Chapter 2

2.0 Vector potential

In India, Galloway (1936) was the first to record occurrence of potato virus. However, authentic records about occurrence of potato viruses X, Y and A were made by the pioneers like (Pal, 1943; Vasudeva and Lal, 1944; Vasudeva and Ramamoorthy, 1946 and Vasudeva and Azad, 1952). The transmission potential of potato viruses in non-persistent mode has been studied by several authors, using both potato colonizing and non-colonizing aphids. Stylet-borne transmission of plant viruses through aphid is now known to be occurring due to mechanical contamination of the vectors’ stylet as was postulated as early as 1928 by Doolittle and Walker and re-substantiated by many workers since 50’s (Bradley, 1952, 1953, 1966; Bradley and Rideout, 1953; Zettler and Wilkinson, 1966; Khurana, 1971, Warren et al., 2005). Stubbs (1955) was one of the pioneer workers observed the efficiency of aphid, *M. persiciace* (Sulzer) in transmitting PLRV and yellow spinach virus. Upreti and Nagaich (1971 a, b) after a detailed investigation observed the varying efficiency of 12 different clones of *M. persiciae* (Sulzer). Sohi and Swenson (1964) compared the transmission efficiency of two biotypes of *Macrosiphum pism* (Harris) and found that both biotypes differed greatly in their ability to transmit been yellow mosaic virus. Transmission efficiency of *M. persicae* (Sulzer) clone with 3 clones of *A. gossypii* (Glover) in transmitting southern cucumber mosaic virus (SCMV) observed by Simons (1958) and pepper vein banding mosaic (PVMV) with 2 clones of *M. persicae* (Sulzer) and 3 clones of *A. gossypii* (Glover) and observed the variable efficiency in transmitting virus and he also observed that PLRV-inoculated individuals of *M. persiace* (Sulzer) from different clones varied their ability to transmit PLRV to different plants. Variation among different clones/races and their different development stages or forms, within the same vector species, have been reported to cause variation in efficiency for the virus transmission. (Bawden, 1954; Nagaich and Upreti, 1972).

*M. persicae* (Sulzer) is considered to be the most efficient vector of PVY and its efficiency varies between 4.7% and 71.1%. Transmission efficiency of *Macrosiphum euphorbae* (Thomas) varies between 4.0% and 29.0% (Ragsdale et al., 2001). Non-colonizing aphids such as *Rhopalosiphon padi* (L.) are also capable of transmitting PVY at a lower efficiency of 0.5-11.5% (Van Hoof, 1980; Sigvald, 1984; Harrington and Gibson, 1989; Cervantes and Alvarez, 2001).
2.1 Aphid monitoring

Davies (1934) provided guidelines for the selection and location of sites for multiplication of seed potatoes and maintenance of their health standards under field conditions in hills. He further standardized the technique for taking aphid counts plucked from near the base of the plant as one traverses diagonally in a potato field. Simpson (1940) suggested an improvement in the technique by taking at random 33 plants and taking three leaves, viz. upper, middle and lower, thus making 99 leaves. To this added an odd lower leaf from a 34th plant, which brings the total to 100 leaves. Pushkarnath and Nirula (1970), Bacon et al., (1976) and Singh et al., (2010) further suggested to combine leaf count with sticky and water traps and found positive coefficients of correlation between all the three systems. Moericke (1951) yellow water traps have been found to save substantial yield loss in Dutch seed industry due to safely delaying the harvest when aphid build up is below the critical level of 20 aphids/100 leaves (Hille Ris Lambers, 1972). The critical level of 20 aphids/100 leaves was developed over sixty years ago in UK (Davies and Landis, 1951) and it still holds well in Indian conditions (Verma et al., 1982). Aphid surveys were carried out by various workers for identifying places suitable for raising healthy seed potato crop (Pushkarnath, 1967; Pushkarnath and Nirula, 1970; Chaudhary et al., 1977; Singh et al., 1977; Ram Gulab, 1987; Verma et al., 1990; Zamudio, 1995; Basky, 2002; Garg et al., 2003; Carli and Baltaev, 2008). So far no systematic surveys on aphid population build-up were carried out in the North Eastern states for selecting suitable locations for potato seed production.

