Characterization of the P1 protein and coding region of the zucchini yellow mosaic virus

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The nucleotide sequence of the 5'-terminal P1 coding region of an aphid-transmissible isolate of zucchini yellow mosaic virus (ZYMV; strain FL/AT), a mild isolate (strain MD) and a severe isolate (strain SV), all from Florida, were compared with two other ZYMV isolates. The ZYMV MD and SV isolates and an isolate from California (ZYMV CA) had 95–98% sequence similarities to FL/AT, whereas an isolate from Reunion Island (ZYMV RU) had a 60% sequence similarity to FL/AT. ZYMV MD had an 18 nucleotide insert following the start codon of the P1 coding region. The P1 proteins of all ZYMV isolates shared conserved amino acids in areas of the C terminus similar to those reported for other potyviruses. Polyclonal antisera were prepared to the P1 proteins of ZYMV FL/AT and RU expressed in Escherichia coli. The FL/AT and RU P1 antisera showed varying degrees of reactivity in Western blots with extracts of pumpkin (Cucurbita pepo L.) singly infected with a number of distinct ZYMV isolates. The reaction of the FL/AT P1 antiserum with isolate RU-infected tissue extracts was very weak compared to the homologous reaction. Neither antiserum reacted with extracts from plants singly infected with three other potyviruses, a potexvirus, or a cucumovirus. The P1 proteins of ZYMV isolates ranged in molecular mass from 33 kDa to 35 kDa. The P1 protein of strain MD was larger (35 kDa) than that of FL/AT (34 kDa). Indirect immunofluorescence tests with FL/AT P1 antiserum indicated that the P1 protein aggregates in ZYMV-infected tissues. The antisera to the ZYMV P1 proteins have potential as serological probes for identifying ZYMV and for distinguishing ZYMV isolates by immunoblotting.

Introduction

Zucchini yellow mosaic virus (ZYMV) is one of several members of the Potyviridae, which cause serious losses of cucurbitaceous crops worldwide. Both serological and biological variations have been reported for ZYMV isolates (Lisa & Lecoq, 1984; Lecoq & Purcifull, 1992; Wang et al., 1992). Serological relationships are often complex and ZYMV has been reported to cross react with watermelon mosaic virus 2 (WMV-2) in serological studies of the coat protein (CP; Davis & Yilmaz, 1984; Huang et al., 1986; Lisa & Lecoq, 1984; Purcifull et al., 1984; Somowiyarjo et al., 1989) and cylindrical inclusion (CI) protein (Suzuki et al., 1988). Biological variants range from strains which induce very mild symptoms to those which induce severe and necrotic symptoms (Petersen et al., 1991; Lecoq & Purcifull, 1992). These types of variants have been detected in geographically distinct regions including France and the USA (Lecoq & Purcifull, 1992). Biological variants have also been observed which differ in the ability to be aphid transmitted (Lecoq et al., 1991; Lecoq & Purcifull, 1992).

Many of the traditional and molecular studies involved in distinguishing potyviruses have been confined to the CP and to sequences of the 3' end (Shukla et al., 1991). This has been based, in part, on the ease of purifying CP or in the ease of cloning this coding region by oligo(dT) priming. The product of the 5' coding region, P1, has not been as well characterized as other regions of the genome. The protein encoded by the 5'-terminal region of the potyvirus genome is the most variable of those that have been sequenced (Shukla et al., 1991) and shows the greatest molecular mass variation, in over 30 potyviruses which have been studied by in vitro translations (Hiebert & Dougherty, 1988), with a size range from 32 to 64 kDa. The C terminus of P1 has been identified as a serine-type protease responsible for the autocatalytic
cleavage between P1 and helper component/protease (HC/Pro; P2) (Verchot et al., 1991). Brantley & Hunt (1993) found that the expressed P1 for tobacco vein mottling virus (TVMV) in Escherichia coli is an RNA-binding protein with a preference for ssRNA. Rodriguez-Cerezo & Shaw (1991) prepared an antiserum to TVMV P1 expressed in E. coli. A 31 kDa protein was detected at low levels in TVMV-infected tissue extracts which had been enriched for endoplasmic reticulum and mitochondria. Yeh et al. (1992), using monoclonal antibodies to a 112 kDa protein product of papaya ringspot virus (PRSV) type P, were able to detect both 51 and 64 kDa proteins, which presumably correspond to the HC/Pro and the P1 proteins, respectively. Canto et al. (1994) reported that monoclonal antibodies to potato virus Y (PVY) HC/Pro were useful in the identification of PVY strains.

