UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRONÔMICAS
CAMPUS DE BOTUCATU

CARACTERIZAÇÃO E IDENTIFICAÇÃO DE VÍRUS EM *Allium* spp.

DAIANA BAMPI

Tese apresentada à Faculdade de Ciências Agronômicas da UNESP - Campus de Botucatu, para obtenção do título de Doutor em Agronomia (Proteção de Plantas).

BOTUCATU – SP

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como alicerce de sua maturidade.

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1. RESUMO

O gênero *Allium* inclui espécies de importância para o consumo humano como o alho, cebola e espécies ornamentais. Algumas destas espécies por serem propagadas vegetativamente, podem ser infectadas por vírus pertencentes aos gêneros *Allexivirus, Carlavirus e Potyvirus*, frequentemente em infecções mistas. *Leek yellow stripe virus* (LYSV) pertence ao gênero *Potyvirus* e é considerada a espécie de maior importância na cultura do alho, de modo que foi sequenciado o genoma completo de um isolado de LYSV brasileiro (LYSV-MG) e avaliada sua diversidade genética. Seu genoma possui 10.341 nucleotídeos e codifica uma poliproteína de 3221 aminoácidos. Baseado na análise da região codificadora para a proteína P1, o isolado LYSV-MG, bem como demais coletados em regiões produtoras de alho, não foram classificados como pertencentes aos grupos S e N. Os isolados brasileiros apesar de não apresentarem a deleção na região da P1 (típica do grupo S), formaram um grupo monofilético muito próximo deste grupo e de um isolado de Okinawa, Japão. Em relação ao gene P1, os isolados brasileiros compartilharam 97-99% de identidade de nucleotídeos entre si e 51-64% com isolados de LYSV de outros países. Os dados sugerem que os isolados brasileiros de LYSV, o isolado de Okinawa e os isolados pertencentes ao grupo S podem ser provenientes de um ancestral comum antes da ocorrência da deleção na P1 e divergência dos isolados no grupo S. Como parte do doutoramento sanduíche, realizado na United States Department of Agriculture, sob orientação do Dr. John Hammond, foram estudados os vírus presentes em quatorze diferentes espécies de Allium ornamental nos Estados Unidos. As plantas foram adquiridas em floriculturas e plantadas a campo no outono de 2013 na região de Beltsville, MD. Folhas sintomáticas foram coletadas após a floração durante a primavera de 2014 e avaliadas por PCR usando primers universais para os gêneros *Allexivirus, Carlavirus e Potyvirus*, e específicos para as espécies em cada gênero. Após a floração os bulbos foram colhidos e armazenados a 4º C durante 60 dias. Os bulbos foram plantados em vasos e mantidos sob temperatura controlada de 4º C até a emergência das folhas e posteriormente transferidos para casa-de-vegetação. As plantas foram testadas por ELISA utilizando antissoro específico para os vírus: *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Shallot virus X* (ShV-X), *Garlic common latent virus* (GarCLV), *Shallot latent virus* (SLV), *Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV) e *Shallot yellow stripe virus* (SYSV). Amostras positivas para presença de vírus foram visualizadas ao microscopio eletrônico de transmissão. Nas plantas de Allium ornamental avaliadas por PCR foi detectada a presença de GarVB, GarVC, *Garlic virus D* (GarVD), *Garlic virus E* (GarVE) em *Allium sphaerocephalon*, GarVB, GarVC e ShVX em *Allium caeruleum*, LYSV em *Allium bulgaricum, Allium flavum* e *Allium atropurpureum*, SLV em *Allium moly* e *Narcissus yellow stripe virus* (NYSV) em *Allium carinatum*. Os testes de ELISA confirmaram a presença de GarVB, GarVC, ShVX, SLV e LYSV. Testes de microscopia eletrônica revelaram a presença de partículas flexuosas de 650-800 nm, típicas de allexivirus, carlavirus e potyvirus. Este foi o primeiro relato de NYSV em espécies de *Allium* spp. Além disto, *A. sphaerocephalon* e *A. caeruleum* não haviam sido previamente descritas como hospedeiras de allexiviruses nos Estados Unidos.

Palavras-chave: *Leek yellow stripe virus*, alho, potyvirus, allexivirus, Allium ornamental, *Narcissus yellow stripe virus*.

CHARACTERIZATION AND IDENTIFICATION OF VIRUS IN *Allium* spp.

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2. SUMMARY

The genus *Allium* includes important species used for human consumption, as garlic,onion and many other species grown as ornamentals. Some of these species are propagated vegetatively and can be infected by viruses belonging to the genera *Allexivirus, Carlavirus* and *Potyvirus*, often in mixed infections. *Leek yellow stripe virus* (LYSV) belongs to the genus *Potyvirus* and is considered the most important virus in Allium species. The complete genomic sequence of *Leek yellow stripe virus* garlic isolate from Brazil (LYSV-MG) has been determined. The LYSV-MG genome consists of 10,341 nucleotides and encodes a deduced polyprotein of 3,221 amino acids. Based on the analysis of the coding region for P1 protein, isolate LYSV-MG and others collected in garlic producing regions, could not be classified as belonging to the groups S and N. Brazilian isolates do not have the deletion present in the P1 from the S-type group but are more closely related to S-type than to N-type isolates. The Brazilian isolates formed a monophyletic group closer to S-type and one isolate from Okinawa, Japan. Brazilian isolates share 97-99% of P1 region nucleotide identity with each other, and 51-64% with different isolates from around the world. The data suggest that Brazilian LYSV isolates are derived from an ancestral source of the Okinawa and S-type isolates, prior to the P1 deletion and divergence in the S-type isolates. As part of the doctorate sandwich in United States Department of Agriculture, under the supervision of Dr. John Hammond, viruses present in ornamental Allium from Beltsville, MD were detected. Bulbs of fourteen different species of *Allium* were purchased from retail nurseries and planted in the field during the fall of 2013. Leaf tissue from the flowering symptomatic plants were collected during spring 2014, and tested by PCR using generic primers for the genus *Allexivirus, Carlavirus* and *Potyvirus*. PCR-positive samples were re-tested using specific primers for specific species described in each genus. After flowering and foliage dieback, the bulbs were harvested and stored at 4º C for 60 days, before being planted in pots and maintained in a cooler until leaves emerged, and then transferred to a greenhouse. The sprouted plants were tested by ELISA using specific antibodie for the *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Shallot virus X* (ShV-X), *Garlic common latent virus* (GarCLV), *Shallot latent virus* (SLV), *Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV) and *Shallot yellow stripe virus* (SYSV). Electron microscopy was performed for positive samples. The presence of GarV-B, GarV-C, *Garlic virus D* (GarV-D), *Garlic virus E* (GarV-E) in *Allium sphaerocephalon*, GarV-B, GarV-C and ShV-X in *Allium caeruleum*, LYSV in *Allium bulgaricum, Allium flavum* and *Allium atropurpureum*, SLV in *Allium moly* and *Narcissus yellow stripe virus* (NYSV) in *Allium carinatum* was confirmed by PCR. ELISA test was positive for GarV-B, GarV-C, ShV-X, SLV and LYSV. Electron microscopy revealed the presence of flexuous particles of 650-800 nm, typical of *Allexivirus*, *Carlavirus* and *Potyvirus*.This was the first report of NYSV in *Allium* spp. Moreover, *A. sphaerocephalon* and *A. caeruleum* had not previously been described as allexiviruses host in the United States.

Keywords: *Leek yellow stripe virus*, garlic, potyvirus, allexivirus, Ornamental Allium, *Narcissus yellow stripe virus*.

3. INTRODUÇÃO

O gênero *Allium* é o mais numeroso da família *Alliaceae* e inclui cerca de 750.000 espécies (BLOCK, 2010). Parte destas espécies são de importância na alimentação humana (alho, celoba, cebolinha e alho poró), outras possuem potencial medicinal e ainda há aquelas utilizadas como plantas ornamentais (KATIS et al., 2012).

O alho é considerado a segunda principal espécie do gênero *Allium* mais amplamente consumida no mundo (FAO, 2015). O alho é propagado de forma vegetativa, propiciando o acúmulo de vírus pertencentes aos gêneros *Potyvirus*, *Carlavirus* e *Allexivirus* (FAJARDO et al., 2001), que podem ocorrer de forma isolada ou em infecções mistas. As espécies descritas em *Allium* spp. são: *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Turnip mosaic virus* (TuMV) e *Shallot yellow stripe virus* (SYSV) pertencentes ao gênero *Potyvirus*; *Garlic common latent virus* (GarCLV) e *Shallot latent virus* (SLV) pertencentes ao gênero *Carlavirus* e *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Garlic virus E* (GarV-E), *Garlic virus X* (GarV-X), *Garlic mite-borne filamentous virus* (GarMbFV) e *Shallot virus X* (ShVX) pertencentes ao gênero *Allexivirus* (ADAMS et al., 2011a; KATIS et al., 2012). Esses vírus causam sintomas de mosaico e estrias brancas ou amarelas. Os maiores danos na produção de alho têm sido atribuídos às infecções causadas por OYDV e LYSV. LYSV é considerado um dos vírus mais importantes na cultura devido sua ampla distribuição e pela redução do rendimento e qualidade dos bulbos (LOT et al., 1998). No Brasil LYSV é considerado um dos vírus predominantes em campos comerciais de produção de alho (FAYAD-ANDRE et al., 2011).

