CHAPTER 1

General Introduction

Viruses and virus diseases of cool season food legumes

Legume crops play a major role worldwide as source of human food, feed and also in crop rotation. Faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.), and grasspea (*Lathyrus sativus* L.), collectively referred to as cool season food legumes (Summerfield et al. 1988) are of particular importance in developing countries of Asia, North and Northeast Africa where they provide a cheap source of seed protein for the predominantly poor population. Diseases including those caused by viruses are among the main constraints reducing their yield. Bos et al. (1988) listed some 44 viruses as naturally infecting faba bean, chickpea, field pea and lentil worldwide. Since then, a number of new viruses were described from these crops including *Faba bean necrotic yellows virus* (FBNYV) (Katul et al. 1993) and *Chickpea chlorotic dwarf virus* (CpCDV) (Horn et al. 1993), which are widespread and economically important. Most of the viruses of cool season food legumes are known to naturally infect more than one host within this group of crops (Bos et al. 1988, Brunt et al. 1996 and Makkouk et al. 2003a).

Virus symptoms in cool season food legumes vary depending on the virus or its strain, host species or cultivar and the prevailing environmental conditions. For practical purposes however, the symptoms are roughly categorized into two broad groups (Makkouk et al. 1994). Mosaic/mottle symptoms mainly caused by mechanically transmitted viruses invading parenchyma tissues represent one group, and yellowing, stunting, leaf roll and necrosis symptoms mostly caused by phloem-limited viruses namely luteoviruses, nanoviruses and a geminivirus form the second one/group. Viruses causing mosaic/mottle symptoms in their host belong to different families including *Potyviridae* (e.g. *Pea seed-borne mosaic virus* and *Bean yellow mosaic virus*), *Comoviridae* (e.g. *Broad bean stain virus* and *Broad bean true mosaic virus*) and *Bromoviridae* (e.g. *Alfalafa mosaic virus*, *Cucumber mosaic virus* and *Broad bean mottle virus*) (Bos et al. 1988, Brunt et al. 1996 and Makkouk et al. 2003a). Most of these viruses are non-persistently transmitted by aphids, may be seed-borne depending on the host (Bos et al. 1988), and are therefore of global concern due to international seed movement as primary source of infection in the field.

Yellowing, stunting and leaf roll diseases primarily caused by luteoviruses are considered to be the most destructive viral diseases of cool season food legumes worldwide (Bos et al. 1988). So far five luteoviruses have been reported to infect cool season food legumes from different parts of the world. These are *Bean leaf roll virus* (BLRV) (Ashby et al. 1984), *Beet western yellows virus* (BWYV) (Bosque-Perez and Buddenhagen, 1990, Fortass et al. 1997), *Soybean dwarf virus* (SbDV) (Tamada and Kojima, 1977), reported first as a synonym Subterranean clover red leaf virus (SCRLV) (Wilson and Close, 1973), *Chickpea stunt disease-associated virus* (CpSDaV) (Naidu et al, 1997) and *Pea enation mosaic virus-1* (PEMV-1) (Demler et al 1995). Pea leafroll virus (PeLRV), Legume yellows virus (LYV) and Michigan alfalfa virus (MAV) which were reported from legumes are considered to be synonymous to BLRV (Francki et al. 1991).

In the 1990's, it became evident that some viruses genetically unrelated to luteoviruses are involved in the etiology of yellowing and stunting diseases of some legumes. These viruses some of which were once considered as luteoviruses (Chu et al. 1995) are later named nanoviruses. These include *Subterranean clover stunt virus* (SCSV), *Milk vetch dwarf virus* (MDV) and FBNYV. In addition, a leafhopper-transmitted *Chickpea chlorotic dwarf virus* (CpCDV, *Mastrevirus, Geminiviridae*) (Horn et al. 1993) was reported to cause similar symptoms in legumes such as chickpea and faba bean, further indicating the diversity of viruses associated with this group of diseases. Despite their taxonomic affinity however, luteoviruses and nanoviruses infecting legumes share important ecological properties since they are phloem-limited and thus cause similar symptoms, share overlapping host range and aphid vectors. Consequently, members of these groups of viruses often occur in mixed infections in the field posing the need for an integrated approach for their study and control.

