Variations of *Leek yellow stripe virus* Concentration in Garlic and Its Incidence in Argentina

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ABSTRACT

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The purpose of this work was to determine variations in titer of *Leek yellow stripe virus* (LYSV) throughout the crop cycle and bulb storage, and to evaluate the incidence of infected plants in the main garlic-production regions of Argentina. One hundred plants with LYSV from each of five cultivars were analyzed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in six different vegetative stages in White- and Red-type garlic cultivars and seven stages in cv. Rosado Paraguayo, throughout the year. In two White-type garlic cultivars, LYSV showed peaks of viral concentration in May, at the beginning of the crop cycle, and in November, just before harvest. In two Red-type garlic selections, an increase was detected in November (period of bulbing). The highest virus titers for these four garlic cultivars were detected in devernalized clove. In Rosado Paraguayo, the peak virus concentration occurred in September prior to harvesting. In a survey at 14 different localities in Argentina, 3,066 random samples were analyzed. LYSV was found in 80 to 98% of the plants from all regions, except in Santa Cruz, where 34% of plants were infected. The importance of this study is that it allows us to recommend the most suitable moment of the year to make the analysis with DAS-ELISA.

Additional keywords: changes in virus concentration, indexing of virus, *Potyvirus*

Argentina has 11,000 ha under garlic cultivation with an average yield of 7,692 kg/ha. It exports 45 to 55% of its production and is the second-largest exporter in the world (3, J. L. Burba, *personal communication*). The principal types of garlic produced in the country are long-cycle White and Red, which are well accepted in the international market. They are mainly produced in the provinces of Mendoza and San Juan. The garlic cv. Rosado Paraguayo, which does not reach the quality required by the European markets, is exported to Brazil. This is a short-cycle garlic that appears very early in the domestic market, and is an important economic alternative for all the central and northern provinces in the country, with Córdoba as its main producing area. All the materials

currently in use have varying levels of virus infection, which affect their development and limit productivity.

Garlic is vegetatively propagated, resulting in widespread infection by viruses. These viruses are passed on to following generations and, even though they do not cause the death of the plant, they produce chronic infections. These infections cause severe yield reductions that vary depending on the cultivars, but in some cases reach 60% (4,15,29). It is clearly important for the control of these pathogens to obtain virus-free material, and to have programs to produce certified virus-free stocks of popular garlic cultivars. These programs require highly sensitive diagnostic tests and the implementation of sampling protocols that specify, among other things, when there is the greatest likelihood of detecting a virus, thus ensuring the efficiency of the analysis.

Viruses of the genera *Potyvirus*, *Carlavirus*, and *Allexivirus* infect and cause mosaic in garlic. The *Potyvirus* species *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) have been identified in all the countries where viruses infecting garlic have been studied (1,7,13,22-26,28).

LYSV was detected in leek in Belgium with a 40% frequency in 1971 and 74% in 1972 (27). In Denmark, 20% of the leek plants were infected in 1976, 12% in 1977, and 43% in 1978 (21). In New Zealand, all the plants tested were infected (20).

In garlic, LYSV caused severe symptoms and a great reduction of bulb weight (15,17). In Argentina, the use of immunosorbent electron microscopy plus decoration (ISEM-D) detected viral particles serologically related to LYSV in garlic and leek (6).

One purpose of this work was to determine variations in the concentration of LYSV throughout the crop cycle and bulb storage to identify the best time to conduct a reliable analysis. A second objective was to determine the incidence of LYSV-infected plants in the principal garlic-producing regions of Argentina.

MATERIALS AND METHODS

Antiserum and serological assays. Antiserum for LYSV was prepared in our laboratory from the purification of a LYSV isolate from leek (16).

Direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed as described by Clark and Adams (5), also using their method for purification of anti-LYSV immunoglobulin (IgG) and preparation of alkaline phosphatase-IgG conjugate. The optimum dilutions were 1:4,000 for coating IgG and 1:5,000 for conjugate, and were used for analysis of samples throughout the study (IgG A₂₈₀ of 2.25 were used for preparing the IgG dilution). Sensitivity of the test was estimated to be 17 ng/ml with purified virus.