Os isolados de LYSV, segundo classificação de Yoshida et al., 2012, podem ser classificados em dois grandes grupos, S e N, analisando-se a região codificadora para a proteína P1. O grupo S possui uma deleção na P1 em torno de 201 nucleotídeos em relação ao grupo N (TAKAKI et al., 2005 e YOSHIDA et al., 2012) e está presente na China, Sul do Japão, Coréia e Austrália, enquanto que os isolados do grupo N foram relatados no Norte do Japão, Europa, Uruguai e Austrália (YOSHIDA et al., 2012). No Brasil, apesar da importância das infecções com LYSV, não há dados moleculares sobre os isolados de LYSV brasileiros. Portanto, o genoma completo de um isolado de LYSV proveniente de alho do Brasil foi sequenciado e a região para a proteína P1 avaliada para este isolado bem como demais coletados em diversas regiões produtoras de alho.

Durante o doutorado sanduíche no United States Department of Agriculture, sob coordenação do Dr. John Hammond, também foram avaliadas as espécies de vírus presentes em plantas de Allium ornamental nos Estados Unidos. Foi identificada a presença de diversos vírus relatados em espécies de alho e cebola e a presença de um potyvirus até então não relatado em *Allium* spp, o *Narcissus yellow stripe virus*.

O presente trabalho foi dividido em dois capítulos intitulados: 1) "***Leek yellow stripe virus*** **isolates from Brazil form a distinct clade based on the P1 gene."** redigido em inglês conforme as normas da revista Journal of Plant Pathology e 2) "***Narcissus yellow stripe virus*** **and different species of the genera** ***Allexivirus*****,** ***Carlavirus*** **and** ***Potyvirus*** **are present in ornamental** ***Allium*** **species available in the United States**" redigido em Inglês conforme as normas da revista Plant Disease.

4. REVISÃO BIBLIOGRÁFICA

4.1 O Gênero *Allium*

O gênero *Allium* inclui cerca de 750.000 espécies (BLOCK, 2010), caracterizadas por apresentarem bulbos envoltos por túnicas membranosas (algumas vezes fibrosas), tépalas livres ou semi-livres (FRITSCH et al., 2006). Algumas dessas espécies podem ser usadas na alimentação, outras possuem potencial medicinal e um maior grupo de espécies são usadas como plantas ornamentais (KATIS et al., 2012). As utilizadas na alimentação humana são: cebola (*Allium cepa*), cebolinha (*Allium schoenoprasum*), alho (*Allium sativum*) e alho poró (*Allium porrum)* (BREWSTER, 1994).

As espécies ornamentais distinguem-se das demais pela diversidade de flores e tamanhos (MIGLINO et al., 2011). Evidências sugerem que o alho e cebola foram domesticadas na Ásia central e então propagadas para o Oriente Médio e Mediterraneo, que é considerado o centro secundário destas espécies (BLOCK, 2010). Porém o centro de origem de todas as espécies de *Allium* spp não foi completamente elucidada.

4.2 A cultura do alho e o cenário produtivo

O alho, é considerado a segunda principal espécie do gênero *Allium* mais amplamente consumida no mundo (FAO, 2015). Sua propagação se dá através do plantio dos bulbilhos (FILGUEIRA, 2007).

É uma hortaliça que possui alto valor nutricional, composta por vitaminas A, B2, B6, C, aminoácidos, adenosina, sais minerais (ferro, silício, iodo), enzimas, como a aliniase (YOSHIDA et al.,1987; RESS et al., 1993; BIANCHI et al., 1997).

O alho é uma cultura de clima temperado, portanto, as temperaturas baixas são ideais para que a bulbificação ocorra (SOUZA e MACEDO, 2009). As temperaturas médias mensais exigidas para formação dos bulbos são de aproximadamente 13 °C e para um bom desenvolvimento das plantas é de 24 °C, sendo que a falta destas temperaturas pode afetar a formação dos bulbos (MASCARENHAS, 1978). No Brasil pesquisadores adaptaram a técnica de vernalização pré-plantio, que consiste em armazenar o alho- semente em câmara com temperatura de 3 a 5 °C, por um período de 40 a 60 dias possibilitando o plantio de cultivares nobres de alho originárias da Argentina e do Sul do Brasil, em regiões onde as condições termo-fotoperiódicas não satisfazem as exigências da planta (SOUZA e MACEDO, 2009).

Atualmente existem no mercado brasileiro basicamente três grupos de cultivares que foram desenvolvidas para atender a demanda do consumidor. Essas cultivares são diferenciadas pela duração do ciclo, exigências de fotoperíodo e de temperatura (FILGUEIRA, 2000). As cultivares precoces ou comuns possuem um ciclo mais curto, em torno de quatro meses, são cultivares menos exigentes em fotoperíodo e temperatura, porém apresentam bulbilhos pequenos e de menor valor comercial (Ex: Branco mineiro e Cateto roxo). As cultivares de ciclo mediano ou semi-nobre, possuem um ciclo de aproximadamente cinco meses, sendo essas cultivares mais exigentes em fotoperíodo e temperatura, porém produzem bulbilhos mais graúdos e alcançam melhor cotação comercial (Ex: Amarante). Para as cultivares nobres ou tardias: o ciclo é mais longo, em torno de seis meses, e são cultivares muito exigentes em fotoperíodo (mínimo de 13 horas) e exigem temperaturas baixas, produzindo bulbilhos graúdos de alta qualidade comercial, portanto obtém alta cotação comercial, o que as torna competitivas ao alho importado (Ex: Chonan, Roxo-Pérola-de-Caçador e Quitéria) (FILGUEIRA, 2007; FAYAD-ANDRÉ, 2011).

O cultivo do alho nobre roxo no Brasil se iniciou no ano de 1977 no estado de Santa Catarina. Sem conhecimento tecnológico, as primeiras produtividades de alho nobre roxo no estado foram em torno de 3.000Kg/ha. (LUCINI, 2008). Hoje com o avanço da tecnologia muitas lavouras conseguem produtividades em torno de 13.000 Kg/ha, mesmo assim, a produção brasileira não é suficiente para suprir o consumo anual. A produção brasileira representa 33% do abastecimento interno, o restante é importado da China (45%) e Argentina (25%) (CARVALHO, 2013). Nos anos de 1990, a produção de alho nacional atingiu quase que a totalidade da demanda consumida no Brasil. Porém, com o aumento do consumo, a importação cresceu em números exorbitantes, devastando a produção interna, devido a desvalorização do alho nacional (CARVALHO et al., 2013).

De acordo com dados obtidos pelo Levantamento Sistemático da Produção Agrícola (2015), em 2014 o Brasil produziu 93.859 toneladas, com rendimento médio de 9.629 Kg/ha, em uma área de 9.748 hectares. Na safra 2014 o Estado de Santa Catarina foi o que mais produziu no País, em uma área de 2.230 hectares, produziu 21.449 toneladas. Em seguida veio o Estado de Minas Gerais, com produção de 21.173 toneladas, seguido de Goias, com 21.040 toneladas e Rio Grande do Sul com 16.614 toneladas. O estado de Minas Gerais apresentou um rendimento médio de 13.538 Kg/ha, seguido de Santa Catarina com rendimento de 9.618 Kg/ha, Goiás 9.277 Kg/ha e Rio Grande do Sul 7,6Kg/ha (Levantamento Sistemático de Produção Agricola, 2015). A China é o país com maior produção, sendo que produz em média 76% da produção mundial (FAO 2015).

4.3 Vírus infectando o gênero *Allium*

Grande parte das espécies do gênero *Allium* são propagadas de forma vegetativa, propiciando o acúmulo de vírus pertencente aos gêneros *Allexivirus* *Carlavirus e Potyvirus* (KATIS et al., 2012).

4.3.1 Gênero *Allexivirus*

O gênero *Allexivirus* pertence à família *Alphaflexiviridae*. As espécies possuem partículas filamentosas flexuosas com 800 nm de comprimento e 12 nm de diâmetro, uma molécula de RNA linear de fita simples senso positiva com 9.0 kb de tamanho, incluindo a cauda poly (A) na extremidade 3‟ e a CAP no terminal 5‟. A organização genômica baseia-se em seis ORFs (“open reading frame” ou fase aberta de leitura). A ORF1 codifica a polimerase viral; a ORF2 e 3 codificam a TGB (Triple gene block) que está envolvida no movimento; a ORF4 codifica uma proteína que não apresenta homologia com nenhuma proteína conhecida; ORF5 correspondente à proteína capsidial e a ORF6 codifica uma proteína com função ainda desconhecida, e possui uma estrutura denominada “dedo de zinco” que tem a capacidade de se ligar a ácidos nucléicos (ADAMS et al., 2011a; KING et al., 2012).

A transmissão dos vírus pertencentes a esse gênero ocorre através de ácaros da espécie *Aceria tulipae* (KANG et al., 2007) e além disto podem ser transmitidos por extrato vegetal para espécies de *Allium* e para *Chenopodium quinoa*, *C. amaranticolor* e *C. murale* que mostram sintomas de lesões locais (KATIS et al., 2012).

Oito são as espécies pertencentes ao gênero que podem infectar *Allium* spp.: GarV-A, GarV-B, GarV-C, GarV-D, GarV-E, GarV-X, GarMbFV e ShVX, porém prevalecem na cultura do alho (KATIS et al., 2012).

