NUCLEOTIDE SEQUENCES OF THE COAT PROTEIN AND READTHROUGH PROTEIN GENES OF THE CHINESE GAV ISOLATE OF BARLEY YELLOW DWARF VIRUS

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Summary. – The nucleotide sequences of the coat protein (CP) and readthrough protein (RTP) genes of the Chinese GAV isolate of Barley yellow dwarf virus (BYDV) were determined. The CP and RTP genes of GAV isolate comprised 600 and 1374 nucleotides, respectively. When the CP and RTP gene sequences of GAV isolate were compared with those of BYDV isolates MAV-PS1, P-PAV, NY-SGV and Cereal yellow dwarf virus RPV (CYDV-RPV), the highest similarity (97.2%) between the CP genes of GAV and MAV-PS1 isolates was observed, while the RTP genes of these two isolates shared a lower similarity (87.8%). The results of the alignment of the deduced amino acid sequences of RTP showed that the sequence diversity observed was located at the C terminus.

Key words: Barley yellow dwarf virus; GAV isolate; MAV-PS1 isolate; P-PAV isolate; NY-SGV isolate; Cereal yellow dwarf virus RPV; coat protein gene; readthrough protein gene; nucleotide sequences; deduced amino acid sequences;alignment; transmission phenotype

Barley yellow dwarf viruses (BYDVs, family *Luteoviridae)* are economically most important pathogens for small grains, especially barley, wheat and oats (Lister and Ranieri, 1995). Luteoviruses are phloem-limited and obligately transmitted by aphids in circulative/persistent manner. According to the currently valid virus taxonomy (Van Regenmortel *et al.*, 2000) there are five BYDV species, namely BYDV-MAV and BYDV-PAV (both in the genus *Luteovirus,* the family *Luteoviridae*) and BYDV-GPV, BYDV-RMV and BYDV-SGV (all unassigned species in the family *Luteoviridae*). CYDV-RPV belongs to the species CYDV-RPV, the genus *Polerovirus*, the family *Luteoviridae.*

Within the BYDV-MAV and BYDV-PAV genomes, six open reading frames (ORFs) have been identified; ORF3 and ORF5 encode CP and a putative RTP that has been found in purified virus particles (Mayo and Ziegler-Graff, 1996). Waterhouse *et al.* (1989) have suggested that this protein is involved in aphid transmission specificity; later on, Wang *et al.* (1995) and Chay *et al.* (1996) have confirmed it.

In China, BYDVs cause mainly yellow dwarf diseases of wheat throughout the northern and north-western provinces. Crop losses of 20–30% have been observed for many years, and even greater losses have been estimated in 1966, 1970, 1973, 1978 and 1999 in some provinces. Most of the work on BYDVs in China has been focussed on the disease epidemiology and control, isolate identification, mechanism of transmission by aphid vector and resistant host breeding.

The Chinese GAV isolate of BYDV can be transmitted efficiently by *Sitobion avenae* Fabr. (*S. avenae*) and *Schizaphis graminum* Rond. (*S. graminum*), and occurs more frequently than the other luteoviruses in cereal crops in China. GAV isolate shows a strong serological reaction with NY-MAV-specific antiserum, but NY-MAV isolate is transmitted specifically only by *S. avenae.*

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Abbreviations: BYDV(s) = barley yellow dwarf virus(es); CP = coat protein; CYDV-RPV = Cereal yellow dwarf virus RPV; ORF = open reading frame; RTP = readthrough protein