2.2 Potato degeneration

Today, several hundred pests and pathogens are recognized as causes of potato disease and decline. Some of them are probably indigenous to areas where the plant was introduced. In many instances, however, the disease agents and pests were carried along with the vegetative propagation material that was transported around the globe. Many important diseases of potato are caused by viruses. Potato is one of the plant species in which the largest numbers of viruses are found. According to Bawden (1956), potato is the oldest sufferer of virus diseases on record next only to tulip. Haynes and Lu (2005) quoted a figure of 22% of potato yield lost each year due to
biotic stress. Economically more important of potato viruses are Polerovirus (Formerly Luteo), Potyvirus, Potex virus and Carla virus groups. According to Salazar (1989), nearly 30 different viruses have been reported infecting potato. This does not include the multiple strains of some of the viruses described. Fortunately, only a few are economically important in causing severe damage in a particular growing environment. For instance, potato virus M (PVM) is not commonly found in South American potatoes (CIP, 1984), whereas it is important virus for potatoes in Europe (De Bokx, 1972).

Studies on the cause of degeneration of seed potatoes in the country showed that aphid, particularly M. persicae (Sulzer), is responsible, as in western countries, for the spread of virus diseases in the fields.

Situations suited for seed production in hills are areas where summer temperatures are low and those exposed to wind currents. In exposed situation in the hills the drifting winged aphids migrants cannot easily alight on the crops and colonize freely. High humidity is an added advantage, as the moisture deposited on the wings of tiny aphids makes it difficult for them to fly (Pushkarnath, 1976). Potato being a vegetatively propagated crop when same stocks are used from year after year results in cumulative infiltration of pathogens, particularly the prevalent viruses, spread both through contact and aphid/vectors (Khurana, 1992). Infections of viruses have strong debilitating effect bringing down the yield potential of the infected plants/crop. It has also been observed that loss in yield following virus infection is genotype/variety specific. The varieties react with different degrees of loss in tuber yield depending on the virus (es)/stage of infection, and period of field exposure of the seed stocks (Nagaich et al., 1969; Killick, 1979; Milosevic and Stoiiljkovic, 1991; Khurana, 1992; Singh et al., 1994; Khurana et al., 1998; Bari et al., 1998; Biswas et al. 2004). A lower incidence (Say up to 5-10%) of the viruses, causing either single or combined infections in the current season or coming from previous season reduces the yields (Reestman, 1972; Hooker, 1981; Khurana and Singh, 1988; Olubayo et al., 2002; Machangi et al., 2004; Salazar, 2006; Pourrahin and Farzadfar, 2007; Potato Aphid, 2008). Also the degeneration and consequent losses in yield vary in different potato growing regions (Vashishth et al., 1981; Garg, 1987; Khurana and Singh, 1988; Bhandal and Naik, 1991; Khurana et al., 1998; Khurana, 1999; Lakra, 2000; Chandla et al., 2001; Basu et al., 2003, Sharma and Kang, 2003; Ayieko and Tirschley, 2006; Kabira et al., 2006; Nyaga, 2008). Rate of degeneration of potato stocks are not known in the crops raised in North Eastern hill states. Present study was an attempt in this direction. Outcome of the findings may help the local farmers to raise their own seed potato crop with minimum degeneration of stocks and better productivity.
2.3 Transmission

Aphids are one of the most important insects/vectors of agricultural and horticultural crops. As aphids feed on potato and can transmit and spread virus in these crops. This has been a subject of extensive research. The role of insect vector as an agent for virus transmission was first reported by a Japanese rice grower, Hatsuzi hashimoto in 1844 (mentioned in Katsura, 1936) long before the discovery of virus as a causal agent of plant diseases in the year 1898. Earlier studies on the transmission of cucumber mosaic virus by aphids were done by Doolittle (1916) and on spinach-blight by McClintock and Smith (1918). One of the first papers on the spread of the potato virus was published by Doncaster and Gregory (1948). Over 4500 species of aphids have been reported world over of which, about 675 occur in India. (Eastop, 1977; Agarwala and Ghosh, 1984). Of all the aphid vectors reported, *M. persicae* (Sulzer) is the most polyphagous and efficient aphid vector (Van Hoof, 1980; Blackman and Eastop, 2000; Halbert *et al*., 2003; Bernhard and Dixon, 2005; Warren *et al*., 2005) known to transmit over 100 plant viruses in about thirty plant families including potato (Kennedy *et al*., 1962). Among potato viruses, most important are PVY and PLRV (Broadbent, 1953, De Bokx, 1972, Khurana and Singh, 1986). Among aphid vectors of important viruses, aphids, *M. persicae* (Sulzer) and *A. gossypii* (Glover) play a vital role in limiting virus free potato seed production (Patkar *et al*., 1969; Hille Ris Lambers, 1972; Verma, 1977; Singh, 1981; 1985, 1993; Woodford, 1992; Verma and Chandla, 1999; Radcliffe and Ragsdale, 2002; Santanu and Konar, 2003). If care is not taken to prevent virus infection(s) in the seed crop, the seed stocks get fully riddled with viruses and yields are substantially get reduced if same seed stock is used for 3 to 4 years, thus regular replacement of virus free stocks is essential (Verma *et al*.,1998; Robert *et al*., 2000; Khurana, 2008).