Os allexivirus estão amplamente distribuídos em todo mundo e podem causar sintomas mais severos quando associados com um potyvirus (TAKAICHI et al., 2001). Entretanto, há falta de informação dos efeitos causados pela infecção, devido à dificuldade de se isolar cada espécie do complexo viral. Segundo Cafrune et al. (2006), GarVA seria a espécie que causa maiores danos à cultura do alho e afeta a sua produção.

4.3.2 Gênero *Carlavirus*

O gênero *Carlavirus* pertence a família *Betaflexiviridae*. São vírus constituídos por uma molécula de RNA fita simples, senso positiva, com partículas virais flexuosas e alongadas com 610 a 700 nm de comprimento e 12 a 13 nm de diâmetro. cotendo seis ORFs: a ORF1 codifica a replicase viral; a ORF2, 3 e 4 formam o Triple Gene Block (TGB), conjunto de proteínas envolvidas no movimento célula a célula (CHEN et al., 2004). A ORF5 codifica a proteína capsidial e a ORF6 codifica uma proteína com função ainda desconhecida, talvez responsável pela transmissão por afídeos, envolvimento na replicação do RNA, e/ou silenciamento gênico (KING et al. 2012).

As espécies de carlavirus mais comuns em *Allium* spp. são GarCLV e SLV (KATIS et al., 2012). Estes virus são transmitidos por afídeos de forma não persistente, sendo as principais espécies transmissoras do SLV: *Aulacorthum solani, Myzus ascalonicus, M. persicae, Neutoxoptera formosana* e *Aphis gossypii* (BOS et al., 1978). Para GarCLV não há relatos de espécies vetoras (KATIS et al., 2012). *Celosia argentea*, *C. amaranticolor, C. quinoa* e *Nicotiana occidentalis* foram relatadas como hospedeiras do SLV e GarCLV (VAN DIJK, 1993a). Infecções com GarCLV e SLV são geralmente latentes, porém os sintomas são mais severos quando ocorrem em infecções mistas com potyvirus ou allexivirus (TAKAICHI et al., 2001).

4.3.3 Gênero *Potyvirus*

O gênero *Potyvirus* pertence à família *Potyviridae*, que inclui seis gêneros (*Potyvirus, Ipomovirus, Maclunavirus, Rymovirus, Tritimovirus, Brambyvirus e Bymovirus*) (KING et al., 2012). A família *Potyviridae* constitui o maior e mais importante grupo de vírus de plantas do ponto de vista econômico, sendo que aproximadamente 30% dos vírus de plantas já caracterizados pertencem a essa família (KING et al., 2012).

O genoma é composto por uma molécula de RNA fita simples, senso positiva e possuem partículas filamentosas flexuosas com 680-900 nm de comprimento e 11-13 nm de diâmetro. A organização genômica baseia-se em uma única ORF responsável por codificar uma poliproteína de aproximadamente 345 kDa. Apresentam a proteína VPg ligada covalentemente ao terminal 5‟, o genoma possui cerca de 9,7 Kb de extensão e apresentam uma cauda poli (A) no terminal 3‟ (ADAMS et. al., 2011b). Possuem uma pequena ORF incorporada na região codificadora para a proteína P3 denominada de *Pretty Interesting Potyviridae* ORF - PIPO (CHUNG et al., 2008).

O RNA genômico é envolto por um capsídeo formado por aproximadamente 2.200 cópias. A poliproteína codificada sofre autoproteólise dando origem a cada uma das proteínas virais: P1, HC-Pro, P3, 6KI, CI, 6K2, VPg, NIa, NIb e CP (Fig. 1) (ADAMS et al., 2011b).

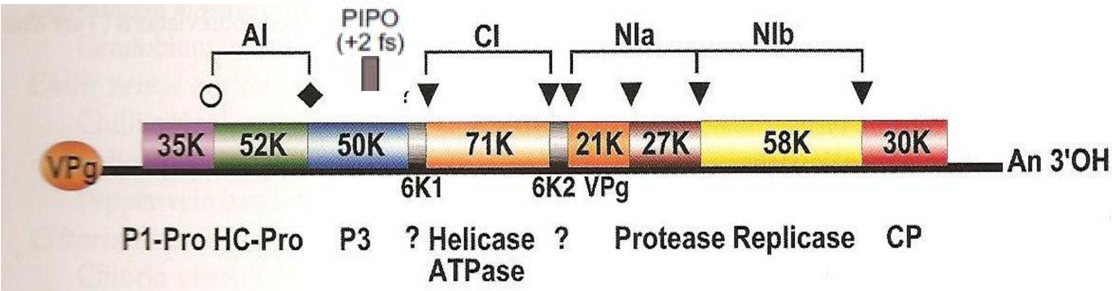

Figura 1. Organização genômica do vírus *Tobbaco etch virus* (TEV), gênero *Potyvirus*. A linha escura representa o RNA sentido positivo e os retângulos coloridos representam as ORFs com suas respectivas massas moleculares (kDa). Os símbolos acima das ORF representam os sítios de clivagem das proteases (Adaptado de BERGER et al., 2005).

Uma importante característica dos potyvirus é que a maioria das proteínas codificadas são multifuncionais (URCUQUI-INCHIMA et al., 2001). A proteína

P1 é a mais variável tanto em tamanho quanto em sequência de nucleotídeos e aminoácidos do genoma dos potyvirus e considerada a proteína mais apropriada para estudos de relacionamento filogenético e estudos de história evolutiva dos potyvirus. (VALLI et al., 2007). O papel da proteína P1 no processo infeccioso não é totalmente conhecido. Estudos relataram seu envolvimento no aumento da patogenicidade (PRUSS et al., 1997), amplificação do genoma (VERCHOT e CARRINGTON, 1995) , acumulação de proteínas (JOHANSEN e CARRINGTON, 2001), autoproteólise (VERCHOT et al., 1991), auxilia a HC-Pro na supressão do silenciamento gênico (RAJAMAKI et al., 2005; VALLI et al., 2007) e interfere na sintomatologia (RYAN & FLINT, 1997).

A proteína HC-Pro está envolvida na transmissão por afídeos, proteinase e autoclivagem do carboxi-terminal, movimento sistêmico, supressão do silenciamento gênico, sinergismo e desenvolvimento de sintomas (MAIA et al., 1996). A proteína P3 apresenta função relacionada à patogenicidade e a proteína 6K1 apresenta função desconhecida (RODRIGUEZ-CEREZO et al., 1993; LANGENBERG e ZHANG, 1997). A proteína CI tem função de helicase e movimento célula-a-célula (LAIN et al., 1990). A proteína 6K2 tem função de ancorar o complexo replicativo às membranas da célula (RESTREPO-HARTWIG e CARRINGTON, 1992). A proteína NIa (VPg-Pro) tem função de localização celular, proteinase e interações proteína-proteína (HONG et al., 1995). A proteína NIb possui função de RNA polimerase dependente de RNA (RdRp) (HONG e HUNT, 1996) e a proteína CP (proteína capsidial) está envolvida na transmissão por afídeos, movimento célula-a-célula e sistêmico e na montagem da partícula viral (SHUKLA e WARD, 1988).

O ICTV (Comite internacional de taxonomia de vírus) utiliza como critérios para demarcação de novas espécies no gênero *Potyvirus* a identidade de aminoácidos inferior a 80% e sequência de nucleotídeos menor que 76% para a proteína capsidial ou o genoma completo (KING et al., 2012). Além disto, diferentes sítios de clivagem da poliproteína, gama de hospedeiros, ausência de proteção cruzada, transmissão ou não por sementes, reações de hospedeiros, diferenças sorológicas e vetores primários diferentes (BERGER et al., 2005).

As espécies frequentemente relatadas em *Allium* spp são OYDV e LYSV, sendo estas consideradas economicamente importantes pela sua ampla distribuição geográfica e pelos danos na produção (CONCI et al., 1992; VAN DIJK, 1993b; CHEN et al., 2001). Os potyvirus são transmitidos por afídeos em uma relação de transmissão do tipo não persistente (COSTA, 1998). As principais espécies de afídeos que transmitem LYSV são *M. persicae*, *A. fabae*, *A. gossypii*, *A. nerii*, *Hyperomyzus carduellinus*, *Rhopalosiphum maidis*, *R. padi*, *Schizaphis graminum*, e *Uroleucon sonchi* (LUNELLO et al., 2002). Para OYDV existem mais de 50 espécies descritas como vetoras, porém as mais importantes são *M. ascalonicus*, *M. persicae*, *R. maidis*, e *Acyrthosiphon pisum* (VAN DIJK, 1993a).

Alguns isolados de LYSV causam lesões locais necróticas em *C. album*, *C. murale*, *C. amaranticolor* e *C. quinoa* (KATIS et al., 2012).

