of serodiagnosis. The specificity of the antigen/antibody relationship provides an extremely versatile tool for virologists for the valid identification of viruses. One problem in serodiagnosis is the supply or availability of antiserum and its production which involves sophisticated techniques with high cost equipment.

Until recently, a range of serological tests are available for virus detection from seed or leaf tissue. Most serological tests are based on the precipitation produced when antibodies and antigens combine. The most commonly used serological tests for virus detection and identification are as follows:

4.2.1 Precipitin Tests

When antigen (virus) and antibody react together at an optimal proportion ratio, a precipitate is formed. That is an indication of positive reaction for the presence of antigen. The precipitation reaction is widely used for detection of plant viruses. Various factors strongly influence the precipitation tests such as electrolyte contents, pH, temperature, and concentration of reactants. These tests are

performed either with purified virus or clarified seed or plant extracts. The precipitation tests are attempted either in liquid media or in semisolid media. Various precipitin tests performed for virus detection are as follows:

4.2.1.1 Precipitin Tests in Liquid Media

In these tests antigen and antibody are allowed to react in liquid environment. It include three types of tests:

4.2.1.1.1 Tube Precipitin Test

In this test small tubes (7 mm) are filled with small volume (0.3-0.5ml) of antigen and are allowed to react with equal volume of antiserum. Usually, this test is performed in water bath at 37° C. The results are recorded against a dark background after 2-3 hr. In order to avoid non-specific reaction due to presence of plant protein in the seed extract, the sample are clarified by centrifugation or boiling at 45 –50° C for 5-10 min. The following procedure described here has been reported by Hill (1984).

Materials

1. Precipitin tubes (7 x 100mm)
2. 15 ml tubes for preparing virus or antiserum dilutions.
3. Racks for holding test tubes.
4. Water bath at 37° C.
5. Pipets, 1 and 5 ml for diluting antigens, antiserum, and adding saline.
6. Saline (0.85%) or phosphate buffer saline (PBS).
7. Light box with slit source.
8. Clarified samples to be tested.

Procedure

1. Arrange the precipitin tubes in the tube holding rack. Make antigen and antibody dilutions as shown in **Fig. 4.1.**
2. In another tube rack, arrange two rows of nine large tubes and add 5 ml of saline solution to all 18 tubes except the first one in each row.
3. For antiserum dilution add 1.25 ml of antiserum to the first tube and make up to 10 ml with saline solution. Mix thoroughly. Transfer 5 ml to the second tube, mix thoroughly and transfer 5 ml to the third tube etc., until eight twofold dilutions are obtained (1/8 to 1/2048).

4. For antigen dilution add 5 ml undiluted clarified seed or plant tissue extract to first tube and add 5 ml saline solution or PBS to give a ½ dilution. Mix thoroughly. Transfer 5 ml from this tube to the next tube and so on, until all eight antigen dilutions are made.
5. Set the precipitation tubes containing antiserum dilutions beginning with the most diluted (1/2048), 0.5 ml to each of the tubes in 8 rows. Repeat with dilution 1/1024 to the tube in row 7, and so on, until all tubes in row 1 are filled with 1/8 dilution. Starting with the most diluted sap (1/512) transfer 0.5 ml to each tube in column 8, repeating with decreasing antigen dilutions in each column until all are added. Add 0.5 ml saline in tube in row 9, and column 9. Mix thoroughly.
6. Incubate the tubes in the water bath at 37° C **(Fig.4.2).**
7. Record the results on light box after 2-3 hr. The precipitation in the tubes is recorded as follows:

- : no reaction + : slight ++ : moderate
+++ : heavy ++++ : very heavy

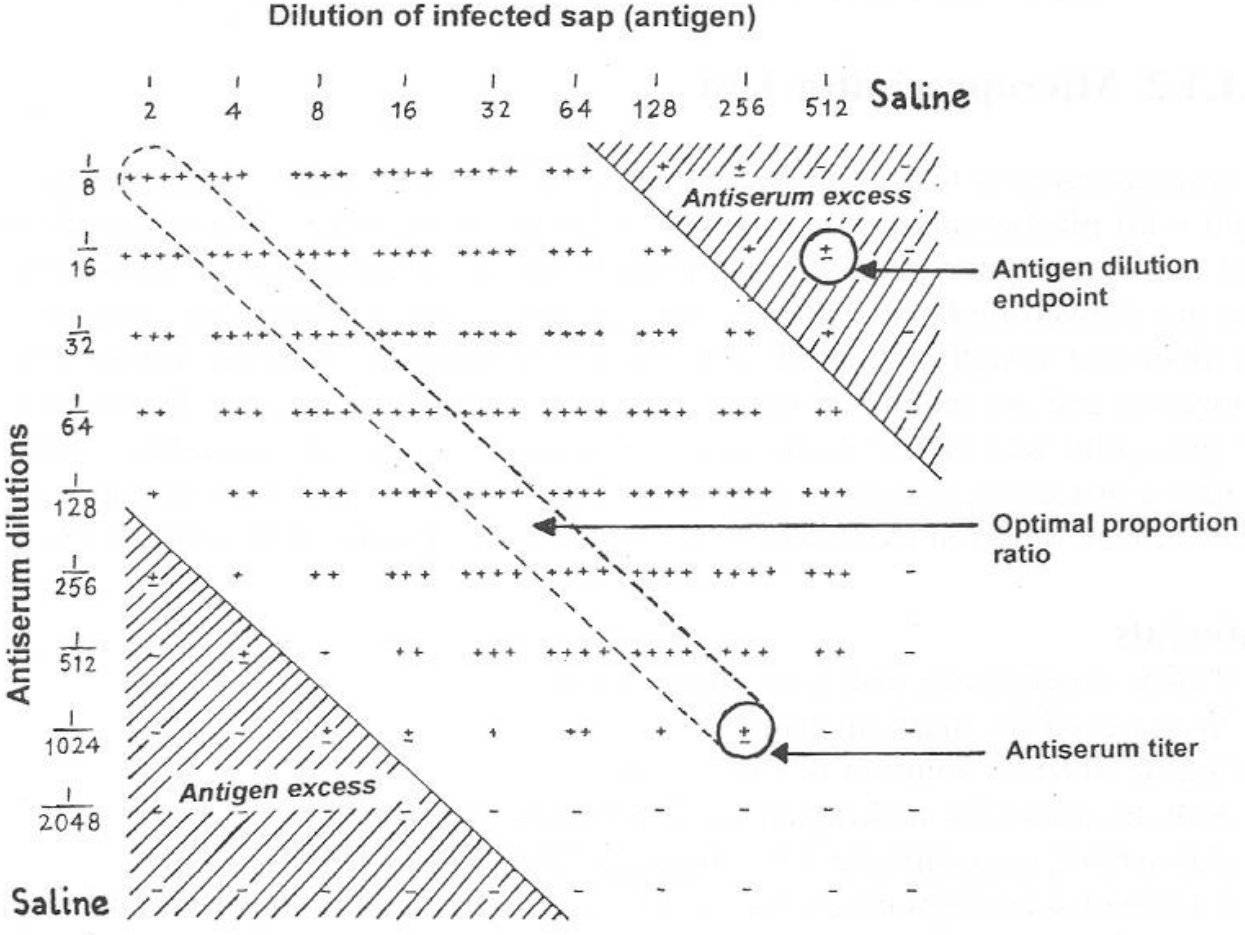


Figure. 4.1: Layout of tube precipitin test (antigen and antibody dilutions).

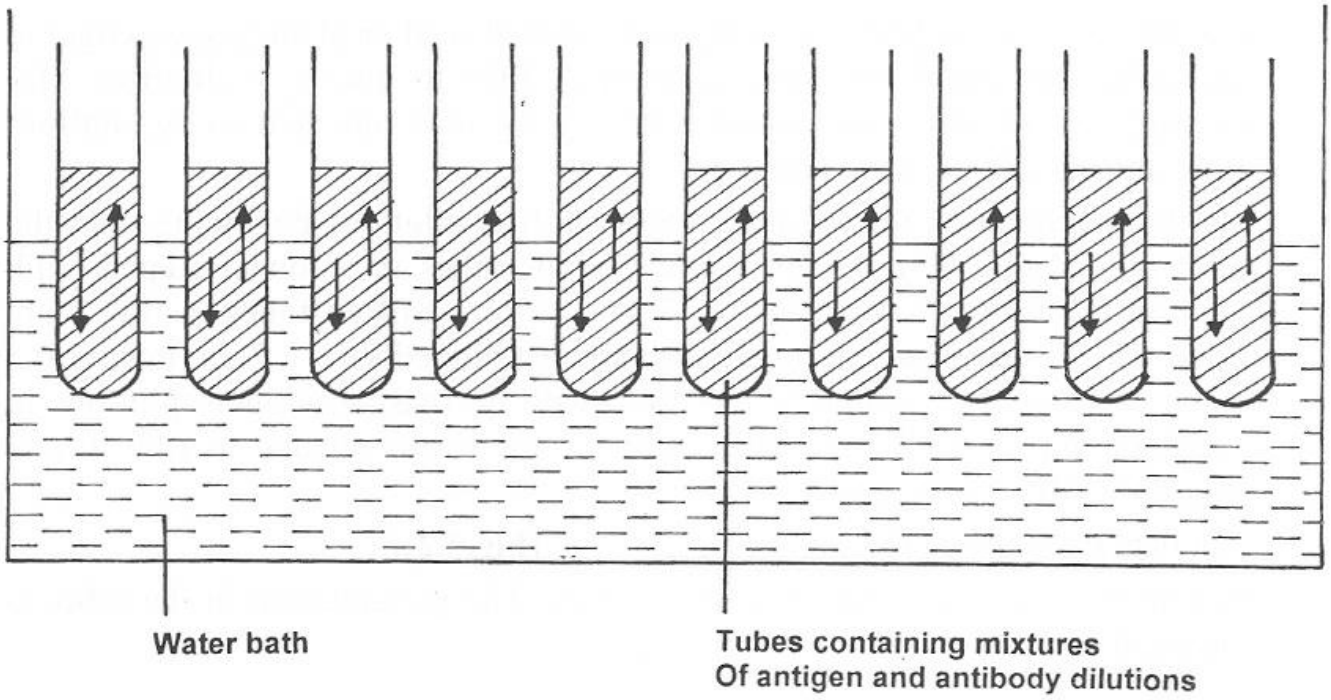


Figure 4.2: Layout and incubation of test tubes with antigen and antibody dilutions under tube precipitin test to promote mixing by convection.

4.2.1.1.2 Microprecipitin Test

The microprecipitin test (Van Slogteren, 1955) is performed in petri dishes pre-coated with plastic coating or in small wells in plastic trays (haemoagglutination trays) or in microtiter plates. Plant or seed extract is added to the antiserum and drops are viewed under the microscope against a dark background. The drops in petri dish are usually covered with liquid paraffin or mineral oil to prevent evaporation and are incubated for 3-6 hr at room temperature. The disadvantage of tube precipitin test is the requirement of large volumes of reactants. This test provides a procedure that conserves reactants. The details and modifications of this test have been reported (Matthews, 1970; Van Regenmortel, 1982; Hill, 1984).

Materials

1. Plastic coated petri dishes or microtiter plates.
2. Wax pencil for marking grids.
3. Saline (0.85%) solution or PBS solution.
4. Suitable tubes for making antigen and antiserum dilutions.
5. Adjustable micropipettes (5-200ul) and 200-100ul.
6. Clarified seed or plant extract to be tested, also include positive and negative control.
7. Mineral oil.

Procedure

1. Prepare grids (8 mm) at the bottom of the petri dish with wax pencil. Also keep separate record of these grids on the paper.
2. Make appropriate series of dilutions of antigen and antiserum as were made in precipitin test, using saline or PBS as dilutant.
3. Add 10ul drop of the appropriate dilution of antigen and antiserum to each space in the grid as shown in the **Fig. 4.3.**
4. Also include positive and negative controls.
5. Carefully flood the dish with mineral oil from one edge until it covers the whole plate, taking care to avoid disturbing the drops.
6. Incubate the plate very carefully at 37° C for 2 hr.
7. Examine the drops after 2 hr under the microscope using dark background.
8. Take observations after 2 hr, transfer the dish to the refrigerator and again take observations after storage overnight.

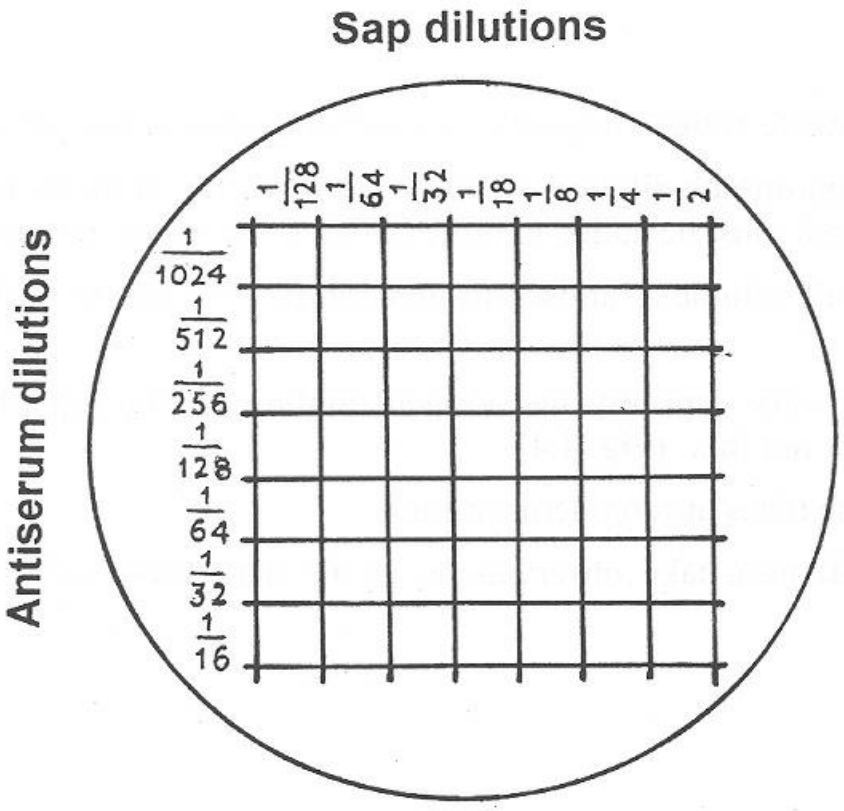


Figure 4.3: Layout of grids for microprecipitin test (sap and antiserum dilutions).

4.2.1.1.3 Ring Interface Test

In this test antiserum at various concentration is mixed with 10% glycerol and 0.1-0.2 ml is pipeted into tubes (2-3 mm). Antiserum must be diluted to 1:10 or greater to give relatively clear solution. The same amount of the clarified antigen is then carefully layered on the top of the antiserum dilutions. The tubes are incubated for 1-2 hr at room temperature. Diffusion occurs at the interface and a disc of precipitation is formed where reactants have reached optimal proportions. Readings are taken in a dark room against a strong light source. This method is sensitive than tube and microprecipitin tests when purified virus preparations are used. The procedure described here has been reported by Ball (1990).

Materials

1. Small glass tubes (0.5 x 5 cm or 0.7 x 7 cm) or microtiter plates for making dilutions.
2. Adjustable pipets: pasteur, hand drawn or long tip 1 ml pipets.
3. Buffers: most suitable (i.e. saline, PBS, Tris etc.)
4. Glycerin

Procedure

1. Prepare suitable antigen dilutions in small test tubes or microtiter plate.
2. Make an appropriate dilution of antiserum in buffer. If dilution is greater than 1:10, glycerin must be added to buffer to give 10% concentration.
3. Place a small volume of antiserum-glycerin (0.1-0.2 ml) in the bottom of each tube.
4. Over layer very carefully the antigen dilution on the top of the antiserum dilution. Do not mix **(Fig. 4.4).**
5. Incubate the tubes at room temperature.
6. After 15-20 min, take observations in the dark room using a strong light source.

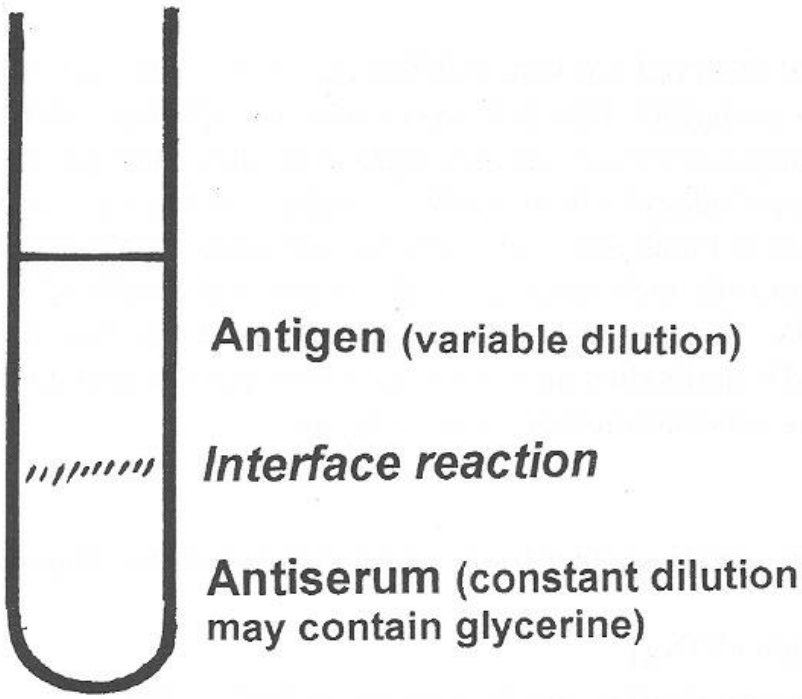


Figure 4.4: Distribution of reactants (antigen and antibody) for the ring interface test.

4.2.1.2 Precipitin Tests in Semisolid Media

In these tests relatively more information is obtained on purity, homogenity, and physical properties of the reactants and on the occurrence of non-specific reactions than precipitin tests in liquid media. The only limitation to these tests is the diffusion of rod-shaped viruses with a tendency to aggregate (Hamilton, and Ball, 1966) and diffuse very poorly in the gels. However, this limitation has been overcome by degrading viruses into fragments by sodium dodecyle sulfate (SDS) (Shepard, 1970). High graded agar (Difco Agar Noble) is widely used as the stabilizing gel. This test has successfully been used to detect BSMV, potato virus X (PVX), potato virus S (PVS), and potato virus M (PVM). There are two types of precipitin tests in semisolid media.

4.2.1.2.1 Gel Double Diffusion Test (GDDT)

This is the most commonly used serological test. It is performed in a petri dish, filled to a depth of 5 mm with agar (approx. 15 ml agar per dish). Wells are cut in agar gel with cork borer in such a pattern that a central well is surrounded by six or more peripheral wells. Antiserum of a known virus is added in the central well, and the antigens (virus infected samples) are added in the surrounding wells. When both antigen and antiserum diffuse from their wells into the agar, a visible

precipitin line is observed (Ackers and Steere, 1967). Agar gel at a concentration of 0.7 to 1% is preferred. This test works best for spherical viruses. Particles of most of the elongated viruses do not diffuse readily through the agar medium, therefore, sodium dodecyl sulfate (SDS) is added in the sap, as SDS causes the elongated viruses to break down into smaller sub-units which can diffuse easily in the agar gel (Purcifull and Gooding, 1970). Slack and Shepherd (1975) were able to detect BSMV directly in individual barley seed by placing them in agar containing BSMV antibodies and observed virus-specific precipitation lines. This test detects virus concentration of 10 to 25 ug/ml.

Materials

1. Flat bottom sterile standard size petri dishes (plastic disposable dishes are preferred).
2. Sodium azide (NaN_3).
3. 0.01M phosphate buffer, pH 7.0 containing 0.85 % NaCl.
4. Agar 0.8 % (Ion Agar No 2, or Agarose or Noble Agar).
5. Pipettes, micropipette for dispensing and for diluting antigens or antibodies.
6. Moist chamber for incubating the plates (a plastic tray with cover with moist paper towels will be adequate for this purpose).
7. Water bath (50^o -55^o C).
8. Small 5.0 ml capacity (120 x 75 mm) tubes for making dilution.
9. Cork borer or gel cutter.
10. Seed samples or leaf tissue.
11. Grinder or mortar and pestle.

Procedure

Agar at a concentration of 0.8% will provide a satisfactory medium for diffusion. To prepare follow these steps:

1. Dissolve 0.8 g of purified agar or agarose in 0.01 M phosphate buffer containing 0.85 % NaCl. For elongated viruses prepare 0.8 % agar containing 0.5 % SDS and 0.01% NaN_3.
2. Autoclave for 10 min or microwave for 3 minutes.
3. Maintain agar at 50-55^o C in a water bath.
4. Pour 15 ml agar with the help of glass cylinder into petri plates.
5. Keep the petri plates on flat surface to ensure uniform spread of agar.
6. Allow the agar to solidify at room temperature.
7. Cut wells into the agar with the help of cork borer or a template and remove agar plugs. It is convenient to use a gel cutter with six circular cutters arranged around the central one of the same size (4 mm diameter), and a distance between the wells of 4 mm is suitable **(Fig. 4.5).**

8. Add suitable dilution of antibody (antiserum) to the central well and antigen (prepared seed samples) to the peripheral wells.
9. Incubate for 24 hr the petri plates at room temperature or at 37°C in a moist chamber.

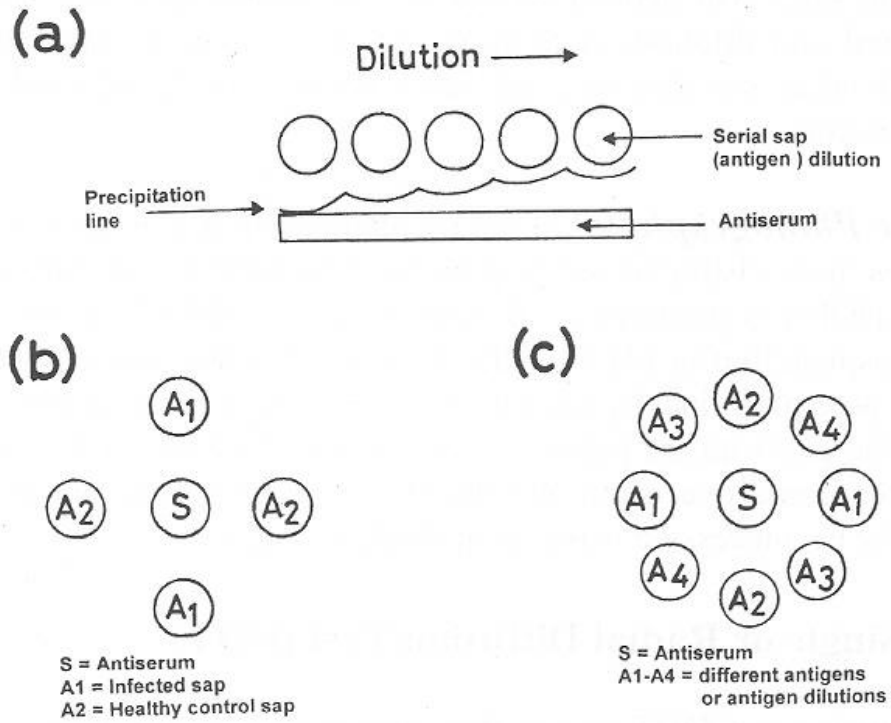


Figure 4.5: Arrangement of wells for gel double diffusion test (a) Useful configuration to determine antigen dilutions (which will give clear precipitation lines) before proceeding to a comparative test (b) A simple confirmatory arrangement which can be repeated several times within a petri dish using different antiserum and sap dilution (c) A more complex antigen wells around a single antiserum well for comparison of relationships between antigens and testing many samples (Hill, 1984).

10. Read the results in a dark room against dark background over box with slit or circular light source. Observe the dishes every day for one to two weeks.

If the antiserum is added to the central well, and sap (antigen) to the wells around it, different precipitation lines may be observed.

Precautions

1. Avoid excess heating of agar.
2. Do not overflow the wells with antigen or antibody dilution.
3. Use healthy plant and normal serum extracts as control.

For certain viruses such as CMV, better results can be obtained if agar is dissolved in 0.05 M dipotassium hydrogen orthophosphate (K_2HPO_4) at pH 7 to

8, containing 0.005 M sodium EDTA (ethylene diamine tetraacetic acid, disodium salt) and 0.02 % NaN_3. This can be prepared by dissolving in one liter of distilled water, 8.7 g K_2HPO_4, 1.86 g Na-EDTA, 0.2 g NaN_3 and 7.5 g agar. The antiserum dilutions can be prepared with 0.9 % NaCl. The dilution required to give precipitin lines will depend on the titer of antiserum concerned, antiserum used, undiluted and dilution. A dilution of 1:5 often gives satisfactory results. Infected fresh crude sap may be used, better results can be obtained if virus is in high concentration.

Staining for Photography: For taking photograph it is necessary to make the precipitin lines more visible by staining with 0.1 M DOPA (3-4 dihydroxy phenyl-DL alanine). DOPA is prepared by dissolving 1.97 g DOPA in 100 ml of 0.1 M potassium phosphate buffer pH 7.4. The stain is applied to the surface of the agar gel above the precipitin line, by saturating a piece of chromatographic paper. Stain diffuses into the gel from the paper. Allow the lines to stain for 6 to 12 hr. During this period they could over-stain and then they may be destained to the required colour intensity by successive washing in distilled water.

