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Short communication

Conformation polymorphism of RNA transcripts derived from various PNRSV isolates is affected by the temperature of electrophoresis*

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Abstract

Polymorphism of heterologous duplexes of RNA transcripts of prunus necrotic ringspot virus isolates was studied at different temperatures of electrophoresis. Duplexes made from complementary RNA transcripts originating from four virus isolates differed in their electrophoretic mobilities. The differential mobilities of double-stranded RNA transcripts increased with increasing temperature of electrophoresis. The mobility of single-stranded RNA, on the other hand, though affected by temperature during electrophoresis, was not simply correlated with sequence divergence. This finding further supports the hypothesis that transcript conformation is the basis for the differential mobility phenomenon.

Abbreviations: PAG – polyacrylamide gel; PNRSV – prunus necrotic ringspot virus; SSCP – single-strand conformation polymorphism; ds-TCP – double-strand transcript conformation polymorphism; ss-TCP – single-strand transcript conformation polymorphism.

Prunus necrotic ringspot virus (PNRSV) is a positive-sense RNA *Ilarvirus* (Family: *Bromoviridae*) with a tripartite genome; it occurs worldwide in *Prunus* spp. (Fulton, 1983). PNRSV exists as numerous isolates or strains that vary widely in their pathogenic, biophysical and serological properties (Aebig et al., 1987). Distinguishing the various strains is, therefore, of prime importance. Direct sequencing of regions of interest from many individual isolates is currently impracticable. In a previous study (Rosner et al., 1998), we applied the principle idea of single-strand conformation polymorphism (SSCP) for demonstrating the differentiation between PNRSV isolates by polymorphism of single-stranded RNA transcripts copied from amplified virus-specific PCR products (ss-TCP). In the present study we demonstrated electrophoretic polymorphism of ss- and ds-RNA transcripts heteroduplexes of four different PNRSV isolates at different temperatures of electrophoresis.

The effect of electrophoresis temperatures (ranging from 4 to 50 °C) on the mobility of heterologous ds-transcript molecules in PAG was studied using PNRSV isolates Apr152, Pe, Ro and Pl138. Homologous and heterologous complementary RNA transcripts were prepared from virus specific PCR products, (Rosner et al., 1998), mixed and following incubation at 50 °C for 15 min annealed at room temperature for 1 h. Fractionation of the transcript heteroduplexes in gel was studied at different temperatures of electrophoresis (Figure 1). The band patterns of the heteroduplexes consisting of either the Apr152 (2) or the Ro (4) transcripts (see Figure 1) clearly differed from each other as well as from those of Pe (3) and Pl138(1) (a homoduplex reference). The Pe-containing duplex (3) had a slightly faster mobility than the Pl138 homoduplex (1). The results were quantified by calculating the migration distance of each heteroduplex band relative to that

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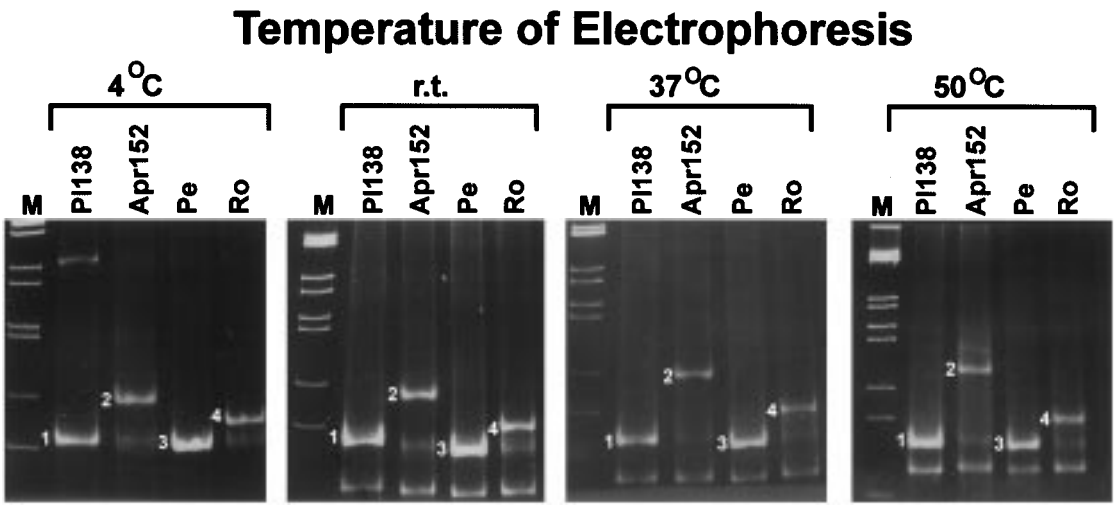

Figure 1. Fractionation of RNA heteroduplexes at different temperatures: RNA transcripts were copied from either the 3′ or the 5′-end of PCR products of isolates Pl138, Apr152, Pe and Ro as in Rosner et al. (1998). The transcript of isolate Pl138, used as a reference, was mixed with equal amounts of complementary transcripts of isolates Apr152 (2), Pe (3) and Ro (4) (heteroduplexes), or with its own complementary transcript (homoduplex) (1). The transcript mixtures were denatured and annealed and the double-stranded RNA duplexes were fractionated by gel electrophoresis at 4 °C, room temperature (r.t.), 37 °C and 50 °C. M, DNA size marker III (Boehringer Mannheim) carried out as described in Rosner et al. (1998).

of the Pl138 homoduplex (Table 1). The migration of the Apr152 heteroduplex, relative to the Pl138 homoduplex, slowed down from 0.84 at 4 °C to 0.65 at 50 °C while the change of migration of the Ro heteroduplex was somewhat smaller: from 0.93 to 0.87. The relative rate of migration of the Pe heteroduplex remained practically unchanged (about 1.02). Thus, all four PNRSV isolates were differentiated. Heteroduplex fractionation at a higher temperature (50 °C) of electrophoresis gave better resolution.