Taxonomy of luteoviruses

The International Committee of Taxonomy of Viruses (ICTV) recognized luteoviruses as a separate virus group in 1975 (Fenner, 1976), as a genus *Luteovirus* in 1995 (Randles and Rathjen, 1995) and later as a separate family *Luteoviridae* (D'Arcy et al 2000). The family *Luteoviridae* is divided into three genera namely *Luteovirus, Polerovirus* and *Enamovirus,* depending on genome organization, sequence similarity and methods of gene expression (D'Arcy et al. 2000). As a group, luteoviruses possess icosahedral particles about 25 nm in diameter consisting of a major ~22 kDa coat protein and a minor component of 52 kDa. They

are naturally transmitted only persitently by aphids and not mechanically, circulate but do not replicate in their aphid vectors and are confined to the phloem tissues of the infected plant.

The genome of luteoviruses is ~5.7 kb single stranded positive sense RNA the 3' end of which is not polyadenylated and without tRNA-like structure (D'Arcy and Domier, 2004). In general, the 5' half of the *Luteovirus* genome is phylogenetically related to that of members of *Tombusviridae* family whereas that of members of the genera *Polerovirus* and *Enamovirus* is closer to the genus *Sobemovirus* (D'Arcy et al. 2000). Three luteoviruses namely BLRV, SbDV and Sugarcane yellow leaf virus (ScYLV) that are suggested to have their genome evolved from recombination between ancestral luteoviruses and poleroviruses (Domier et al. 2002, Rathjen et al. 1994, Smith et al. 2000) are recently assigned to genera based primarily on the phylogenetic affinity of their polymerase gene. Accordingly, BLRV and SbDV are classified as members of genus *Luteovirus* whereas as ScYLV is assigned as a member of genus *Polerovirus* (D'Arcy and Domier, 2004). At present, there are 26 species in the family *Luteoviridae* of which 15 are assigned to one of the genera (D'Arcy and Domier, 2004) while the others are not yet assigned mainly due to lack of sufficient sequence information.

Members of family *Luteoviridae* share five to six major open reading frames (ORFs) designated ORF0 through 5, the same number corresponding to those ORFs coding for proteins of similar arrangement and possible functions (Fig. 1). These ORFs are separated by an intergenic region of ca. 100 nt in members of *Luteovirus* and ca. 200 *in Polerovirus* and *Enamovirus* into a gene cluster divergent among the genera (ORF 0, 1 and 2) at 5' half and conserved (ORF 3, 4 and 5) at 3' half of the genome. Members of genera *Polerovirus* and *Enamovirus* have genome-linked protein (VPg) and ORF0 which is absent in members of the genus *Luteovirus* (Fig 1). PEMV-1, the sole member of genus *Enamovirus* does not possess ORF4 (Fig. 1). Some luteoviruses such as *Barley yellow dwarf virus-PAV* and *Potato leafroll virus* (PLRV) are shown to have minor ORFs named ORF6 and/or ORF7 in the 3' half of their genome (Miller et al. 1995, Ashoub et al. 1998). The genome organization of type members of the three luteovirus genera and the corresponding major ORFs are presented in Fig. 1.