Samples were homogenized 1:5 (wt/vol) in the extraction buffer (phosphate buffer + 0.05% Tween-20 + 2% polyvinylpyrrolidone + 2% nonfat dried milk). Volume used was 200 µl/well. Each DAS-ELISA plate included extracts from five healthy plants (virus-free garlic plants obtained according to Conci and Nome [8] and tested by ISEM-D [18] with LYSV antiserum) and extract from two positive controls (LYSV positive plants by ISEM-D). To compare the absorbance values obtained at different months throughout the year, a "relative concentration" value (RC) was calculated. The RC value was obtained by dividing the absorbance of each sample by the average of the five healthy controls tested on the same ELISA plate, plus twice the standard deviation of the healthy controls. Plants were considered positive with an RC value higher than 1.

Variation of LYSV concentration. Five cultivars of garlic were tested; two White

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types, 'Blanco Mendoza' and 'Norteño INTA', and two Red-type garlic cultivars belonging to the germ plasm bank of the La Consulta INTA Experimental Agricultural Station, AR-I-108 ('Colorado El Nevado') and AR-I-107 ('Colorado Payén'). These were evaluated in the province of Mendoza. In the province of Córdoba, cv. Rosado Paraguayo, a short-crop-cycle cultivar, also was tested. Plants from the five cultivars were tested by ISEM-D with LYSV antiserum and 100 positive plants of each cultivar were selected as LYSV-infected plants. These were marked and the RC values were recorded throughout the crop cycle and during bulb storage.

The cultivars of White-type garlic were planted on 21 March and harvested on 20 November (cycle of 244 days). Samples were taken in the following stages after planting: 60 days (20 May); 98 days, vegetative growth stage (27 June); 143 days, bulbing stage (11 August); 227 days, end of the crop cycle (3 November); 266 days, dormant clove (after harvest, 12 December); 378 days, devernized clove (2 April).

The Red garlic cultivars were planted on 27 April and harvested on 6 December (232-day cycle). Samples were taken in the following stages after planting: 60 days (27 June); 105 days, plants in the vegetative growth stage (11 August); 189 days, bulbing stage (3 November); 219 days, the end of the crop cycle (3 December); 229 days, dormant clove (after harvest, 13 December); 340 days, devernized clove (2 April).

Rosado Paraguayo was planted on 1 March and harvested on 16 September (199-day cycle). Samples were taken in the following stages after planting: 65 days (5 May); 97 days, plants in the vegetative growth stage (6 June); 125 days (4 July); 159 days, bulbing stage (7 August); 189 days, the end of the crop cycle (6 September); 276 days, dormant clove (after harvest, 2 December); 367 days, devernized clove (2 March).

During the first four sampling dates for the White and Red garlic, and the first five dates for Rosado Paraguayo, the youngest leaf was analyzed by DAS-ELISA for LYSV because previous results have shown that these have the greatest virus concentration (*unpublished data*). Bulbs were analyzed in the last two samplings of all the cultivars studied. One clove was removed from each bulb for the first analysis and another clove for the second. A cube of the basal portion of the clove (1 by 0.5 by 0.5 cm), homogenized 1:5 (wt/vol) in extraction buffer, was used for the test.

From the RC values, the average and standard error were calculated for each stage at which the material was analyzed. Although all plants tested each month were infected with LYSV, the number of positive plants was determined by DAS-ELISA (RC higher than 1). The percentage of

DAS-ELISA-positive plants was calculated for each stage analyzed.

Incidence of LYSV in Argentina. A survey was conducted in the largest garlic-producing areas of Argentina. Samples were collected from different regions in five provinces: San Juan (Rawson, Santa Lucía, and Pocito), Mendoza (Valle de Uco and Uspallata), Córdoba (Jesús María), Buenos Aires (Bahía Blanca, Hilario Ascasubi, and Médanos) and Santa Cruz (El Calafate, Cancha Carrera, San Rafael, Mansilla, and Gobernador Gregores). In these provinces, the cultivars Colorado, Chino, Violeta Francés, Blanco, Rosado Paraguayo, Violeta Santacrucense, and Castaño-INTA were sampled. Samples were analyzed in the devernized clove stage based on results from a previous test on the variation of virus concentration throughout growth stages.

