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Evaluation of the National Plant Germplasm System's Garlic Collection for Seven Viruses

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Abstract

Garlic (*Allium sativum* L. and *A. longicuspis* Regel.) germplasm maintained by the USDA-ARS Western Regional Plant Introduction Station (WRPIS) was screened for four viruses commonly infecting garlic [*Garlic common latent carlavirus* (GCLV), *Leek yellow stripe potyvirus* (LYSV), *Onion yellow dwarf potyvirus* (OYDV), and *Shallot latent carlavirus* (SLV)] plus three others [*Iris yellow spot tospovirus* (IYSV), *Tobacco rattle tobravirus* (TRV), and *Tomato spotted wilt tospovirus* (TSWV)]. Over 200 accessions were rated for the incidence of symptomatic plants during two production seasons, in July 2005 and June 2006. In both years, incidence of symptomatic plants ranged from less than 10% to 100% in individual accessions. Laboratory testing of selected accessions showed that the accessions were predominantly infected with OYDV (60% of infections), followed by GCLV (25%) and LYSV (15%).

Introduction

Four states — California, Nevada, Oregon, and Washington — account for most of the garlic (*Allium sativum* L.) production in the United States. Commercial acreage in the USA in 2000 was between 16,000 and 20,000 ha with a farm-gate value of more than $200 million (USDA National Agricultural Statistics Service). Of the over 50 diseases that affect cultivated alliums (6,25), there are four viruses consistently documented as infecting garlic: *Onion yellow dwarf potyvirus* (OYDV), *Leek yellow stripe potyvirus* (LYSV), *Garlic common latent carlavirus* (GCLV) and *Shallot latent carlavirus* (SLV) (5,8,11,13,14,15,16,17,21). The National Plant Germplasm System (NPGS) has a collection of more than 200 garlic (*A. sativum* and *A. longicuspis*) accessions from over 40 countries. This is one of the largest publicly available garlic collections in the world. The collection is maintained at the Western Regional Plant Introduction Station (WRPIS) in Pullman, WA, which collects, regenerates, and distributes this material. Little is known about the prevalence or identity of specific viruses in the NPGS garlic collection. In 2004, we confirmed the occurrence of three viruses (OYDV, LYSV, and GCLV) in the WRPIS garlic accessions grown at the USDA Pullman Farm (17). Certain cultivars had up to 90% infection. Considering the high incidence of virus infection and the potential impact on germplasm conservation and distribution, there is a need to evaluate all garlic accessions for virus infection. This study was undertaken to determine the extent and identity of viruses in the collection. This information can aid in continued monitoring of the collection for viruses, including emerging viral diseases, identification of sources of resistance or tolerance in the germplasm, and development of a virus elimination program. Preliminary data was presented as an abstract elsewhere (18).

Multiplication of Garlic Germplasm in the Field

At WRPIS, 30 to 35 garlic cloves per accession are planted by hand annually in mid-October. Hand harvest of bulbs is initiated in mid-July and continues into early August. Harvested garlic plants are dried down at ambient air temperatures in an enclosed drying shed, after which roots and tops are removed and clean bulbs are stored at ca. 14°C and 40 to 50% RH. Adjacent crops encompass a variety of legumes, grasses, other *Allium* species such as *A. ampeloprasum* L., and mustard cover crops. Rotation of garlic away from ground previously cropped to *Allium* species is followed to the extent permitted by available planting space with typical rotation being 3 to 4 years.

Sampling and Testing for Viruses

Garlic plots (Fig. 1) were surveyed during two consecutive seasons during 2005 and 2006. Each accession was rated for presence or absence of symptoms. Leaves showing symptoms suggestive of virus infection such as mosaic, chlorotic spots or streaks, stunting were collected for testing in July 2005 and 2006. Final disease incidence was recorded as percent incidence of symptomatic plants per 20-plant sample in each accession. Accessions were then grouped into arbitrary classes based on percent incidence. One symptomatic leaf each from two plants from at least ten accessions for each group was tested for seven viruses. While latent infections could be present, only symptomatic plants were rated and tested. Where symptomatic plants were not present in a given accession, leaf samples from the asymptomatic plants were tested by ELISA. Samples were stored at 4°C for up to 1 week until they were processed for ELISA. For storage longer than 1 week, samples were kept at -80°C.