The high sequence variability of P1 in potyviruses makes it an interesting protein coding region for study and a target for the development of probes for distinguishing potyviruses. The primary objectives of this study were to (i) characterize the P1 protein and coding region of an aphid-transmissible isolate of ZYMV from Florida (FL/AT); (ii) compare the P1 protein and coding region of ZYMV FL/AT to those of other ZYMV isolates and other potyviruses; (iii) evaluate antisera to the P1 protein as serological probes for studying the variability of ZYMV isolates; (iv) evaluate the serological relationship of this P1 protein to those of other potyviruses infecting the Cucurbitaceae; and (v) localize the P1 protein in ZYMV-infected tissues.

Methods

**Virus isolates.** An isolate of ZYMV from Florida (FC-1119; Purcifull et al., 1984), which was maintained in a greenhouse by aphid transmission, was used as the type isolate in this study and is hereafter designated as ZYMV FL/AT. The other Florida isolates were a culture of FC-1119 maintained by mechanical inoculation in the greenhouse (FL/GH), a severe, necrotic isolate from Florida (FC-2088) designated as SV, a mild isolate from Florida (FC-1994) designated as MD, isolates FC-2000, -2050, -2154, -3179 through -3183, from the collection of D. E. Purcifull and G. W. Simone, and isolate 81-25 from the collection of W. C. Adlerz. The ZYMV isolate from Reunion Island (RU; Baker et al., 1991, 1992) and Florida isolates SV and MD, both of which were sequenced in this study.

**Culture of virus isolates.** Host plants were either maintained in a growth room with an approximately 16 h day length and an average temperature of 23°C or in a greenhouse. Isolates obtained from outside the state of Florida were kept under quarantine conditions in a locked growth room. Host plants used for routine assay and maintenance were pumpkin (Cucurbita pepo L. ‘Small Sugar’), squash (C. pepo L. ‘Early Prolific Straightneck’), watermelon (Citrullus lanatus (Thunb.) Matsumi & Nakai ‘Crimson Sweet’) and cantaloupe (Cucumis melo L. ‘Hales Best Jumbo’).

**Virus purification and RNA extraction.** The protocol used for virus purification was similar to that described by Lecoq & Pitrat (1985). Virion RNA was isolated from a virus preparation (3 mg/ml) by adding an equal volume (1 ml) of RNA dissociating solution (200 mm-Tris-HCl, 2 mm-EDTA, 2% SDS pH 9.0) and 6 μl of protease K (20 mg/ml). After a 10 min incubation at room temperature, the RNA was isolated by a linear-log sucrose density gradient as described elsewhere (Dougherty & Hiebert, 1980).

**Synthesis of ZYMV clones.** The initial cDNA library of the ZYMV FL/AT RNA was made by using Lambda gt11 (Lambda Librarian; Stratagene). Purified viral RNA (8.3 μg) was used as the template in the first-strand (reverse transcription) synthesis reaction. Second-strand synthesis and the ligation of EcoRI/NorI linkers were performed according to the manufacturer’s instructions (Stratagene). The cDNA preparations with linkers were ligated to EcoRI-digested, calf intestinal alkaline phosphatase-treated Lambda gt11 DNA (Protocolone Lambda gt11 system; Promega) and then packaged and titrated according to manufacturer’s instructions using the Packagene Lambda DNA packaging system (Promega).

A library made specifically to represent the 5′ terminus of the ZYMV genome was constructed using the Lambda ZAP II EcoRI cloning kit (Stratagene). A primer, with the sequence 5′ CGGTGTGTGCGC-TAC 3′, which corresponded to the CI protein-encoding region, was used in this cloning experiment and was synthesized at the University of Florida Interdisciplinary Center for Biotechnological Research (ICBR) DNA Synthesis core. The host strain used for this vector system was E. coli XLI-Blue.