4.4 Métodos para identificação e detecção de vírus

Os vírus que infectam o gênero *Allium* ocorrem muitas vezes em infecções mistas dificultando a determinação dos sintomas relacionados a cada vírus (WARD et al., 2009). Várias técnicas podem ser utilizadas na detecção de vírus, incluindo microscopia eletrônica, hibridização de ácidos nucléicos, ELISA (Enzyme Linked Immuno Sorbent Assay), RT-PCR (Transcriptase Reverse - Polymerase Chain Reaction) e métodos biológicos, sendo as técnicas moleculares as mais utilizadas e precisas (MARTIM et al., 2000). As técnicas moleculares, como de RT-PCR são altamente específicas e sensíveis, permitindo a detecção de vírus a partir de uma pequena quantidade do RNA alvo (LUNELLO et al., 2004). Para os principais vírus de *Allium* spp. já estão disponível na literatura oligonucleotídeos para identificação dos gêneros e específicos para as espécies (Tabela 1). Além da técnica de PCR tradicional, o PCR quantitativo já tem demonstrado alta eficiência para algumas espécies (LUNELLO et al., 2004; MITUTI, 2013).

Também foram relatadas algumas plantas indicadoras para vírus que infectam alho como *C. quinoa, C. murale, C. amaranticolor* e *N. occidentalis* (VERBEEK et al., 1995).

O teste ELISA é o principal método para o diagnóstico utilizado em larga escala na detecção de vírus em espécies de *Allium*. Antissoros específicos comerciais são disponíveis para as duas espécies de carlavirus (GarCLV e SLV), além de ser empregado também o antissoro universal para potyvirus comercializado pela empresa Agdia (MITUTI, 2013).

Tabela 1: Oligonucleotídeos utilizados para identificação de vírus em *Allium* spp.

Primer	**Sequência (5'- 3')**	**Gênero ou espécie**	**Referência**
SLV/GCLV 7303 SLV/GCLV 7665	GGNTKKGAAWCTGGGAGDCC CATKTMATTCCAAACAACNGGYGC	Carlavirus	Mituti et al., 2015
PV1 WCIEN	GAT TTA GGT GAC ACT ATA GT_{16}- ATG GTT TGG TGY ATY GAR AAT	Potyvirus	Gibbs and Mackenzie, 1997 Mota et al., 2004
Cpallexi-senso2 Cpallexi-anti 1	CTACCACAATGGTTCCTC GATTTCTTTAACGCAGTG	Allexivirus	Oliveira, 2013
OYDV-F OYDV-R	CRCCARTTCTGGATAAYGC CTCCGTGTCCTCATCCG	OYDV	Mituti et al., 2015
P1-1170 LY5P	CTTCMTCRCASTCATGKTCC AATCTCAACACAACTTATRC	LYSV	Mituti et al., 2015 Yoshida et al., 2012
SLV 7044 SLV 8004	CTTTTGGTTCACTTTAGG GCACGCAATAGTCTACGG	SLV	Mituti et al., 2011
GCLV 7303 SLV/GCLV 7665	GGSTTTGARACTGGGAGGCC CATKTMATTCCAAACAACNGGYGC	GarCLV	Mituti et al., 2015
GarV-A1 GarV-A2	CCCAAGCTTACTGGAAGGGTGAATTAGAT CCCAAGCTTAGGATATTAAAGTCTTGAGG	GarV-A	Melo Filho et al., 2004
CPBS2 CPBA1	GCAGAATAARCCCCCYTC RAAGGGTTTATTCTGTTG	GarV-B	Oliveira, 2013
GarV-C1 GarV-C2	CCCAAGCTTCATCTACAACAACAAAGGCG CCCAAGCTTATAAGGGTGCATGATTGTGG	GarV-C	Melo Filho et al., 2004
GarV-D1 GarV-D2	CCAAGCTTAAGCAAGTGAAGAGTGTAAG CCAAGCTTTTGGAAGAGGAGGTTGAGA	GarV-D	Melo Filho et al., 2004
CPShS1 CPShA1	GAATGCATCAGGRGAYCTC GCRGGRGGTTTCTTCTG	ShV-X	Oliveira, 2013
CPES1 CPEA1	GGRTCGTCACGATTYGTTAC YTTGAACCTCATACCYCC	GarV-E	Oliveira, 2013
CPXS2 CPXA1	GCCTTCTGAAAATGACTTAG CTAGGATTTGCTGTTGGG	GarV-X	Oliveira et al., 2013

4.5 Sintomatologia e controle de viroses em *Allium* spp.

Os vírus interferem diretamente nos fenômenos vitais e nos processos bioquímicos e fisiológicos da planta, na síntese de proteínas, na fotossíntese, no transporte de água e de elementos minerais através do sistema vascular ocasionando reduções significativas na concentração de clorofila, vigor vegetativo e prejudicando a formação dos bulbos, devido a redução na produção de carboidratos (ZAMBOLIM et al., 2007).

Alguns vírus formam inclusões citoplasmáticas afetando diretamente os componentes celulares, como nucléolo, núcleo, citoplasma e suas organelas (EL-ELA et al., 2006), como os potyvirus que formam inclusões citoplasmáticas do tipo cata-vento no citoplasma das plantas infectadas (HULL, 2002). Os vírus em alho causam sintomas de mosaico, amarelecimento, estrias e clorose levando a grandes perdas econômicas e danos na produção, principalmente quando há infecção por OYDV e LYSV (CONCI et al., 1992; VAN DIJK, 1993a; CHEN et al., 2001; LUNELLO et al., 2007). Sintomas típicos de infecção viral por allexivirus em alho incluem o subdesenvolvimento da planta e folhas retorcidas devido o ataque de ácaros (KANG et al. 2007). Os carlavirus apresentam-se aparentemente assintomáticos (PALUDAN, 1980). Os allexivirus e carlavirus quando em infecção mista com potyvirus induzem sintomas mais severos e maiores perdas de produção (CONCI et al., 2003; LOT et al., 1998; CAFRUNE et al., 2006).

Os vírus em alho devem ser controlados preventivamente, principalmente pela utilização de bulbos livres de vírus. A associação da termoterapia e a cultura de tecido propicia a obtenção de bulbos livres de vírus e tem sido empregada com sucesso (PAVAN, 1998). Evitar cultivos sucessivos e próximos à lavouras mais velhas, realizar o plantio em áreas com altitudes elevadas, controlar plantas hospedeiras de vírus e dos afídeos, eliminar restos culturais também são medidas preventivas indicadas para o controle dos vírus em alho (DUSI, 1995).

O controle de afídeos através da aplicação de inseticidas, visando redução de incidência de potyvirus e carlavirus não é uma prática recomendada. Os afídeos transmitem os vírus de maneira não persistente, desta forma o vírus é transmitido em poucos segundos após a aquisição e muitas vezes a aplicação do inseticida acelera o metabolismo dos pulgões, aumentando a transmissão logo após a aplicação (ZAMBOLIM et al., 2007). Para o gênero *Allexivirus*, o ácaro vetor pode ser controlado através do tratamento por imersão dos bulbos ou bulbilhos em acaricidas antes do plantio (MENEZES SOBRINHO, 1997), bem como durante seu armazenamento (OLIVEIRA et al., 2014).

4.6 Danos provocados por viroses na cultura do alho

As viroses são consideradas um dos principais problemas na cultura do alho devido sua propagação vegetativa (VAN DIJK 1993a; CHEN et al., 2001; FAJARDO et al., 2001). Os maiores danos na produção em alho têm sido atribuídos às infecções causadas por OYDV e LYSV, porém estes se agravam na presença de infecções mistas entre os diferentes vírus do complexo viral que infectam o alho (FAYAD-ANDRÉ, 2011).

De acordo com Mituti et al. (2015), 81 % das plantas de alho nobre coletadas em campos comerciais nas safras de 2007 a 2011 nas principais regiões produtoras do Brasil encontraram-se infectadas por potyvirus. Destas, 41 % estavam infectadas somente com potyvirus, 16% apresentaram infecção mista com LYSV e OYDV, 25 % apresentaram infecção mista de potyvirus e allexivirus, 6 % estavam infectadas com potyvirus e carlavirus e 9% apresentaram infecção mista com os tres gêneros, LYSV e OYDV foram encontrados com a mesma frequência.

A ocorrência de infecções mistas torna difícil determinar as perdas e danos atribuidos a cada vírus (WARD et al., 2009). No entanto, alguns estudos mostram que infecções com LYSV reduziram o peso de bulbos em até 28 %. Enquanto que com o OYDV a redução foi de 48 a 65%. Já em infecções mistas com LYSV e OYDV houve uma redução no peso de bulbos de 56% a 84% (LOT et al., 1998). Os *Allexivirus* são o segundo gênero com maior prevalência em campos comerciais no Brasil, tendo sido detectados em 49 % das amostras avaliadas de 2007 a 2011 com predominância das espécies GarVD e GarVA (MITUTI et al., 2015). Cafrune et al., 2006 verificaram que plantas de alho infectadas por GarVA e GarVB apresentaram reduções do peso de bulbo de 14 a 32% e 9 a 15%, respectivamente. Plantas infectadas por OYDV, LYSV, GarV-A, GarV-C, GarV-D e GarCLV apresentaram redução de até 61% no peso de bulbos em relação às plantas sadias.

Mituti et al. (2015) relatou baixa prevalência de carlavirus no Brasil. Em alho cultivado, em campos comerciais, 21% das plantas avaliadas apresentaram infecção com carlavirus, sendo GarCLV a espécie predominante. SLV foi detectado em apenas 3% das amostras.

