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TRANSMISSION OF JOHNSONGRASS CHLOROTIC STRIPE MOSAIC VIRUS BY EMBRYO INJECTION

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ABSTRACT

Johnsongrass chlorotic stripe mosaic virus, a virus for which transmission has not been demonstrated before, was readily transmitted by injection of embryos in presoaked seeds. Transmission was achieved by inoculation of crude infected plant extracts, purified virus preparations and extracted RNA. The virus remained stable in purified preparations or plant tissues stored at -18°C with frequent thawing over many years, and in leaf extracts kept at room temperature for over 3 wk Maize, sorghum and wheat are reported as new experimental hosts of the virus. The virus was also detected in field samples of maize, *Setaria* sp. and *Digitaria* sp. by enzyme-linked immunosorbent assay (ELISA).

Key words: Cereal viruses, Embryo inoculation, Seed transmission.

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انتقال ویروس موزائیک نوار سبزرد قیاق با تزریق به رویان

1. Professor and Researchers, respectively.

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به ترتیب استاد و پژوهشگران مرکز تحقیقات بیماری های ویروسی غلات دانشکده کشاورزی، دانشگاه شیراز، شیراز، جمهوری اسلامی ایران.

چکیده

ویروس موزائیک نوار سبزرد قیاق که پیش از این روشی برای انتقال آن شناخته نشده بود، به آسانی از راه تزریق به رویان بذرهای خیس شده انتقال یافت. انتقال با تزریق عصاره خام برگ های آلوده گیاه، آموده خالص شده ویروس و آر. ان. ای استخراج شده حاصل شد. ویروس در آموده خالص شده یا بافت گیاهی که برای چند سال در ۱۸- درجه سانتی گراد نگاه داری شده بود، با وجود یخ زدن و یخگشایی های مکرر، ثبات خود را حفظ کرد و در عصاره خام گیاه در دمای اتاق بیش از سه هفته پایدار ماند. در این پژوهش، ذرت، سورگوم و گندم به عنوان میزبانان جدید گلخانه ای این ویروس معرفی می شوند. افزون براین، ویروس در نمونه های ذرت *Setaria* sp. و *Digitaria* sp. از مناطق آلوده با روش ELISA تشخیص داده شد.

INTRODUCTION

Johnsongrass chlorotic stripe mosaic virus (JCSMV) is an isometric virus infecting Johnsongrass (*Sorghum halepense* L.) at two locations in the Fars province of Iran (2, 3). The virus resembles tombusviruses in particle morphology and the size of the genome and coat protein (4). However, it is serologically unrelated to a number of tombusviruses tested and in contrast to tombusviruses it was not transmissible by conventional mechanical inoculation, despite its unusually high concentration in plants (2, 3). Likewise, the use of purified virus preparation or its phenol extracts as inoculum failed to transmit the virus (3, 4). Non-transmissibility of the virus left many of its biological properties undetermined. Recently, Koohi-Habibi (5) showed that the virus can produce local lesions on mechanically inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa*. The virus was assigned to the genus *Aureusvirus* in the family *Tombusviridae* on the basis of genome sequence and organization (5). In the present paper, efficient transmission of JCSMV by injection of infected sap as well as

purified virus or RNA preparations into maize embryo and certain other tissues are reported. Information is also presented on the stability and hosts of the virus.

MATERIALS AND METHODS

Sources of Inoculum

The virus was obtained from naturally infected Johnsongrass as described previously (3,4). The following inocula were used for injection: Fresh leaf extract obtained by grinding infected leaves in 0.07M neutral phosphate buffer, extract of frozen infected leaves stored at -18 °C for over 11 yr with repeated thawing and freezing, purified virus preparations kept in the same conditions as frozen tissue, and viral RNA preparation obtained from purified virus by phenol extraction (4).

Test Plants

Maize (*Zea mays* L., a local hybrid) was the main test plant used for inoculations. Other plant species used were sorghum (*Sorghum bicolor* L.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.).

