PATHOLOGY AND MOLECULAR COMPARISON
OF A RANGE OF PEA SEED-BORNE MOSAIC
VIRUS ISOLATES

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TABLE OF CONTENTS

Summary.................................................................................................................i
Publications.............................................................................................................v
Statement...............................................................................................................vi
Acknowledgments...................................................................................................vii
Abbreviations..........................................................................................................viii

Chapter 1  General Introduction

1.1 The pea crop.....................................................................................................1
1.2 Pea viruses.........................................................................................................1
1.3 Discovery of PSbMV........................................................................................1
1.4 Geographical distribution................................................................................2
1.5 Agricultural importance....................................................................................2
1.6 Yield losses........................................................................................................3
1.7 Pathotypes.........................................................................................................4
1.8 Purification.........................................................................................................5
1.9 Biological properties.........................................................................................6
   1.9.1 Symptoms of PSbMV infection..................................................................6
   1.9.1.1 On leaf................................................................................................7
   1.9.1.2 On seed...............................................................................................7
   1.9.1.3 On infected seedlings .........................................................................8
   1.9.2 Intracellular symptoms..........................................................................8
   1.9.3 Host range...............................................................................................9
   1.9.4 Transmission............................................................................................9
       1.9.4.1 Seed transmission............................................................................9
       1.9.4.2 Aphid transmission........................................................................11
1.10 Serological properties....................................................................................11
1.11 Physical properties.........................................................................................12
1.12 Chemical properties.......................................................................................12
1.13 Molecular properties.....................................................................................13
   1.13.1 Genome structure and organisation......................................................13
   1.13.2 Gene products and functions...............................................................14
   1.13.3 The coat protein gene..........................................................................16
1.14 Sequenced pathotypes.....................................................................................16
Chapter 2 General Materials and Methods

2.1 Materials...........................................................................................................18
2.1.1 Seed sources..................................................................................................18
2.1.2 Virus sources...............................................................................................18
2.1.3 Antisera........................................................................................................18
2.1.4 Synthetic oligodeoxyribonucleotides..........................................................19
2.1.5 Bacterial plasmid system for cloning.......................................................19
2.1.6 Biochemicals, gels, media, stains and solvents........................................19

2.2 Methods............................................................................................................20
2.2.1 Plant culture.................................................................................................20
2.2.2 Virus inoculation........................................................................................20
2.2.3 Quantification of virus and nucleic acids..................................................20
2.2.4 Electron microscopy....................................................................................21
2.2.5 Western blot analysis...................................................................................21
2.2.6 Avoiding ribonuclease (RNase) contamination.........................................21
2.2.7 Phenol:chloroform extraction and ethanol precipitation...........................22
2.2.8 Isolation of RNA........................................................................................22
  2.2.8.1 From virus particles.................................................................................22
  2.2.8.2 From plant tissue.....................................................................................22
  2.2.9 Preparation of cDNA probes......................................................................23
  2.2.9.1 32P-labelled cDNA probes......................................................................23
         2.2.9.1.a Purification.........................................................................................23
         2.2.9.1.b Measurement of radioactivity..........................................................23
  2.2.9.2 DIG-labelled cDNA probes....................................................................24
2.2.10 Nucleic acid hybridisation.......................................................................24
  2.2.10.1 Dot blot hybridisation..........................................................................24
  2.2.10.2 Tissue print hybridisation.................................................................24
  2.2.10.3 Northern blot hybridisation.................................................................25
  2.2.10.4 Southern blot hybridisation.................................................................25
  2.2.10.5 Prehybridisation and hybridisation assays........................................26
  2.2.10.6 Detection...............................................................................................26

2.2.11 Gel electrophoresis......................................................................................27
  2.2.11.1 Agarose gel electrophoresis..................................................................27
  2.2.11.2 Polyacrylamide gel electrophoresis (PAGE)......................................28
  2.2.11.3 Sodium dodecyl sulphate-PAGE (SDS-PAGE)..................................28
         2.2.11.3.a Staining.........................................................................................28
Chapter 3 Purification and Serological Detection of PSbMV