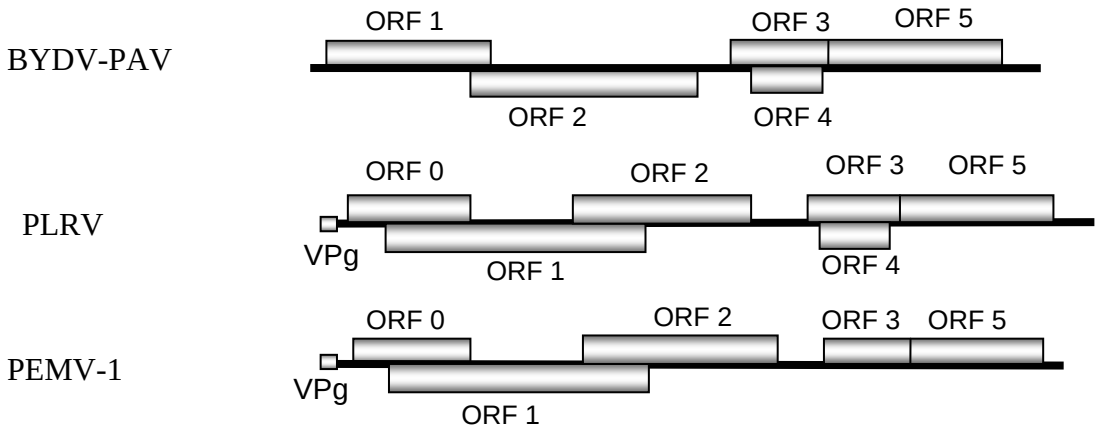

Fig. 1. Genome organization of the three genera of the family *Luteoviridae*

Taxonomy of nanoviruses

Nanoviruses have multipartite ssDNAs encapsidated within small isometric particles (18-20 nm) and are persistently transmitted by aphids. They were formerly referred to as non-geminated ssDNA plant viruses and later assigned as members of family *Circoviridae* (Chu et al. 1995) and then reclassified to a floating genus *Nanovirus* (Randles et al. 2000). Recently, the differences in genome size and sequence homology, number of components and biological properties (host range and vectors) led to their classification to the family *Nanoviridae* consisting of two genera: *Nanovirus* and *Babuvirus* (Vetten et al. 2004). Genus *Nanovirus* presently consists of three members, SCSV, MDV and FBNYV all of which are mainly limited naturally to legume hosts and transmitted naturally mainly by *Aphis craccivora. Banana bunchy top virus* (BBTV) is currently the only member of *Babuvirus* recognized by ICTV.

The genomic information of members of *Nanoviridae* is distributed in over at least 6-8 molecules of circular ssDNA (Burns et al. 1995, Katul et al. 1998, Sano et al. 1998, Vetten et al. 2004) (Fig. 2 and Table 1). Each of these ssDNA components is approximately 1 kb in size and appear to be structurally similar (Fig. 2) in having a positive sense DNA containing a conserved stem-loop structure (and other conserved domains) in the non-coding region and a coding region individually encapsidates as virus particles. Each of the DNAs of members of *Nanoviridae* encode only a single protein with the exception of a second virion sense ORF nested within the master rep encoding protein of BBTV (Vetten et al. 2004). A further peculiarity of nanoviruses is the occurrence of additional autonomously replicating DNAs in addition to the putative genomic DNAs which are considered to be satellites (Vetten et al. 2004).