For laboratory studies *M. persicae* (Sulzer) is the favored vector of PLRV (Williams and Ross, 1957) noticed that this species varied in efficiency to transmit PLRV. Aphids that were efficient vectors produced offsprings which performed efficiently, whereas the offsprings of aphids that performed poorly in transmitting a virus were also poor vectors. Hinz (1965, 1966) found differences in transmission efficiency of six different taxa of this species. Virus transmission efficiency also depends upon the virus source/cultivars/test hosts used (Messieha, 1967; Khurana *et al*., 1973). In laboratory studies comparing *Physalis floridana* (Rydb.) and *S. tuberosum* (L.) as source plants for PLRV (Kirkpatrick, 1948; Kirkpatrick and Ross, 1952) found *P. floridana* (Rydb.) to be a better virus source than potato. Watson and Roberts (1939) proposed a system to clarify insect transmitted plant viruses into two groups, non-persistent and persistent. Sylvester (1958) later improved the system by introducing the term semi-persistent viruses, whose persistence in the vectors is
intermediate between non-persistent and persistent. Kennedy et al., (1962) used the term circulative, for those viruses that are ingested, pass through the gut wall into the haemolymph, and then pass through the salivary glands. Harris (1977) proposed and ingestion-egestion mechanism for transmission of both non-persistently and semi-persistently aphid transmitted viruses.

### 2.3.1 Non-persistent transmission

The efficiency of the transmission of the non-persistently transmitted potato viruses has been studied by a number of authors. These studies have been done with colonizers as well as with non-colonizers. PVY is the one of the most important virus of potato and was first reported in *S. tuberosum* L. from UK (Smith, 1931) and then different strains were characterized according to local and systemic infection each strain induces in diagnostic plants (Weidemann, 1981; Jones, 1990; Blanco-Ergoiti et al., 1998; Carnegie and van der Haar, 1999; Kerlan et al., 1999; Ohshima et al., 2000; Nie and Singh, 2002; Warren et al., 2005) and transmitted non-persistently (Singh et al., 1984; Difonzo et al., 1996; Suranyi, 1999; Gray and Gildow, 2003; Cervantes, 2008; Cervantes and Alvarez, 2011). PVY is the most common *potyvirus* virus affecting potatoes because it is easily transmitted and cause major yield losses (Singh et al., 1982; Beemster and De Bokx, 1987; Ward and Shukla, 1991) either in combination of different strains of PVY or combination of virus Y and other viruses such as virus X, A and S (Nagaich et al., 1974; Khurana and Singh, 1988). These are known as the complex diseases. All such combinations may cause severe symptoms, disastrous for the crop and resulting in heavy yield losses depending upon the combination of the virus (strain) and potato variety. There are three main strains of PVY in potato: common or ordinary strain (PVY\(^0\)), tobacco veinal necrosis strain (PVY\(^N\)\(^,\)) (Smith and Dennis, 1940) and stipple streak strain (PVY\(^C\)). PVY\(^C\) was first to be recognized and was identified (Salaman, 1930) has not spread worldwide and has less economically importance than PVY\(^0\) and PVY\(^N\). More recently, new variants have been reported: PVY\(^{NTN}\), PVY\(^{NW}\), and PVY\(^Z\). PVY\(^{NTN}\) causes the potato tuber necrosis ring spot disease, now present in the most potato-producing areas. PVY\(^{NW}\) causes a typical necrosis in tobacco and was first reported infesting potato in cv. “Wilga” in Poland in 1984 (Chrzanoswska, 1987) but is now rather widespread (Kerlan et al., 1999). The severe strain of PVY reduces the yield (Radcliffe, 1982; Radcliffe and Ragsdale, 2002; Halbert et al., 2003) sometimes up to 80% (Beemster and de Bokx, 1987) and 14-29% in autumn (Singh et al., 1982). Laboratory studies have shown that besides *M. persicae* (Sulzer) and other potato colonizing species, a large number of aphid species can transmit strains of PVY (Karl 1971; Van Hoof 1980; De Bokx and Piron 1984; Sigvald 1984; Harrington et al., 1886; Harrington and Gibson, 1989).
2.3.2 Persistent transmission

