Genetic Diversity of a Natural Population of Apple stem pitting virus Isolated from Apple in Korea

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Apple stem pitting virus (ASPV), of the Foveavirus genus in the family Betaflexiviridae, is one of the most common viruses of apple and pear trees. To examine variability of the coat protein (CP) gene from ASPV, eight isolates originating from 251 apple trees, which were collected from 22 apple orchards located in intensive apple growing areas of the North Gyeongsang and North Jeolla Provinces in Korea, were sequenced and compared. The nucleotide sequence identity of the CP gene of eight ASPV isolates ranged from 77.0 to 97.0%, while the amino acid sequence identity ranged from 87.7 to 98.5%. The N-terminal region of the viral CP gene was highly variable, whereas the C-terminal region was conserved. Genetic algorithm recombination detection (GARD) and single breakpoint recombination (SBP) analyses identified base substitutions between eight ASPV isolates at positions 54 and 57 and position 771, respectively. GABranch analysis was used to determine whether the eight isolates evolved due to positive selection. All values in the GABranch analysis showed a ratio of substitution rates at non-synonymous and synonymous sites (dNS/dS) below 1, suggestive of strong negative selection forces during ASPV CP history. Although negative selection dominated CP evolution in the eight ASPV isolates, SLAC and FEL tests identified four possible positive selection sites at codons 10, 22, 102, and 158. This is the first study of the ASPV genome in Korea.

Keywords: apple stem pitting virus, apple, CP, genetic diversity

Recombination is the process by which segments of genetic information switch between nucleotide strands of different genetic variants during replication (García-Arenal et al., 2001). Plant RNA viruses are known to have high mutation and recombination rates due to the absence of proofreading activity in their RNA replicase (Drake and Holland, 1999; Bruyere et al., 2000; Malpica et al., 2002). However, this mutability does not necessarily result in a high degree of genetic variability within their populations, as distribution in the virus population of genetic variants depends on two major evolutionary processes: genetic drift and selection (García-Arenal et al., 2001). Genetic drift is a random effect that occurs during the transmission of genetic traits to new generations, while selection is a directional process by which variants that are fittest in a certain environment will increase their frequency in the population (positive selection) and less fit variants will decrease their frequency (negative or purifying selection) (García-Arenal et al., 2001).

We analyzed the genetic diversity of a natural population of Apple stem pitting virus (ASPV) isolated from apple trees in Korea. ASPV is the type member of the Foveavirus genus in the family Betaflexiviridae (Martelli et al., 2007; Martelli and Jelkmann, 1998), and is one of the most common viruses of apple and pear trees worldwide (Kundu, 2003). ASPV is known to cause disease associated with stem pitting, vein yellowing, and graft incompatibility such as top-working disease in apple (Jelkmann, 1997). Previous studies have reported the genetic diversity of ASPV isolates (Komorowska et al., 2011; Schwarz and Jelkmann, 1998; Yoshikawa et al., 2000). Recently, Komorowska et al. (2011) detected recombination events and evolutionary pressures in ASPV isolates from apple and pear in Poland. In Korea, stem pitting symptoms were first reported in 1973 (Oh et al., 1973); however, genomic studies of ASPV have not been performed. To explore the evolutionary history of ASPV spread in apple trees in Korea, we surveyed ASPV infections in 251 apple trees collected from 22 apple orchards...
Table 1. Information on the identified Apple stem pitting virus (ASPV) isolates from apple orchards in Korea. M24 was used to compare variations in the coat protein (CP) amino acid sequences