3.1 Introduction .................................................................................35
3.2 Materials and methods .................................................................36
  3.2.1 Virus isolates and propagation ..................................................36
  3.2.2 Purification of PSbMV particles ...............................................36
  3.2.3 Virus quantitation and storage ..................................................37
  3.2.4 Extraction of RNA from PSbMV particles ...............................38
  3.2.5 Detection of PSbMV by dot-immunobinding assay (DIBA) ...........38
    3.2.5.1 Preparation of sample ......................................................38
    3.2.5.2 Preparation of blocking solution L ....................................38
    3.2.5.3 Preparation of blocking solution S ....................................39
    3.2.5.4 DIBA procedure ............................................................39
      3.2.5.4.a Blocking of membrane ..............................................39
      3.2.5.4.b Specific antibody binding .........................................39
      3.2.5.4.c Conjugate antibody binding .....................................39
      3.2.5.4.d Substrate ...............................................................40
  3.2.6 Comparison by DIBA ..........................................................40
  3.2.7 Western immunoblotting .......................................................40

3.3 Results .........................................................................................41
  3.3.1 Virus purification .................................................................41
3.3.2 Size measurement of PSbMV particles..........................41
3.3.3 Infectivity assay..................................................41
3.3.4 Size of the PSbMV RNA.............................................41
3.3.5 Development of a reliable DIBA for diagnosis of pea viruses....42
3.3.6 Sensitivity of DIBA..................................................42
3.3.7 Comparison by DIBA...............................................42
3.3.8 Size of the coat protein of PSbMV..................................43

3.4 Discussion.......................................................................43

Chapter 4 Diagnosis of PSbMV by Nucleic Acid Hybridisation

4.1 Introduction.......................................................................45
4.2 Materials and methods.....................................................46
  4.2.1 Viral RNA preparation...............................................46
  4.2.2 Synthesis of radioactive and non-radioactive cDNA probes.....46
  4.2.3 Size measurement of cDNA probes.................................46
  4.2.4 Sample preparation for dot blot hybridisation....................47
  4.2.5 Dot blotting..................................................................47
  4.2.6 Tissue printing..........................................................48
  4.2.7 Hybridisation and detection assays.................................48

4.3 Results..............................................................................48
  4.3.1 Purity of RNA and size of the cDNA probes.......................48
  4.3.2 Detection by radioactive probes......................................49
  4.3.3 Detection by non-radioactive probes.................................49
  4.3.4 Detection by tissue print hybridisation.............................50
  4.3.5 Detection of PSbMV in dry seed.....................................50

4.4 Discussion.......................................................................50

Chapter 5 Pathology of PSbMV

5.1 Introduction.......................................................................53
5.2 Materials and methods.....................................................54
  5.2.1 Plant growth and virus culture.......................................54
  5.2.2 Detection of PSbMV....................................................54
  5.2.3 Assay of sample........................................................54
  5.2.4 Generation 1 (G1) plants...............................................55
  5.2.5 Generation 2 (G2) plants...............................................55
  5.2.6 Generation 3 (G3) plants...............................................56

5.3 Results..............................................................................56
Chapter 6 Survey of PSbMV in Pakistan

6.1 Introduction.................................................................................................................. 62
6.2 Materials and methods.............................................................................................. 63
   6.2.1 Seed, antisera and virus sources........................................................................ 63
   6.2.2 Survey area.......................................................................................................... 63
   6.2.3 Sampling............................................................................................................. 63
   6.2.4 Sample preparation............................................................................................ 64
   6.2.5 Detection by DIBA.......................................................................................... 64
   6.2.6 Bioassay of PSbMV isolates and determination of pathotype......................... 65
   6.2.7 Screening of Pakistani pea varieties for susceptibility to PSbMV isolates S6 and US................................................................. 65
6.3 Results......................................................................................................................... 65
   6.3.1 Detection of BYMV, CMV and PSbMV in surveyed crops............................ 65
   6.3.2 Detection of PSbMV in seed and seedlings....................................................... 66
   6.3.3 Pakistani PSbMV isolates.................................................................................. 67
   6.3.4 Reaction of Pakistani PSbMV isolates on pea differential genotypes................. 68
   6.3.5 Screening of pea varieties for resistance to isolates S6 and US......................... 68
6.4 Discussion.................................................................................................................... 69