Clones identified in Lambda gt11 were digested with EcoRI and extracted from an agarose gel using a Prep-A-Gene DNA purification kit (Bio-Rad). Fragments were then subcloned by ligating into EcoRI-digested pGEMEX-1 (Promega). Plasmid clones [pBluescript SK(−)] were isolated from Lambda ZAP II with the use of the helper phage R408 according to manufacturer’s instructions (Stratagene).

**Immunoscreening of ZYMV clones.** Immunoscreening for clones expressing specific regions of the ZYMV genome was conducted essentially according to manufacturer’s instructions as described in the picoBlue immunoscreening kit (Stratagene) and by Short et al. (1988). Antisera to the CP and CI proteins of ZYMV, to the small nuclear inclusion protein (NIA) of tobacco etch virus (TEV), the HC/Pro (provided by T. Pirone, Kentucky, USA) of TVMV and HC/Pro (previously described as amorphous inclusion protein; de Mejia et al., 1985) of PRSV W were used for primary antibody screening. The antisera to the non-structural proteins used in screening were known to react with corresponding proteins of ZYMV and other potyviruses.

**DNA sequencing of ZYMV clones.** The nucleotide sequences of plasmid preparations and PCR products were determined using the standard Sanger dideoxynucleotide chain termination method (Sanger et al., 1977) employed in both USB and Pharmacia LKB sequencing kits. Sequence analysis and comparisons were made using the University of Wisconsin Genetics Computer Group sequence software.
The P1-encoding regions of ZYMV SV and MD were cloned and sequenced after amplification of the cDNA by PCR using primers specific for P1 of FL/AT, following procedures similar to those described by Robertson et al. (1991).

Amplification of P1 by PCR for subcloning and expression. Based on the nucleotide sequence of the P1 coding region of FL/AT, primers were made that corresponded to the N- and C-terminal regions. The specific primers for production of P1 were, on the 5′ terminus, 5′ CATGAGAATTCAGCTACAGGCTCTATCATG 3′, and on the 3′ terminus, 5′ CTGACCTTTAGACCTGTTCCAGCGGCTTGATC 3′. Primers used for sequencing and PCR amplification were obtained from the University of Florida ICBR DNA Synthesis facility. For cloning of P1 of ZYMV FL/AT, restriction sites with five flanking bases on the 5′ end were incorporated into the primers to provide for in-frame directional cloning into the pETH vector (McCarty et al., 1991; Studier et al., 1990) at HindIII and BglII sites on the pETH polylinker. The expressed protein contained 11 amino acid residues from the N-terminal peptide of T7 gene 10 protein in the vector.

Induction and expression of the P1 protein. The pETH plasmid from cultures, which were identified as having P1 in the correct orientation and reading frame was used to transform the appropriate host to provide for in-frame directional cloning into the pETH vector (McCarty et al., 1991; Studier et al., 1990) at HindIII and BglII sites on the pETH polylinker. The expressed protein contained 11 amino acid residues from the N-terminal peptide of T7 gene 10 protein in the vector.

Antigen preparation and antibody production. Cultures (50 ml) were induced for large scale P1 protein production. The expressed P1 protein was partially purified after sonication by three cycles of centrifugation of the insoluble fraction at 10,000 g and resuspension with TE buffer. The protein in the precipitate was further purified by preparatory SDS–PAGE. The protein band was visualized by incubating the gel in 0.2 M-KCl for 7 min at 4 °C after SDS–PAGE. The protein band was excised, washed three times in cold distilled water, frozen at -20 °C, and eluted using a Bio-Rad Electro-eluter at 10 mA/tube, with constant current for 5 h. The purified protein was dialysed overnight against distilled water and then lyophilized. Purity of the eluted protein was checked by analytical SDS–PAGE. Coomassie blue staining of the purified P1 protein in SDS–PAGE revealed a single band of 36 kDa. The antisera to ZYMV FL/AT (#1181) and RU (#1186) P1 proteins were prepared in rabbits by an initial immunization with 2 mg of antigen emulsified in Freund’s complete adjuvant. Subsequent injections with Freund’s incomplete adjuvant consisted of intramuscular injections of 1 or 2 mg at 2 weeks, 3 weeks and 4–7 months after the first injection.

