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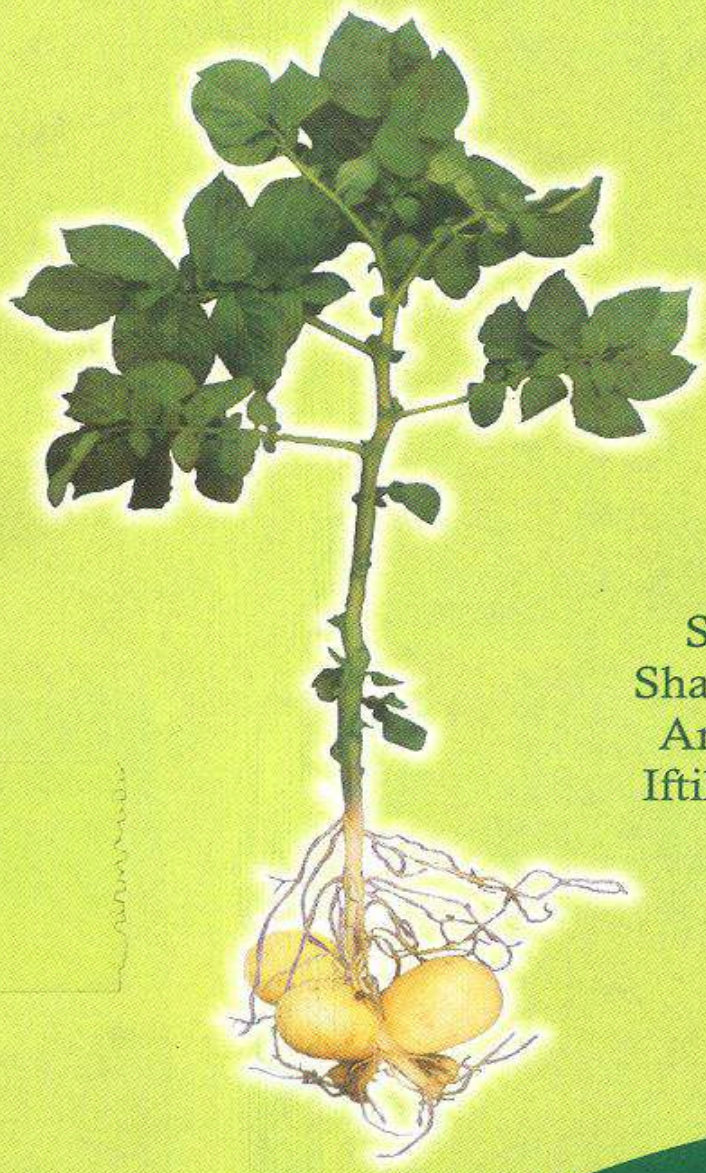

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2000

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"Dedicated to Dr. Abdul Hafiz
as a small token of gratitude
for his contribution to the
Pakistan's Agriculture"

CONTENTS

ABBREVIATIONS

%	Percent
µm	Microemeter
@	At the rate of
°C	Degree centigrade
A	Absorbence
a.m.	Ante meridiem
AMV	Alfalfa mosaic virus
c.	Circa-about
c.	Approximately
*c*DNA	Complementary deoxyribonucleic acid
cm	Centimeter
Cs_2SO_4	Cesium sulphate
CsCl	Cesium chloride
cv	Crop variety
Da	Dalton
DAS	Double antibody sandwich
DEP	Dilution end point
dist	Distil
DNA	Deoxyribonucleic acid
ds	Double stranded
e.g.	Example given
ELISA	Enzyme-linked immuno sorbent assay
EM	Electron microscopy
f.sp.	Farm species
g	Gram
gcm^{-3}	Gram per centimeter cube
gm	Gram
ha	Hectare
hrs	Hours
ICTV	International committee on taxonomy of viruses
IFAS	Indirect immuno fluorescence antibody staining
J2	Second stage juvenile
lb	Pounds
LIC	Laminated inclusions component
lit	Litre
LIV	Longivity in vitro
log	Logarithm

m	Meter
M.wt.	Molecular weight
MF	Marginal flavescence
mg/l	Milligram per litre
mid	Middle
min	Minute
ml	Milli litre
MLO	Mycoplasma like organisms
mm	Millimeter
Mr	Relative molecular weight
MR	Metalaxyl resistance
nm	Nanometer
NCM	Nitrocellulose membrane
nt	Nucleotide
NWFP	North West Frontier Province
ORF	Open reading frame
p.m.	Post meridiem
PCN	Potato cyst nematode
PCNB	Pentachloronetrobenzene
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pH	-ive logarithm of hydrogen ion concentration
PLRV	Potato leaf roll virus
PMTV	Potato mop top virus
PP	Potato phyllody
PTA	Phosphotungistic acid
PTR	Potato top roll
PTW	Purple top wilt
PVA	Potato virus A
PVM	Potato virus M
PVS	Potato virus S
PVX	Potato virus X
PVY	Potato virus Y
PVY^{C}	Potato virus Y, strain C
PVY^{N}	Potato virus Y, strain N
RNA	Ribonucleic acid
S	Sedimentation coefficient
SDI	Serological differentiation index
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
sp	Species

ss	Single stranded
SSEM	Serological specific electron microscopy
ssp	Subspecies
TCN	Tryosine-casein-nitrate medium
TEM	Transmission electron microscope
TIP	Thermal inactivation point
TMV	Tobacco mosaic virus
TRV	Tobacco rattle virus
TTC or TZC	Tetrazolium medium
U.K.	United Kingdom
USA	United States of America
USSR	Union of Soviet Socialist Republicans
var	Variety
viz	Namely
VPg	Genome-linked protein
WA	Water agar
WB	Witches' broom

FOREWORD

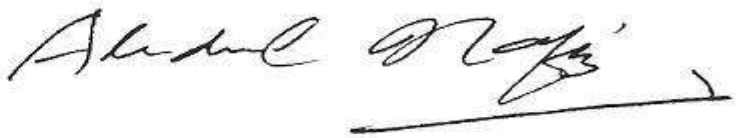
Evidence is available that man's advance from food-gathering stage to food-producing took place, simultaneously, in five to six widely dispersed places. These include Fertile Crescent, Balochistan (in Pakistan), East Indies and South China, Nigeria and Middle America. Primitive agriculture in Fertile Crescent and Balochistan depended upon wheat and barley. But in rain forest areas the beginnings of agriculture were made with tubers like cassava and yam. Other grains came later and were also diversified-rice in China and East Indies, sorghum in Kenya and Nigeria. In middle America, beginning was made with potato and sweet potato and ended up with maize. So potato is one of the ancient crop of old civilizations. It went through many changes spread over a long period of domestication, assuming a very important position in cropping systems of a large number of countries. It has now become a valuable food and industrial crop with numerous advantages. Above all, potato is the highest yielder per unit area (upto 100 tones/ha in Holland).

Pakistan occupies about 80,000 hectares under potato, out of which two-thirds are in the Punjab and one-third in NWFP. In the planes, potato is grown as autumn and spring crops while it is sown during summer in the hilly areas. Average yields are very low (13 tonnes/ha) due to many reasons, particularly use of infested seed tubers, faulty production techniques and ineffective control measures against insect pests and diseases. There is a big scope of increasing the production by about two to three times, if improved potato production and protection technologies are developed and implemented. Number of diseases caused by viruses, fungi, bacteria and nematodes, which attack the potato crop causing huge economic losses. Many of the diseases are tuber-borne, needing implementation of healthy seed production and distribution system. This step alone can contain numerous diseases.

It is heartening to mention that Dr. Saif Khalid and his other colleagues has compiled a complete and exhaustive account of all the damaging diseases, detailing the pathogens, symptoms (alongwith colored photographs), incidence, mode of perpetuation and control measures both preventive and curative. At the end a detailed methodology of disease-free seed potato production as well as a basic

package of crop production technology has been added to promote enhanced per unit area production of healthy potato tubers. This book will prove highly useful for scientists, teachers, students, extentionists, farmers and administrators in their respective spheres of activities. No doubt, the book will prove very useful in the long run not only in enhancing potato production in Pakistan but also in enriching the farmers, and decreasing the seed imports' and increasing the potato exports, improving the country's economy.

I congratulate Dr. Saif Khalid and his colleagues on producing the book under review and appreciate their sincere efforts following the publication of his first book on **"Research on Plant Viral Diseases in Pakistan".** Undoubtedly the crop-wise or pathogen-wise approach can be highly productive in a thorough, in depth analysis of integrated control of plant diseases.



Dr. Abdul Hafiz
Former Director, Food and Agriculture
Organization of the United Nations

Islamabad, 2000

PREFACE

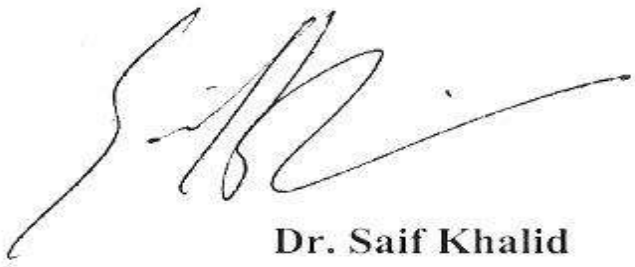
Apart from the books of Dr. Abdul Hafiz in 50's and a book by Kamal and Mughal in mid 60's, there is a scarcity of books on plant pathology. The last book "Plant Diseases" by Dr. A. Hafiz was published in 1986, which was a compilation of all knowledge available on the subject upto that year. This book has long been out of print and stock exhausted. The book is still in demand as there has been nothing new produced. Since then a lot of new knowledge has been generated that need to be compiled for the benefit of researchers, extensionists, teachers, students and progressive farmers. Considering that writing a single book on diseases of all crops is a huge task, we initiated writing the books on diseases of individual crops, and potato is the first in this series.

The book is completed now and we would like to thank to those involved in its production. Special thanks are due to Dr. Abdul Hafiz, Dr. Samina Khalil and Malik Mohammad Mushtaq for their valuable suggestions for improving the book. Thanks are also due to Mrs. Khurshid Burney for some of the photographs; Mr. Shahid Aslam Siddiqui for proof reading and collection of data and, Mr. Abdul Khaliq for composing the book.

From early 80's potato production has gained momentum in Pakistan and its production has increased from 448.5 thousand tonnes in 1980 to 1810.4 thousand tonnes in 1999. This was due to awareness among the growers of the potential and importance of potato as food source. However, cultivation of potato to new areas helped trafficking diseases to new localities. On the one hand, non-availability of disease-free seed potato helped in dissemination of diseases and on the other hand new diseases have been introduced with imported seed potatoes further aggravating the situation. Meaningful disease management suggestions can only be offered if the pathogens are quickly and correctly diagnosed alongwith their modes of perpetuation. We tried to gather as much information as possible on these diseases for the benefit of reader and hope that the book will help provide information on these diseases to reduce losses caused by them.

The diseases have been dealt within descending order of importance in four parts, each dealing with specific group of pathogens i.e. viral, bacterial, fungal and nematodal diseases. Besides, a package of seed

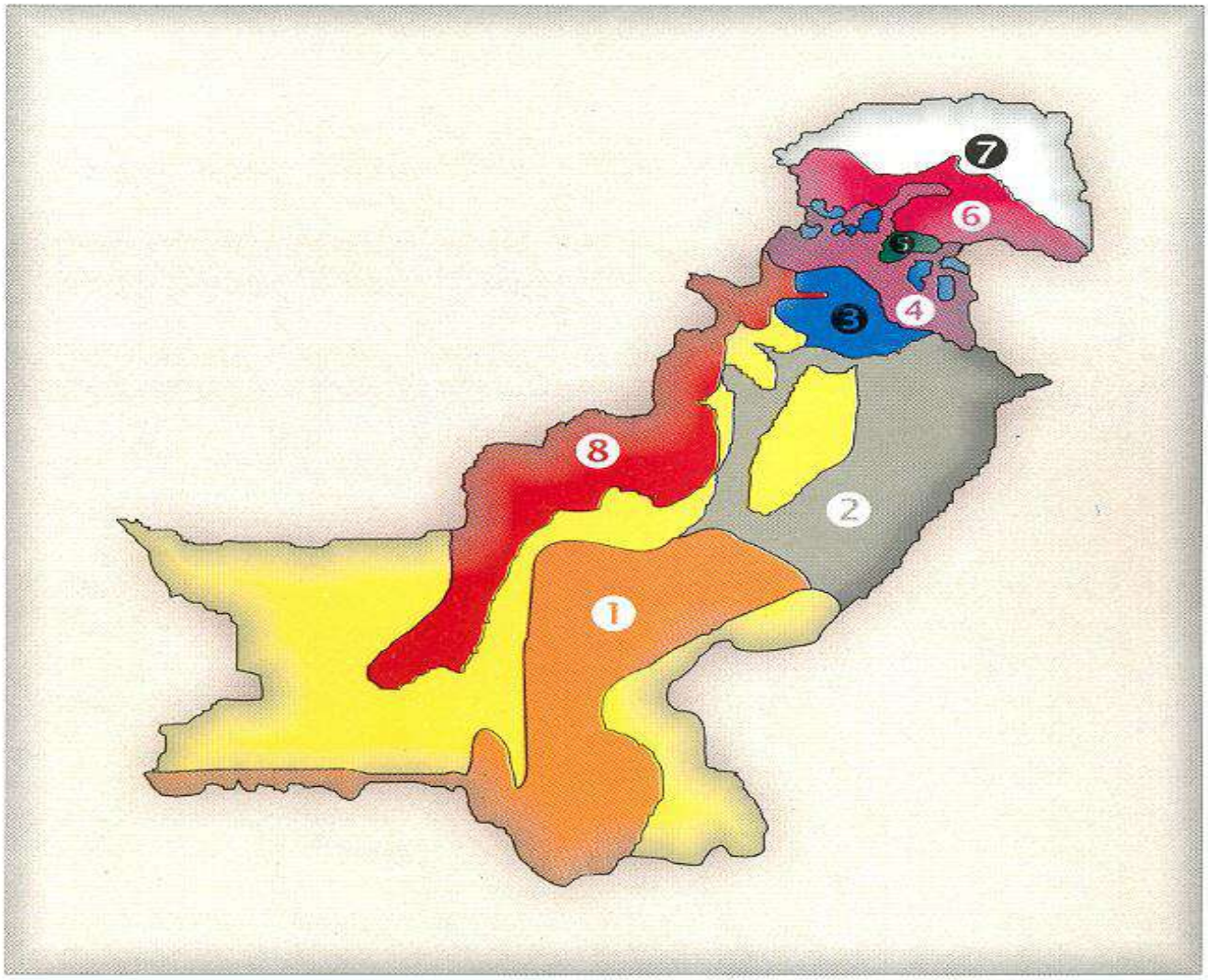
potato production and protection technologies to contain diseases within economical level is also included.



Dr. Saif Khalid
NARC, Islamabad

Islamabad, 2000

POTATO PRODUCTION ZONES OF PAKISTAN



1. Irrigated plains of Sindh, Southern Punjab and Balochistan
2. Irrigated plains of Central Punjab and South East NWFP
3. Irrigated and rainfed plains of NWFP and Northern Punjab
4. Irrigated lower valleys of NWFP
5. Rainfed high valleys and hillsides of NWFP, Northern Punjab and Azad Kashmir
6. Irrigated high valleys of NWFP, Northern Areas around Chilas and Azad Kashmir
7. Irrigated high valleys of Northern Areas and NWFP around Mastung
8. Irrigated high valleys of Balochistan, South and North Waziristan

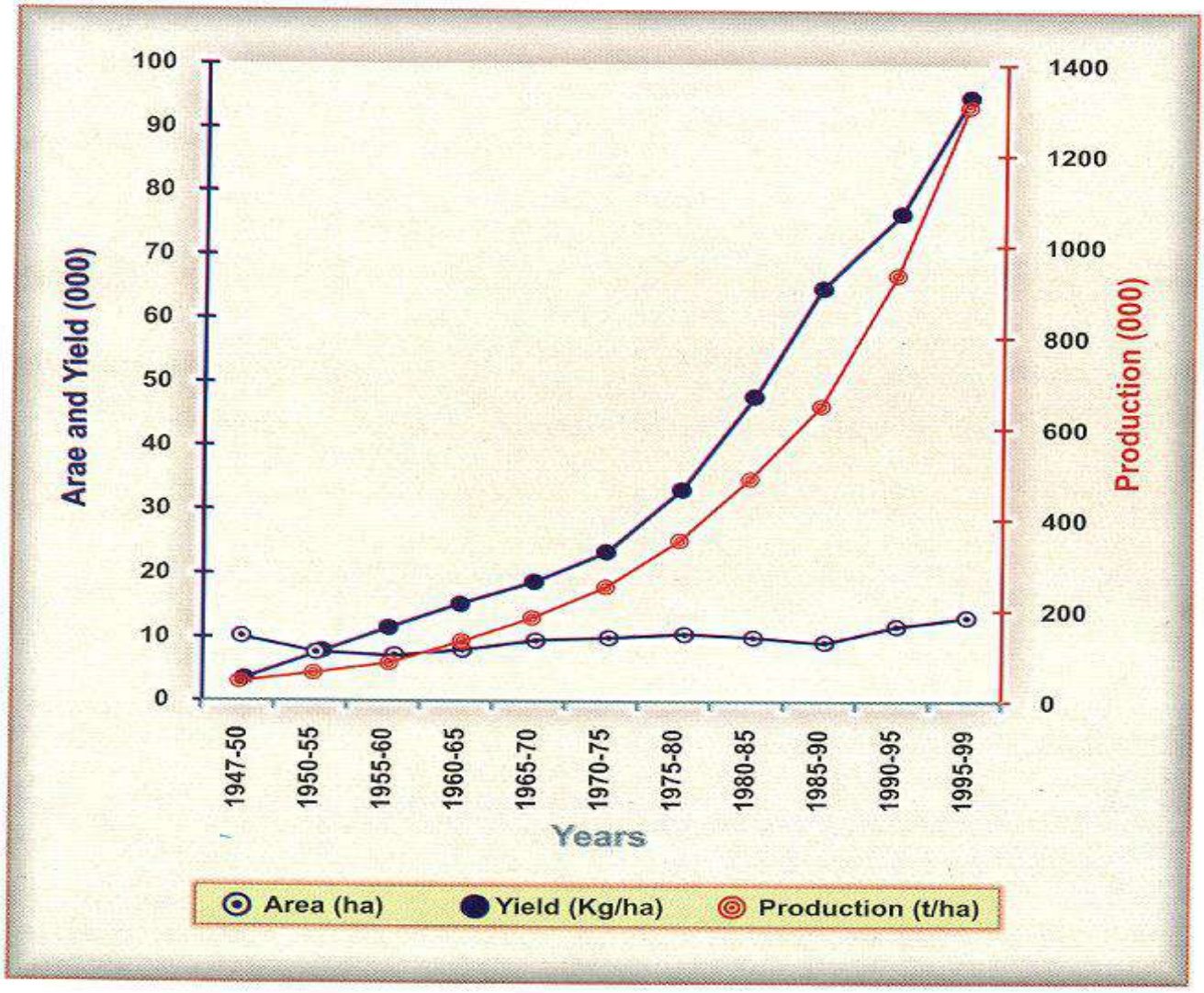
(Source: Zanoni, 1991)

POTATO AREA, PRODUCTION AND YIELD IN PAKISTAN

Year	Area (000 ha)	Production (000 t)	Yield (000 kg/ ha)
1947-48	2.8	28.0	10.0
1948-49	3.2	37.0	11.6
1949-50	5.3	52.0	10.0
1950-51	4.9	47.0	9.8
1951-52	7.3	50.0	7.0
1952-53	7.7	61.0	8.1
1953-54	7.7	69.0	9.1
1954-55	10.9	73.0	5.9
1955-56	9.7	72.0	7.6
1956-57	10.5	69.0	6.6
1957-58	12.6	99.0	8.0
1958-59	12.6	102.0	8.3
1959-60	12.6	89.0	7.2
1960-61	15.0	110.0	7.5
1961-62	14.2	126.0	9.3
1962-63	13.0	116.0	9.1
1963-64	15.0	134.0	9.1
1964-65	18.6	170.0	9.3
1965-66	17.0	151.0	9.0
1966-67	19.0	165.0	8.8
1967-68	20.2	186.0	9.3
1968-69	21.1	227.0	11.0
1969-70	17.0	176.0	10.5
1970-71	20.2	228.6	11.3
1971-72	23.0	253.7	11.0
1972-73	23.4	241.3	10.3
1973-74	23.4	238.8	10.2
1974-75	27.7	289.5	10.5
1975-76	28.6	320.8	11.2
1976-77	25.7	318.0	12.3
1977-78	29.8	293.5	9.9
1978-79	37.7	392.4	10.4
1979-80	42.9	448.5	10.4
1980-81	38.0	394.3	10.4
1981-82	45.3	476.6	10.5
1982-83	51.5	518.1	10.1
1983-84	49.6	509.8	10.3
1984-85	54.5	543.3	10.0
1985-86	62.9	618.4	9.8
1986-87	60.5	594.3	9.8
1987-88	58.1	563.2	9.7
1988-89	63.9	644.8	10.1
1989-90	80.0	830.9	10.4
1990-91	72.0	751.3	10.4
1991-92	75.6	859.8	11.4
1992-93	76.0	932.8	12.3
1993-94	79.0	1050.2	13.3
1994-95	79.0	1105.0	13.9
1995-96	78.9	1063.5	13.5
1996-97	85.8	963.6	11.2
1997-98	104.7	1425.5	13.6
1998-99	109.5	1810.4	16.5

Source: Agricultural Statics of Pakistan

AVERAGE TRENDS OF POTATO AREA, PRODUCTION AND YIELD IN PAKISTAN (1947-2000)



INTRODUCTION

INTRODUCTION

The genus *Solanum*, to which cultivated potato belongs, is large consisting of about 1000 species. More than 200 species of potato have been found, but just eight are cultivated (Huaman, 1986). It is found all over the world, except for the far south and north. It is concentrated in South and Central America on the one hand and Australia on the other (Hawkes, 1978). The cultivated potato *Solanum tuberosum* has its origin in the South American Andes. Various species of wild tuber-bearing *Solanums* are found in Central America, Mexico and as far north as Colorado. Potato has been in cultivation for at least 2000 years (Smith, 1977). Several wild, tuber-bearing *Solanum* species are grown on the Andean highlands from which the parent stocks of our domestic potato came.

Potato was introduced into Europe by Spaniards in Spain in 1570 and came to England during 1588-93 (Hawkes, 1967). From here, potato spread nearly to every part of the world. From Spain it reached to continental Europe and some parts of Asia. From the England it spread to Ireland, Scotland, Wales and parts of the northern Europe as well as to British overseas colonies.

Thus a crop confined to South America for many centuries has been spread to all over the world in a span of 400 years and is now ranked 4th in the world in economic importance and is used for human consumption, animal feed and as a source of starch and alcohol (Horton, 1992).

At the start of this century world potato production was about 135 million tonnes, by the middle of this century it was 250 million tonnes and in the late 1980's, it was about 200 million tonnes. Europe produced about 90% of the world potato during the first half of the century. Potato production began to decrease in Europe after World War II. Since 1960, the total world area under potato cultivation has fallen by 20%.

Of total world production, Eastern Europe and the USSR produce just under half, and Western Europe about 17%. About 15% of world potato crop is grown in the Far East, 10% in the America and Oceania and less than 5% in Africa and Middle East. Potato production in India and Pakistan has quadrupled since 1960. As a result of these

divergent trends more potatoes are grown now in Asia than in Western Europe.

The balance of world potato production is gradually shifting form the developed to the developing countries. Only 40% of the world potatoes are now grown in Europe, 35% are grown in other developed countries and 25% in developing countries (Horton & Anderson, 1992).

Potato (*Solanum tuberosum*) is an important crop of Pakistan, covering an area of about 109.5 thousand ha with an annual production of 1810.4 thousand tonnes (Anonymous, 1999). In different agro-ecological zones of Pakistan, three crops are cultivated annually viz., spring, summer and autumn. Spring and autumn crops are cultivated in the plains and summer plantings in the uplands at elevation ranging from 1,500 to 3,000 m.

For a country like Pakistan having a population of about 130 million with a growth rate of 2.61%, which is expected to lead to a population of 140 million by the year 2000, yield per unit area of land is of great significance. It is a major source of starch for human consumption and potentially of great benefit in meeting the food requirements of the ever-increasing population. In Northern Pakistan, potato is the only major source of income and only one crop is grown per year. The farmers are poor and majority of them have small land holdings ranging from 1/4 to 1 ha. The whole family, particularly the women, provide the labor force doing land preparation, planting, digging etc. It is the most profitable crop and grown year after year. Therefore, it has become a high priority crop for this community. Northern Pakistan is the main seed producing area, supplying seed to rest of the country.

The potato crop in Pakistan is affected by many pests and diseases, and of these potato viruses, powdery scab, potato cyst nematode (PCN), aphids and white grub (*Phyllophaga* sp.) are the most damaging ones. Surveys done by Pakistani workers have revealed that viruses, powdery scab and aphids are wide spread, whilst PCN and white grub are serious problems in the northern hilly areas.

Pakistan lies between 24-36° north and 61-76° east. Much of Pakistan has a sub-tropical climate which extends into a warm temperate zone in the north. It covers an area of 79.61 million hectares of that 54.97 million hectares are reported to be under agricultural utilization.

Agriculture is the backbone of Pakistan's economy. Almost two thirds of the total population derives their livelihood from it. The main industries of the country are also based on agriculture. Rice, cotton, wheat, sugarcane, tobacco, maize and potato are the major crops.

Pakistan is generally dry and has low rainfall, apart from Southern slopes of Himalayas. Much of the rain is brought by the Monsoon winds in summer starting in mid July and lasting up to mid September. The northern and north-western regions also receive winter rainfall. Potato farming systems in Pakistan are quite complex and diverse due to the wide variability of climatic, socio-economic, cultural and agronomic conditions. For potato production, Pakistan can be divided into eight agro-ecological zones which differ in altitude, longitude, latitude, topography, climate, soils and irrigation infrastructure (Zanoni, 1991). The crops grown vary considerably between zones. Potatoes in Pakistan are grown in areas having altitudes upto 3100 m. In the high altitude valleys, potatoes are grown as a summer crop, with planting starting in mid March and lasting up to July depending on crop variety and altitude. Harvesting in these areas start from late July and continues until late November. In the plains and lower valleys, three consecutive crops can be grown per year.

DISEASES CAUSED BY VIRUSES

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Introduction

Viruses are unique and among the smallest class of disease causing agents. They are obligate parasites (multiply only in living cell), cannot grow on artificial media and are inactive outside host cells. Their existence was reported as early as sixteen century, but it was late eighteen and beginning of nineteenth century when researchers started to understand even the rudiments of plant virology. The infection nature of viruses was proved in 1886 (Mayer, 1886) by showing the transmissibility of the tobacco mosaic virus (TMV). Its submicroscopic nature was demonstrated by Iwanowski (1892). He showed that TMV is so small that it can pass through smallest bacteria retaining filters. The ability of plant viruses to multiply only *in vivo* was demonstrated by Beijerinck (1898).

The shape of plant viruses may be isometric, bacilliform, rod, bullet or filamentous. Size of plant viruses can vary from 17-2000 nm. Some viruses have two or more particles of different lengths. For instance alfalfa mosaic virus (AMV) has bacilliform particles of at least four sizes; mostly 30, 35, 43 and 65 nm in lengths. Particles (virions) consist of two parts i.e. infectious nucleic acid, which is encapsidated (covered) with protein coat. The nucleic acid is referred as genome and carries the genetic information needed for the replication of virus. Nucleic acid part of most plant viruses, is ribonucleic acid (RNA) but some have deoxyribonucleic acid (DNA). It can be single (ss) or double (ds) stranded. The function of the coat protein is to protect the viral genome between infection cycles.

Some viruses have a genome that consists of more than one molecule of nucleic acid, known as multipartite. All molecules of multipartite genome are needed for the production of new virion. The viral genome may be assembled in one particle or divided over different particles, therefore, all are needed to ensure infectivity. Some viruses possess a multipartite genome, each part of which is embodied in a nucleocapsid. These nucleocapsids are surrounded by a membrane to form one virus particle. In this case all particles are identical, although the genome is multipartite.

Plant viruses in nature are transmitted through different means such as grafting, insect, soil-inhabiting organisms (nematodes & fungus), seed, pollen, dodder and by vegetative propagation. Among the insect vectors, aphids (*Aphididea*), leaf hoppers (*Cicadellidae*), planthoppers (*Delphacidae*), treehoppers (*Membracidae*), whitefly (*Aleyrodidae*), mealy bugs (*Pseudoccoccidae*), beetles (*Coleoptera*), thrips (*Thysanoptera*) and mites (*Eriophyidae*) are the important ones. Generally, if a virus is transmitted by one of these insect groups, it is unlikely to be transmitted by any other group of insect, but exception does occur. Tobacco ringsopt virus is usually transmitted by nematode vector (*Xiphinema americanum*), but it also reported to be transmitted by thrips (Messieha, 1969) and spider mites (Thomas, 1969).

Plant viruses can induce a wide range of symptoms in infected plant, ranging from latency, color deviation (chlorosis, mosaic, mottling, flecking, spotting, line pattern, ring-spotting, vein banding, vein yellowing, vein mosaic, striping, streaking, color-breaking, yellowing, blanching), growth reduction, necrosis, malformation, leaf rolling, leaf curling, puckering, leaf distortion, leaf narrowing, rugosity, rossetting, profuse branching, enation formation (outgrowth on leaves), swellings, wilting, anatomical deviations, physiological and biochemical deviations to death of the plant.

Many substances from plants and other organisms origin, as well as synthetic organic chemicals, have been tested against plant viruses, but none has achieved any practical importance. General control measures to minimize crop losses caused by plant viruses includes:

> Elimination of source of infection (eradication of weeds and other alternative hosts, roguing within the crop, eradication of volunteer/ground keepers plants); avoidance of source of infection (use of virus free seed/planting material, modification of cropping procedures, cultivation in isolated areas, crop hygiene); avoidance of the vectors (cropping in vector-free areas, change in cropping practices); chemical control of vectors (insects, nematodes, fungal); non-chemical control of vectors (barriers and reflective mulches, oil sprays, biological control by predators); plant resistant to vectors, control by cross-protection and genetic protection (use of resistant/tolerant cultivars).

Potato Leaf Roll Virus

Occurrence and Importance

This virus has great importance in the history of potato, and perhaps the beginning of potato viruses. It was first found *in Solanum tuberosum* sp. *Tuberosum* in the Netherlands (Brunt *et al.*, 1990) but virions were first isolated by Peters (1967). Probably distributed world-wide in potato growing areas. Quality of seed tubers is badly affected due to development of phloem necrosis. That is why it is also known as phloem necrosis virus. Alongwith PVY, both these viruses were the causes of potato degeneration. Diseased plants produce fewer and smaller tubers than the normal plants resulting in significant yield reduction, which may exceed half of the yield. If the plants are 100% infected, the yield losses vary between 40-70%. In Pakistan, yield reduction upto 70% is estimated (Mughal & Khalid, 1985).