Appel (1906) first described potato leaf roll in Germany and distinguished it from the “curl” complex. He described various forms of the disease, such as showing stunted growth, leaf curl and crinkled leaflets, and concluded that all are due to the same unidentified cause. He further observed that the disease is readily transmitted through the tubers and the virulence apparently increased from generation to generation. PLRV is transmitted in a persistent manner, characterized by minimum access periods for acquisition and inoculation of c.1h each. There is a latency period between acquisition and transmission of the virus, and the minimum time for transmission is c.12 hr. Transmission frequency increases with an increase in the access-feeding period of up to 2 days or more. Both larvae and adults can transmit the virus. Aphids remain infective after moulting and remain viruliferous for life time.

PLRV is transmitted by several aphid species, of which M. persicae (Sulzer) is the most efficient vector (Botjes, 1920; Schultz and Folson, 1921; Cottier, 1931; Williams and Ross, 1957; Close, 1965; Claridge, 1972; Lowe, 1973; Miln, 1978; Sylvester, 1980; Raman, 1985; Webby, 1988; Jayasinghe, 1988; Halbert et al., 1995; Woodford et al., 1995; Suranyi, 1999). Several aphid species have been reported to transmit PLRV (Kennedy et al., 1962). PLRV is not transmitted by mechanical inoculation, by seed or pollen. it is transmitted experimentally by grafting. In spite of intensive investigations into the mechanism of virus life cycle, the biochemical and physiological processes of the interactions between the viruses and plants are still poorly understood. Research in this area has been reviewed by Ross, 1986; Kegler and Friedt, 1993; Swiezynski, 1994; Valkonen, 1994; Valkonen et al., 1996 and Solomon-Blackburn, 2001 a, b). These authors summarized the information available and suggested strategies for breeding viral resistance into potato. M. euphorbiae (Thomas) transmits potato strains of the virus less efficiently, but is an efficient vector of the Australian tomato yellow top isolates. In Brazil, Myzus nicotianae (Blackman) was found to be an efficient experimental vector (Cupertino et al., 1995). PLRV is circulative in the vector aphids and can be found in the haemolymph of M. persicae (Sulzer). It was shown that endosymbiotic bacteria (Buchnera sp.) are involved in the persistent transmission of PLRV by M. persicae (Sulzer) (Hogenhout et al., 1998).

Besides, M. persicae (Sulzer), there are other potato colonizing aphids viz. Aphis fabae (Scop.), A. gossypii (Glover), A. nasturtii (Kaltenbach), Macrosiphum euphorbiae (Thomas), Myzus certus, M. humuli (Phorodon pruni), and Rhopalosiphum insertum (Walker) that are less effective in transmitting virus (Van Hoof, 1980; Halbert et al., 2003). Several authors found that Aulacorthum solani (Kaltenbach) is poor at transmitting PLRV as could also be concluded from data compiled by Hinz (1970). Some authors failed to show transmission by this species (Heinze, 1960).
Aphis nasturtii (Kaltenbach) which is not always present but has sometimes been very abundant in potato crops of Central and Eastern Europe is known to be only slightly effective at spreading PLRV (Kostiw, 1980; Gabriel et al., 1987). Several authors found that Macrosiphum euphorbiae (Thomas) is a poor vector of PLRV (Murphy and Mckay, 1929; Hinz, 1970). Although it is the adult of apterous form that tend to be used in virus transmission studies, some authors have reported that larval instar are more efficient at transmitting virus (Robert et al., 1969). Miyamoto and Miyamoto (1966) and Singh, et al., (1988) contended that variation in transmission of potato leafroll virus by M. persicae (Sulzer) was due to environmental conditions. Miyamoto and Miyamoto (1971) further showed that larvae are as good as adults of M. persicae (Sulzer) in transmitting PLRV. Ragsdale et al., (1994) suggested that wingless aphids have not yet been linked to the spread of PVY in the potato fields. In plants grown from infected tubers (secondary infection), yields may be reduced by 40-70% and under stress conditions, e.g. high temperature, the reduction can be even greater (van der Zaag 1977).
2.4 Potato Virus Y (PVY; Genus: Potyvirus; Family: Potyviridae; Group: IV (+) sense RNA virus (Smith, 1931)

2.4.1 Genomic organization of PVY (Jakab et al., 1997)

Fig.1.