Inoculation Method

For embryo injection, the seeds of test plants were presoaked in water for various periods of time (Table 2). Approximately 5µlof the inoculum was forced into the embryo with a syringe and a 28-gauge (ca. 0.35 mm thick) needle while holding the seed with a pair of tweezers in one hand and the syringe in the other. In each experiment, unless otherwise stated, 50-100 seeds were injected per treatment. Alternatively, endosperm, hypocotyl and young stem were injected. Attempts were also made to inoculate the plants by puncturing the seeds or stems with a needle or slashing them with a blade dipped in the inoculum. In some experiments the seed coat was removed before injection.

Virus Detection

Inoculated plants were assessed visually for symptoms and assayed by testing against JCSMV antiserum (3) in agar-gel double diffusion test or ELISA. Meaas were compared using Duncan's new multiple range test (DNMRT).

RESULTS

Germination of Injected Seeds

Injection of whole maize seeds with water had little effect, if any, on the rate of germination as compared to non-injected seeds. Some reduction in the rate of germination was observed when JCSMV- infected sap was used instead of water (Table 1). Germination was greatly reduced when the seed coat was removed before inoculation.

Table 1. Comparison of germination rates in water-injected, JCSMV-injected and non-injected maize seeds.

Treatment	% Germination ± standard deviation†
Non-injected	64.00 ± 11.88a§
Injected with H_2O	64.00 ± 14.29a
Injected with JCSMV-infected sap	52.50 ± 15.00b

† Average of 20 replications each with 20 seeds.

§ Means followed by the same letter are not significantly different at 5% level using DNMRT.

Transmission

JCSMV was successfully transmitted to maize by injection of freshly prepared infected sap into the embryo with or without seed coat removal. Early symptoms consisted of delayed germination and severe seedling stunting, distortion and necrosis. Seedlings which survived, developed leaf distortion and striping (Fig. 1). All symptomatic plants reacted strongly with JCSMV antiserum.

The rate of transmission varied from experiment to experiment and ranged from 7 to over 60% for whole seeds presoaked for 4 hr (Transmission rate decreased when the seeds were presoaked for longer periods (Table 2).

Attempts were also made to transmit JCSMV to maize by other inoculation methods. Occasional transmission was obtained by injection of the virus into endosperm and hypocotyl, by puncturing the embryo with a needle, slashing it with a blade, slashing the young stems with a needle, or submerging newly germinated seeds in soil extracts containing the virus for several hours.

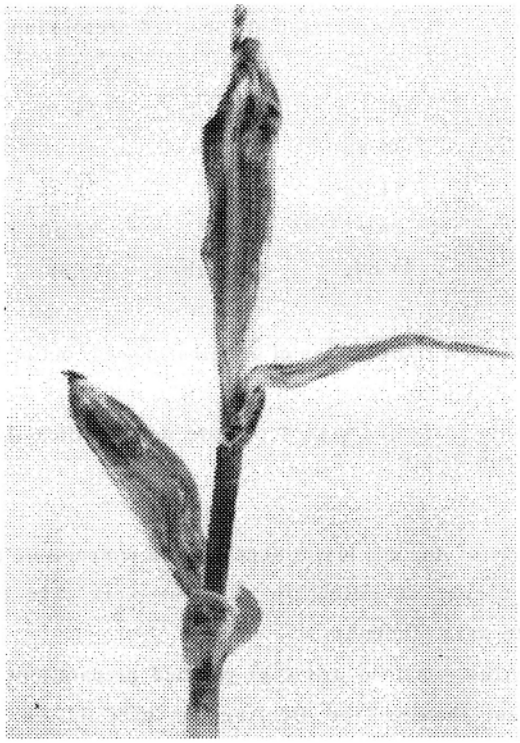

Fig. 1. Maize seedling showing stunting, leaf distortion and chlorotic stripes after inoculation of Johnsongrass chlorotic stripe mosaic virus by embryo injection.