Western blotting procedure. The Western blotting procedure was conducted essentially as described by Towbin et al. (1979) using a Bio-Rad Mini-PROTEAN II electrophoresis cell and Bio-Rad Trans-Blot electrophoretic transfer cell. Blocking solutions contained E. coli lysozyme at 1 mg/ml and extracts from non-infected plants (prepared by triturating leaf tissue in water; 1:9 w/v).

Young, symptomatic leaves of inoculated test plants were harvested between days 5 and 21 post-inoculation. Extracts for immunoblots were prepared by triturating leaf tissue in extraction buffer (ES buffer; Rodriguez-Cerezo & Shaw, 1991). The ES buffer consisted of 75 mM-Tris–HCl pH 6.1 containing 9 M-urea, 7.5% 2-mercaptoethanol and 4.5% SDS. One part plant tissue was triturated in a mortar and pestle with 2 parts of ES buffer. The triturate was squeezed through a single layer of moistened cheesecloth, boiled for 2 min and centrifuged at 5000 g for 5 min. Centrifuged samples were stored at -20 °C. Each isolate was tested from at least two different sources of tissue.

In vitro translation and immunoprecipitation. The wheat germ in vitro translation procedure was the same as described by Cline et al. (1985). RNA (3 μg) from ZYMV FL/AT, in a 50 μl wheat germ extract mixture containing 40 μCi of [3H]leucine was incubated at 25 °C for 60 min. Immunoprecipitation analyses were performed as described by Dougery & Hiebert (1980). Precipitated products were separated by SDS–PAGE on a 10% polyacrylamide gel and detected on dried gels by fluorography as described by Bonner & Laskey (1974). Antiserum was used for immunoprecipitation of in vitro translation products were to P1 and CP of ZYMV and to HC/Pro of PRSV W.

Light microscopy and immunofluorescence tests. Indirect immunofluorescence tests were conducted as described by Hiebert et al. (1984) with some modifications. Six μl of 10% DMSO in PBS, 27 μl of healthy plant extract (diluted 1/10 in PBS containing 1% ovalbumin) and 27 μl of antiserum were incubated together for 30 min prior to addition of epidermal strips from plant tissue. Epidermal strips were incubated in the antibody preparation in a 1.5 ml microtube after vortexing for 10 s. Tissue was incubated in the antibody solution on a shaker for 3–4 h at room temperature in the dark. Rinsing between steps was done twice in 1 ml of TBST (20 mM-Tris–HCl, 150 mM-NaCl, 0.1% Tween 20 pH 7.2) after vortexing for 10 s and once for 1 h in PBS while shaking at room temperature in the dark. Rhodamine-conjugated Protein A (Sigma) was used as a fluorescent probe. The rhodamine conjugate was diluted 1 g/ml in PBS. The conjugate (8 μl) was then mixed with 40 μl of 10% DMSO and 352 μl of PBS. After vortexing, rinsed tissue was incubated in this solution at room temperature for 3–4 h in the dark while shaking. After a final rinse, tissue was mounted on microscope slides using Aqua-mount (Lerner Laboratories). ‘Crimson Sweet’ watermelon was used as the host for immunofluorescence tests. Tissue sections were photographed with epifluorescence optics using a Nikon Fluophot microscope with a G2A filter.

Results

Similarities between the P1 of ZYMV isolates

Sequence comparisons were made between the P1-encoding regions of five ZYMV isolates, CA, RU, FL/AT, SV and MD. The sequences of P1 from ZYMV SV and MD were derived from clonal produced by reverse transcriptase (RT)–PCR using custom primers for P1. An agarose gel comparison of P1 products from RT–PCR for MD (mild) and SV (severe) isolates showed a size difference between the two, with the mild being slightly larger (Fig. 1). Amino acid sequence comparisons revealed a six residue insert (corresponding to a larger P1, see below) in the MD isolate immediately following the initiation codon (Fig. 2). The P1 sequences for the ZYMV FL/AT, SV and MD isolates were quite similar, with a nucleotide sequence similarity of 98% between FL/AT and SV, and a 95% sequence similarity between FL/AT and MD. The P1 of ZYMV CA had a high degree of similarity compared to FL/AT (96%), whereas RU was highly divergent, with only a 60% nucleotide sequence similarity compared to the P1 of FL/AT (Table 1). The ZYMV FL/AT P1 was also compared to the P1
of five distinct potyviruses and similarities ranged from 37–42% (Table 1).