The PVY presents a single-stranded positive sense RNA genome of 10,000 bases in length (9703 bases for the PVYN-605 isolate,), 680-900 nm in length and 11-15 nm in width (Edwardson, 1947). Morphologically potyvirus consists of approximately 2000 copies of coat protein (CP) which forms a cylindrical inclusion body (CIb) (Talbot, 2004). The CIb encapsulates a single strand of positive sense RNA which is in the order of 10 kb in length and has a non-translated 5'-terminal region (5'-NTR) as well as 3' poly tail (Dougherty and Carrington, 1988; Van der Vlugt et al., 1989). The 5'-end of the viral genome is covalently linked to the viral encoded VPg protein. The 3'-end is constituted by a polyadenylated sequence. A single Open Reading Frame (ORF) is present on the PVY genome. This ORF encodes a polyprotein (3063 residues) cleaved by three viral protease (P1, HC-Pro and Nla) to produce nine functional proteins (P1, HC-Pro, P3, 6k1, CIb, 6k2, Nla-Pro, VPg, Nlb and CP) (Talbot, 2004). These viral proteins are involved in different steps of the viral cycle.
2.4.2 Symptoms in Potato

PVY has high genetic variability, with several distinct strain groups infecting the potato crop. (De Bokx and Huttinga, 1981; Kerlan et al., 1999). These strains are recognized by the symptoms that they produce in naturally infected potato and tobacco \textit{(N. tabacum L.)} (De Bokx and Huttinga, 1981; Kerlan et al., 1999). The most common strain is PVY$^0$, which induces mild to severe mosaic and leaf-drop streak in potato and systemic mottle on tobacco. PVY$^N$, the tobacco veinal necrosis strain, induces very mild mottling on most potato cultivars, with occasional necrotic leaves on some cultivars (Chachulska et al., 1997; Kerlan, 1999). However, it induces severe systemic necrosis of leaf veins and petioles on tobacco (De Bokx and Huttinga, 1981). PVY$^{NTN}$, a strain belonging to necrotic group of PVY$^N$ causes potato tuber ring necrotic disease in potato tubers. (Kus, 1992). It induces chlorotic mottle to mosaic symptoms on potato plants and superficial to deeply sunken necrotic rings on the tubers (Le Romancer et al., 1994). PVY$^{NO}$, a new recombinant strain between O and N strain, is serologically similar to PVY$^0$, but produce necrosis on tobacco (Nie and Singh, 2002; Crosslin et al., 2005) and necrotic rings on potato tuber of some cultivars.

2.4.3 Natural host range:

PVY has a wide range of host plants besides the potato, tobacco \textit{(Nicotiana tabacum L.)}, tomato \textit{(Lycopersicum esculentum Mill.)} and pepper \textit{(Capsicum annuum L.)} (McDonald and Singh, 1996), ornamentals such as dahlia \textit{(Dahlia spp.)}, and petunia \textit{(Petunia spp.)}, and the weeds bitter sweet \textit{(Solanum dulcamara L.)} and black night shade \textit{(S. nigrum L.)}.

2.4.4 Experimental Hosts

2.4.4.1 Diagnostic species: \textit{Chenopodium amaranticolor} (Coste & Ryne), \textit{C. quinova}, and \textit{Physalis floridana} (Rydb.).

2.4.4.2 Propagation species: \textit{N. tabacum} (L.) cv. Samsun, \textit{N. glutinosa} (L.) and \textit{Datura metel} (L.).
2.5 *Potato leafroll virus* (PLRV; Genus: *Polerovirus*; Family: *Luteoviridae*; Group: V (+) sense RNA virus) (Quanjer, Van der Lek & Oortwijn Botjest, 1916)

2.5.1 Genomic organization of PLRV (Loebenstein, 2001)

![Genomic organisation of PLRV (Loebenstein et al., 2001)](image)