Potato leaf roll virus (PLRV) is among the important potato viruses in Pakistan and has been reported from throughout the country with an incidence of 15-65% (Mughal *et al.*, 1988). However, for the first time, its prevalence was reported in 1978 (Mirza, 1978). Recent survey also showed that PLRV is among the most prevalent and economically important viruses of potato in Pakistan (Ahmad *et al.*, 1995_{a-d}; Jan & Khan, 1995; Ahmad & Ahmad, 1995). Some basic properties of a field isolate of PLRV were studied by Arif (1988) and Arif *et al.* (1995).

Symptoms

Symptoms of primary infection results in characteristic rolling of the upper leaves at the top of the plants (Figure 1). Such plants are dwarf, upright in habit and their leaves become thick and pale. The leaves of some varieties are often rolled upwards, especially at the base. Infection in late growing season usually let the plants symptomless and yield losses are nominal. However, tubers

Figure 1. Symptoms of primary infection, rolling of upper leaves

from such plants are partially infected.

In case of secondary infection, whole plant looks erect and may be smaller than the healthy ones. Older plant shows rolling of lower leaves while upper leaves are pale (Figure 2). Basal leaves are thick, leathery, brittle and crackle when squeezed in hand, due to heavily accumulation of starch. A purple pigment at the base of young leaflets may also develop. Some cultivars of *S. tuberosum* ssp. *Andigena* and other wild species do not show leaf rolling, but rather stunting and severe chlorosis (Salazar, 1996). Infected tubers of some varieties develop internal necrosis, known as net necrosis, while other develop thin sprouts from such tubers.

Figure 2. Symptoms of secondary infection, rolling of lower leaves

Virus

The virus belongs to the genus *Luteovirus* with approved acronym PLRV. Other synonyms are potato phloem necrosis virus, solanum yellow and tomato yellow top strain (Harrison, 1984).

Relation with Cells and Tissues

The virus is apparently confined to the phloem tissue of intact plants. The greatest number of virus particles is found in the cytoplasm of phloem parenchyma and companion cells.

Morphology

Virions are not enveloped and particle shape is isometric (Figure 3), measuring 24 nm in diameter (Peters, 1967; Takanami & Kubo, 1979); with hexagonal outline.

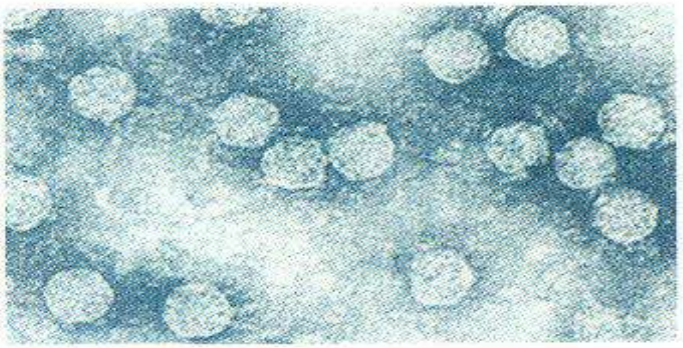

Figure 3. Particles of potato leaf roll virus stained with uranyl acetate

Physicochemical and Physical Properties

Buoyant density is 1.39 g cm^{-3} in CsCl (Rowhani & Stace-Smith, 1979); density 1.34 g cm^{-3} in Cs_2SO_4 (Thomas, 1984). Sediment as single component in purified preparations. Sedimentation coefficient is 115 *S*. Infectivity of sap not changed by treatment with di-ethyl ether and retained when deproteinised with proteases, phenol or detergent.

- TIP (Thermal inactivation point) = 70-80°C (in *Physalis floridana* sap)
- DEP (Dilution end point) = 10^{-4}
- LIV (Longivity in vitro) = 5-10 days at 2°C (Murayama & Kojima, 1965)

Nucleic Acid

Virions contain 30% nucleic acid and one molecule of linear single stranded RNA of particle weight (M. wt.) 7000.

Proteins

Virions contain 70% protein. Two structural virion proteins found. Size of the larger protein is 26000 Da and size of 2nd is 7000 Da which is a genome-linked protein (VPg). Method of preparation is described by Rowhani & Stace-Smith (1979). Virus-coded non-structural proteins have been isolated (Mayo *et al.*, 1982; Mayo & Barker, 1984)). Three non-structural proteins are found. Size of the largest is 125000 Da (Mayo *et al.*, 1982); 2nd largest 71000 Da (Mayo *et al.*, 1982) and size of 3rd is 29000 Da (Mayo & Barker, 1984; ICTV, 2000).

Function of Helper and Satellite Viruses

Virions associated with helper virus, but independent from its functions during replication.

Cytopathology

Virions found in phloem, companion cells, in cytoplasm and in cell vacuoles (Kojima *et al.*, 1969). Vesicles occur in the cytoplasm attached to the nucleus and fused with the nuclear envelope. Inclusions present in infected cells. Inclusions are crystals in the cytoplasm and contain virions. Other cellular changes are thickening of walls in primary phloem cells of stems and petioles, and callose accumulation in some sieve tubes of tubers. The presence of callose is the basis of various staining tests (e.g. with 1% Resorcin Blue), used before serological methods were developed (de Bokx, 1967; ICTV, 2000).

Serological Properties

Virus(es) with serologically related virions are tobacco necrotic dwarf (SDI 1), beet western yellows/beet mild yellowing (SDI 2-4), bean leaf roll, subterranean clover red leaf and barley yellow dwarf viruses (Roberts *et al.*, 1980; Tamada *et al.*, 1984; Thomas, 1984; ICTV, 2000).

Transmission

In nature, PLRV is transmitted to potato only by insect vectors, in a persistent manner *by Myzus persicae* which is economically most important and efficient vector. *Macrosiphum euphorbiae* transmits potato strains less effectively. Virus is retained when vector moults, does not multiply in the vector. Experimental transmission is by grafting and through aphids. The virus could not be transmitted mechanically, by seed or pollen (ICTV, 2000).

Host Range

The virus produces dwarfing, chlorosis, leaf rolling and phloem necrosis on various solanaceous hosts including *D. stramonium, P. floridana, S. dulcamara* and *S. villosum. Physalis floridana* is the best indicator plant. Non-solanaceous hosts are *G. globosa, Amaranthus candatus* and *Nolana lanceolata.* Other hosts are listed in Table 1.

Detection Methods

PLRV can be detected by serology (ELISA - a useful method for routine large scale testing). Molecular methods such as nuclear acid based and PCR can also be applied. Serological specific electron microscopy (SSEM) is another useful method. The best indicator hosts are *P. floridana* and *D. stramonium.*

Control

- Use virus free, certified seed potato
- Control the aphid vectors
- Roguing
- Heat treatment of the tubers (e.g. 10-20 days in air at 37.5°C)
- Assess the health of tuber stocks by serological tests through ELISA, after harvest
- Use resistant varieties. Currently used varieties in the country are susceptible, however, resistant varieties are available.

Table 1. Hosts of Potato Leaf Roll Virus

Host Plants	Symptoms
Natural Hosts	
Potato *(Solanum tuberosum)*	Pallor or reddening of the tip leaves, which roll and become erect. Plants grown from infected tubers are stunted, leaflets upwardly rolled, oldest leaves first.
Solanum tuberosum ssp. *andigena* in South America	Stunting and marginal yellowing of tip leaves.
Lycopersicon esculentum	Stunting of plants, marginal yellowing and curling of leaflets, and death of flower buds.
Diagnostic Hosts	
Datura stramonium	Systemic interveinal yellowing.
Physalis floridana	Systemic interveinal chlorosis, older leaves slightly rolled and stunting of plant.
Solanum tuberosum ssp. *tuberosum*	Stunting and leaves rolling.
Insusceptible Hosts	
Brassica campestris ssp. *pekinensis, Raphanus sativus, Vicia faba*	
Maintenance and Propagation Hosts	
Physalis floridana, Solanum tuberosum	
Assay Host	
Physalis floridana (W)	Local lesions or whole plants.
Other Susceptible Hosts	
Capsella bursa-pastoris, Celosia argentea, Montia perfoliata and *Nicotiana clevelandii.*	

Sources of host-range data: Natti *et al.* (1953); Braithwaite & Blake (1961); Rodriguez & Jones (1978); Harrison (1984); Thomas (1984); Tamada *et al.* (1984); Brunt *et al.* (1990) & ICTV (2000).

Potato Mop Top Virus

Occurrence and Importance

First found in *Solanum tuberosum* in Northern Ireland, U.K. (Calvert & Harrison, 1966). The virus occurs and spreads in the Eurasian region, South and Central American region (in the Andean region), Israel, Japan and U.K. Also found in Chile and Taiwan, but there is no evidence of its spread. The virus is self-eliminating in potato growing on virus-free soil. Virus does not spread to all tubers of infected plants, thus the virus is maintained by the vector.

In Pakistan, Potato mop top virus (PMTV) was first reported in traces by Mughal *et al.* (1988). It is still among the less important viruses of potato and is found in localized areas (Khalid, unpublished). However, its vector i.e. *Spongospora subterranea* has been reported from Astak valley in Northern Areas (Ahmad *et al.*, 1996); from Balochistan and Baltistan (Iftikhar & Ahmad, 1999); Sawat and Kagan (Rattu *et al.*, 1999); Gilgit and Hunza (Iftikhar & Ahmad, 2000) and from Chitral (Iftikhar, 2000).

Symptoms

The symptoms induced by PMTV vary widely in different cutlivars and they are greatly affected by environmental conditions. Transmission of the virus from one generation to the next by tubers is erratic. Aucuba and mop-top symptoms have often been attributed to chilling in the early stages of growth. Plants with secondary symptoms may produce three main types of symptoms, mostly on one stem only per hill. The type of symptoms induced depends greatly on the cultivar and environmental conditions. They develop best in cool conditions (5-15°C).

Stems of infected plants have short internodes and leaves, whose leaflets are reduced in size and have wavy or rolling margins. The infected plants show a dwarfed and bunched growth habit, which is typical of some cultivars.

Aucuba symptoms: irregular bright-yellow blotches, rings or line patterns, usually in the middle leaves (Figure 4). The aucuba is the most common symptom of PMTV in many cultivars.

Chlorotic chevrons which may be distinct or diffuse and ultimately become a distinct mosaic in the upper leaves (Calvert, 1968).

Primary tuber symptoms, which differ markedly from secondary symptoms, consist of slightly raised, necrotic concentric rings 1-5 cm in diameter on any part of the tuber surface. Internally, spraying in the form of necrotic areas may be present. Secondary symptoms frequently consist of cracks of different sizes, so that malformation occurs. In tubers, secondary symptoms are found in cultivars that develop mop-top in the stems like Patrones.

Figure 4. Symptoms of potato mop top virus

Virus

Virus belongs to the genus *Furovirus* with approved acronym PMTV. Recent studies on variation of PMTV from Andes showed that three isolates contained between three and six amino acid change in the N terminal of the coat protein (Salazar, 1996).

Morphology

Virions are not enveloped and rod-shaped with clear hollow core. They are usually straight with clear modal length 100-150 or 250-300 nm long and 18-20 nm in diameter. In some isolates uncoiling of the terminal protein helix is obvious (ICTV, 2000).

Physicochemical and Physical Properties

Particles sediment as three components in purified preparations with sedimentation coefficient 236 *S*, 126 *S* and 171 *S*. The infectivity is specific with 236 S (ICTV, 2000).

- TIP = 75-80°C
- DEP = 10^{-3}
- LIV = up to 200 days

Nucleic Acid

Linear single stranded RNA.

Proteins

One structural virion protein with 18500-20000 Da is found.

Cytopathology

Virus particles are found in leaves and in the cytoplasm. Inclusions found in infected cells are viroplasma and small loose sheaves containing virus particles. They are of no diagnostic values and are not seen under an optical microscope. Other cellular changes are necrosis in arcs or lines in potato tubers (ICTV, 2000).

Serological Properties

Virus(es) with serologically related virions are soil borne wheat mosaic virus and also tobacco mosaic virus, but very distantly. Virus(es) with serologically unrelated virions includes peanut clump, sugarbeet necrotic yellow vein, hypochoeris mosaic and oat golden stripe viruses. There is no homology (using *c*DNA or RNA hybridization) between potato mop top and peanut clump, soil borne wheat mosaic and beet necrotic yellow vein viruses (ICTV, 2000).

Transmission

PMTV is transmitted naturally by the zoospores of the fungal vector belonging to the Plasmodiophorales: potato powdery scab fungus *Spongospora subterranea*, but virus-free Spongospora has not been experimentally infected (Jones, 1988). PMTV can survive in the resting spores of the fungus for several years. The fungus and hosts other than potato may be responsible for the persistence of the virus in soil, even when no potatoes are grown. The virus probably spreads from field to field in spore balls of *S. subterranea* carried by tubers. Non-vector transmission is by mechanical inoculation and by grafting.

Host Range

Hosts are listed in Table 2.

Table 2. Hosts of Potato Mop Top Virus

Host Plants	Symptoms
Natural Hosts	
Potato (*Solanum tuberosum*)	Dwarfing (mopping); chlorotic and necrotic chevrons and blotching, tuber cracking and necrotic conchoidal layers in tubers especially in cool weather (15°C).
Chenopodiaceae, Solanaceae	Weeds species are susceptible and commonly infected in the Andean region of South America, but not elsewhere.
Diagnostic Hosts	
Nicotiana debneyi	Systemic necrotic oak leaf patterns.
Chenopodium amaranticolor	Concentric brown local lesions.
Maintenance and Propagation Hosts	
Nicotiana benthamiana, Nicotiana debneyi, Nicotiana clevelandii	
Assay Hosts	
Chenopodium amaranticolor (L)	Local lesions or whole plants.
Nicotiana debneyi (W)	Local lesions or whole plants.
N. benthamiana (W)	Local lesions or whole plants.
Other Susceptible Hosts	
Beta vulgaris, Capsicum annuum, Atriplex hortensis, Chenopodium album, C. foliosum, C. quinoa, Datura ferox, D. stramonium, Hyoscyamus niger, Lycopersicon esculentum, Nicandra physalodes, Nicotiana glutinosa, N. rustica, N. tabacum, Petunia hybrida, Physalis floridana, P. peruviana, Solanum nigrum, Tetragonia expnsa.	

Sources of host-range data: Jones (1974); Brunt *et al.* (1990) & ICTV (2000).

Detection Methods

Symptoms produced in potato resemble those caused by tobacco rattle virus (TRV). Both PMTV and TRV induce somewhat different

symptoms in *Chenopodium amaranticolor*. PMTV induces concentric fine necrotic ringspot lesions on inoculated leaves. A single lesion may spread to cover half a leaf, while TRV produces necrotic local lesions, some tending to spread. In both cases no systemic infection take place.

Different forms of ELISA and nucleic acid based methods can be used for routine large scale testing.

PMTV can be distinguished through EM studies from other viruses occurring in potato by its particle shape and size (Kassanis *et al.*, 1972; Brunt *et al.*, 1990).

Control

- Use disease free potatoes in soil which is not infested with *S. subterranea*
- Rogue seed potato fields carefully
- Crop rotation such as oil seed rape as main or in between crop, reduce disease incidence (Winter & Winiger, 1983).

Potato Virus A

Occurrence and Importance

This disease was first found in *Solanum tuberosum* in Eire (Murphy & McKay, 1932). It occurs in Europe and North and in most of the other potato-growing areas also, where it has been introduced through infected seed potatoes. It causes yield losses upto 40%. In Pakistan, Potato virus A (PVA) was first isolated in 1978 from Punjab (Mirza, 1978). Later reports suggested that PVA was one of the most important potato diseases in Punjab (Anwar & Mirza, 1984). During 1988, PVA incidence in Pakistan was reported between 2-15% (Mughal *et al.*, 1988). Currently PVA only occurs in low percentages in all potato production zones, however, occasionally higher incidence is also reported (Ahmad *et al.*, 1995c).

Symptoms

Symptoms first appear on terminal leaves showing blochy mottle, mild mottling and slight crinkling. In many cultivars it induces mosaic, sometime severe. Symptoms remain masked and difficult to detect in bright sunlight. Tuber symptoms are usually unrecognizable, however, they may be smaller in size. Leaves of the susceptible varieties may show mild mosaic, roughness of surface and waviness of the leaf margins. Some hypersensitive varieties develop top necrosis. Such cultivars possess a form of field immunity. When infected in combination with PVY or PVX, it produces crinkle symptoms.

Virus

Virus belongs to the genus *Potyvirus* family *Potyviridae* with approved acronym PVA. Other synonyms are *Marmor solani*, Potato mild mosaic virus, Potato virus P and Solanum virus 3 (Bartels, 1971).

Morphology

Particles are flexuous filaments with a clear modal length of 730 nm long and 11 nm in diameter (Figure 5). Virions are not enveloped (Brandes & Paul, 1957).

Physicochemical and Physical Properties

- TIP = 44-52°C
- DEP = 1:10-1:40
- LIV = 0.5-0.75 days (12-18 hrs at 18°C. MacLachlan *et al.*, 1953)

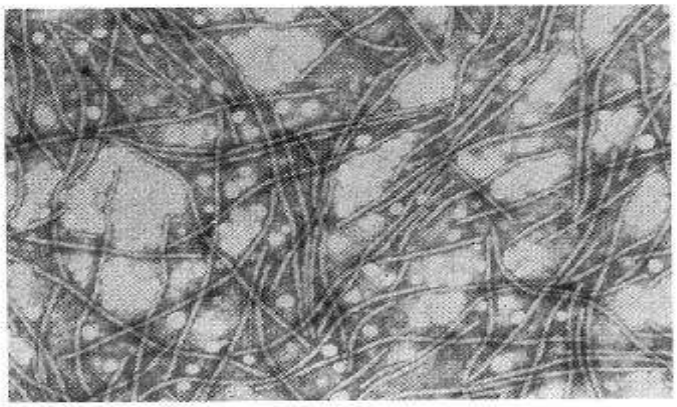

Figure 5. Virions of potatos virus A stained with 2% PTA

Nucleic Acid

Single stranded RNA.

Function of Helper and Satellite Viruses

Virions associated with helper virus, but independent from its functions during replication.

Cytopathology

Virions are found in cytoplasm of all parts of the host plant. Inclusions are present in infected cells.

Serological Properties

Purified preparations are weakly antigenic. Serologically related viruses are PVY (common and tobacco veinal necrosis strain), henbane mosaic and tobacco etch viruses (ICTV, 2000).

Transmission

Virus is sap transmissible and stylet-borne in a persistent manner by insect vectors belonging to the *Aphididae* including species: *Aphis fragulae*, *A. nasturii* and *Myzus persicae*. Helper virus is not required for transmission, but *M. persicae* can transmit potato aucuba mosaic virus when the source plant also contains potato virus A (Clinch *et al.*, 1936). Not transmitted by true potato seed.

Host Range

Other than solanum, many solanaceous hosts have been practically observed. Some of the local lesion hosts of PVA are *Datura* spp., *Nicotiana* spp., *Lycium* spp., *Lycopersicon* spp. and *Solanum* spp. Other hosts are listed in Table 3.

Table 3. Hosts of Potato Virus A

Host Plants	Symptoms
Natural Hosts	
Potato (*Solanum tuberosum*)	Mild mosaic, leaf surface rough with wavy margins, or symptomless.
Diagnostic Hosts	
Nicotiana tabacum cv. Samsun,	Systemic vein-clearing and diffuse mottling.
N. tabacum cv. White Burley	Systemic vein-clearing and dark green vein-banding.
Nicandra physalode	Systemic slight vein-clearing and mottle to severe necrosis, rugosity and stunting.
Lycopersicon pimpinellifolium	Systemic necrosis and death.
Solanum demissum, *S. tuberosum* cv. Aquila (=A6 hybrid), *S. demissum* SdA	Many local lesions.
Maintenance and Propagation Hosts	
Nicotiana tabacum cv. Samsun	
Assay Hosts	
Solanum demissum, *S. tuberosum* cv. Aquila (L)	Local lesions or whole plants.
S. demissum SdA (L)	Local lesions or whole plants.
Nicandra physalodes (W) is useful for aphid transmission tests with *Myzus persicae*.	Local lesions or whole plants.

Sources of host-range data: MacLachlan *et al.* (1953); Kohler (1953); Bartels (1970); Bartels (1971); Brunt *et al.* (1990) & ICTV (2000).

Detection Methods

Difficult to distinguish from PVY by symptoms in many test plants and EM. However, may be distinguished serologically and on test plant *Solanum demissum* SdA in which PVA induces local lesions

(Bartels, 1971). ELISA method is mostly used for routine large scale testing. Molecular methods can also be applied.

Control

- Roguing
- Use virus free seed potato
- All commonly cultivated potato varieties in Pakistan are susceptible to PVA, however, resistant varieties are available elsewhere.

Potato Virus M

Occurrence and Importance

This disease was first found in *Solanum tuberosum* in the USA (Schultz & Folsom, 1923; Brunt *et al.*, 1990). Probably it is distributed worldwide. In Pakistan, it is not of great importance. First report of its occurrence in traces was by Mughal *et al.* (1988). Potato virus M (PVM) was also recorded from Sindh, where its incidence was 10% and 6% in Ghotki and Babar, respectively (Ahmad, *et al.*, 1995d). Generally PVM occurs in low percentage (1%) in NWFP (Ahmad, *et al.*, 1995a), however, occasionally its high incidence is also reported in upper Kaghan valley (Jan & Khan, 1995).

Symptoms

Symptoms in potato, range from very slight to very severe and are influenced by virus strain, potato cultivar and environmental conditions. These are prominent in young plants and older plants may not show symptoms. An interveinal mosaic accompanied by clearing of the veins is a common symptom. Necrotic spots may develop on the upper leaf surface, accompanied by brown streaks on the veins of the under surface, petioles and stems.

Virus

Virus belongs to the genus *Carlavirus* with approved acronym PVM. Other synonyms are Kartoffel - K virus, Kartoffel - Rollmosaik virus, Potato paracrinkle virus, Potato interveinal mosaic virus, Potato leaf rolling mosaic virus, Potato virus E, Solanum virus 7, Solanum virus 11. Four strains are also reported i.e., Potato leaf rolling mosaic and interveinal mosaic, Paracrinkle, Dutch isolates and Fortuna isolates (Wetter, 1972).

Morphology

Virions are not enveloped. Particles are filamentous, usually straight to slightly curve, with a clear modal length of 650 nm long and 12 nm in diameter (Brandes *et al.*, 1959). Basic helix obscure.

Physicochemical and Physical Properties

- TIP = 65-71°C
- DEP = 10^{-2} - 10^{-3}
- LIV = Several days at 20 C

Nucleic Acid

Virions contain 6% nucleic acid and one molecule of single stranded RNA.

Genome Organization and Replication

The sequence of the 3′ proximal 2630 nucleotides of the genomic RNA has been determined; five ORF's have been recognised which encode polypeptides of *c.* 11000, 34000, 25000, 12000 and 7000 (ICTV, 2000).

Function of Helper and Satellite Viruses

Virions are associated with helper virus, but remain independent from their functions during replication.

Cytopathology

Virions are found in cytoplasm of all parts of the host plant. Inclusions, present in infected cells, are amorphous X-bodies and are of diagnostic values which can be seen using an optical microscope (Wetter, 1987).

Serological Properties

Virus is strongly antigenic and high-titre antiserum can be prepared. Virus(es) with serologically related particles are carnation latent, potato S, chrysanthemum B, passiflora latent, cactus 2 and red clover vein mosaic viruses, but distantly.

Transmission

PVM is transmitted in nature by insect vectors belonging to the *Aphididae* in a non-persistent manner by *Myzus persicae* (Wetter & Volker, 1960) but less efficiently *by Aphis frangulae, A. nasturtii* and *Macrosiphum euphorbiae* (ICTV, 2000). Some isolates are not aphid-transmitted (Kassanis, 1961). Non-vector transmission occurs by mechanical inoculation and by grafting. The virus is not transmitted by seed or pollen.

Host Range

N. debneyi, C. quinoa, Phaseolus vulgaris, D. stramonium, Vigna sinensis, Beta vulgaris, Lycopersicon esculentum. Other hosts are listed in Table 4.

Table 4. Hosts of Potato Virus M

Host Plants	Symptoms
Natural Hosts	
Potato (*Solanum tuberosum*)	Symptoms range from very slight (*e.g.* in cv. King Edward) to severe (*e.g.* in cv. Arran Victory). Causes mottles, mosaic, crinkling and abaxial rolling of leaves, and stunting of shoots.
Diagnostic Hosts	
Datura metel	Chlorotic or necrotic local lesions then systemic rugose chlorotic mottle, leaves abscissed, plants stunted and may die.
Gomphrena globosa	Chlorotic spots with reddish borders; not systemic.
Lycopersicon esculentum	Symptomless systemic infection.
Nicotiana debneyi	Irregular brown necrotic ring-like local lesions; not systemic.
Solanum rostratum	Systemic necrotic streaking of stem, petioles and leaf-veins.
Assay Hosts	
Datura metel (L)	Local lesions on whole plants.
Gomphrena globosa (L)	Local lesions on whole plants.
Phaseolus vulgaris cv. Red Kidney bean (L)	Local lesions on whole plants.
Other Susceptible Hosts	
Chenopodium album, C. amaranticolor, Cyamopsis tetragonoloba, Dianthus barbatus, Dianthus caryophyllus, Nicotiana occidentalis, Solanum demissum, S. demissum × S. tuberosum, S. melongena and *Vigna unguiculata.*	

Sources of host-range data: Wetter (1972); Brunt *et al.* (1990) & ICTV (2000).

Detection Methods

Potatoes are often infected with both potato viruses M and S; PVM can be freed from PVS by inoculating tomato, which is immune to PVS, or the potato cultivar Saco, which is very resistant to PVS and PVX.

ELISA kits and reagents are commercially available from a number of companies. Molecular methods are also used for detection.

Control

- Use virus free seed tubers
- Grow seed potato in aphid free environment
- Sanitation
- Infected potato can be freed from potato M, S and other viruses by apical meristem culture (Kassanis, 1957).

Potato Virus S

Occurrence and Importance

This virus was first found in *Solanum tuberosum* in the Netherlands (de Bruyn, 1952; Rozendaal, 1952) and probably is distributed worldwide. In Pakistan, the virus is reported from all potato growing areas (Anwar & Mirza, 1984; Ahmad *et al.,* 1995a-c). Yield losses upto 10-20% with an incidence of 2-12% were reported by Mughal *et al.* (1988). In recent years, its incidence have been on increase (Khalid, unpublished).

Symptoms

Generally symptoms on most cultivars are very mild. Typical symptoms are deepening of the veins on the upper side of the leaves, which may become rugose (Figure 6). Slight mottling and faint banding of veins may also be visible. Sensitive varieties turn brown and may develop necrotic spots. Symptoms are more conspicuous under cloudy weather. The only symptom in tuber is size reduction which may reduce yield by 10-20%.

Virus

Contact transmitted virus belongs to the genus *Carlavirus* with approved acronym PVS. Other synonym is Pepino latent virus (Jones *et al.*, 1980; Dolby & Jones, 1988). Different strains evoke similar symptoms in potato but differ in host range. In Netherlands, a virulent strain has occasionally been found that evokes bronzy necrosis in many cultivars, later accompanied by withering and dropping of lower and middle leaves (Wetter, 1971; de Bokx & van der Want, 1987).

Morphology

Virions not enveloped, filamentous, usually straight to slightly curved with a clear modal length of 650 nm long and 12 nm in diameter.

Physicochemical and Physical Properties

- TIP = 55-60°C
- DEP = 10^{-2} - 10^{-3}
- LIV = 3-4 days at 20°C

Figure 6. Mottling and deepening of veins caused by potato virus S

Proteins

One structural virion protein found.

Genome Organization and Replication

The sequence of the 3′ 3553 nucleotides of the genomic RNA has been determined; six ORF's recognised which encode for polypeptides, respectively of Mr 10734, 32515, 7222, 11803, 25092 and 41052 (ICTV, 2000).

Function of Helper and Satellite Viruses

Virions associated with helper virus, but independent from its functions during replication.

Serological Properties

The virus is fairly antigenic and high titre antiserum can be prepared. Virus(es) with serologically related virions are carnation latent, potato M, chrysanthemum B, passiflora latent, cactus 2 and red clover vein mosaic viruses, but distantly.

Transmission

It is tuber perpetuated. Transmitted in nature by an aphid vector, *Myzus persicae,* in a non-persistent manner. Isolates may differ in their transmissibility by *M. persicae* (Brunt *et al.*, 1990); an isolate from the U.S. seedling 41956 could not be transmitted under conditions in which the related potato virus M was transmitted (Wetter & Volker, 1960). Natural transmission occurs by leaf contact, machinery, animal and man. Mechanical inoculation is helpful in experimental/non-vector transmission, true potato seed transmission is not reported.

Host Range

Physalis philadelphica, *Datura metal* and *Solanum villosum* are symptomless hosts where as *Chenopodium album* is a weed host. Other hosts are listed in Table 5.

Detection Methods

In some potato cultivars PVS is found with the related PVM. PVS may be obtained from mixture by inoculating *Nicotiana debneyi* which is systemically infected by PVS, but only locally by PVM.

Table 5. Hosts of Potato Virus S

Host Plants	Symptoms
Natural Host	
Potato (*Solanum tuberosum*)	Causes few or no symptoms, but decreases yield of potato tubers by upto 20%.
Diagnostic & Assay Hosts	
Chenopodium amaranticolor, C. quinoa, C. album	Chlorotic local lesions which, in older leaves, may develop a green halo; not systemic.
Solanum rostratum	Small necrotic local lesions, systemic necrotic spots.
Nicotiana debneyi	Symptomless in inoculated leaves; systemic vein clearing and mottling and necrosis.
Cyamopsis tetragonoloba	Small brown necrotic local lesions in cotyledons, not systemic.
Maintenance and Propagation Host	
Nicotiana clevelandii	

Sources of host-range data: Wetter (1971); Burnt *et al.* (1990) & ICTV (2000).

ELISA kits and reagents are commercially available from many companies.

Control

- Use virus-free seed potatoes
- Sanitation
- Use resistant cultivars such as Saco which is an immune variety, while cultivars Loshitsaii, Kandidat and Ogonek are resistant. Non of these cultivars are cultivated in Pakistan
- Apical meristem culture can be used for virus elimination.

Potato Virus X

Occurrence and Importance

First found in *Solanum tuberosum* in the U.K. (Smith, 1931). The disease prevails in all potato growing areas of the world. Potential losses generally range between 10-25%.