Chapter 7 PCR Amplification and Cloning of Regions of the PSbMV Genome

7.1 Introduction................................................................................................................. 72
7.2 Materials and methods............................................................................................. 73
   7.2.1 Reverse transcription (RT) and PCR primers.................................................... 73
   7.2.2 First strand cDNA synthesis by reverse transcription...................................... 73
   7.2.3 Second strand cDNA synthesis and PCR amplification................................... 74
   7.2.4 Analysis, elution and cloning of PCR products................................................. 74
7.3 Results......................................................................................................................... 74
Chapter 8 Sequence Comparisons of Cloned Regions from PSbMV

8.1 Introduction..........................................................78
8.2 Materials and methods............................................79
  8.2.1 Sequencing primers.............................................79
  8.2.2 Clones sequenced..............................................80
  8.2.3 Preparation of plasmid DNA for sequencing..............79
  8.2.4 DNA sequencing strategy....................................79
  8.2.5 DNA sequencing analysis using computer programs......80
8.3 Results......................................................................80
  8.3.1 The genomic sequences of PSbMV clones (S6 1.7, US 1.7, PK9 1.3 and S6 1.8) ........................................80
  8.3.2 Comparison of the partial NB sequence.....................81
  8.3.3 Comparison of the complete CP sequence..................82
  8.3.4 Phylogenetic relationship on the basis of CP sequence ...82
  8.3.5 Comparison of the CP amino acids by hydrophathy plots..83
  8.3.6 Comparison of the UTR sequence............................83
  8.3.7 Comparison of the partial P1-Pro sequence.................84
  8.3.8 Comparison of the complete HC-Pro sequence.............84
8.4 Discussion............................................................84

Chapter 9 Identification of Genomic Heterogeneity using the Ribonuclease Protection Assay (RPA)

9.1 Introduction..........................................................87
9.2 Materials and methods............................................88
  9.2.1 Isolation of PSbMV RNA.......................................88
  9.2.2 Source of clones...............................................88
  9.2.3 In vitro transcription of plasmid clones by RNA polymerase ..88
    9.2.3.1 Preparation of template DNA...........................88
    9.2.3.2 Preparation of radiolabelled cRNA probes...............89
SUMMARY

This thesis describes the development of serological and nucleic acid based diagnostic methods for pea seed-borne mosaic virus (PShMV); isolate specific effects on infected pea plants; the collection and biological comparison of new PShMV isolates from Pakistan; the cloning and sequencing of specific parts of the genome of selected isolates; nucleotide and amino acid sequence comparisons between selected isolates; and, the development of a ribonuclease protection assay (RPA) for identifying genomic differences among the PShMV isolates.

The dot-immunoassay (DIBA) was modified to detect PShMV. For virus, the DIBA detection threshold was 1 ng per μl and in sap extracted from infected leaf the dilution endpoint was 1/4096. DIBA was used for the detection of PShMV in samples collected from the field, dry pea seed and in seedlings germinated in the glasshouse. Nucleic acid-based hybridisation assays using dot-blot and tissue-print formats were developed to detect PShMV. These assays used randomly primed cDNA probes (32P- and Digoxigenin-labelled) representing the full genome of PShMV. For virus, the dot blot hybridisation assay detection threshold was 160 pg per μl and in sap extracted from infected leaf the dilution endpoint was 1/3125. For RNA, the dot blot hybridisation assay detection threshold was 51 fg per μl and in total nucleic acid extracted from infected leaf the dilution endpoint was 1/3125. PShMV RNA was detected in leaf and embryo squashes by tissue print hybridisation assay.

To study the pathogenesis of PShMV, the South Australian isolate S6 and the North American type isolate US were classified by pathotype specific pea genotypes and used as representatives of the PShMV pathotypes P-4 and P-1 respectively. Under glasshouse conditions, Dundale pea plants inoculated mechanically with S6 showed severe mosaic
symptoms on leaves, the premature emergence of axillary shoots at 21 days post inoculation (at the 11-12 node plant stage), delayed flowering, delayed pod set, delayed pod maturation, and the senescence of plants was delayed by 14 days. Seed yield was reduced by 82% and the virus was transmitted to 31% of seedlings. Isolate US showed mild leaf rolling of leaves, the emergence of axillary shoots at 28 days post inoculation (at the 19-20 node plant stage) and senescence of plants was delayed by 7 days. Seed yield was reduced by 35% and the virus was transmitted to 8% of seedlings. Axillary shoots were produced in healthy plants from 35 days at the 22-23 node stage when the plants had already started to flower. Both viruses showed petal colour break. Plants infected via seed with S6 were symptomless, they senesced 7 days late, seed yield was reduced by 52% and 12% of their seed contained virus. Some of the plants infected via seed with US showed mild leaf rolling, there was no significant effect on yield and 7% of seed contained virus.