It possesses a 5.9 kb nucleotide long genome consisting of a single stranded RNA with plus orientation that encodes eight Open Reading Frames (ORFs) (Martin *et al.*, 1990; Ashoub *et al.*, 1998). The six major ORFs are separated by a small intergenic region into two clusters (van der Wilk *et al.*, 1997). Three ORFs located near the 3’ end encode, through sub-genomic RNA molecules, a 23kDa coat protein (CP), a 17 kDa floematic movement protein (MP) and a 56 kDa protein involved in the virus-aphid interactions (Chay *et al.*, 1996). The ORFs located near the 5’ end encode the following proteins directly from genomic RNA: VPg of 70 kDa protein is encoded by first ORF, a 28 kDa protein of unknown function is encoded by the second ORF while 3rd ORF encodes a 69kda replicase. ORF3 encodes the capsid protein. Initiation of an internally located AUG codon within the CP gene, but in a different reading frames, codes for the movement protein (ORF 4). Suppression of the CP amber stop codon results in formation of an ORF 3/ORF 5 read–through protein, which is supposedly involved in aphid transmission (Ashoub *et al.*, 1998). In addition two sub-genomic RNAs have been observed-sgRNA1 (~2.3kb) and sgRNA2 (~0.8kb) (Smith and Harris, 1990; Ashoub *et al.*, 1998). sg RNA 1 serves as mRNA for ORF3,ORF 3/5 and ORF 4.
sg RNA 2 may code for two viral proteins of 7.1 kDa (ORF 6) and 14 kDa (ORF 7), respectively (van der Wilk et al., 1997).

2.5.2 Symptoms in Potato

Symptoms of primary infection by PLRV consist of pallor and upward rolling of young leaves, especially at the base, with an upright habit. The edge of young leaflets of some cultivars may develop reddening and also has a purple discoloration (Wales et al., 2008) Secondary symptoms in plants grown from infected tubers, are stunting of the shoots and upward rolling of leaflets, especially of basal leaves, which become rigid and leathery (Rodriguez and Jones, 1978).

Necrosis may develop in the phloem tissue of stems and petioles, and excessive callose occurs in the sieve tubes of stems and tubers. Callose staining with resorcin blue, the Igel-Lange test, (de Bokx, 1967) was often used for detecting PLRV in tubers before the introduction of ELISA. Carbohydrates accumulate in the infected leaves-sometimes 2-3 times more than in healthy leaves, with a corresponding reduction in the tubers-due to impaired phloem transport. This is probably due not only to necrosis in the phloem cells, but perhaps also because of blockage of photoassimilate movement from the chloroplast into the cytosol by the triose-phosphate translocator (TPT). Thus, in transgenic plants in which cDNA for TPT was expressed in reverse orientation, leaves accumulated five times as much starch as leaves of control plants. This could also be due to blockage of sucrose loading into the phloem of PLRV-infected plants by the sucrose transporter protein. Thus, in sucrose transporter antisense potato plants starch content increased up to 10-fold.

It was suggested that the p28 (p0) protein of PLRV may be involved in leaf symptom expression (van der Wilk et al., 1997) as healthy potato plants transformed with the PLRV p28 protein gene displayed an altered phenotype resembling virus-infected plants.

2.5.3 Natural Host Range

This is mainly restricted to species of the family Solanaceae and can also infect some non-solanaceous plants.

Datura stramonium (Kohler), Solanum villosum (L.) Mill., P. floridana,(Rydb.). L. esculentum (Mill.) (Braithwaite and Blake, 1961), D. stramonium Var. Tatula (L.), S. aculetissimum (Jacq.), S. dulcamara (L.), S. erianthum (D.Don), S. paniculatum (L.), Nicotiana clevelandii (A. Gray) and Nicandra physalodes (L.) Gaertn. are also alternative hosts. Non-solanaceous host species include Amaranthus caudatus (L.), Capsella bursa-pastoris (Moench.),
Celosia argentea (L.), Gomphrena globosa (L.), Claytonia perfoliata (John), and Sisymbrium altissimum (L.) (Natti et al., 1953; Thomas, 1984; Tamada et al., 1984; Ellis, 1993; Loebenstein et al., 2001).

2.5.4 Experimental Hosts

2.5.4.1 Diagnostic Species

D. stramonium (Kohler), P. floridana (Rydb.), P. heterophylla (Nees), Physalis spp., S. tuberosum ssp. Tuberosum (L.).

Brassica campestris var. pekinensis (L.) (Chinese cabbage), Raphnus saivus (L.) (radish) and Vicia faba (L.) (broad bean) are non hosts.