| ASPV isolates | Original host | Region collected | Orchard where collected | CP gene length (nt) | GenBank accession no. |
|---------------|---------------|------------------|-------------------------|--------------------|-----------------------|
| M24           | Apple         | -                | -                       | 1,191              | AF438522              |
| JGH-HR-YC     | Apple cv. Hongro | Young-cheon     | JGH                     | 1,191              | KC791783              |
| SJY-HR-YC1    | Apple cv. Hongro | Young-cheon     | SJY                     | 1,191              | KC791784              |
| SJY-HR-YC2    | Apple cv. Hongro | Young-cheon     | SJY                     | 1,191              | KC791785              |
| KJH-HR-JS1    | Apple cv. Hongro | Jang-su         | KJH                     | 1,191              | KC791786              |
| KJH-HR-JS2    | Apple cv. Hongro | Jang-su         | KJH                     | 1,191              | KC791787              |
| KJH-HR-JS3    | Apple cv. Hongro | Jang-su         | KJH                     | 1,191              | KC791788              |
| KHS-HR-SJ     | Apple cv. Hongro | Sang-ju         | KHS                     | 1,191              | KC791789              |
| NJS-HJ-GW     | Apple cv. Huji   | Gun-wei         | NJS                     | 1,191              | KC791790              |
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Thus, to determine whether the ASPV isolates evolved due to positive selection, the dNS/dS ratios in the CP coding regions were estimated using the GABranch method (Kosakovsky Pond et al., 2006). This method can automatically partition all branches of the phylogeny describing non-recombinant data into groups according to the dNS/dS ratio. Based on our results, all values in this GABranch analysis showed dNS/dS ratios below 1, indicative of negative selection during the history of ASPV CP (Fig. 4). The dNS/dS values calculated for the CP gene from the eight ASPV isolates were in the range of those calculated for CP sequences of other members of the family Flexiviridae (Komorowska et al., 2011). Despite the high variability of the CP gene sequences, all values in this GABranch analysis showed omega values below 1. Thus, high levels
of variability in the CP gene sequences resulted from a high rate of synonymous mutations. Other viruses showed low dNS/dS values in the family Flexiviridae, despite the high level of variability in the CP sequences (Chare and Holmes, 2005; Teycheney et al., 2005).

To identify sites under positive or negative selection for each codon, SLAC, FEL, and REL were performed (Kosakovsky Pond and Frost, 2005a, 2005b). These methods can be used to estimate the ratio of non-synonymous and synonymous substitutions at each site. A consensus scoring approach was applied to determine the site most likely experiencing positive selection. Although purifying (negative) selection dominated the evolution of the CP gene in the eight ASPV isolates, all three methods showed that codons 10, 22, 102, and 158 were under positive selection (Table 2). These results suggest that various codons are under different selection forces in ASPV Korean isolates.

Here, we reported the genetic diversity of a natural population of ASPV originating from apple orchards located in intensive apple growing areas in Korea. Komorowska et al. (2011) reported that the majority of mutations in CP gene sequences from ASPV isolates collected from Poland were deleterious. In agreement with these results, although the Korean ASPV isolates showed strong variation in their CP gene sequences (even those originating from the same orchard), purifying (negative) selection dominated during evolution of the CP gene from eight ASPV isolates. Meanwhile, our results also show that although purifying selection dominated the evolution of the CP gene in the eight ASPV isolates, various codons were under different selection forces. This is the first study of the ASPV genome in Korea.

Table 2. Results obtained from SLAC, FEL, and REL analyses, which were used to identify sites under positive or negative selection in the CP gene of ASPV. For each method, two values were provided (dNS/dS and p-values), with the exception of the REL method, which generates a Bayes factor instead of a p-value. The consensus column indicates a certain codon under positive selection: “P”, positive selection; “N”, negative selection.

| Codon | Consensus | SLAC | | | REL | Bayes factor |
|-------|-----------|------|----------|------|----------------|---------------|
|       |           | dNS/dS$^1$ | p-value | dNS/dS | p-value | dNS/dS | p-value |
| 10    | P         | 1.45839 | 0.444444 | 1.11281 | 0.135212 | 0.0670287 | 64.0498 |
| 22    | P         | 1.42796 | 0.463589 | 1.01134 | 0.156472 | 0.0347429 | 55.9968 |
| 102   | P         | 1.43413 | 0.517591 | 0.856537 | 0.17512 | 0.0276572 | 52.1857 |
| 158   | P         | 1.39762 | 0.455587 | 1.02478 | 0.156074 | 0.026598 | 54.6577 |

$^1$Normalized value

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