4.2.1.2.2 Single or Radial Diffusion Test (RDT)

This test is similar to GDDT except that antiserum is incorporated into agar gel. Wells are cut in the agar that contain antigen, diffusion occurs and where the concentration of the reagents reaches the right proportions, a precipitate is formed. This test has 10-fold greater sensitivity than DDT. This method is sensitive, rapid, ad can detect as little as 1ug/ml of degraded virus. This method has been used for the detection of seed-borne BSMV. Slack and Ball (1990b) described the procedure of this test as follows:

Materials

1. Agar (Difco Noble) or Agarose.
2. Borate, Tris, or phosphate buffer
3. Flat-bottom glass plastic petri dishes.
4. Gel punch (cutter)
5. Humid chamber for incubation of plates. A plastic box with wet towels can serve this purpose.
6. Water bath (45-50° C) to hold gel in liquid state.

Procedure

1. Dissolve agar in suitable buffer by autoclaving for 10 min or heating in microwave oven until just melted.

2. Mix sodium azide to buffer or melted agarose to give 1:1000 dilution. Dispense the agar (10-12 ml) into the tubes. Keep the tubes in water bath.
3. Add appropriate amount of antiserum, mix by gently inverting the tubes several times and pour or pipet into a plate which is on a leveled table.
4. Make wells into the solidified agar gel.
5. Fill the wells with clarified antigen (seed extract/plant tissue) at appropriate dilution. Also include positive and negative control.
6. Incubate plates at constant temperature e.g. 22-25° C.
7. Take observations in the dark room against dark background using slit light or circular light source.

4.2.2 Agglutination Tests

The term "*agglutination*" is used instead of precipitation when the size of the reacting antigenic particle is approximately equal to that of a cell. There are three types of agglutination tests.

4.2.2.1 Slide Agglutination Test

It is also called chloroplast agglutination test. It is a very simple test. One drop of the virus infected crude sap prepared from seed or leaf tissue is mixed with one drop of antiserum on a microscopic slide, and is observed under the microscope. If the chloroplast and other sap debris clump together, this is an indication that the test is positive. The test is particularly useful for rapid detection of virus-infected potatoes in the field. This test is employed only with elongated plant viruses which occur in high concentration in sap such as TMV and PVX.

4.2.2.2 Latex Agglutination Test

In this test the antibodies are adsorbed onto commercially available polysterene latex particles (0.8 u diameter). The sensitized latex is then mixed with antigen (sample to be tested) and flocculation of the latex beads indicates antigen-antibody interaction **(Fig. 4.6).** Best results are obtained if purified immunnoglobulin (IgG) are adsorbed to the latex particles. Using antibody-coated latex, several workers succeeded in detecting 100 to 1000 fold smaller quantities of virus than was possible by gel double diffusion test (van Regenmortel, 1982). The advantages of using latex coated with IgG is that smaller volumes of antiserum are used. The sensitized latex can be stored and sensitivity is increased over tube or microprecipitin tests. The latex test is rapid, specific, and sensitive. The results can be obtained within 15 min to I hr. The method can detect one infected seed per

100 seeds of 1 ug/ml of virus. The procedure followed here has been reported by Slack and Ball (1990b).

Materials

1. Chemicals
   a. Tris (hydroxymethyl) aminomethane (0.1 M Tris, pH 7.4).
   b. Sodium chloride (0.85%) solution.
   c. Polyvinyl pyrrolidone (Mr. 40,000 (PVP).
   d. Bovinne serum albumin (Sima, Catalog: # A 4378)
2. Polysterne latex beads (0.8 um dia) (sigma: catalog: # LB-8)
3. Watman filter paper #2.
4. Centrifuge machine.
5. 100 Lambda micropipets or 96 well slide tissue culture plate, or test tubes or ringed slides.
6. Mortar and pestle.
7. Small vials or test tubes.
8. Variable speed rotator for shaking tubes or plates.
9. Dissecting microscope.

Procedure

A. Latex Conjugation

1. Use immunoglobulin (IgG) fraction of serum in 0.1 M Tris, pH 7.4, and prepare several two-fold dilutions (e.g. 1:50 - 1:160). The dilutions of 1:200 to 1:800 of IgG fraction have proved to be best for use with rabbit polyclonal antibodies to several different antigens.
2. To each antibody dilution (5 ml), add an equal volume of latex beads diluted 1/15 (v:v) in 0.85% saline. Gently shake latex beads to disperse before making dilutions. Latex beads should be stored at 4° C and can be used unless aggregate.
3. Mix and let stand at room temperature (22° C) for 1 hr. Shake several times.
4. Centrifuge for 30 min at 4000 g to get pellet, carefully pipet off the supernatant, but quickly.
5. Wash the pellet twice with 0.02% PVP in 0.85% saline in order to stabilize the sensitized latex beads.
6. Resuspend the final pellet in 5 ml of 0.1 M Tris, pH 7.4, containing 0.02% sodium azide.
7. Filter preparation through Watman filter paper # 2. Use a Bachner funnel and gentle vacuum (vacuum, ca 15 psi, is required to filter preparations).
8. Store at 4° C. Do not freeze. Stability varies, but preparations usually can be used for 8-12 months.

B. Antigen Preparation and Testing

1. Seed extract or plant tissue to be tested, dilute the sample as 10X in 0.1 M Tris, pH 7.4, containing 0.85% saline or other suitable buffer. Better to prepare 100x antigen dilution if the antigen occurs in high concentration.
2. Also include positive and negative control.
3. Take 10ul sensitized latex by capillary action into 100-Lambda micropipets. Wipe the pipet tip with a disposable wipe and then draw another 10ul (20ul total) antigen into pipet.
4. Select at least two dilutions of sensitized latex and mix each with the two antigen dilutions to optimize the reactant concentration.
5. Attach pipet to arm of rotator and rotate end-over-end for 15 min at 7 rpm, or droplets can be mixed in serum depression plates, or microtiter plates or test tubes, and oscillated at 100-200x/min on plate form rotator for 20-30 min.
6. Score the reaction with a dissecting microscope with dark background illumination (10-100x).
7. Positive reactions are observed with distinct clumping or agglutination and a clear suspension of the background.

The components and principle of latex agglutination test are shown in **Fig. 4.6.**

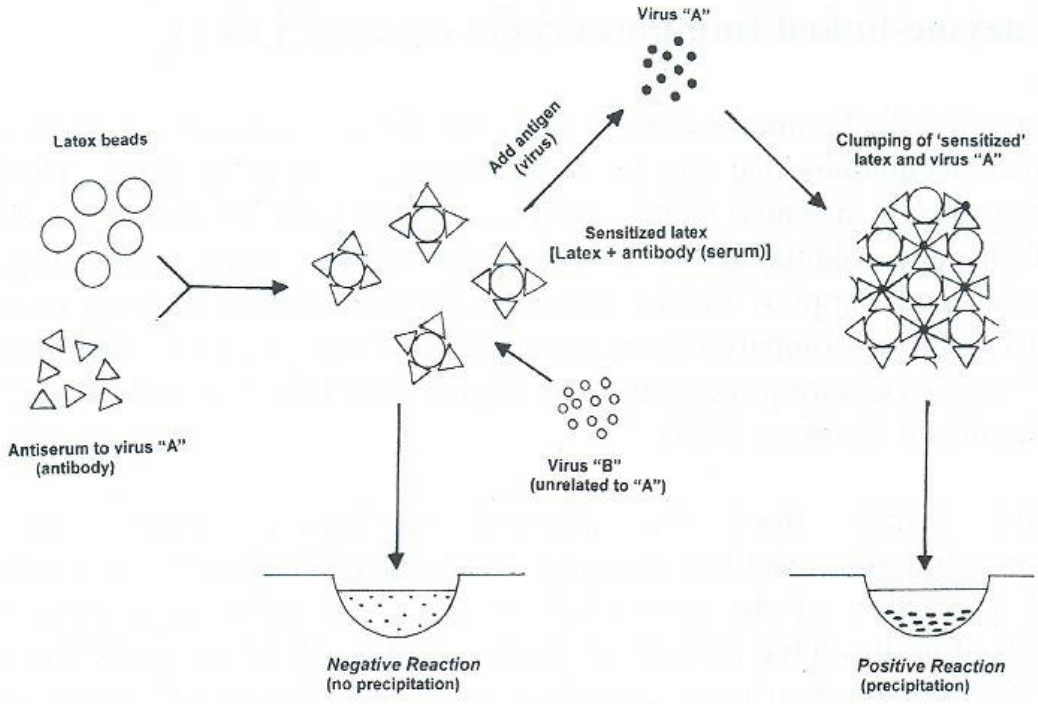


Figure 4.6: Components and principle of latex agglutination test.

4.2.2.3 Virobacterial Agglutination Test

This test was first described by Chirkov et. al., (1984) for the identification of plant viruses and used by Walkey et. al., (1989). The technique is simple and

allows rapid detection of viruses which occur in low concentration in seed or plant tissue.

Procedure

1. Mix formalin-treated *Staphylococcus aureus* (a kind of bacterium) (1 vol) with diluted virus antiserum (1 vol, 1:1 antiserum/glycerol mixture + 24 vol phosphate buffer saline- PBS, pH 7.2 containing 2 mg/ml sodium azide (5 vol).
2. This bacterial-antibody conjugate is coloured by the addition of several drops of saturated alcohlic basic fuchsin.
3. Approximately 4ul of this conjugate is mixed with 2ul of crude sap to be tested on a Welled multi-test slide (Flow Laboratories).
4. Also include positive and negative control.
5. A positive reaction is indicated by agglutination of the bacterial particles within 1-3 min.

4.3 Enzyme-linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is one of the most suitable serological techniques that can be used to detect viruses in seeds. The ELISA method is one of the most widely used serological tests, originally developed in 1976 (Clark and Adams, 1977). The test is particularly useful for testing a large number of seed samples. ELISA generally detects concentration of virus lower than 0.01ug/ml as compared to immunodiffusion test (1 ug/ml), microprecipitin test (0.5 ug/ml), electron microscopy (0.1ug/ml) and infectivity assay (0.05 ug/ml) (Bar-Joseph and Garnsey, 1981).

The test differs from the classical serological methods in which immunoprecipitation reactions are used. Immunosepecific reactivity is recognized through the action of the associated enzyme label rather than observing the formation of an insoluble antigen-antibody complex. There are numerous variants of ELISA, but the two main categories of ELISA procedures, which are most commonly used in virology laboratories are direct ELISA and indirect ELISA.

In direct ELISA (Clark and Adams, 1977) the wells of the microtiter plate are first coated with immunoglobulin (IgG) purified from the antiserum. The test sample is then added to the adsorbed antibody. The enzyme-labeled antibody is then added to the trapped virus. The attached enzyme subsequently digests an added enzyme

substrate which results in a colour change. In this case the antigen is sandwiched between two immunoglobulin (IgG) and that is why this ELISA is also called as "double antibody sandwich ELISA", and is designated as DAS-ELISA. It is highly strain specific. One disadvantage of DAS-ELISA is that a specific conjugated antibody is required for each virus to be detected.

In indirect ELISA (Edwards and Cooper, 1985) (also called as direct antigen coated ELISA: DAC-ELISA) procedure, the immobilized antigen is the target for unconjugated specific antibody. The trapped antibody is detected by a secondary antibody (produced in a second animal against the antibody of the first animal) conjugated to an enzyme, called universal conjugate (goat anti rabbit). The main advantage of indirect ELISA is that one single conjugate can be used with all systems. This type of ELISA is particularly suitable for virus detection in disease survey and for testing the presence of viruses in seeds. It is relatively more economical to perform than DAS-ELISA. Moreover, for the screening of a large number of seed samples, DAC-ELISA is more convenient and effective. DAC-ELISA is not highly specific, high background reactions are often encountered. In addition, it works well only in case of viruses that are present in plant tissues at high concentration.

Keeping in view the importance of ELISA as a tool for assessing seed transmitted viruses in seed lots, the procedure and various types of ELISA are discussed below:

4.3.1 Double Antibody Sandwich ELISA (DAS-ELISA)

This type of ELISA is also called as "direct ELISA". As already mentioned, DAS-ELISA is more specific as compared to DAC-ELISA, therefore it is mainly suitable to distinguish strains of a virus and to develop serological relationships among viruses. In particular, this test is not suitable for (a) virus detection in disease surveys (b) when adequate quantities of antiserum are not available for immunoglobulin extraction and conjugation and (c) for probing a single antigen with several different antisera.

Preparation of Buffers: The following buffers must be prepared and stored at 4^{o}C before starting DAS-ELISA.

1. Antibody coating buffer: Prepare in one liter distilled water

Sodium carbonate (Na_2CO_3):	1.59 g
Sodium bicarbonate ($Na\ HCO_3$):	2.93 g

First dissolve the chemicals in 900 ml distilled water, and check pH, it should be 9.6, make the volume up to one liter.

2. Virus (antigen) extraction buffer: (Also called virus buffer or conjugate buffer).

a. First prepare phosphate buffer saline (PBS) with 0.05 % Tween-20 (PBST) by dissolving the following salts in one liter of distilled water

Sodium chloride (NaCl):	40 g
Potassium phosphate (KH_2PO_4, monobasic):	2.0 g
Sodium phosphate (Na_2HPO_4, anhydrous dibasic):	11.5 g
Potassium chloride (KCl):	2.0 g
Sodium azide (NaN_3) :	2.0 g

First dissolve the chemicals in 800 ml distilled water, then make the volume up to one liter. It is called 5X PBS (stock solution). Store at room temperature.

b. Now take 200 ml 5 X PBS (stock solution) as prepared above and add to it the followings:

Polyvinylpyrrolidone (PVP: MW: 40,000):	20 g
Tween-20 (polyoxy ethylene: Sorbitan monlaurate):	0.5 ml/liter
Egg ovaalbumin:	2 g
Distilled water:	800 ml

Dissolve and make the volume up to one liter, adjust pH to 7.4. Store at 4°C.

***Note*:** Extraction buffer is used both for grinding virus samples and for making conjugate solution.

3. *Washing buffer (WB):* In one liter distilled water

5 X PBS :	200 ml (prepared as above)
Distilled water:	800 ml
Tween - 20 :	1 ml

Stir well to mix. Store at room temperature.

4. Substrate buffer (SB): In one liter distilled water

Diethanolamine :	97 ml
Distilled water :	800 ml

Add slowly 97ml diethanolamine to 800ml distilled water on an electric stirrer. Add concentrated HCl drop by drop to adjust pH to 9.8, then make the volume up to one liter.

Note: Substrate buffer stored for a longer period, even in a refrigerator is not suitable. It is always better to use fresh buffer.

Materials

ELISA plates, antiserum, micropipettes (10-100 ul and 100-1000 ul), 200ul micropipette (fixed) or adjustable multichannel micropipette, ELISA reader, beakers, washing squeeze bottle, magnetic bars, electric stirrer, humidity chamber (plastic box with moist tissue papers), mortar and pestles.

Procedure

1. Add 200ul of purified IgGs at concentration of 1ug/ml (optimum concentration.of IgG can be determined by standardization trials, which may vary with each conjugate or IgG produced) to each well of ELISA plate with fixed volume micropipete.
2. Incubate antibody-coated plate at 37°C for 4-5 hr or over night at 4°C.
3. Wash plate three times, allowing 3 min soaking for each wash with washing buffer.
4. Add 200ul antigen samples (crude plant or seed extracts, or purified virus preparation) prepared in virus extraction buffer (PBST containing 2 % PVP) to each well.
5. Incubate at 37°C for 4-5 hr or overnight at 4°C.
6. Wash plate as mentioned under step-3.
7. Add 200ul alkaline phosphatase (ALP) labeled IgG diluted normally up to 1: 1000 in conjugate buffer pH 7.4.
8. Incubate at 37°C for 4-5 hr or overnight at 4°C.

9. Wash plate as before.
10. Prepare substrate solution by dissolving substrate tablets (1 mg of p-nitrophenyl phosphate/1 ml buffer) in 25 ml substrate buffer, pH 9.8. These tablets are available in 5 mg concentration and one tablet is adequate for each plate. Add 200ul substrate solution in each well.
11. Incubate plate at room temperature for 1-2 hour. In case of positive reaction the substrate develops yellow colour (as a result of conversions of p-nitrophenyl phosphate to p-nitrophenol).
12. Plates can be scored visually or if ELISA reader is available take absorbance reading at 405nm in case of ALP. Test readings are considered positive if they were twice or more times greater than readings from healthy control plant extract.

The layout of direct ELISA (DAS-ELISA) have been shown in **Fig. 4.7.**

4.3.2 Direct Antigen Coating ELISA (DAC-ELISA)

This type of ELISA is also called as "indirect ELISA". This is the simplest test of all ELISA procedures and is widely used for detecting seed-borne viruses. Antigens prepared in 0.02 M carbonate buffer, pH 9.6, are used for coating ELISA plates. This is followed by the addition of crude antiserum (not purified IgG as in case of DAS-ELISA) with its optimal concentration. As majority of the virus antisera are produced in rabbit therefore, the rabbit immunoglobulin attached to antigens are probed with enzyme labeled anti-rabbit immunoglobulins produced in goat. In place of immunoglobulins, the use of Protein-A conjugate is equally good as that of anti-rabbit conjugate.

Materials

Buffers used in this ELISA are the same as listed under DAS-ELISA, the antigen buffer used in DAC-ELISA to extract sap from samples is prepared as follows. The other buffers are the same.

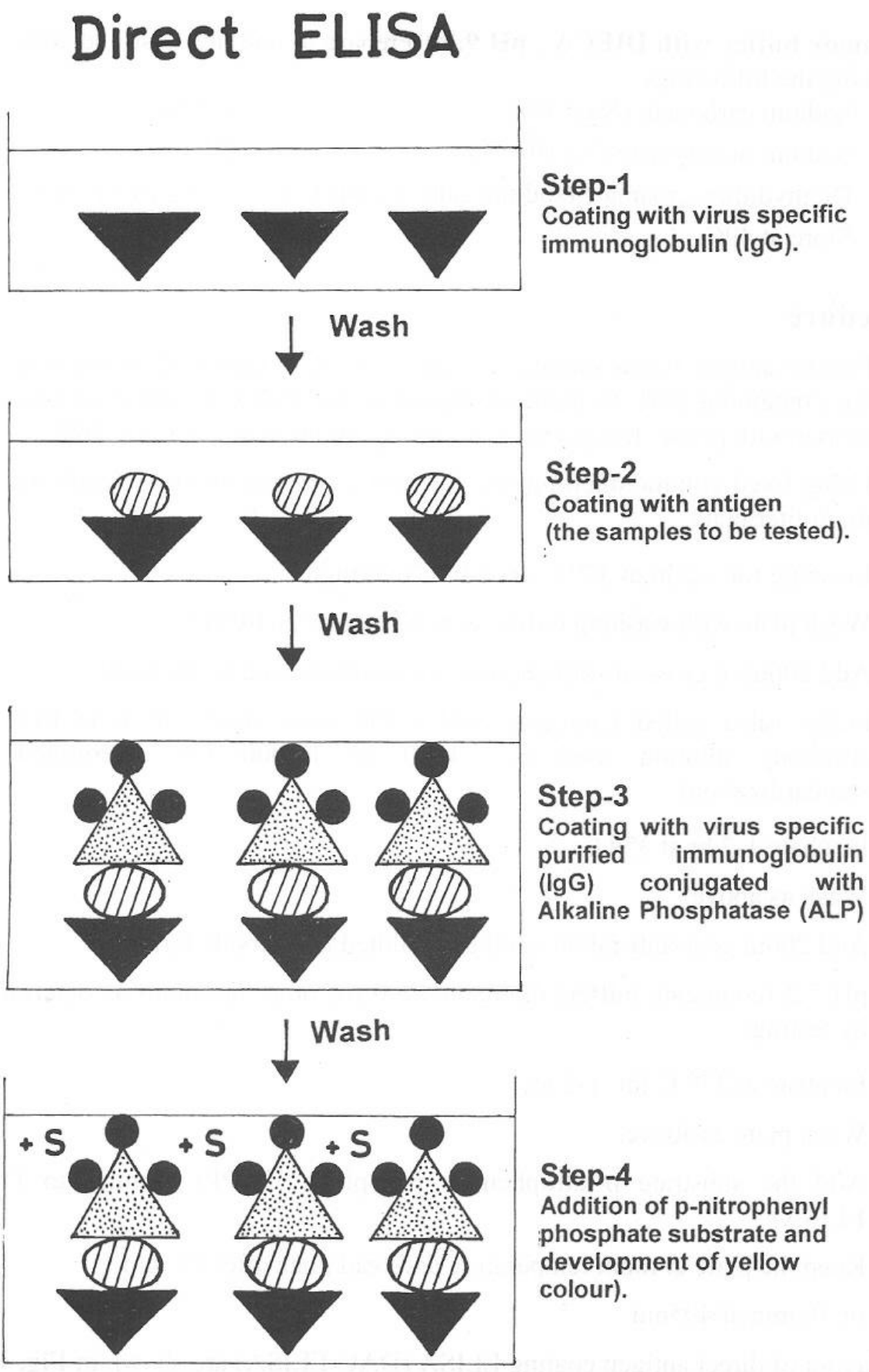


Figure 4.7: Layout of double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

Carbonate buffer with DIECA , pH 9.6 : Prepare in one liter distilled water by dissolving the followings:

Sodium carbonate (Na_2CO_3):	1.59 g
Sodium bicarbonate ($Na\ HCO_3$) :	2.93 g
Diethydithiocarbamate (sodium salt) Na-DIECA :	1.71g (0.01 M Conc),

Store at 4°C.

Procedure

1. Prepare antigen (virus samples extracts) in 0.02 M carbonate buffer with pH 9.6 containing 0.01 M diethyldithiocarbamate (DIECA) while grinding in mortar with pestle. It is good to use antigen dilution of 1:50 or 1:100.
2. Using fixed volume micropipette add 200 ul antigen extract to each well of microtiter plate.
3. Incubate for 1-2 hr at 37° C or at 4° C overnight.
4. Wash plate with washing buffer as in case of DAS-ELISA.
5. Add 200ul of cross-absorbed crude antiserum diluted in antibody

   buffer (also called conjugate buffer, the same used in DAS-ELISA). Antibody dilution used is 1:1000 or 1:5000 (or determined by standardization).
6. Incubate 1-2 hr at 37° C
7. Wash as above.
8. Add 200ul goat-anti-rabbit conjugate diluted in antibody buffer,

   pH 7.2 (conjugate buffer) diluted 1:1000 (or other optimum as determined by testing).
9. Incubate at 37° C for 1-2 hr.
10. Wash plate as above.
11. Add the substrate p-nitrophenyl phosphate (PNPP) (similar to DAS-ELISA).
12. Keep the plate at room temperature and read plate after 15 min

    or 30 min at 405nm.

The layout of direct antigen coating ELISA (DAC-ELISA) are shown in **Fig. 4.8.**

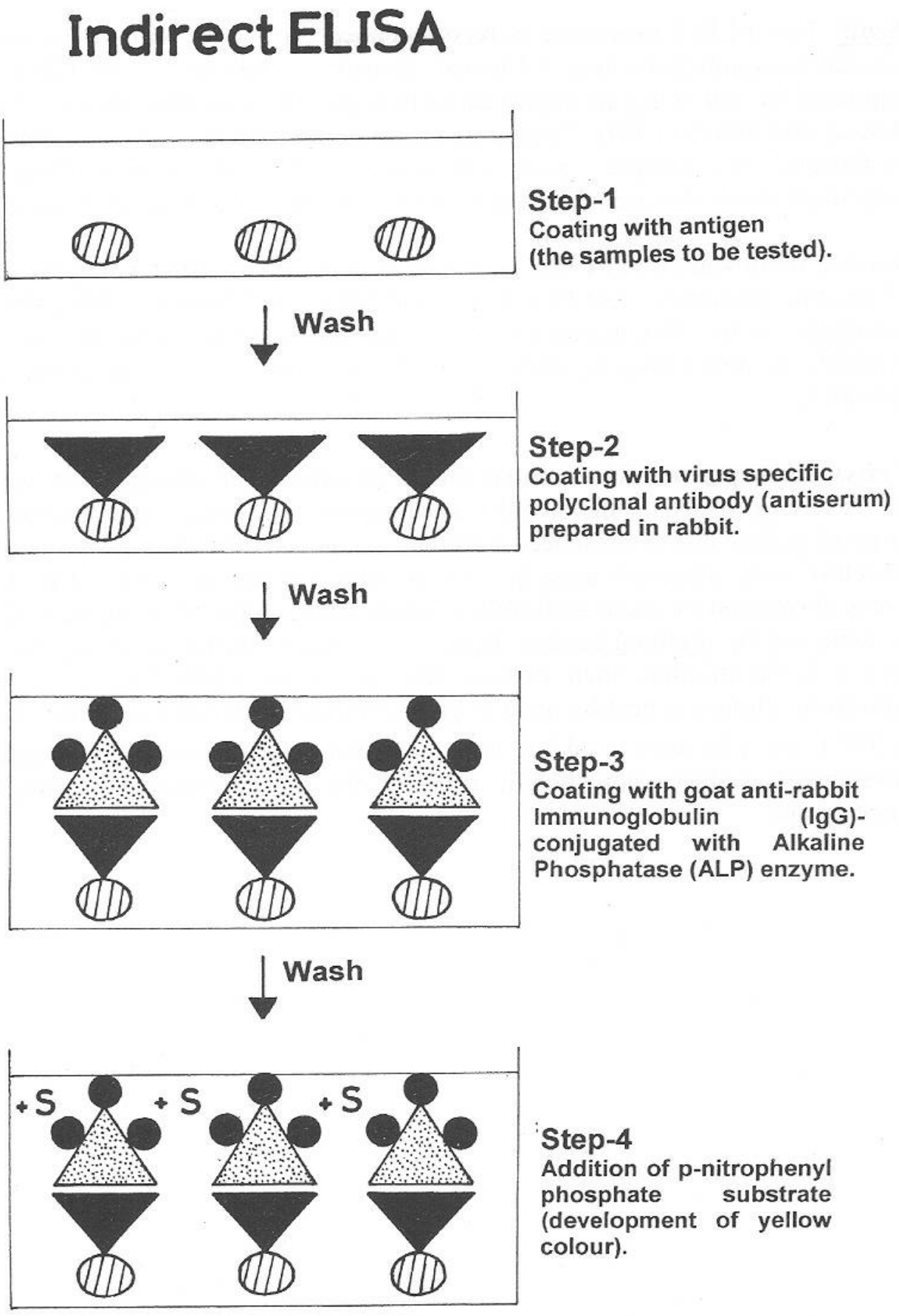


Figure 4.8: Layout of direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA).