Samples were taken from 15 to 50% of the area planted in each garlic region, for a total of 115 lots. The sampling unit was one each of the crop lots of each farmer, designating a lot as garlic of the same origin planted in the same field. Between 20 and 100 bulbs were randomly collected from each lot. One clove was removed from each bulb and analyzed by DAS-ELISA for LYSV as described above.

RESULTS

Variations of LYSV concentration.

Fluctuations of the RC of virus were detected throughout the crop cycle and bulb storage and were different for each garlic cultivar from Mendoza and Córdoba. The percentage of plants testing positive in DAS-ELISA also varied by garlic cultivar and growth stage. (Fig. 1).

White-type garlic. Cv. Blanco Mendoza had the greatest virus concentration in May, at the start of the crop cycle (RC = 9.7), then fell sharply by June, followed by a gradual rise until the end of the crop cycle in November (Fig. 1A). The RC continued increasing during the clove stage until it again reached a high concentration in the devernized clove (RC = 9). In this cultivar, it was possible for DAS-ELISA to detect the virus in more than 97% of infected plants in all of the months analyzed.

In the cv. Norteño INTA, as in the case of Blanco Mendoza, the peaks of greatest RC occurred in May, at the beginning of the crop cycle (RC = 40) and in devernized cloves (RC = 32; Fig. 1B). Only in these two stages was it possible to detect the virus in 100% of the diseased plants by DAS-ELISA (0% of escape). Although the virus RC was low in June, in August, and in dormant cloves, an increase was observed in November with a small standard error, and it was possible to detect the virus in 99% of the plants in this stage. In dormant cloves, the virus could be detected in only 5% of the infected plants.

Both cultivars showed peaks of highest virus concentration in May and in devern-

alized clove, and it was possible to detect 100% of infected plants by DAS-ELISA.

Red-type garlic. In cvs. Colorado El Nevado and Colorado Payén, the same variations were recorded in the virus concentration (Fig. 1C and D). During cultivation, the virus concentrations were low, with a small increase detected in November (the bulbing stage, 189 days from planting), where it was possible to detect the virus in 100% of infected plants, with another fall toward the end of the crop cycle in December. The highest virus concentration occurred in the devernized clove stage, with an RC value of 27, and LYSV was detected in all plants. During June, August, and December and in the dormant cloves, the percentage of plants detected by DAS-ELISA varied from 50 to 90%.

Rosado Paraguayo garlic. The peak RC (40.5) occurred at the end of the crop cycle (September, 189 days from planting), when the virus was detected in 100% of the plants (Fig. 1E). In May, June, July, August, and in the devernized clove, virus was detected in more than 93% of the plants. Virus was detected in 58% of the dormant cloves.

Survey of LYSV in Argentina. Samples (3,066) from major garlic-production areas of Argentina were analyzed and LYSV was detected in cloves from all geographical regions studied. Of the plants infected, 80 to 98% were infected, except in the province of Santa Cruz, where 34% of plants were infected (Table 1). The actual percentages by cultivar were: Blanco, 84.5%; Colorado, 94.3%; Chino, 98.6%; Violeta Frances, 81.8%; Violeta Santacrucense, 34.5%; Rosado Paraguayo, 100%; and Castaño-INTA, 100%.

DISCUSSION

The results show that concentration of LYSV in garlic differs according to the sampling time, cultivar, and region. This is probably due to the physiological state of the crop, virus-cultivar relation, and the ambient temperature. Similar results also have been observed in other studies, which report that significant fluctuations in concentration of virus occur in different plant species. This has mainly been determined for *Prunus necrotic ringspot virus* in cherry and peach (9,19), *Cowpea chlorotic mottle virus* in soybeans (2), and *Sweet potato feathery mottle virus* in sweet potato (30). In garlic plants, Koch and Salomon observed changes in OYDV concentrations over time when they analyzed plants from meristem and callus culture (14). However, there are no reports about fluctuations in virus concentration in garlic plants at different stages of crop development or in dormant and devernized cloves.