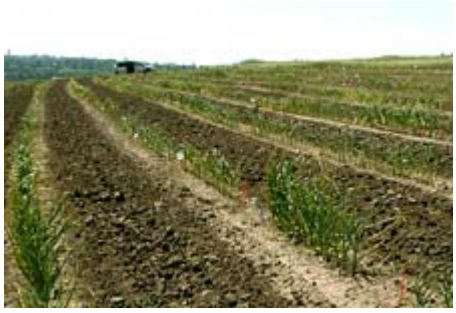

Fig. 1. Garlic accessions at the USDA-ARS Western Regional Plant Introduction Station Farm, Pullman, WA, in June 2006.

Samples were tested for the following viruses using commercially available antisera (Agdia Inc., Elkhart, IN): GCLV, *Iris yellow spot virus* (IYSV), LYSV, OYDV, SLV, *Tobacco rattle virus* (TRV), and *Tomato spotted wilt virus* (TSWV). Samples were tested for IYSV since natural infection of garlic by IYSV was reported (22). Considering the wide host range of TSWV, samples were tested to investigate the possibility of TSWV infection in garlic. Where necessary, ELISA results were verified by reverse transcription-polymerase chain reaction (19) using virus species-specific primers (Table 1).

Table 1. Primers used in the reverse transcription-polymerase chain reaction for the detection of *Garlic common latent virus* (GCLV), *Leek yellow stripe virus* (LYSV), and *Onion yellow dwarf virus* (OYDV). For each virus specific primer pair, the first one is the forward primer and the second one is the reverse primer. Primer sequences are given in 5' to 3' direction.

Virus	**Primers**
GCLV WAF	GCG GGG GAT GAT ATG TGT GC
GCLV WAR	TGT GAT CTC TGT TCC TCC GT
OYDV cpF WA	TGG TGC ATT GAG AAT GGG ACA TC
OYDV V300	AAT TTG GAC TAT GAT GGA TGG
LYSV-WA	TCA ATG CCG GCC ACA GTG GT
LYSV-WAR	CTT ACT GCA ACA TAA GAA CG

Table 2. Percent incidence of symptomatic plants per 20-plant sample in garlic (*Allium sativum* and *A. longicuspis* [denoted by asterisk]) accessions at WRPIS Farm, Pullman, WA.

Percent symptomatic plants	**Garlic accessions and cultivars**
Asymptomatic and virus free*	PI 250662, PI 383817, PI 383819, PI 383820, PI 383824, PI 383831, PI 493106, PI 493107, PI 493112, , PI 497947, PI 497948, PI 540315*, PI 540319, PI 540375, PI 540376, PI 543048, PI 543049, PI 576914*, PI 615424 PI615428, PI 615434, D-126, W6-12820*, W6-12840, W6-12912, W6-14858*, W6-18723, W6-18724, W6-18726, W6-18727, W6-18729, W6-24371*, W6-24414*, W6-26171, Z006, Z008, Z048, Z052, Z053, Z054, Siberian, Red Rezan, Pskem, Maiskij, Carpathian, Brown Rose, Brown Tempest, Red Grain, Lorz Italian, Ukrainian White Inferno
<10	PI 383822, PI 383823, PI 497941, PI 497944, PI 540316, PI 540327, PI 540359, PI 540360, PI 540361, PI 540362, PI 540370, PI 615416, PI 615426, PI 615432, PI 615433, W6-50, W6-670, W6-672, W6-1861, W6-1961, W6-2562, W6-2563, W6-8415, W6-8417, W6-8420, W6-10729, W6-10736, W6-12816, W6-12825, W6-12829, W6-12830, W6-12836, W6-12837, W6-26170, W6-26172, W6-26183, DX-127, DX-134, Asian Tempest, Japanese, Darcheli, Israeli, Shatili, Wild Buff
10-40	PI 383821, PI 383830, PI 383833, PI 493101, PI 493104, PI 493105, PI 493110 , PI 497942, PI 497943, PI 497946, PI 540331, PI 540363, PI 540365, PI 540367, PI 540368, PI 540377, PI 540378, PI 540379, PI 540380, PI 568882, PI 615417, PI 615425,W6-1880*, W6-1903*, W6-8410, W6-8413, W6-10730, W6-12842, Chargali, Floha
41 to 80	PI 493115, PI 493116, PI 493117, PI 493097*, PI 497949, PI 540318, PI 540364, PI 615423, W6-12824, W6-12828, W6-12834, Rose Du Var, Orting
>80	PI 493099*, PI 497945, PI 540320, PI 540373, PI 615418, PI 615419, PI 615420, PI 615427, PI 615431, W6-715, W6-1884, W6-1885, W6-2561, W6-8404, W6-8409, W6-10735, W6-10737, W6-11052, W6-12821, W6-12823, W6-12833, W6-12839, W6-12844, W6-18725, W6-26182, Chesnok Red, Georgian Crystal, Georgian Fire, Pitarelli, Jerome's French Rose, German Red, Romanian Red, Zemo

* Based on ELISA testing.