These differential rates of migration among heteroduplexes of the virus isolates may originate from the formation of mismatched regions within the dsRNA heteroduplex, caused by sequence divergence. Such regions create flexible single stranded points along the heteroduplex molecule, which allow specific secondary structure foldings – in contrast to the rigid double strand structure of homologous sequences. Electrophoretic polymorphism may arise from these conformational variations. It may be speculated that a higher temperature of electrophoresis would prevent non-specific base pairings thus increasing the effect of the mismatched regions on the conformation of the heteroduplex and hence causing the larger differential mobility observed.

The mobility of ssRNA transcripts at various temperatures of electrophoresis was also studied (Figure 2).

Table 1. Relative migration rates of transcript heteroduplexes* at different temperatures of electrophoresis

Temperature of electrophoresis (°C)	Virus isolate			
	Pl138	Apr152	Pe	Ro
4	1.00	0.84	1.02	0.93
r.t.	1.00	0.80	1.03	0.93
37	1.00	0.74	1.01	0.88
50	1.00	0.65	1.02	0.87

*The relative rate of migration was defined as the ratio between the distance of migration of each heteroduplex band and that of the Pl138 homoduplex (which served as reference). Data were taken from Figure 1. These values were calculated for each gel separately.

Two PNRSV isolates: Pl138 and Apr152 – which exhibited the largest polymorphism of ssRNA transcripts (Rosner et al., 1998) were chosen. At room temperature, the transcript of isolate Pl138 moved slower than that of Apr152, while at 37 °C their relative positions in the gel were reversed and the Apr152 transcript was the slower one. At a low temperature (4 °C) the transcript bands were each split into several minor ones: two for Apr152 and three for Pl138. At a high temperature of 50 °C, the mobilities of both Pl138 and

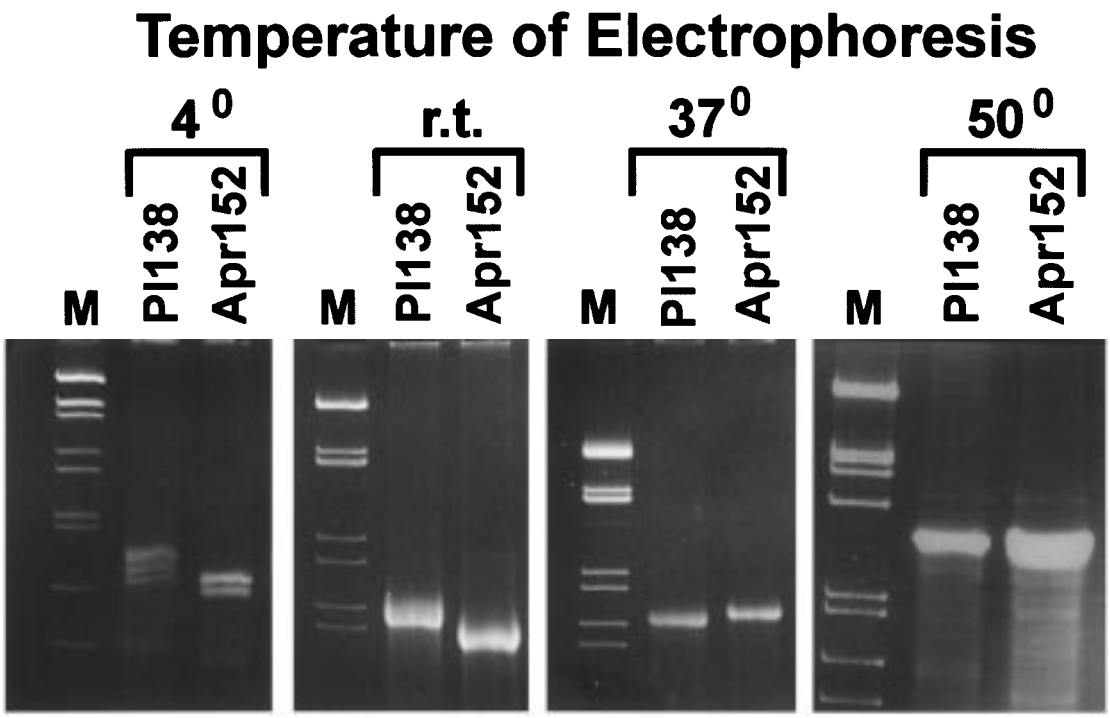

Figure 2. Effect of temperature on the electrophoretic mobility of single-stranded RNA transcripts. Transcripts of Pl138 and Apr152 were fractionated by polyacrylamide gel electrophoresis at 4 °C, r.t., 37 and 50 °C.

Apr152 transcripts were the same, presumably because of the breakdown of the secondary structure. It seems, therefore, that in comparison with that of double-stranded transcripts, the differential mobility of single-stranded transcripts is more complex and is not simply correlated with the sequence divergence. It is assumed that changes in the nucleotide sequences of amplified products of the virus genome have an effect on the conformation and, thereby, on the electrophoretic mobility of the RNA transcripts (Goldrick et al., 1996; Sarkar et al., 1992).

The finding that the relative mobility of RNA transcripts of various PNRSV isolates was differentially affected by the temperature of electrophoresis strongly supports the hypothesis that it originates from conformation variations in the RNA transcripts, which may be strongly influenced by temperature.

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