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CAPÍTULO I

Leek yellow stripe virus ISOLATES FROM BRAZIL FORM A DISTINCT CLADE BASED ON THE P1 GENE[1]

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Leek yellow stripe virus ISOLATES FROM BRAZIL FORM A DISTINCT CLADE BASED ON THE P1 GENE.

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Abstract

The complete genome sequence of a garlic isolate of *Leek yellow stripe virus* (LYSV) from Brazil (LYSV-MG) was determined and its phylogenetic relationships with other LYSV isolates from other parts of the world were inferred. The LYSV-MG genome consists of 10,341 nucleotides and encodes a polyprotein of 3,221 amino acids. Brazilian LYSV isolates are more closely related to S-type than to N-type isolates but do not have a deletion in the P1 gene. Partial nucleotide sequence analysis of the P1 gene revealed 97-99% identity among Brazilian isolates, and 51-64% identity between Brazilian isolates and isolates from other countries for which sequence information is available in Genbank. The Brazilian isolates formed a monophyletic group closest to the S-type isolates and one isolate from Okinawa (O-type). These data suggest that Brazilian LYSV isolates are derived from an ancestral source of the Okinawa and S-type isolates, prior to the occurrence of the P1 deletion and divergence of the S-type isolates.

Keywords: *Allium sativum, Potyvirus,* variability.

Introduction

Garlic (*Allium sativum* L.) can be infected by multiple viruses, mainly of the genera *Allexivirus, Carlavirus* and *Potyvirus* (Conci et al., 2003; Melo-Filho et al., 2006; Lunello et al., 2007; Katis et al., 2012). *Leek yellow stripe virus* (LYSV) belongs to the genus *Potyvirus* in the family *Potyviridae*, with flexuous and filamentous particles containing a single-stranded, positive sense RNA genome. It was first reported in 1987 in garlic by Walkey et al. (1987). LYSV is considered the most important virus in garlic due to the wide distribution and severe symptoms (Van Dijk, 1993, 1994). Infected garlic plants exhibit mosaic and yellow stripe symptoms on leaves and may produce smaller, malformed bulbs resulting in severe losses of yield and quality that vary depending on the cultivar (Lot et al., 1998; Takaki et al., 2005). LYSV is transmitted by aphids and may easily re-infect virus-free garlic plants in the fields (Yoshida et al., 2012).

Mituti et al. (2015) found that 81 % of field-grown noble garlic in Brazil were infected with potyviruses of which 41% were infected only by potyviruses (*Onion yellow dwarf virus* (OYDV) either LYSV), 16% were mixed infected with OYDV and LYSV, 25% were infected with potyviruses and allexiviruses, 6% were infected with potyviruses plus carlaviruses, and 9% were infected with potyviruses, allexiviruses, and carlaviruses. Previous studies showed that LYSV was the most prevalent virus from garlic in Brazil (Fayad-Andre et al., 2011). The occurrence of multiple virus infections in the same plant makes it difficult to determine and predict yield loss attributed to each virus (Ward et al., 2009). Single infections of LYSV showed a maximum reduction in bulb weight of 17-59%, depending on the cultivar; single infections of OYDV reduced bulb weight by up to 48-65% and mixed infections with OYDV and LYDV had a synergistic effect with bulb weight reduced by as much as 56- 84% in different cultivars (Lot et al., 1998).

The P1 coding region has been demonstrated to be the most variable of the potyvirus genes. The roles of P1 in potyvirus infection are still emerging, although there are indications that P1 may enhance the ability of HC-Pro to suppress RNA silencing (Rajamäki et al., 2005; Valli et al., 2006), and to increase viral pathogenicity (Pruss et al., 1997). One of the first functions identified was self-cleavage at the P1/HC-Pro junction (Verchot et al., 1991); further work identified stimulation of genome amplification (Verchot and Carrington, 1995), and increasing mRNA and protein accumulation (Johansen and Carrington, 2001). RNA binding activity has been reported for the P1 protein of *Turnip mosaic virus* (Soumounou and Laliberte, 1994) and *Potato virus A* (Merits et al., 1998), while RNA binding has been attributed to involvement of P1 in viral movement (Arbatova et al., 1998). More recent work has shown that two single amino acid mutations in the P1 of *Plum pox virus* (PPV) were associated with milder symptoms, and that one mutation (W29R) reduced both virus accumulation and symptom severity in *Prunus persica*, while the other (V139E) caused symptom attenuation in herbaceous hosts (Maliogka et al., 2012); this, combined with the studies on P1 diversity of Valli et al. (2007), indicates that P1 variability is associated with host adaptation, and that particular viral variants are adapted to specific hosts or even specific host genotypes.

The N-terminal region of P1 shows a high variability in both length and amino acid sequence, and host-dependent regulation of P1 self-cleavage may function to modulate the efficiency of establishment of infection, and to minimize induction of host defense responses; Pasin et al. (2014) showed that an infectious clone of PPV from which P1 was deleted was still able to replicate and spread systemically in *Arabidopsis*, and that this was unrelated to any direct effects on RNA silencing suppression. However, P1 was shown to be indirectly involved in RNA silencing suppression, because when P1 was not able to self-cleave, HC-Pro function was limited and infectivity adversely affected in wild-type

Arabidopsis, but not in an *Arabidopsis* mutant defective in RNA silencing; these authors also demonstrated that the N-terminal 164 residues of PPV P1 are not required for P1 self-processing, but act as a negative regulator of self-cleavage in an *in vitro* assay (Pasin et al., 2014). Deletion of this regulatory region led to enhanced early amplification of the PPV genome, but reduced overall viral accumulation; it was therefore proposed that P1 acts to fine-tune potyviral regulation by interacting with specific host-plant effectors and activating the P1 protease activity to release HC-Pro with its RNA silencing suppression activity. As HC-Pro may itself induce further host defenses, the combined effects of P1 negative regulation and HC-Pro silencing suppression activity may maintain higher long-term replication in hosts to which the virus is adapted (Pasin et al., 2014).

The P1 protein of *Tobacco etch virus* (TEV) has both a nucleolar localization signal and a nuclear export signal, and to traffic in and out of the nucleus and nucleolus during infection (Martinez and Daròs, 2014). When an infectious clone of TEV was separately labeled with different fluorescent proteins fused to P1 and NIb, the labeled P1 was only detected close to the infection front, whereas NIb was detected in cells from early to late stages if infection, suggesting that P1 is rapidly degraded. P1 was also shown to bind specifically to 60S ribosomal subunits during infection, and it was suggested that P1 stimulates protein translation, whereas HC-Pro inhibits translation, potentially suppressing cap-dependent translation of host mRNAs in favor of viral synthesis (Martinez and Daròs, 2014). P1 may thus play multiple roles in adaptation of potyviruses to specific hosts, and by virtue of this role in host adaptation, it is therefore an appropriate sequence to utilize for phylogenetic comparisons to understand the evolutionary history of a potyvirus species (Valli et al., 2007).

In this work, we determined the complete nucleotide sequence of a representative LYSV isolate collected from garlic crops in Brazil and compared it with the complete sequences of LYSV isolates from China, Japan and Australia. We have further analyzed the nucleotide sequence of the partial P1 gene of various LYSV isolates from garlic collected in several locations in Brazil, and compared these with other isolates for which sequence information is available in Genbank. Our results indicate that LYSV isolates from Brazil form a monophyletic clade distinct from isolates from other countries.

Material and Methods

LYSV-infected garlic plants from various regions of Brazil were initially identified by PCR using the WCIEN/PV1 generic potyvirus primers (Mota et al., 2004; Gibbs and Mackenzie, 2007) to amplify part of the coat protein (CP) gene, followed by sequencing. Identification of LYSV was confirmed by PCR with the LY5P/LY2M specific primers to amplify part of the 5′ untranslated region and the N-terminal domain of the P1 gene as described by Yoshida et al. (2012). Plants were also tested by PCR for viruses of the genera *Allexivirus* and *Carlavirus*, and for the presence of *Onion yellow dwarf virus* (OYDV) using the primers described by Oliviera et al. (2013), Mituti et al. (2011), and Mituti et al. (2015), respectively. LYSV isolate MG (LYSV-MG) was identified from a singly-infected garlic plant from Santa Juliana, Minas Gerais State, and was selected for full genome sequencing. The variability analysis was carried out with isolates from garlic plants collected in the most important production areas of Brazil, located in the South, Southeast and West Central regions. Total RNA was extracted from infected leaves using the Total RNA Purification Kit (Norgen Biotek). The cDNA was prepared using the PV1 primer (Gibbs and Mackenzie, 1997) with AMV reverse transcriptase (Promega), following the manufacturer‟s instructions. The PCR reactions were performed using either degenerate or specific upstream and downstream primers, with a total of 5 μL of 10x High

Fidelity PCR Buffer, 2 mM of $MgSO_4$, 0.4 μM of each primer, 0.2 mM of dNTPs (2.5 mM each), 1 μL of Platinum Taq High Fidelity, 3 μL of cDNA and water per complete 50 μL reaction. Partial CI and HC-Pro genes were amplified using the universal primers described for the family *Potyviridae* (Ha et al., 2008). Partial CP and nuclear inclusion b (NIb) genes were amplified using universal primer WCIEN paired with PV1 (Mota et al., 2004; Gibbs and Mackenzie, 2007) and with NIB2F/NIB3R primers described by Zheng et al. (2010) respectively.