Stability of the Viurs

JCSMV was successfully transmitted to maize when purified preparation of 2.5-10 optical density units were injected into the embryo. Likewise, the virus was efficiently transmitted by injection of viral RNA extracted by phenol-chloroform treatment. Average infection rates obtained with purified preparations and RNA extracts in several trials were 28 and 24%, respectively.

Table 2. Effect of presoaking duration of maize seed on the rate of JCSMV transmission by embryo injection.

Experiment	%Transmission at specified presoaking durations†			
	4 hr	8 hr	12 hr	24 hr
1	53.10	38.88	28.99	8.47
2	57.89	35.09	23.68	17.02
3	51.78	30.50	28.33	13.46
4	49.23	Md§	22.50	Md
Mean	53.00a¶	34.82b	25.88c	12.90d
SD	± 3.63	±4.20	± 3.26	± 4.29

† Each figure is % transmission of germinated seeds out of 100 planted.

§ Md=missing data.

¶ Means followed by the same letter are not significantly different at 1% level, using DNMRT.

Extracts of infected leaves kept at room temperature (30°C) showed no significant change of infectivity over one week. The infectivity of sap declined afterward (Table 3).

The virus remained active in purified preparations stored at -18°C, with frequent thawing and freezing for over 11 yr. Average rate of transmission for preparations stored under these conditions for 3 and 11 yr was 23.8 and 28.2%, respectively.

Hosts

In addition to maize, wheat and sorghum were infected with JCSMV after embryo injection. Of four cultivars of wheat tested only one (cv. Roshan) and of 9 cultivars of sorghum tested five became infected. The symptoms in these plants consisted of striping in the first or second leaf, followed by stunting and distortion of subsequent leaves. In wheat the new leaves often appeared filiform. Occasionaly, infected plants recovered after initial development of symptoms. Barley seeds injected under the same conditions did not give rise to infected seedlings. Attempts to apply the method to Jonsongrass seeds were unsuccessful as the seeds germinated poorly and were difficult to inject.

Table 3. Percent transmission of JCSMV by embryo injection of maize seed with infected leaf sap left at room temperature for various periods of time after extraction.

Experiment	% Transmission at specified days after sap extraction†				
	0	7	14	21	28
1	34.25¶	33.93	26.00	14.81	12.00
2	37.18	34.15	17.39	13.04	11.76
3	38.00	37.04	15.94	14.47	6.25
4	39.13	MD§	MD	17.19	MD
Mean	37.14a¶	35.04a	19.78b	14.88b	10.00b
SD	± 2.08	± 1.74	± 5.74	± 1.72	± 3.25

† Each figure is % transmission of germinated seeds out of 100 planted.

§ MD=Missing data.

¶ Means followed by the same letter are not significantly different at 1% level, using DNMRT.

ELISA detected JCSMV in maize, sorghum, *Setaria* sp. and *Digitaria* sp. with chlorotic stripes, collected in the fields in the same general area where infected Johsongrass was found. The rate of incidence, however, was very low.

DISCUSSION

The particles associated with Johnsongrass chlorotic stripe mosaic were referred to as "viruslike particles" because of the failure to demonstrate their transmissibility (3). They were later regarded as virus by analogy to other viruses (4). The information presented in this paper is further proof of the viral nature of these particles. Recent sequence data confirm taxonomic position of the virus in the family *Tombusviridae* and assign it to the newly established genus *Aureusvirus* (5). The virus can cause a similar disease in certain other gramineous species.

JCSMV appears to be a biologically stable plant virus. It survives in frozen tissues or purified preparations for many years without apparent loss of infectvity. Failure to transmit the virus to other gramineous species by ordinary rubbing method suggests that it must be introduced into certain non-epidermal cells for successful transmission. Louie (7) obtained maize white line mosaic virus (MWLMV) transmission in maize only when the virus was introduced in vascular tissues of the seed. Louie *et al.* (9) reported similar results with newly discovered maize necrotic streak virus (MNeSV), a virus with many similarities to JCSMV. MNeSV was transmitted by vascular puncture but not by leaf rubbing method (9). Using ELISA, Izadpanah *et al.* (4) detected JCSMV in 100% of the seeds from infected Johnsongrass. None of the seeds, however, developed into infected plants when grown under greenhouse conditions. These findings and the present data suggest that JCSMV is confined to Johnsongrass seed coat and that the virus is excluded from the embryo during developmental stages of the seeds. Another possibility is a rapid decline in transmissible virus, but not viral antigen, in the course of seed maturation as reported for alfalfa mosaic virus (1).