In Pakistan, Potato virus X (PVX) is prevalent in all potato growing areas (Mughal & Khalid, 1985; Jan *et al.*, 1994; Ahmad & Ahmad, 1995). However, increase in its incidence in recent years has been noticed (Khalid, unpublished). Anwar & Mirza, (1984) mentioned PVX among the most important viruses in the Punjab. Apart from potato, PVX has been reported from tomato (Mughal, 1985; Irshad, 1991; Hassan *et al.*, 1993); tobacco (Mughal, *et al.*, 1986) and chilies (Hameed *et al.*, 1995). High-titre antiserum was produced against PVX and ELISA kit developed (Khalid, *et al.*, 1989).

Symptoms

Symptoms vary considerably with the cultivar, virus strain and the growing conditions and thus range from complete latency to severe mosaic. Many of the older cultivars were carrying the virus latently, hence PVX was also known as healthy potato virus. Leaves of infected plants show mottling, interveinal mosaic (Figure 7), mild mosaic and super mild mosaic. Infected plants with mild symptoms in the upper leaves may show typical symptoms in the older leaves shaded by the top ones. Some virulent strains cause rugosity and crinkling of the foliage. In chronic cases plants show dwarfing. Yield losses could be 10-25%, depending on the cultivar and virus strain (Rich, 1983). In combination with PVA or PVY may cause serious complex diseases.

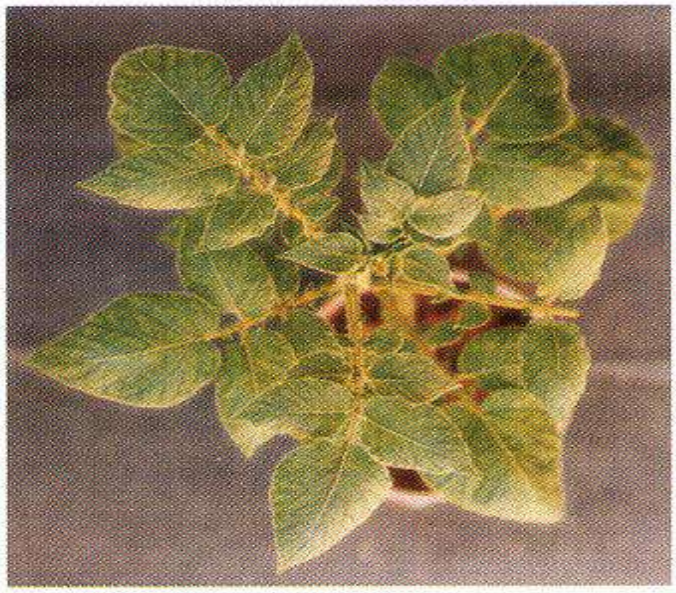

Figure 7. Mosaic symptoms in potato caused by PVX

Virus

Virus belongs to the genus *Potexvirus* with approved acronym PVX. Other synonyms are Potato latent virus, Potato mild mosaic virus and Solanum virus 1. Many minor strains can be distinguished, mainly by the symptoms they induce in tobacco. Strains fall into four groups on the basis of serology, into four groups on the basis of their infectivity for different potato varieties and into further three groups on the basis of thermal inactivation points (Bercks, 1970; Koenig & Lesemann, 1989). A turnip strain is also reported from India (Koenig & Lesemann, 1989; Samad *et al.*, 1991).

Morphology

The virions are flexuous filamentous (Figure 8), with a clear modal length 515 nm long and 13 nm in diameter (Brandes, 1964). Axial canal is obscure and 3.4 nm in diameter. Basic helix is obvious and its pitch is 3.4 nm (Varma *et al.*, 1968).

Figure 8. Potato virus X particles, stained with 2% PTA

Physicochemical and Physical Properties

Sediment as single component in purified preparations with sedimentation coefficient 117.7 *S*. Isoelectric point (pH) is 4.4. Infectivity retained when deproteinised with proteases, phenol or detergent.

- TIP = 68-76°C
- DEP = 10^{-5} - 10^{-6}
- LIV = Several weeks (40-60 days) at 20°C

Nucleic Acid

Virions contain 6% nucleic acid (Knight, 1963) and one molecule of linear single stranded RNA. Total genome length is 6435 nt.

Protein

Virions contain 94% protein. One structural virion protein with 30,000 Da is found (Shaw & Larson, 1962; Shaw *et al.*, 1962; Miki & Knight, 1968).

Function of Helper and Satellite Viruses

Virions are associated with helper virus, but independent from its functions during replication.

Cytopathology

The virions occur, mostly in large aggregates, in the cytoplasm and may fill the greater part of the cells. This is the only potexvirus known to induce the formation of cytoplasmic laminated inclusions component (LIC) in the cytoplasm. The function of LIC is not known and they are not related serologically to viral coat protein (Koenig & Lesemann, 1989).

Serological Properties

PVX has strong antigenic properties and antisera with high titre can be produced. Other related viruses are white clover mosaic, hydrangea ringspot, cactus X and clover yellow mosaic viruses, but distantly. A high titre antiserum to PVX has been produced by Khalid *et al.* (1989).

Transmission

Transmission in nature is without help of a vector. Virus is easily perpetuated through infected tubers. The other ways of easy transmission are infectious sap, field implements and the mechanical contact of roots or leaves but not by seed or pollen. This virus retains its infectivity on rubber, iron, human skin and unpainted wood. It could remain viable 6 hrs on painted wood, cotton and jute and in soil upto 24 hrs.

Host Range

It is very wide and distributed in 15 families. Test hosts are *Gomphrena globosa*, *Datura stramonium* and *Chenopodium amaranticolor*. *G. globosa* shows necrotic local lesions where as *D. stramonium* develops a systemic mottle. Other susceptible hosts are *Nicotiana tabacum, N. glutinosa, Solanum nigrum, Petunia* spp. and *Trifolium incarnatum*. Other hosts are listed in Table 6.

Table 6. Hosts of Potato Virus X

Host Plants	Symptoms
Natural Host	
Solanum tuberosum	Symptoms vary, some strains symptomless, others induce necrotic streaks.
Brassica campestris ssp. *rapa*	Mild mosaic mottling and distortion of leaves, plant stunting.
Diagnostic Hosts	
Datura stramonium	Systemic chlorotic rings, then mosaic and mottling.
Nicotiana tabacum	Systemic ringspot or mottle. Some strains symptomless at high glasshouse temperatures.
Maintenance and Propagation Host	
Nicotiana tabacum	
Assay Host	
Gomphrena globosa (L)	Local lesions or whole plants.

Sources of host-range data: Koenig & Lesemann (1989); Brunt *et al.* (1990) & ICTV (2000).

Detection Methods

ELISA method is mostly used for routine large scale testing. Leaf dip preparation generally contains many virions (Francki & McLean, 1968). Molecular methods can also be used.

Control

The virus is highly infectious and spread by mechnical contact, especially ôn agricultural and horticultural equipments. The most effective means of control is the use of resistance conferred by a major gene. Cultivars possessing some forms of resistance have been widely grown, however, a strain (HB) known to break all forms of resistance was identified from South America (Koenig & Lesemann, 1989). Progress has been made in engineering transgenic tobacco

against PVX by expressing the viral coat protein gene (Koenig & Lesemann, 1989). In Pakistan, all commercially grown potato varieties are susceptible to PVX, therefore, following measures can be helpful.

- Adaptation of sanitary measures
- Growing virus-free seed potatoes.

Potato Virus Y

Occurrence and Importance

First found in *Solanum tuberosum* in the U.K. (Smith, 1931). Probably distributed worldwide and in outdoor crops of pepper, tobacco and tomato in warmer countries. It is also known as severe mosaic virus. Losses range from 58-83%. Alongwith PLRV it is the most important virus of potato in Pakistan. The virus was first isolated by Mirza (1978) and was among the most important diseases of potato in the plains of Punjab (Anwar & Mirza, 1984). Losses in Pakistan are estimated from 40-70% (Mughal & Khalid, 1985).

In Pakistan, this virus is distributed throughout the country with an incidence of 2-25% (Mughal *et al.*, 1988). Other natural hosts of Potato virus Y (PVY) in Pakistan are tobacco (Mughal *et al.*, 1986; Hameed *et al.*, 1991), tomato (Mughal, 1985; Irshad, 1991; Hassan, 1995) and chili (Hameed *et al.*, 1995). An antiserum to PVY with high titre has been produced (Khalid, unpublished).

Symptoms

Current season symptoms are characterized by the development of brown necrotic streaks along the veins of the leaves, the petioles and the stems. In severe cases the leaves and petioles die and hang downward from the stems (Figure 9). Diseased plants show chlorotic mottling, severe rugose, wrinkling, dwarfing and frequently premature death of plants. Infected tubers are always smaller than normal. Necrosis is usually most severe after primary than secondary infection which results in dwarfing and bunchy appearance and rugosity of plants. The differences between the primary

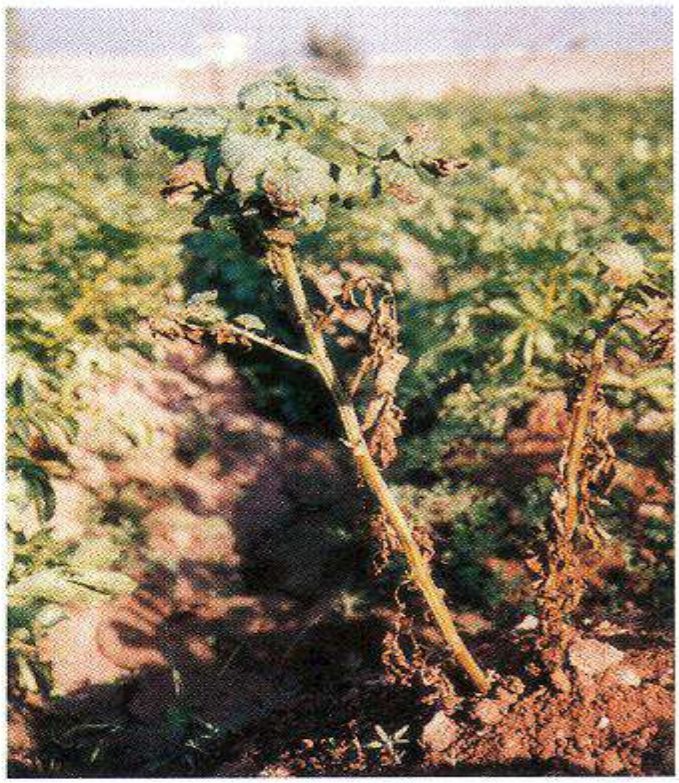

Figure 9. Necrosis and dropping of leaves caused by potato virus Y° strain

and secondary symptoms induced by PVY are often uncertain because of the diversity of potato cultivars, virus strains and effect of climatic conditions (de Bokx & Pirone, 1977). Generally, a variety of symptoms may develop varying from light green, mottling, rugosity of leaves to severe necrosis, curling of leaves to dwarfing of plants, depending on the potato cultivar involved.

Virus

Virus belongs to the genus *Potyvirus* family *Potyviridae* with approved acronym PVY. Other synonyms are Potato acropetal necrosis virus, Potato severe mosaic virus, Tobacco vein-banding virus (Brunt *et al.*, 1990), Solanum virus 2, Marmor upsilon brinjal mosaic virus (Varma, 1988; Brunt *et al.*, 1990), Datura 437 virus.

Five strains of PVY are reported i.e., Potato virus Y° group (common strain-PVY°, spread worldwide), Potato virus Y^{N} group (tobacco veinal necrosis strain-PVY^{N}, occurs in Europe including the USSR, parts of Africa and South America), Potato virus Y^{C} group (stipple-streak strain including potato virus C), probably present in Australia, India and some parts of the U.K. and continental Europe. Potato virus C itself is not transmitted by the aphid *M. persicae* (Watson, 1956) but other strains of this group are (Brunt *et al.*, 1990). In Pakistan, PVY° is the most prevalent strain (Khalid, unpublished).

Morphology

Virions are not enveloped. Particles are flexuous filamentous (Figure 10) with a clear modal length of 684 nm long, from purified preparation (Delgado-Sanchez & Grogan, 1966), or 730 nm long and 11 nm in diameter. Axial canal is obscure and 2-3 nm in diameter. Basic helix is obscure and its pitch is 3.3 nm (Varma *et al.*, 1968).

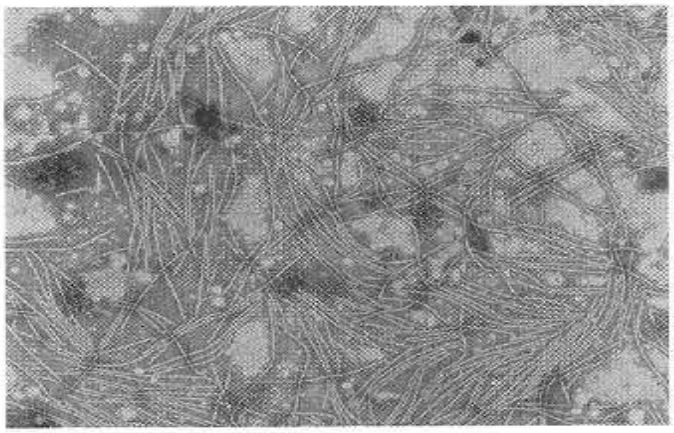

Figure 10. Particles of potato virus Y, stained with 2% PTA

Physicochemical and Physical Properties

Buoyant density 1.323 g cm^{-3} in CsCl (strain Y°), or 1.326 g cm^{-3} in CsCl [strain Y^{N}]. Sediment as one component in purified preparations

with coefficient 145 *S*. A260/A280 ratio 2.3 [corrected for light-scattering (Leiser & Richter, 1978)], or 2.9 uncorrected (Stace-Smith & Tremaine, 1970; Leiser & Richter, 1978). Infectivity of sap is not changed by treatment with di-ethyl ether. Infectivity is retained when deproteinised with phenol or detergent (ICTV, 2000).

- TIP = 50-62°C for 10 min
- DEP = 10^{-2}-10^{-3}
- LIV = 48-72 hrs (7-50 days)

Nucleic Acid

Virions contain 5.4-6.4% nucleic acid (Stace-Smith & Tremaine, 1970; Leiser & Richter, 1978). Virions contain one molecule of linear single stranded RNA. Complete nucleotide sequence has been determined (Brunt *et al.*, 1990).

Proteins

Virions contain 93.6-94.6% protein. Viral coat protein migrates as two bands in SDS-polyacrylamide gel. The M.Wt of the slower migrating polypeptide is 33000-34000 Da (Hiebert & McDonald, 1973; Huttinga & Mosch, 1974; Mughal & Francki, 1976). Amino acid composition has been determined by Stace-Smith & Tremaine (1970); Miki & Oshima (1972); Makkouk & Gumpf (1975); Mughal & Francki (1976).

Function of Helper and Satellite Viruses

Virions are associated with helper virus, but independent from its functions during replication.

Cytopathology

Particles are found in epidermis; in cytoplasm and in cell vacuoles. Inclusions present in infected cells. Inclusions are crystals in the nucleus, when infected by certain strains (Kitajima *et al.*, 1968), or are pinwheels which occur mainly in epidermal tissue in the cytoplasm (Brunt *et al.*, 1990). Other cellular changes found, include: mitochondria often being surrounded by filaments with a diameter of 9-10 nm but of indeterminate length (Brunt *et al.*, 1990) when infected with strain Y^N in *Datura metel*.

Serological Properties

Virus is good immunogenic and good quality high titre antisera can be obtained. Viruses with serologically related virions are tobacco etch, henbane mosaic, PVA, pepper veinal mottle and bidens mottle viruses, but distantly (Bartels, 1964; Brunt *et al.*, 1978). In Pakistan, an antiserum to PVY° strain with 1:256 titre in gel diffusion test has been prepared (Khalid, unpublished).

Transmission

PVY is transmitted by sap inoculation, by stem and core grafting and atleast by 25 species of aphids in a non-persistent manner. *Myzus persicae* is the most efficient vector transmitting the virus; others are *Aphis fabae, Macrosiphum euphorbiae, M. certus, Phoroden humuli and Rhopalosiphum insertum* (Kennedy *et al.*, 1962; Van Hoof, 1980). Helper component is required for transmission. Other means of transmission are; mechanically, by grafting, through tubers and a mite spp. *Tetranychus telarius* is also known for transmitting the virus (Schultz, 1963).

Host Range

It is wide including: *Nicotiana tabacum, N. glutinosa, S. nigrum, Lycopersicon esculentum, Petunia* spp. and *Chenopodium amaranticolor.* Diagnostic test plant is *Physalis floridana. C. amaranticolor* is the best local lesion plant. *N. tabaccum* shows systemic infection of vein clearing and vein banding. Other hosts included are *Dahlia* spp., *Physalis* spp. and *Capsicum frutescens.* Similarly many species which fall in eight different families are affected. Other hosts are listed in Table 7.

Detection Methods

Best tests for diagnosis: *Tinantia erecta* is immune to potato aucuba mosaic virus, PVM and PVS and hence may be used to separate PVY from these viruses (Brunt *et al.*, 1990). Most strains of PVY induce no local lesions in *Solanum demissum* 'A', which gives necrotic local lesions with PVA (Cockerham, 1958). Potato cultivars resistant to PVX (*e.g.* cv. Saco), can be used to separate PVY from PVX. Detached leaves of *Solanum demissum* × *S. tuberosum* `A6' are commonly used to detect PVY, but this host does not distinguish between several viruses which infect it (Bartels, 1970).

Table 7. Hosts of Potato Virus Y

Host Plants	Symptoms
Natural Hosts	
Potato (*Solanum tuberosum*)	Mild to severe leaf mottling, or streak or `leaf-drop streak' with vein necrosis (`stipple-streak').
Capsicum ssp.	Mild leaf mottling, but severe in complex with other viruses.
Nicotiana ssp.	Mild mottle or veinal necrosis.
Lycopersicon esculentum	Mild leaf mottling but severe in mixed infections.
Diagnostic Hosts	
Nicotiana glutinosa	Mild to severe systemic mottling.
N. tabacum	Systemic vein-clearing and leaf mottling.
Solanum tuberosum	Y^c strain local lesions in cv. Duke of York (Eersteling); Y^N and Y^O strains no local lesions.
Tinantia erecta	Severe systemic mottling.
Diagnostic Insusceptible Hosts	
Datura stramonium	
Solanum demissum A	
Maintenance and Propagation Host	
N. tabacum cv. Samsun NN	
Assay Hosts	
Chenopodium amaranticolor (L)	Local lesions or whole plant.
C. quinoa (L)	Local lesions or whole plant.
Physalis floridana (L)	Local lesions or whole plant.
Solanum tuberosum cvs Duke of York, Saco (L)	Local lesions or whole plant.
Lycium ssp. (L)	Local lesions or whole plant.
Solanum chacoense (TE1) (L)	Local lesions or whole plant.
S. demissum `Y' (L)	Local lesions or whole plant.
S. demissum × *S tuberosum* 'A6'(L)	Local lesions or whole plant.

Sources of host-range data: Beemster & Rozendaal (1972); Edwardson (1974); de Bokx & Huttinga (1981); Brunt *et al.* (1990) & ICTV (2000).

ELISA kits are commercially available and are routinely used for detection/indexing. All molecular methods can also be applied. In electron microscopic studies, leaf sap contains few virions. SSEM can be useful in mixed infection.

Control

Use of insecticides to control virus spread through vectors has been ineffective. The main control methods are:

- Sowing of virus-free seed potatoes.
- Avoidance of infection, *i.e.* growing crops when vectors are absent or numbers are low.
- Not growing crops near established crops of the same species
- Destroying haulms of seed-potato crops before maturity to restrict virus spread at the end of the growing season.
- Spraying mineral oils can help in reducing frequency of transmission.
- Use of reflective surface and sticky yellow sheets which can reduce virus spread.
- Early roguing can be beneficial.
- Remove all potato plants after harvesting as these (ground-keepers) can be the reservoir of PVY.
- Keep field clean from weeds, especially belonging to *Solanaceous* family, they can act as important virus sources.
- Known resistant cultivars are Avon, Katahdin, Kennebec, Monona, Nordak, Norgleam, Oromonte, Saco, Snowflake, York Caribe and Russette have also shown resistance. However, all currently grown potato cultivars in Pakistan are susceptible to PVY.

Phytoplasma

Occurrence and Importance

Phytoplasma, previously known mycoplasma like organism (MLO), are associated with and are believed to be the causative agent of many plant diseases including potato. They were considered to be the viruses with unusual characteristics. Plants showing yellowing, phyllody, witches broom and dwarfing were shown to contain MLO in phloem tissue (Doi *et al.*, 1967). Many phytoplasma-like diseases are reported in potato (Table 8), however, three possible groups i.e. Aster Yellow, Witches broom and Stolbur are more common.

Table 8. Phytoplasma diseases of potato

Disease	Yield loss (%)	Main vector(s)	Tuber transmi-ssion (%)
Purple top roll (PTR)	50-75	*Alebroides dravidanus* *Orosius albicinctus* *Seriana equata*	20-70
Purple top wilt (PTW)	<10	*Macrosteles fascifrons* *Elymana virescence*	3-8
Marginal flavescence (MF)	50-90	*O. albicinctus* *S. equata*	0-65
Witches' broom (WB)	15-50	*A. dravidanus* *O. albicinctus* *S. Ophiola flavopicta*	50-100
Potato phyllody (PP)	Upto 65	-	50-100
Stolbur	10-80	*Hyalesthes absoletus* *Aphrodes bicinctus* *Euscelis plebejus*	-

- *Data not available*

Source: Salazar, L.F., 1996. Potato Viruses and their Control.

Generally these diseases are of minor importance, however, some times they can significantly reduce the yield. Purple top roll, marginal flavescence and witches broom causing yield reduction ranging from 50-75, 50-95 and 15-65%, respectively, has been reported from India (Nagaich *et al.*, 1982).

In Pakistan, Mycoplasma-like symptoms in potato were first observed in early 90s (Khalid & Mughal - unpublished) in the district of Shahiwal. Latar similar symptoms were also recorded by Shafiq *et al.* (1995a) in autumn crop in the Punjab. They were of two types:

a) Infected plants has long and thin stems, having swollen nodes, lush green foliage with slight effect of late blight and frost, numerous tubers with deep eyes.

b) Plants showing stunted growth with purple discoloration of leaves with numerous small size tubers.

The incidence of type *a* was higher in Sahiwal (0-80%), while that of *b* was higher in Okara (0-95%). In an other experiment, significant yield reduction was recorded (Shafiq *et al.*, 1995b). Recently (May, 2000), potato plants showing identical symptoms were also noticed in Mansehra area (unpublished) and in tomato (Aug. 2000) from Sawat Valley (Khalid, unpublished). Although no studies have been carried out on phytoplasma diseases of potato and other crops in Pakistan but type of symptoms observed in potato suggests that witches broom and purple top are present in Pakistan.

Symptoms

Generally infected plants show chlorosis along the margins of leaflets. Tubers from these plants are small, malformed and produce hairy sprout. Young infected plants produce aerial tubers (Figure 11) and swollen stem nodes (Figure 12). In case of witches broom infection, plants are severely stunted and produce many axillary and basal branches giving bushy appearance to the plant (Figure 13) and producing many small tubers (Figure 14).

Figure 11. Aerial tubers induced by phytoplasma infection

Pathogen

Phytoplasmas are small pleomorphic (having various shapes: spherical, filamentous, spiral or amoeboid) organisms lacking true cell wall, and are bounded by a single triple-layered membrane with a diameter upto 1000 nm. Phytoplasma bodies contain a fibrillar network of strands, assumed to be DNA and areas showing ribosomal-like granules. Their propagation seems to be by binary fission, budding or fragmentation (Hooker, 1981). They are found in the phloem sieve cells and occasionally in the phloem parenchyma cells of infected plants.

Figure 12. Swelling of stem nodes induced by phytoplasma

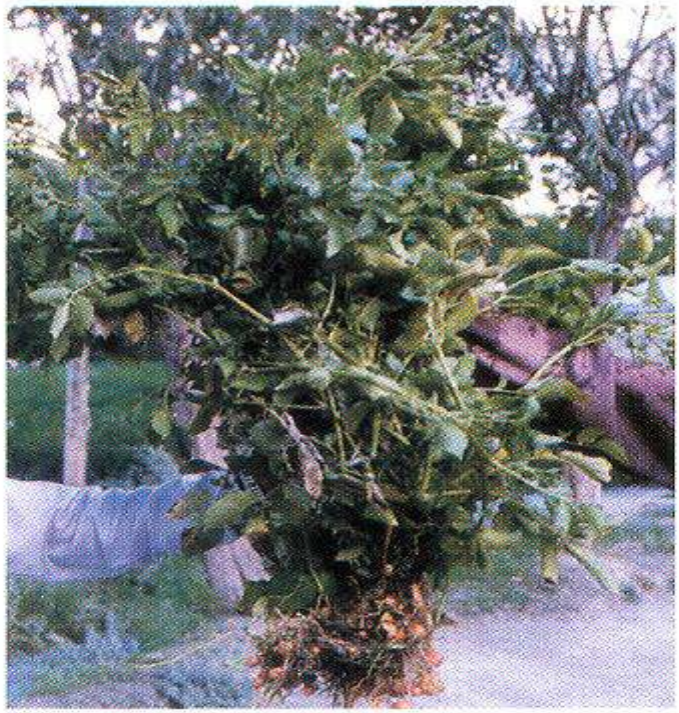

Figure 13. Witches broom symptoms caused by phytoplasma

Transmission

They can not be transmitted by sap inoculation or cultured *in virto*, but are graft transmissible. In nature they are transmitted and spreaded mainly through leafhoppers, in which they also propagate. Leafhoppers do not complete life cycles on potato. Neither nymphs nor adults can acquire phytoplasma from potato. They acquire it from other infected hosts and then transmit them to potato, while feeding on them.

Host Range

Although phytoplasmas have never been a major problem in potato, but they have many vegetables, ornamental field crops and weeds as susceptible hosts.

Control

- Experimentally they can be controlled by using antibiotics of tetracycline group.
- Aureomycine @ 200 mg/ suppressed symptoms of aster yellows.
- Heat therapy can be used to cure infected plants, but is not practical.
- Clonal selection combined with control of vector is more useful.
- Leafhopper can not acquire phytoplasma from infected potato, therefore, they can be controlled by killing the hosts such as convolvulaceous weeds, grasses and small grains.
- Change the planting date; after the migration of leaf hopper ends.

Figure 14. Numerous small tubers produced by phytoplasma infection

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DISEASES CAUSED BY BACTERIA

DISEASES CAUSED BY BACTERIA

Introduction

Bacteria are prokaryotic microorganisms that occur nearly everywhere in nature. About 1600 bacterial species are known, out of which 80 species have been found to cause diseases in plants.

Bacteria may be rod-shaped (bacillus), spherical (coccus), ellipsoidal, or filamentous. Almost all plant pathogenic bacteria are rod-shaped, the only exception being *Streptomyces*, which is filamentous. Depending on the shape their size vary between 0.5-1.0 µm in diameter in cocci, 0.5-1.0 µm wide and 2-3 µm in length in bacillus forms; however, some filamentous forms may exceed 100 µm in length. Most plant-pathogenic bacteria have flagella and can move through liquid medium. The cell walls of most species are enveloped with a viscous, gummy material, forming a relatively large mass around the cell called capsule (Pelezar, *et al.*, 1977).

Bacteria multiply asexually (binary fission) with astonishing rapidity, under favorable conditions it may divide every 20 minutes, one bacteria becoming two, two becoming four and so forth, reaching one million in 10 hours. Hence, significance of bacteria as pathogens stems primarily from the fact that they can produce tremendous numbers of cells in a short period of time that causes changes in the environment and hence develop disease overnight.

Majority of plant-pathogenic bacteria are parasitic in nature and develop in the host plant and partly in plant debris or in the soil as saprophytes. However, the degree of their development in one environment or the other varies, e.g. *Erwinia amylovora*, which cause fire blight of pear and apple, produces their population in the host plant and their number in the soil is insignificant, where as *Ralstonia* (*Pseudomonas*) *solanacearum*, which causes the bacterial wilt of solanaceous crops are rather typical soil inhabiting, these bacteria multiply in the host and are gradually released into the soil. If susceptible host is grown repeatedly in such soils, sufficiently high number of bacteria are released to cause a net increase of bacterial populations in the soil from season to season. In nature dissemination of plant pathogenic bacteria is primarily through water, insects, animals and humans.

Most common symptoms produced in plants are leaf spots, blights, soft rots of fruit, root and storage organs, wilts, overgrowths, scabs and cankers.

Control of bacterial diseases is often very difficult; usually a combination of control measures is required to combat specific bacteria. Use of healthy disease free seeds, sanitation aimed at reducing inoculum in soil, adjustment of watering and fertilizing regimes helps in checking infection chances. The use of resistant crop varieties is best and preferred choices of avoiding heavy losses caused by bacterial diseases. Frequently used chemicals as foliar sprays include copper compounds, Bordeaux mixture, fixed coppers and Kocide. Zineb is used for spray on young plants, which may be injured by the copper compounds. In certain cases antibiotics and biological control has been successful by treating seeds of nursery stock, tubers, seeds etc. with antagonistic bacteria. Resistant varieties, supplemented with proper cultural practices and chemical applications, are the most effective means of controlling bacterial diseases, especially when environmental conditions favor the development of disease (Agrios, 1988).

The important bacterial diseases of potato (bacterial wilt, black leg, soft rot and common scab) in Pakistan will be discussed in detail. Less important bacterial diseases of potato are Ring rot (*Corynebacterium sepedonicum*) and Pink eye (*Pseudomonas fluorescens*). Their incidence, mode of perpetuation and general control measures are given in table 9, 10 and 11, respectively.

Table 1. Incidence of Bacterial Diseases in Pakistan

S#	Disease	Plain Crops		Hill Crops
		Autumn	Spring	
1.	Bacterial Wilt [*Ralstonia (Pseudomonas) solanacearum*]	*	*	*
2.	Black Leg *(Erwinia carotovora ssp. carotovora & E. carotovora ssp. atroseptica)*	*	*	*
3.	Common Scab *(Streptomyces scabies)*	**	**	**

* traces **medium ***severe
(Data based on visual observation)

Table 2. Mode of Perpetuation and Plant Parts Affected by Bacteria

S#	Disease	Modes of Infection			Plant Parts Affected				
		Plant debris	Diseased seed tubers	Infested soil	Leaves	Stems	Stolen	Tubers	Roots
1.	Bacterial Wilt [*Ralstonia (Pseudomonas) solanacearum*]	+	+	+	-	+	+	+	-
2.	Black Leg *(Erwinia carotovora ssp. carotovora & E. carotovora ssp. Atroseptica)*	+	+	+	-	+	+	+	+
3.	Common Scab *(Streptomyces scabies)*	+	+	+	-	-	-	+	-

+ = Yes - = No

Table 3. Recommended Control Measures

Preventive Measures

(a) Use of healthy seeds tubers.
(b) Cultivation in un-infested fields.
(c) Destruction of diseased plant debris.
(d) Cultural practices to stop spread.
(e) Long rotations.
(f) Destruction of host plants etc.

