DNA fingerprinting of reference strains of *Puccinia striiformis* f. sp. *tritici* in China*

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The rapid development of molecular biology resulted in the wide application of population genetics theory to plant pathology. It is the development of efficient molecular DNA marker systems that gives a great insight into the population genetics of a number of fungal plant pathogens such as rice blast fungus *Magnaporthe grisea*[1], late blight fungus *Phytophthora infestans*[2], barley powdery mildew fungus *Erysiphe graminis* f. sp. *hordei*[3] and wheat blotch fungus *Septoria tritici*[4]. The knowledge on population biology of plant pathogens is essential for screening and identification of disease resistance genes, for development of resistance genes, and for development of more effective disease control measures[5]. In view of the economic importance of wheat stripe rust much effort has been given to it since the 1950s in China. This greatly improved wheat production via development of effective control of wheat stripe rust. However, extensive understanding of the mechanisms of virulence variation and evolution of wheat stripe rust was limited by a lack of suitable genetic markers. Our goal was therefore to develop molecular DNA markers so as to facilitate population biological and epidemiological studies of wheat stripe rust. We previously identified a family of moderately repetitive DNA sequences (PSR) in *P. striiformis*[1)]. In this report, we described DNA fingerprinting of the reference strains of epidemic races of *P. striiformis* with PSR331S3, a subclone of PSR331.

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1) Shan, W. X., Chen, S. Y., Zhang, G. Y. *et al.*, Identification of a family of dispersed, moderately repetitive DNA sequence in an obligate fungal plant pathogen *Puccinia striiformis*, 1995.

1 Materials and methods

1.1 Strains

The origin of the fungal strains and their virulence patterns on 17 differential wheat cultivars were as described previously[6].

1.2 DNA isolation

The method for isolation of rust fungal genomic DNA was as described in Shan *et al.*'s paper[1].

1.3 Southern hybridization

Standard procedures used for restriction enzyme digestion, agarose gel electrophoresis and Southern hybridization were according to the method of Sambrook *et al.*[7]. Random oligonucleotide primer procedure was used for preparation of ^{32}P-labeled DNA probes. Hybridization and membrane washing (three times for 15 min each in: 2×SSC, 0.1% SDS; 1×SSC, 0.1% SDS; 0.5×SSC, 0.1% SDS) were done at 65℃.

1.4 Statistical analysis

Each hybridizing band was considered to be a genetic locus, and the presence and absence of each band were scored 1 and 0, respectively. Cluster analysis was performed with group-average method using Euclidean Distance as a measure of genetic dissimilarity between strains.

2 Results

In previous work, we identified firstly a family of moderately repetitive DNA sequences which were named PSR sequences. Among the four PSR sequences, PSR331 and PSR389 were able to generate distinct fingerprinting patterns[1]. PSR331S3, a subclone of 2.1 kb of PSR331, was demonstrated to be more effective than PSR331 in generating fine DNA fingerprints.

The results from DNA fingerprinting of Chinese reference strains of *Puccinia striiformis* f. sp. *tritici* using PSR331S3 as a probe indicated that PSR sequences were effective in detecting genetic variation within as well as among races (fig. 1, for fingerprinting patterns, see table 1).

Cluster analysis based on fingerprint data grouped the tested strains into several lineages, which were not correlated with virulence variation. Strains of the same races, for example, CY31 strains 29-Shui-11, 93-6-5-16 and 93-7-1-18, CY30 strains Chuan28 and 91-6 2-10, were shown to be genetically distinctive. On the other hand, strains of different

1) See the footnote 1) on page 2078.

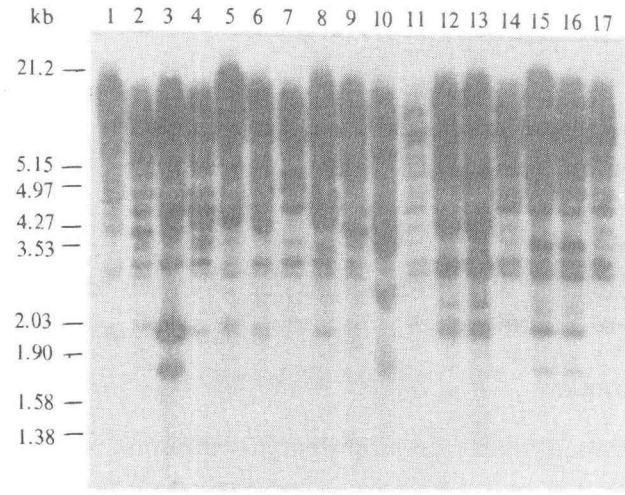

Fig. 1. Southern hybridization of PSR331S3 to *Bgl* II-digested genomic DNAs of the reference strains of *Puccinia striiformis* f. sp. *tritici*. Strain abbreviations are as shown in table 1. Lambda DNA digested with *EcoR* I and *Hind*III was used as standard size markers.