The partial P1 gene was amplified using LY5P/LY2M specific primers for LYSV (Yoshida et al., 2012). The gaps between these regions were covered using specific primers designed from the sequences previously generated (Fig. 1). The amplified fragments were cloned using the p-GEM T Easy Vector system (Promega), and the cloned fragments were sequenced in both directions by Macrogen Inc. (Republic of Korea). To obtain a consensus sequence three clones of each fragment were sequenced. The final LYSV-MG genome sequence and the partial P1 gene sequences of various Brazilian isolates were deposited in the DDBJ/ EMBL/ GenBank database under the accession numbers KP258216 (full genome of LYSV-MG), KP236094, KP236095, KP236096, KP236097, KP236098, KP236099, KP236100, KP236101, KP236102 and KP236103. Comparisons were made between the LYSV-MG sequence and the available complete sequence of other garlic isolates as follows: Chinese (NC_004011); (DD462959), Australian (JX429967); (JX429965) and Japanese (AB194623); (AB194621) and (AB194622) isolates. Variability between isolates was evaluated by phylogenetic analysis of the P1 amino acid sequence encoded by the 846 nucleotides corresponding to part of the P1 gene of LYSV-MG, and the corresponding regions of other isolates. OYDV (AB219834) was used as the outgroup sequence for the phylogenetic analysis. The sequences were aligned using ClustalW

(Thompson et al., 1994) with default parameters. Neighbor-joining phylogenetic trees were prepared using MEGA 6.0 (Tamura et al., 2013) with 1,000 bootstrap replicates.

Results and Discussion

LYSV-MG was selected for cloning and sequencing of the full genome because the source plant was determined by PCR to be infected by LYSV, but free of any detectable allexivirus, carlavirus, or OYDV infections. The complete genome of LYSV-MG consisted of 10,341 nucleotides (nt), excluding the 3′ terminal poly (A) tail (Fig. 1). The overall ssRNA nt composition of LYSV-MG was adenine (A) 32.2%, cytosine (C) 19.4%, guanine (G) 22.8%, and uracil (U) 25.5%. The AUG codon located at nt position 83-85 is likely to be the translation initiation codon, since it was in a context (AAC<u>AUG</u>GCT) that is similar to the context of the initiation codon of other isolates of LYSV. The 5′ non-coding region (NCR) was 82 nt in length, and AU-rich. The 3′-NCR was 592 nt in length and also AU-rich. The stop codon (UGA) was at position 9,746–9,748. The putative translation product contained 3,221 amino acids (aa). According to the conserved cleavage sites predicted by Adams et al. (2005a), a total of ten proteins are expected: P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa, NIb and CP (Fig. 1). The predicted cleavage sites of LYSV-MG were consistent with the known sites of other potyviruses (Adams et al., 2005a). Several previously identified, highly conserved amino acid sequence motifs described in potyviruses (Shukla et al., 1994) were identified in LYSV-MG, including PTK, IGN and CSC motifs of HC-Pro, which are known to be required for aphid transmission, RNA amplification and systemic movement, respectively. The GDD motif in the NIb region associated with viral RNA replication (Urcuqui-Inchima et al., 2001; Ha et al., 2008) and a DAG motif in the CP region associated with aphid transmission (Lopez-Moya et al., 1999) were also identified. The PIPO (pretty interesting potyviridae ORF) with the $G_{1-2}A_{6-7}$ motif (Chung et al., 2008) was found at nucleotides 3230 to 3470 and contains 80aa.

Phylogenetic analysis based on the full genome sequence of garlic isolates of LYSV showed that LYSV-MG falls into a clade with one Australian (JX429965) and two Chinese isolates (including NC_004011), whereas three other Australian isolates (including JX429967) and three Japanese isolates (including AB194623) form a separate clade, with both clades supported by bootstrap values of 100% (Fig. 2). The P1 region of the two Chinese isolates and one Australian isolate (JX429965) contains a deletion of 201-nucleotides (67 aa) with respect to the isolates in the other clade (including JX429967 and AB194623). LYSV-MG does not have this deletion, but shows the highest genomic nt and aa identities with the isolates which do have the deletion.

We therefore compared the complete genomic nt and aa sequences, and those of the individual genome segments of LYSV-MG and Chinese (NC_004011), Australian (JX429965 and JX429967) and Japanese (AB194623) isolates from the two clades. LYSV-MG shares highest identity of both the complete genomic nt and polyprotein aa sequences with the Chinese isolate (NC-004011) and one Australian isolate (JX429965) having the deletion in P1 (Table 1). Both the whole genome identities (76-77% nt; 82-84% aa) and CP identities (79-83% nt; 83-85% aa) of all isolates were above the species discrimination values for the genus *Potyvirus* (Adams et al., 2005b). Comparison of individual LYSV-MG cistrons with those of other isolates revealed that the regions encoding CP and CI were the most highly conserved, whereas the P1 region was the most variable (Table 1). However, despite the absence of a deletion in the LYSV-MG P1, the P1 region was most closely related to those of NC_004011 and JX429965, both of which have a 201 nt/67 aa deletion (Table 1). In addition, while the cleavage sites between all proteins (Fig. 1) were identical between LYSV-MG and these two most closely-related isolates, there were differences at the sites 6K1/CI, CI/6K2, 6K2/VPg and NIb/CP when compared to the less-related Australian (JX429967) and Japanese (AB194623) isolates (data not shown).

Because differences were found among the P1 coding regions of LYSV-MG and isolates from Japan, China and Australia, we decided to analyze the partial P1 coding region from a wider range of garlic isolates collected from different locations in Brazil and compare their nucleotide sequence with that of other garlic isolates that is available in Genbank. We analyzed ten isolates of LYSV from Brazil, together with 25 LYSV partial P1 sequences obtained from GenBank. The phylogenetic analysis showed that Brazilian isolates form a clade distinct from the other isolates of other countries, but are phylogenetically closest to the S-type isolates and an isolate from Okinawa (AB636327) (Fig. 3). Brazilian isolates share 97-99% nt identity and 51-64% with isolates from other geographic origin. The position of LYSV-MG within the clade of Brazilian isolates demonstrates that this fully-sequenced isolate is representative of the garlic LYSV population in Brazil.

In agreement with the previous reports by Chen et al. (2001), Takaki et al. (2005), and Yoshida et al. (2012), our phylogenetic analysis revealed that LYSV partial P1 nt sequences from garlic formed two main groups, the N-type (including isolates from Northern Japan, and some from Uruguay and Australia) and the S-type (Southern Japan, China, Korea and other Australian isolates). The Sp-type clade (Spanish isolates) and monotypic U-type clade (Uruguay) of Yoshida et al. (2012) were on the same major branch as the N-type isolates (Fig. 3). As the Sp-type and U-type isolates group closely with the N-type isolates, and also lack the deletion present in the S-type isolates, we considered this major branch as essentially „N-type‟. The S-type group has large P1 sequence deletions relative to the N-type group (around 201 nt; Yoshida et al., 2012). None of the isolates from Brazil (B-type) have this deletion, but are phylogenetically closer to S-type isolates than N-type isolates.

There is evidence that garlic originated in Central Asia, then spread in two directions, to China and India and to Europe and Russia (Yoshida et al., 2012). It is likely that an ancestral LYSV population was similarly divided resulting in the emergence of the N-type group and the S-type group during the evolution of LYSV, with two separate introductions into both Japan and Australia. It is possible that the S-type group derived from the N-type group after extensive deletion because insertions are much rarer than deletions (Takaki et al., 2005). It is likely that the distinct variability of those LYSV isolates can be explained by both their host‟s geographical origin and unique adaptation to a particular garlic cultivar, resulting in formation of the various clades observed (Takaki et al., 2005; Yoshida et al., 2012).

The P1 protein has been demonstrated to be the most variable protein among those encoded by the Potyvirus genome (Shukla et al., 1994; Valli et al., 2007). As P1 diversity may be associated with adaptation to particular hosts or even specific host genotypes, the P1 region is therefore an appropriate sequence for use in studies of phylogenetic relationships to understand the evolutionary history of LYSV isolates in garlic (Takaki et al., 2005; Yoshida et al., 2012). We believe that the Brazilian isolates have a common origin due to the high identity (97-99%) observed among them. The data suggest a possible common origin for Brazilian and O-type Okinawa (AB636327) LYSV isolates, suggesting that Brazilian LYSV isolates are derived from an ancestral source of the Okinawa and S-type isolates, prior to the occurrence of the P1 deletion and divergence in the S-type isolates. A second possibility is that isolates from Brazil, Australia and Okinawa were introduced via China prior to the divergence of the S-type.

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Table 1 Comparison of the full genomic nucleotide (nt) and amino acid (aa) sequences between Brazilian *Leek yellow stripe virus* isolate MG (LYSV-MG) and representative LYSV isolates from China (NC_004011), Australia (JX429965), Australia (JX429967), and Japan (AB194623).