Curative Measures

(g) Dis-infection of seed tubers.
(h) Spray.

Diseases	a	b	c	d	e	f	g	h
Bacterial wilt [*Ralstonia (Pseudomonas) solanacearum*]	+	+	+	+	+	+	-	-
Black Leg *(Erwinia carotovora ssp. carotovora & E. carotovora ssp. atroseptica)*	+	-	+	+	+	+	+	-
Common Scab *(Streptomyces scabies)*	+	+	+	+	+	+	+	-

+ = Yes - = No

Bacterial Wilt [*Ralstonia (Pseudomonas) solanacearum*]

Occurrence and Importance

Bacterial wilt, also known as brown rot of potato, is most common disease in warm-temperate, semitropical and tropical areas of the world. In Pakistan, the disease was first reported in 1968 from Sindh (Kamal & Mughal, 1968). Later in 1980s, the disease with low incidence (0.1-0.5%) was reported from Punjab and NWFP. The disease incidence ranged from 3.6-6% in the districts of Jhang, Faisalabad, Sialkot and Lahore, reducing yield by about 4.3-5.7% (Bhatti, *et al.*, 1984). In NWFP the disease was reported from the district of Swat in 1984 (Khan, *et al.*, 1985a). Later it was reported from Kaghan, Kalam and Gilgit but with low incidence (Turkensteen, 1986). Disease introduction in the hilly areas was suspected through seed potatoes from plains (Zanoni, 1991).

Symptoms

Plants start wilting at any stage of growth. Wilting, stunting and yellowing of the leaves are the main symptoms. These resemble the symptoms produced under water stress conditions and those produced by *Rhizoctonia* or *Fusarium* spp. However, wilting of one branch at initial stage is characteristic of bacterial wilt, followed by wilting of the entire plant (Figure 15). Wilted leaves may fade to a pale green and finally turn brown without rolling of the leaflet edges as they dry.

Figure 15. Bacterial wilt: Plant infected with *Ralstonia (Pseudomonas) solanacearum*

Underground stem, stolon and roots also show symptoms in advance stage. Tubers may or may not show symptoms. Cross section of infected tubers show distinct, grayish brown vascular discoloration that may extend into the pith or cortex from the xylem tissues (Figure 16).

Causal Organism

Brown rot or bacterial wilt is caused by *Ralstonia (Pseudomonas) solanacearum*. It is non-spore forming non-capsulate, gram negative, nitrate-reducing, ammonia forming, aerobic, rod shaped bacterium. In liquid media, the wild type is non-motile. In culture, avirulent is flagellate. Pathogen has different races or biotypes. Preliminary studies indicated the occurrence of race-1, having wide host range in solanaceous crops and is more adaptable to warmer areas and lower elevations (Hafiz, 1986).

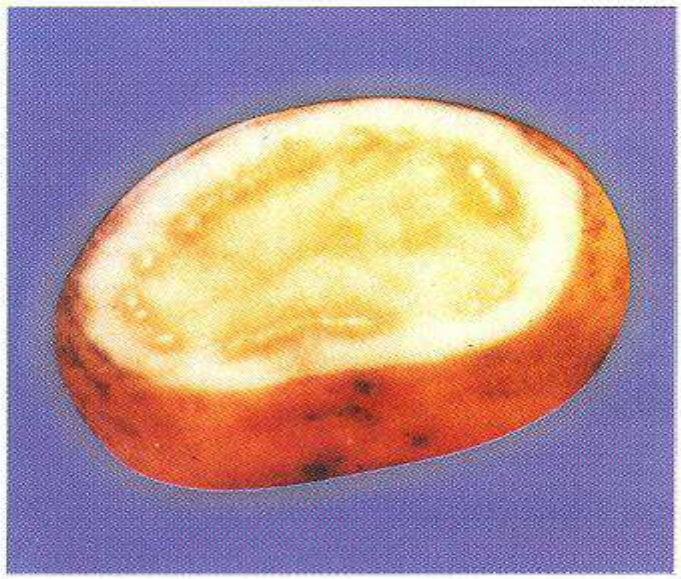

Figure 16. Transverse section of tuber infected with *R. solanacearum* showing bacterial ooze in vascular ring

R. solanacearum looses its virulence and viability in unaerated liquid media. Colonies of virulent wild types are irregularly round and fluidal white with pink center, whereas, colonies of avirulent variants are uniformly round, butyrous and deep red (Figure 17).

Figure 17. Virulent and avirulent colonies of *R. solanacearum* on TZC medium

Disease Cycle

Pathogen survives in infected seed tubers and in infested soil. Under favorable (warm and humid) conditions, it causes infection through wounds on roots that may occur during cultural operations. After penetration, it multiplies and spread throughout the xylem vessels of stem and petioles, thus blocking their water supply and causing wilting.

Epidemiology

Infected seed potatoes are an important source of inoculum. Disease development is mainly dependent on temperature and humidity but pathogen can also survive and infect potato crop at relatively low temperature. Disease spreads by irrigation, water and insects or by use of soil contaminated implements. The disease occurs in soil types ranging from sandy to heavy clay and over a wide range of soil pH. It usually develops in localized areas with poor drainage.

Other Hosts

Banana, tomato, eggplant, pepper, tobacco, wide range of weeds and ornamental plants are among the hosts of *R. solanacearum*.

Detection Methods

i. Visual observation:
   - Initially, only one branch of the infected plant shows wilting (see Fig. 15).
   - If the cut surfaces of a sectioned infected stem is placed in contact and then drawn apart slowly, fine strands of bacterial slime become visible and stretch a short distance before breaking.
   - Sharply cut infected stem suspend in a beaker of clear water and keep undisturbed. Within a few minutes milky white thread like bacterial mass oozes out from the cut end of the stem.
   - When tubers are cut in half and pressed, grayish white, shinny droplets of bacterial slime ooze out of the vascular ring (see Figure 16).

ii. Microscopic observation:
   - Bacterial streaming can be seen microscopically in thin section of infected tissue mounted in water under a cover slip.

iii. Isolation on specific medium.

Specific media

Tetrazolium Medium (TTC or TZC) (Kelman, 1954).

Peptone	10.0 g
Casein Hydrolysate	1.0 g
Glucose	5.0 g
Agar	17.0 g
Distilled Water	1000 ml
Tetrazolium Chloride (Aqueous solution)	2.5 ml of 1%

iv. Gram staining technique (Fox, 1993).
v. Indirect immuno fluorescence antibody staining (IFAS) (OEPP/EPPO, 1990).
vi. Post-enrichment enzyme-linked immunosorbent assay (ELISA) on nitrocellulose membrane (NCM-ELISA) (Priou *et al.*, 1999) for detection of latent infection in potato tubers.
vii. Nucleic acid-based method (Hybridization) (Fox, 1993).

Control

- Follow strictly quarantine measures to eliminate the seed-borne inoculum.
- Disinfect the knives before and after cutting the tubers.
- Manage the crop by good tillage; growth and minimal injury.
- Expose soil to high temperature in hot weather by leaving it fallow.
- Follow crop rotation with rice for disease management (Malik, 1995).

Black Leg and Soft Rot (*Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica*)

Occurrence and Importance

Black leg and bacterial soft rot had minor importance in Pakistan. On the basis of field symptoms, the disease was reported in 1984 from Swat valley (Khan, *et al.*, 1985a). From hilly areas and plains of Punjab, it was reported in 1985 and 1986, respectively by Turkensteen (1986 & 1987). The disease was more frequent in the districts of Sialkot, Gujranwala and Faisalabad with an incidence ranging between 0.2-2.9% in Desiree, Ultimus, Multa, Patrons and Cardinal varieties (Hafiz, 1986).

Bacterial soft rot causes severe economic loss by reducing quantities of produce available for sale. Disease also reduces germination of seed potatoes.

Symptoms

The disease can appear at any stage of plant development. Black leg affects stem and produces soft rot in tubers. When plant becomes infected from the soil, it leads to wilting and death of the above ground parts. Shoots from infected potato tubers are stunted and often appear blackened at the base, known as black leg (Figure 18). Tubers rot may develop in the field, storage or after planting. Infection occurs through lenticels and wounds or through the stolon end of the tuber. Lesions associated with lenticels appear as slightly sunken, tan to brown, circular water soaked areas (Figure 19). During dry environment they become hard and dry. Whereas,

Figure 18. Black leg: *Erwinia carotovora* spp. *atroseptica* infection on underground part of the stem

the lesions associated with injuries are irregular in shape, sunken and dark brown. Rotting tissue is usually odorless in the early stages of decay but develops a foul odor and a slimy or ropy consistency, as secondary organisms get involved.

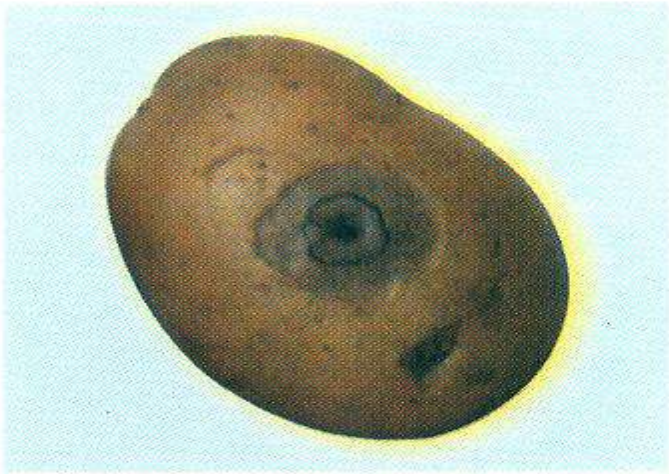

Figure 19. Bacterial soft rot symptoms on tuber having sunken, brown, circular water soaked lesions

Causal Organism

Erwinia carotovora ssp. *Carotovora (Ecc)* and *E. carotovora* ssp. *atroseptica (Eca)* cause basal stem rot known as blackleg and soft rot in tubers. The bacteria are rod shaped, gram negative, motile and opalescent, approximately 0.7×1.5 µm in size. They are non-spore forming and are facultatively anaerobic.

Disease Cycle

Soil, seed tubers and plant debris are the primary source of inoculum. Tools and field implements can also spread the pathogen. The bacteria cannot directly attack plants as they can only enter through wounds, bruises, cracks and damages by insects. The presence of abundant moisture around the wounded areas is necessary for the development and spread of infection. Bacteria multiply and break down the surrounding tissues. The slimy mass, produced in decayed tissues, contains numerous bacteria, which can cause re-infection upon contact with wounded portion (illustrated life cycle of the pathogen is given in Figure 20).

Epidemiology

Pathogen survives in cool (lower than 18-19°C) and wet conditions. Generally more frequent in northern production areas. It survives for 80-110 days at 2°C but for shorter times at higher temperatures. Bacteria may be present in the soil or in decaying plant refuse, which is responsible for the seasonal carry over of the disease. It is also found in storehouses. Cool wet soils at planting time followed by higher temperature favor post emergence blackleg symptoms. Higher

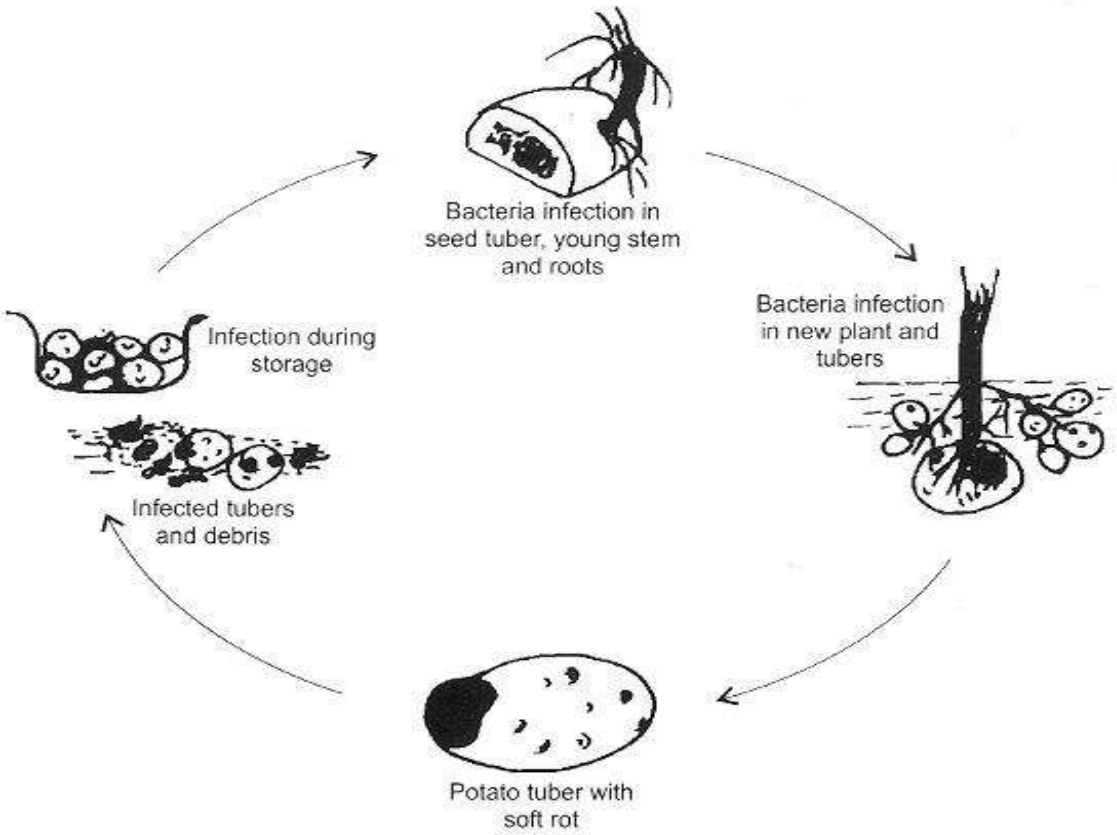

Figure 20. Disease cycle of bacterial soft rot caused by *Erwinia* spp.
(Adapted from G.N. Agrios)

soil temperature favor seed decay and pre emergence death of shoots. Greater losses due to blackleg occur in warm areas than in cooler ones. Optimum temperature for decay is 25-30°C, which prevails after summer crop harvesting in hilly areas and autumn crop in plains.

Other Hosts

Other hosts are vegetables (sweet potato, tomato, onion, carrot, turnip, radish, peas) and fruits (mango, pomes and stone fruits).

Detection Methods

i. Visual observation:
Observe swollen, circular, brown, water soaked lesions on tubers (see Figure 19).

ii. Isolation on specific medium (Tuite, 1969).

Specific media

Simmons citrate agar	23.0 g
Calcium chloride	3.0 g
Bile salt	5.0 g

Crystal violet	0.001 g
Pectate solution	4 ml
Distilled water	1 lit

iii. Gram staining technique (Fox, 1993).
iv. Transmission electron microscopy-TEM (Fox, 1993).
v. DAS-ELISA (Helias, *et al.*, 2000).
vi. PCR (Helias, *et al.*, 2000).

Control

- Storehouses should be cleaned and fumigated and provided with adequate ventilation to prevent humidity, which can provide suitable conditions to soft rot.
- Try to minimize the mechanical injuries, wounds and bruises to tubers to reduce the degree of infection.
- Infected plants and plant debris of potatoes should be removed from the field to avoid the spread of disease.
- Avoid over irrigation and water logging.
- Allow potato tubers to fully mature before harvest to minimize the disease.
- Avoid harvesting during warm rainy season.
- Use resistant to tolerant varieties e.g. Desiree (moderately resistant) (Helias, *et al.*, 2000) to minimize the disease losses.
- Follow crop rotation with other crops such as cereals, corn or legumes.

Common Scab (*Streptomyces scabies*)

Occurrence and Importance

Common scab was first observed in Swat valley, with maximum incidence in Gujar Gabral (Khan *et al.*, 1985a). Now it is present in almost all potato growing zones of the country. It is more severe in the areas where potatoes are monocultured. Scabby-tubers affect the grade quality, reduce the market value of the crop but have little effect on total yield and storing.

Symptoms

Pathogen mainly affects the tuber and produces two types of scab i.e. shallow or superficial and deep-pitted. Shallow scab may consist of superficial cork like layer (russet scab) or an extension into the tuber (raised scab) 1-2 mm high (Figure 21). Whereas, pitted scab has depth up to 7 mm and dark brown in color. Tissue under the lesion is straw colored and somewhat translucent. In russet scab such tissue may not be evident, brown to tan stem and stolon lesion originates at lenticels as elongate lens-shaped lesions or at natural wounds as approximately circular lesions.

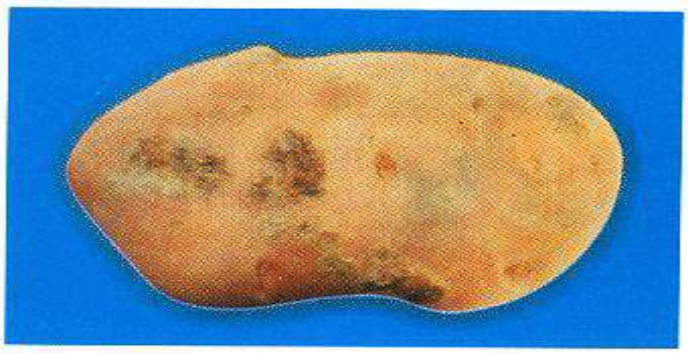

Figure 21. Common scab *(Streptomyces scabies)* infection of russet type on tuber surface

Causal Organism

Streptomyces scabies (Thaxter) Waksman & Henrici [syn. *Actinomyces scabies* (Thaxter) Güssow] is gram-positive, filamentous bacteria belonging to the genus Streptomyces. It has barrel-shaped conidia 0.8-1.7 × 0.5-0.8 µm. Conidiophore is branched, having septa with long, spirally coiled terminal branches. When the cross wall constrict, spores are pinched off at the tip and eventually break away from the hyphae, the spore germinates by means of one or two germ tubes. Streptomyces are classified with bacteria because they are prokaryotes and have cell wall, closely resembling those of bacteria than of fungi. They do resemble fungi in their filamentous morphology but differ in the small diameter of their vegetative filaments.

Streptomyces scabies is aerobic and produces colorless vegetative filaments and pale aerial mycelium on a number of media at 25-30°C. It sporulates best on potato agar media with low sucrose (0.5%). Pathogen differs from other bacteria by the radiating streptomyces filaments.

Disease Cycle

Pathogen survives on plant debris, on tubers and in soil for indefinite period under favorable conditions (continuous cropping of potato and other hosts). It penetrates tissues through lenticels, wounds, stomata and in young tubers directly. Young tubers are more susceptible than older ones. After penetration, it grows between few layers of cells. Meanwhile secretes substances that stimulate the surrounding cells and produces several layers of cork cells that isolate the pathogen. Group of cork cell layers are produced, pushed outward and sloughed off. Pathogen multiplies in dead cells and thereby large scab lesions develop (illustrated life cycle of the pathogen is given in Figure 22).

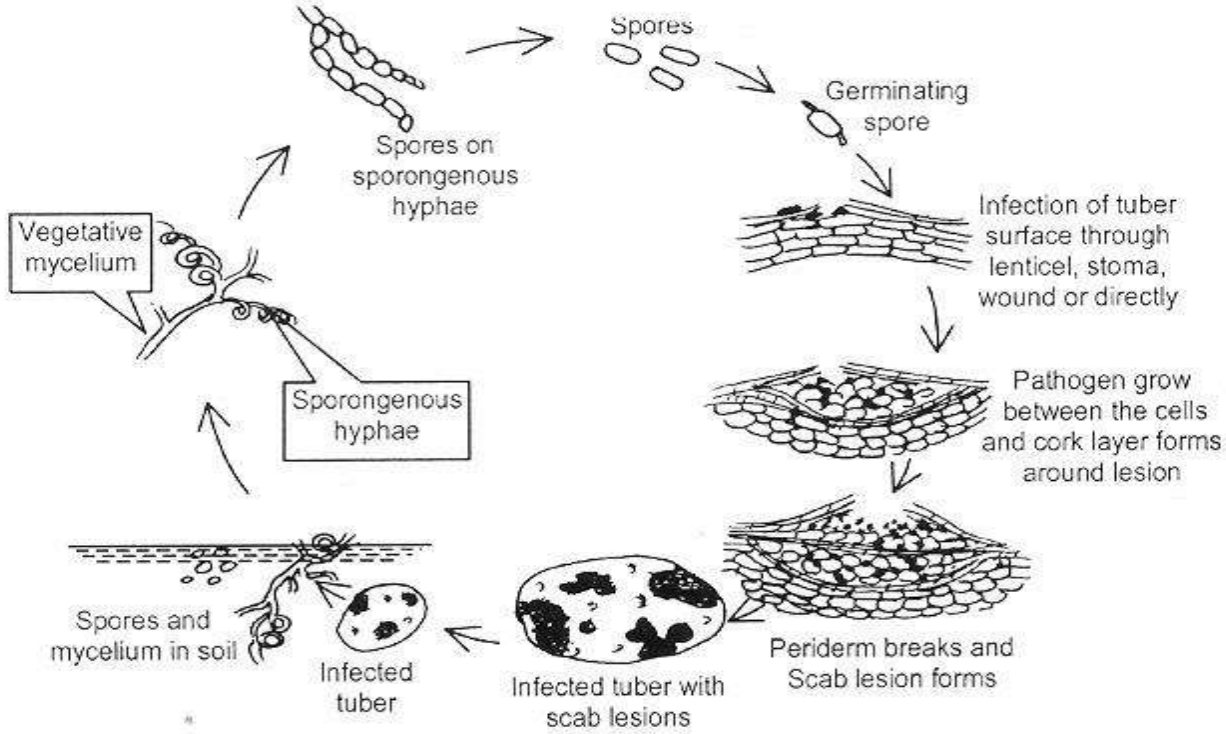

Figure 22. Disease cycle of common scab caused by *Streptomyces scabies*
(Adapted from G.N. Agrios)

Epidemiology

Pathogen spread through soil water, wind-blown soil and infected seed tubers. Common scab increases as the pH of the soil increases (5.2-8.0). Optimum temperature is about 20-22°C but it can grow between 11-30°C. Dry soil favors its development especially at the time of tuber initiation and early stages of bulking. Disease decreases in field after certain crop rotations and plowing under certain green manure crop, probably as a result of inhibition of the pathogen by antagonistic microorganisms.

Other Hosts

Carrot, radish, beets and turnip are among the hosts of *S. scabies*.

Detection Methods

i. Visual observations:
Scabbed lesions (russet or pitted) on tuber surface (see Figure 21).

ii. Isolation of pathogen from tubers and soil on specific media:
Specific media:
Tryosine- casein- nitrate agar medium (TCN) (Menzies & Dade, 1959).

Sodium caseinate	2.5%
Sodium nitrate	1%
L. tryosine	0.1%
Agar	1.5%
Distilled water	1 lit.

Disease Assessment

The assessment is made on the basis of tuber area covered with the scab on 0-5 scale (Figure 23) (Anonymous, 1976).

0 = no symptoms on tubers.
1 = less than 1% area affected.
2 = 1-10% area affected.
3 = 11-20% area affected.
4 = 21-50% area affected.
5 = 51% or more area affected.

Control

- Use certified seed potatoes.
- Treat the seeds with mercuric chloride (0.1%) or 5% formaldehyde (Hafiz, 1986) or with Pentachloro-nitrobenzene (PCNB) (Agrios, 1988) to reduce the disease.

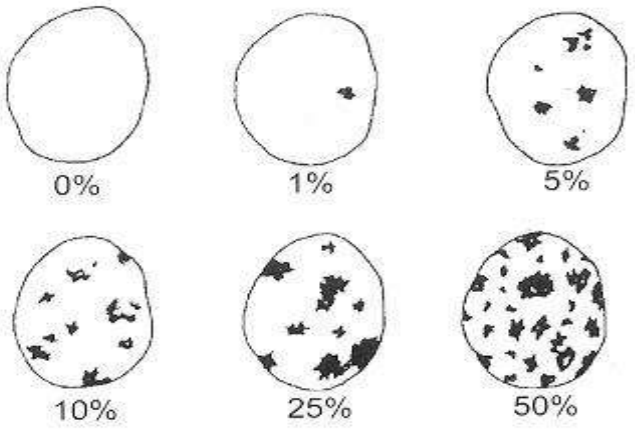

Figure 23. Standard area diagram for assessment of common scab of potato

- Irrigate the crop for about 6 weeks during the early stages of tuber development (Agrios, 1988).
- Use tolerant varieties (smooth skinned like Cardinal and Desiree) to reduce the disease (Hafiz, 1986).
- Follow crop rotation for long period with non-host crops like maize.

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DISEASES CAUSED BY FUNGI

DISEASES CAUSED BY FUNGI

Introduction

Fungi belong to plant kingdom but lack chlorophyll; they have filamentous, branched somatic structure surrounded by cell wall containing cellulose or chitin or both, called hyphae or mycelium. These hyphae may be non septate as in phycomycetes or septate as in Ascomycetes, Basidiomycetes and Deuteromycetes or fungi imperfecti (Alexopoulos, 1962).

Fungi are reproduced by means of spores that may be asexual or sexual. In lower fungi asexual spores are produced inside a sac called sporangium and are released through an opening on sporangium or upon its rupture. These spores can move through flagella and are called zoospores. Other fungi produce non-motile spores called conidia on special hyphae (conidiophore). In some cases, hyphae produce terminally or intercalary, rounded thick walled chlamydospores. In some fungi asexual spores (conidia) can occur in a thick-walled structure called pycnidia (Agrios, 1988). Sexual spores are formed by the fusion of two gametes. In one group of fungi (Ascomycets) the sexual spores (ascospores) are formed within zygot cell called ascus. In another group of fungi (Basidiomycetes) spores are produced outside the zygot cell (basidium) called basidiospores (Agrios, 1988). These spores require favorable temperature and moisture to germinate. The fungi are disseminated primarily in the form of spores and to some extent as fragments of hyphae and hard masses of mycelium known as sclerotia. In lower fungi, zoospores are the structure that can move by themselves, while majority of the pathogenic fungi spread from plant to plant and to different parts of the same plant through different agents such as wind, birds, insects, animals and humans.

Fungi can cause diseases and produce symptoms on different parts of the infected plant i.e. on leaf, stem, root and tuber.

Generally control of plant diseases is possible when all parameters for controlling the disease are taken into account. The most common control measures include, the use of healthy (free of pathogen) seeds or propagating stocks, destruction of infected plant parts or volunteer plants, use of clean tools and containers, proper drainage of field,

crop rotation with non host crops and use of resistant plant varieties. Chemicals are effective but they are expensive and hazardous. Chemicals may be applied as sprays or dusts on the plants, seeds or into the soil. In case of seeds, treatment with systemic fungicides or hot water is effective. Fungal diseases are probably much easier to control than any other group of plant pathogens but losses caused by these are still very high.

In Pakistan potato crop is affected by a number of fungal diseases. As a result of these diseases, a high percentage of loss occurs due to reduced yield and rotting of potato tubers. Turkensteen identified twelve fungal pathogens from plains of Pakistan and eighteen from hilly areas (Turkensteen, 1986, 1987).

So far 18 fungal diseases of potato have been reported from Pakistan, of which most important are black scurf, early blight, fusarium dry rot, fusarium wilt, late blight, powdery scab and verticillium wilt. These will be discussed in detail, while the less important are listed below (Table 12).

Table 12. Less Important Fungal Diseases of Potato

S#	Disease	Pathogen
1.	Wart	*Synchytrium endobioticum*
2.	Pink Rot	*Phytophthora erythoseptica*
3.	Powdery Mildew	*Erysiphe cichoracearum*
4.	White Mold	*Sclerotinia sclerotiorum*
5.	Stem Rot	*Sclerotium rolfsii*
6.	Black Rot	*Rosellinia* sp.
7.	Thecaphora Smut	*Thecaphora (Angiosorus) solani*

Fungal diseases incidence, mode of perpetuation and control measures are given in Tables 13, 14 and 15, respectively.

Table 13. Incidence of Fungal Diseases

S#	Disease	Plain Crops		Hill Crops
		Autumn	Spring	
1.	Black Scurf *(Rhizoctonia solani)*	***	***	**
2.	Early Blight *(Alternaria solani)*	*	**	*
3.	Fusarium Wilt (*Fusarium spp.*)	*	**	*
4.	Late Blight (*Phytophthora infestans*)	**	*	**
5.	Powdery Scab (*Spongospora subterranea*)	-	-	**
6.	Wilt/Vertcillium Wilt *(V. albo-atrum & V. dahliae)*	*	**	**

* traces ** medium *** severe (Data based on visual observation)
Detected from soil through bioassay (tomato bait plants)
- Data not availbale

Table 14. Mode of Perpetuation and Plant Parts Affected by Fungi

S#	Disease	Modes of Infection			Plant Parts Affected				
		Plant debris	Diseased seed tubers	Infested soil	Leaves	Stems	Stolen	Tubers	Roots
1.	Black Scurf *(Rhizoctonia solani)*	+	+	+	-	+	+	+	+
2.	Early Blight *(Alternaria solani)*	+	+	+	+	+	-	+	-
3.	Fusarium Wilt (*Fusarium spp.*)	+	+	+	-	+	-	+	+
4.	Late Blight (*Phytophthora infestans*)	+	+	-	+	+	+	+	-
5.	Powdery Scab (*Spongospora subterranea*)	+	+	+	-	-	-	+	+
6.	Wilt/Vertcillium Wilt *(V. albo-atrum & V. dahliae)*	+	+	+	+	+	-	+	-
7.	Fusarium Dry Rot *(Fusarium spp.)*	+	+	+	-	-	-	+	-

+ = Yes - = No

Table 15. Recommended Control Measures

Preventive Measures

(a) Use of healthy seed tubers.
(b) Use of un-infested fields.
(c) Destruction of diseased plant debris.
(d) Cultural practices to stop spread.
(e) Long rotations.
(f) Destruction of host plants etc.

Curative Measures

(g) Dis-infection of seed tubers.
(h) Spray.

Diseases	a	b	c	d	e	f	g	h
Black Scurf *(Rhizoctonia solani)*	+	+	+	+	+	+	+	-
Early Blight *(Alternaria solani)*	+	+	+	+	-	+	+	+
Fusarium Wilt *(Fusarium spp.)*	+	+	+	+	+	+	-	-
Late Blight *(Phytophthora infestans)*	+	-	+	+	-	+	+	+
Powdery Scab *(Spongospora subterranea)*	+	+	+	+	+	+	+	-
Wilt/Verticillium Wilt *(V. albo-atrum & V. dahliae)*	+	-	+	+	+	+	-	-
Fusarium Dry Rot *(Fusarium spp.)*	+	+	+	+	+	+	+	-

+ = Yes = No

Black Scurf (*Rhizoctonia solani*)

Occurrence and Importance

Black scurf was first mentioned by the name of stem rot and black spot of tubers (Hafiz, 1955). Later disease was reported from Swat (Khan, *et al.*, 1985a and Turkensteen, 1986). Now the disease is present in all potato growing areas and is emerging as a disease of major economic importance. Disease causes tuber malformation, pitting and stem end necrosis that leads to poor quality tubers.