Note: This ELISA procedure is recommended for routine virus detection. The test can be completed within 3-4 hours. Sensitivity and rapidity of ELISA can be improved by following an improved ELISA procedure as described by Van den Heuvel and Peters (1989). Using this technique, 50-100pg virus was reported to be detected in a sample. Such a procedure could prove very instrumental in detecting viruses that are present in very low concentration in seeds or seedlings.

Another modification in ELISA is the use of penicillinase (PNC) enzyme in place of alkaline phosphate (ALP) system (Sudarshana and Reddy, 1990). The main advantage of the PNC-based ELISA is that the substrate, penicillin is readily available in developing countries, and at much lower cost than p-nitrophenyl phosphate.

Cross absorption: An essential factor governing the detection of very low levels of virus in seed is the quantity of antiserum. Sometimes the antiserum is not of good quality due to presence of antibodies against host plant components, and therefore such antiserum must be cross-absorbed before its use in DAC-ELISA. Cross absorption of crude antiserum is made with healthy plant extract. This can be achieved by grinding healthy leaves in conjugate buffer (antibody buffer) to give a 1:100 dilution, then filtering through cheese cloth. Prepare a suitable antiserum dilution in healthy plant extract suspended in conjugate buffer. Incubate at 37^{o} C for 1 hr prior to adding it to microtiter plate. Cross absorbed antiserum substantially reduces non-specific reaction due to precipitation of antigens of plant origin.

Table-2: List of seed-borne viruses reported to be detected in seed by enzyme-linked immunosorbent assay (ELISA).

Virus	Host	References
Apple mosaic virus	*Prunus dulcis*	Barba (1986)
Alfalfa mosaic virus	*Medicago sativa*	Halk et. al. (1982)
Barley stripe mosaic virus	*Hordeum vulgare* *Triticum aestivum*	Faris-Mukhayyiosh et. al. (1983) Lister et. al. (1981)
Bean common mosaic virus	*Phaseolus vulgaris*	Jafarpour et. al. (19779)
Broad bean stain virus	*Vicia faba*	Barbara (1979)
Broad bean true mosaic virus	*Vicia faba*	Barbara (1979)
Brome mosaic virus	*Triticum aestivum*	Von Wechmar et. al (1984)
Cowpea aphid-borne mosaic virus	*Vigna unguiculata*	Konate & Neya (1996)
Cucumber green mottle mosaic virus	*Cucumis sativus*	Faris-Mukhayyiosh et. al. (1983)
Cucumber mosaic virus	*Phaseolus vulgaris*	Davis et. al. (1981)
Lettuce mosaic virus	*Lactuca sativa L.*	Falk & Purcifull (1982)
Maize dwarf mosaic virus	*Zea mays*	Hill et. al. (1984)
Pea early browning virus	*Pisum sativum*	Van Vuurde & Maat (1985)
Pea seed-borne mosaic virus	*Pisum sativum*	Hamilton & Nichlos (!978) Mink & Parsons, (1978)
Peanut mottle virus	*Arachis hypogea*	Bharathan et. al. (1984)
Peanut stripe virus	*Arachis hypogea*	Demski et. al. (1984) Demski & Warwick (1986)
Prunus dwarf virus	*Prunus spp.*	Halk et. al. (1982)
Prunus necrotic ringspot virus	*Prunus spp.* *Prunus dulcis*	Mink & Aichelle (1984)
Ryegrass seed-borne virus	*Lolium spp.*	Chester et. al. (1983)
Soybean mosaic virus	*Glycine max*	Hill et. al. (1984)
Squash mosaic virus	*Cucurbit spp.*	Nolan & Campbell (1984)
Tobacco ringspot virus	*Glycine max* *Solanum tuberosum* (in true seed)	Lister (1978) Torrance & Jones (1982)

4.4 Tissue Blot Immunobinding Assay (TBIA)

Since the development of ELISA for the detection of plant viruses (Clark and Adams, 1977) several modifications have been introduced to improve sensitivity and specificity to simplify the procedure (Van Regenmortel, 1982). The recent development of a tissue-blot immunoassay (TBIA) was a further improvement which permitted sensitive detection of several plant viruses and most importantly eliminated the need for sample extraction (Hsu and Lawson, 1991).

In TBIA, a secondary labeled antibody is used, usually goat anti-rabbit conjugated to alkaline phosphate. In place of ELISA plates, nitrocellulose membrane that have high affinity for binding proteins are used. The antigen is immoblized on these membranes. The immobilized antigen is then exposed to solutions of unconjugated virus specific antibody. Mostly crude antiserum is used. Trapped antibody is detected with alkaline phosphatase (ALP) or horseradish peroxidase (HRP)-labeled protein A, anti-Fc, or anti-IgG. ALP is preferred because it is easier to prepare conjugates with ALP than HRP. For ALP, naphthol phosphate is used as a substrate, followed by the addition of a diazonium salt such as Fast Blue RR or Fast Red RR. Naphthol phosphate in the presence of ALP is converted to phosphoric acid and naphthol. Naphthol is detected by adding diazonium salt, which together form an insoluble product which can be detected visually.

The TBIA is an in-expensive, sensitive and quick test, that does not require expensive equipment. It permits the detection in extremely small volume such as seed extracts. Membranes are easier to process than ELISA plates. The TBIA is recommended for laboratories that lack sophisticated facilities.

Materials

Nitrocellulose membranes, antiserum, micropipette, glass dish (approx. 10 x 12 cm) or disposable square plastic petri dishes (9.5 x 9.5 cm), shaker, blunt ends forceps.

Solutions required: Keep ready the following buffers before start. Store buffers at 4°C.

1. Coating buffer, pH 9.6

Sodium carbonate (Na_2CO_3) :	1.59 g
Sodium bicarbonate ($NaHCO3$) :	2.93 g

Dissolve the above salts in 900 ml distilled water adjust pH to 9.6, make the volume to one liter.

2. Tris-buffered saline (TBS)

Tris : 4.84 g
NaCl : 58.48 g

Dissolve these salts in 1.9 liter distilled water, adjust pH to 7.5 and make up the volume to 2 liters. It is useful to make a 5X concentration stock solution and dilute this when required.

3. TBS-Tween (TBST)

TBS : 1 liter
Tween-20 : 0.5 ml
Mix Tween-20 in one liter TBS

4. Blocking solution

TBS : 1 liter
Non-fat dry milk powder: 10 g

5. Antibody buffer:

TBS : 1 liter
Non fat dry milk powder: 10 g

6. Alkaline phosphatase labeled goat anti-rabbit IgG or Fc-specific dilute in antibody buffer. Make approprite dilution such as 1 : 1000.

7. ***Solution A*** **:** Add 50 mg of naphthol phosphate AS-B1: 20 ml of dimethyl formamide to 20 ml distilled water. Adjust pH to 8.00 with 0.1 M Na_2CO_3.

8. Substrate solution: Fast Red or Fast Blue RR salt, 50 mg: Tris HCl (0.2 M, pH 8.3). 18 ml; distilled water; 13 ml; solution A, 2 ml. Substrate solution should be prepared fresh each time and filtered through an ordinary filter paper (Whatman No.3).

9. Washing buffer: Prepare in 50 ml PBST

PBST :	50 ml
Bovine serum albumin :	1% (0.5 g)
Non-fat dry milk :	4 % (2 g)

First dissolve the reagents, then make the volume up to 50 ml, store at 4^{o} C.

Procedure

Always wear gloves when handling nitrocellulose or nylon membranes. A rotary shaker is needed to perform various steps in the procedure. As nitrocellulose membranes are fragile therefore, these should be handled with blunt tipped forceps. This procedure involves the following steps:

1. Prepare appropriate dilution of antigen (seeds extracts etc.) in carbonate coating buffer. Usually 10^{-1} to 10^{-5} (10 to 5000) (at 10-fold intervals) are used. Extract from healthy tissue should serve as control.
2. With the help of a micropipette apply gently one or 2ul antigen dilution onto membrane to avoid excess spreading.
3. Air dry the membrane at least for 15 minutes.
4. Transfer membrane to a plastic or glass dish containing blocking solution so that the membrane is fully emersed.
5. Shake the membrane in blocking solution at room temperature for 1 hour.
6. Grind healthy seeds to make 1: 20 dilution in antibody buffer (1 g in 19 ml buffer). Filter through cheese cloth. Now use this healthy sap to make appropriate dilution of virus-specific antiserum. Normally a dilution of 1:250 or 1:500 (0.05 ml serum diluted in 12.5 ml (1/250) or 0.05 ml diluted in 25.0 ml (1/500). After incubating the diluted antiserum at 37° C for 45 minutes, it is now ready to use.
7. Remove the membrane from blocking solution and transfer to diluted antiserum. Shake at room temperature for 1 hr.
8. Pour off antibody solution. Wash the membrane in TBS-Tween + milk powder thrice, shaking 5 minutes during each wash.
9. Dilute the alkaline phosphatase conjugate (goat antirabbit IgG or FC) to a dilution of 1:500 (0.05 ml in 25 ml buffer in antibody buffer and add to dish containing membrane).
10. Shake at room temperature for 1 hr.
11. Pour off conjugate solution, wash the membrane as in step 8.
12. Add substrate solution and shake till colour develops (15 -30 min are sufficient). Do not allow excess colour development.
13. Pour off the substrate solution and wash membrane in distilled water.
14. Dry the membrane with paper towels and record the results.

4.5 Monoclonal Antibodies for Virus Detection

Recently monoclonal antibodies have been developed against some seed-borne viruses. Their use for detection purposes depends on their affinity for the virus. On the other hand, a monoclonal antibody is likely to be more strain specific than

a polyclonal antiserum, however, a monoclonal antibody against BCMV has been obtained that reacts with a wider range of strains than several polyclonal antiserum (Wang et. al., 1984). In case of monoclonal antibody an ELISA known as TAS-ELISA (triple antibody sandwich ELISA) is used.

4.5.1 TAS-ELISA Procedure

Materials

1. Antibody dilution buffer (10 X).
2. Anti-mouse IgG alkaline phosphatase conjugate.
3. Blocking solution (10 X, you can use skimmed milk for making blocking solution).
4. ELISA coating buffer (i.e. carbonate buffer).
5. Monoclonal antibody specific for the virus to be tested.
6. PBS-PVP (10 X), this is the serum buffer or antibody buffer.
7. Washing buffer.
8. Polyclonal antibody –N (rabbit) to virus to be tested.
9. Positive control (may be purified virus or virus infected plant tissue), ELISA plate.
10. Substrate tablets and substrate solution.

The solution are sometimes provided in form of a kit in a dropper bottle, use three drops from each bott le to make 100ul.

Procedure

1. Dilute the anti-N (polyclonal) antibody in 1 X ELISA coating buffer at 1 : 100 (three drops from the bottle in 1 ml of buffer). Add 100ul to each well using 3-5 wells per sample if possible. Incubate for 2 hr at room temperature or at 37°C.
2. Wash wells three times with BPS-Tween (i.e. washing buffer). Invert plate and pat dry onto absorbent paper.
3. Add 125ul of 1 X blocking solution to each well. Incubate for 1 hr at room temperature. Invert plate and empty the wells by shaking.

4. Make antigen solution using 0.1 g of positive sample powder (or 1g fresh weight tissue) in 10 ml of 1X antibody dilution buffer. Grind and spin to remove the debris.
5. Add 100ul of each sample to 3-5 wells and incubate for 2 hr at room temperature or at 37°C.
6. Wash wells 3 times with PBS-Tween. Invert plate and pat dry onto absorbent paper.
7. Add 100ul of anti-N monoclonal antibody (undiluted) to the well. Incubate for 2 hr at room temperature or at 37°C.
8. Wash wells 3 times with washing buffer (PBS-Tween). Invert plate and pat dry onto absorbent paper.
9. Dilute anti-mouse IgG alkaline phosphatase 1:5000 in 1X antibody dilution buffer (plants). Add 100 ul to the well and incubate for 2 hr.
10. Wash wells 4 times with washing buffer (PBS-Tween). Invert plate and pat dry onto absorbent paper.
11. Make substrate solution using substrate buffer (pH 9.8) by adding one tablet each of buffer and substrate in 20 ml of water. Add 100ul to the well. The yellow colour should be visible in about 20-30 minutes. Read the absorbance at 405nm with ELISA reader.

The layout of the TAS-ELISA procedure are presented in **Fig. 4.9.**

4.6 Serologically Specific Electron Microscopy (SSEM)

Derrick (1973) was the first to introduce electron microscopy (EM) serology when he trapped virus particles to an antibody coated EM carbon grids. It is also called as "immunospecific Electron Microscopy" (ISEM). This method combines the high resolution of electron microscopy and the specificity of serological reaction. In these techniques virus and antiserum are reacted together and the results are viewed under the electron microscope. Generally the principles of SSEM are similar to other methods such as ELISA, radio immunoassy (RIA), that involve adsorption of antiserum to plastic surface, except that virus particles are detected with an EM. instead of using antibody-linked to enzyme or radioisotopes. Essential steps in the SSEM are as follows:

1. The copper grids are first coated with carbon film in a special machine for this purpose

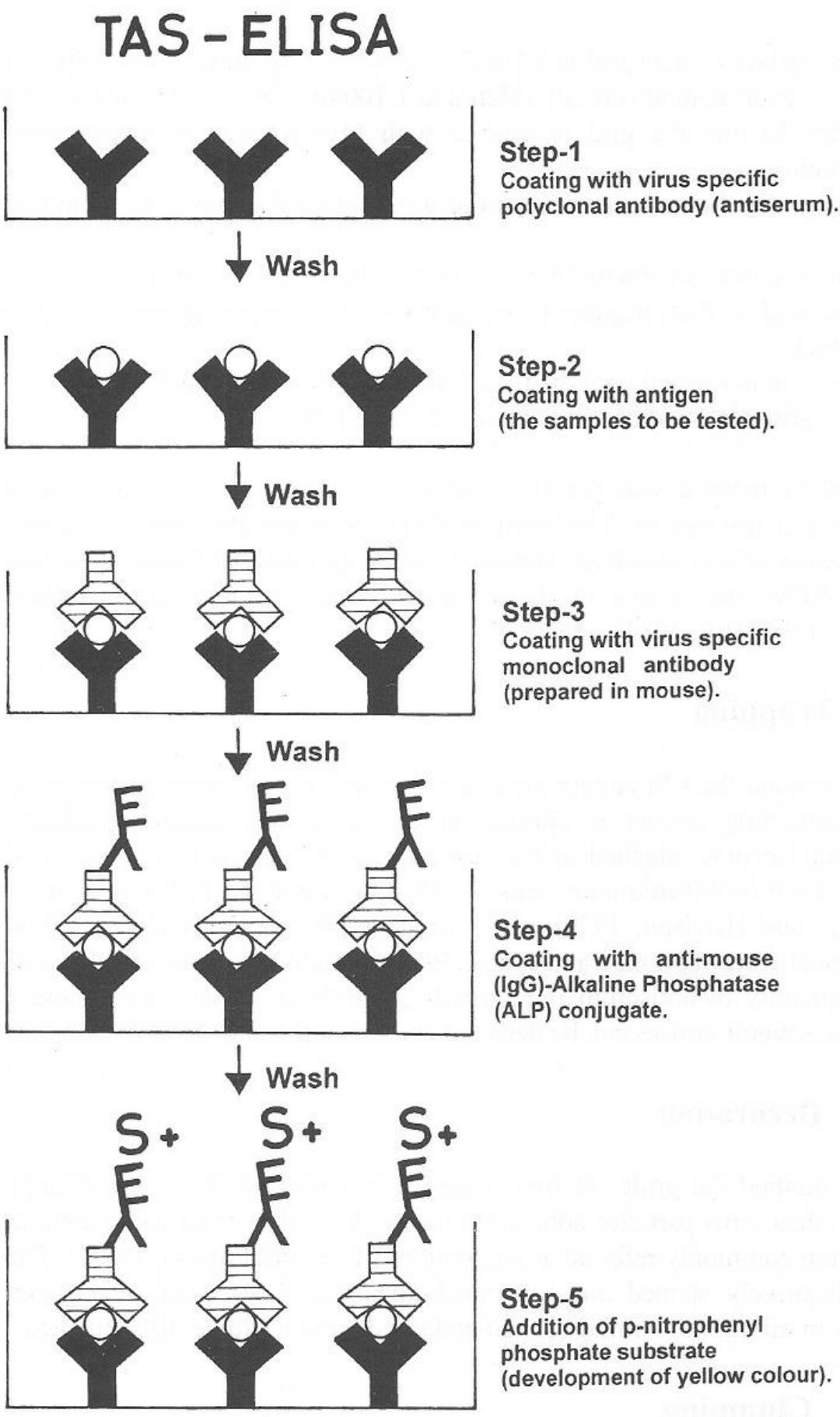


Figure 4.9: Layout of triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA).

2. The carbon-coated grid is floated on antiserum or antigen or mixture of both (what ever method is used) diluted to 1:100 to 1:5000 in Tris buffer of PBS.
3. After 30 min the grid is washed with PBS to remove unabsorbed serum proteins.
4. The grid is floated on an extract of virus-infected tissue from 10 minutes to 1 hr.
5. The grid is washed with PBS to remove cellular debris and salts.
6. The grid is then negatively stained with 5% uranyl acetate for 1.5 min or longer.
7. The grid is washed with distilled water or 95% ethanol, and finally
8. The grid is blotted dry and examined in the EM.

The SSEM method was sensitive enough to detect the two soybean-infecting viruses in a mixture of 1 infected seed per 1000 healthy seeds. The method is rapid, selective and sensitive. Generally, three methods have been employed under these SSEM, the details of these, methods have been described (Bashir and Hassan, 1998; Hill, 1984).

4.6.1 Trapping

In this method the EM copper grids are first coated with virus antiserum, then the virus-containing extract is applied to the grids. The viruses reacting to the antiserum become attached to the grid and can be detected by negative staining. This method is 100-fold more sensitive than conventional EM in detecting viruses (Roberts and Harrison, 1979). It is probably the most sensitive method of all serological tests (Torrance and Jones, 1981). Results are rapid (1-4 hr) and only a small quantity of antiserum is required. If grids are treated with more than on antisera, several viruses can be detected at a time (Thomas, 1980).

4.6.2 Decoration

In this method the grids are first coated with virus-containing sap (sample to be tested), then virus particles adhered to the grids are decorated while applying virus antiserum commonly referred as decoration (Milne and Luisoni, 1975). The grid is then negatively stained and examined under the EM. This technique allows viruses in mixture of similar-shaped and sized particles to be distinguished.

4.6.3 Clumping

In this technique virus-containing sap is first mixed with virus antiserum ad then copper grid is touched to the virus-antiserum mixture. The grid is washed and then

negatively stained. In this method clumps of virus particles are produced and attracts about tenfold more virus particles on the grid (Milne and Luisoni, 1975). This method is used for sensitive detection and identification of virus particles.

4.7 Polymerase Chain Reaction (PCR)

Sometimes the virus concentration in the sample to be tested is too low to be detected by routine serological tests such as ELISA, TBIA, SSEM, and GDDT. ELISA is sensitive enough to detect one infected-seed in 100 (Jones and Proudlove, 1991). Limitation to current ELISA includes a requirement for greater sensitivity, the need for high quality antiserum (free of cross reactivity with plant proteins) and its dependence on operator skill to avoid test failure. An assay based on Polymerase Chain Reaction (PCR: Innes et. al., 1990) involves the enzymatic amplification of DNA or RNA fragment defined by two oligonucleotide primers is potentially able to overcome these limitations. It has greater sensitivity and does not require antiserum. A PCR assay has been successfully used to detect CMV in seed samples of dry lupin (Wyli et. al., 1993) and PSbMV in pea seeds (Kohnen et. al., 1992). The following three essential steps to PCR are: (a) Melting of the target DNA (b) Annealing of two oligonucleotide primers and (c) Primer extension by thermostable DNA polymrase (Saiki et. al., 1988). Newly synthesized DNA strands serve as targets for subsequent DNA synthesis as the three steps are repeated up to 50 times. Although, the reagents used in PCR are expensive and these facilities are not available in every laboratory, but it has several advantages over traditional virus detection techniques: the technique possess high sensitivity, detect minute quantity of virus, and it is rapid and versatile (Hensen and French, 1993). The details of PCR procedures have been reported (Innis et. al., 1990; Wylie et. al., 1993; Kohnen et. al., 1992; Arif, 1998). For detection of a RNA virus from seeds, the PCR assay involves the following steps (Wylie et. al., 1993).

Procedure for PCR

1. **Preparation of Samples:** Take virus-infected seed samples to be tested, grind in a mill fitted with a 2 mm sieve.

2. **RNA Extraction:** In involves the following steps:
   a. Take 100 mg of ground seed flour, place the sample in a 1.5 ml microcentrifuge tube with 400ul of extraction buffer (200mM Tris-HCl pH 7.5, 250mM Sodium Chloride, 25 mM EDTA, 0.5% SDS). Vortex for 5 seconds.
   b. Centrifuge at 1300 g for 1 min, and transfer 300ul of extract to a fresh microcentrifuge tube.

c. Precipitate the nucleic acid (RNA) by adding 1 volume ice-cold isopropanol and then cool to – 80° C for 15 min.
d. Centrifuge (3000 g for 5 min) to get pellet, discard the supernatant, air dry the pellet and then resuspended the pellet in 50ul TE (10mM Tris HCl pH 7.4, ImM DTA).

3. Primers: Oligonucleotides are usually 18-30 bp in size with similar G + C (approximately 50%). Working concentration of primers are generally 25-100p mol of each primer for 50 ml reaction. If added in excess, they can inhibit the reaction. Add 1 ml of a 1 mg/ml conc. of primer in a 100 ml reaction sample. Select a suitable primer based on the virus dealing with.

4. Reverse Transcription: It involves the following steps:
a. Use Perkin Elmer Cetus reagents and optimize the conditions. Use the following conditions: 4mM $MgCl_2$, 50 mM KCl, 10mM Tris-HCl pH 8.3, 1 mM deoxynucleotide triphosphate (dNTP); 0.4 ul pf primer (96um, 5 units RNASE inhibitor and 12.5 units M-MLV reverse transcriptase.
b. Add RNA extract (0.5ul) to aliquots (9.5ul) of the reaction mixture.
c. Layer on drop of paraffin oil and incubate at 42° C for 15 min, 99° C for 5 min, in a Perkin Elmer Cetus Thermal Cycler (Model TC1) or the other available DNA-cycler.
d. Following reverse trancription, the reaction volume will be increased to 50 ul maintaining conditions of 4 mM Mg Cl2, 50 mM KCl, 10 mM Tris-HCl pH 8.3, and with the addition of 1.25 units AmpliTaq DNA polymerase and 0.5 ul primer (10um).
e. Centrifuge the tubes to combine the aqueous phases.

5. PCR Amplification: The following thermal cycling is used: initial template denaturation at 92° C for 3 min followed by 35 cycles of primer annealing and extension at 60° C for 1 min and strand separation at 95° C for I min. An additional period of 7 min at 60° C followed cycling.

6. Analysis of PCR Product: Aliquots (10ul) of PCR-Product is analysed by electrophoresis in a 2.0% agarose gel containing 0.5ug/ml ethidium bromide in Tris-Borate EDTA buffer as described by Sambrook et. al. (1989).