Isolated compared		**5'UTR**	**Whole genome**	P1	HC-Pro	P3	CI	NIa-VPg	NIa-Pro	NIb	CP	3'UTR
					Range of nt and aa identity (%)							
China[a]	nt	68	77	73	76	77	81	81	79	81	83	89
China	aa		84	63	81	78	94	93	93	93	85	
Australia[b]	nt	40	77	70	76	78	82	81	79	81	81	90
Australia	aa		84	63	82	80	95	93	92	92	84	
Australia[c]	nt	50	76	65	78	75	80	76	78	79	80	83
Australia	aa		82	55	87	71	93	84	90	90	83	
Japan[d]	nt	53	76	64	77	75	81	76	78	79	79	83
Japan	aa		82	55	87	71	92	84	91	91	83	

[a] NC_004011, isolate Yugang GYH; [b] JX429965, isolate SG2; [c] JX429967, isolate AG1; [d] AB194623, isolate 1A31

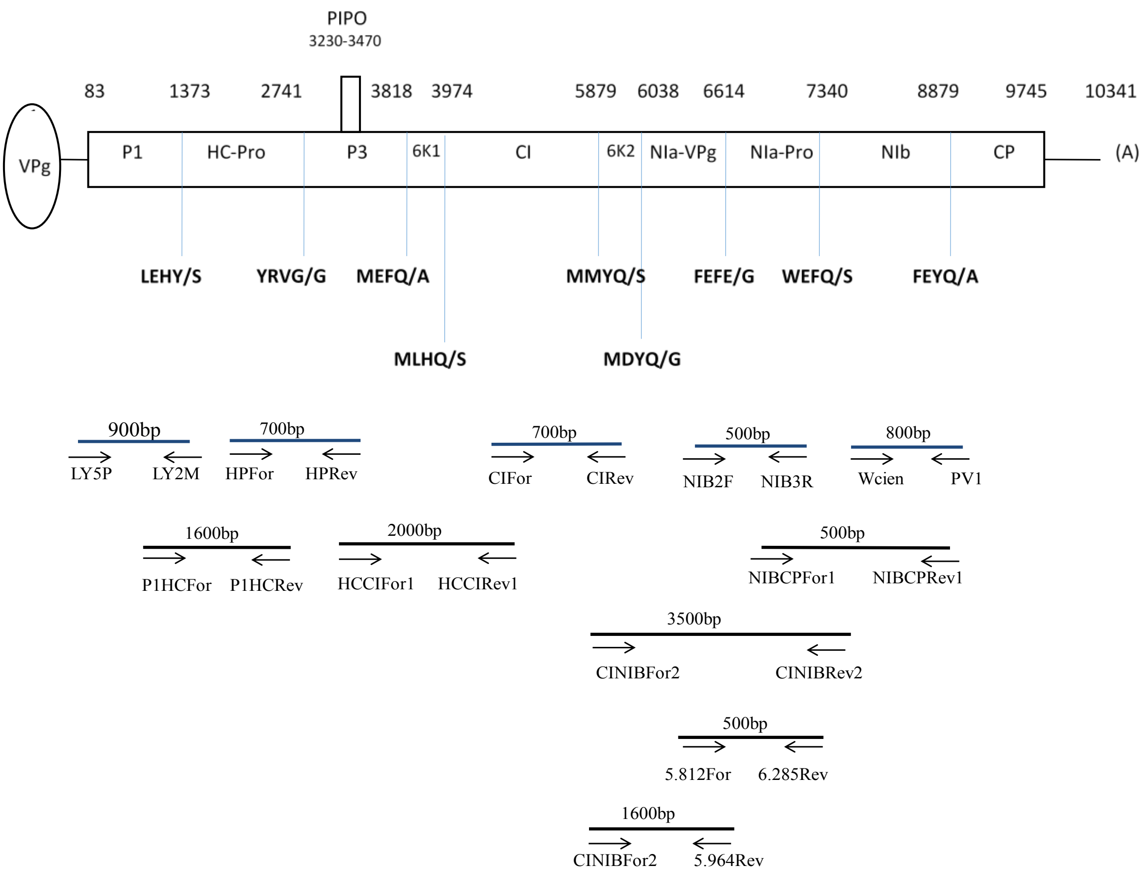

Fig. 1 Schematic representation of the genome organization of *Leek yellow stripe virus* (LYSV-MG). The terminal untranslated regions (UTR) are depicted by lines, and the open reading frame is shown as an open box with the different polyprotein domains (proteins). The small box above P3 represents the ORF PIPO. The numbers above the genome indicate the start of each region. The predicted proteinase cleavage sites in the polyprotein are indicated below the genome. The primers used to amplify the genome are indicated below the arrows with the estimated size of amplicon indicated above lines (not to scale).

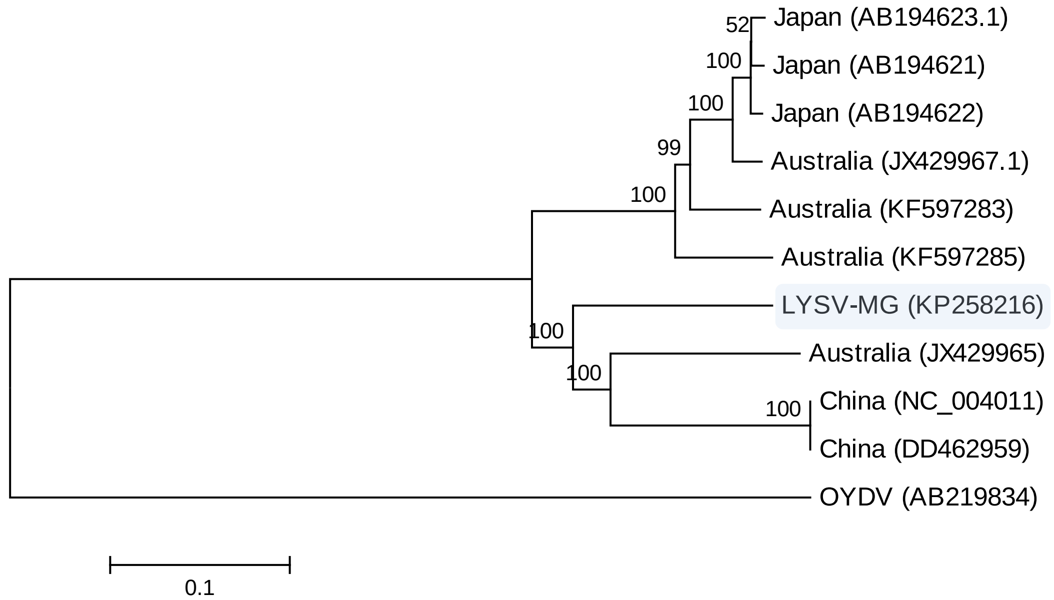

Fig. 2 Phylogenetic analysis using the complete nucleotide sequences of *Leek yellow stripe virus* MG-isolate (LYSV-MG) and garlic LYSV isolates from China, Australia and Japan.The tree was constructed the Neighbor-joining method implemented in the MEGA 6.0 software. Bootstrap values of 1000 replicates are shown. OYDV (AJ510223) was used as an outgroup.