Inoculation of embryonic tissues appears to be a suitable method for trasmission of certain viruses which are difficult to transmit otherwise (7, 11, 12). Louie (7) used this method to transmit several viruses. One of the critical factors in embryo inoculation is the presoaking time of the seeds. In our experiments as in those of Louie (7) and Zhang *et al.* (12) with MWLMV, the highest transmission rate was obtained with 4 hr presoaking.

Longer presoaking periods lowered the transmission rate. It would be of value to determine what physiological or structural changes are responsible for reduced transmission rate when the presoaking time is prolonged.

The means of natural transmission of JCSMV remains to be determined. However, in view of the high virus concentration in the plant, the possibility of a vectorless transmission should be considered. Pereliminary experiments suggest both release and uptake of JCSMV by maize root (unpublished data). Root transmission without the involvement of vector has been suggested for MWLMV (8), MNeSV (9), southern bean mosaic virus (10) and many members of the family *Tombusviridae* (6) to which JCSMV belongs.

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LITERATURE CITED

1. Bailiss, K.W. and S.K. Offei. 1990. Alfalfa mosaic virus in lucerne seed during seed maturation and storage, and in seedlings. Plant Pathol. 39: 539-547.
2. Izadpanah, K. 1982. Difference in the etiology of maize mosaic in Shiraz and Karaj. Iran. J. Plant Pathol. 18: 3-6.
3. Izadpanah, K. 1988. Johnsongrass chlorotic stripe mosaic and its associated viruslike particles in Iran. J. Phytopathol. 121: 209-216.
4. Izadpanah, K., W. Huth, D.E. Lesemann and H.J. Vetten 1993. Properties of Johnsongrass chlorotic stripe mosaic virus. J. Phytopathol. 137: 105-117.
5. Koohi-Habibi, M. 2001. Genome characteristics and taxonomic position of Johnson grass chlorotic stripe mosaic virus. Ph.D. Thesis. Tarbiat Modarres Univ., Tehran, Iran (in Farsi.).
6. Lommel, S.A., G.P. Martelli, and M. Russo. 2000. Family *Tombusviridae*. In: M.H.V. Van Regenmortel, C.M. Fauquet, D.H.L.

Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D.J. McGeoch, C.R. Pringle and R.B. Wickner, (eds.), Academic Press, New York, U.S.A.

7. Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses. Louie, R. 1995. Vascular puncture of maize kernels for the mechanical transmission of maize white line mosaic virus and other viruses of maize. Phytopathology 85:139-143.
8. Louie, R., J.J. Abt and J. K. Knoke. 1989. Transmission studies of maize white line mosaic virus. Phytopathol. 79: 1188 (Abst.).
9. Louie, R., M.J. Redinbaugh, D.T. Gordon, J.J. Abt and R.J. Anderson. 2000. Maize necrotic streak virus, a new maize virus with similarity to species of the family *Tombusviridae*. Plant Disease 84: 1133-1139.
10. Teakle, D.S. 1986. Abiotic transmission of southern bean mosaic virus in soil. Aust. J. Biol. Sci. 39: 353-359
11. Zhang, L. and T.A. Zitter. 1989. Infection of sweet corn and wheat with maize white line mosaic virus by artificial inoculation. Phytopathology 79: 1197 (Abst.).
12. Zhang, L., T.A. Zitter and E.J. Lulking. 1991. Artificial inoculation of maize white line mosaic virus into corn and wheat. Phytopathology 81: 397-400.