Some time causes heavy loss of seed potatoes particularly when seed germination and plant growth is slow. Significant yield losses have been reported from many parts of Sahiwal and Okara districts. Among the seed and soil-borne diseases black scurf is posing a threat to potato production in Pakistan (Malik, 1995).

Symptoms

Fungus produces black to dark brown sclerotia on the surface of mature tubers (Figure 24). These sclerotia may be flat and superficial or large, irregular lumps resembling soil. The tuber periderm under such sclerotia is usually unaffected. Other tuber symptoms include cracking, malformation, pitting and stem-end necrosis. It produces dark brown lesions on roots, stolons and sprouts (Figure 25), killing these parts or delayed emergence especially in cool, wet soils. When it girdles the young stem, plant dies. The sexual stage of the pathogen occurs on stems just above the soil line as a whitish gray mat on which basidiospores are formed, giving the surface a powdery appearance.

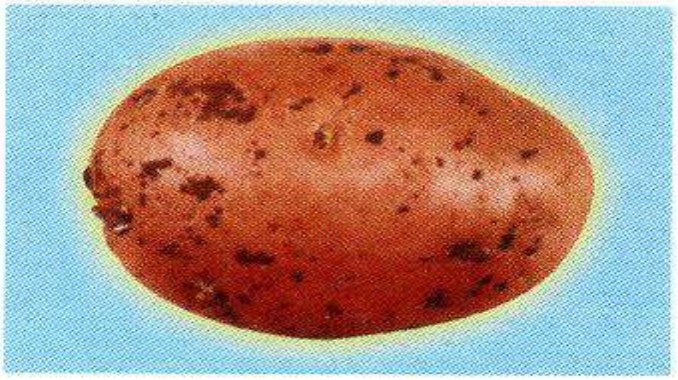

Figure 24. Black scurf : Sclerotia of *R. solani* on tuber surface

Causal Organism

The pathogen in its imperfect stage is *Rhizoctonia solani* and in perfect stage is *Thanatephorus cucumeris*, which produces basidiospores. The mycelium is generally tan to dark brown and hyphae are rather large (generally 8-10 µm in diameter). Young

vegetative hyphae have multi nucleate cells, branched near the distal septum of a cell. Right angle branching and constriction of branch hyphae at the point of origin or formation of a septum in the branch near the origin is the characteristic of *R. solani* (Figure 26).

Pathogen produces a growth-regulating toxin that may be partially responsible for tuber malformation.

Figure 25. Dark brown lesions of *Rhizoctonia* canker on roots and underground stem
(Photo: Pennsylvania Agric. Extension Service)

Disease Cycle

The fungus lives as mycelium on plant debris in the soil or in the form of sclerotia on tubers and in soil. Under favorable conditions, sclerotia germinate and infect emerging sprouts and plant parts at or below the soil line. Basidiospores appear as grayish-white powdery mass on stem just above the soil line (illustrated life cycle of the pathogen is given in Figure 27).

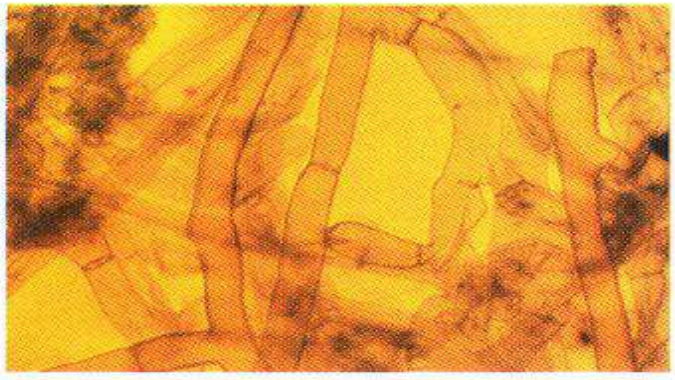

Figure 26. *Rhizoctonia solani (Thanatephorus cucumeris)*: mycellium at right angle branching with septa

Epidemiology

Fungus survives as mycelia or sclerotia in the soil, plant debris and on potato tubers. Once established in soil, remains there for indefinite period. In soil it can be found up to a depth of 10-15 cm. The fungus spreads with rain, irrigation or floodwater, with tools or propagative materials. Favorable conditions for the pathogen are low soil temperature and high moisture levels. Some races have best infection at 15-18°C, but others are more active at much higher temperature-up to 35°C. Disease is more severe in soils that are moderately wet than soils that are waterlogged or dry. Deficiencies of potassium, nitrogen, calcium or excess of nitrogen can increase the disease.

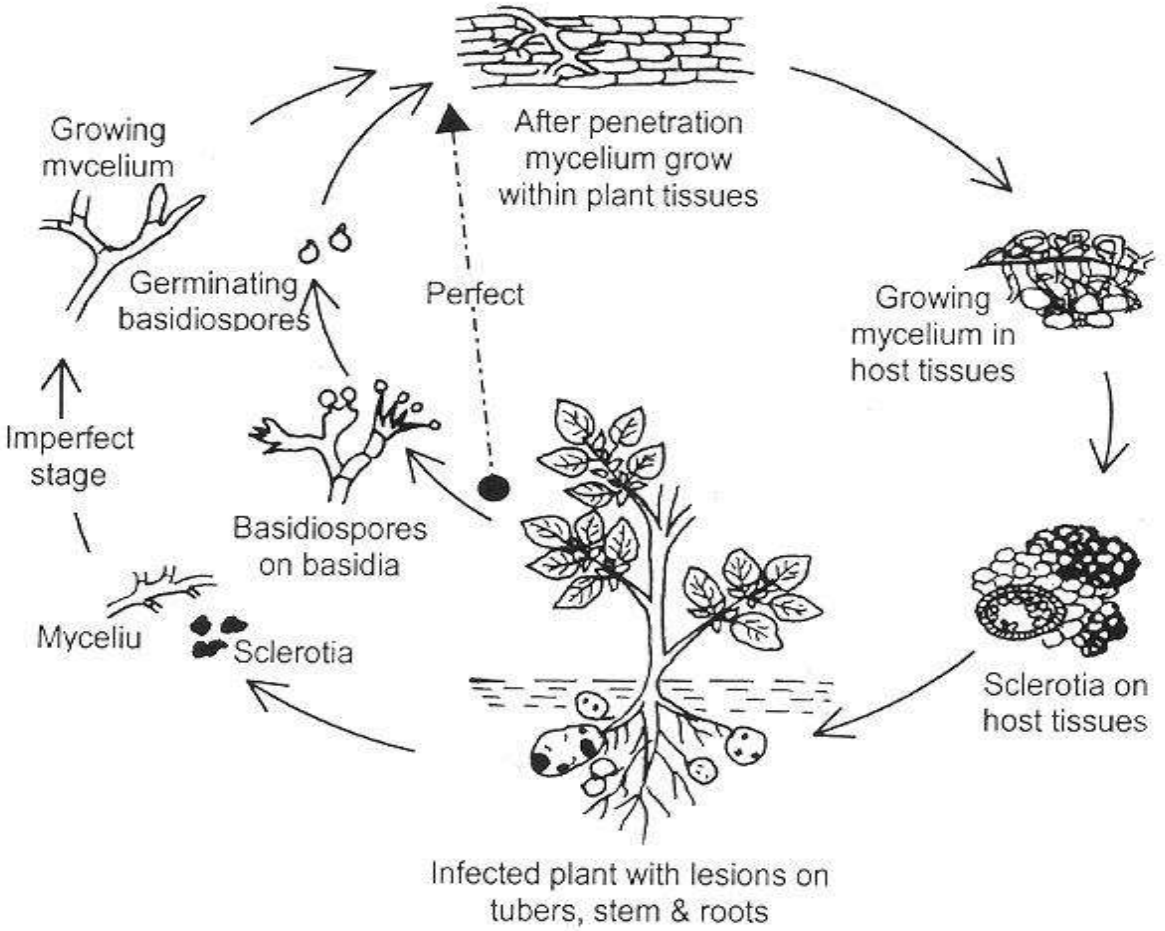

Figure 27. Disease cycle of *Rhizoctonia* canker or black scurf caused by *R. solani*
(Adapted from G.N. Agrios)

Other Hosts

R. solani is a pathogen of numerous crops including almost all vegetables, several field crops, turf grasses, ornamentals, flowers and weeds.

Detection Methods

i. Visual observation:
Observe presence of brown to black sclerotia on tubers (see Figure 24) or dark brown lesions on roots and on stem (see Figure 25).
ii. Light microscopy (see Figure 26).
iii. Isolation on potato dextrose agar medium (PDA) or water agar (WA).

Potato Dextrose Agar (PDA) (Tuite, 1969).

Potato peeled sliced	200 gm
Agar	12 gm
Dextrose	10 gm
Water	1 lit

Disease Assessment

On the basis of affected tuber surface the assessment is made on 0-5 scale (Figure 28) (Anonymous, 1976).

0 = no symptoms on potato tubers.
1 = 1% or less area affected.
2 = 1-10% area affected.
3 = 11-20% area affected.
4 = 21-50% area affected.
5 = 51% or more area affected.

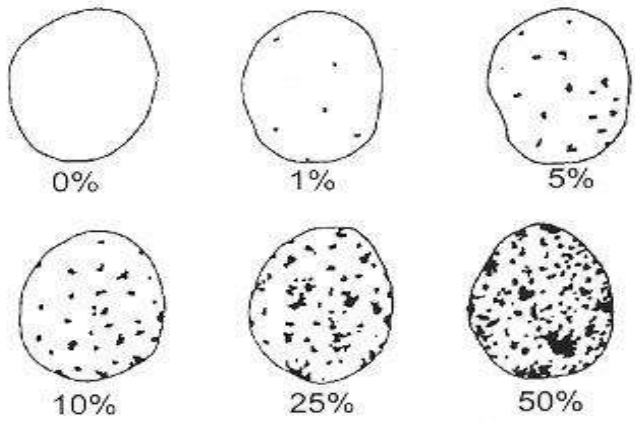

Figure 28. Standard area diagram for assessment of black scurf

Control

- Wet, poorly drained areas should be avoided for potato cultivation.
- Use disease free seed combined with seed treatment with Benomyl (Hooker, 1981).
- Seed should be planted on raised beds and in the well-drained soil to encourage fast growth of the seedlings.
- Harvest the crop as soon as the tubers are mature, which can reduce the number of sclerotia on tubers.
- Apply boric acid (1.0%) before storage (Somani, 1988).

Early Blight (*Alternaria solani*)

Occurrence and Importance

The disease is prevalent throughout the world. In Pakistan, it was first mentioned in 1955 (Hafiz, 1955) and later observed in Baluchistan (Shafiq, 1986). Now, early blight is prevalent in most potato growing areas of the country. However, it is more of a problem in drier areas (Malik, 1995) as compared to irrigated lower valleys of NWFP and high valleys of Northern Areas.

Losses, varies in different areas and depend on degree of infestation. Disease reduces the size of tubers and quantum of yield, especially in the early season infection.

Symptoms

Mainly it is foliar disease but tubers are also affected. Infection starts first on lower, older leaves. Lesions are brown in color, oval or circular in shape, appear first as small spots of 1-2 mm in diameter and gradually enlarge. A concentric ring of raised and depressed necrotic tissue within lesions give spots a target appearance and is characteristic of early blight (Figure 29). As new lesions develop, the older ones expand and the entire leaf becomes necrotic. Later, necrotic leaves dry up and droop or fall off. When tubers are affected, lesions are dark, slightly sunken and circular to irregular in shape, having up to 2 cm diameter and 5-6 mm depth, often surrounded by a raised border of purplish to gunmetal color. The underlying flesh is dry leathery to corky and usually brown. Tissue in advanced decay is often water-soaked and yellow to greenish yellow. Lesions can increase in size during storage and tubers become shriveled in advanced cases.

Figure 29. Early blight, *Alternaria solani* infection on potato leaves

Causal Organism

Alternaria solani soraner is causal pathogen of disease. It produces conidia of 15-19 × 150-300 µm with 9-11 transverse septa and with longitudinal septa (Figure 30). Spores are usually borne singly but may be catenulate. Spores are straight or slightly bent, tapering gradually to a long beak. Color varies from pale to light tan to olive-brown. The beak is flexuous, occasionally branched, and 2.5-5.0 µm wide. Conidiophore occurs singly or in small groups, pale to olive brown, 6-10 µm in diameter and up to 100 µm long.

Figure 30. Spores of *Alternaria solani*

Disease Cycle

Alternaria survives as mycelium in infected plant debris and as mycelium or spores on infected tubers or on other solanaceous hosts. Fungal spores penetrate the leaves directly through the epidermis and after infection it produces new conidia on leaf surface that are further spread by wind, splashing rains or through tools (illustrated life cycle of the fungus is given in Figure 31).

Epidemiology

Mycelium growth in pure culture occurs at 28°C. Optimum temperature for formation of conidiophore and conidia is 19-23°C. Conidiophores develop in light, whereas, light inhibit conidia formation above 15°C. Maximum spore production in the field occurs between 3.00 a.m. and 9.00 p.m. Optimum temperature for tuber infection is 12-16°C but varies with cultivar. Disease progresses during alternating period of wet and dry weather. Early blight can be severe in irrigated desert regions because of prolonged periods of dew. Stresses such as injury and poor nutrition increase the severity of disease. Foliage infection depends on plant maturity. Late maturing varieties are usually more resistant.

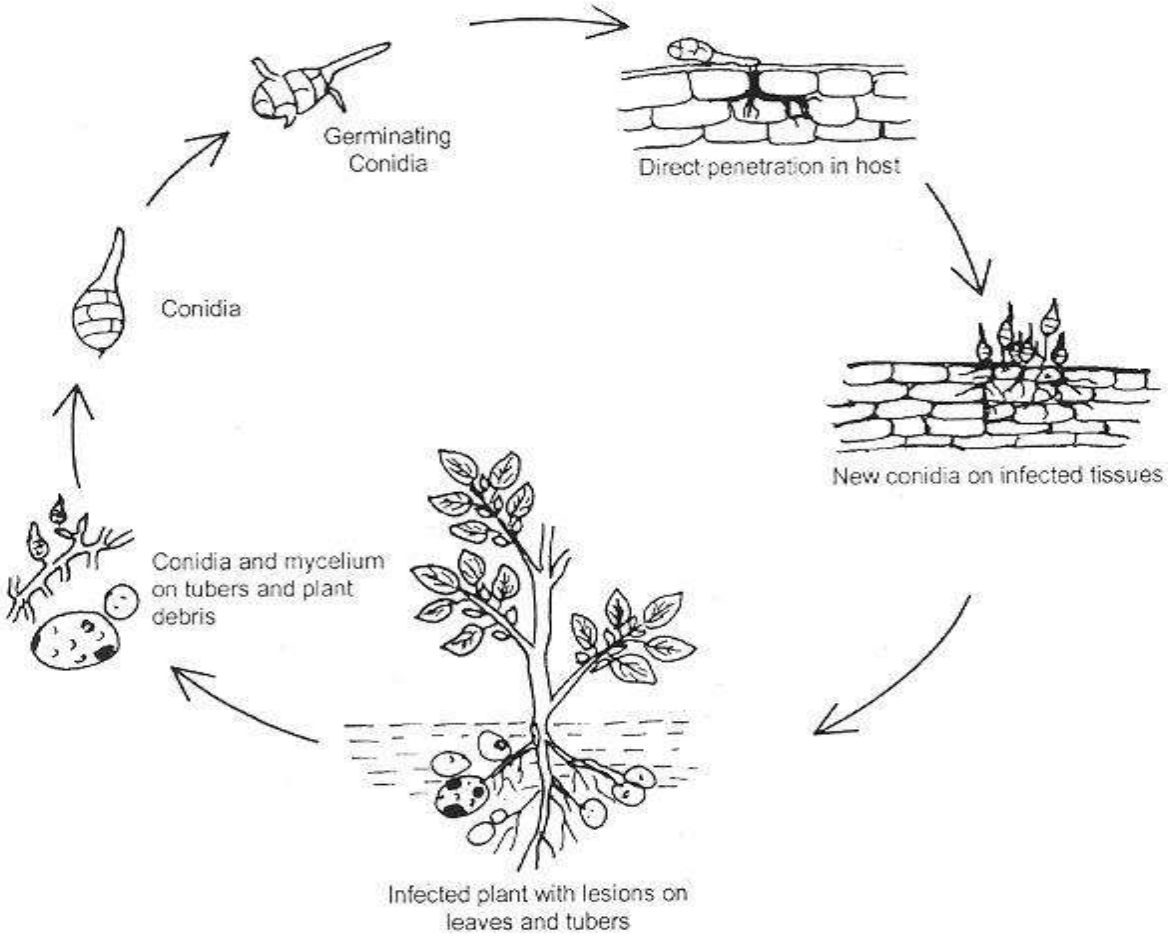

Figure 31. Disease cycle of early blight caused by *Alternaria solani*
(Adapted from G.N. Agrios)

Other Hosts

Other host plants of *A. solani* include tomato, eggplant and brassica species.

Detection Methods

i. Visual observation:
The leaves of infected plants develop brown, oval to circular lesions having concentric rings of raised and depressed necrotic tissue (see Figure 29).

ii. Microscopy:
Direct from samples.
After isolaton on blotter paper or on potato dextrose agar medium (PDA) (see Figure 30).

Disease Assessment

As it is mainly a foliar disease, therefore, assessment is made on account of leaf area covered by the disease on 0-5 scale (Figure 32) (Anonymous, 1985a).

0 = no symptoms on leaves.
1 = less than 1% area affected.
2 = 1-10% area affected.
3 = 11-20% area affected.
4 = 21-50% area affected.
5 = 51% or more area affected.

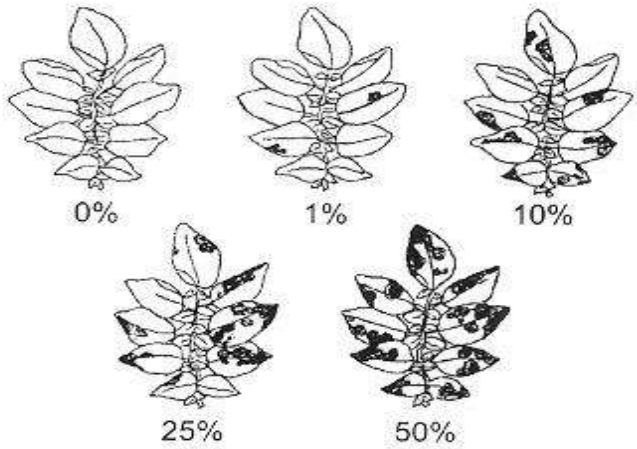

Figure 32. Standard area diagram for assessment of early blight

Control

- Use healthy seed tubers to control the source of primary infection.
- Destroy the diseased plant debris.
- Apply the fungicide for foliar spray. Effective chemicals for controlling the disease are Antracol, Bordeaux mixture, Cupravit and Dithane M-45. Three sprays at 5-days interval are effective in reduction of disease intensity up to 90% (Hafiz, 1986). After disease appearance the crop should be sprayed fortnightly.
- Permit tubers to mature in the ground before digging and avoid brushing in the handling.

Fusarium Dry Rot (*Fusarium* spp.)

Occurrence and Importance

Fusarium dry rot of potatoes is worldwide and usually occurs at lower temperature. In Pakistan two Fusarium species; *F. angustum* and *F. oxysporum* have been found to cause the disease. However, *Fusarium solani* was also found to be associated with *F. oxysporum* at Tandojam (Hafiz, 1986).

Symptoms

Dry rot mainly affects the tubers in storage and planted seed tubers. Brown to black lesions are developed on the surface of tubers which slowly enlarges and become sunken, shriveled or concentrically wrinkled, occasionally fungal growth appear on the surface (Figure 33). On cutting, such tubers show cavities lined with white mycelium of fungus. Rotted tubers become hard and dry. During storage, at saturation of relative humidity, secondary infection of *Erwinia* spp. may occur.

Figure 33. Dry rot: Infected tuber with *Fusarium* spp.

Causal Organism

Two species of Fusarium; *F. angustum* sherb. and *F. oxysporum* schi. are dominant causal agent of dry rot. In some areas, *F. solani* is also associated with main pathogen (*F. oxysporum*). These species have macro as well as micro conidia and they can grow and maintained on potato-dextrose agar (PDA). *F. angustum* give creamy white colony on media, whereas, *F. oxysporum* become pinkish on PDA and *F. solani* forms a denser, white mycelial mat that exhibits a purple pigment with age.

Disease Cycle

Fusarium species can survive for long period in soil but the primary inoculum is generally tuber borne. Contaminated containers and equipment used in handling or storing potatoes are often the source of infection. The infected seed tubers and infested soil that adhere to the surfaces of tubers are also source of infection.

Epidemiology

Harvested potato tubers are tolerant to infection but become more susceptible during storage. Dry rot develops rapidly in high relative humidity at 15-20°C. Disease development continues at the coldest temperature, which is safe for potatoes. Wound healing can reduce infection. Wound periderm forms in 3-4 days at 21°C with adequate humidity and aeration and prevent infection.

Detection Method

i. Symptom observation on tubers (see Figure 33).
ii. Light microscopy from direct infected tubers. Presence of white mycelium is an indication of fungal infection.
iii. Light microscopy after isolation on potato-dextrose agar medium.

Control

- Use healthy mature tubers without bruises and injuries to reduce the losses.
- Potato tubers should be surface sterilized with formalin solution in water (1:320 v/v) to check the disease (Hafiz, 1986).
- Produce seed potatoes from certified seed during autumn in the plains.
- Potatoes harvested late in summer (hilly areas), store at cooler places and use as seed for growing autumn crop in the plains (Hafiz, 1986).
- Disinfect the store rooms either by spraying with formalin solution or by burning sulfur at the rate of 1 lb/1,000 cubic feet space or any other suitable chemicals like Dithane M-45 (Hafiz, 1986).
- Store seed potatoes in well aerated store at 12-15°C.

Fusarium Wilt (*Fusarium* spp.)

Occurrence and Importance

Fusarium wilt has been reported from all parts of the country. However, it was first mentioned in 1955 (Hafiz, 1955) and later its presence was reported from Balochistan (Shafiq, 1986). Generally, it is more common in spring as compare to autumn crop due to low soil temperature. In Pakistan two species of Fusarium i.e. *F. oxysporum* and *F. radicola* have been isolated and proved to be pathogenic under artificial conditions of inoculation (Kamal & Mughal, 1968; Hafiz, 1986).

In most parts of the country Fusarium has been found with Vertcillium in the soil. The interaction of Vertcillium with Fusarium and Rhizoctonia in soil causes early dying syndrome, in which plants prematurely die (Malik, 1995).

Symptoms

Symptoms are visible at the age of 4-6 week. Under dry conditions, plant starts yellowing, wilting, drying and death of the leaves within two weeks of symptom appearance. Yellowing begins at the lower leaves and progresses upwards. A cross-section of the stem and tuber shows discoloration of the vascular tissues (Figure 34). At advance stages of infection in wet cold soil, the underground part of the stem may rot and plant die early in the season. A transverse section of the affected rootlets under the microscope show the presence of delicate filaments of the fungus in the xylem bundles. The disease makes rapid progress in the presence of high humidity and temperature. Under this condition, tuber infection through wounds or lenticel causes circular lesions and a dry rot in storage.

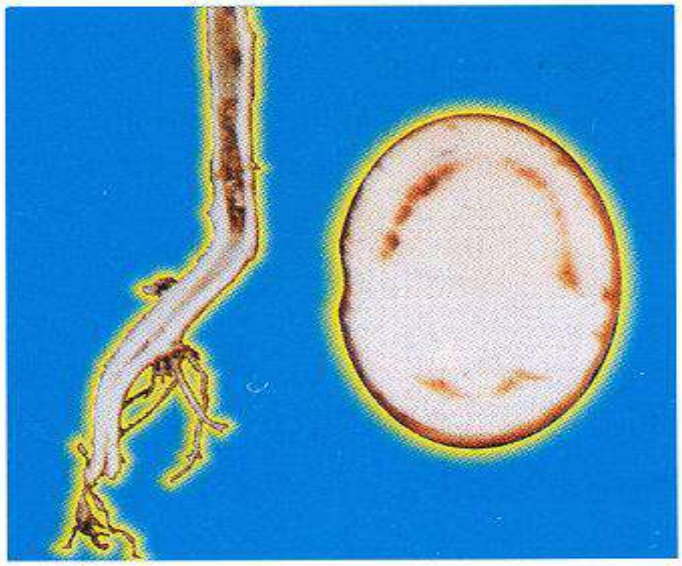

Figure 34. Fusarium wilt: A typical discoloration of the vascular system in stems and tubers

Causal Organism

Two species of Fusarium i.e. *F. oxysporum* and *F. radicola* have been found to cause the disease. Pathogen causes varying degrees of infection depending upon the crop season. Mostly, it is found in the spring, rarely noticed in autumn crop due to low soil temperature. Pathogen is easily isolated from vascular discolored stem tissue below or close to the soil line.

Initially, colorless mycelium becomes pale-yellow, however, under certain conditions it produces pink or purplish coloration. The fungus produces three kinds of asexual spores. Micro conidia, which are one or two celled and abundant, produced frequently inside the vessels of infected plants. Macro conidia are the typical 'fusarium' spores, 3-5 celled, gradually pointed and curved toward both ends (Figure 35). Chlamydospores are one or two celled, thick walled and produced terminally or intercalary on older mycelium or in macroconidia. All these types of spores are produced in cultures and probably in the soil. Chlamydospores survive in the soil for long period.

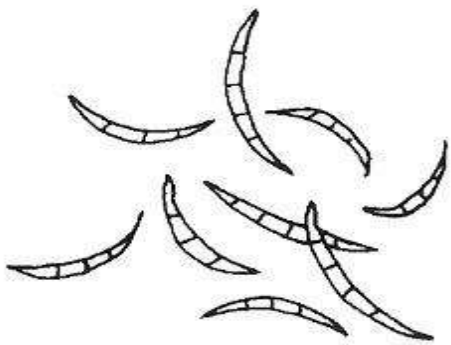

Figure 35. Macroconidia of *Fusarium oxysporum*

Disease Cycle

Fusarium spores reside in plant debris in soil or on tubers in storage. During the growing period, infection starts through invasion of roots and spread into the tubers. Inoculum is introduced into new fields primarily through the planting of infected seed tubers. Continual potato production, particularly replanting infected tubers, accelerates inoculum build up. Inoculum is dispersed from infested fields by surface drainage of water, wind and through soil carried on implements (illustrated lifecycle of the fungus is given in Figure 36).

Epidemiology

Wilt is most severe at high temperature and particularly when plants are under stress in dry and hot growing conditions. The maximum temperature for the growth of the fungus is between 35-40°C, the minimum is between 5-10°C and the optimum is 25-30°C. Spore germinates at wide range of pH values.

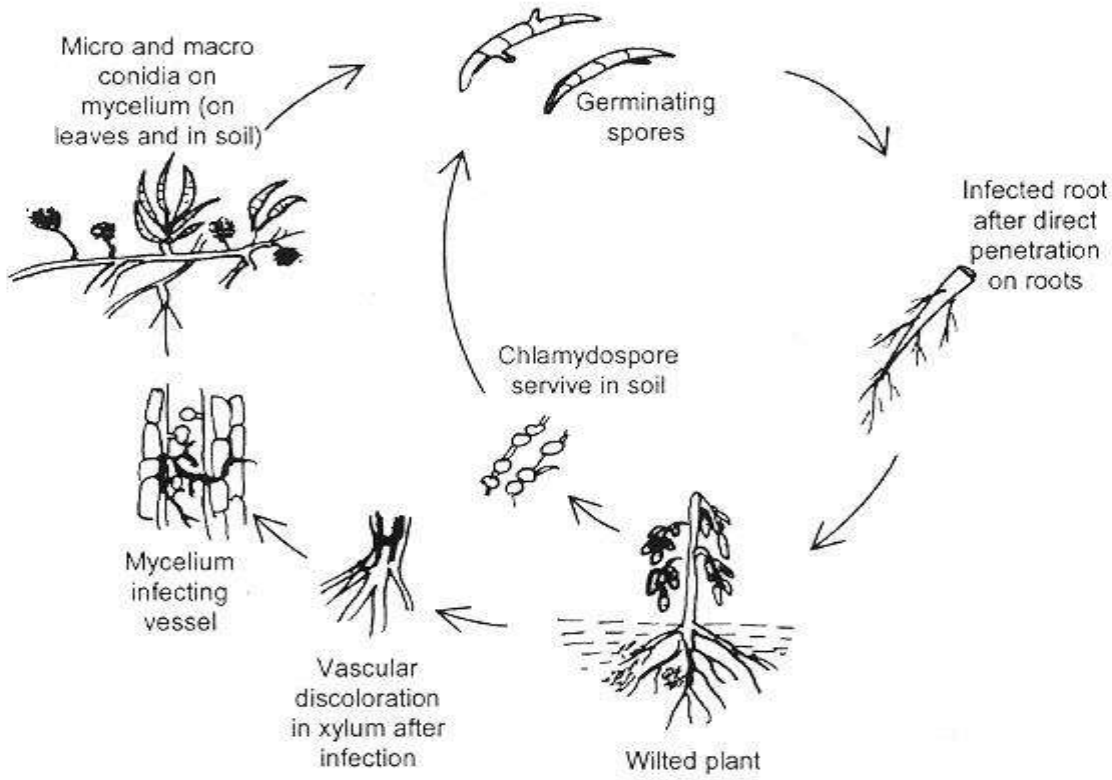

Figure 36. Disease cycle of Fusarium wilt caused by *Fusarium oxysporum*

Other Hosts

Fusarium spp. has wide range in solanaceous and non-solanaceous crops and causes severe losses in most vegetables, flowers and field crops, such as cotton, tobacco, banana and sugarcane etc.

Detection Methods

i. Visual observation:
A cross section of infected tuber shows discoloration of vascular tissues (see Figure 34).

ii. Light microscopy (see Figure 35).

iii. Isolation on PDA and Specific media.
Specific media:
Tochinai solution (*F. oxysporum*) (Tuite, 1969).

Peptone	100 g
KH_2PO_4	0.5 g
$MgSO_4.7H_2o$	25 g
Maltose	20.0 g

Dist. water to make one lit.

iv. Scanning electron microscopy (SEM) (Fox, 1993).

Control

- Select seed potatoes from healthy crop grown in disease-free areas, to minimize the disease.
- Disinfect the seed potatoes with 2% formalin.
- Select seed potatoes from autumn crop and store them in cold houses for next sowing.
- Destroy and burn the plant debris before sowing.
- Harvest carefully to avoid bruises and injuries to potato tubers.