Table 1 Data set showing presence (1) or absence (0) of 24 *Bgl*II positions hybridizing to PSR331S3 in the tested strains of *Puccinia striiformis* f. sp. *tritici*

Label	Strain/race	Place	PSR331S3/*Bgl*II
1	CY17	–	000111011001011001011101
2	CY19	–	000010011101011001111101
3	CY21	–	001100001001111001111101
4	CY22	–	100100011110111001111111
5	CY23	–	100110011010111101101101
6	CY25	–	000110111011111101111101
7	CY26	–	100001111010011001011101
8	CY27	–	100100111100111101101111
9	CY28	–	100000111011011111111101
10	Hx/CY29	–	001100011101111101111110
11	L13/CY29	–	000010011011011011011111
12	Shui-11	–	010110111001011011011101
13	91-6-2-10/CY30	Pingshan, SC	010110111001011101111101
14	Chuan-28/CY30	Pingshan, SC	000010011011011001111101
15	93-6-5-16/CY31	Anyue, SC	101101011100011001101101
16	93-7-1-18/CY31	Gangu, GS	101101011100011001101101
17	29-Shui-11/CY31	Taibai, SX	000010011011011001111101

SC, Sichuan Province; GS, Gansu Province; SX, Shaanxi Province.

virulence patterns may share the same genetic background (for example, CY30 strain Chuan28 and CY31 strain 29-Shui-11 had the same DNA fingerprinting patterns) (fig. 2). It was therefore questionable for understanding mechanisms involved in virulence and systematic evolution of pathogens solely based on evolutionary relationship among races

deduced from virulence analysis.

Genetic variation between two subcultures Hx and L13 of a reference strain of CY29 was detected with PSR331S3 as well. Preliminary experiments suggested that the PSR fingerprints were genetically relatively stable. The observed polymorphisms therefore might be caused by a contamination event because subculture Hx was multiplied on wheat cultivar Huixianhong which was susceptible to all known races, whereas subculture L13 was multiplied on wheat cultivar Lovrin13 susceptible to very limited number of races. Subculture Hx is more susceptible to contamination.

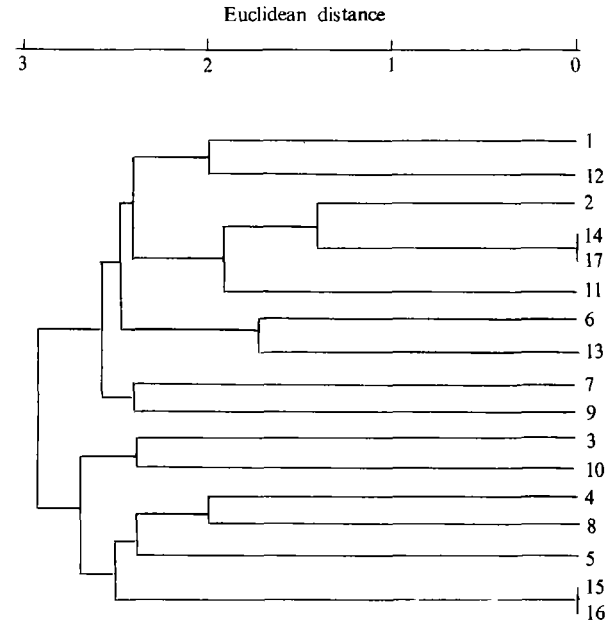

Fig. 2. Dendrogram constructed by group-average cluster analysis of the reference strains of *Puccinia striiformis* f. sp. *tritici* based on fingerprint data as shown in table 1.

It was shown that strain 93-6-5-16, sampled from Anyue, Sichuan Province, was genetically of the same origin with strain 93-7-1-18 sampled from Gansu Province based on PSR fingerprints. In the case of strain Chuan28 of Pingshan, Sichuan Province, and strain 29-Shui-11 of Taibai, Shaanxi Province, a similar result was obtained. The results suggested that gene flow of *P. striiformis* f. sp. *tritici* might be more frequent than expected, though the pathogen populations were separated distantly and even with mountain ranges. However, more isolates would be needed before obtaining firm conclusion.

Under our experiment conditions, there were 24 distinct *Bgl* II fragments homologous to PSR331S3. Among the 24 *Bgl* II fragments hybridizing PSR331S3, 18 were shown to be polymorphic among the tested strains. The potential of PSR sequences in the generation of strain (clone)-specific fingerprints implied their future use for population biological and epidemiological studies of *P. striiformis* f. sp. *tritici*.

3 Discussion

The knowledge on populations of an organism will be essentially necessary for understanding its evolution. A lack of genetic markers was responsible for our poor understanding of the population biology of fungal plant pathogens, of which our knowledge was almost exclusively based on virulence surveys. The virulence genes were

assumed to be not suitable for understanding molecular mechanisms involved in virulence variation because of some limitations such as strong host selection subjected to virulence genes. Results from DNA fingerprinting of a number of plant pathogenic fungi indicated that DNA polymorphism was not correlated with virulence variation, i.e. the evolution of virulence in pathogens was independent of DNA polymorphism. Although the molecular mechanisms of virulence evolution in pathogens were not clear yet, it was quite possible that the diversity of population genetic structures of pathogens was closely correlated with their potential to adapt to changing environments, i.e. populations of complex genetic structures have great potential to generate new virulence genes or gene combinations to breakdown newly introduced host resistance genes.

Results from DNA fingerprinting of epidemic races of *P. striiformis* f. sp. *tritici* using PSR331S3 as a probe indicated that PSR sequences were effective in detecting genetic variation within as well as among races. Preliminary results suggested that DNA fingerprints based on PSR sequences were genetically relatively stable. In view of their species-specificity, highly variable and moderately repetition, it was possible that PSR sequences were components of transposable elements. Therefore, genetic loci (hybridizing bands) homologous to PSR sequences may be independently inherited. In addition, the independences of PSR-based DNA markers with virulence diversity suggested that PSR fingerprints, unlike virulence genes that are subject to strong host selection, were genetically neutral. These features of PSR fingerprints implied that PSR sequences would facilitate population genetic studies of *P. striiformis*. In view of the potential of PSR sequences in generation of strain (clone)-specific fingerprints, PSR sequences would be useful for monitoring specific strain in the environment and for understanding population biology of host-pathogen interactions. It is anticipated that PSR sequences will greatly facilitate future studies on population biology and epidemiology of *P. striiformis*.

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