The outlines and principle of the PCR assay have been shown in **Fig. 4.10.**

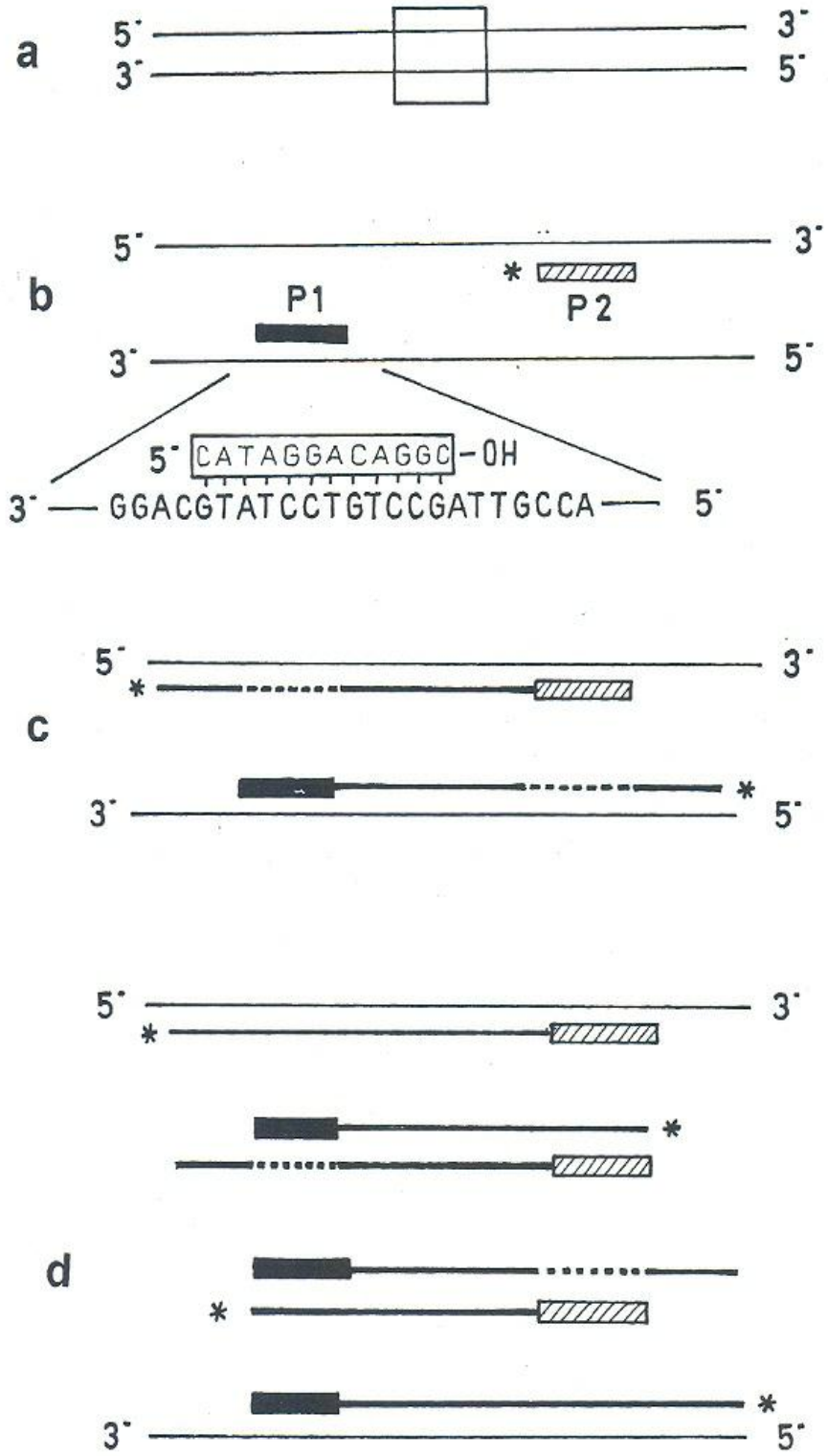


Figure 4.10: Layout and principle of Polymerase Chain Reaction (PCR). (a) DNA double helix with boxed target segment. (b) Hybridization of PCR primers to opposite strands of the region flanking the target. The 3' end of each primer is indicated by an *. The base-base interacting of one primer (boxed) with the region flanking the target is shown below. (c) Results of extending each primer with DNA polymerase. The dotted region of each extension product indicates the position that is complementary to the other primer. (d) PCR product after the material in (c) was subjected to another round of amplification.

Chapter 5 Quarantine and Genetic Resources

5.1 Introduction

The seed movement from one place to another started with man,s early civilization and since then has become important for his survival. Presently, collection, conservation, utilization of plant genetic resources and their global distribution are essential components of crop improvement programmes. This movement of germplasm involves a risk of accidentally introducing new viruses (that are often symptomless). In order to minimize this risk, effective testing procedures are required to reduce the chances of introducing new viruses or their strains to new geographic regions. The volume of germplasm exchanged by each country has created a pressing need for crop specific overviews of the existing knowledge regarding phytosanitary safety of germplasm transfer. In Pakistan, the various research institutes at federal and provincial level receive more than 10,000 lines of breeding material every year which goes directly to the field, and may pose a great risk of pathogen introduction. To meet the demand of increasing population, there is need for both plant introduction and quarantine. Quarantine is one means of keeping pests or diseases under control. They are usually government acted laws designed to regulate the entry of plant material that might carry pests. Nearly all the countries regulate the importation of plant material. The following premises are fundamental to plant quarantine measures:

1. These measures should be based on sound biological principles.
2. Quarantine must derive from adequate law and authority.
3. Quarantine laws should be modified as conditions change and more facts become available.
4. Both professional workers and the general public must cooperate.
5. Those responsible for quarantine measures must be well informed.

All the seed or plant material that enter a country should be classified into following three categories:

A. Restricted: Genera in this category can be readily imported by anyone with few restrictions on how or where the material can be grown or used.

B. Postentry: The species under this category from certain countries pose a high risk of damage to local crops. The species under this category must be grown under close observation.

C. Prohibited: Species in this category tend to have a high incidence of latent viral infection; since such species pose a serious threat, specific requirements must be met before the species can be imported.

5.2 Organizations Involved in Genetic Improvement

For the last three decades, there has been a tremendous increase in the flow of plant genetic resources at national and global level. A significant portion of this material is vital to the crop improvement programmes of many countries. The seed health quarantine implications of international and adaptive testing of plant genetic resources represent a major concern to International Agricultural Research Centres (ARC,s) and other institutions involved in such efforts. Large germplasm collections, often referred to as gene banks have been established for different food crops in many countries of the world. In recent years, these implications have attracted the attention of a number of organizations including Food and Agriculture Organization (FAO of the United Nations), the Consultative Group on International Agricultural Research (CGIAR) and International Plant Genetic Resources Institute (IPGRI). These and other national and international organizations are actively involved in genetic improvement of the world,s major food crops in a number of developing countries. The flow of genetic material at international level is regulated by quarantine laws.

5.3 International Recognition of Germplasm Health Issue

The demand of germplasm for breeding programmes, particularly in developing countries, and awareness of the risks of genetic erosion in those countries has rapidly increased since the early 1960s. FAO soon realized the issue of germplasm collection and brought to the notice of world community, and in 1974 the International Board for Plant Genetic Resources (IBPGR), now International Plant Genetic Resources Institute (IPGRI) of CGIAR was established within FAO headquarters in Rome for the development of a world network of plant genetic resources activities.

The international attempts for improvement of health status of germplasm and controlling seed-borne pathogens been been reviewed by Bos and Vermeulen (1991) and with special reference to viruses, by Bos (1989b). The attempts have led to IBPGRI/FAO ***Global Program for the Safe Movemnt of Germplasm***

(Frison and Putter, 1988). The germplasm material should go through quarantine, either while leaving the gene banks, or after entry in the recipient country, and seeds or plant grown in isolation from the imported seeds should be tested for seed-borne pathogens. The program to publish a series of the International Guidelines for the safe movement of germplasm was initiated jointly by Plant Protection Division of FAO, and IBPGR (now re-organized as International Plant Genetic Resources Institute-IPGRI) in 1988. The Inter-Centre meeting on germplasm health was held in 1991 under the initiative of IPGRI with participation of several International Agricultural Research Centres (IARC,s) of CGIAR. Through these efforts, the necessity of sanitation of seed stock has been internationally recognized. ***"To eliminate seed-borne pests"*** is referred to as a target of research to be undertaken in Global Plan of Action for the conservation and sustainable utilization of plant genetic resources for food and agriculture adopted by the International Technical Conference on Plant Genetic Resources, Leipzig, Germany, June 1996.

Quarantine regulations restrict the entry of plants, plant products, seeds, soil, cultures of living organisms, packing materials, and commodities, as well as their containers. The aim is to protect agriculture and environment from avoidable damage by hazardous organisms introduced by man. Quarantine regulations governing the import and export of plant or seed material usually do not allow the presence of any pathogen. Therefore, adequate elimination procedures such as chemotherapy, fumigation, hot-water treatment and shoot-tip culture, in conjunction with reliable detection (indexing) procedures, are pre-requisites to the safe and efficient transfer of plant genetic resources.

5.4 Quarantine and Seed Health Testing in Pakistan

In Pakistan, the Plant Protection Department under the Federal Ministry of Food, Agriculture and Livestock, has the responsibility for quarantine implications. The department has quarantine offices headed by a Quarantine Officer at each international airport in the country. The imported plant or seed material is required to be accompanied by a phytosanitary certificate issued by the exporting country. The imported seeds or plants upon arrival are inspected visually by the quarantine officer. The dry seeds if necessary are subjected to test the seed-borne pathogens by blotter method. Similar procedures are followed for the material to be exported. The fumigation or seed-treatment is necessary for the material to be exported before issuing phytosanitary certificate. In both cases the consignment is thoroughly examined before phytosanitary certificate is issued by the Plant Protection Department.

As far as the seed health testing is concerned, it is carried out in the country under different research institutes/centres at federal and provincial level. The seeds are mainly tested for detection and identification of seed-borne pathogens. The seed samples collected from seed lots produced in the country under seed certifications schemes are tested in laboratories working in various cities (Islamabad, Peshawar, Karachi, Quetta, Lahore) under the control of Seed Certification and Registration Department (SCRD), Government of Pakistan., Ministery of Food, Agriculture and Livestock. The SCRD department has 17 seed testing laboratories in the country. The laboratories in Karachi and Lahore also test imported seeds for seed-borne pathogens. One laboratory is working at Karachi under Pakistan Agricultural Research Council (PARC). Various Agricultural Research Institutes working at provincial level such as Ayub Agricultural Research Institute, Faisalabad (Punjab), Agricultural Research Institute, Tarnab, Peshawar (NWFP), Agricultural Research Institute, Tandojam (Sind) and Agricultural Research Institute, Quetta (Baluchistan) perform routine seed health testing for locally produced seeds. Generally, the blotter and agar plate methods for fungi, nutrient agar plate method for bacteria and Baerman funnel technique for nematode is followed for testing seeds. Inspection for viral diseases is mainly based on symptoms. Although a few laboratories under CSRD conduct seed testing of imported as well as locally produced seeds for viruses, but there are no facilities in these laboratories for testing seeds or plant material by serological techniques such as ELISA, TBIA, ISEM or DDT for the detection and identification of seed-borne viruses other than potatoes. These laboratories also lack the facilities of greenhouses for testing incoming consignments. All these laboratories need further strengthening with trained staff, modern equipment and greenhouses which are essential for effective plant quarantine in Pakistan.

5.5 Role of Plant Genetic Resources Institute (PGRI), at Islamabad.

This institute was established in March, 1993 at Islamabad under Pakistan Agricultural Research Council (PARC) with the cooperation of Japan International Cooperation Agency (JICA). The institute has one component of Plant Introduction and Seed Health Laboratory equipped with modern equipment and greenhouse facilities. The staff is highly qualified with foreign training in seed health testing both for fungal and viral pathogens. The Seed Health Laboratory in PGRI was originally planned for facilitating the inspection of incoming germplasm in cooperation with quarantine system. With the international trend as above in sight, however, the role of this unit was expanded to cope with a broader area of managing germplasm sanitation i.e. inspection of seed stocks for pathogen contamination, developing the methods to get clean

seeds from contaminated seed, research on effect of pathogen contamination on the longevity of stored seeds, and develop strategies to control seed-borne pathogens. Since the establishment of the institute a number of imported consignments were tested. Crop germplasm preserved in gene bank have been tested under field and greenhouse conditions for detection and identification of seed-borne pathogens. The institute has all the facilities for virus detection in seed and plant material. Observations and studies regarding seed-borne viruses under this laboratory are as follows:

5.5.1 Observations on Seed-borne Pathogens by Seed Health Laboratory, Islamabad, Pakistan

Several events were encountered in Seed Health Laboratory regarding seed-borne pathogens which dipicted the importance of diagnosis and control of systemic phytopathogens in the management of plant genetic diversity.

1. Some of the seed stocks preserved in the cold cache were found to be infected by pathogens in a systemic manner.
2. Legumes seeds received from supposedly reliable international organizations turned out to habore a type of virus not recorded in Pakistan.
3. *Pyrus* spp. collected from northern areas of Pakistan and shipped to Japan were found to be infected with viruses such as apple stem grooving virus (ASGV), pear spot virus (PSV) or pear vein yellows virus (PVYV).
4. The cotton germplasm (*Gossypium* spp.) preserved at Central Cotton Research Institute, Multan in form of prennial plants was heavily infected with cotton leaf curl virus (CLCuV). Although the CLCuV is a Geminivirus and not seed-transmitted. but the phenomenon suggests, that when the germplasm accessions are preserved in a perennial state, they may not only be ruined by perpetual infection by this virus but also become a source of yearly outbreak of the pathogen in epidemic form.
5. The lawns of Bermuda grass in Islamabad were showing symptoms of "white-leaf " disease. On testing samples from diseased plants Mycoplasma-like Organisms (MLO) were detected with the help of a Japanese research group (Ahmad et. al., 1995).
6. Fig plants imported from Syria, when grown in the greenhouse expressed virus-like symptoms. On testing by ELISA, CMV was detected in all the plants.

5.5.2 Germplasm Health

The facets of the involvement of the systemic infection by the pathogens in the genetic diversity conservation may be still wider.

1. Seed sanitation as suggested by FAO/IPGRI is, of course, of prime importance in the *ex-situ* conservation of germplasm. Pathogens infecting seeds may pose the following problems:
    a. Intrusion of pathogens to otherwise clean areas (often crossing the national borders)
    b. Shortening of longevity of seed stocks
    c. Cross infection of germplasm accessions during their rejuvenation while planted in the same area. .

    Improvement technology is needed in the following aspects:
    a. Pathogen indexing methods.
    b. Methods to harvest clean seeds
    c. Standardize procedures to keep seed stocks in a gene bank clean from pathogen contamination.
2. Control of systemic as well as non-systemic pathogens in the movement of vegetative organs/tissues requires further technological improvement. Plant quarantine has been targeted especially on the movement of pathogens carried by vegetative organs/tissues. Further technical development is required in the following aspects.
    a. *In vitro* culture, especially aseptic, is now widely used to transfer vegetative organs/tissues across the borders. The technology still needs to be expanded.
    b. Methodology to inspect the cleanliness of the plant material has to be further developed, especially for under-utilized plant species.
3. Germplasm preserved in the field (such as *Gossypium* spp.) need to be kept clean from systemic pathogens. The perennial state of vegetative organisms may bring about complications even by normally non-seed-transmissible pathogens.
4. *In situ* preservations has become emphasized since the environmental aspects came in the genetic diversity issue. Strategy of how to manage germplasm *in situ* is, however, yet to be established. Survey and monitoring the genetic diversity in the natural habitat seems to be the main tools currently employed. A question is whether recording the natural habitation of germplasm is sufficient for a guide for future actions. We may have to look more carefully into biotic and abiotic stresses potentially threatening the survival of the targeted plant species. Contamination of natural habitat as in the case with *Pyrus* spp. in the northern areas and in Bermuda grass in Islamabad warns us to take this issue more seriously.

5.6 Virus Indexing

Through various facets of germplasm health problems, indexing and controlling systemic pathogens is of primary importance. Ways to index plant viruses have rapidly advanced in recent few decades. Biotechnological devices have become widely used along with classical assay methods. How to detect, how to identify and how to control seed-borne viruses employing various tools from a wide optional methodology may offer a guide to the strategy of genetic diversity conservation in a wider scope.

Virus indexing consists of transmitting the infectious agents from a plant in which it may or may not induce symptoms to healthy indicator plants known to show symptoms. The transmission may be based on mechanical or grafted procedures or the insect vector or dodder. Indexing tests may require only days or weeks with herbacious plants, but may require months or years with woody plants.

5.7 Seed-borne Viruses Reported From Pakistan

Although the subject of seed-borne viruses and their transmission in seeds has not been studied in detail in Pakistan mainly due to lack of trained manpower and well equipped laboratory facilities for the detection of such pathogens, but with the establishment of PGRI at Islamabad, a number of legume crop germplasm accessions of lentil, pea, groundnut, cowpea, and soybean were evaluated under field as well as greenhouse conditions for the detection and identification of seed-borne viruses in Seed Health Laboratory. The germplasm evaluated and viruses detected from seeds are as follows:

5.7.1 Seed-borne Viruses in Pea germplasm

Thirty eight local collections of pea (*Pisum sativum*) were evaluated by growing-on test under greenhouse conditions during 1993. Fifty percent lines were found contaminated with PSbMV **(Fig. 5.1a).** The natural seed transmission rate by growing-on test was determined to be 2.8 to 25.8 percent. When the seed extracts of the same lines were tested by ELISA, 60% lines were found contaminated with PSbMV and the seed transmission rate was 2 to 15% (Mehmood et. al., 1995; Mehmood et. al. 1996). According to a survey conducted in three districts of Punjab (Lahore, Kasoor, Rawalpindi) and one in NWFP (Haripur), 984 samples from farmer,s fields of pea and experimental plots at research stations were collected and tested by ELISA. PSbMV was detected from samples collected from research stations and not from farmers fields (Mehmood. et. al., 1997). The PSbMV probably has been introduced in Pakistan from exotic seeds and was

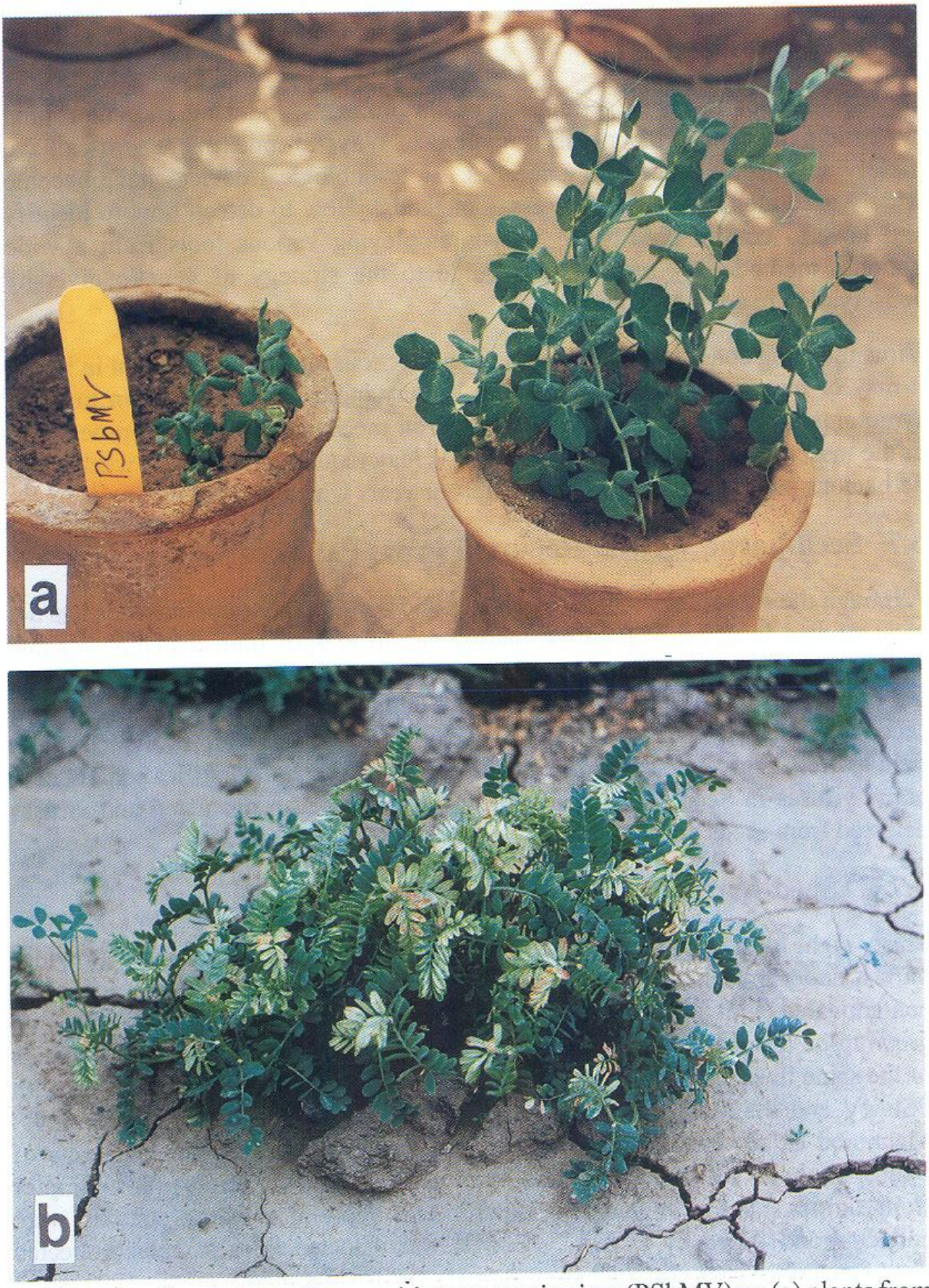


Figure 5.1: Symptoms of pea seed-borne mosaic virus (PSbMV) on (a) plants from diseased seeds (left), healthy (right) (b) on lentil plants under natural infection conditions.

first reported in 1993 in peas (Bashir et. al., 1993a). Sixty five lines were screened against PSbMV by mechanical inoculation method and the following 12 lines were found highly resistant to PSbMV: 90-19, 93-9, Bonneville, Dual, 90-12, PMR-80, 88P223-9, 88P-226-29, 88P-038-10-13, 89P117-5, 89P134-2, and 89P166-5.

5.7.2 Seed-borne Viruses in Lentil Germplasm

During 1993-94, 1002 lentil (*Lens culinaris*) germplasm accessions received from International Centre for Agricultural Research in Dry Areas (ICARDA), Syria and 62 from gene bank of PGRI, National Agricultural Research Centre (NARC), Islamabad, were evaluated both under field and glasshouse conditions. One hundred thirty lines were showing virus-like symptoms **(Fig.5.1b).** The samples from these lines were collected and tested by ELISA against CMV, PSbMV and bean yellow mosaic virus (BYMV) antiserum. About 35 % samples reacted with PSbMV antiserum showing positive reaction. Lentil strain of PSbMV was detected in only exotic material (Bashir et. al., (1995). Local lentil collections were found free of any virus (Riaz et. al., 1994). Although lentil strain of PSbMV has been reported from naturally infected plants in Pakistan (Aftab et. al., 1992), but there was no report of germplasm contamination with PSbMV before this study.

During 1997, an extensive survey of chickpea and lentil crops was conducted and a large number of samples (more than 3000) were collected and tested by Tissue Immunobinding Assay (TIBA). PSbMV and CMV were the most common seed-borne viruses in lentil (Bashir et. al., 1998). Although, CMV has been reported in naturally infected lentil from Pakistan (Bashir et. al., 1994), but no information is available on its seed transmission in lentil. The other seed-borne virus reported in lentil is AMV **(Fig. 5.2a)** (Bashir et. al., 1998). AMV has been reported to be seed transmitted in lentil at a very low rate (1-2%) (Jones and Coutts, 1996).

5.7.3 Seed-borne Viruses in Chickpea Germplasm

Chickpea (*Cicer arietinum*) germplasm has not been evaluated in a systematic way, but when the samples collected from field-grown virus-infected plants were tested by ELISA, two seed-borne viruses i.e. PSbMV and CMV were detected

Figure 5.2: Symptoms of alfalfa mosaic virus (AMV) on naturally infected plants of (a) lentil (b) chickpea.

(Bashir et. al. 1998). Although CMV was reported in chickpea in 1993 (Bashir and Malik, 1993), but was not detected in seed when more than 2000 seeds were evaluated in the greenhouse. Another seed-borne virus detected in naturally infected chickpea plants was AMV (**Fig.5.2b**). AMV has been reported to be seed-transmitted in chickpea at a rate of 0.1 to 0.5% (Jones and Coutts, 1996). Although CMV has been reported to be seed-borne in chickpea (Jones and Coutts, 1996), but we could not detect in seeds.

5.7.4 Seed-borne Viruses in *Vigna* spp. Germplasm

During 1994, 235 accessions of *Vigna* species (*Vigna radiata, Vigna mungo, Vigna aconitifolia*) were evaluated by growing-on test under glasshouse conditions for detection and identification of seed-borne viruses. Eighteen accessions of urd bean (*Vigna mungo*) were found to be contaminated with ULCV **(Fig. 5.3a).** (Ahmad et. al., (1997). The rate of seed-transmission varied from 2.7% to 46% (Riaz et. al., 1994; Ahmad et. al., 1997). The ULCV has already been reported from Pakistan and could cause 81% yield reduction when infection occurs at early stage of plant development (Bashir et. al., 1991)

5.7.5 Seed-borne Viruses in Cowpea Germplasm

During 1990-91 a detailed survey of cowpea (*Vigna unguiculata*) crop was conducted in 13 districts of Punjab and NWFP, Pakistan. About 151 samples collected from virus-infected plants were collected and tested by ELISA. Seed samples were also collected and tested. The following five seed-borne viruses were detected from seed and plant tissues: BlCMV, CABMV, SBMV, CSMV and cowpea mottle virus (CPMoV). From seven cowpea seed lots representing two commercial seed types collected from Punjab and NWFP, the same seed-borne viruses were detected. CABMV was seed-transmitted at a rate of 1 to 7 %. (Bashir and Hampton, 1993).

Recently BlCMV and CABMV have been detected from imported seed lots of cowpea from Nigeria (Bashir et. al., 1999). During 1999 two cowpea trials (cowpea early maturity trial and cowpea medium maturity trail) each consisting of 25 test lines were received from International Institute of Tropical Agriculture

Figure 5.3: Symptoms of urdbean leaf crinkle virus (ULCV) on urdbean plants (b) symptoms of blackeye cowpea mosaic virus (BlCMV) on cowpea.

(IITA), Ibadan, Nigeria for their evaluation at NARC, Islamabad. These lines were evaluated both under field and greenhouse conditions. At seedling stage some accessions were showing virus-like symptoms **(Fig. 5.3b)**, both under greenhouse and field conditions. The samples from symptomatic plants were collected and tested by ELISA. Based upon ELISA results two seed-borne viruses i.e. BlCMV and CABMV were detected and the following cowpea lines from IITA were found contaminated with seed-borne viruses: IT93K 686-2, IT96D-618, IT95K 1092-12, IT95K 1093-5, IT95K 1381, IT95K 207-15, IT95K 333-3, IT95K-637-1, IT97K-1038-94, IT95K 207-15, IT95K-1453-47, IT97K-819-132, IT93K 452-1, IT96D-651 and IT97K 400-3. Both these viruses (BlCMV and CABMV) were also detected from local seeds collected from Sialkot district (Bashir et. al., 1999).