Of 11 amino acid differences in P1 of the ZYMV SV isolate compared to FL/AT, five were polar (Fig. 2; positions 2, 4, 7, 23, 24, 86 and 129). In addition there was a deletion of a histidine residue at P1 position 41 in the SV isolate. There were six additional amino acids at the N terminus, plus nine different amino acids, for MD when compared to FL/AT (Fig. 2). Seven of these contributed to the charge or polarity of the protein (Fig. 2; positions 2, 4, 7, 23, 24, 86 and 129). In spite of the variability seen among the P1 coding regions for five ZYMV isolates, certain consensus sequences of amino acids believed to be involved or required for protease activity of P1 were conserved. For example, all five isolates had the conserved histidine residue at position 235, and the serine residue at position 276 within the P1 coding region.

Detection of P1 protein in plants infected with ZYMV

Extracts of ZYMV-infected tissues with ES buffer gave satisfactory reactions in Western blots using antiserum to P1 of ZYMV FL/AT or RU as a probe. The FL/AT antiserum reacted specifically to a protein of around 225 kDa, which is close to the molecular weight of P1 (Verchot-Vila et al., 1991).
ZYMV P1 protein characterization

34 kDa in plant tissue infected with ZYMV FL/AT (Fig. 3). No protein was detected in extracts from healthy plant tissues. Western blots with preimmune serum for ZYMV FL/AT did not result in a detectable protein reaction, whereas a prominent band of approximately 68 kDa was observed for RU preimmune serum (data not shown) as well as for the immune serum #1186 (Fig. 4a). Heterogeneity was seen in the size of the P1 protein among some of the other ZYMV isolates used in this study (Fig. 3). The approximate size for the P1 protein ranged from 35 kDa (five isolates) to 33 kDa (five isolates). A larger protein of around 35 kDa was noted

for three Florida isolates including MD, and for the three isolates from France. P1 products that were slightly smaller than that of ZYMV FL/AT included the Florida isolate SV and the isolates from Italy, Reunion Island, Taiwan, Egypt and Connecticut. In addition to size differences of the P1 protein, some isolates showed possible breakdown products of about 31–33 kDa and 26–27 kDa, whereas for other isolates (FC-3182, weak and E15) some extracts showed an 88 kDa product (Figs 3 and 5) presumed to be due to incomplete processing of P1 and HC/Pro. The 88 kDa product was not detected consistently, however (for example, isolate FC-3182 in Fig. 4). This product for FC-3182 also reacted with antiserum to the HC/Pro of PRSV W (Fig. 5).
G. C. Wisler, D. E. Purcifull and E. Hiebert

Fig. 6. Specificity of antiserum (#1181) to P1 of ZYMV FL/AT in Western blots. Extracts from samples infected with ZYMV FL/AT show a prominent band at approximately 34 kDa and a weak band at around 26 kDa. Note the lack of reactivity with extracts from pumpkin singly infected with any of three potyviruses (PRSV W, WMV-2, 2932), a cucumovirus (CMV), a possible potexvirus (FC-1860) or from non-inoculated pumpkin leaves (Mock).

Fig. 7. Immunoprecipitation of wheat germ in vitro translation products. TP, total products; P1, products immunoprecipitated with ZYMV FL/AT P1 antiserum; AI, products immunoprecipitated with antiserum to the HC/Pro of PRSV W; CP, products immunoprecipitated with antiserum to the CP of ZYMV FL/AT; NS, products immunoprecipitated with preimmune serum. The positions of molecular mass markers (lane M) are indicated on the left.

The size differences between the P1 proteins of ZYMV FL/AT, MD and SV were consistent regardless of the host used for Western blot assays. These three isolates were tested in pumpkin, watermelon, cantaloupe and squash (data not shown).