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1    V D S S T P E P T P Q P Q P E P K P D P Q P T P E P Q H K R F F Y V V G T P Y V  GAV
     - - - - - - - - S - - - - - - - - - - - - - - - - - R Q - - - - - - - - - - - -  MAV-PS1
41   I I Q T R E S S D S I A V K S M N D Q S F R Y I E N E T S E Q R T V Q A W W T S  GAV
     V - - - - - - - - - - - - - - - - - - A - - - - - - - - - - K - - - - - - - N -  MAV-PS1
81   N N G V Q A Q A A F V F P I P A G E Y S V N I S C E G L Q S V D H I G G N R D G  GAV
     - - S - - - - - - - I - - - - - - - - - - - - - - - S - - - - - - - - I - - - - - - - - -  MAV-PS1
121  Y W I G L I A Y Q N Q S G D Y W G V G Y W A G C D I T N L L G T N T W R P G H E  GAV
     - - - - - - - - - - S - - - - - - - - - - - - - V - - - - - - - - - - - - - - - - - - - -  MAV-PS1
161  D L E L N G C K F T N G Q I V E R D A V I S F H L K A Q G A D P K F Y L M A P K  GAV
     - - - - - - S - - - - D - - - - - - - - - - - - - - - V - - R - - - - - - - - - - - - - -  MAV-PS1
201  T M K S D K Y N Y V V S Y G G Y T D K R M Q F G T I S V T V D E S D V E A Q R Y  GAV
     - - - A - - - - - - - - - - - - - - - - - - - - E - - - - - - - - - - - - - - - - - E - -  MAV-PS1
241  N R H T S A V R K T E N L D Y G W M S V L P P Y D P N Q V P E Q E E E Q P M V D  GAV
     S - - - - T - - R - - - - - - - - - - N - - - - - N - D - - - - - - D - - - V - -  MAV-PS1
281  K G M D S R S P V E P P S P T S D T E A E R A F D L R E E K L T R A R L E Y E A  GAV
     - E - - A G - - I D T A - L - - - - - - - - K - - - - K - - E - - I - - - - - - - -  MAV-PS1
321  A T E S I P D A A P D I L P S K S E M S S R P I D H D G R S L P K P Q S K E V L  GAV
     - - V - - - - - - - - - - - - - - - - - - - - - K - - - R - - - - - - - - S - T - - - -  MAV-PS1
361  G T Y Q G Q N I T P D D V P P V I A E K L R E V N R A P S T L L Y D R Q P K T P  GAV
     - - - - - - - - - - S - - - - - - - - - - E - - - - - - - - - - - - S - - - - - - - - - Q -  MAV-PS1
401  K S F L S R F V E T N K T S L A S P G S Q S S T S G M T R E Q A S E Y T R I R K  GAV
     - N P - T - - - L S - - - - T - - - - - - - - - - - A - - - - - - - - - - - - - - - - - -  MAV-PS1
441  S M G L T A A K E Y K A S L A D T                                              GAV
     - L - - - - - - Q - - - - - D - -                                              MAV-PS1
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Fig. 1
Alignment of deduced amino acid sequences of RTP genes of GAV and MAV-PS1 isolates of BYDV
For the source of sequences see Materials and Methods.

Table 1. Nucleotide sequence similarity (%) of CP and RTP genes of GAV isolate to those of other isolates of BYDV and CYDV-RPV

Isolate/Virus	GAV	MAV-PS1	P-PAV	NY-SGV	CYDV-RPV
GAV	–	97.2	76.6	67.6	52.2
MAV-PS1	87.8	–	76.7	52.5	53.7
P-PAV	60.8	61.5	–	69.0	56.4
NY-SGV	49.1	48.7	50.7	–	52.5
CYDV-RPV	41.7	42.5	42.7	47.8	–

The upper right values refer to RTP gene, while the lower left values to CP gene.
For the abbreviations and sources of data see Materials and Methods.

To understand differences in transmission phenotype of GAV and NY-MAV isolates of BYDV, we compared the nucleotide and deduced amino acid sequences of CP and RTP genes of isolates GAV, MAV-PS1, P-PAV and NY-SGV of BYDV and CYDV-RPV (species CYDV-RPV).

The Chinese GAV isolate was maintained in *Avena sativa* plants (cultivar Coast Black) as described earlier (Zhou *et al.*, 1984). The virus was purified from leaves of systemically infected oats plants two weeks after inoculation by a modified enzyme-assisted procedure as described by D'Arcy *et al.* (1989). Viral RNA was extracted from purified virus preparations by a SDS-phenol method and was ethanol-pre-