Late Blight (*Phytophthora infestans*)

Occurrence and Importance

Late blight is a notorious disease of potato and tomato. The disease is believed to have originated from Andes (North America) and become increasingly worse in Europe and was responsible for the famous famine in Europe (Ireland) in 1845 (Large, 1940). In Pakistan, the disease was first reported from Kalam and Malam Jaba valley of district Swat (Khan *et al.*, 1985b). However, it is believed that it was present in Pakistan before that. Since then the disease has been reported from the Punjab and NWFP. More recently, it has been found in areas of Balochistan and Northern Pakistan where macroclimate for disease is apparently unsuitable (Ahmad, *et al.*, 1995). Out of eight production zones of Pakistan (see page vii); zone 4, 5, 6a and 6b has been declared as the area of common occurrence of late blight (Ahmad, *et al.*, 1995).

The pathogen can attack potato crop at any stage of growth at 10-20°C in humid weather. It is devastating when at the early growth stage. However, it is not equally severe every year. Attack on summer crop of the hilly areas of NWFP starts by the end of July to mid-August. In the high valleys of Kalam and Kaghan, crop is usually attacked at the early bulking stage (growth stage IV). The mid-hills crop planted in April is usually attacked at the late bulking stage by the end of July. In the spring crop, attack starts by the end of March and the crop is at risk up to the third week of April, if temperatures remain favorable (Malik, 1995). In autumn crop of Punjab, late blight appears in December when crop is almost mature, thus yield losses are not significant.

Disease can cause yield losses upto 75% and also affect the quality of tubers (Malik, 1995). In severe cases, it causes total destruction of plants within a week or two. Losses, however, vary from one race to another and from one year to other, depending on the prevailing temperature, moisture and on control measures practiced. Potatoes may become infected during harvest and may rot in storage.

Symptoms

Disease symptoms first appear at tips or edges of the lower leaves in the form of circular or irregular water soaked lesions. Under cool and humid climatic conditions the lesions enlarge and form blighted areas

having whitish fluffy margins on the lower leaf surface (Figure 37). Under continuous wet conditions, the brown lesions, turn black and expand and cover the entire foliage. Blight extend and affect the all above ground parts of the plants, rot away quickly, giving off characteristic odor. Harvesting in wet weather or when tubers come into contact with infected foliage may also result in tuber infection. Infected tubers have a brownish surface (Figure 38) and after some time rot start from skin and extend inward. The depth of rot depend upon the time after infection, variety and temperature. Fresh tuber shows brown, necrotic rot but dry tubers show secondary infection by other fungus and bacteria.

Figure 37. White aerial growth of *Phytophthora* infestans at the margins of the lesion
(Photo: W. Fischer, Dept. Plant Path., Cornell Univ.)

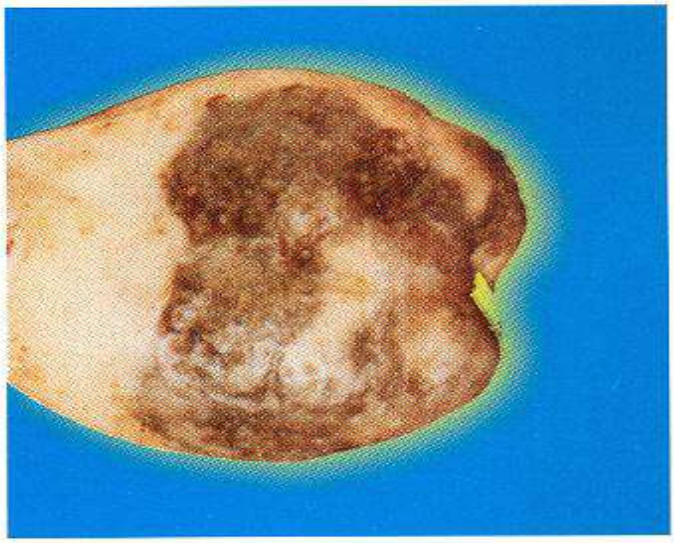

Figure 38. Late blight: Brown dry rot on the surface of potato tuber
(Photo: R.S. Kirby, Pennsylvania State Univ.)

Causal Organism

Phytophthora infestans belongs to oomycetes class of fungi. It produces lemon-shaped, papillate sporangia on branched sporrangiophore (Figure 39). At the places where sporangia are produced, the sporangiophore form swellings that are characteristic of this fungus. Sporangia may germinate by means of germ tube but most commonly they form about eight biciliate zoospores that swim freely in water, encyst on solid surface, germinate by germ tube and enter the host via leaf stomata. Sexual reproduction results in oospores (Figure 40), formed by the union of oogonia and anthridia produced by opposite mating types. Oospores within oogonia are 24-46 µm in diameter and

germinate by a germ tube with a terminal sporangium, which in turn, either liberates zoospores or forms another germ tube and infect the host.

P. infestans has two mating types, A1 and A2 (Rowe & Easton, 1981). Sexual structures (anthridia and oogonia) are induced only in the presence of the opposite mating type, and genetic fusion results in oospores, which are survival structures, whereas, infection of foliage or tubers is initiated by asexual sporangia and zoospores. Mycelium of the fungus most commonly survive as mycelium in infected tubers. After plant emergence, the fungus invades a few of the growing sprouts and sporulates under moist conditions, producing primary inoculum. Once primary infection has occurred, further spread of *P. infestans* takes place by rain splash mechanism.

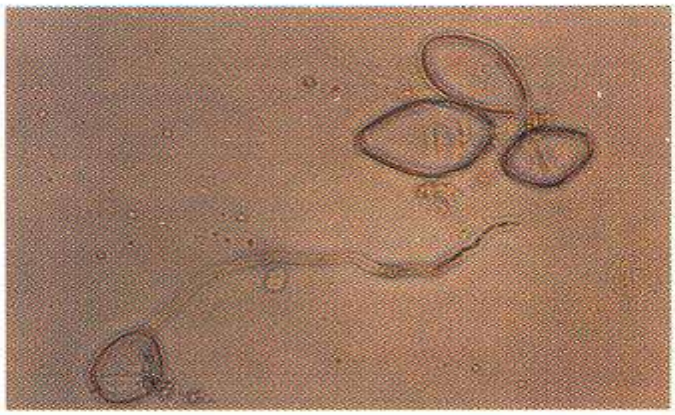

Figure 39. Papillate sporangia of *Phytophthora* infestans

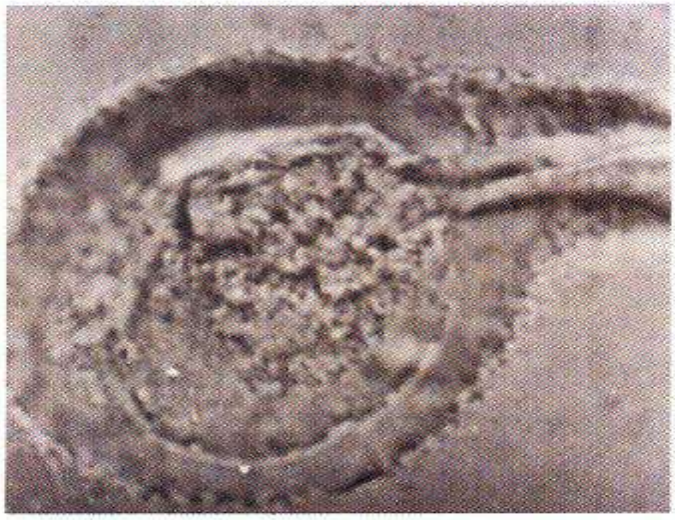

Figure 40. Oospore of *Phytophthora* infestans

Two mating types (A1 and A2) of *P. infestans* were reported previously from Mexico (Gallegly & Galindo, 1958). Until early 1980s, it was believed that only A1 mating type is distributed throughout the world. However, A2 isolates were discovered in Switzerland (Hohl & Iselin, 1984), United Kingdom (Tantius *et al.*, 1986) and elsewhere. In Pakistan presence of two mating types A1 and A2 was reported in 1995 (Ahmad & Mirza, 1995). Both A1 and A2 mating types of *P. infestans* are widely prevalent in almost all potato production zones of Pakistan (Batool, 1999; Batool *et al.*, 1999a).

Metalaxyl resistance studies in Pakistan revealed that three phenotypes of the fungus, i.e., sensitive, intermediate and resistant are

present among the isolates collected from all potato production zones of the country (Ahmad & Batool, 1999; Batool *et al.*, 1999b).

Disease Cycle

The pathogen resides as mycelium in infected potato tubers. The mycelium spread in the tissues of the tubers and finally reaches few of the shoots. The mycelium spreads up the stem most rapidly in the cortical region causing discoloration and collapse of the cells. The mycelium grows through the stem and travels up to the surface of the soil. When it reaches the aerial parts of plants, it produces sporangiophore, which emerge through the stomata of the stem and leaves and project into the air. It produces sporangia, which become detached and drift off when ripe, or are dispersed by rain. When sporangia land on wet potato leaves or stem, they germinate and cause new infection. The germ-tube penetrates the leaf cuticle or enters through a stoma and produces mycelium. The cells on which mycelium feeds are killed, and as they begin to decay, the mycelium spreads into fresh tissue. A few days after infection, new sporangia emerge from the stomata of the leaves and produces sporangia, which are spread by the wind and infect new plants. Under favorable weather, the period from infection to sporangia formation may be four days, therefore, a large number of asexual generations and new infection may occur in one season.

Infection of tubers begins in the field during wet weather; sporangia are washed down from the leaves and are carried into the soil. The zoosporangia, liberate from sporangia under cooler environment, in the presence of free water, germinate and penetrate the tubers through lenticels or wounds. Most of the blighted tubers rot in the ground or during storage (The life cycle of fungus is diagrammatically explained in Figure 41).

Epidemiology

Late blight epidemic depends greatly on the humidity and temperature during different stages of the life cycle of the fungus. The fungus sporulates abundantly near 100% relative humidity and at temperature between 16-22°C. Temperature above 30°C checks the growth of the fungus in the field but do not kill it, and start sporulation again when the temperature becomes favorable with sufficient humidity. Systems for forecasting late blight and timing of fungicide applications rely on

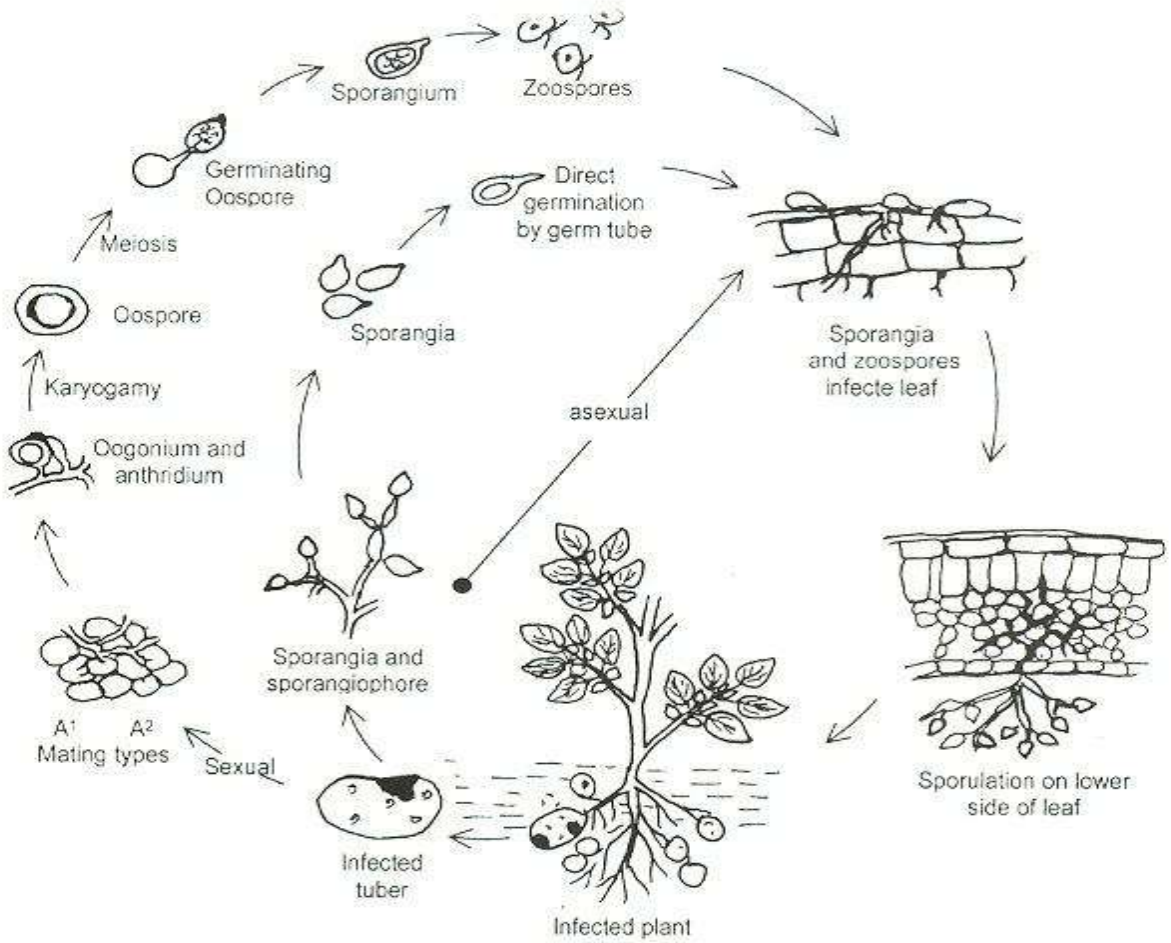

Figure 41. Disease cycle of late blight of potato caused by *Phytophthora infestans*
(Adapted from G.N. Agrios)

records of temperature and rainfall or temperature and relative humidity and predict the probability of late blight development, assuming the presence of inoculum.

Other Hosts

P. infestans infect few other members of the Solanaceae family like tomato, pepper, eggplant and nightshade.

Detection Methods

i. Visual observation:
Observe circular to irregular, brown, water soaked spots with whitish fluffy mycelial masses along the margins on the lower side of the leaves (see Figure 37).

ii. Light Microscopy.
Direct from lesions (see Figure 39).

iii. Isolation on specific media (Rye Agar media with antibiotics) - (Ahmad & Mirza, 1995).

Rye broth	1 lit
Glucose	5 gm/lit
Agar	20 gm/lit

iv. Aerial photography with natural and infrared color film (Blazquez, 1990).

v. Serology (ELISA) (Harrison *et al.*, 1990).

vi. Nuclic Acid-Based method (PCR) (Trout *et al.*, 1997).

Disease Assessment

The disease assessment is made on the basis of leaf area covered with the lesions on 0-5 scale (Figure 42) (Anonymous, 1985).

0 = no symptoms on leaves.
1 = less than 1% area affected.
2 = 1-10% area affected.
3 = 11-20% area affected.
4 = 21-50% area affected.
5 = 51% or more area affected.

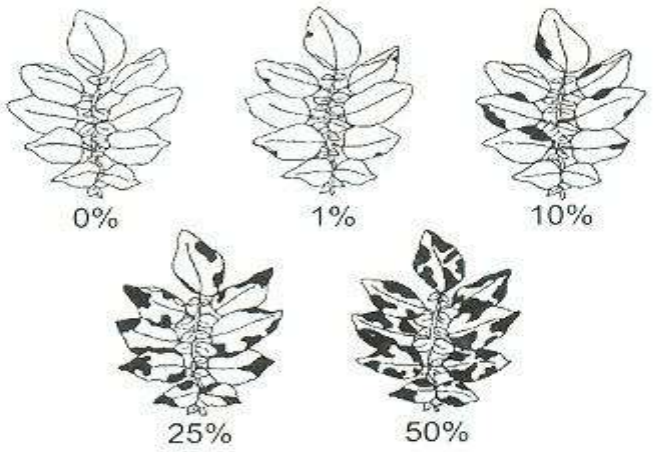

Figure 42. Standard area diagram for the assessment of late blight of potato

Control

- Use certified seeds to reduce the primary inoculum.
- Burn the plant debris such as vines and rotted tubers from the field to reduce the primary source of inoculum.
- Destroy the volunteer potato and tomato plants from the field. They usually appear in hill crops and emerge from tubers and seed potatoes left in soil after previous harvesting.
- To protect tuber infection, ridges should be properly dressed up.
- Apply protectant fungicides like Mancozeb (Diathane M-45), Antracol, Captan and Cupravit (Malik, 1995). The number of sprays depend on the crop duration, usually 7-10 days interval is recommended between each spray and is stopped two weeks before harvesting.

- Use Ridomil M2-78 in combination with contact fungicide e.g. Dithane M-45 to control the pathogen (Malik, 1995).
- Follow crop rotation with non-solanaceous crops for at least 3 years.
- Remove all infected tubers before storage and maintain adequate air circulation and temperature during storage.

Powdery Scab (*Spongospora subterranea*)

Occurrence and Importance

Powdery scab of potatoes originated from Andean region of South America is known to occur throughout the world. However, it is common in temperate region. In Pakistan, on the basis of visual observation, it was first reported from isolated pockets in the districts of Attock, Okara and Faislabad (Turkensteen, 1987) and later in some parts of Kalat division, Balochistan (Turkensteen, 1988). Disease was confirmed in 1996 through microscopic, bioassay and serology from Astak valley in Northern Areas (Ahmad *et al.*, 1996). Since then, its presence has been confirmed in Balochistan and Baltistan (Iftikhar and Ahmad, 1999), Gilgit and Hunza valley (Iftikhar & Ahmad, 2000), Swat and Kaghan valley (Rattu *et al.*, 1999) and Chitral valley (Iftikhar, 2000).

Powdery scab has a cosmetic effect on potato tubers, making them appear unsightly and their market value is reduced. The disease can have an important effect on the export trade, particularly of seed tubers. The amount of highly scabbed tubers can reduce more than half the yield. Sorting the tubers is labor-intensive and further reduces the financial returns from the crop. The resting spores or cystosori of the pathogen are vector of potato mop-top virus (PMTV), which can lead to poor plant growth and causes sprang in tubers. PMTV probably survive between potato crop, in resting spores and is introduced into plants during infection by viruliferous zoospores. Tubers with powdery scab lesions have been reported to be particularly susceptible to various other diseases, possibly because the affected tissue acts as an infection court for other microorganisms. Powdery scab has been implicated in increasing the susceptibility to late blight (*Phytophthora infestans*), pink rot (*Phytophthora erythreseptica*), dry rot (*Fusarium cacruleum*) during storage and rot caused by *Colletotrichum atramentarium*.

Symptoms

Pathogen produces scab (blisters) on the tuber surface (Figure 43) and cause severe tuber distortion (or cankers). Scab spot begins as a small pimple up to 2 mm in diameter on the surface of the growing tubers, over which the skin is at first unbroken, later, the skin ruptures, exposing a dark powdery mass beneath (Figure 44), which consists of

rounded to oval spore balls or resting spores (Figure 45a,b). Normally it affects only the outer tissues but occasionally they may penetrate more deeply, effectively destroying a large proportion of a tuber. The margins of mature scabs are smooth in outline and slightly raised while the scab itself feels slightly spongy, cork like, hence the alternative name for the disease is corky scab. Although individual scabs are roughly circular in shape with a diameter of usually less than 10 mm, they frequently merge, especially if a tuber is heavily infected, resulting in large lesions with irregular outlines. Scab symptoms are also particularly severe at the rose end of tubers. Nodules like galls (Figure 46) may also develop on stolons and roots. Severe root galls after maturity release cystosori, causing young plants to wilt and die.

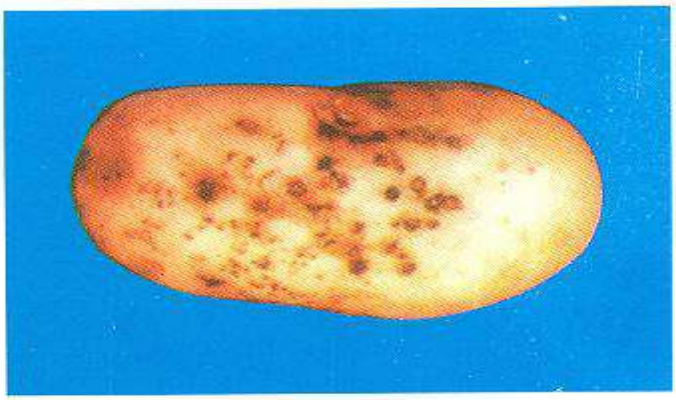

Figure 43. Powdery scab: Potato tubers with typical symptoms

Figure 44. A single lesion showing the powdery mass of resting spores inside and the remnants of epidermis
(source: Spongospora web site)

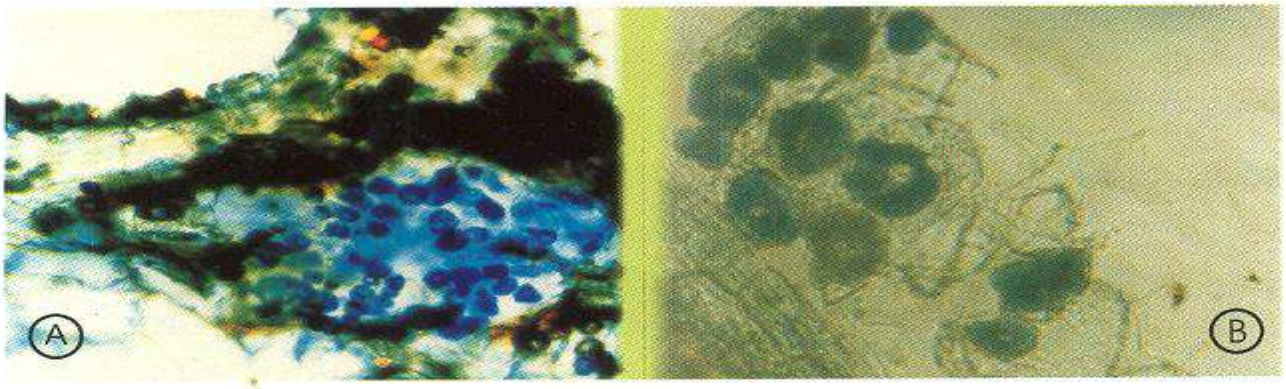

Figure 45.

A) Cystosori or resting spores of *S. Subterranea* within lesion of infected potato tubers

B) Light micrograph of spore balls of *S. Subterranea*

Causal Organism

Spongospora subterranea f. sp. *subterranea* causes the disease in potatoes. It is obligate parasite and belongs to family plasmodiophoraceae. Spore balls or sporosorus are usually spongy, often hollow, or with numerous irregular channels and openings, 19-85 µm mostly 40-80 µm in diameter and each sporosorus consists of 500-1000 resting spores (cysts) 3.5-4.5 µm in diameter (Figure 47). Primary and secondary zoospores are uninucleate, ovoid with two flagella (2.5-4.6 µm).

Figure 46. Potato roots with nodule like galls

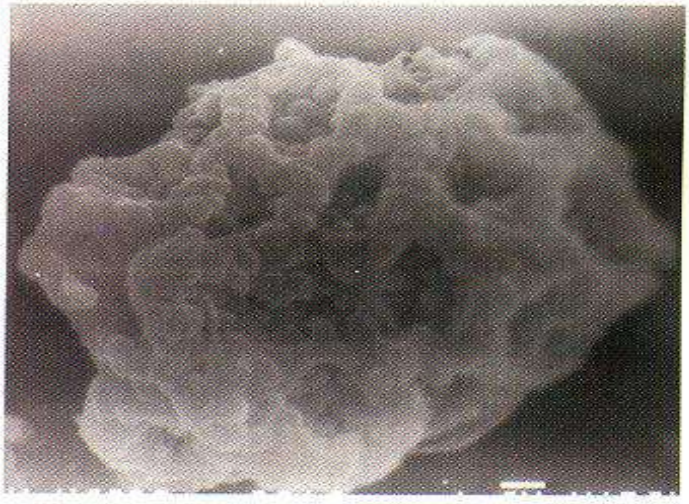

Figure 47. Electro-micrograph of spore balls with numerous spores

The fungus survives in soil in the form of cystosori made up of resting spores. These resting spores are stimulated by the presence of roots of susceptible plants. Resting spores germinate to produce primary zoospores. Penetrate epidermal cells of roots and stolons or root hairs, ultimately producing multi-nucleate zoo-sporangia (Figure 48), which gives secondary zoospores that further spread infection to roots and tubers. Invasion by secondary zoospores stimulates the host cells to become larger and numerous, leading into formation of galls. Within these galls, balls of resting spores are ultimately formed (illustrated lifecycle of fungus is given in Figure 49).

Epidemiology

The initial inoculum for the disease in a particular field may arise from fungal resting spores, present in the soil or are imported with seeds or sludge. No information is available on the level of soil infestation necessary for an outbreak of the disease. Highly infested soils may have not more than 1000 spore balls/g. The resting spores

are able to survive in a dormant state in soil for a number of years (up to 10) and are difficult to kill.

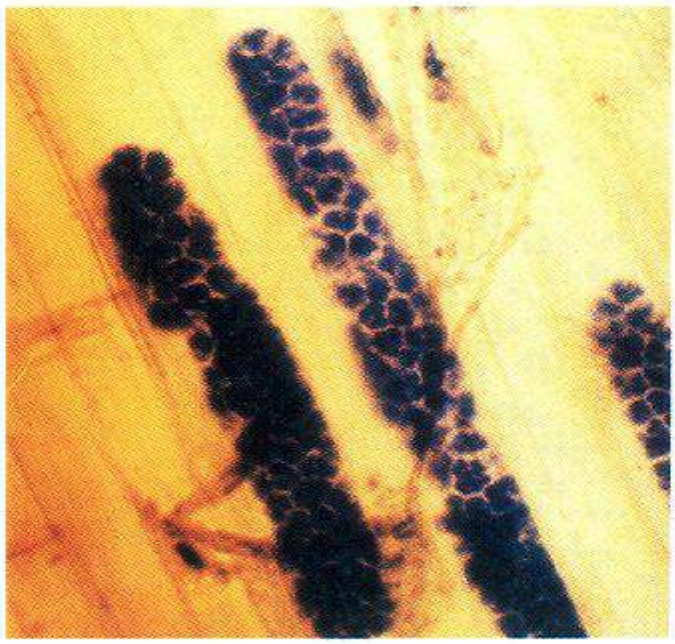

Figure 48. Multinucleated zosporangium of *S. subterranea* in epidermal cells of roots

Disease is predominant in wet and poorly drained soils at low temperature (16-18°C). On the other hand there are reports of its presence in well-drained sandy soil at moderate temperature in Israel and South Africa with 40°C soil temperature (Sopongospora home page, 2000). The nutritional state of the soils had no influence on the occurrence of the disease. The same is true for the pH. In laboratory experiments no relationship between pH of nutrient solution and root infection by zoospores has been found (Merz, 1989).

Other Hosts

The fungus infects other tuber bearing *Solanum* spp. and roots of non tuber-bearing hosts such as *Datura stramonium*, *Solanum nigrum* L., *Lycopersicon esculentum* and *Nicotiana rustica* L without formation of resting spores. Other hosts are Maize (*Zea mays*), sorghum (*Sorghum vulgare*) and pea (*Pisum sativum*) (unpublished).

Detection Methods

Pathogen is identified from tubers and soil through different methods.

i. Light microscopy (see Figure 45).
ii. Electron microscopy (see Figure 47) (Jones, 1978).
iii. Bioassay test (Merz, 1989).
iv. Serology (ELISA) (Walsh *et al.*, 1996).
v. PCR (Polymerase chain reaction) (Bell *et al.*, 1999).

Disease Assessment

The assessment is made on the basis of tubers area covered with the scab on 0-5 scale (Figure 50) (Anonymous, 1985a).

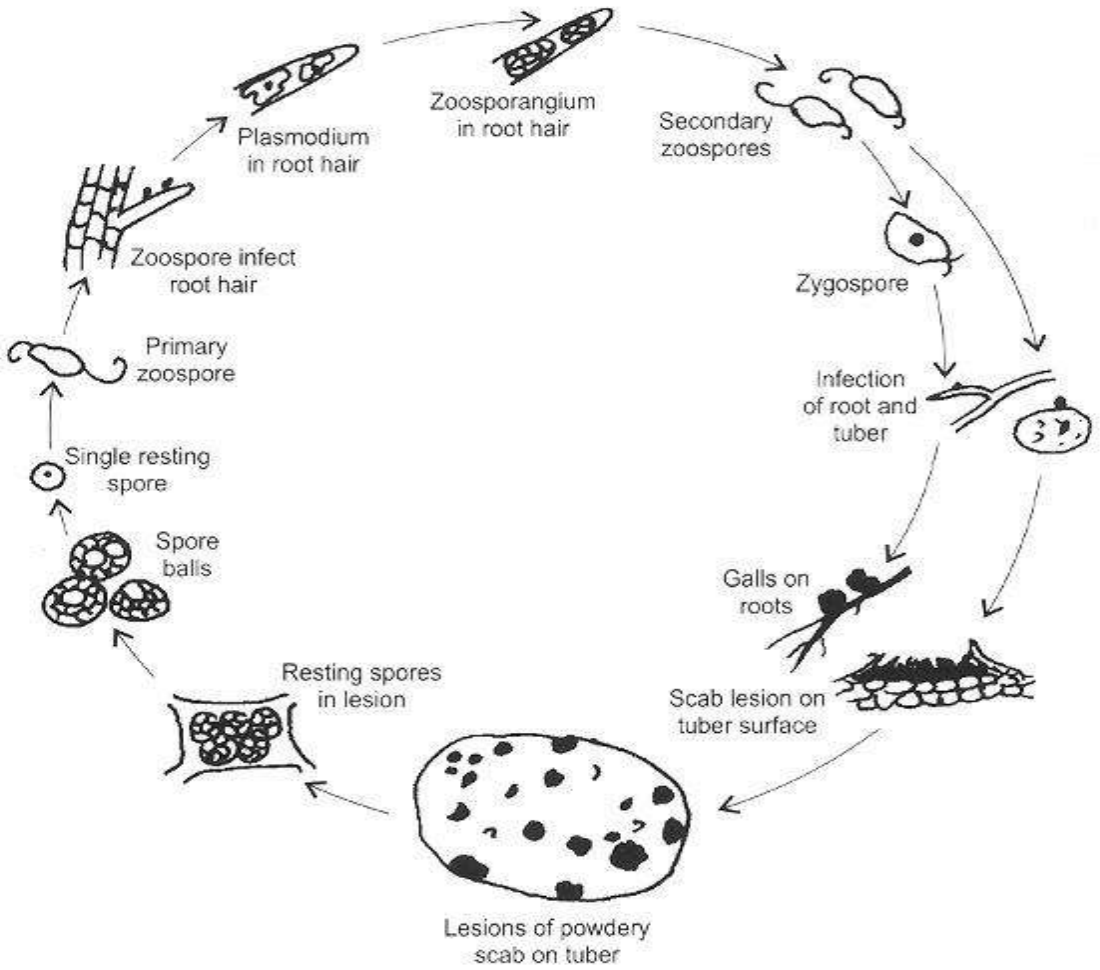

Figure 49. Disease cycle of powdery scab caused by *S. Subterranea*
(Adapted from G.N. Agrios)

0 = no symptoms on potato tubers.
1 = 1% or less area affected.
2 = 1-10% area affected.
3 = 11-20% area affected.
4 = 21-50% area affected.
5 = 51% or more area affected.