Results and Conclusions

The most common symptom was yellow streaks on leaves. Symptoms first appeared as yellow specks or spots which then spread rapidly, coalesced and became more pronounced (Figs. 2 and 3). Visual ratings of the plots in 2005 and 2006 showed extensive incidence of symptomatic plants. In both years, symptoms became pronounced in July. There was considerable variation in the incidence of symptomatic plants among various accessions in the germplasm collection. A majority of the accessions had symptomatic plants. Incidence varied from 10 to 100% (each accession had an average of 20 plants), while a limited number of accessions had fewer than 5% symptomatic plants (Table 2).

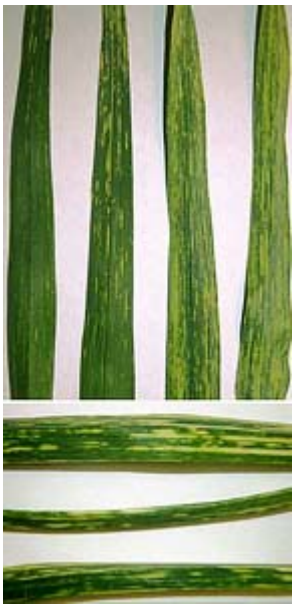

Fig. 2. Symptoms in garlic infected by *Leek yellow stripe virus* (top) and *Onion yellow dwarf virus* (bottom).

Fig. 3. Effect of *Onion yellow dwarf virus* on garlic. Infection leads to drying of the leaves (left); close-up of symptoms on garlic leaves (right).

Based on ELISA, OYDV was the most prevalent (60% of sampled plants), followed by GCLV (25%) and LYSV (15%). Only one sample from one accession tested positive for SLV. Two samples from one accession were infected with both GCLV and LYSV. None of the samples were infected with IYSV, TRV, or TSWV. Certain accessions had high incidence of infection with one or more viruses based on both severity of symptoms and ELISA results. For example, Floha, Georgian Crystal, and Georgian Fire had severe symptoms and 100% of the plants were infected with OYDV (Fig. 2). The identity of each virus-species detected by ELISA was verified for at least one ELISA positive sample for each of these viruses by RT-PCR followed by cloning and sequencing of the amplicons (*data not shown*).

Testing for other garlic-infecting viruses such as allexiviruses remains to be conducted to estimate their incidence if any. IYSV, transmitted by *Thrips tabaci* and a serious pathogen of onion in the Pacific Northwest (10,19), was recently reported to be infecting garlic (22). However, our testing of the garlic accessions did not identify any IYSV-positive plants. GCLV, OYDV, LYSV, and SLV are transmitted in a non-persistent manner by aphids (12,25). No aphid activity was noticed in either year and the virus infections found in the field were likely to have mostly resulted from bulb-borne infection. Though mixed infections were reported to be common in garlic (3,8,16), we did not find evidence of extensive mixed infections in our study.

Information on the identity and incidence of viruses in garlic germplasm, both commercially and non-commercially distributed, is needed to prevent virus spread, since several studies have shown the impact of various garlic viruses on yield (2,13,15). The two-year screening of the national germplasm collection provided important information on the relative incidence and distribution of selected viruses in the collection. It should be noted that the incidence of these viruses in various accessions could change over time since each accession has to be grown in the field every year in order keep the collection viable and this approach would invariably expose the accessions to vector-borne viruses that could be introduced into the collection during the season. However, information generated in this study based on both visual rating and laboratory testing of the accessions would lead to an increased awareness among the users of this collection. This information is also useful in developing targeted, virus-specific testing procedures to establish and maintain virus-free planting material.

Infection of seed cloves by viruses continues to be a concern, and testing to ensure their virus-free status would prevent inadvertent distribution and spread of virus-infected material. Continued screening of accessions that showed lower virus incidence will potentially lead to identification of sources of resistance to these viruses. Development and use of cultural, genetic, and biotechnological measures to eliminate viruses from accessions that are found to be highly susceptible would lead to reduced virus incidence (1,4,7,9,20,23,24,26).

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