cipitated. A RT-PCR was employed for synthesis of cDNAs of CP and RTP genes. First-strand cDNAs were synthesized from GAV RNAs with minus strand oligonucleotide primers GAVCP1 (5'-CTATTTGGGAGTCATGTTGGC-3') and GAVRTP1 (5'-TTGATCTTCACTATG-3') using Moloney murine leukemia virus reverse transcriptase (Promega). Double-stranded cDNAs were synthesized from the first-strand GAV cDNAs using oligonucleotide primer pairs GAVCP1 (5'-CTATTTGGGAGTCATGTTGGC-3') and GAVCP2 (5'-ATGAATTCAGTAGGCCGTAGA-3') (for CP) and GAVRTP1 (5'-TTGATCTTCACTATG-3') and GAVRTP2 (5'-GTAGACTCCTCAACA-3') (for RTP) by a PCR method. The oligonucleotide primers were designed and synthesized according to the RNA sequence of the MAV-PS1 isolate of BYDV (Ueng *et al.*, 1992). The primer pairs P31, P32 and P51, P52 were used to amplify CP and RTP genes, respectively. The obtained PCR products were made blunt-ended with T4 DNA polymerase and were subjected to agarose gel (0.8%) electrophoresis. The fragments of choice were inserted into the *Sma*I site of pGEM-7zf(+) vector (Promega). *Escherichia coli* JM109 strain was used for transformation. After screening by selective media, recombinant plasmids were isolated and purified by the alkaline method, electrophoresed, isolated and subjected to restriction analysis. Nucleotide sequencing of inserted genes was performed by the dideoxynucleotide chain termination method using an automated sequencer (Perkin Elmer). The nucleotide and deduced amino acid sequences of the CP and RTP genes of GAV isolate were compared and aligned with sequences of other BYDVs isolates accessible in the GenBank database. Multiple sequence alignment was performed using the DNasis 2.5 Software (Hitachi Software Engineering Co. Ltd.) and DNAStar MegAlign (DNAStar, Inc). The CP and RTP gene sequences of the following BYDVs isolates were included in the comparison: MAV-PS1 and P-PAV (Ueng *et al.*, 1992; Acc. No. X17260), NY-SGV (Lei *et al.*, unpublished data; Acc. No. U06865) and CYDV-RPV (Larkins *et al.*, 1991; Acc. No. NC001599).

The nucleotide sequences of CP and RTP genes of GAV isolate were determined from four recombinant clones and deposited at GeneBank (Acc. No. AF338909). CP and RTP genes consisted of 600 and 1374 nucleotides, respectively, identically with MAV-PS1 isolate. The nucleotide sequences of GAV isolate showed a low similaritiy to other isolates except MAV-PS1 (52.2–76.6% for CP gene and 41.7–60.8% for RTP gene) (Table 1). Because the similarity of CP gene of isolates GAV and MAV-PS1 was very high (97.2%), we suggest that CP gene might not be the reason for the difference in transmission phenotype between isolates GAV and NY-MAV.

As RTP gene showed a lower similarity of GAV and MAV-PS1 isolates (87.8%), and it is generally accepted that each gene performs its function through its protein product, a multiple sequence alignment was conducted with the deduced amino acid sequences of RTP of GAV and MAV-PS1 isolates (Fig. 1). A similarity of 87.09% was obtained; 59 of total of 457 amino acids were substituted in GAV isolate. These substitutions were located mainly in the region of amino acid positions 238–346. There were 24 substitutions in total of 108 amino acids in this region. These substitutions might be the reason for difference between GAV and NY-MAV isolates in their transmission phenotype.

A RTP consisting of ORF3 and ORF5, which can be the result of non-functional stop codon in ORF3, has been detected in preparations of purified particles of many luteoviruses (Mayo and Ziegler-Graff, 1996). The region immediately downstream of the ORF3 stop codon is very rich in proline and shows considerable homology in the readthrough domain of all luteoviruses. This proline hinge may serve as a loose tether joining CP (presumably anchored in the virion capsid) and the rest of the readthrough domain (Bahner *et al.*, 1990; Guilley *et al.*, 1994). However, the C-terminal region of RTP is more diverse; Guilley *et al.* (1994) have proposed that this region is involved in the specificity of transmission by vectors. The results presented here support this hypothesis.

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Note of the Editor-in-Chief. The terms "the Chinese GAV isolate of BYDV", "the MAV-PS1 isolate of BYDV", "the P-PAV isolate of BYDV", and "the NY-SGV isolate of BYDV" are not in accord with the currently valid virus nomenclature, taxonomy and classification (see Van Regenmortel *et al.*, 2000 in the enclosed References and the part of this article dealing with five BYDV species).

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