Our results show significant variations in the RC of LYSV throughout the crop cycle and bulb storage and suggest that these variations affect the possibility of making a reliable diagnosis. All the plants

tested were infected with LYSV before starting the studies. Nonetheless, at particular moments in the crop cycle, the fall in viral concentration prevented the detection of disease in some plants through DAS-ELISA. For example, in cv. Norteño INTA, the 100 plants tested in May showed LYSV DAS-ELISA positive; whereas, in June and August, only 74 and 91% of the same plants showed DAS-ELISA positive and, later (November), these same plants were positive in 99% of the cases. Similar variations to these were found in all the cultivars analyzed (Fig. 1). This demonstrates the importance of know-

ing the moment of highest viral concentration, so as to obtain reliable results and reduce the possibility of diagnosing diseased plants as healthy.

The results suggest that, to obtain the most reliable results for detecting virus in White and Red garlic in Mendoza, it is best to survey either in the devernized clove stage, or in May for White garlic and in November for Red. On the other hand, for Rosado Paraguayo in Córdoba, the highest concentration was detected at the end of the crop cycle (September), which suggests that this is the best moment to make reliable analyses. However, all the stages

tested in this cultivar, except that of dormant clove, showed a high percentage of positive plants, which means that it should be possible to obtain reliable results at any stage.

The survey showed that the percentage of infection in Argentina is proportionally higher than that observed in other parts of the world. Van Dijk (26) detected LYSV in 20% of garlic plants from different parts of the world and in 45% of wild garlic plants from gene collections. Barg et al. (1) analyzed 290 garlic samples from seven Asian countries and detected the presence of *Potyvirus* spp. in all of them; however, in

Fig. 1. Mean relative concentrations of *Leek yellow stripe virus* (LYSV) and percentage of positive plants by direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) from known LYSV-infected plants detected in different months during the crop cycle, in dormant clove (dc) and devernized clove (dvc), in garlic cvs. A, Blanco Mendoza, B, Norteño INTA, C, Colorado El Nevado, D, Colorado Payén, and E, Rosado Paraguayo. Bars indicate the standard error; bars do not appear where they are too small for the scale used.

Table 1. Survey of *Leek yellow stripe virus* in garlic-producing regions in Argentina

Provinces surveyed	Area planted (ha)	Fields analyzed	Area analyzed (ha)	No. samples analyzed	Positive samples	Percent positives
Córdoba ^a	70 ^a	2	30	220	198	90
San Juan	1,280 ^b	46	299.5	989	793	80
Mendoza	8,763 ^c	38	1,300	770	753	98
Buenos Aires	650 ^d	22	141	464	429	92
Santa Cruz	130 ^e	7	65	623	215	34
TOTAL	10,893	115	1,835.5	3,066	2,388	78

^a The Jesús María locality; area under garlic in this Province is about 600 ha.

^b Gonzalez (12).

^c Estévez and Vanin (10).

^d García and Rivas (11).

^e Horacio Gábel (personal communication).

that study, LYSV was detected in only very few samples of garlic from Taiwan or of leek from Indonesia.

The lower percentage (34%) of LYSV infection in the province of Santa Cruz is probably because of this region's low temperatures, together with the strong winds, which may hinder the development and movement of the aphids that vector this virus. The cv. Violeta Santacrucense is a special ecotype adapted to the extreme climatic conditions of Santa Cruz, and was therefore sampled only in this area. For this reason, the percentage of infected plants for this cultivar coincides with the percentage of infected plants for the province of Santa Cruz.

The importance of this study is that it allows us to recommend the most suitable moment of the year to make the analysis with DAS-ELISA. Knowing the behavior of each viral species is an important tool when making a reliable diagnosis or when planning field survey studies. Moreover, the results show that it is possible to diagnose the virus with DAS-ELISA based on cloves. The RC of the LYSV increased in the devernized clove and it was possible to reliably detect all the infected plants at this stage. The great advantage of this is that the analysis can be made with bulbs, in order to know the health of a lot of "seed-bulbs" immediately before planting or even before buying them.

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