5.7.6 Seed-borne Viruses in Peanut Germplasm

One hunded and fifty peanut (*Arachis hypogea*) germplasm accessions maintained by Oilseed Programme, NARC, Islamabad were evaluated by growing-on test under greenhouse conditions. All plants were tested by ELISA against all possible seed-borne virus antisera e.g. peanut stripe virus (PStV), peanut mottle virus (PMoV) and peanut clump virus (PCV). Although seed-borne PCV has been reported from naturally infected peanut plants **(Fig. 5.4a)** in Pakistan (Delfosse et. al., 1995a), but fortunately all the germplasm accessions tested under greenhouse conditions were found free of any seed-borne virus (Bashir et. al., 1995). Originally these lines were received from International Crops Research Institute for Semiarid Tropics (ICRISAT), India.

5.7.7 Seed-borne Viruses in Lathyrus Germplasm

During 1997, three Lathyrus (*Lathyrus sativus*) accessions: 41224, 41203, and 41204 were received from ICARDA, Syria and tested by growing-on test in the glasshouse. Hundred seeds of each accession were grown in plastic pots and each plant was tested against three seed-borne virus antisera: PSbMV, CMV and BYMV. All the three accessions were found contaminated with two seed-borne viruses: CMV and PSbMV **(Fig. 5.4b)** (Bashir et. al., 1997a). PSbMV was also detected from 34 samples of Lathyrus showing virus-like symptoms collected

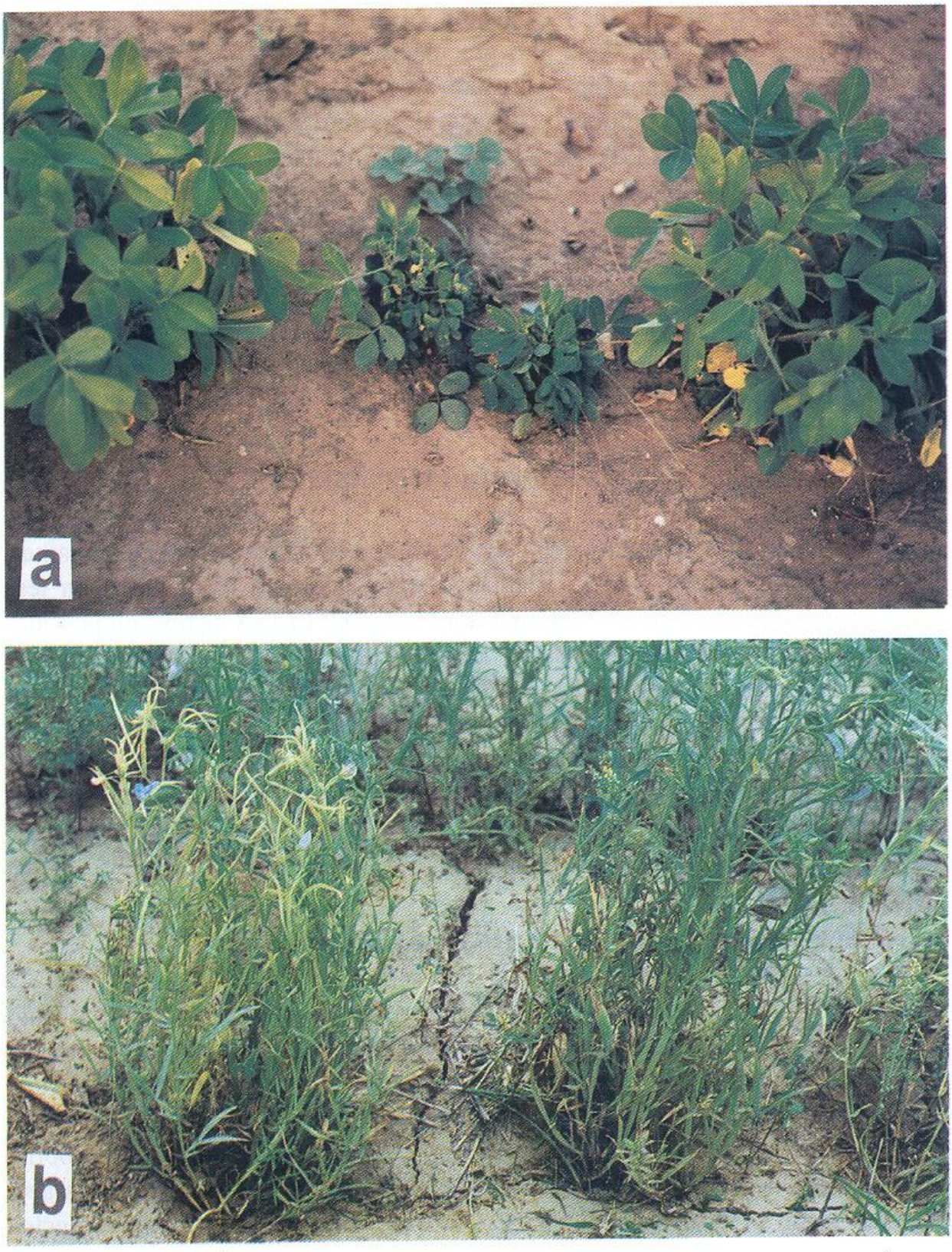


Figure 5.4: (a) Peanut plant infected with peanut clump virus (PCV) (b) symptoms of pea seed-borne mosaic virus (PSbMV) on *Lathyrus sativus* under field conditions.

from Lathyrus International Trial from ICARDA, planted at Barani Agricultural Research Institute (BARI), Chakwal (Punjab) during 1997 (Bashir et. al, 1997a).

5.7.8 Seed-borne Viruses in Soybean Germplasm

Natural infection of SMV has been reported in soybean in Pakistan and SMV is a serious disease of soybean in NWFP (Ali and Hassan, 1992). During 1993, seeds of 30 different soybean varieties from local sources as well as 20 germplasm accessions from Asian Vegetables Research and Development Centre (AVRDC), Taiwan were obtained and evaluated for seed-borne viruses under controlled conditions. Eighteen out of 30 varieties, and 14 out of 20 germplasm accessions were found contaminated with SMV **(Fig. 5.5a).** The highest seed transmission rate of SMV in local collections was 60%, whereas it was as high as 95% in soybean exotic germplasm collections from AVRDC (Ali and Hassan, 1993). This study was conducted in the Department of Plant Pathology, NWFP Agricultural University, Peshawar, Pakistan.

5.7.9 Seed-borne Viruses in Garlic Germplasm

During 1997, six garlic (*Allium sativum*) cultivars were evaluated for adaptation at NARC, Islamabad, under Vegetables Programme, NARC, Islamabad. The local garlic was planted as check. All the exotic lines were showing virus-like symptoms **(Fig. 5.5b).** The samples from these lines were collected and tested by ELISA. All the 16 samples were positive for garlic yellow streak potyvirus (GYSV) (Bashir et. al., 1997b). The local garlic variety was found free of virus infection.

5.7.10 Seed-borne Viruses in Eggplant

During 1995, a few eggplants (*Solanum melongena*) of variety "Hari" grown in plastic tunnel at NARC, Islamabad under Vegetable Programme were observed with virus-like symptoms **(Fig. 5.6a).** Twenty samples from symptomatic plants were collected and tested by ELISA. All these samples reacted positively with CMV antiserum. Based on ELISA results CMV was detected in all the virus-infected plants (Bashir et. al., 1996).

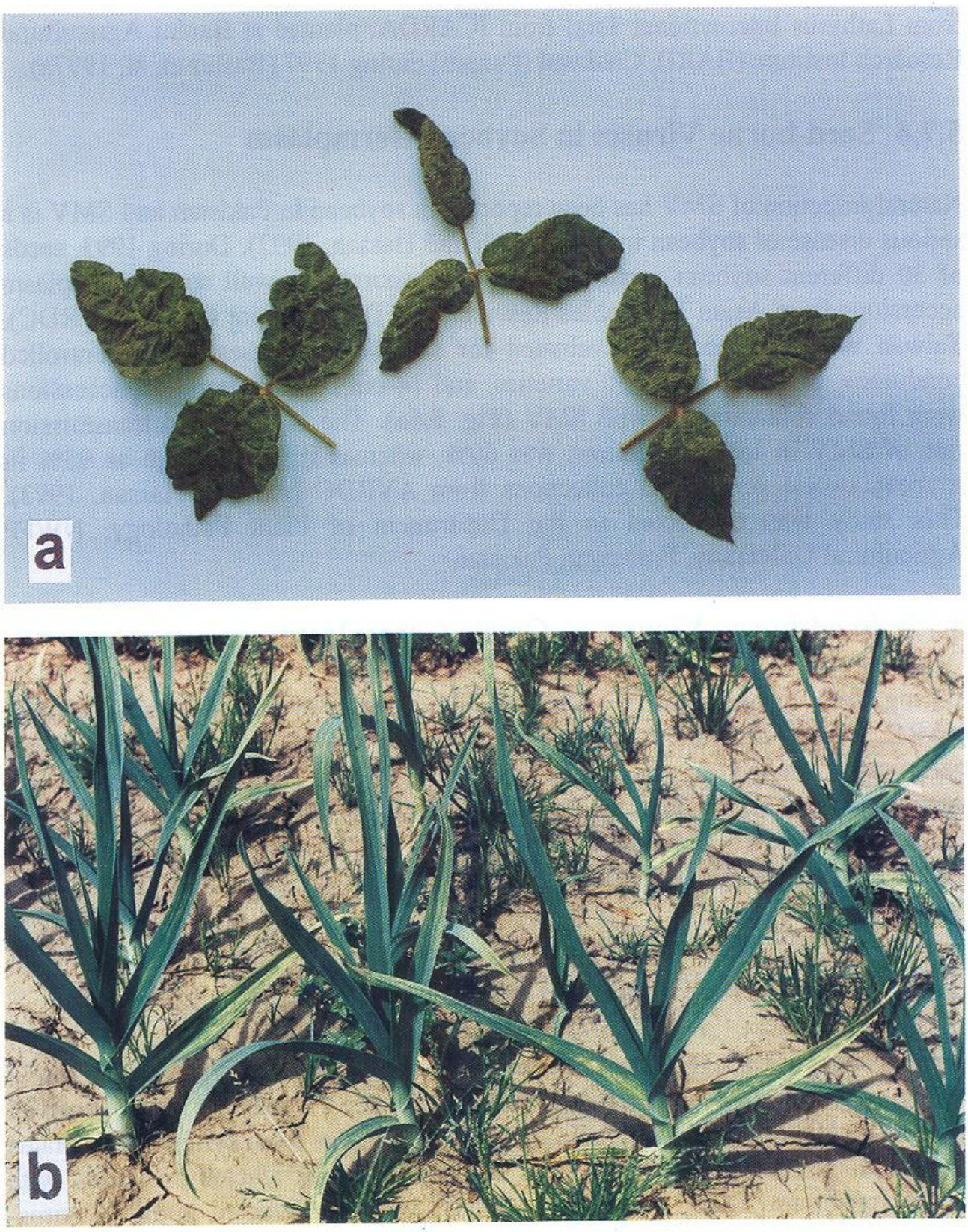


Figure 5.5: (a) Symptoms of soybean mosaic virus (SMV) (GYSPV) on soybean leaves (b) symptoms of garlic yellow streak potyvirus on garlic under field conditions.

5.7.11 CMV in Exotic Fig Plants

During, 1998, ten cuttings of fig (*Ficus carica*) were imported from Syria. When these were planted under controlled conditions, all the plants expressed virus-like symptoms **(Fig. 5.6b).** Based on ELISA results, CMV was detected in all the plants.

5.8 Quarantine Requlations

Almost every country in the world has quarantine regulations implemented effectively or not. Briefly, these regulations have the following features in common:

1. Specify prohibitions.
2. Grant exception to prohibitions for scientific purpose.
3. Require import permit
4. Require phytosanitary certificate and or certificate of origin.
5. Stipulate inspection upon arrival.
6. Prescribe treatment upon arrival to eliminate a risk.
7. Prescribe quarantine, post-entry quarantine, isolation, or other safeguards.

5.9 Suggested Approaches to Avoid Introduction of Foreign Pathogens

The following are some suggestions for the parties involved in exchange of germplasm in order to accelerate the flow of genetic material without increasing risks of pathogen introductions:

1. Try to avoid the introduction of genetic material already existing in the country. Do not duplicate the material which may involve the risks of pathogen introduction.
2. If the breeder plan to import the world collections of various varieties of a crop which must be passed through quarantine, he should be very specific while selecting genotypes. Does he really need the world collections to improve crop productivity?
3. Set up priorities for country or region to ensure that quarantine facilities and services are utilized in high priority areas.

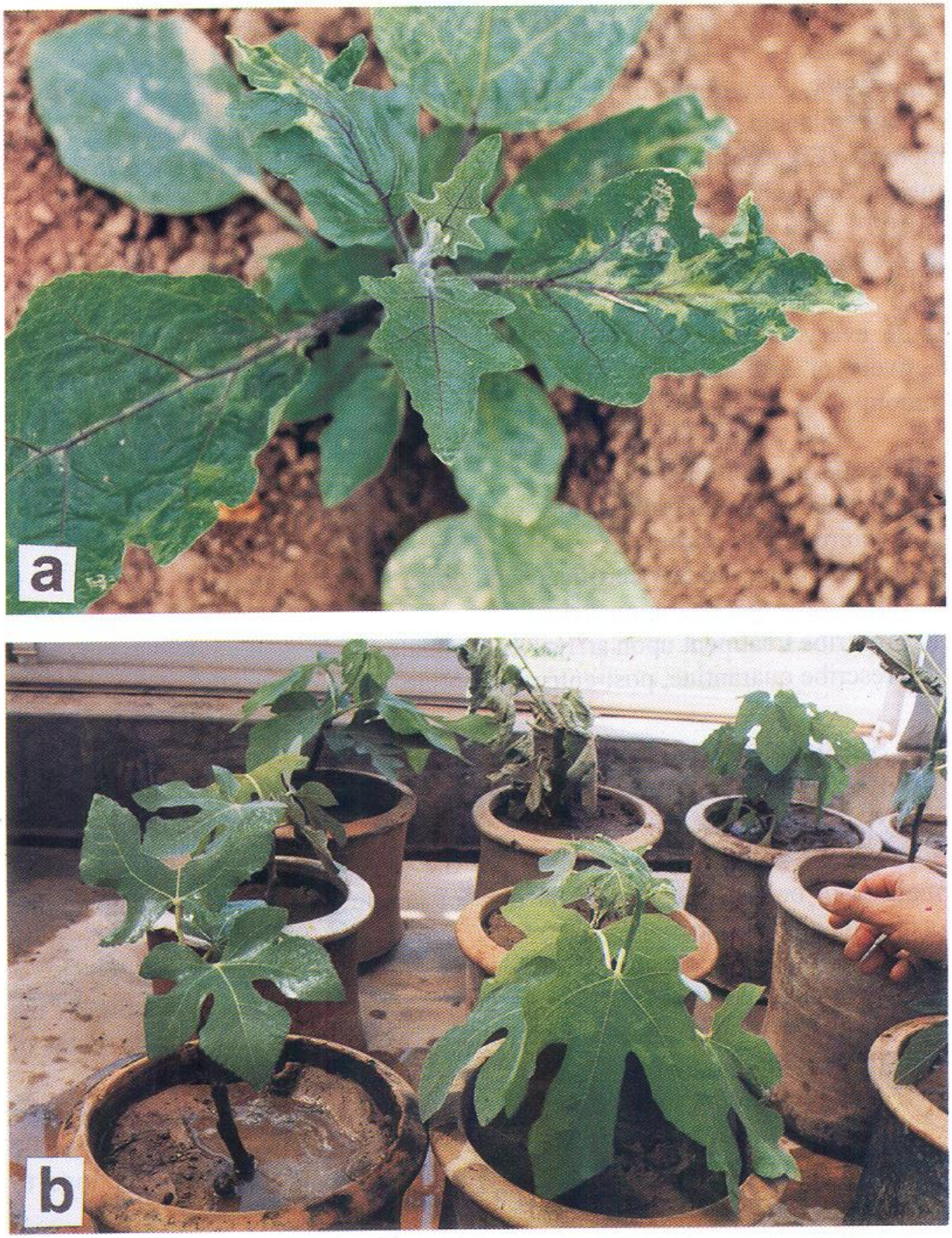


Figure 5.6: Symptoms of cucumber mosaic virus (CMV) on (a) eggplant and on (b) fig plants.

4. Establish a plant introduction accessions system so that all accessions of germplasm are fully documented to genetic background and origin.
5. Work closely with country (s) or regional plant quarantine officer.
6. Provide treatment and other safeguards to facilitate the enterance of germplasm rather than routine denying entry.
7. Exchange information about the geographical distribution of pests and pathogens.
8. All the imported material should be tested for seed-borne pathogens under close observations in the greenhouse.

5.10 Suggestions for the Improvement of Seed Health Testing in Pakistan

Training of Staff: The plant pathologists and seed technologists associated with national and regional gene banks, seed testing laboratories should undergo extensive training in seed-pathology, including techniques of seed health testing.

Laboratory Facilities: Well-equipped laboratory facilities should be available at the national Plant Genetic Resources Institute and in Quarantine Centres in order to enable implementation schemes aiming at establishing data base through screening of seed collections.

Greenhouse Facilities: In order to examine the imported seed or plant material, growth chamber, greenhouse facilities with controlled temperature, humidity and light should be established at quarantine stations, seed testing laboratories and institutes utilizing plant germplasm for breeding purposes.

5.11 Future Research Needs for Seed-borne Viruses

The following aspects are suggested for future research and development.

1 To conduct extensive survey to detect, identify and listing of seed- borne viruses in Pakistan.

2. Application of improved methods for the detection, identification and characterization of seed-borne viruses in various crops.

3. Improvement of methods of detecting seed-borne viruses in the standing crops and in bulk seeds samples.

4. Development of more realistic sampling procedures in the light of current methods of detecting viral antigens and nucleic acids.

5. Since seeds are efficient carriers of viruses to long geographical distances, each virology laboratory in the country, particularly the laboratories working under Federal Seed Certification department be well equipped for testing samples of various crops.
6. There is also a need to set tolerance limits for seed-borne viruses in various crops for seed health testing.
7. The present crop germplasm maintained at various places in the country must be evaluated to know the lines contaminated with seed-borne viruses, and virus-free seed should be produced for distribution to the researchers.
8. Screening of crop germplasm for identification of resistant sources and incorporation of resistant genes to develop new varieties which do not allow the virus to infect or seed-transmission.
9. A system should be developed to evaluate and test the imported seed of crops or breeding material trials/nurseries for the detection of seed-borne viruses and to follow strict quarantine measures.

Chapter 6 Viruses of Quarantine Significance

6.1 Introduction

Due to increased population pressure particularly in developing countries, the demand for crop germplasm has been increased many fold for the improvement of crop productivity. Movement of crop germplasm and breeding material (trials/nurseries) may facilitate the long distance dissemination of seed-borne viruses, some of which are of quarantine significance. In order to maintain high crop productivity in modern agriculture, it is essential to minimize the risk of introducing seed-borne viruses, especially to geographical locations known to be free of such viruses.

Distribution of seed from gene banks involves the risks of introduction of new pathogen in different regions or locations. The viruses that are potentially harmful and still of limited distribution should be prevented from spreading to other countries and regions and they should receive quarantine status. In most countries a list of viruses of quarantine importance is issued and such list is updated on regular basis (Holdeman, 1986). In developing countries, the informations on such a aspect are lacking, and it is not easy to decide which virus is of quarantine significance or not. In such cases it is suggested that extensive in-country surveys be conducted and a list of seed-borne viruses of quarantine importance be prepared. In Pakistan no such list of seed-borne viruses exist, however according to the previous surveys conducted from time to time, it was found that PSbMV is the most prevalent seed-borne virus in lentil and peas (Bashir et. al., 1998: Mehmood et. al., 1996), and five seed-borne viruses: BlCMV, CABMV, SBMV, CSMV and cowpea mottle virus (CMoV) have been reported in cowpea in Pakistan (Bashir and Hampton, 1993), but their percent seed transmission has not been determined. Similarly there may be possibility of the occurrence of other seed-borne viruses in the country, but no information is available. In this section the characteristics of seed-borne viruses of quarantine significance are described.

6.2 Alfalfa mosaic virus (AMV)

Alfamovirus genus, particles are of four types in bacilliform shape, 18 nm wide x 57, 43, 35, and 30nm long.

Host range: The virus has very extensive natural host range and includes over 150 species in 22 families of dicotyledons. Symptomless infection is common in many legumes.

Symptoms: The symptoms shown by naturally infected plants persist, or disappear soon after infection and the plants apparently recover. In lucern it causes mosaic and mottle, the symptoms are masked at higher temperature. In soybean, brilliant yellow mottle or mosaic (calico); in common bean, cowpea and mung bean, systemic yellow mosaic. Lethal systemic necrosis may occur in pea, and wilting in chickpea. Red and white clover often exhibit mosaic.

Transmission: Mechanically transmitted. At least 14 species of aphids can transmit the virus in a non-persistent manner. Up to 50% seed transmission is reported in alfalfa seeds and lucern (Beczner and Manninger, 1975; Pesic & Hiruki, 1986).

Geographical distribution: Worldwide

Indexing: The virus induce necrotic lesions by mechanical inoculation on *Chenopodium amaranticolor* and *C. quinoa*. Can be indexed by ELISA, but testing whole of seeds may reveal antigen in seed coat of which the embryo is free of virus (Pesic and Hiruki, 1986).

6.3 Bean common mosaic virus (BCMV)

Potyvirus genus, flexuous rod shape particles about 720 to 770 nm.

Host range: Mainly found in *Phaseolus* species, mungbean (Kaiser et. al., 1968), also reported from *Lupinus luteus* (Frencel & Pospieszny, 1979) and several other legumes.

Symptoms: Vein-banding mosaic of dark green areas along with main veins, sometimes curling or blisters with malformation. Bud necrosis has been observed in some bean genotypes (Drijfhout, 1978).

Transmission: Mechanically transmitted. Several aphid species may transmit the virus in a non-persistent manner. *Aphis fabae* and *Myzus persicae* are the major vectors. Seed transmission in common bean (*Phaseolus vulgaris)* and mung bean (*Vigna rdiata*) is up to 25% (Kaiser et. al., 1968), in phasey bean (*Macroptilium*

lathyroides) and tepary bean (*Phaseolus acutifolius*) up to 22% (Provvidenti & Cobb., 1975), and urdbean (*Vigna mungo*) from 2-10% (Agarwal et. al., 1979).

Geographical distribution: World wide.

Indexing: The virus induces necrotic lesions on common beans cvs. Top Crop and Widusa when inoculated mechanically.

6.4 Bean yellow mosaic virus (BYMV)

Potyvirus genus, flexuous rod shape particles about 750 nm. Various strains of virus have been reported.

Host range: Infects common bean, faba bean, chickpea, cowpea, soybean, and some perennial legumes, non-legume hosts are squash, spinach, freesia and gladiolus.

Symptoms: Symptoms shown by naturally infected plants vary reasonably. The virus induces apical necrosis mosaic in beans and necrotic local lesions in some legume hosts.

Transmission: Mechanically transmitted. By many aphid species in a non-persistent manner, also through seed in some legume species such as faba bean, pea, white sweet clover and white yellow lupin. The rate of seed transmission varies from 0.1 to 6% (Fiedorow, 1980; Zschu, 1962; Cobett, 1958).

Geographical distribution: World wide.

Indexing: Selected cultivars of common bean, faba bean, pea *Chenopodium amaranticolor*, and *C. quinoa*.

6.5 Blackeye cowpea mosaic virus (BlCMV)

Potyvirus genus, flexuous rod shape particles about 750 nm. Although closely related with cowpea aphid-borne mosaic virus (CABMV) but is quite distinct from CABMV (Bashir & Hampton, 1996a).

Host range: Naturally infects cowpea, asparagus bean (*Vigna unguiculata* var. *sesquipedalis*), common bean, mung bean and soybean.

Symptoms: In susceptible cowpea it causes mosaic, mottle, green-vein banding and distortion. When occurs in mixed infection of CMV, causes stunting in

cowpea and "rugose mosaic" in asparagus bean (Chang, 1983: Pio-Ribeiro et. al., 1978).

Transmission: Mechanically transmitted. Vectored by aphid species such as *Aphis crassivora*, *Macrosiphum euphorbiae* and *Myzus persicae* in a non-persistent manner. Seed transmission has been reported from 3.5 to 55% in cowpea (Mali & Kulti, 1980; Mali et. al., 1983; Bashir & Hampton, 1994a).

Geographical distribution: Worldwide, wherever cowpea is grown.

Indexing: Induce necrotic local lesion in *Chenopodiun amaranticolor* and cowpea cvs. UCR 524B, UCR 7964 and UCR 8517 (Bashir & Hampton, 1996c).

6.6 Blackgram mottle virus (BMoV)

Carmovirus genus, particles are isometeric about 28 nm.

Host range: Blackgram (*Vigna mungo*) in seeds of which it was first detected (Phatak, 1974). Also infects Soybean and mungbean,

Symptoms: Causes mosaic, leaf mottling usually with puckering, vein clrearing and leaf distortion in blackgram (*Vigna mungo*).

Transmission: Mechanically transmitted. Vectored by beetles (*Cerotoma trifurcata and Epliachna varivestis*), and via seed of blackgram up to 8% (Phatak, 1974).

Geographical distribution: Australisia, India, Indonesia and Thailand.

Indexing: By mechanical inoculation induces local lesions on *Cyamopsis tetragonoloba, Macrotyloma uniflorum, Phaseolus lunatus, Phaseolus vulgaris.*

6.7 Broad bean stain virus (BBSV)

Comovirus genus, angular isometeric particles about 28 nm. Pea green mosaic virus and pea seed-borne symptomless virus are strains (Musil et al., 1983).

Host range: Found in faba bean (*Vicia faba*), lentil, pea, vetch, and hybrid clover (*Trifloium* spp.) Also infects chickpea, some cultivars of common beans (*Phaseolus vulgaris*) and mostly symptomless to a number of wild legumes. Does not infect non-legumes (Makkouk et al., 1987).