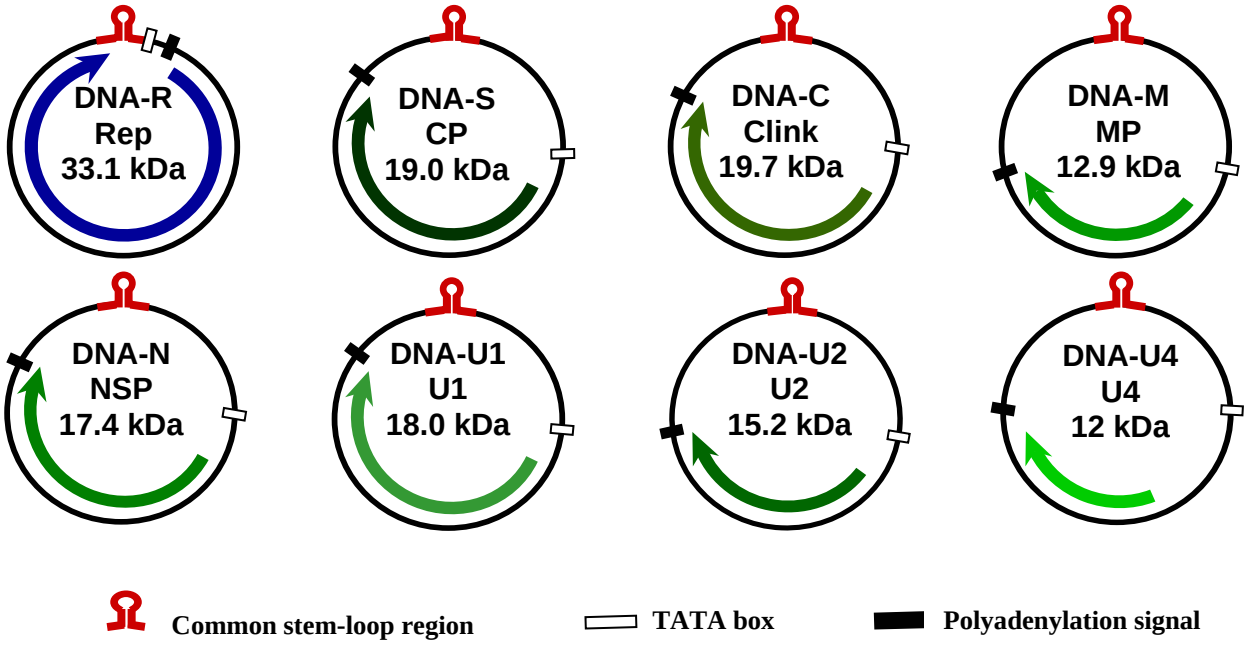

Fig. 2. Diagram illustrating the putative genomic organization of *Faba bean necrotic yellows virus* (an Egyptian isolate). Each of the DNAs has a size of about 1000 nt. Arrows refer to the approximate size of encoded protein (also given in kDa) and direction of transcription. *Milk vetch dwarf virus* (MDV) and *Subterranean clover stunt virus* (SCSV) have similar structure although DNA-U4 has not been identified in both viruses and U2 has not been is not identified in SCSV (see also Table 1) (modified from Vetten et al. 2004).

Until recently, the naming of nanovirus DNAs has led to confusion as different authors independently gave names to the different DNA molecules when they were first identified. To solve this problem, ICTV has recently adopted a standard nomenclature of the genomic DNAs of members of *Nanoviridae*. The DNAs were named in such a way that their designation should reflect the function of the protein they encode and those encoding similar putative proteins be given the same name in individual viruses within the family (Vetten et al. 2004, Table 1). DNAs encoding genes with unknown function are named tentatively as U1 through U5 (U derived from the term Unknown).

Table 1. Designation, size and functions of the proteins encoded by the various DNA components of members of family *Nanoviridae.*

Protein*	Protein Size (kDa)	Encoding DNA component	Identified from				Protein function(s)§
			FBNYV	MDV	SCSV	BBTV	
M-Rep	33.1-33-6	DNA-M	+ (2)	+ (11)	+ (8)	+ (1)	**R**eplication initiator
CP	18.7-19.3	DNA.S	+ (5)	+ (9)	+ (5)	+ (3)	**S**tructural (capsid)
Clink	19.0-19.8	DNA-C	+ (10)	+ (4)	+ (3)	+ (5)	**C**ell cycle link
MP	12.7-13.7	DNA-M	+ (4)	+ (8)	+ (1)	+ (4)	**M**ovement
NSP	17.3-17.7	DNA-N	+ (8)	+ (6)	+ (4)	+ (6)	**N**uclear shuttle
U1	16.9-18.0	DNA-U1	+ (3)	+ (5)	+ (7)	-	**U**nknown
U2	14.2-15.4	DNA-U2	+ (6)	+ (7)	-	-	**U**nknown
U3	10.3	DNA-U3	-	-	-	+ (2)	**U**nknown
U4	10 or 12.5	DNA-U4	+ (12)	-	-	-	**U**nknown
U5	5.0	DNA-R	-	-	-	+ (1)	**U**nknown

Legend: *Master replication initiator protein (M-rep), coat protein (CP), cell cycle link protein (Clink), movement protein (MP), and nuclear shuttle protein (NSP). U1 to U5 are temporary designations until the protein function has been determined. A + (yes) or – (no) indicates whether a protein has been described from the virus species or not. The former designation number of the encoding DNA is given in parantheses. § The underlined and bold letters indicate how the DNA component designation is derived and may help as a memory guide (after Vetten et al. 2004)