2.5.4.2 Propagation Species

P. floridana (Rydb.), D. stramonium (Kohler) and S. tuberosum (L.).

A few viruses are totally dependent on potato for survival and spread while majority are independent of potato for survival and spread. Viruses that depend on the potato for survival and spread usually show a restricted or moderate host range, whereas those that do not depend on the potato for survival and spread have a wide host range and may also cause economically important losses in other crops (Salazar, 1977).

A review of national and international literature has identified up to 28 aphid species as actual or potential vectors of up to seven potato viruses in New Zealand (Fletcher, 1997). Nine of these are PLRV vectors. The green peach aphid is the most efficient vector of PVY and PLRV (Radcliffe et al., 1993; Salazar, 1996). Another species, the melon or cotton aphids, A. gossypii (Glover), have recently become an important virus vector in potato in Europe, partly due to its resistance to various pesticides (Rongai et al., 1998). This aphid a vector of several potato viruses but not known to transmit PLRV.

2.6 Enzyme Linked Immuno Sorbent Assay (ELISA)

The ELISA technique developed by Engvall and Perlmann (1971) was first applied to plant virus detection by Voller et al., 1976; Clark and Adams, 1977; Bar-Joseph & Garnsey, 1981. It has significantly increased the ability to detect and study plant viruses, and is currently the most widely used method for the detection of potato viruses due to its simplicity, adaptability, rapidity, sensitivity and accuracy. ELISA has earlier been reviewed by Converse and Martin (1990). The double antibody sandwich (DAS-ELISA) test on a solid phase (usually plastic) is most commonly used. Virus is first selectively trapped by a specific antibody adsorbed on a solid
surface, a specific enzyme-labeled antibody (conjugate) is added to the immobilized virus, and the reaction is measured visually or spectrophotometrically, after adding a suitable enzyme substrate. A variation of the above method is the indirect ELISA, in which plates are coated with antigen, and the primary antiviral antibody of one animal species (e.g. rabbit) is added. A secondary commercial antibody (e.g. goat anti-rabbit) enzyme conjugate which reacts with the first antibody is then added.

DAS-ELISA is especially useful for detecting antigens in complex mixtures. This is because the bound antibody specifically traps the antigens of interest, while non-specific antigens are removed in the wash step. Although the indirect ELISA is considered less strain-specific, the test is simple to perform and the same enzyme-antibody conjugate can be used for detecting many different viruses. Commercial kits for PLRV, PVM, PVS, PYX, PVY, and other viruses are available, and give reliable results when potato leaves are tested. For routine testing it is preferable to use polyclonal antisera. Care has to be taken to include both positive and negative controls in ELISA tests. The minimum level of virus detection by ELISA is about 2ng/ml. Torrance and Robinson (1989) have reported that the Swiss now routinely test 20,000 seed potato tubers per day for PVY and PLRV by ELISA under certification program. Thousands of microplants and polyhouse grown potato plant samples are routinely tested for virus freedom with ELISA in India at CPRI, Shimla under the breeder seed production programme. Shiv Kumar et al., (2003) suggested that ELISA and ISEM detection of viruses for better crop health in potato and marked reduction in the virus incidence of the seed stocks accompanied with increased tuber yield.

2.7 Electron Microscopy

Plant viruses in infected tissues often exist in sufficient concentrations that they can be extracted and examined by standard electron microscopy (EM). Observation of the shape and size of a virus particle is a basic step towards virus identification. In many cases, EM provides information on virus morphology to be obtained within minutes after sampling a diseased plant. EM is used to examine viruses in crude extracts from infected plants (Derrick, 1973; Hill, 1984; Khurana and Garg, 1993). In recent years, cytological, techniques have been developed for the detection of virus induced inclusions. These intracellular structures are characteristic for the virus inducing them and have proved to be valuable agents in the diagnosis of plant viral diseases (Christie et al., 1995). EM alone can be used for detection of viruses at various stages of growth (Vertaccini and Marani, 1982).
2.8 Immuno Sorbent Electron Microscopy (ISEM)