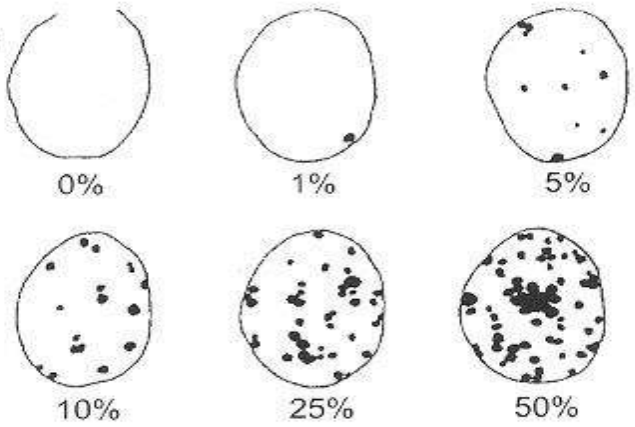

Figure 50. Standard area diagram for assessment of powdery scab

Control

a. Direct control

- Soak infected seed tubers in mercuric chloride to reduce seed borne inoculum but it should be avoided because of its environmental impact. Whereas, zinc can be applied in small plots (Burgess *et al.*, 1992

- Treat the seed tubers with warm water for 10 min at 55°C two month before planting (Mackay & Shipton, 1983).

b. Indirect control

- Use certified seed to minimize the disease.
- Use resistant cultivars. All commercially grown potato varieties are susceptible, however, high resistance is reported in cv. Gladiator from New Zealand (Falloon *et al.*, 1997).
- Irrigation increases powdery scab during tuber initiation so check the irrigation to reduce it significantly (Taylor & flett, 1981; Taylor *et al.*, 1986).
- Follow crop rotation to check and reduce the spread of disease at least 10 years if possible. Use catch crop, like oil seed rape as main or in-between crop to reduce disease incidence (Winter & Winger, 1983).

Wilt/Vertcillium Wilt (*V. albo-atrum* & *V. dahliae*)

Occurrence and Importance

Disease is seed and soil borne. It has been reported in all potato growing areas of the country (Malik, 1995), however, it was first observed in 1985 from hilly areas of Pakistan (Turkensteen, 1986). It may be confused with other diseases that cause early maturity. The disease attack is more severe under stress conditions (low moisture, low fertility, especially low nitrogen in the soil) and affect the yield.

Symptoms

Verticillium wilt is almost identical to Fusarium wilt. In some cases Verticillium induces wilt at lower temperature than Fusarium. Wilting starts from lower leaves and progress upwards. Single stem or leaves on one side of the stem may wilt first. Severely affected plants are stunted and die quickly. Tubers of infected plants may develop a light brown discoloration in the vascular ring. Severe vascular discoloration may extend over halfway through the tuber. Cavities may develop inside the severely affected tubers. Pinkish or tan discoloration may develop around the eyes or as irregular blotch on the surface of affected tubers. This may be confused with mild late blight infection.

Causal Organism

Two species of Verticillium, *V. albo-atrum* and *V. dahliae* have been reported from all potato growing areas of Pakistan (Turkensteen, 1986). *V. albo-atrum* develops septate resting dark mycelium on stem in the field and also in culture in contrast to *V. dahliae* which forms dark mycelial strands with black, thick walled pseudo sclerotia, called micro sclerotia, 30-60 µm in diameter. Vegetative hyphae of both are similar (2-4 µm diameter and colorless). Conidiophore is septate with side branches, swollen at the base and arranged in a whorl (Figure 51). Conidia of *V. albo-atrum* are 6-12 × 2.5-3 µm and those of *V. dahliae* are 3-5.5 × 1.5-2 µm. Conidia are usually single celled or may have one septa. Both types may be present within same potato plant.

Disease Cycle

The disease develops from infected seed or soil. Infection is through root hairs, wounds or through sprout and leaf surface. Hyphae progress intracellularly and intercellularly to the xylem. Transport of conidia within vessels of potato is probable. Both species survive poorly in soil in the absence of suitable hosts. Infectious propagules of dark mycelium and pseudo sclerotia survive in soil, depending on soil type; they germinate and produce conidia within hours.

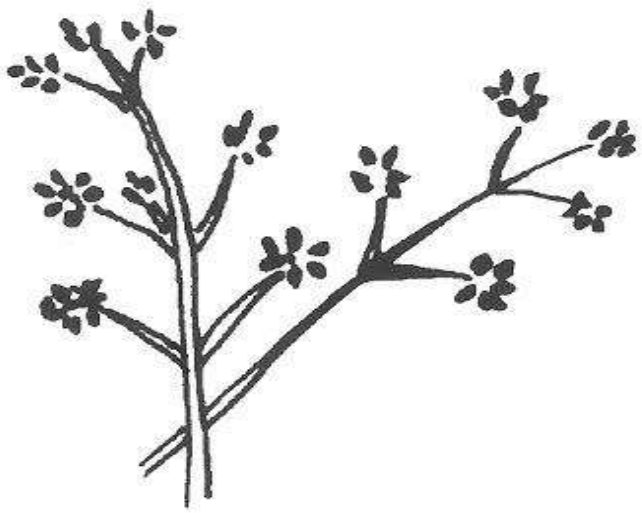

Figure 51. Culsters of conidia on conidiophores of *Verticillium albo-atrium*

Epidemiology

V. albo-atrum is tuber-borne and inoculum remains in soil up to one year. Whereas, *V. dahliae* is soil-borne and may remain up to eight years even though potatoes or related crops are not planted. Warm soil temperature (22-27°C) favors growth of *V. dahliae*, whereas, *V. albo-atrum* is more pathogenic at 16-27°C. Crop rotation (3 years) reduces the inoculum build up in the soil. Inoculum in field soil or in soil adhering to the surface of potato tubers is more important in initiation of wilt symptoms than is inoculum from seed tubers with vascular discoloration. Inoculum spreads from field to field by contaminated equipment or irrigation water. Inoculum may also be air-borne or spread from plant to plant by root contact.

Other Hosts

Both species of Vertcillium have wide host range. Verticillium attacks more than 200 species of plants, including vegetables (such as eggplant, pepper, tomato), flowers (chrysanthemum, aster and dahlia), fruits (apricot, cherry and peach etc.), field crops (cotton, alfalfa and peanut) and forest trees.

Detection Methods

i. Visual observation:
A cross section of stem and of a tuber shows discoloration of vascular bundles.

ii. Light microscopy after isolation on PDA and Specific media (see Figure 51).

Specific media:

Tallioy medium (*V. albo-atrum* & *V. dahliae)* (Tuite, 1969).

Prune extract	100 ml
Lactose	5 g
Difco yeast extract	1 g
Agar	30 g
Dist. Water to make a liter	

iii. Scanning electron microscopy (SEM) (Fox, 1993).

Control

- Use certified seeds.
- Seed tubers contaminated with infested soil should be surface sterlized with 2% formalin before sowing.
- Use systemic fungicide (Benomyl) and non- systemic (Captan, Mancozeb) for seed treatment (Hooker, 1981).
- Follow crop rotation (3-4 years) with cereals to reduce the inoculum.

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DISEASES CAUSED BY NEMATODES

DISEASES CAUSED BY NEMATODES

The plant parasitic nematodes are microscopic worms which live in soil and in plant roots. They attack on roots, buds, stems, crowns, leaves and seeds of crop plants. The degree of damage depends on nematode population, crop stage, and environmental factors. Visible symptoms of nematode attack often include reduced growth of foliar parts and sometime death of plants. The nematodes are known to effect the yield, quality and limit the utilization of nutrients. The infested plants are exposed to be invaded by fungi and bacteria. Some nematode species serve as virus vectors as well.

Plant parasitic nematodes can be divided into three major groups: tylenchs, longidorids and the trichodorids. Tylenchs are ranging from 0.2 to 1 mm long but occasionally over 3 mm. In some genera the female loses the vermiform shape and becomes obese or even globose. The longidorids are much longer and range from 0.9 to 12 mm in size. The trichodorid are short ranging from 0.5 to 1.1 mm, cigar shaped. Based on their habitat they can be divided into three groups:

1. Ectoparasitic – this type of nematodes does not enter the root system but feed by using their stylet to puncture cells.
2. Semi-endoparasitic – in this type of nematodes the interior part of the nematode stays inside the root while the posterior part remains in the soil.
3. Endoparasitic – the entire nematode penetrate in the root.

Morphologically the nematode has a head or labial region that has a central orifice, the mouth through which stylet is protruded. Various sensory structures including the amphids occur on the head. The body is enclosed in a cuticle, which is usually transversely annulated. Beneath the cuticle is hypodermis and muscles. The central cavity of the nematode, the pseudocoelom contains a viscous fluid, within this cavity are three major organs – digestive, reproductive and excretory.

The digestive system comprises stylet; oesophagus; intestine and rectum. The stylet is a protrusible cuticular tube pointed anteriorly and with a sub-terminal aperture and generally swelling posteriorly to form three basal knobs. The oesophagus comprises a narrow cylinder or procorpus, which expand to form the median bulb and then narrows to form isthmus before expanding into the oesophageal

glands. The intestine is a largely undifferentiated tube, which opens via the rectum at the anus or in adult male, the cloaca.

The reproductive system in both sexes is tubular. The female genital system composed of ovary, oviduct, uterus and vagina. The vagina opens to exterior via the vulva. The male system consists of testis, seminal vesicle and vas deferens. The copulatory organ consists of the paired spicules with a guiding ring the gubernaculum. The male tail often has cutiuclar expansions, the caudal alae or bursa, which helps in copulation.

The excretory system consists of a uni-nucleate gland cell connected via an excretory canal to the ventrally situated excretory pore.

Reproduction of the nematode is either amphimictic or parthogenetic. Eggs are either laid singly or stuck together in masses to form a gelatinous matrix. Egg sacs and cysts serve to protect the eggs. The eggs of cyst nematodes require the presence of root exudate from the host to promote hatching. Nematodes have typically four juvenile stages between the egg and adult stage.

In the absence of a live host nematodes may survive in the soil or in plant residues. In a number of genera the eggs are survival stage and are protected in a gelatinous matrix or within the hardened cyst. Thus can survive for a very long time the maximum could be 39 years as in case of earcockle of wheat.

Nematodes found associated with potato crop all over the world are *Globodera* spp., *Meloidogyne* spp., *Ditylenchus* spp., *Nacobbus* spp. and *Pratylenchus*. In Pakistan *Globodera* is wide spread in the Northern area while *Meloidogyne* spp., (root knot) is encountered occasionally. In this chapter we will only discuss potato cyst and root-knot nematode (*Meloidogyne* spp.).

Potato Cyst Nematode

Occurrence and Importance

Potato cyst nematode (PCN) species are believed to have evolved in South America but now have a world wide distribution and are major and persistent pests except in warmest soils (Evans & Stone, 1977). It probably spread to Europe with the breeding material brought for blight resistance in 1850's (Evans *et al.*, 1975). The nematode was first reported from Germany (Kuhn, 1881) and since then it has spread all over Europe. It is believed that golden cyst nematode was introduced in USA on military equipment brought back after the first world war to Long Island, New York but the nematode was not recognized until some 20 years later (Evans & Brodie, 1980). It reached the Indian sub-continent before 1970 being first reported from India in 1972 (Evans & Stone, 1977) and from Pakistan in 1980 (Maqbool, 1980).

PCN was first reported in Pakistan in 1980 from Abbottabad where the infestation level was 80 cysts/100 gram of soil (Maqbool, 1980). Its economic effect was not felt until 1985. Serious damage was not first reported from Abottabad, but from Kalam (Khan *et al.*, 1986) where population of more than 894 cysts/100 gram of soil was recorded. Damage appeared to be restricted to the Utror valley stretching up to 5 kilometres upstream with the river. With the passage of time and without phytopathological restrictions, PCN has spread to the adjoining valleys (Gul & Saifullah, 1990; Maqbool & Shahina, 1989). Recent studies showed that PCN has spread not only on both sides of the river in the valley but also as far north as Sust in the Hunza valley bordering with China (Munir, unpublished).

Symptoms

The above ground symptoms are not very specific, however root injury causes stress and reduced uptake of water and nutrients results in yellowing and discoloration of leaves. Stunting and wilting of plants is prominent under drought conditions. Small immature females of white and yellow colour can be seen on the roots at flowering stage (Figure 52). When females die they become cysts, and their cuticle become brown that contains 300-500 eggs.

Causal Organism

Stone (1972) reported potato cyst nematodes into two species *Globodera (Heterodera) rostochiensis* and *G. pallida*. Prior to that only one specie, *Heterodera rostochiensis* has been described.

Figure 52. Females parasitizing the roots of potato

The discovery that some potato cultivars does not allow a certain population to multiply whereas other population continue to multiply lead to the speculation that only a restricted part of the South American gene pool has been introduced to many countries. *G. pallida* is common to north of Lake Titicaca and *G. rostochiensis* common to the south of this lake. From these regions both species have spread to many of the potato growing areas of the world. The discovery of resistant genes H1, H2, and H3 also lead to the identification of pathotypes of these two species. Kort *et al.* (1977) recognised five pathotypes of *G. rostochiensis* (Ro1-5) and three of *G. pallida* (Pa1-3) on the basis of their multiplication on different cultivars. There are small differences in the life cycles of the two species (McKenna & Winslow, 1972) but it is thought that their biology is essentially similar.

Disease Cycle

The active part of the life cycle begins, when the second stage juveniles (J2) emerge from the eggs after stimulation by substances emanating from host plant roots. The J2 enter the host roots near the tip and use their stylet to cut through the cell walls, leaving a trail of ruptured cells. Finally they come to rest with their heads towards the stele and begin feeding from one cell. Their hollow stylet pierces the cell, injects saliva, and later withdraws some cell contents. The saliva induces cell enlargement and breakdown of surrounding cell walls to form a large, syncytial transfer cell with dense, granular cytoplasm (Jones & Northcote, 1972). Once the juvenile is sedentary, it undergoes three molts to become the adult. Sex is distinguishable at the start of the third juvenile stage. The nematode continues to feed until its development is complete, a period which takes 2 to 3 months depending upon temperature. Fourth-stage males remain coiled within the sac-like third-stage cuticle and emerge from the root after the final

moult. The males are vermiform, about 1 mm long. They live for about 10 days in the soil and apparently do not feed (Evans & Stone, 1977). Adult females enlarge as their gonads increase in size, ultimately rupturing the root cortex and exposing their bodies outside with only their heads embedded in the root. At this stage the female releases a pheromone that attracts the males. Fertilization is accomplished when the males coil around the vulval areas of the female. Each female may undergo multiple mating with many different males (Evans & Stone, 1977).

The female accumulates all her eggs within her body and the embryo develops within the egg upto the second stage juveniles while still in the female's body. When the female dies, the cuticle tans to form a tough, leathery cyst that can contain up to 500 embryonated eggs. When the potatoes are harvested, the cysts are detached from the roots into soil where they over-winter. When the next potato crop is planted, exudates from the roots stimulate the juveniles and life cycle is again initiated (Figure 53).

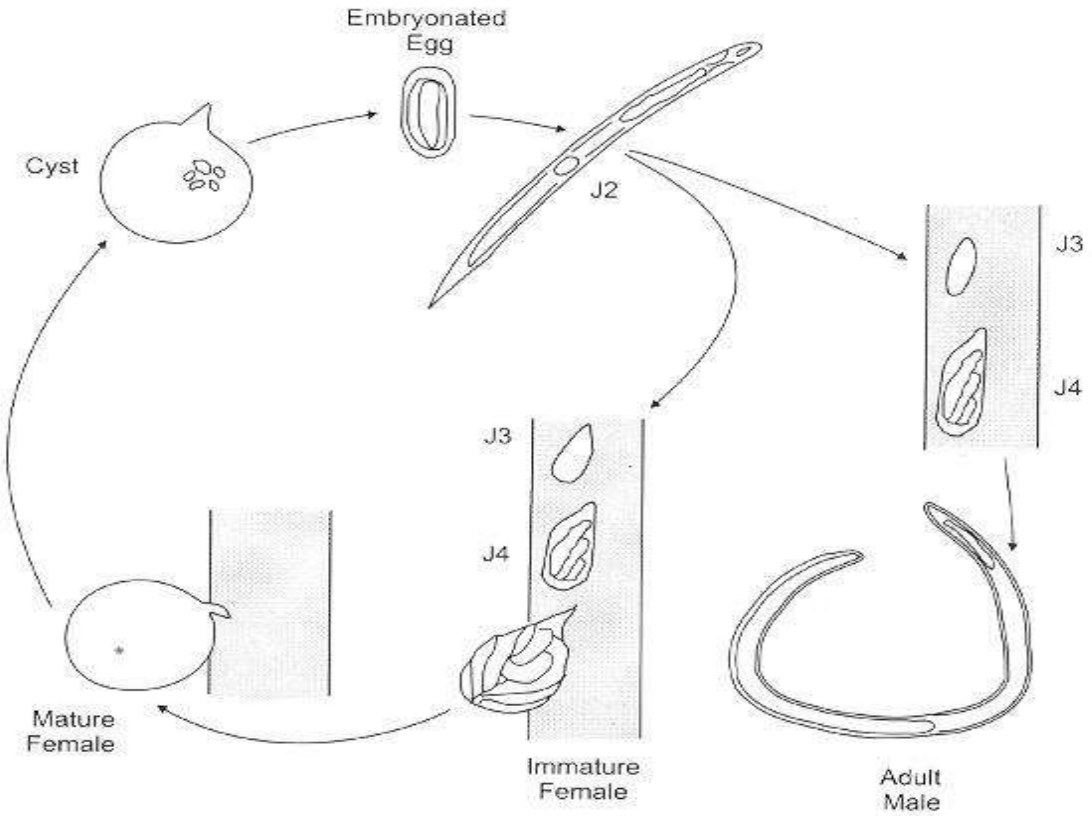

Figure 53. Life cycle of potato cyst nematode

The life cycles of the two species are basically the same-except for differences in temperature adoption, a fact that might have influenced their geographical distribution. *G. pallida* is better-adapted at temperatures between 10 and 18°C than is *G. rostochiensis* (Franco, 1979). At 20°C there appears to be no measurable differences in activity of the two species, but at 25°C, *G. rostochiensis* is better adapted. When soil temperature rise above 30°C for a lengthy period, neither species can survive (Evans & Trudgill, 1978).

Epidemiology

From patterns of trade it is almost certain that PCN in Pakistan came from western Europe. The history of potato cultivation in Kalam and Kaghan valley is not clear. The evidences suggest that potato has been cultivated in these areas since very long time, but cultivation of improved cultivars started in early 80's. This led to the assumptions that PCN has been introduced through seed source. The dominance of *G. rostochiensis* in northern areas is due to the fact that it is better adapted to soils having temperatures ranging from 10–18°C. The mean monthly temperature in Kalam valley during the growing season ranges from 22°C in May reaching a maximum of 27°C in June and then falling to 22°C in September. The conditions, which favour potato production are also favorable for nematode multiplication and survival (Table 16). PCN multiplies well in cool temperatures, and at high soil temperatures for prolonged periods will limit its development and reproduction (Jones *et al.*, 1970). Eggs will remain viable in cysts for 20 years in soils under severe environmental stress (Oostenbrink, 1966). They withstand extreme cold and long periods of desiccation. PCN disseminates by movement of infested soil, farm implements and contaminated tubers as well as by irrigation water (Table 17). A large portion of eggs hatch only in the presence of potato root diffusates. In soils not planted with potato, PCN populations decline between 33% (Huijsman, 1961) and 20% per annum (Cole & Howard, 1962).

Other Hosts

The other hosts of PCN are tuber bearing solanums and tomato. Eggplant is known as poor host of PCN.

Table 16. Incidence of Nematode Diseases

S#	Disease	Plain Crops		Hill Crops
		Autumn	Spring	
1.	Potato Cyst Nematode (PCN) (*Globodera* spp.)	-	-	**
2.	Root Knot (*Meloidogyne* spp.)	*	*	*

* traces **medium ***severe
(Data based on visual observation)

Table 17. Mode of Perpetuation and Plant Parts Affected by Nematodes

S#	Disease	Modes of Infection			Plant Parts Affected				
		Plant debris	Diseased Seed Tubers	Infested soil	Leaves	Stems	Stolen	Tubers	Roots
1.	*Globodera* spp.		+	+				+	+
2.	*Meloidogyne* spp.		+	+				+	+

+ = Yes - = No

Detection Method

Nematode cysts on root can be observed at flowering stage. Soil analysis for extraction of cysts also provides an excellent mean of diagnosis. It is important to note, however, that it takes 7-8 years from introduction until the nematodes become established and reach the detectable level. Microscopic observation provides distinguishable characters for differentiation among the two species of PCN.

Jones *et al.* (1970) also described differences in juvenile length and stylet length, female colour, surface pattern of cuticular ridges around the vulva and anus and stressed the need for further studies to determine whether PCN are one species or two. Bouwman & Ross (1972) also found differences in stylet length between pathotypes of *G. rostochiensis*.

Root-knot Nematode (*Meloidogyne* spp.)

Occurrence and Importance

Root-knot nematodes are cosmopolitan in distribution attacking almost all-major crops and many weed species. Initially all root-knot nematodes were considered to belong to one extremely polyphagous specie, *Heterodera marioni* until Chitwood (1949) re-established the genus *Meloidogyne* Goeldi, 1987 (Netscher & Sikora, 1990). More than 51 species of *Meloidogyne* has been reported from all over the world on different crops (Jepson, 1987). From Pakistan only four species are found infesting more than 100 plant species. Although many species are known to attack potato, only five species are considered to be of global importance. In Pakistan *Meloidogyne arenarea, M. hapla* and *M. javanica* are reported on potato. First report of root knot from Pakistan is of *M. hapla* from Sialkot and Murree hills in 1973 (Riaz & Khan, 1973). *M. hapla, M. arenarea* and *M. javanica* were reported from Gujranwala, Hazara, Lahore, Mardan, Muzaffargarh and Peshawar with different severity levels (Maqbool & Ghaffar, 1986).

Symptoms

The above ground symptoms are manifestation of damage caused to the root system. They are very general, non-specific and, like other soil-borne pathogens appear as clear yellow patches in the field. These symptoms resembles those associated with mineral deficiency, stunted growth, wilting, decline in fruit production and loss in yield. Below ground symptoms include rather more characteristic galling most usually associated with root-knot nematodes. Galling incidence and size depends on nematode density and the nematode species. *M. hapla* galls are usually smaller than those caused by other species and have extensive lateral root formation. Under favorable environmental conditions tuber of all shapes and sizes can become infected (Jatala, 1975). Infested tubers have galls, which give a warty appearance or can become completely deformed (Figure 54). In tubers, nematode females are usually found 1-2 cm below skin feeding on vascular elements.

Causal Organism

The causal organism is species of *Meloidogyne*. The most important among them are *M. arenarea*, *M. hapla*, *M. incognita* and *M. javanica*. There are several races of *Meloidogyne* species and they all attack potatoes in varying degrees (Jatala & Bridge, 1990).

Figure 54. Tuber infested with root-knot nematode

Disease Cycle

The life cycle of this nematode is very much similar to that of cyst nematode. Both roots and tubers are infected, however, the first generation occurs mainly on the root systems, while the succeeding generations attack tubers. There are upto five generations on the susceptible host under favorable conditions (Jatala & Bridge, 1990).

Epidemiology

As *Meloidogyne* species attack a number of plant species, their population can be maintained on weeds and volunteer crops. However, in the absence of a suitable host, their populations are drastically reduced. They over-winter usually in the form of eggs. Infected tubers, plant parts and planting material, as well as movement of infested soil by machinery and irrigation water are the main sources that disseminate this nematode (see Table 17). *M. arenarea*, *M. incognita* and *M. javanica* develop better in higher temperatures and cannot withstand cool temperatures, therefore, they are of economic importance in the plains of Punjab, lower valleys of NWFP and Sindh. *M. hapla* on the other hand develops well in cool temperatures and have an optimum temperature of 20°C (Taylor & Sasser, 1978). They are distributed in northern areas, Balochistan and hills of Murree.

Other Hosts

Meloidogyne species have a wide host range and attack many agriculturally important crops and weeds. Most of the tubers bearing *Solanum* species are susceptible to *Meloidogyne*.

Detection Methods

Roots of infested plants are found having galls formed by the nematode. Staining of tuber and root tissues with 1:1 solution of Lacto-glycerol having 0.05% acid fuchsin (Bridge *et al.*, 1982) help in identifying the females on the roots. The cuticular markings (parinneal pattern) surrounding the vulva and anus of females of *Meloidogyne* spp. are used in the identification of species (Figure 55).

Control Methods

Plant parasitic nematodes invade roots and cause damage to them, which results in the reduction of root growth, uptake of nutrients and tuber yield. Yield loss is proportional to nematode population density at planting (Seinhorst, 1965) but environmental factors such as soil type has also some effects (Trudgill, 1986).

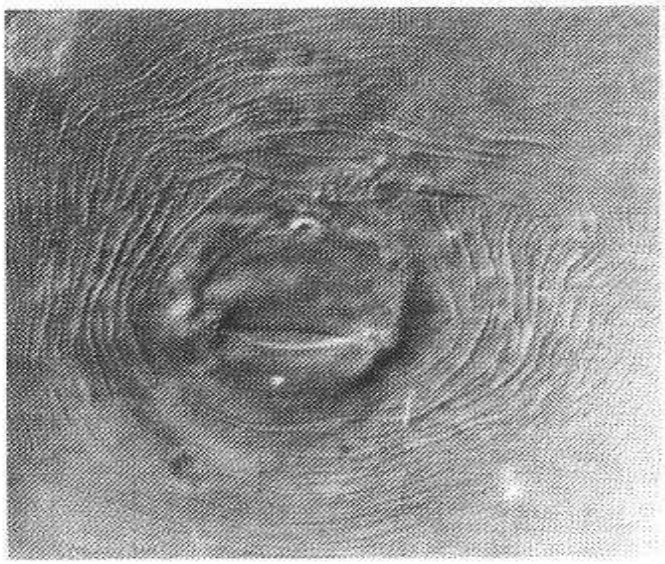

Figure 55. Parinneal pattern of *M. hapla*

PCN can be controlled by chemical, biological or cultural methods, including the use of resistant or tolerant cultivars, and the integration of all three is becoming increasingly important (Trudgill *et al.*, 1992).

Chemical Control

According to Trudgill (1986) in 1982 in Britain alone 21,000 hectares of land was treated with nematicides (c. £200/ha) compared with 6800 hectares in 1976. The increased use of nematicides is partially due to the increased incidence of *G. pallida*. In Pakistan use of nematicides is almost nil as the farmers have small holdings and poor resources.

Nematodes can be controlled by fumigant and non-fumigant nematicides. However, none of these nematicides are completely effective. Fumigants kill 75 - 80% of PCN (Whitehead, 1986). Non-fumigant nematicides such as aldicarb act as nematostats inhibiting hatch (Osborne, 1973), and preventing juveniles from locating and

penetrating host (Hague & Pain, 1970). But these measures are often insufficient to prevent an overall increase in population density. Furthermore, as these nematicides remain active for only a few weeks, some juveniles may escape their effects or recover and attack roots. There have been no reports of chemical control of PCN or root knot in potato in Pakistan.

Biological Control

Biological control of nematodes mainly involves fungi and bacteria, which attack eggs and females. However, so far none have been found which are particularly effective against PCN (Trudgill *et al.*, 1992), Crump & Irving (1992) used *Verticillium chlamydosporium* in their experiments and observed 76% control of first generation eggs of *G. pallida*. Hackenberg & Sikora (1994) reported significant reductions (25%) in *G. pallida* root invasion rates in green house studies with the use of *Agrobacterium radiobactor*. In Pakistan Saifullah *et al.*, (1988) used *Paecilomyces lilacinus* and observed some promising results. Munir *et al.* (1988) isolated a number of fungi from cysts and eggs. Since they isolated fungi from surface-sterilised eggs they suggested further work on these fungi to determine their potential as bio-control agents. Effective control of root-knot nematode using *Paecilomyces lilacinus, Pasturia penetrnase, Verticillium chlamydosporium* and *Bradyrhyzobium* sp. has been reported but in crops other than potato crop (Zaki & Maqbool, 1992; Abid *et al.*, 1992; Zaki & Maqbool, 1993; Haque *et al.*, 1994; Parveen & Ghaffar, 1998).

Natural Compounds

Materials with high phenolic content are often used as soil amendments for nematode control. The nematicidal activity of marigold (*Tagetes spp.)* has been recognised for many years, which has polythienyls as toxic compound (Uhlenborek & Biljoo, 1958, 1959). Rich *et al.*, (1989) has shown in their work that ricin, a protein derived from castor bean, has an adverse effect on the motility of *Meloidogyne incognita*. Juices extracted from a number of plants contain compounds toxic to nematodes. Zurreen & Khan (1984) tested latices from a number of wild and cultivated plants against *M. javanica*. They observed a significant reduction in egg hatch. Nandal & Bhatti (1986) also carried out similar studies and found it effective. Oil cakes of sunflower, peanut and neem were also been tested

against many root-knot species and were reported as having nematicidal properties. Munir (1998) observed that neem oil at 4% is phytotoxic, but at 0.065% the 51% reduction in PCN cysts occurred. Different parts of neem plant have also received the attention of many nematologists who tested its efficacy and reported as having nematicidal properties. Olive pomace against root-knot nematode have also been found effective (D'Addabbo, *et al.*, 1997 and Rodriguez-Kabana, *et al.*, 1995).

Resistance/Tolerance

Trudgill (1991) defined resistance as being conferred by host genes that restrict or prevent nematode multiplication in a host species. In contrast, tolerance relates to the ability of a host genotype to withstand or recover from the damage caused by nematode attack and to yield well.

Growing resistant potatoes usually prevents nematode reproduction and the remaining population comprises those eggs, which have not hatched. Partially resistance decreases the rate of multiplication compared with that on susceptible cultivars, but at low population densities some population increase may still occur. The use of resistant cultivars to control PCN has several advantages over other methods; these are: economy, less technical knowledge required, no toxic effects and, if control is effective rotations can be shortened. However, if they are to be grown in heavily infested soil exact knowledge of their tolerance must be known, as intolerant cultivars will suffer extreme damage. In contrast, tolerant cultivars that are not resistant tend to increase nematode population densities to damagingly high numbers (Trudgill, 1991).