In addition to size differences noted for the P1 proteins for the various ZYMV isolates, some differences were also noted in the reactivity of the two P1 sera. Of the ZYMV isolates tested in this study, RU-, SV- and 'Italy'-infected tissue extracts reacted weakly or not at all with the antiserum (#1181) to the P1 of FL/AT (Fig. 3 and 4b). The RU isolate extracts did react strongly with antiseras to CP of FL/AT, and to the CI protein and HC/Pro of PRSV W in Western blots (data not shown). The antiserum (#1186) to P1 of ZYMV RU (after a booster immunization) reacted strongly with RU-infected tissue extracts as well as with extracts from ZYMV isolates FL/AT, Italy, MD and SV (Fig. 4a).

Extracts from pumpkin singly infected with any of several other viruses that infect cucurbits but are distinct from ZYMV were also tested in Western blots. These included PRSV W, WMV-2, an unnamed potyvirus (FC-2932) that is antigenically different from ZYMV, PRSV W and WMV-2 (Purcifull et al., 1991), CMV and a possible potexvirus of cucurbits (FC-1860; Purcifull et al., 1988). All of these extracts were negative in Western blot tests when tested against the antiserum to the P1 of ZYMV FL/AT (Fig. 6) and RU (data not shown).

Immunoprecipitation analysis of in vitro translation products

Translation products obtained in the wheat germ in vitro translation system, immunoprecipitated with antiseras to P1 and CP of ZYMV FL/AT, to HC/Pro of PRSV W, and with preimmune serum, were analysed by SDS-PAGE. Only the P1 and HC/Pro were found to be present in total translation products (Fig. 7). The antiseras to P1 and HC/Pro precipitated products of the appropriate molecular mass for each, 34 and 52 kDa, respectively. Neither the CP nor preimmune serum precipitated a protein product. Interestingly, the P1 antiserum also precipitated a smaller product of 25 kDa which may be similar to the possible breakdown product usually seen in immunoblots for that isolate.

Detection of P1 protein by indirect immunofluorescence

Fluorescence microscopy, using the P1 protein antiserum labelled with rhodamine-conjugated Protein A, showed the presence of aggregates in the cytoplasm of epidermal strips from ZYMV-infected watermelon. ZYMV isolate FL/AT showed accumulation of amorphous aggregates with particulate fluorescing bodies in cells of epidermal tissues when tested with the P1 antiserum of FL/AT (Fig. 8a). Similar results were seen with ZYMV isolates SV and FC-3182. Epidermal strips of plants infected with ZYMV FL/AT showed no fluorescence with preimmune serum as a probe (Fig. 8b), but aggregates could be seen unstained in epidermal tissue. Epidermal strips of ZYMV SV- and FC-3182-infected plants also did not show fluorescence when treated with preimmune serum. Tissues of watermelon infected with ZYMV RU and healthy watermelon (mock-inoculated) were nega-
Fig. 8. Immunofluorescence of watermelon stem tissue infected with ZYMV FL/AT and probed with antiserum (#1181) to ZYMV FL/AT P1 (a) and to normal serum (b). The antigen–antibody reaction was detected with rhodamine–protein A conjugate and photographed with epifluorescence optics. The localization of P1 is shown by the specific immunofluorescence of granular aggregates in (a). The scale bars represent 100 μm.

ZYMV P1 protein characterization

The P1 coding region for four ZYMV isolates, three from Florida and one from California, showed a high nucleotide and deduced amino acid sequence similarity. However, the P1 coding region for a ZYMV isolate from Reunion Island had a low (60%) nucleotide sequence similarity compared to these four ZYMV isolates. In contrast, the HC/Pro (P2) and P3 genes of ZYMV RU had 88% and 84% nucleotide sequence similarities, respectively, compared to FL/AT and CA (Table 1; Baker et al., 1992). In addition, the sequence of the CP gene of RU is 88% similar to that of CA (Baker et al., 1991). According to the criteria set out by Shukla et al. (1991), the nucleotide sequence similarity of the P1 gene from RU compared to the P1 gene of other ZYMV isolates (60%) could classify it as a distinct potyvirus, whereas the sequence similarities of other regions of the ZYMV RU genome (84–88%) are close to those expected for an isolate of the same virus. It appears that the sequence similarity from one area of the potyvirus genome does not always predict the degree of sequence similarity in other coding areas.