During the early growing season of 1995, a survey was made for PSbMV in the major pea-growing areas of North West Frontier Province (NWFP) of Pakistan. Of 713 samples collected from 62 commercial pea fields or experimental plots, 11 samples were positive by DIBA to PSbMV with an average incidence of 1.5%. PSbMV was also detected in 1 to 5% of dry seed from five of the 12 Pakistani pea varieties tested and in 8 to 20% of seedlings raised from seed of three of these varieties. Twelve Pakistani PSbMV isolates (PK1-PK12) were recovered from the field samples, DIBA positive seed extracts and from seedlings germinated in the glasshouse. These isolates were compared with isolates S6 and US and were not distinguishable by serology, capsid protein size, RNA size or dot blot hybridisation using randomly primed cDNA probes to isolates S6 and US. The ability of isolates PK1-PK12, S6 and US to infect a standard range of pathotype specific pea differential genotypes showed that they could be classified into 4 distinct groups. One group contained isolates US, PK2, PK3, FK6, PK7 and PK10 and was classified as a pathotype P-1 group. Another group contained
isolates S6, PK8 and PK12 and was classified as a pathotype P-4 group. The remainder could not be classified by the existing PShMV pathotyping system and were tentatively placed into two other groups named U-1 (unknown 1) and U-2 (unknown 2). Isolates PK1, PK4, PK5 and PK11 were placed in the U-1 group and PK9 was placed in the U-2 group.

Nucleotide and amino acid sequences of the 3' and 5' parts of the RNA of isolates S6 (P-4), US (P-1) and PK9 (U-2) were compared. The 3' part included some of the NIB gene, the full coat protein (CP) gene and the untranslated region (UTR). The 5' part included some of the P1-Pro gene and the full helper component (HC-Pro) gene. Both parts were amplified by the reverse transcription polymerase chain reaction (RT-PCR) using specific primers designed from the published sequences of the P-1 DPD1 and P-4 NY isolates of PShMV. All PCR products were cloned into the pGEM®-T transcription vector and sequenced in both directions. Nucleotide and amino acid sequence alignments and phylogenetic relationships among the PShMV isolates S6, US and PK9 were compared with the previously fully sequenced (P-1 DPD1 and P-4 NY isolates) and partially sequenced (P-1 NZ (New Zealand); CAN (Canada) and GER (Germany)) isolates of PShMV. Phylogenetic comparisons based on nucleotide sequences divided these isolates into two main groups. The first group contained isolates CAN, DPD1, GER, NZ, PK9 and US. However, much diversity was found among the isolates in this first group, and three clusters of isolates were observed. The clusters included isolates DPD1, NZ, and PK9, isolates CAN and US, and the third cluster contained only isolate GER. The second group contained isolates S6 and NY.

Increased discrimination between isolates was achieved by the ribonuclease protection assay (RPA). A comparison of all of the 14 PShMV isolates (S6, US and PK1-PK12) quantified their genomic heterogeneity within defined 3' parts of isolate S6 (1737 nt), US (1739 nt) and PK9 (1366 nt). The RPA banding patterns obtained for cRNA probes
from isolates S6, US and PK9 were used for a phylogenetic analysis which placed the 14 PSbMV isolates into three distinct groups. The first group contained isolates US, PK5, PK6 and PK7. The second group contained isolates S6, PK8 and PK12. The third group contained isolates PK1, PK2, PK3, PK4, PK9, PK10, and PK11.

This is the first comparison of a range of geographically different isolates of PSbMV on the basis of both biological and molecular properties. The biological comparison has put the Pakistani PSbMV isolates into four groups and RPA into three distinct groups. Identification of a previously undescribed cluster of isolates of PSbMV including representatives from Pakistan only, may be evidence for a centre of evolution of PSbMV in or near Pakistan, an area which is also considered to be near the centre of origin of pea, the principal host of PSbMV.