Symptoms: It causes systemic leaf mild mottling, sometimes necrosis and distortion. Seeds of infected faba bean may show a chracteristic necrotic pattern of the testa around the periphery of the seed.

Transmission: Mechanically transmitted. Vectored by weevils (*Apion vorax* and *Sitoma* spp.). Via seed of faba bean, transmission is up to 10% (Gibbs & Smith, 1970) or 2.7% (Jones, 1978). Also reported in seeds of pea, lentil and *Vicia palaestinae* as a symptomless carrier (Kowalaska & Beczner, 1980: Makkouk & Azzam, 1986; Makkouk et al., 1987).

Geographical distribution: Europe, North Africa, Sudan and West Africa.

Indexing: ELISA is used to detect virus from leaves, seeds or developing embryos of faba bean. Virus sometimes detectable in cotyledons while not in embryonal axis (Makkouk et al., 1987).

6.8 Broad bean true mosaic virus (BBTMV)

Comovirus genus, angular isometeric particle about 28 nm.

Host range: Only found in fab bean and pea. Artificially transmitted to several legumes but not to non-legumes (Gibbs & Paul, 1970).

Symptoms: Malformation of leaves, mottle and mosaic. At high temperature the symptoms are masked.

Transmission: Mechanically transmitted. By weevils (*Apion vorax* and *Sitona spp.*), and through seeds of faba bean up to 17% (Jones, 1980; Cockbain et al., 1976).

Geographical distribution: Europe and North West Africa, China. Also found in South Australia in crops grown from imported seed (Boswell & Gibbs, 1983).

Indexing: Assay hosts are faba bean and pea, detected by ELISA.

6.9 Cowpea aphid-borne mosaic virus(CABMV)

Potyvirus genus, flexuous rod shaped particles about 750 nm. CABMV is closely related to blackeye cowpea mosaic virus (BlCMV), but is distinct potyvirus (Bashir & Hampton,1996a; Huguenot et. al., 1993).

Host range: Naturally occurs in cowpea, but also infects some other legumes.

Symptoms: Severe mosaic, mottle and distortion in susceptible genotypes. Several strains based on symptomatology of the virus have been reported. The symptoms of CABMV on artificially inoculated plants have been shown in **Fig. 6.1.**

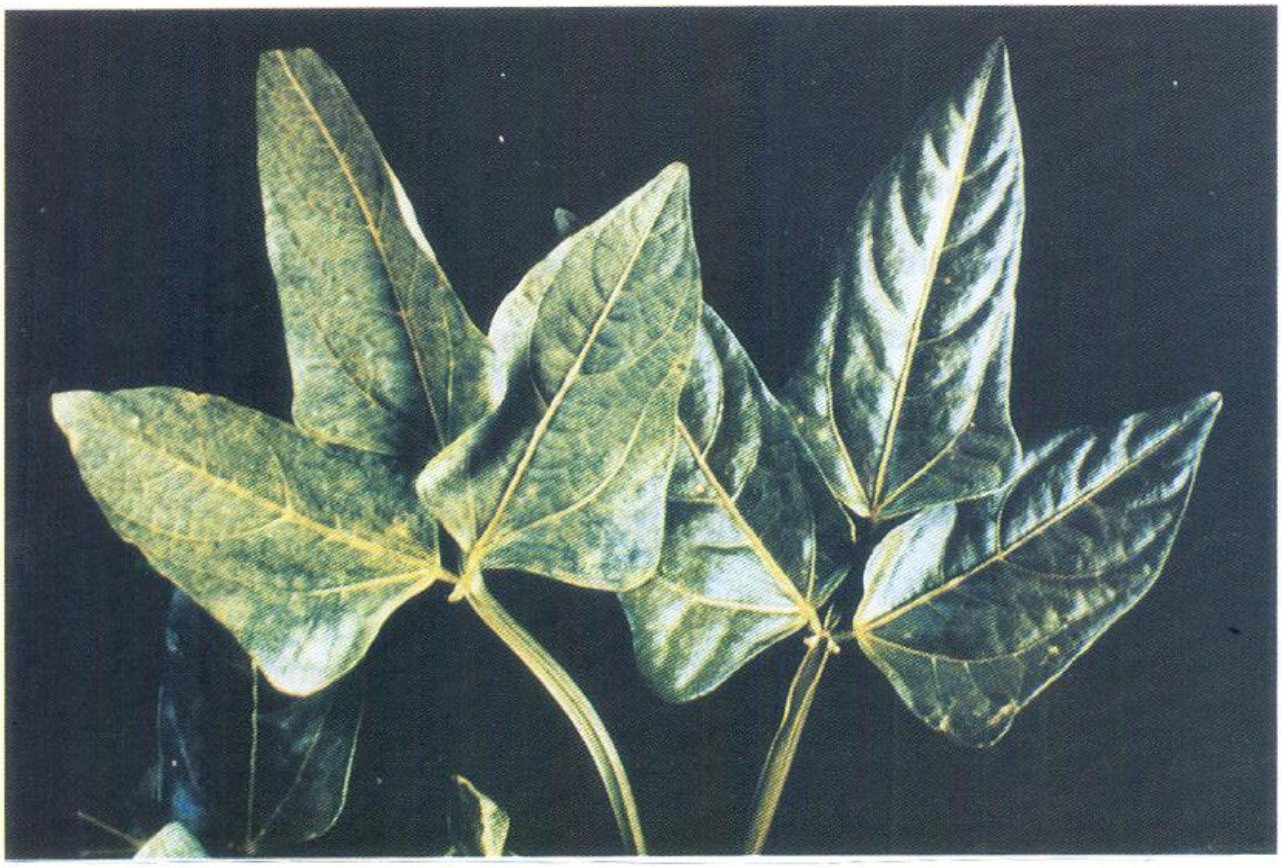


Figure 6.1: Symptoms of cowpea aphid-borne mosaic virus (CABMV) on naturally infected cowpea leaves.

Transmission: Readily transmitted by sap inoculation. Under field conditions several species of aphids can transmit the virus but *Aphis crassivora* is an efficient vector. Also through seed up to 40% (Kaiser & Mossahebi, 1975).

Geographical distribution: Worldwide, wherever cowpea is grown.

Indexing: Induces local lesions in *Chenopodium amaranticolor*, basil (*Ocimum basicilicum*), and some cowpea cvs. Worthmore, Magnolia and Serodo (Bashir & Hampton, 1996c; 1996d). Also detected by ELISA.

6.10 Cowpea mild mottle virus (CMMoV)

Carlavirus genus, filamentous, particles are about 650 nm.

Host range: Reported from cowpea (*Vigna unguiculata*), bambara groundnut (*Vigna subterranea),* soybean, winged bean (*Psophocarpus tetragonolobus*), groundnut, mungbean, and some other legume species. Commonly found in common bean (*Phaseolus vulgaris*), and lima bean (*Phaseolus lunatus*) in Nigeria in which it causes prominent disease symptoms. Also reported from tomato (Brunt & Philips, 1981).

Symptoms: In cowpea it causes diffuse chlorotic blotches on primary leaves and systemic mottling and leaf distortion. Also causes mild mosaic and mottling in soybean. Symptoms in soybean are mild. Chlorosis, stunting and rugose symptoms in common bean. Some strains cause bright yellow mosaic in soybean, vein mosaic and general leaf chlorosis followed by apical necrosis, distortion and stunting.

Transmission: Transmitted by whitefly (*Bemisia tabaci*). Seed transmission is from 0.5 to 100% in cowpea, soybean and common bean (Brunt & Kenten, 1974).

Geographical distribution: Spreads in Ghana, Ivory Coast, Nigeria, Kenya, Israel, India, Tanzania, Thailand, Indonesia and Fiji.

Indexing: Serologically detected by ELISA, and biologically test plants are *Nicotiana clevelandii* and *N. megalosiphom* (Anno-Nyako, 1984).

6.11 Cowpea mosaic virus (CPMV)

Comovirus genus, particles are isometeric about 25 nm, This virus was orginally described as cowpea yellow mosaic virus (Chant, 1959; Swaans & van Kammen, 1973).

Host range: Occurs in cowpea, groundnut, soybean, *Crotarlia juncea,* pigeon pea (*Cajanus cajan*) and *Crotalaria juncea,*

Symptoms: Causes severe mosaic, vein yellowing, mottle and leaf distortion in susceptible cowpea genotypes.

Transmission: Beetle vector (*Ootheca mutabilis*, and *Paraluperodes quaternus*, and by *Nematocerus acerbus* (Curculionidae) (Chant, 1959; Bock, 1971: Whitney & Gilmer, 1974). Seed transmission is 1 to 5% (Gilmer et al., 1973).

Geographical distribution: Spreads in Nigeria, West Africa, Kenya Tanzania Suriname, Cuba and USA (Bock, 1971; Patel & Kuwite, 1982),

Indexing: Test plants are *Chenopodium* spp. and *Nicotiana benthamiana*. Serologically detected by ELISA.

6.12 Cowpea mottle virus (CMoV)

Carmovirus genus, particles are spherical, about 27 nm.

Host range: Occurs in cowpea and bambara groundnut (*Vigna subterranea*) and *Voandzeia subterranea* (Rossel, 1977; Shoyinka et al., 1978).

Symptoms: Causes mottling or bright yellow mosaic and stunting.

Transmission: Mechanically transmitted. By beetle (*Ootheca mutabilis*). Seed transmission in cowpea is up to 10% (Shoyinka et. al., 1978; Allen et. al., 1982), also reported in common bean (Shoyinka et al., 1978) and bambara groundnut (Robertson, 1966).

Geographical distribution: Occurs in Nigeria (Shoyinka et al., 1978) and Pakistan (Bashir & Hampton, 1993)

Indexing: Test plants are *Chenopodium* spp. Serologically detected by ELISA.

6.13 Cowpea ringspot virus (CRSV)

Comovirus genus, particles are spherical, about 25-30 nm.

Host range: Naturally infects cowpea. Also reported in lima bean (*Phaseolus lunatus*) and winged bean (*Psophocarpus tetragonolobus*).

Symptoms: Generally induces very mild symptoms in form of chlorosis or mottle.

Transmission: Mechanically transmitted. By several species of aphids in a non-persistent manner. Also through seed in cowpea from 10 to 30% (Phatak, 1974; Phatak et al., 1976).

Geographical distribution: Occurs wherever cowpea is grown.

Indexing: Test hosts are *Chenopodium* spp., *Nicotiana glutinosa*, and *N. benthamiana*.

6.14 Cowpea severe mosaic virus (CSMV)

Comovirus genus, isometeric particles about 25 nm.

Host range: Occurs naturally in cowpea. Also reported in common bean and soybean (Thongmeearkom & Goodman, 1976).

Symptoms: In cowpea it causes severe mosaic, mottle and leaf distortion. Systemic vein clearing of first trifoliate leaves, distortion and blistering of younger leaves in susceptible cowpea genotypes has been reported. The symptoms of CSMV on artificially inoculated plants have been shown in **Fig. 6.2.**

Transmission: Mechanically transmitted. Beetle vectors are mainly by *Cerotoma ruficornis* and *C. trifurcata*. Transmitted in cowpea seeds up to 10% (Shepherd, 1964; Haque & Persad, 1975) and asparagus bean (*Vigna sesquipedalis*) up to 8% (Dale, 1949).

Geographical distribution: Occurs in cowpea and common bean in Latin America and USA. The virus is also reported from Pakistan (Bashir & Hampton, 1993).

Figure 6.2: Symptoms of cowpea severe mosaic virus (CSMV) on cowpea under filed conditions.

Indexing: Serologically detected by ELISA.

6.15 Cucumber mosaic virus (CMV)

Cucumovirus genus, particles are isometeric, about 29 nm. Several strains have been reported.

Host range: CMV has a very wide host range. It infects a number of legumes and non-legumes species. Among the legume hosts it has been reported from adzuki bean, chickpea, cowpea, faba bean, groundnut, lentil, lucern, lupins, common bean, peas, and clovers.

Symptoms: Symptoms vary depending upon host species and virus strain. Generally it causes mosaic, rugose mosaic and mottling. In case of mixed infection with BlCMV it causes severe stunting in cowpea and asparagus bean (Pio-Riberio et al., 1978; Chang, 1983.

Transmission: Mechanically transmitted. By several species of aphids in a non-persistent manner. Transmission through seed of cowpea, common bean, groundnut, mung bean, and yellow and blue lupin is from 5 to 100% (Green, 1985; Bos & Maat, 1974; Phatak, 1974; Jones, 1988).

Geographical distribution: World wide.

Indexing: Test plants *are Chenopidium amaranticolor, C. quinoa, Cucumis sativus, Vigna unguiculata*. Detected by ELISA in seed.

6.16 Pea early-browning virus (PEBV)

Tobravirus genus, Particles are tubular with two lengths of about 105 and 215 x 21 nm. Some serotypes are reported (Robinson & Harrison, 1985).

Host range: Infects pea, common bean, faba bean, and yellow lupin. Symptomless infection has been reported in lucern and some other legumes (Bos & van der Want, 1963).

Symptoms: It causes irregular leaf, stem and pod necrosis in peas, and entire shoots may be killed. May cause leaf mottling. In common bean it causes irregular leaf and stem necrosis with severe plant stunting. The broad bean yellow serotype causses yellow vein banding in faba bean (Russo et al., 1982). Symptomless infection in faba bean is also reported (Fiedorow, 1980).

Transmission: Transmitted by nematode (*Trichodorus* spp.). Transmission through pea seeds is up to 37% (Harrison, 1973), and in faba bean up to 10% (Fiedorow, 1983).

Geographical distribution: Europe and Morocco.

Indexing: The virus induces local lesions in *Chenopodium amaranticolor*, cucumber and common bean. ELISA is used for virus detection in seed.

6.17 Pea seed-borne mosaic virus (PSbMV)

Potyvirus genus, the particles are flexuous rods about 770 nm.

Host range: Occurs naturally in peas, lentil, faba bean and vetch (*Vicia villlosa*).

Symptoms: It causes vein clearing to marked rosetting of main stem and side branches with small dark green leaves folded upward along the midribs. Flower become distorted, often sterile, pods reduced in size with few mis-shaped seeds. A lentil strain of PSbMV was non-pathogenic to some pea genotypes (Hampton, 1982), whereas another isolate was much more severe on peas (Musil, 1980).

Transmission: Mechanically transmitted. Naturally by several species of aphids in a non-persistent manner. Seed transmission in peas ranged from 2.5% to 95% (Mink et. al., 1969; Mehmood et., al., 1996), in lentil up to 44% (Hampton & Muehlbauer, 1977) and in faba bean up to 3% (Musil, 1980).

Geographical distribution: Asia (India, Pakistan, Japan, Taiwan), Europe, New Zealand, North Africa and North America.

Indexing: Test plants are *Chenopodium amaranticolor*. ELISA detects virus in seeds.

6.18 Peanut clump virus (PCV)

Furovirus genus, particles are rod shaped, bipartite and about 245 nm and 190 x 22 nm.

Host range: Naturally infects groundnut, chillies (*Capsicum annuum*) and millet (*Sorghum arundinaceum*), *Sorghum bicolor, Setaria italica.*

Symptoms: Causes severe stunting, mottle, mosaic, chlorotic ring spots and chlorotic rings on young leaves. The leaflets become small. The symptoms of PCV on naturally infected plants have been shown in **Fig. 6.3.**

Figure 6.3: Peanut plant infected with peanut clump virus (PCV) (left), healthy (right).

Transmission: Mechanically transmitted. Soil-borne fungus vector is *Polymyxa graminis*. Seed transmission in groundnut is up to 20% (Thouvenel & Fauquet, 1981).

Geographical distribution: Burkia Faso, Ivory Coast, India, Senegal, South Africa, and Pakistan (Delfosse et. al., 1995)

Indexing: The Indian isolates of PCV induces necrotic lesions or veinal necrosis in *Phaseolus vulgaris*. For West African isolates the test plant is *Chenopodium amaranticolor*.

6.19 Peanut mottle virus (PMoV)

Potyvirus genus, particles are flexuous rods about 750 nm.

Host range: Naturally infects groundnut, wild groundnut (*Arachis chacoense*), common bean, lupins (*Lupinus angustifolius* and *L. albus*), mung bean, pea, soybean, and some forage legumes.

Symptoms: Causes mild mottle on the younger leaves, the older leaves show upward curling of edges and mottling.

Transmission: Mechanically transmitted. By aphids in a non-persistent manner. *Aphis crassivora* is the efficient vector. Through seed up to 20% in groundnut (Bock, 1973), and less than 1% in cowpea and L*upinus albus (*Demski et al., 1983a; Demski et. al, 1983b).

Geographical distribution: Worldwide. Spreads in East Africa, South-East Asia, India, Philippines, Taiwan, Malaysia, South America and South East USA, but not yet reported from Pakistan.

Indexing: Induces local lesions in *Phaseolus vulgaris* cv. Topcrop. ELISA detects virus in seed.

6.20 Peanut stripe virus (PStV)

Potyvirus genus, particles are flexuous rods about 750 nm.

Host range: Natural hosts are groundnut, cowpea, soybean and *Dolichos lablab, Lupinus albus,* and *Sesamum spp.* .

Symptoms: Causes prominent stripes or bloches on young leaflets. Older leaves show mosaic in form of green islands or oak-leaf patterns, and conspicuous leaf chlorosis. The symptoms of PStV on naturally infected peanut leaves have been shown in **Fig. 6.4.**

Transmission: Transmitted mechanically. By aphids in a non-persistent manner. *Aphis crassivora* is the efficient vector. Through seeds of groundnut up to 40% ((Demski et al., 1984). Under field conditions seed transmission is usually low (1-5%).

Geographical distribution: China, India, Indonesia, Japan, Malaysia, Mynamar (Burma), North America, Philippines, Thailand and Vietnam. Not yet reported from Pakistan.

Indexing: Test plants are *Chenopodium amaranticolor* and *C. quinoa*. ELISA for virus detection in seed.

Figure 6.4: Symptoms of peanut stripe virus (PStV) on peanut under field conditions.

6.21 Southern bean mosaic virus (SBMV)

Sobemovirus genus, particles are isometeric about 30 nm. Strains of the virus are reported.

Host range: Naturally infects in common bean, cowpea, black *gram (Vigna mungo*), mung bean, and soybean.

Symptoms: Causes mosaic, mottle and occasionally stunting and distortion.

Transmission: Mechanically transmitted. By beetles (Chrysomelidae) in a circulative manner. The bean and cowpea strains are transmitted by beetle (*Cerotoma trifurcata* and *Epilachna variverstis*). In Africa the main beetle vector is *Ootheca mutabilis*. Through seeds of cowpea up to 40% (Shepherd & Fulton, 1962; Givord, 1981), also in common bean from 1-30% (Morales & Castano, 1985).

Geographical distribution: Africa, India and U.S.A. SBMV has also been reported in Pakistan (Bashir & Hampton, 1993).

Indexing: Test plants are *Phaseolus vulgaris* cvs. Topcrop and Pinto and Cowpea cv. Clay. ELISA for detection in seeds.

6.22 Soybean mosaic virus (SMV)

Potyvirus genus, particles are flexuous rods about 750 nm.

Host range: Infects soybean, faba bean (*Vicia faba*) and white lupin.

Figure 6.5: Symptoms of soybean mosaic virus (SMV) on soybean (right), healthy (left).

Symptonms: Severity of symptoms depend upon host genotype and virus strains. Causes leaf rolling, mild mottle and rugosity. Some isolates cause severe mosaic,

distortion, flower abnormality and necrosis. The symptoms of SMV on naturally infected plants have been shown in **Fig. 6.5.**

Transmission: Mechanically transmitted. By aphids in a non-persistent manner. Through seeds of soybean up to 95.2% (Ali & Hassan, 1993), also through seeds of white lupin at rate of 1.2% (Vroon et al., 1988).

Geographical distribution: Worldwide, wherever soybean is grown. SMV has been reported from Pakistan (Ali & Hassan, 1992; 1993).

Indexing: Test plants are *Chenopodium spp.* ELISA is useful for virus detection in seeds.

6.23 Soybean stunt virus (SSV)

Cucumovirus genus, particles are isometeric about 28-30 nm.

Host range: Generally infects soybean, but may infect 14 species of legumes by artificial inoculation.

Symptoms: In soybean causes yellow vein banding or mottling, leaf crinkling and severe stunting. In some cultivars may cause vein necrosis and leaf apex margin and top necrosis.

Transmission: Mechanically transmitted. Through seeds of soybean up to 50% (Koshimizu & Iizuka, 1963).

Geographical distribution: China, Indonesia, Japan, USA, and USSR.

Indexing: Test plants are *Chenopodium amaranticolor, Nicotiana tabacum* (White Burley), *Phaseolus vulgaris* (Monroe). Detected by ELISA in seed.

6.24 Tobacco ringspot virus (TSRV)

Nepovirus genus, particles are isometeric about 28 nm.

Host range: Wide host range. It infects annual and prennial herbaceous and woody species. Main host is soybean. Also found in common bean, sweet clover (*Melilotus* spp.), red clover, peas, and *Lupinus polyphyllus*.

Symptoms: Causes necrotic spots, mottling, chlorotic ring spots and vein banding, pod size is reduced.

Transmission: Mechanically transmitted. By nematodes (*Xiphinema americanum*). *Thrips tabaci* may be a natural vector. Through seeds of soybean up to 100% (Athow & Bancroft, 1959).

Geographical distribution: North America. Also reported from Egypt, Turkey, India, and Sri Lanka.

Indexing: Induces local lesions in *Nicotiana clevelandii, N. tabacum, C. amaranticolor* and cowpea (*Vigna unguiculata*). ELISA detects virus in seed.

6.25 Tobacco streak virus (TSV)

Ilarvirus genus, particles are isometeric about 27-35 nm.

Host range: First found in *Nicotiana tabacum* in USA (Johnson, 1936). Infects soybean, cowpea and common bean. Also reported from pea and some clovers. Causes disease in a wide range of non-legume crops.

Symptoms: Causes stunting, leaf mottling, systemic necrosis, reddening of nodal tissue and bud blight in soybean. Irregular chlorotic spots on leaves which may be dwarfed in appearance.

Transmission: Mechanically transmitted. Thrip vectors are *Frankliniella oocidentalis* and *Thrips tabaci.* Through seeds of soybean up to 30% (Ghanekar & Schwenk, 1974; Kaiser et al., 1982). Less than 1% in cowpea (Kaiser et al., 1982) and up to 26% in common bean (Thomas & Graham, 1951). Also transmitted in several non-legume hosts.

Geographical distribution: Australia, Europe, Japan, North and South America and New Zealand.

Indexing: ELISA for detection of virus in seed and plant tissue.

6.26 Tobacco aspermy virus (TAsV)

Cucumovirus genus, particles are sphecical about 25-30 nm.

Host range: Naturally infects tomato and chrysanthemum.

Symptoms: Causes mottle, mosaic and distortion of young leaves of common bean (*Phaseolus vulgaris*). In some genotypes induces yellow spots along the veins.

Transmission: Mechanically transmitted. By aphids in a non-persistent manner. Through seeds of beans up to 18.7% (Wang, 1982a).

Geographical distribution: Australia, Europe, India, Japan, New Zealand and North America.

Indexing: Test plants are *Chenopodium amaranticolor, C. quinoa, Nicotiana glutinoisa* and *Phaseolus vulgaris*. ELISA for serological tests.

6.27. Urdbean leaf crinkle virus (ULCV)

Ungrouped, particles are spherical about 25-30 nm.

Host range: Urdbean (*Vigna mungo*) (Beniwal et. al., 1980), cowpea, mungbean, pigeonpea (*Cajanus cajan*), and tepary bean (*Vigna aconitifolius*).

Symptoms: Causes leaf crinkling, rugosity and distortion of leaves. The infected leaves become dark green. Also causes flower sterility. The symptoms of ULCV on naturally infected plants have been shown in **Fig. 6.6.**

Transmission: Mechanically transmitted. By beetles (*Henosepilachna*

dodecastigma). Transmission through urd bean seeds varies from 2.7 to 46% (Kolte & Nene, 1972; Riaz et. al., 1994), also in mung bean seed up to 15% (Beniwal et al., 1980a).

Figure 6.6: Symptoms of urdbean leaf crinkle virus (ULCV) on urd bean (*Vigna mungo*) under field conditions.

Geographical distribution: India and Pakistan.

Indexing: Test plants are cucumber "National Pickling", *Lagenaria cylinderica, Vigna aconitifolia*, and cowpea (*Vigna unguiculata*).

Chapter 7 Control of Seed-borne Viruses

7.1 Introduction

Many plant diseases caused by fungi, bacteria and nematodes can be effectively controlled by chemicals, but this is not true in case of viral diseases. Although efforts were made to find out chemicals for the control of viral diseases, but no such chemicals have been found. Therefore, control measures other than chemicals use for viral pathogens must be adopted.

In order to have effective control measures of seed-transmitted viruses two types of approaches may be employed i.e. preharvest and postharvest control. Postharvest control of seed-borne viruses may be considered only as a least resort, since it is better to prevent the occurrence of seed-borne viruses than to eradicate seed-infection. Once seed-infection has occurred by a virus, it becomes very difficult to eliminate it without destroying seed viability. Therefore, preference should be given to avoid seed-infection by adopting preharvest control strategies.

As seed transmission facilitates viruses to perpetuate from one season to the next and long distance dissemination, therefore the production of virus-free seed is an important step to control seed-borne viruses. Other factors that maintain and help virus spread in crop under field conditions must be managed while controlling such pathogens. Sometimes obtaining and propagating virus-free seed may be a problem or it may be difficult to prevent re-infection of virus-free seed stock, therefore, control of seed-borne viruses focuses on all the sources of the viruses and means which help spread.

The following measures are adopted to prevent virus infection at preharvest stage.