In Pakistan there is no published work on resistance, however (Soomro, unpublished) carried out some varietal assays against PCN and found six potato clones viz Skirze, Alhamra, Stemstern, Kenzy, 278072-10 and 299142-12 resistant to PCN.

As in Pakistan the *Globodera rostochiensis* is prevalent, therefore, varieties having H1 genes can be used to mange the PCN problem. Cardinal is commercially grown in Pakistan that contain H1 gene.

Cultural Control

Cultural control includes rotational growing of non-host or resistant varieties and soil management to decrease nematode survival. Whitehead (1995) reported a decline of 12.8-40.5% in eggs of *G. pallida* and *G. rostochiensis* when spring barley was grown for four consecutive years. In Pakistan in the PCN-infested areas, a decrease of 30% cyst population was observed when non-host crops such as turnip and cabbage were used as rotational crops (Munir *et al.*, 1995).

Ellis (1968) determined that *Lycopersicon hirsutem* var. *glabratum* produce root diffusates of high-hatching activity and this feature would make the plant a good trap crop. Use of potato cultivar before actual planting is also tested by Whitehead (1977, 1985), Brodie (1982) LaMondia & Brodie (1986) as trap cropping. Munir (1998) during his thesis research found that tobacco plants provide moderate hatch of PCN at the same time it is a non-host crop.

Other Important Nematodes

Nacobbus aberrans (The false root-knot nematode)

The nematode is found in tropical and temperate regions of South American continent, USA, USSR, It is considered the most important constraint to potato production in southern Peru and Columbia (Mai *et al.*, 1981). In England and Netherlands in glass houses (Franklin, 1959). Although there is report of this nematode from India but its presence in Pakistan can not be confirmed (Sher, 1970).

Ditylenchus destructor (Potato rot or tuber nematode)

Potato rot nematode occurs in many potato producing countries, but the damage is only apparent in temperate zones.

Less Important nematodes associated with Potato in Pakistan

Agelenchus, Aphelenchus, Dolichorhynchus, Helicotylenchus, Hoplolaimus, Merlenius, Paktylenchus, Paratylenchus, pratylenchus, Psilenchus, Quinisulcius, Rotylenchulus, Tylenchus, Tylenchorhynchus and *Zygotylenchus* (Maqbool, 1986).

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PACKAGE OF SEED POTATO PRODUCTION

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Introduction

The potato crop degenerates rapidly in the plains of Pakistan. Just in two or three seasons of successive propagation of the stocks reduce its yield potential by more than 50 percent. In this context highest research priority at PARC was given to develop a technology which would permit production of reasonably disease-free seed by avoidance of vector (aphids) populations in growing season and adoption of appropriate plant protection measures. The following procedure will help to produce quality seed locally, at a price that even small farmers can afford. This also ensures the supply of disease-free seed of right physiological age at the planting time to growers and the present average yields of 13-15 tonns per hectare may be at least doubled.

Causes of Degeneration of Seed Stocks and its Consequences

Potato, a vegetatively propagated crop, degeneration is mainly caused by tuber-borne diseases i.e viral and phytoplasma-associated maladies. The infected stocks produce weak and sick plants having different symptoms on leaves, ranging from negligible mottling to severe mosaic, crinkle, venial necrosis, and rolling. Their combined effect brings about reduction in plant vigor, tuber size and yield. Virus infected stocks do not respond well to fertilizers and cultural practices. Once infected, the viruses permeate to all vegetative parts and also to the tubers. Infectious nature of the viruses explains that why the seed stocks degenerate rapidly and the yield progressively.

Physiological degeneration, especially under tropics, which is a manifestation of physiological aging, chronological age, warmer temperature during growing season and longer storage also assumes an acute problem in seed production. The physiological age of the tuber affects the rate of sprout growth, plant emergence, plant growth, its pattern of tuber initiation, its build up, tuber number, tuber size, and crop maturity. Plants grown from physiologically older seed

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[1] *Article contributed by Khalid Farooq, SSO, Potato Pogram, NARC, Islamabad.*

known as stage of senility emerge earlier, have numerous main stems with poor/low vitality and the tubers produced are small in size. Such plants are subject to many pests and diseases. On the other hand, physiologically younger seeds, known as partially dormant are slow in emergence; plant stand is erratic and subsequently a longer period is required for tuber sizing. Besides, such a crop is subject to a longer epiphytic period and higher aphid incidence towards maturity.

Mechanical mixture during storage or growing season also reflects on the varietal purity and thus lowers the seed value. Volunteer plants (groundkeepers) especially in summer crop lead to some problems.

Basic Principles of Quality Seed Production

The basic approach for raising healthy seed has wider applicability; the cultural details differ from area to area depending upon environmental conditions and disease build up. It is in this context that main ingredients of seed production technology are described here based on practical experience. These include:

Selection of suitable sites and varieties for seed production

Selection of eligible areas and growing periods based on studies on appearance and build up of aphid *(Myzus persicae)*, efficient vector, as well as incidence of tuber and soil borne diseases and pests are the pre-requisites for growing a healthy seed crop. These areas are well exposed, wind-swept where the climatological factors allow the required period for potato production and avoidance of vector populations. Once pathogen free stocks are developed, they are introduced into the system of growing in the vector free period with other precautions, such as timely roguing and removal of haulm before the critical aphid build up, but critical levels of aphid catch vary with the system used. For visual counts 20 aphids per 100 compound leaves and 3 for sticky traps. The health standards of seed stock can be maintained for several generations. Observations have shown that annual rate of degeneration by following this technique is less than 0.5 percent.

Note: *In Pakistan autumn and summer seasons are suitable for seed potato multiplication.*

Development of nucleus stocks and production of basic seed

a) Start with basic seed (produced by tissue culture techniques). The basic seed is available from the following organizations:

   i) Tissue Culture Lab., NARC, Islamabad
   ii) Tissue Culture Lab., AARI, Faisalabad
   iii) Punjab Seed Corporation, Sahiwal
   iv) Tissue Culture Lab., Agriculture Department, Northern Areas, Gilgit
   v) Potato Research Station, Abottabad

b) If basic seed is not available then select healthy plants. It comprises selection of apparently healthy hill units and true to type, and tuber indexing to check their disease status. Single eyes from the rose-end of each tuber is scooped out and planted in a screen house. Such raised plantlets are visually examined (when about 15 cm high) for visible mosaics, leaf roll and any other such abnormalities.

   In locations where climatic factors do not allow development of nucleus stocks as described above, the basic stocks of the adapted varieties may be imported in small quantities to feed the national seed programs. These initial valuable stocks are multiplied in the following manner:

   - **First year clones:** Nucleus tubers are planted at 1 × 1 m spacing to avoid transmission of contagious viruses, PVX and PVS. The crop is inspected thrice during a crop season for genetic purity and visible mosaic symptoms, if any, to be rouged out.

   - **Second year clones:** The produce of the first year selection is grown (10 tubers selected per clone) at inter row spacing of 1m with 30 cm plant to plant. The crop is visually checked thrice for genetic purity and viral diseases during the crop season.

   - **Third year clones:** The clones of the second year selection are planted at spacing of 75 × 30 cm, separately about 100 tubers per clone. The crop is observed vigorously for genetic purity and disease freedom. Only high yielding clones, true to type are bulked after harvesting and designated as basic seed.

Seed increase programs during autumn season

The basic seed should be increased subsequently into certified grade I and II under supervision of technical staff. Roguing of the crop should be attended as early as possible when the symptoms are exhibited. First inspection of the crop for genetic purity and freedom from diseases should be done when the crop is 40-45 days old. Second one is carried out before the plants in the adjacent rows touch each other. In the situations where flowering occurs, the crop may be examined for the 3rd time at the flowering stage to remove off types and genetic mixture. All such plants should be carefully removed and destroyed including their daughter tubers.

Disease and pest management

Major diseases encountered in Pakistan are late blight (*Phytophthora infestans*), bacterial wilt/brown rot [*Ralstonia (Pseudomonas) solanacearum*], viruses, in some areas phytoplasma and, cyst as well as root-knot nematodes. Among the insect pests; mites, aphids, cutworms and whitegrubs are important.

Late blight: It is effectively checked by growing field resistant varieties and prophylactic sprays of suitable fungicides like Dithane M-45 and Ridomil @ 2-4 gm/l when the crop cover is complete. Subsequent periodic sprays at 10-15 days interval may be resumed with the onset of epiphytotic. Later in season, when it is not feasible to control, the crop should be dehaulmed and the ridges properly dressed up to avoid tuber infection. This is a very important step to avoid carrying over of late blight inoculum to the next generation.

Bacterial wilt/brown rot: Basic seed program should not be located in an area endemic for the bacterial pathogen. At least 3-4 years rotation with cereal crops is advisable to keep the soils free from this pathogen. Green manuring is also very helpful.

Viruses and phytoplasma: For insect transmitted diseases, use of systemic insecticides, which help in arresting and lowering the vector population, helps in preventing vector damage upto 6-8 weeks. Thereafter, the crop may be sprayed periodically with systemic insecticides like Karate, Thiodon, Match, etc. Roguing and dehaulming have to be undertaken as already described with the exhibition of symptoms and build up of critical number of

aphid vectors. In fact dehaulming is the main ingredient of the Seed Plot technology. Less important pests like cutworms and white grubs can be effectively controlled by application of soil insecticides at the time of planting (Furadon, Basudin 5-6 kg/ha).

Further, avoiding the use of implements that have been employed in the table potato crop can also check the spread of such viruses.

Timely lifting also helps to prevent infection with black scurf to some extent. Black scurf is widely prevalent in all potato growing areas, could be successfully controlled by seed treatment with 3% boric acid. Common scab (*Streptomyces scabies*), which is now assuming wider importance can also be controlled by seed treatment (3% boric acid, dip tuber ½ hours before planting).

Package of improved crop production technology

The following improved production technology is recommended to enhance yield of healthy tubers:

- The field should not have had the potato crop in the preceding year. Hot weather cultivation should be adopted to minimize the root inocula of different pathogens.
- Plough the fields during summer months. Keep the land open in July and August, plough once or twice to reduce the incidence of soil-borne diseases and also control the perennial weeds.
- The common aphid host plants like mustard, radish and other crucifers, malvaceous, and cucurbitaceous crops as well as weeds should not be allowed nearby. Complete isolation of the basic seed multiplication area is a must.
- **Green manuring:** Sow green manure crops during kharif in June. Burry the crop after 7-8 weeks to allow for proper decomposition before potato planting.
- **Seed source:** The seed should be obtained from a reliable source, preferably from a Government seed producing agency. Only basic or certified seed should be used. It is better to replace seed stock every 3-4 years.
- **Field preparation:** After green manuring, prepare the field for planting. The field should be leveled and provision made for drainage. Plough the fields with a mould-board plough or disc

harrow followed by one or two tillage with a desi plough. Plank the soil after each round of tillage.

- **Seed size and rate:** Use seed tubers each of 35-45 gm and having multiple sprouts. The seed rate vary from 2-2.5t/ha depending upon seed size. The multisprout tubers have the ability to produce large number of seed size tubers, leading to high yield.
- **Seed preparation:** Seed potatoes should be removed from the cold store at least 10 days before planting. Keep the seed bags in pre-cooling chamber of the cold store for at least 24 hours. Do not bring the bags directly outside as this will result in rottage due to immediate exposure to high temperature. The tubers should be spread in a thin layer under shade in diffused light for pre-sprouting. Unsprouted and rotten tubers should be removed periodically. Sprouted tubers should be taken to the field in seed trays or baskets for planting to avoid sprout damage.
- **Planting time:** Plant the crop between 25 September to 10 October (autumn) and 1-30 May (summer).
- **Manuring:** Broadcast farmyard manure (FYM) @ 12-20 t/acre before ploughing. Chemical fertilizer @ 100:50:50 NPK/acre. The application of NPK with placement or banding method is more economical than broadcast method.
- **Irrigation:** Pre-sowing irrigation is advantageous for uniform germination. If it has not been given, then the first irrigation should be given immediately after planting. It should be a light one to avoid damage to the ridges. Second irrigation should be given after about a week. Subsequent irrigations are given as and when required. Light and frequent irrigations are better than heavy irrigations at greater interval. Stop irrigation about 10 days before haulm killing.
- **Roguing:** During the crop season, examine the seed plot thrice to remove off-type and diseased plants showing mosaics, mottling, veinal necrosis, crinkling, rolling of leaves, marginal flavescence and purple top roll symptoms. First roguing is done 25-30 days after planting and immediately before earthing up. Second roguing should be done 45-50 days after planting. Third roguing, if needed, should be done 3-4 days before haulm killing. Care

should be taken to ensure that all the tubers of the diseased and off type plants are also removed.

- **Haulm killing:** Kill the haulms of the crop in the last week of December or when 3-5 aphids/100 compound leaves are observed in the field. Ensure that re-growths do not appear on the stumps after dehaulming, as tender and succulent leaves are even more attractive to the aphid vectors.
- **Harvesting and grading:** Harvesting should begin 10-15 days after haulm killing when the skin of the tubers has become firm. Harvesting should not be delayed under any circumstances. Keep the freshly harvested tubers in heaps in a cool place for about 10 days. The size of the heap should be about 1.5 m high and 3.5 m broad. Cover the heaps with paddy or wheat straws to protect them from direct sunlight. If it rains during the period, the heaps should be covered with tarpauline. Grade the tubers according to their size preferably in four grades, small (below 35 gm), medium (35-55 gm), large (55-75 gm) and extra large (above 75 gm). At the time of grading, cut and cracked tubers should be sorted out and removed.
- **Seed storage:** Store the seed bags in a cold store latest by 5th March, otherwise, the rising temperature will deteriorate the quality.

APPENDIX - 1

GLOSSERY

GLOSSARY

Alternative host	One of two kinds of plants on which a parasitic fungus (e.g., rust) must develop to complete its life cycle.
Amorphous X-bodies	Inclusion, of irregular shape, usually consisting mainly of protein.
Amphids	Sensory organ in nematodes. (Chemoreceptors)
Anatomical	Related with internal structure of an organ or organism.
Anatomical deviations	A change in the internal structure of an organ or organism, e.g., as provoked by a pathogen of other factors.
Antagonistic	Organism can either inhibits or kill the growth of an organism.
Anthridia	The male sexual organs of fungi.
Antibiotics	A chemical compound produced by micro-organism which inhibit or kills other micro-organisms.
Antigenic virus	A virus able to induce antibodies when introduced into an animal.
Antiserum	An animal blood serum containing specific antibodies.
Apical meristem culture	Laboratory cultivation of the zone of active dividing cells at the shoot or root tip of a plant.
Artificial medium	Any nutrient system for the cultivation of cells of plants, bacteria or other organisms; usually a complex mixture of organic and inorganic nutrients.
Asexual	Type of reproduction that does not involve union of gametes or meiosis.
Avirulant	Lacking virulence.
Bacillus	A rod shaped bacterium with rounded ends.

Bacteria	Extremely small, relatively simple prokaryotic microorganisms traditionally classified with the fungi as Schizomycetes.
Basidiospore	Spors formed on a club shaped basidium.
Bioassay	A test to measure the relative infectivity of a pathogen.
Biochemical	Chemistry of plants and animals and their life processes.
Biological control	The control of pests or diseases by exploiting some biological features, such as parasites, predators, natural enemies or antagonists.
Biotype	A subgroup within a species usually characterized by the possession of a single or a few characters in common.
Blanching	An extreme chlorosis; the absence of all tissue pigments.
Blight	Disease characterized by general and rapid killing of leaves, flowers and stems.
Blotch	Disease characterized by large and irregular in shape, spots or blots on leaves, shoots and stems.
Buoyant density	The intrinsic density which a molecule, virus or sub-cellular particle has when suspended in an aqueous solution of a salt, such as CsCl, or a sugar, such as sucrose.
Callose	A carbohydrate in plant cells that plugs the sieve tubes and stops them functioning.
Canker	A necrotic, often sunken lesion on a stem, branch, twig of a plant.
Capsule	A relatively thick layer of mucopolysaccharides that surrounds some kind of bacteria.
Catenulate	Formed in chains or in an end to end series.
Chlamydospore	A thick walled asexual spore form by the modification of a cell of a fungal hypha.
Chlorosis	Yellowing or bleaching of laminae or other normally green organs of plants due to destruction or decrease of chlorophyll in the chloroplasts.

Chlorotic	Lacking chlorophyll. Light green or yellow color due to destruction of chlorophyll.
Chlorotic chevrons	Yellowing or bleaching of laminae or other normally green organs forming V shaped lesions.
Clonal selection	Selection of favorable individuals from a progeny originating by asexual multiplication from one individual.
Coat protein	The protein forming the outer surface coat of virus particle.
Color-breaking	A streaking of the pigmentation of petals due to viruses or genetic abnormalities.
Companian cell	A specialized parenchyma cell occurring in close developmental and physiologic association with a sieve-tube member.
Conidia	Asexual fungus spores formed from the end of a conidiophore.
Conidiophore	A specialized hypha on which one or more conidia are produced.
Cross-protection	Prevention of infection or suppression of the symptoms due to a second virus inoculation by prior infection with a related strain.
Cultures	To artificially grow microorganisms on a prepared food material.
Cuticle	A membranous layer on outer wall of epidermal cells consisting primarily of wax and cutin.
Cyst	A sac, especially a resting spore, in case of nematodes hardened female body.
Cystosori	Encysted compact mass of spores.
Cytoplasm	The protoplasm of an animal or plant cell external to the nucleus.
Deoxyribonucleic acid (DNA)	An organic substance of high molecular weight composed of nucleotides linked with deoxyribose, for the most part, in cell nucleus; the genetic material of organisms and some viruses.

Disease	Any disturbance of a plant that interfere with its normal structure and functions.
Disinfecting	A phenomenon in which a physical or chemical agent that frees a plant, organ or tissue from infection.
Dissemination	Trasfer on inoculum from its source to healthy plants.
Double stranded	Consisting of two strands twisted into a double helix and joined by hydrogen bonds between the organic bases.
Ellipsoidal	Plane sections having circular shape.
Enation formation	An abnormal outgrowth on a leaf, usually growing from the veins of the leaf's underside and causing a 'rasp leaf'.
Epidemic	A wide spread and severe outbreak of a disease.
Epidermis	The superficial layer of cells occurring on all plant parts.
Eradication	The elimination of a pathogen or pest from the host or from the host's environment, or both.
Facultative anaerobic	An organism usually required oxygen can live in the absence of molecular oxygen.
Filament	A thread like, filiform structure.
Flagella	Whiplike structures projecting from a bacteria or zoospore and functioning as organs of locomotion.
Flecking	Spots of different size and color on the leaves, fruits or other parts of a plant.
Fungicide	A compound toxic to fungi.
Fungus	Nucleated, usually filamentous, sporebearing organism devoid of chlorophyll.
Gall	A swelling or over growth produced on a plant as a result of infection by certain pathogens.
Genetic protection	Protecting plants from diseases by introducing/ incorporating resistant genes.
Genome	The sum of all genes in chromosomes.

Genomic RNA	The RNA of viruses containing the collection of genes.
Host	A plant that is invaded by a parasite and from which the parasite obtains its nutrients.
Hybridization	The act or process of producing hybridization. Species races, varieties and so on, among plants or inter breeding of animals; a process of forming a hybrid by cross pollination of plants or by mating animals of different types.
Hypersensitive	An organism having abnormal sensitivity to infection or to some substances, forming quick necrosis of cells or tissues immediately around the point of inoculation.
Hyphae	A single branch of a mycelium.
Immunity	The highest degree of resistance; plant species or cultivars are not hosts, i.e. they cannot be infected under any circumstances.
Immunogenic	The antigen causing immune reaction.
In vitro	In an artificial medium; outside the living organism; in culture.
In vivo	Within the living organism.
Inclusions	A proteinaceous material produced in the virus-infected cell consisting of arrays of virus particles or sites of DNA or RNA synthesis or a product of the reaction of the cell to virus penetration; it occurs in the cytoplasm and/or in the cell nucleus.
Infection	Establishment of infectious agents in the body of a susceptible host.
Infestation	Plant surface or soil contaminated with bacteria, fungi, etc.
Inoculum	The pathogen or its parts that can cause disease.
Intercalary	Formed along and within the mycelium, not at the hyphal tips.
Intercellularly	Within or through the cells.

Invasion	The spread of pathogen into the host.
Leaf curling	The wrinkling of plant laminae.
Leaf distortion	Deformation of leaf shape.
Leaf narrowing	Reduction in the leaf width; reduction in growth, where midrib and main veins are almost normal.
Leaf rolling	Abnormal upward or downward rolling of the leaves due to a change in the leaf turgor, poor translocation of assimilates from leaves to roots due to blockage or disruption of the vascular bundles; uneven growth of the palisade or the spongy mesophyll.
Lesions	A localized area of discolored, diseased tissue.
Line pattern	Forming of various patterns in which narrow stripes or lines prevail.
Local lesions	Discrete necrotic, chlorotic or discolored areas, usually around the site of inoculation.
Malformation	An unnatural shape or abnormal development of certain organs in the diseased plant.
Mechanical inoculation	The transmission of a virus by the sap of infected plant through mechanical injury of the host cells.
Mosaic	A symptom of some plant virus diseases characterized by chlorosis or mottling of the leaves; normal green and yellowish spots or flecks on the lamina.
Mottling	The discoloration of leaves in the form of large flecks or spots.
Mulches	Any material such as straw, dust, leaves, plastic film and loose soil that is spread upon the surface of the soil to protect the soil and plant roots from the effects of rain, soil crusting, freezing or evaporations.
Mycelium	The hyphae that make up the body of a fungus.
Necrosis	The death and subsequent darkening of cells, tissues or organs of the infected parts.
Nematode	The usually microscopically small worms of

	orders *Dorylaimidae* and *Tylenchidae* living as saprophytes in water or soil, or as parasites on plants; some of them are vector of viruses (genera *Trichodorus, Xiphinema, Longidorus, Paratrichodorus*).
Non-persistent manner	In which virus is not retain for long (more than a few hours) by its insect vector. Such viruses are probably carried superficially on the stylets of aphids and do not multiply in the vector.
Nucleic acid	The macromicules involved in protein synthesis and in the genetical mechanism of all cells; are composed of pentose, phosphorus, pyrimidine and purine bases.
Nucleocapsid	A complex of subunits or capsomers incorporating nucleic acid; the name for the capsid (protein coat) and the associated infectious core of a virus particle.
Nucleotide sequence	The sequence in which the individual nucleotides are arranged in the linear molecules of a nucleic acid.
Nucleotides	The structural unit of a nucleic acid always having one of four heterocyclic bases linked to pentose (ribose or deoxyribose) and phosphoric acid.
Obligate parasite	A parasite that only lives on or within another living organism.
Oogonia	The female gametangia of some phycomycetes conaining one or more gametes.
Oospore	A sexual spore produced by the union of two different gametangia.
Papillate	Bearing a papilla i.e., a hump or swelling.
Parasites	Organisms living on or in another living organism (host) and obtaining its food from the latter.
Parenchyma	A tissue of higher plants consisting of living cells with thin walls that are agents of photosynthesis and storage.
Pathogen	An entity that can incite disease.
Perfect stage	The sexual stage in the life cycle of a fungus.

Persistence	The period of time in which vector retains a virus.
Persistent manner	In which virus is retained by its vector for weeks or months and sometimes throughout the whole life of the vector.
Phenotype	The external visible appearance of an organism.
Phloem necrosis	The discoloration and death of vascular bundle phloem cells; the pathogen may be present in the affected tissue.
Phytoplasma (Mycoplasma)	A group of pathogenic microorganisms resembling the L-forms of bacteria; they are of spherical, elongated or irregular shape enclosed in a three-layered membrane.
Pleomorphic	Having more than one shape.
Pollen	The small male reproductive bodies produced in pollen sacs of the seed plants.
Predators	An animal that preys on other animal as a source of food.
Primary Infection	The first infection of a plant is the spring by the over-wintering pathogen.
Profuse branching	Excessive branching from same/one origin.
Prokaryote	No nuclear membrane is present in a single cell.
Propagules	Any part of an organism capable of independent growth.
Pseudo sclerotia	Sclerotia like structures.
Pseudocoelome	Cavity like structure.
Puckering	Abnormal curvature of the leaf blade due to uneven growth as a consequence of disease.
Quarantine	Control of import and export of plants to prevent spread of diseases and pests.
Race	A genetically and often geographically distinct mating group within a species; also a group of pathogen that infect a given set of plant varieties.

Resistance	The power of an organism to overcome completely or in some degree the effect of a pathogen or other damaging factor.
Resistant	Possessing qualities that hinder the development of a given pathogen.
Ribonucleic acid (RNA)	The nucleic acid with a glycide ribose in its molecule; it takes part in protein synthesis and in many protein functions as genetical material.
Ring-spotting	Formation of more or less regular, lightgreen, yellow or necrotic (often concentric) rings on the leaf blade or other plant parts; a symptom of a plant disease.
Roguing	The removal of virus infected plants in order to obtain virus-free planting material.
Rosetting	An abnormal growth of leaves at the shoot apex due to shortening of the internodes or to increased leaf production.
Rot	The softening, discoloration of a succulent plant tissues as a result of fungal or bacterial infection.
Rugosity	Crinkling of the laminae or bark due to unequal growth.
Russet	Brownish, roughened areas on skin as a result of cork formation.
Sanitation	The removal and burning of infected plant parts, decontamination of tools, equipments, hands, etc.
Sap transmissible virus	A virus which can be transmitted by sap inoculation from infected to healthy plants.
Saprophyte	An organism that uses dead organic material for food.
Scab	A roughened, crustlike diseased area on the surface of a plant organ.
Sclerotia	Compact mass of hyphae and capable to survive under unfavorable conditions.
Secondary infection	Any infection caused by inoculum produced as a result of a primary or a subsequent infection; an infection caused by secondary inoculum.

Secondary symptom	The appearance of additional, secondary reactions of the host to a pathogen infection; they are mostly systemic symptoms.
Sediment	Matter that settles to the bottom of a liquid.
Sedimentation coefficient	A quantitative expression of the motion velocity of a given type of molecule in a unit gravitational fields; depends on the size, shape and weight of the molecule.
Seed	A fertilized ovule containing an embryo which forms a new plant upon germination.
Septa	A cross wall (in a hypha or spore).
Sequencing	The determination of the order of nucleotides in a DNA or RNA molecule, or that of amino acids in a polypeptide chain.
Serology	A method using the specificity of the antigen-antibody reaction for the detection of antigenic substance.
Sexual	Produced as a result of a union of nuclei in which meiosis takes place.
Single stranded	Consisting of a single strand twisted into a helix.
Sporangia	Container or case of asexual spores
Sporangiophore	A hypha or fruiting structure bearing spores.
Spores	The reproductive unit of fungi consisting of one or more cells.
Sporosorus	Group of reproductive body of fungus that formed within the tissue and that may erupt through the surface.
Sporulates	To produce spores.
Spotting	The small, roundish, usually necrotic punctate spots on various plant parts.
Stoma	(Plural=stomata). A minute organized opening on the surface of leaves or stem through which gases pass.

Streaking	A disease characterized by necrotic or chlorotic lines or spots on the leaf veins or on other parts of the plant.
Stripping	A plant disease characterized by long, narrow and elongated necrotic or differently colored stripes of tissues.
Stunting	The general growth of entire plant effected, resulted shorter size of plant.
Stylet	Piercing apparatus in plant parasitic nematodes.
Stylet-borne virus	A virus transmitted by vectors with sucking mouthparts (mostly aphids), and carried superficially at the tips of maxillary stylets.
Susceptible	Lacking the inherent ability to resist disease or attack by a given pathogen; nonimmune.
Swelling	The enlargement of some plant tissues or organs.
Symptom	A characteristic external sign or an expressive, typical property; a phenomenon according to which the cause of a disease and sometimes also the pathogen species may be identified.
Systemic	Spreading internally throughout the plant body (pathogen or chemical).
Tissue	A group of cells of similar structure which performs a special function.
Titre	The highest possible dilution at which infection still occurs on the test plant; in serology it means the greatest dilution of an antigen still reacting with homologous antibody.
Tolerant	Plant that sustain the effects of a disease without dying or crop loss.
Toxin	A compound produced by a microorganism and being toxic to a plant or animal.
Vascular	Plant tissue or region consisting of conductive tissue.
Vector	An animal able to transmit the pathogen.

Vector	An organism able to transmit a pathogen from an infected to a healthy host; for plant viruses these are mainly animal species (insects, mites, nematodes, etc.) but also some species of dodder or fungi.
Vegetative	A sexual; somatic.
Vegetative propagation	Production of a new plant from a portion of another plant, such as a stem or branch.
Vein banding	A systemic symptom in which the decoloration of the tissue is localized along the main veins.
Vein mosaic	A light-green or yellow mosaic along the leaf veins.
Vein yellowing	A plant disease in which only the veins of diseased plants or their parts turn yellow-green or yellow.
Vesicles	A small, thin-walled bladder like cavity, usually filled with fluid.
Vessel	A xylem element or series of such elements which conduct water and mineral nutrients.
Viron	The complete, mature virus particle.
Virulence	The degree of pathogenicity of a given pathogen.
Viruliferous	Vector containing a virus and capable of transmitting it.
Virus	A submicroscopic obligate parasite having in its nucleic acid component a complex genetical information necessary for independent reproduction in the host cells.
Wilt	Loss of rigidity and drooping of plant parts.
Wilting	Drooping of stems and foliage due to loss of water and decreased turgidity of cells. May be caused by water stress or by disease.
Wrinkling	Undulation or rugosity of the surface of leaves or bark caused by uneven growth.
Xylem	A plant tissue consisting of tracheids, vessels, parenchyma cells.

Yellowing	A change in the plant green coloration to a yellow shade due to a gradual loss of chlorophyll.
Zoosporangia	Sporangia which contains or produces zoospores.
Zoospore	An independently motile spore.

APPENDIX - 2

COMPANIES PRODUCING ELISA KITS AND REAGENTS OF PLANT VIRUSES

LIST OF COMPANIES PRODUCING ELISA KITS AND REAGENTS OF PLANT VIRUSES

S#	Company Address	Contacts
1.	Bioreba AG Chr. Merian-Ring 7 CH-4153 Reinach BL 1 Switzerland	Tel: +41 61 7121125 Fax: +41 61 7121117 Email: admin@bioreba.ch Web: www.bioreba.ch
2	Agdia 30380 County Road 6 Elkhart, Indiana 45614 U.S.A.	Tel: +1 800 62 AGDIA +1 219 264 2014 Fax: +1 219 264 2153 Email: info@agdia.com Web: www.agdia.com
3.	Adgen Limited Nellies Gate, Auchincruive Ayr KA6 5HW Scotland, U.K.	Tel: +44 1292 525275 Fax: +44 1292 525477 Email: info@adgen.co.uk Web: www.adgen.co.uk


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