Nucleotide sequence similarities between the P1 gene of ZYMV FL/AT and those of five other potyviruses ranged from 37% to 42% (Table 1). These similarities also were lower than those for the HC/Pro (50–52%) and P3 (43–45%) genes compared to ZYMV FL/AT (Table 1). These data are in agreement with Shukla et al. (1991) in that the percentage sequence similarity between distinct potyviruses is low, and that P1 is less conserved than the HC/Pro- or P3-encoding region. The hypervariable regions of the potyviral genome, based on comparisons of different potyviruses, also show variability at the virus strain/isolate level (Thole et al., 1993). Antibodies produced to these hypervariable regions in the potyviral genome have a potential to serve as very specific probes for rapid identification of potyviral strains (Purcifull & Hiebert, 1992).

Despite the high degree of sequence similarity among four ZYMV isolates in the P1 coding region, significant differences were detectable by SDS–PAGE and these may be exploited in distinguishing these isolates. For example, the additional 18 nucleotides in the 5' terminus of the P1 gene of ZYMV MD code for six additional amino acids and result in a P1 product which is readily resolved in Western blot analysis using antiserum to P1 of FL/AT. Some ZYMV isolates also had a slightly smaller P1 product. The SV isolate P1 differs from the type isolate by the absence of a single histidine residue as well as having a slightly smaller P1 by SDS–PAGE. Since the P1 protein of ZYMV SV has only one less amino acid residue, the smaller size of P1 seen in SDS–PAGE may
be due to the charge effect on mobility as a result of the loss of the histidine. Sequence information is not available for the other isolates with a smaller P1 protein so we do not know whether this is due to deletions or to amino acid residue differences which affect the mobility in SDS-PAGE analysis. Other variations such as incomplete processing of the P1-CH/Pro polypeptides and breakdown products of the P1 protein were evident for some ZYMV isolates in the Western blot analysis and are presumably due to sequence variations in the P1 coding region.

The amino acid residues for the N-terminal region of P1 proteins of the five ZYMV isolates in this study were less conserved than those in their C-terminal regions. The additional six amino acids of ZYMV MD directly followed the methionine at the N terminus. Most of the changes in amino acids in both ZYMV MD and SV are also in their respective N-terminal region, including the histidine missing from the SV isolate.

The amino acid residue histidine, the serine residue within the amino acid consensus sequence (GXSGS) and a five amino acid motif (LVIRG) (Fig. 2) in the C-terminal half of P1, determined by Verchot et al. (1991) to be essential for protease activity, were conserved in all sequenced ZYMV isolates. Although the consensus sequence LVIRG was the same for all ZYMV isolates, it was slightly different from that (FIVRG) for 5 distinct potyviruses (Verchot et al., 1991). The Y/S or D cleavage sites between P1 and P2 (HC/Pro) were also conserved among the ZYMV isolates.

The ZYMV P1 accumulates in infected cells to form inclusions (Fig. 8) as do other non-structural potyviral proteins (for example CI proteins). Further analysis by electron microscopy is needed to determine the precise morphology of the aggregates and their possible association with other viral proteins or with host components.

The antisera developed in this study have been useful for detecting variability of ZYMV isolates by immunoblotting. There were differences between isolates in the size of the P1 proteins in the reactivity of the P1 and HC/Pro polyprotein. The antisera to the P1 of ZYMV FL/AT or RU did not react with extracts from plants infected individually with three other potyviruses, one cucumovirus, or one potexvirus. Serological (polyclonal) probes prepared to ZYMV P1 protein not only enable the clear distinction of ZYMV from other potyviruses but may also be used to characterize ZYMV strains.

The variation in the P1 coding region of the ZYMV isolates also has been noted for other potyviruses (Verchot et al., 1991; Thole et al., 1993; Duan et al., 1993) and has led to speculation that the P1 may be associated with virus-host interactions. Further studies to elucidate this possible involvement of P1 in host response will involve the use of full-length infectious transcripts. With these transcripts the P1 from different isolates could be interchanged to determine the possible effects on the host response.

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References


ZYMV P1 protein characterization


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