7.2 Pre-harvest Control Strategies

These methods help to prevent virus infection, reduce disease severity and incidence, and avoid spread of disease and to produce virus-free seeds.

7.2.1 Planting in Isolation

If the seed crop is planted in an area that is at sufficient distance from the source of infection or vector reservoirs, this will delay or reduce the virus disease severity. The incidence of LMV was 3% if the new crop was sown at 0.5 mile away from old lettuce field, but was 60% if grown adjacent to the old filed. Beet mosaic and beet yellows were markedly reduced by isolating beet filed by 12 to 15 and 15 to 20 miles respectively from an infected beet crop (Mandahar, 1987). Isolation as a control measure is effective only against non-persistent viruses, whereas not much effective against the viruses that are vectored persistently.

In case of pollen-transmitted viruses, the only solution would appear to be absolute exclusion of pollen-dispersed viruses from planting stock in isolation to spatial isolation between infected planting and young orchards.

7.2.2 Control Through Weed Eradication

Many weeds act as reservoirs of virus or virus vectors within the crop or in areas adjacent to crop plants, and serve as initial source of inoculum from which viruses are carried by the vectors to healthy crop. If these sources can be eradicated by clean cultivation or by the use of herbicides, the potential reservoirs of virus are eliminated. Effective control of CMV in lettuce crop and celery mosaic virus (CeMV) in celery crop was obtained by removal of weeds around the crops (Townsend, 1947; Tomlinson et. al., 1970).

Some of the nematode transmissible viruses of economically important crops might be controlled effectively while controlling weeds in which these viruses are seed-borne. Lister and Murant (1967) reported that a brief period of fallowing of soil before planting coupled with weed control, gave excellent control of tomato black ring virus (TBRV) and raspberry ring spot (RRSV) viruses. The elimination of weeds in which the viruses are seed-borne might eradicate them from vector infested soils

7.2.3 Clean Cultivation

Clean cultivation will ensure the destruction of volunteer plants from the previous crop. Such plants are common in potato crops where "volunteer" plants may be infected with aphid transmitted potato virus Y and leaf roll viruses (Doncaster and Gregory, 1948). The shattered virus-infected seeds may provide inoculum at initial stage of crop growth. Sugar beet plant regenerating from root debris or previous crop may be a source of beet mosaic and yellows viruses (Howell and

Mink, 1971). The eradication of these plants by clean cultivation will reduce virus incidence and severity.

7.2.4 Roguing Within the Crop

The removal of the virus-infected plants during crop growth period is another effective control method. If the virus induces symptoms which are quite visible it becomes easy to rogue the plants, but not possible in case of latent infection. Removal of infected plants has been successful control of Cacoa swollen shoot disease in West Africa (Kenton & Legg, 1971) and to limit the spread of plum pox virus in Britain.

7.2.5 Control of Insect Vectors

As majority of the plant viruses is spread by insect vectors, therefore, the easy way to control spread of insect-transmitted viruses would be through elimination of their vectors by spraying crop with chemicals. This would kill the vector during acquisition period or transmission to plants. In case of viruses that are transmitted non-persistently, the spraying of chemicals may not be effective as in case of persistent viruses, because the viruses may be transmitted within seconds and the plants become infected before the insect die. Destruction of weeds also helps to check vector population. This is particularly effective in lettuce necrotic yellows virus (LNYV) in Australia (Stubbs et. al., 1963).

Nematode and fungal transmitted viruses may persist longer time in the vectors, so chemical treatment to kill the vectors is probably the most effective means of control. The only difficulty is with the vectors, which occur at considerable soil depths which may be beyond the effectiveness of the chemicals. Harrison et. al., (1963) demonstrated effective control of strawberry crops against arabis mosaic virus (AbMV) by killing nematode (*Xiphinema diversicaudatum*) vector with methyl bromide. The incidence of fungal vector (*Spongospora subterranea*) of potato mop top virus (PMTV) has been controlled by chemical treatment (Cooper et. al., 1976).

7.2.6 Use of Resistant Varieties

Concerted efforts should be made to identify lines in the germplasm collections of major crops, which are resistant to a collection of strains of the predominant viruses. This is particularly true for viruses, which are effectively transmitted through seed. The basis of resistance in most cases appears to be hypersensitive reaction or host resistance to virus replication (i.e. symptomless systemic

infection), rarely in an immune response. In several instances (e.g. in germplasm collections of *Glycine max*, *Lens culinaris* and *Pisum sativum*) a systematic analysis has revealed excellent resistance in the existing collection (Goodman et. al., 1979; Hampton and Braveman, 1979). Additional references on sources of resistance to seed-borne viruses are reported by Meiners (1981). Although, conventional breeding have been used for incorporating virus resistance, other possibilities of genetic modifications using gene vectors (e.g. plasmids, DNA plant viruses) may have potential use. There is also a great scope to develop coat protein (CP) mediated resistance using CP-viral gene against some seed-borne viruses.

7.2.7 Host Resistance to Seed Transmission

The viruses which are unable to infect embryo of the seed may be due to host resistance to seed transmission. The resistance to seed transmission results from physical as well as physiological/biochemical barriers to virus entry into, and replication in reproductive tissues. Such barriers may be due to specific host-resistance mechanisms, which have evolved to exclude pathogens under certain conditions. Either virus specific or host specific limitations or virus movement or replication may influence viral entry into and transmission through seeds. The effect of physiological variation among tissue types on viral infection was studied by Citovsky et. al., (1993). The ability of TMV to invade different tissues may be effected by phosphorelation of P_{30} by a cell wall-associated protein kinase. Cultivars with specific resistance to seed transmission has been reported in barley against BSMV (Carroll et. al., 1979), in pea against PSbMV (Wang et. al., 1993) and in alfalfa against AMV (Bailiss and Offei, 1990). Resistance to seed transmission of BSMV in barley cv. Modjo is conditioned by a single recessive gene (Carroll et. al., 1979), whereas resistance to seed transmision in peas against PSbMV seems to be multigenic (Wang and Maule, 1993).

Another mechanism of host resistance to seed transmission is restricted cell-to-cell movement of virus within the host plant. Slow movement may not only limit virus accumulation in floral and microgametophyte tissues (Walter et. al., 1992), but could also impede viral establishment in the embryo meristem, generally considered essential for seed transmission (Hanada and Harrison, 1977). The host resistance may inactivate virus to infect embryo during seed maturation and storage.

Some viruses may not be seed transmissible because of inability to replicate during seed germination due to specific host resistance. In some cases the presence of viral inhibitors in seed have been reported (Hajj and Stevens, 1979:

Hanada and Harrison, 1977). Some legume species which are slow transmitter or even non-transmitter to SMV infection have been reported (Cho and Goodman, 1979: Goodman and Oard, 1980).

7.2.8 Production of Virus-free Seed

In order to produce virus-free seed, seed certification schemes should be followed to produce seeds on commercial basis. During multiplication for commercial seed production, re-infection must be prevented as much as possible. Virus freedom must be guaranteed by certification. Screening of germplasm and breeding material under greenhouse and field conditions be carried out to have promising lines free of virus. Regular monitoring and crop inspection for the presence of seed-borne viruses and elimination of virus infected plants from the standing crop be followed at appropriate time. ELISA will be helpful for such purpose. Virus-free seed should be produced in virus-free areas or under controlled conditions.

In order to produce virus-free certified seed the following measures must be adopted:

1. Avoidance of initial sources of infection by
    a. Starting from virus-free seed stock.
    b. Removal of virus infected plants during cultivation.
    c. Avoidance of nearby sources of infection, such as eradication of alternate hosts of virus, weeds, and wild vegetation.
    d. Avoidance of soil containing soil-borne viruses or their vectors.
2. Prevention of virus introduction and spread through contact and vectors by
    a. Care in handling crop
    b. Cultivation in vector-free area
    c. Direct vector control with insecticides
3. Application of reliable methods of routine seed testing for virus infection and development and improvement of such techniques.

Absolute freedom from virus infection under field conditions is not possible, but seed certification programme must focus to reduce the seed infection and threshold level within tolerant limit.

7.2.9 Seed Certification Schemes

A constraint against the production of high quality seed is disease resulting from infection by seed-borne viruses. The application of seed certification schemes against seed transmitted viruses of commercial seed lots and germplasm collections of

different crops is an important method to minimize the spread of destructive viral diseases (Hamilton, 1983). Seed certification programmes against seed-borne viruses must be at the basic level of germplsm collection, available to the plant breeders (Hampton et. al., 1992) and continue through the development of varieties and the increase of seed through breeders seed (pre-basic seed), Foundation seed (basic seed), Registered seed (Certified seed, first generation) and Certified seed (certified seed, second generation). Such programmes should also be accompanied by ways that help to control virus dissemination in standing crops. Regular monitoring of the presence of seed-borne viruses should include inspection of the standing crop, rouging or elimination of virus-infected plants, preventing of sowing of susceptible genotypes and detection of viruses with reliable serological tests such as ELISA. A few seed certification programmes have been adopted in various countries such as Canada, U.S.A., Brazil, India, and Nigeria with PSbMV in pea (USA, Canada), BCMV (Brazil), PMV in peanut (USA), PMV and PStV in peanut (India), CABMV in cowpea (Nigeria) and CMV in cowpea (USA). For such programmes the following aspects are suggested for future research and development:

1. Application of improved methods of detection, identification and characterization of seed-borne viruses.
2. Improvement in methods for detecting seed-borne viruses in standing crops and seed lots.
3. Development of more realistic sampling procedures in the light of essential methods of detecting viral antigens and nucleic acids.
4. Development of new varieties resistant to seed-borne infection/ transmission.

7.3 Post-harvest Control

As already mentioned the post-harvest control of seed-borne viruses should be considered as a least resort. Once the seed is infected with virus it becomes difficult to eradicate virus from seed. However, post-harvest control strategies include the followings:

7.3.1 Removal of Infected Seeds

The seeds showing morphological and physical abnormalities due to virus infection should be separated from normal and healthy seed by mechanical or other means if possible. Generally the virus-infected seeds are small in size and less in weight, but this is not always true, as the seed abnormalities may be due to other factors. Sieving may help to separate such seeds from abnormal seeds.

Stevenson and Hagedorn (1970) could reduce seed transmission of PSbMV in a given seed lot from 10 to 40% by separating abnormal seeds from normal seed. Normal seed cleaning techniques may reduce infection in certain cases but never completely eliminate it. The manual or mechanical removal of infected seeds is ineffective because of the frequent absence of any visible seed abnormality and a lack of absolute correlation with infection.

7.3.2 Seed Treatment

Generally it is not possible to eradicate virus from infected seeds by chemical or heat seed treatment, as the seed germination is adversely affected. Although some workers reported elimination or inactivation of some viruses from infected seeds, but this is not true in all the cases. Heat treatment has been more widely studied to inactivate seed-borne viruses. It had no effect on viruses that have been demonstrated to pass via embryo infection. Viruses can resist much higher temperature in seed than expressed in sap. Howles (1961) was the first to report that heat treatment at 72°C for 22 days of tomato seeds infected with tomato mosaic virus (ToMV) reduced seed transmission without any adverse effect on the seedlings aside from delayed germination. Reddick and Steward (1919) applied elevated temperatures to bean seeds carrying BCMV. Infected seeds were treated with dry heat at 80°C for 10 min, or 75°C for periods up to 40 min, showed 50% virus reduction, but seed germination was drastically reduced. Owusu et. al., (1968) could not eradicate TRSV in seeds of soybean without destroying seed viability. BBSV was inactivated in lentil seeds when seed were exposed to 80°C for 8 days. However, this treatment caused 52% reduction in seed germination, which is not acceptable for commercial seed lots (Kumari & Makkouk, 1996). Haque et. al., (1993) attempted hot water and dry heat treatment to control SMV in soybean seeds. Hot water treatment caused a drastic reduction in seed germination with complete failure at 80°C. However, virus was completely eliminated at 70°C. Dry heat treatment caused reduction in severity of infection and affected badly seed germination. It appears that viruses transmitted through seed in embryonic manner cannot be inactivated by external agents without destroying seed viability.

The viruses which contaminate seeds externally are easier to control by suitable treatment than the viruses carried in the embryo. For example, TMV was inactivated by soaking the tomato seeds in hydrochloric acid or exposing seeds to 1% aqueous solution of tri-sodium orthophosphate for 15 min and then 0.525% sodium hypochloride for 30 min. Treatment with either chemical alone was insufficient (Gooding, 1975).

The cowpea banding mosaic virus (CBMV) was completely inactivated while soaking with malic hydrazide at 40 to 400 ppm for 90 min, 2-thiouracil at 500 and 700 ppm for 4 hr, and Teepol (5 to 10%) for 4 hr without affecting seed viability (Sharma & Verma, 1975). Direct seeding of tomato and pepper, rather than transplanting may be useful in controlling TMV.

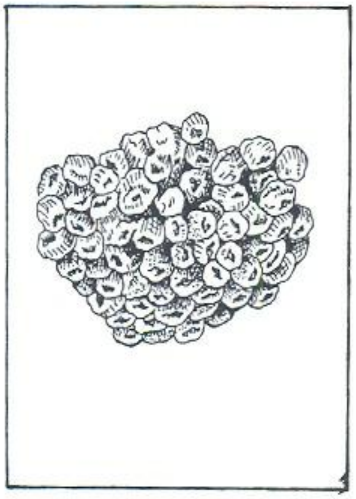
7.3.3 Germplasm Reclamation Technology

The examples of germplasm contamination with seed-borne viruses preserved in gene banks in many countries have already been mentioned. In order to prevent the spread of seed-borne viruses from infected germplasm, it is essential that steps should be taken to produce germplasm free of seed-borne viruses for storage and distribution. Such goals can be achieved by eliminating seed-borne viruses from germplasm seed lots, and this can be done by a simple approach which involves the following four steps (Hampton et. al., 1993).

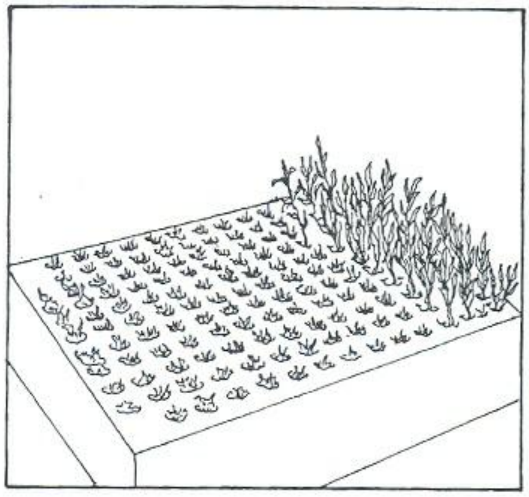
1. Selection of seed or seeds or mother plant candidates that are free of observable disease symptoms.
2. Critical observations of each selected mother plant from seed or seedlings to plant maturity. Test these plants by ELISA to be sure of virus free.
3. Production of seed under carefully controlled greenhouse conditions, including precise irrigation, and control of foliar pathogens.
4. Scrutiny of harvested seeds for any traces of disease symptomatology.

The following procedure is adopted to reclaim the germplasm from seed-borne viruses:

Fig. 7.1 shows various steps involved for the reclamation of germplasm for seed-borne viruses.

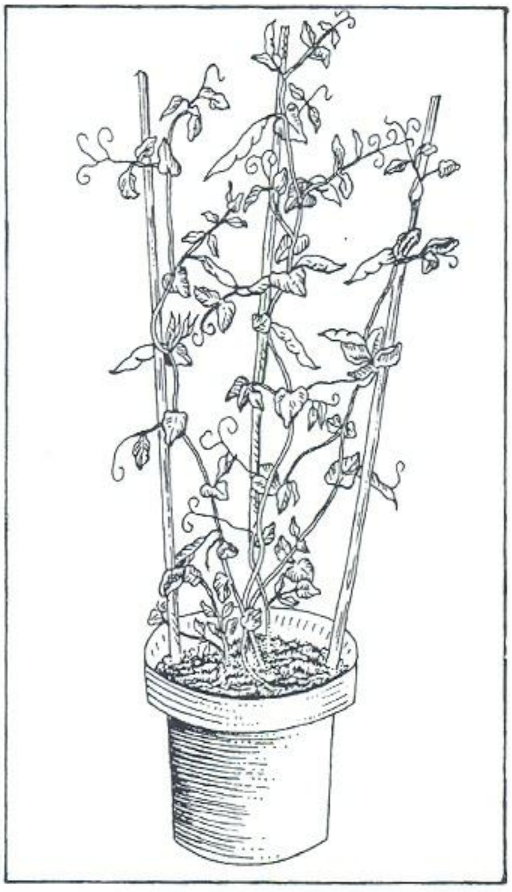


Step 1
Collect 50 to 100 seeds of each available germplasm accession.

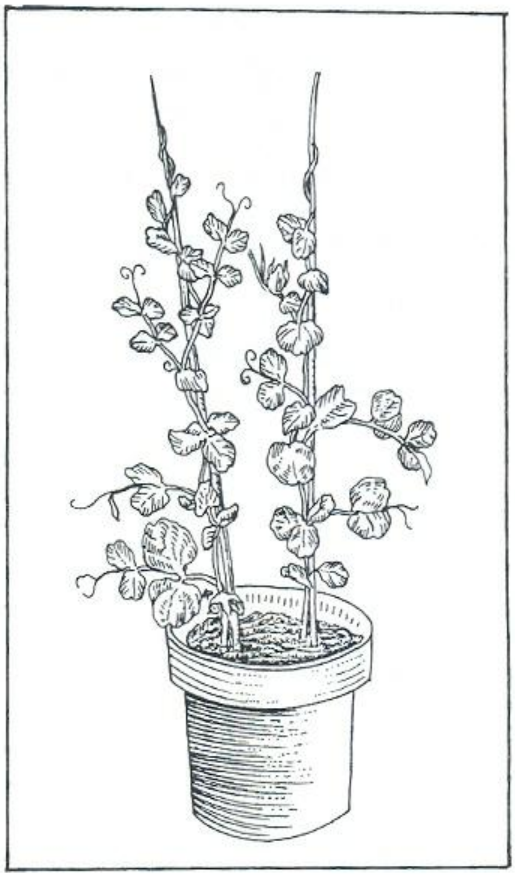


Step 2
Sow individual seed of each accession, one seed per cup, in Styrofoam block planters filled with vermiculate (fertile soil). Discard the plants with virus-like symptoms.

Step 3
Select 25 candidate mother plants representing both seed and plant phenotype and plant them in 8-inches plastic pots.



Step 4
Assay each plant during early growth stage for all possible seed-borne viruses by DAS-ELISA. Again assay at later growth stage. Discard the virus infected plants.



Step 5
Collect seeds from virus-free mother plants at maturity. Bulk the seeds to have virus-free seed source.

Figure 7.1: Various steps involved in germplasm reclamation technology for seed-borne viruses.

Procedure

1. Select available seed-lot of germplasm accessions consisting of 50-100 seeds each.
2. Plant seeds in Styrofoam blocks filled with sterile fertile soil.
3. Observe the growing seedlings carefully and discard the plants showing virus-like symptoms.
4. From the remaining seedlings select 25 candidate mother plants phenotypically identical.
5. Transfer the plants in 8 inches diameter plastic pots containing fertile soil.
6. Test each plant taking leaf samples (2-3 samples from each plant) by ELISA for seed-borne viruses.
7. Discard the infected plants and keep the healthy ones.
8. Use insecticides to keep greenhouse free of insect vectors. Spray insecticides at weekly interval. Also use yellow sticky traps.
9. Harvest 500 seeds from plants found free of any seed-borne virus.
10. Check these seeds to verify that each genotype is free of any seed-borne virus.
11. Increase the seed of these accessions under greenhouse conditions and store in the gene bank.

Chapter 8

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Appendix 1

List of seed-borne viruses

Virus	Host		Distribution	Percent seed transmiss-ion
	Common name	Botanical name		
1	2	3	4	5
Abutilon mosaic virus	Velvetleaf	*Abutilon spp.* *Abutilon thompsonii* *Abutilon mulleri*	?	1 0.7 *
Adzuki bean mosaic virus	Common bean (kidney bean)	*Phaseolus vulgaris*	Japan, Korea	15
Alfalfa mosaic virus	Pepper Soybean Alfalfa, lucerne Burclover Barrelclover	*Capsicum annum* *Glycine max* *Medicago sativa* *Medicago polymprpha* *Medicago truncatula*	Word-wide	1-5 1-3 6-55 * 2
Apple chlorotic leafspot virus	Raspberry, blackberry	*Rubus spp.*	Europe, North America	*
Apple chlorotic latent virus	Raspberry,blackberry	*Rubus spp.*	?	30-40
Apple mosaic virus	Cowpea	*Vigna unguiculata*	N. America, Europe, North & South Africa, Australia, India, New Zealand	2
Apricot gommosis	Apricot Sweet cherry Plum	*Prunus armeniaca* *Prunus avium* *Prunus domestica*	?	* 15 *
Arabis mosaic virus	Beet Shephardspurse Lambsquarters G. strawberry Soybean Hops G. lettuce Henbit Tomato Petunia Violet petunia Broadleaf plantain Annual bluegrass Ladysthumb Common groundsel Starwort	*Beta vulgaris* *Capsella bursapastoris* *Chenopodium album* *Fragaria ananassa* *Glycine max* *Humulus Spp.* *Lactuca sativa* *Lamium amplexicaule* *Lycopersicum esculentum* *Petunia hybrida* *Petunia violacea* *Plantago major* *Poa annua* *Polygonum persicaria* *Senecio vulgaris* *Stellaria media*	Europe & New Zealand	13 33 80 7 6 10 60-100 1-25 2 19-95 20 34 5-28 4.0 22-100 2 57.7

Appendix-1 (Continued)

1	2	3	4	5
Arracacha A & B-viruses	Quinoa Tobacco Potato	*Chenopodium quinoa* *Nicotonia clevelandii* *Solanum tuberosum*	Peru & Bolivia	* * *
Artichoke yellow ringspot virus	Quinoa Jimson weed Tobacco Garden petunia Dill Fennel Weld Starwort	*Chenopodium spp.* *Datura stramonium* *Nicotiana spp.* *Petunia hybrida* *Anethum graveolens* *Foeniculum spp.* *Reseda alba* *Stellaria media*	Greece & Italy	* 15-100.0 * * * * * *
Asparagus bean mosaic virus	Asparagus bean	*Vigna sesquipedalis*	?	35
Asparagus latent virus	G. asparagus	*Asparagus officianalis*	Denmark and Germany	65
Asparagus virus -2	G. asparagus	*Asparagus officinalis*	Europe, Japan, & USA	40-65
Avocado pear sunblotch virus	Avocado	*Persea americana*	USA, Israel	75
Barley mottle mosaic virus	Barley	*Hordeum vulgare*	?	2-45
Barley stripe mosaic virus	Goatgrass Wheatgrass Wild oat Oat Smooth brome Barley Ryegrass Wheat	*Aegilops spp.* *Agropyron elongatum* *Avena fatua* *Avena sativa* *Bromus inermis* *Commellina communis* *Hordeum depressum* *H. glaucum* *H. stebbinsil* *H. vulgare* *Lolium spp.* *Triticum aestivum* *Agrotriticum, Bromus, Cutandia, Cynosurus, Hordeum, Koeleria, Phalaris, Pholiurus spp.*	World-wide	* * 22 0-10 * 8 4 3 2 58 4-86 3-8 7-81
Bean common mosaic virus	Phasey bean Tepary bean Common bean Cowpea Blackgram Greengram Asparagus bean Savibean	*Macroptilium lathyroides* *Phaseolus acutifolius* *Phaseolus vulgaris* *Vigna unguiculata* *Vigna mungo* *Vigna radiata* *Vigna sesquipedalis* *Vigna sinensis*	World- wide	5-33 2-89 25-40 2-10 8-32 37 37 25-40

Appendix-1 (Continued)

1	2	3	4	5
Bean pod mottle virus	Soybean	*Glycine max*	U.S.A	0.08
Bean southern mosaic virus	Common bean Savibean Cowpea	*Phaseolus vulgaris* *Vigna sinensis* *Vigna unguiculata*	Africa, USA	21 3-4 1-3
Bean western mosaic virus	Common bean	*Phaseolus vulgaris*	?	2-3
Bean yellow mosaic virus	White lupin Yellow lupin Sweet clover Pea Alsike clover Red clover Broad bean Asparagus bean Savi bean	*Lupinus albus* *Lupinus luteus* *Melilotus alba* *Pisum sativum* *Trifoilum hybridum* *Triflium pratense* *Vicia faba* *Vigna sesquipedalis* *Vigna sinensis*	World-wide	6 7-21 5 10-30 5 47 0.1-2.4 1 1-37
Beet cryptic virus	Beet	*Beta vulgaris*	?	*
Berseem mosaic virus	Berseem, clover	*Trifolium alexandrinum*	India	70
Blackeye cowpea mosaic virus	Cowpea	*Vigna unguiculata*	World-wide	31
Blackgram leaf crinkle virus (urdbean leaf crinkle virus)	Blackgram	*Vigna mungo*	India, Pakitan	20-42
Blackgram mottle virus	Blackgram	*Vigna mungo*	Asia, Australia, India, Thailand, Indonesia	8
Black raspberry latent virus	Raspberry Common chickweed	*Rubus spp.* *Stellaria media*	North America	10 8-26
Blueberry leaf mottle virus	Quinoa Highbush blueberry Fox grape	*Chenopodium quinoa* *Vaccinium corymbosum* *Vitis labrusca*	USA	12-20 29 5
Brinjal mosaic virus	Brinjal	*Solanum melongena*	?	*
Broad bean mild mosaic virus	Broad bean	*Vicia faba*	?	*
Broad bean mosaic virus	Broad bean	*Vicia faba*	?	*
Broad bean mottle virus	Blackgram Broad bean	*Phaseolus mungo (Vigna mungo)* *Vicia faba*	UK, Germany, India, North Africa, Portagal, Syria, Sudan,	6-7 *
Broad bean stain virus	Broad bean Lentil	*Vicia faba* *Lens culinaris*	Australia, China, Europe, Sudan, Syria,	1 14