Objectives and scope of the study

Food legumes such as faba bean, chickpea and lentil are affected by yellowing and stunting diseases mostly caused by viruses (and nanoviruses) singly or in mixed infection. These diseases result in significant yield losses in countries like Ethiopia, Sudan, Egypt and Morocco where these crops are economically very important. However, the knowledge on the exact identity and genetic diversity of the viruses is limited since virus identification has been based mostly on mere serological diagnosis of field samples with no information on other virus properties. In most previous serological surveys, a significant portion of samples showing typical virus-like symptoms gave no serological reaction with the available antibodies and thus the causal agents remained unidentified (e.g. Abraham et al. 2000, Fortass and Bos, 1991, Makkouk et al. 2003b, Tadesse et al. 1999). This was due to the fact that some of them belonged to yet unrecognized viruses for which specific antibodies were not available. Although nucleotide sequence information is the most reliable means of identifying and charac-

terizing virus isolates, no such data is available in the database for any legume luteovirus species from countries of Northeast and North Africa and West Asia. In the case of nanoviruses for which reliable serological and sequence information is available, the number of samples used in previous studies is often too low and not representative in terms of distribution of virus distribution within a country or a region. Therefore, the main objectives of this work as presented in this dissertation were:

- To identify and characterize the major virus(es) associated with yellowing and stunting diseases of cool season food legumes at biological, serological and molecular level and to develop reliable diagnostic tools.
- To generate partial and if possible complete nucleotide sequence data for representative luteovirus and nanovirus isolates in order to assess their genetic diversity and taxonomic status.

The results of the different aspects of this study are presented in this dissertation as independent but related manuscripts (Chapters 2-7) followed by general discussion and conclusion (Chapter 8).

REFERENCES

Ashby, J.W. 1984. Bean leaf roll virus. CMI/AAB Description of Plant Viruses. No. 286. 5 pp.

Ashoub, A., Rohde, W. and Puffer, D. 1998. *In planta* transcription of a second subgenomic RNA increases the complexity of the subgroup 2 luteovirus genome. Nucleic Acids Research 26:420-426.

Bos, L., Hampton, R.O. and Makkouk, K.M. 1988 Viruses and virus diseases of pea, lentil, faba bean and chickpea. p 591-615. In: RJ Summerfield (ed.). World Crops; Cool Season Food Legumes Kluwar Academic Publishers, Dordrecht/Boston/London.

Bosque-Perez, N. A. and Buddenhagen, I.W. 1990. Studies on epidemiology of virus disease of chickpea in California. Plant Dis. 74:372-378.

Brunt, A.A., Crabtree, A.A., Dallwitz, M.J., Gibbs, A.J. and Watson, L. 1996. Viruses of Plants. Descriptions and Lists from the VIDE Database. CAB International. 1484 pp.

Burns, T.M., Harding, R.M. and Dale, J.L. 1995. The genome organization of banana bunchy top virus: analysis of six ssDNA components. J. Gen. Virol. 76:1471-1482.

Chu, P., Boevnik, P., Surin, B., Larkin, P., Keese, P. and Waterhouse, P. 1995. Non-geminated single stranded DNA plant viruses. pp 311-341. In: Singh, R.P., Singh, U.S., Kohmoto, K. (eds). Pahogenicity and Host Specificity in Plant Diseases, Vol. 3, Viruses and Viroids, Pegamon Press, Kidlington, Oxford, UK.

D'Arcy, C.J. and Domier, L.L. 2004. *Luteoviridae.* pp 343-352. In: Virus Taxonomy, VIIIth Report of the ICTV (C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, and L.A. Balls eds.).

D'Arcy, C.J., Domier L.L. and Mayo, M.A. 2000. Family *Luteoviridae*. pp 775-784. In: van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estess, M.K; Lemon, S.M.; Maniloff, J; Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B.(eds). Virus Taxonomy: Seventh report of the International Committee of the Taxonomy of Viruses. Academic Press, San Diego, CA.

Demler, S.A., De Zoeten, G.A., Adams, G. and Harris, K.F. 1995. Pea enation mosaic virus: properties, and aphid transmission. pp. 303-344. In: B.D. Harrison and A.F. Murant (eds), The Plant Viruses 5: Polyhedral virions and bipartite genomes Plenum, New York and London.

Domier, L.L., McCoppin, N.K., Larson, R.C. and D`Arcy, C.J. 2002. Nucleotide sequence shows that *Bean leaf roll virus* has a *Luteovirus*-like genome organization. J. Gen. Virol. 83:1791-1798.