(Roberts et al., 1982) combines the sensitivity of electron microscope with the specificity of serological reaction. ISEM was introduced by Derrick (1973) for the detection of plant viruses, has been subsequently further improved (Milne and Luisoni, 1975, 1977; Milne and Lasemann, 1984; Garg et al., 1989) and is as or more sensitive than ELISA for some viruses (Roberts et al., 1980; Garg and Khurana 1991) and thousand times more sensitive than conventional electron microscopy (Roberts and Harrison, 1979; Garg et al., 1989). Potato leafroll virus (PLRV) is a small, phloem-restricted virus occurring in very low concentration and poses problem in detection with conventional electron microscopic detection. Optimum parameters were determined for the reliable and sensitive immune electron microscopic diagnosis of potato leaf roll virus along with other important potato viruses and PLRV was best detected when the virus and its antibodies interacted in liquid phase followed by trapping on the grid coated with protein A and homologous antibodies (Garg and Khurana, 1991). PLRV can be detected in potato leaves by serology, using commercial ELISA kits. However, concentration of PLRV varies and, in plants grown at temperatures of 30°C or in older plants, ELISA may not always detect infection. It is also difficult to detect PLRV by ELISA in un-sprouted tubers (Hill and Jackson, 1984).

2.9 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) as detection method is rapid, versatile, specific, and sensitive. PCR employs an enzymatic and an exponential amplification of a specific segment of DNA. This goal may be achieved through multiple cycles of three steps performed at different temperatures to: (i) denature the DNA, (ii) anneal two oligonucleotide primers to the denatured (opened) DNA strands, and (iii) primer extension by thermostable DNA polymerase to synthesize the target sequence whose ends are defined by the primers (Wilson and Walker, 2000). The presence of amplified DNAs can be determined by gel electrophoresis analysis. PCR has been widely used in plant pathology for the detection and diagnosis of pathogens such as viroids, viruses, bacteria, phytoplasma, fungi, and nematodes (reviewed by Henson and French, 1993). Since PCR needs a DNA segment for amplification and most of the plant virus posses RNA genomes, it is absolutely essential to first convert the RNA genome into a cDNA through reverse transcription (RT). RT-PCR is known to be at least 100-105 folds more sensitive than traditional ELISA in terms of detection sensitivity for potato viruses (Leone et al., 1997; Mumford et al., 2004). Large scale testing of both pre harvest & post harvest samples is possible through NASH and RT-PCR as described by (Singh et al., 1999).
PCR specificity and success in pathogen diagnosis depends upon the design of specific primers used to initiate DNA synthesis. Primer sequences are designed from the pathogen genome sequences available in gene bank (NCBI). Oligonucleotide primers must be 18-25 nucleotide residues in length, with a 50% G+C content, no annealing 3' end, no secondary structures, and high G+C content at the 3' ends. Primers may be targeted either to conserved regions (to amplify sequences from groups of pathogens) or to variable regions (to discriminate between strains). The annealing temperature of primers will affect specificity of PCR and successful reaction depends on primer length, its G+C content. Primers around 20 nucleotides require increase of up to 2°C for every addition of A or T and 4°C for G or C. With potato viruses, RT-PCR was applied in detecting Potato virus Y (PVY), Potato leaf roll virus (PLRV), Potato virus X (PVX), Beet western yellow virus (BWYV), Sugarcane yellow leaf virus (ScYLV), Potato virus A (PVA), Potato virus S (PVS), Potato virus M (PVM) and Potato apical leaf curl virus (PALCV) (Prill et al., 1988; Singh and Singh, 1998; Shalaby et al., 2002; Nagata et al., 2004; Suluja et al., 2005).

RT-PCR has been applied for cloning, molecular detection and sequence analysis of CP gene of PLRV. PLRV CP gene of different isolates was cloned in sequencing vectors e.g., an Indian isolate was cloned in pGEM-T vector (Mukherjee et al., 2003), Fujian isolate in pGEM-T vector (XingQuan et al., 2006) and complementary DNA of PLRV in pUC9 (Smith et al., 1988). Polymerase chain reaction linked automated sequencing was used to compare the relatedness of Brazilian potato leafroll luteovirus (PLRV) isolates amongst themselves with each other (Souza-Dias et al., 1999).

Although unquestionable in advantages, PCR is very expensive requiring costly equipment (thermocycler) and costly molecular biology grade consumables. In addition, PCR-based techniques are prone to render false positives due to its extremely high sensitivity coupled with the ease of contamination by aerosols, hair, skin, gloves, contaminated reagents, commercial preparations of TaqDNA polymerase, or even autoclaved material containing target sequences (Dwyerd et al., 1992 and reviewed by Henson and French, 1993). Moreover, only a limited number of samples can be tested in one run. Therefore it might not be a 'first choice' for large scale indexing.
Nevertheless, high sensitivity and rapid response make PCR a convenient approach for testing 'mother' seed stocks/plants. Various methods based on PCR or nucleic acid probes are now being developed and evaluated.