Appendix-1 (Continued)

1	2	3	4	5
Broad bean stain virus	Broad bean	*Vicia faba*	Australia, China, Europe, North & West Africa	1-10
Broad bean true mosaic virus	Broad bean Lentil	*Vicia faba* *Lens culinaris*	Europe North & West Africa, Syria	3-15 14
Cacao necrosis virus	Soybean Lima bean Common bean	*Glycine max* *Phaseolus lunatus* *Phaseolus vulgaris*	West Africa	1-24
Carrot red leaf virus	Carrot	*Daucus carota*	Uniked Kingdom and Germany	25
Cassava green mottle virus	Tobacco	*Nicotiana clevelandii*	Solomon Island	30-35
Cauliflower mosaic virus	Shephardspurse	*Capsella bursa-pastoris*	?	*
Celery latent virus	Quinoa Inca-wheat	*Chenopidium chinoa* *Amaranthuscaudatus*	Italy, Netherlands	* *
Cherry leaf roll virus	Goosefoot Soybean Tobacco C. bean Red elderberry Pansy American elm	*Chenopodium amaranticolor* *Glycine max* *Nicotiana tabacum* *Phaseolus vulgaris* *Sambucus racemosa* *Viola tricolor* *Ulmus americana*	UK, Holland, Germany, & North America	100 100 1 12-40 13-44 1-6 1-4
Cherry necrotic rusty mottle virus	Sour cherry	*Prunus cerasus*	UK, USA	*
Cherry ring mottle virus	Sour cherry	*Prunus cerasus*	?	33.3
Chicory yellow mottle virus	Chicory	*Cichorium intybus*	Italy	3
Citrus psorosis virus	Sweet orange	*Citrus sinensis*	?	19
Citrus xyloporosis virus	Lime	*Citrus aurantifolia*	?	66
Clover (white) mosaic virus	Red clover	*Trifolium pratense*	?	6
Clover yellow mosaic virus	Red clover	*Trifolium pratense*	?	7.6
Cocoa necrosis virus	Common bean	*Phaseolus vulgaris*	?	1-24
Cowpea aphid borne mosaic virus	Adzuki bean Savi bean Cowpea	*Vigna angularis* *Vigna sinensis* *Vigna unguiculata*	World-wide (Italy, Egypt, India, Pakistan, East Africa, USA, Japan, Iran)	1-35

Appendix-1 (Continued)

1	2	3	4	5
Cowpea banding mosaic virus	Cowpea	*Vigna unguiculata (Vigna sinensis)*	India	15-31
Cowpea chlorotic spot virus	Cowpea	*Vigna unguiculata (Vigna sinensis)*	?	3-16
Cowpea isometeric virus	Cowpea	*Vigna sinensis (Vigna unguiculata)*	India	5-16.
Cowpea little leaf virus	Cowpea	*Vigna unguiculata*	India, Philippines	18
Cowpea mild mottle virus	Soybean Common bean Cowpea	*Glycine max* *Phaseolus vulgaris* *Vigna unguiculata*	Africa, Israel India, Indonesia, & Thailand	90 6 90
Cowpea mosaic virus	Catjang bean Cowpea	*Vigna cylindrica* *Vigna unguiculata*	North & South America, Netherlands, Trinidad, & Nigeria	1-23 *
Cowpea mottle virus	Cowpea	*Vigna unguiculata*	Nigeria	0.2-10
Cowpea ring spot virus	Cowpea	*Vigna unguiculata*	Iran	10-30
Cowpea severe mosaic virus	Cowpea Asparagus bean	*Vigna unguiculata* *Vigna sesquipedalis*	USA, Central & South America	3-10 8
Cucumber green mottle mosaic virus	Preserving melon Cucumber Calabash	*Citrullus lanatus* *Cucumis sativus* *Lagenaria siceraria*	Asia & Europe	3-4 44 *
Cucumber mosaic virus	Peanut Waxgourd Chickweed Cantaloupe Pumpkin Cucumber Squashes Pumpkin Soybean Purple deadnettle White lupine Blue lupine Yellow lupine Tomato Green gram Common bean Corn Spurry Chickweed Catjang bean Green gram Cowpea Asparagus bean Savi bean	*Arachis hypogea* *Benicasa hispida* *Cerastium holosteoides* *Cucumis melo* *Cucumus pepo* *Cucumus sativus* *Cucurbita maxima* *Cucurbita moschata* *Echinocystis lobata* *Glycine max* *Lamium purpureum* *Lupinus albus* *Lupinus angustifolius* *Lupinus luteus* *Lycopersicon esculentum* *Vigna radiata* *Phaseolus vulgaris* *Spergula arvensis* *Stellaria media* *Vigna cylinderica* *Vigna radiata* *Vigna unguiculata* *Vigna sesquipedalis* *Vigna sinensis*	World- wide	2 1 2 2 * * 0.7 4-55 0.7 0.2 30-100 4 ** 3-34 30-100 5 0.2 1-40 4-28 2 * 36-100 10 4-28 * 10

Appendix-1 (Continued)

1	2	3	4	5
Dulcamara mottle virus	Bitter night shade	*Solanum dulcamara*	?	0.1
Desmodium mosaic virus	Kaimi clover	*Desmodium canum*	U.S.A.	8
Desmodium mottle virus	Three flowered beggarweed	*Desmodium triflorum*	?	*
Dodder latent mosaic virus	Dodder Field dodder	*Cuscuta californica* *Cuscuta campestris*	?	2 5
Echtes Ackerhbohnenm osikvirus (isosynm broad bean true mosaic virus)	Broad bean	*Vicia faba*	Europe and North West Africa	15
Egg plant mosaic virus	Tobacco Garden petunia	*Nicotiana clevelandii* *Petunia hybrida*	?	* *
Elm mosaic virus	American elm	*Ulmus americana*	?	48
Elm mottle virus	Elm	*Ulmus glabra*	Europe (Germany, UK, & Bulgaria, Czechoslovakia	*
Grape vine Bulgarian latent virus	Quinoa Goosefoot Fox grape	*Chenopodium quinoa* *Chenopodium amaranticolor* *Vitis labrusca*	Bulgaria & USA	5 * *
Grape yellow vein virus	Goosefoot	*Chenopodium amaranticolor*	?	5 8 7
Grape yellow mosaic virus	Goosefoot	*Chenopidium amaranticolor*	?	0.7
Grapevine fanleaf virus	Goosefoot Wine grape Soybean	*Chenopodium spp. amaranticolor* *Vitis vinifera* *Glycine max*	World-wide	1 * *
Groundnut rosette virus	Peanut (Groundnut)	*Arachis hypogea*	Argintina, Africa, South Asia, including Philippines, Australia, & Fiji.	*
Guar symptomless virus	Guar	*Cyamopsis tetragonoloba*	Africa, Australia, India , Pakistan & USA	12-70
Hippeastrum mosaic virus		*Hippeastrum hybridum*	Worldwide	*
Hop chlorotic disease virus	Hops	*Humulus lupulus*	?	27
Lettuce mosaic virus	Quinoa Garden lettuce Prickly lettuce Common groundsell	*Chenopodium quinoa* *Lactuca sativa* *L. scariola* *Senecio vulgaris*	World-wide	0-1 1-16 1-50 2-3

Appendix-1 (Continued)

1	2	3	4	5
Lettuce yellow mosaic virus	Garden lettuce	*Lactuca sativa*	?	30
Lima bean mosaic virus	Lima bean	*Phaseolus lunatus*	?	25
Lucern Australian latent virus	Medics	*Chenopodium spp.* *Medicago sativa*	Australia & North Zealand	* *
Lychnis ringspot virus	Beet Shephardspurse Chickweed Floweing catchfly Night floweing catchfly	*Beta vulgaris* *Capsella bursapastoris* *Cerastium viscosum* *Lychnis divaricata* *Silene gallica* *Silene noctiflora*	USA	10 9 58 28 41 *
Maize dwarf mosaic virus	Maize	*Zea mays*	Australia & USA	1
Melon necrotic spot virus	Cantaloupe	*Cucumis melo*	Japan, Netherland, UK & USA	1-15
Mulberry ringspot virus	Soybean	*Glycine max*	Japan	10
Mungbean isometeric yellow mosaic virus	Mung bean	*Phaseolus aureus* *(Vigna radiata)*	Philippines	*
Mungbean mosaic virus	Blackgram Greengram	*Vigna mungo* *Vigna radiata*	India, Iran	8-32 3
Muskmelon mosaic virus	Cantaloupe Gourd Pumpkin	*Cucumus melo* *Cucurbita flexuous* *Cucurbita moschata*	?	12-93 * *
Nicotiana-veluntina mosaic virus	Tobacco	*Nicotiana glutinosa*	?	72
Onion mosaic virus	Onion	*Allium cepa*	?	*
Onion yellow dwarf virus	Onion	*Allium cepa*	Worldwide	*
Peach mosaic virus	Quinoa	*Chennopodium quinoa*	USA	*
Pea early browning virus	Pea Broad bean	*Pisum sativum* *Vicia faba*	Netherlands, UK, Europe, Morocco	4-50 *
Pea enation mosaic virus	Pea	*Pisum sativum*	Bulgaria, Poland, Yugoslavia	1.5
Pea leaf roll virus	Pea	*Pisum sativum*	Europe, Middle East, Iran, New Zealand, North America, Europe.	2-15

Appendix-1 (Continued)

1	2	3	4	5
Pea mosaic virus	Pea Sweet pea Clover	*Pisum sativum* *Lathyrus odoratus* *Trifolium hybridum*	?	* * 0.7
Pea mild mosaic virus	Pea Sweet pea Alsike clover Red clover	*Pisum sativum* *Lathyrus odoratus* *Trifolium hybridum* *Trifolium pratense*	New Zealand	15 * 0.7 47
Pea seed borne mosaic virus	Pea Lentil Broad bean	*Pisum sativum* *Lens culinaris* *Vicia faba*	World-wide	4-90 5-44 *
Peach latent virus	Cherry	*Prunus avium*	?	2.4
Peach necrotic virus	Peach	*Prunus persica*	?	3-9
Peach ringspot virus	American plum Peach	*Prunus americana* *Prunus persica*	?	* 3-9
Peach rosette mosaic virus	Quinoa Common dandelion Vetch Grapes	*Chenopodium quinoa* *Taraxacum officinale* *Vicia labrusca* *Vitis vinifera*	USA	90 4 9.5 10
Peanut bunchy top virus	Peanut	*Arachis hypogea*	?	*
Peanut chlorosis	Peanut	*Arachis hypogea*	?	*
Peanut clump virus	Peanut	*Arachis hypogaea*	Burkina Faso, W. Africa, India, Pakistan, Nigeria, Senegal,	5-20
Peanut marginal chlorosis virus	Peanut	*Arachis hypogaea*	New Guinea	30-100
Peanut mild mottle virus	Peanut	*Arachis hypogea*	Worldwide	5
Peanut mottle virus	Peanut White lupine Common bean Cowpea	*Arachis hypogea* *Lupinus albus* *Phaseolus vulgaris* *Vigna unguiculata*	USA (Georgia), Australia,	2-20 0.4 1 1
Peanut rosette virus	Peanut	*Arachis hypogea*	India, Phillippines	*
Peanut stripe virus	Peanut	*Arachis hypogea*	China, India, Indonesia, Japan, Malaysia, Mynamar (Burma), Philippines, Thailand, USA, Vietnam,	10-43
Peanut stunt virus	Peanut Soybean	*Arachis hypogea* *Glycine max*	Africa, China, Europe, North America & Japan	0.2 4

Appendix-1 (Continued)

1	2	3	4	5
Pelargonium zonate spot virus	Tobacco	*Nicotiana glutinosa*	Italy	*
Potato virus-T	Jimson weed Apple-of-Peru	*Datura stramonium* *Solanum demissum* *Nicandra physalodes*	Bolivia & Peru	72 10-39 28
Potato virus -X	Potato	*Solanum tuberosum*	World-wide	0.6-2.3
Potato virus-Y	Potato	*Solanum tuberosum*	?	14, 16
Prune dwarf-virus	Sour cherry Peach	*Prunus cerasus* *P. persica*	North America	9-15 *
Prunus necrotic ringspot virus	Pumpkin American plum Sweet cherry Sour cherry Mahalab cherry Pin cherry Peach	*Cucurbita idaeus* *Prunus americana* *Prunus avium* *Prunus cerasus* *Prunus mahalb* *Prunus pennsylvanica* *Prunus persica*	World-wide	40-50 * 6 20-91 70 37 3-9
Rasberry bushy dwarf virus	Red raspberry Wineberry Woodland stawberry	*Rubus idaeus* *R. phonicolasius* *Fragaria vesca*	Europe, North America, New Zealand	40-70 15 1-2
Raspberry ringspot virus	Beet Shephardspurse Garden strawberry Soybean Red raspberry Chickweed Violet petunia	*Beta vulgaris* *Capsella bursapastrois* *Fragaria ananassa* *Glycine max* *Glycine soja* *Rubus idaeus* *Stellaria media* *Petunis violacea*	Europe	50-55 2-3 35 7 20 2 9 8-20
Raspberry latent virus (black raspberry latent)		*Rubus sp.*	?	10
Red clover mosaic	Red clover Broadbean	*Trifolium pratense* *Vicia faba*	?	12-18 *
Redclover vein mosaic virus	Red clover Broadbean	*Trifolium pratense* *Vicia faba*	North and South Ameriac, Europe & South Africa	Up to 100 Up to 100
Runner bean mosaic virus	Scarlet runner bean	*Phaseolus coccineus*	?	42
Satsuma dwarf-virus	Common bean	*Phaseolus vulgaris*	Japan & Turkey	8-6
Sincamas mosaic virus		*Pachyrrhizus angulatus*	Philippines	5-26
Southern bean mosaic virus	Cowpea Common bean Soybean	*Vigna unguiculata* *Phaseolus vulgaris* *Glycine max*	N. America, Africa & Japan	1-4 5 2

Appendix-1 (Continued)

1	2	3	4	5
Tomato aspermy virus	Common bean Chickweed	*Phaseolus vulgaris* *Stellaria media*	China, North America, Europe, Asia, Australia, New Zealand.	19 *
Tomato bushy stunt virus	Apple	*Malus pumila*	?	Up to 17
Tomato bunchy top virus	Peruvian cherry	*Physalis peruviana*	South Africa	*
Tomato ringspot virus	Strawberry Soybean Globe amaranth Tomato Tobacco Geranium Red raspberry	*Fragaria vesca* *Glycine max* *Gomphrena globosa* *Lycopersican esculentum* *Nicotiana tabacum* *Pelargonium hortorum* *Rubus idaeus*	Australia, North America.	68 76 76 3 11 11 *
Tomato spotted wilt virus	Groundsel	*Senecio spp.* *Senecio cruentus*	Worldwide	96 up to 70
Tomato streak virus	Tomato	*Lycopersicon esculentum*	?	66
Turnip mosaic virus	Wild radish	*Raphanus raphanistrum*	?	4
Urdbean leaf crinkle virus	Black gram Green gram	*Vigna mungo* *Vigna radiata*	India, Pakistan & Sri Lanka	18 15
Vicia cryptic virus	Broad bean	*Vicia faba*	U.K.	75
Wtermelon mosaic virus		*Eechinocytis lobata*	North America, Europe, South Africa, Japan & New Zealand	2
White clover mosaic virus	Clover	*Trifolium pratense*	World-wide	6
White clover yellow mosaic virus	Clover	*Trifolium pratense*	North America	8

G: Garden C: Common *: Not known ?: Not reported

Appendix 2

List of viruses, their abbreviations, family and genera

Plant Virus name	Abbrev.	Family	Genus
Abutilon mosaic virus	AbMV	Geminiviridae	Begomovirus
Adzuki bean mosaic virus	AdBMV	Potyviridae	Potyvirus
Alfalfa mosaic virus	AMV	Bromoviridae	Alfamovirus
Apple chlorotic latent virus	ApCLV	?	?
Apple chlorotic leaf spot virus	ACLSV	?	Trichovirus
Apple stem grooving virus	ASGV	?	Capillovirus
Apple mosaic virus	ApMV	Bromoviridae	Ilarvirus
Arabis mosaic virus	ArMV	Comoviridae	Nepovirus
Arracacha virus – A	AVA	Comoviridae	Nepovirus
Arracacha virus – B	AVB	Comoviridae	Nepovirus
Artichoke yellow ringspot virus	AYRSV	Comoviridae	Nepovirus
Asparagus bean mosaic virus	AsBMV	?	?
Asparagus latent virus	AsLV	?	?
Asparagus virus-2	AV-2	Bromoviridae	Ilarvirus
Barley mottle mosaic virus	BMMV	?	?
Barley stripe mosaic virus	BSMV	?	Hordeivirus
Bean common mosaic virus	BCMV	Potyviridae	Potyvirus
Bean pod mottle virus	BPMV	Comoviridae	Comovirus
Bean southern mosaic virus	BSMV	Comoviridae	Sobemovirus
Bean western mosaic virus	BWMV	?	?
Bean yellow mosaic virus	BYMV	Potyviridae	Potyvirus
Beet cryptic virus-1	BCV-1	Partitiviridae	Alphacryptovirus
Berseem mosaic virus	BMV	?	?
Blackeye cowpea mosaic virus	BlCMV	Potyviridae	Potyvirus
Blackgram mottle virus	BMoV	Tombusviridae	Carmovirus
Blackrasberry latent virus	BRLV	?	Ilarvirus
Bringal mosaic virus	BrMV	?	?
Broad bean mild mosaic virus	BBMMV	?	?
Broad bean mosaic virus	BBMV	?	?
Broad bean stain virus	BBSV	Comoviridae	Comovirus
Brome mosaic virus	BMV	Bromoviridae	Bromovirus
Cacao necrosis virus	CNV	?	Nepovirus
Carrot red leaf virus	CrRLV	Luteoviridae	?
Cassava green mottle virus	CsGMV	Comoviridae	Nepovirus
Cauliflower mosaic virus	CaMV	Caulimoviridae	Caulimovirus

Appendix-2 (Continued)

Plant Virus name	Abbrev.	Family	Genus
Celery mosaic virus	CeMV	Potyviridae	Potyvirus
Cherry leaf roll virus	CLRV	Comoviridae	Nepovirus
Cherry necrotic rusty virus	CNRV	?	?
Cherry ringspot mottle virus	CRSMV	?	?
Cherry yellow mottle virus	CYMV	?	?
Citrus psorosis virus	CPsV	?	Ophiovirus
Citrus xyloporosis virus	CXV	?	?
Clover yellow mosaic virus	CYMV	?	Potexvirus
Cocoa necrosis virus	CoNV	Comoviridae	Nepovirus
Cowpea aphid-borne mosaic virus	CABMV	Potyviridae	Potyvirus
Cowpea banding mosaic virus	CBMV	?	Cucumovirus
Cowpea chlorotic spot virus	CCSV	?	?
Cowpea little leaf virus	CLLV	?	?
Cowpea isometeric virus	CPIV	?	?
Cowpea mild mottle virus	CPMMV	?	Carlavirus
Cowpea mosaic virus	CPMV	Comoviridae	Comovirus
Cowpea mottle virus	CPMoV	Tumbusviridae	Carmovirus
Cowpea ringspot virus	CPRSV	Bromoviridae	Cucumovirus
Cowpea severe mosaic virus	CSMV	Comoviridae	Comovirus
Cotton leaf curl virus	CLCuV	Geminiviridae	Begomovirus
Cucumber green mottle mosaic virus	CGMMV	?	Cucumovirus
Desmodium mosaic virus	DeMV	Potyviridae	Potyvirus
Desmodium yellow mottle virus	DYMoV	?	Tymovirus
Dodder latent mosaic virus	DLMV	?	?
Eggplant green mosaic virus	EGMB	Potyviridae	Potyvirus
Elm mosaic virus	EMV	?	?
Elm mottle virus	EMoV	Bromoviridae	Ilarvirus
Garlic yellow streak potyvirus	GYSPV	Potyviridae	Potyvirus
Grapevine Bulgarian latent virus	GBLV	Comoviridae	Nepovirus
Grapevine yellow vein virus	GYVV	?	?
Grapevine yellow mosaic virus	GYMV	?	?
Grapevine fanleaf virus	GFLV	Comoviridae	Nepovirus
Guar symptomless virus	GSLV	Potyviridae	Potyvirus
Hippeastrum mosaic virus	HiMV	Potyviridae	Potyvirus
Hop chlorotic virus	HCV	?	?
Lettuce mosaic virus	LMV	Potyviridae	Potyvirus
Lettuce necrotic yellow virus	LNYV	?	?
Lettuce yellow mosaic virus	LYMV	?	?
Limabean mosaic virus	LiMV	?	?
Lucern Australian latent virus	LALV	?	Nepovirus
Lychnis ringspot virus	LRSV	?	Hordeivirus
Maize dwarf mosaic virus	MDMV	Potyviridae	Potyvirus
Melon necrotic spot virus	MNSV	Tumbusviridae	Carmovirus

Appendix-2 (Continued)

Plant Virus name	Abbrev.	Family	Genus
Mulberry ringspot virus	MRSV	Comoviridae	Nepovirus
Mung bean isometeric yellow mosaic virus	MIYMV	?	?
Mung bean mosaic virus	MbMV	Potyviridae	Potyvirus
Muskmelon mosaic virus	MuMV	?	?
Nicotiana veluntina mosaic virus	NVMV	?	?
Onion mosaic virus	OMV	?	?
Pea early browning virus	PEBV	?	Tobravirus
Pea enation mosaic virus	PEMV	Luteoviridae	Tobravirus
Pea leaf roll virus	PLRV	?	Luteovirus
Pea mosaic virus	PeMV	?	?
Pea mild mosaic virus	PeMMV	?	?
Pea seed-bone mosaic virus	PSbMV	Potyviridae	Potyvirus
Pea rosette mosaic virus	PRMV	?	?
Peach latent virus	PLV	?	?
Peach necrotic leaf spot virus	PNLSV	?	?
Peach ringspot virus	PRSV	?	?
Peach rosette mosaic virus	PRMV	?	Nepovirus
Peanut bunchy top virus	PBTV	?	?
Peanut chlorotic spot virus	PCSV	?	?
Peanut clump virus	PCV	?	Furovirus
Peanut marginal chlorotic virus	PeMCV	?	?
Peanut mottle virus	PeMoV	Potyviridae	Potyvirus
Peanut rosette virus	PeRV	?	?
Peanut stripe mosaic virus	PeStMV	Potyviridae	Potyvirus
Peanut stunt virus	PSV	Bromoviridae	Cucumovirus
Pelargonium zonate spot virus	PZSV	?	?
Pear spot virus	PrSV	?	?
Plum pox virus	PPV	Potyviridae	Potyvirus
Potato mop top virus	PMTV	?	Pomovirus
Potato virus-M	PVM	?	Carlavirus
Potato virus-S	PVS	?	Carlavirus
Potato virus-T	PVT	?	Trichovirus
Potato virus-X	PVX	?	Potexvirus
Potato virus-Y	PVY	Potyviridae	Potyvirus
Prune dwarf virus	PDV	Bromoviridae	Ilarvirus
Prunus necrotic ring spot virus	PNRSV	Bromoviridae	Ilarvirus
Rasbperry bushy dwarf virus	RBDV	?	Idaervirus
Rasbperry ring spot virus	RpRSV	Comoviridae	Nepovirus
Rasbperry latent virus	RLV	?	?
Redclover mosaic virus	RCMV	?	?
Redclover vein mosaic virus	RCVMV	?	Carlavirus
Runner bean mosaic virus	RBMC	?	?

Appendix-2 (Continued)

Plant Virus name	Abbrev.	Family	Genus
Satsuma dwarf virus	SDV	Comoviridae	Nepovirus
Sincasma mosaic virus	SinCMV	?	?
Southern bean mosaic virus	SBMV	?	Sobemovirus
Sowbane mosaic virus	SoMV	?	Sobemovirus
Soybean mosaic virus	SbMV	Potyviridae	Potyvirus
Soybean stunt virus	SbSV	?	?
Soybean stunt mottle virus	SbSMV	?	?
Squash mosaic virus	SqMV	Comoviridae	Comovirus
Sunhemp mosaic virus	SHMV	?	Tobamovirus
Strawberry latent ring spot virus	SLRSV	Comoviridae	Nepovirus
Sugarcane mosaic virus	SCMV	Potyviridae	Potyvirus
Tobacco rattle virus	TRV	?	Tobravirus
Tobacco ring spot virus	TRSV	Comoviridae	Nepovirus
Tobacco streak virus	TSV	Bromoviridae	Ilarvirus
Tobacco mosaic virus	TMV	?	Tobamovirus
Tobacco black ring virus	TBRV	?	?
Tomato aspermy virus	TAV	Bromoviridae	Cucumovirus
Tomato bushy stunt virus	TBSV	Tombusviridae	Tobamovirus
Tomato bunchy top virus	TBTV	?	?
Tomato mosaic virus	ToMV	?	Tombusvirus
Tomato ring spot virus	ToRSV	Comoviridae	Nepovirus
Tomato spotted wilt virus	TSWV	Bunyaviridae	Tospovirus
Tomato streak virus	ToSV	?	?
Turnip mosaic virus	TuMV	Potyviridae	Potyvirus
Urdbean leaf crinkle virus	ULCV	?	Carmovirus
Vicia cryptis virus	VCV	Partitiviridae	Alphacryptovirus
Watermelon mosaic virus	WMV	Potyviridae	Potyvirus
White clover mosaic virus	WCLMV	?	Potexvirus
White clover yellow mosaic virus	WCYMV	?	?

? : unassigned

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