Fenner, F. 1976. Classification and nomenclature of viruses. Second Report of the International Committee of Taxonomy of Viruses. Intervirology 7:1-116.

Fortass, M.F. and Bos, L. 1991. Survey of faba bean (*Vicia faba* L) for viruses in Morocco. Neth. J. Pl. Path. 97:369-380.

Fortass, M.F., van der Wilk, F., van den Heuvel, J.F.J.M. and Goldbach, R.W. 1997. Molecular evidence for the occurrence of beet western yellows virus on chickpea in Morocco. European J Plant Path. 103:481-484.

Francki, R.I.B., Fauquaet, C.M., Knudson, D.L. and Brown, F. 1991. Classification and nomenclature of viruses. Fifth report of the International Committee on Taxonomy of Viruses. Arch Virol (Suppl) 2:309-311.

Horn, N.M., Reddy, S.V., Roberts, I.M. and Reddy, D.V.R. 1993. Chickpea chlorotic dwarf virus, a new leafhopper transmitted geminivirus of chickpea in India. Ann. Appl. Biol. 122:467-479.

Katul, L., Vetten, H.J., Maiss, E., Makkouk, K.M., Lesemann. D.-E. and Casper, R. 1993. Cha-racterization and serology of virus-like particles associated with faba bean necrotic yellows. Ann. Appl. Biol. 123:629-647.

Katul, L., Timchenko, T., Gronenborn, B. and Vetten, H.J. 1998. Ten distinct ssDNA components, four of which encode putative replicase-associated proteins, are associated with the faba bean necrotic yellows virus genome. J. Gen. Virol. 1998. 79:3101-3109.

Makkouk, K.M., Kumari, S.G., Hughes, J. d'A., Muniyappa, V. and Kulkarni, N.K. 2003a. Other legumes. pp. 447-476. In: Loebenstein, G. and Thottappilly, G. (eds). Virus and Virus-like Diseases of Major Crops in Developing Countries. Kluwer Academic Publishers, Dordrecht/ Boston / London. 800 pp.

Makkouk, K.M., Kumari, S.G., Shahraeen, N., Fazlali, Y., Farzadfar, S., Ghotbi, T. and Mansouri, R.A. 2003b. Identification and seasonal variation of viral diseases of chickpea and lentil in Iran. J Plant Dis Prot. 110:157-169.

Makkouk, K.M., Rikallah, L., Madkour, M., El-Sherbeny, M., Kumar, S.G., Amriti, A.W. and Solh, M.B. 1994. Survey of faba bean (*Vicia faba* L) for viruses in Egypt. Phytopath Mediter. 33:207-211.

Miller, W.A., Dinesh-Kumar, S.P. and Paul, C.P. 1995. Luteovirus gene expression. Critical Reviews in Plant Sciences 14:179-211.

Naidu. R.A., Mayo, M.A., Reddy, S.V., Jolly, C.A. and Torrance, L. 1997. Diversity among coat proteins of luteoviruses associated with chickpea stunt disease in India. Ann. Appl. Biol 130:37-47.

Randles, J.W. and Rathjen, J.P. 1995. Genus Luteovirus. In: Murphy et. al.(Ed.), Virus Taxonomy, Six Report of the International Committee of Taxonomy of Viruses. Archives of Virology (Suppl.) 10:379-383.

Rathjen, J.P., Karageorgous, L.E., Habili, N., Waterhouse, P.M. and Symons, R.H. 1994. Soybean dwarf virus contains the third variant genotype in the luteovirus group. Virology 198:671-679.

Sano, Y., Wada, M., Hashimoto, Y., Matsumoto, T., and Kojuma, M. 1998. Sequence of ten circular ssDNA components associated with the milk vetch dwarf virus genome. J. Gen. Virol. 79:3111-3118.

Smith, G.R., Borg, Z., Lockhart, B. E. L., Braithwatie, K.S., and Gibbs, M.J. 2000. Sugarcane yellow leaf virus: a novel member of *Luteoviridae* that probably arose by inter-species recombination. J. Gen. Virol. 81:1865-1869.