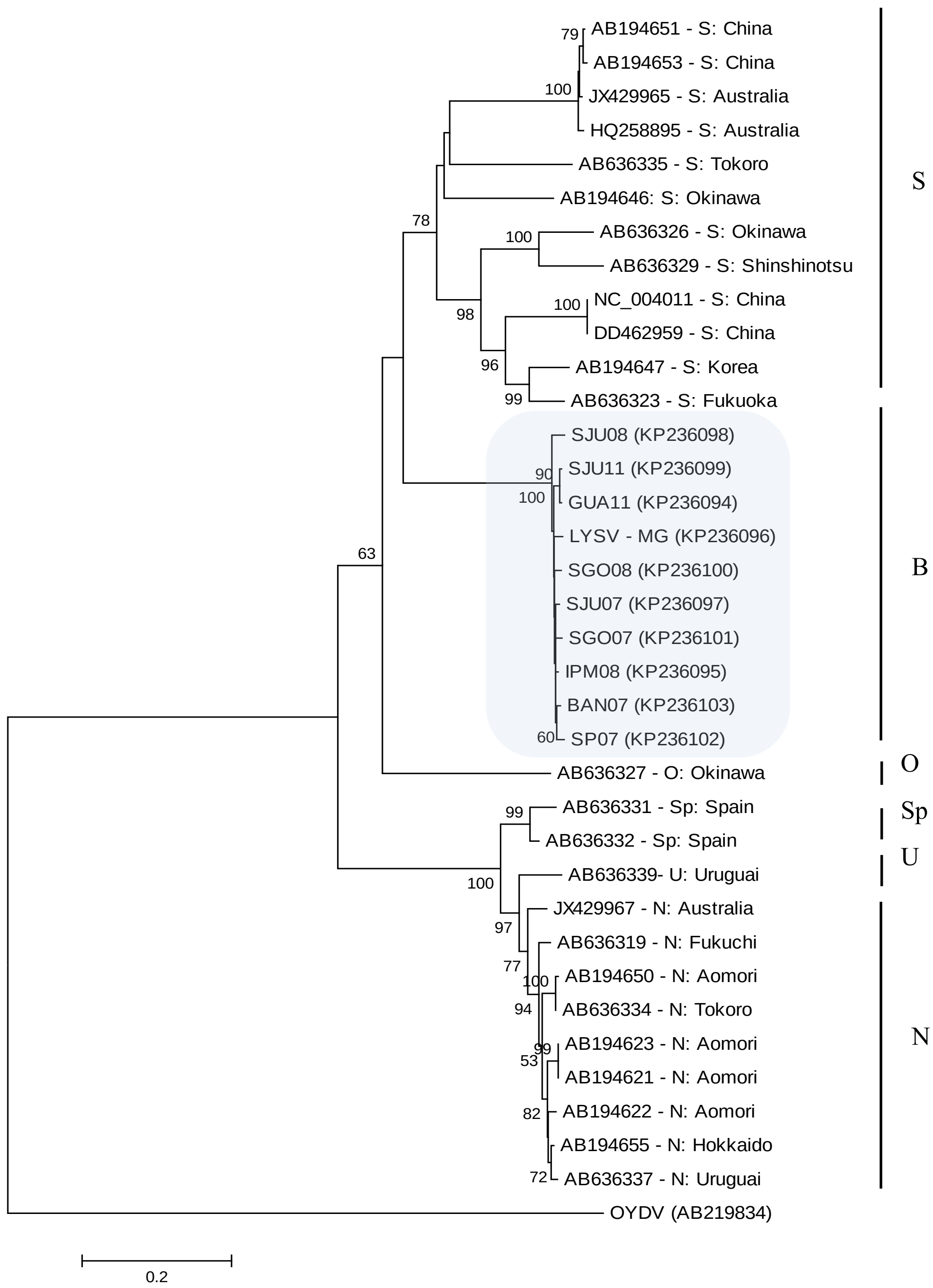

Fig. 3 Phylogenetic relationships of garlic LYSV isolates based on the partial P1 amino acid sequence. The tree was constructed the Neighbor-joining method implemented in the MEGA 6.0 software. Bootstrap values of 1000 replicates are shown. OYDV (AB219834) was used as an outgroup. The Brazilian isolates originated from Bandeirantes (BAN), Ipameri (IPM), São Gotardo (SGO), Santa Juliana (SJU), São Paulo (SP), and Guarapuava (GUA). The fully-sequenced isolate LYSV-MG originated from Santa Juliana. Clades of isolates are shown as „S" (Southern Japan), „O" (Okinawa), „Sp" (Spain), „U" (Uruguay), and „N" (Northern Japan) as identified by Yoshida et al. (2012), with the Brazilian clade „B".

CAPÍTULO II

Narcissus yellow stripe virus and different species of the genera *Allexivirus*, *Carlavirus* and *Potyvirus* are present in ornamental *Allium* species available in the United States[2]

[2] Redigido conforme as normas da revista *Plant Disease*

Narcissus yellow stripe virus and different species of the genera *Allexivirus*, *Carlavirus* and *Potyvirus* are present in ornamental *Allium* species available in the United States

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Abstract

Plants of the genus *Allium* can be infected by several viruses of the genera *Allexivirus, Carlavirus* and *Potyvirus*, often in mixed infections. Bulbs of thirteen different species of *Allium* were purchased from retail nurseries and planted in the field in the fall of 2013; one additional species was sampled from plants established in a home garden. Leaf tissue from the flowering plants were collected in spring 2014, and tested by PCR using generic primers for the genus *Allexivirus, Carlavirus* and *Potyvirus*. PCR-positive samples were re-tested using specific primers for *Allium*-infecting virus species described for each genus. After flowering and foliage dieback, the bulbs were harvested and stored at 4º C for 60 days, before being planted in pots and maintained in a cooler until leaves emerged, and then transferred to a greenhouse. The sprouted plants were tested by ELISA using antibodies specific for the *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Shallot virus X* (ShV-X), *Garlic common latent virus* (GarCLV), *Shallot latent virus* (SLV), *Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV) and *Shallot yellow stripe virus* (SYSV). RT-PCR detected the presence of GarV-B, GarV-C, *Garlic virus D* (GarV-D), and *Garlic virus E* (GarV-E) in *Allium sphaerocephalon*; GarV-B, GarV-C and ShV-X in *Allium caeruleum*; LYSV in *Allium bulgaricum* and *Allium atropurpureum*; SLV in *Allium moly* and *Narcissus yellow stripe virus* (NYSV) in *Allium carinatum*. ELISA testing with available antibodies confirmed the identity of viruses previously detected by PCR. Electron microscopy revealed the presence of flexuous particles of 650-800 nm, typical of *Allexivirus*, *Carlavirus* and *Potyvirus*. To our knowledge this is the first report of *Narcissus yellow stripe virus* in *Allium* spp.

Keywords: Allium spp., vírus detection, mechanical transmission.

Introduction

The genus *Allium* is one of the largest plant genera, including more than 750 species, and most species in the genus are bulb forming (Block, 2010). This genus includes important species used for human consumption, including onion (*Allium cepa*), chive (*Allium schoenoprasum*), garlic (*Allium sativum*), shallot (*Allium ascalonicum*), and leek (*Allium ampeloprasum,* syn. *A. porrum*) and many other species grown as ornamentals (Katis et al. 2012). The ornamental Allium species distinguish themselves by their great diversity in color, inflorescence and flowering height (Miglino et al. 2011). Most species belonging to this genus are vegetatively propagated. This feature allows the accumulation of viruses, which are perpetuated by bulbs from one growing cycle to the next (Katis et al. 2012).

The species of flexuous viruses previously reported to infect *Allium* spp. include *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Shallot yellow stripe virus* (SYSV) and *Turnip mosaic virus* (TuMV) of the genus *Potyvirus, Garlic common latent virus* (GarCLV) and *Shallot latent virus* (SLV) of the genus *Carlavirus, Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Garlic virus E* (GarV-E), *Garlic virus X* (GarV-X), *Garlic mite-borne filamentous virus* (GarMbFV) and *Shallot virus X* (ShV-X) of the genus *Allexivirus* (Katis et al. 2012). Recently, a new *Potexvirus* infecting ornamental Allium was reported in Netherlands, for which the name *Allium virus X* was proposed (Miglino et al. 2011).

In addition to the flexuous viruses listed above, *Tobacco rattle virus* (e.g. Miglino et al., 2006) and a few isometric viruses are also known to infect *Allium* spp., of which the most important worldwide are probably the tospoviruses *Iris yellow spot virus* (Katis et al. 2012), *Impatiens necrotic spot virus* (Hall et al. 1993), and *Groundnut bud necrosis virus* (Sujitha et al. 2012); some other isometric viruses are of regional importance (Katis et al. 2012; Miglino et al. 2006).

Viruses of *Allium* spp. typically induce foliar mosaic and yellow stripe symptoms and may cause both yield losses and deterioration in crop quality (Lot et al. 1998). The occurrence of frequent mixed virus infections in the same plant makes it difficult to determine the symptoms and attribute yield loss caused by each virus (Ward et al. 2009). Members of the genus *Potyvirus* are usually the most prevalent and most economically important, while carlaviruses and allexiviruses are typically latent (Katis et al. 2012). However, coinfection of carlaviruses or allexiviruses with the potyviruses can have synergistic effects, increasing the symptoms and losses (Conci et al. 2003; Lot et al. 1998).

The viruses present in vegetatively propagated species may be transported long-distances with infected bulbs. The accurate knowledge of the viruses present within a country is important to ensure that phytosanitary controls for imported and exported plants are appropriate, and to enable disease control measures to be implemented (Pearson et al. 2009). As the information on the viruses affecting ornamental *Allium* grown in the United States (but largely imported as bulbs) is scarce, in this work we analyzed fourteen ornamental *Allium* species for the presence of viruses. We detected the same virus species commonly found in crops used for human consumption (garlic, onion, chive, shallot and leek) but also identified *Narcissus yellow stripe virus* (NYSV), a *potyvirus* in *Allium carinatum* as a first report of this species in the genus *Allium*.

Material and methods

Plant Material

Bulbs of *Allium amplectens* "Graceful Beauty", *A. carinatum* ssp. *pulchellum, A. flavum, A. ostrowskianum, A. triquetrum, A. caeruleum, A. sphaerocephalon, A. moly, A. atropurpureum, A. aflatuense* „Purple Sensation", *A. schubertii, A. karataviense* „Ivory Queen", and *A. christophii* were purchased from retail nurseries and planted in the field in the fall of 2013; all of these bulbs were labeled as originating from the Netherlands. Leaves of *A. bulgaricum* were collected from an established planting in a private garden. Leaf tissue from the flowering symptomatic plants were collected in spring 2014 and tested by RT-PCR using generic and specific primers for viruses of the genera *Allexivirus*, *Carlavirus* and *Potyvirus*. After flowering and foliage dieback, the bulbs were harvested and stored at 4º C for 60 days, before being planted in pots and maintained in a cooler until leaves emerged, and then transferred to a greenhouse. The sprouted plants were tested by Enzyme-linked immunosorbent assay (ELISA) as described below. Electron microscopy assays were performed for positive samples.

Host Range Studies

Various common indicator plants (*Nicotiana benthamiana, N. glutinosa, N. tabacum*, *Chenopodium quinoa*, *C. amaranticolor, Tetragonia tetragonioides*) and other Allium species, *(Allium sativum, Allium cepa* and *Allium ampeloprasum)* grown from seed were used to test mechanical transmission. Mechanical inoculations were typically made by grinding leaf tissue in 1% K_2HPO_4 in distilled water containing a small amount of Celite, and gently rubbing the sap extract to young leaves of the test plants. Plants were observed for symptom development in the greenhouse over at least four weeks after inoculation.

Polymerase Chain Reaction (PCR) Assays

The Total RNA of all species was extracted from 100 mg of fresh tissue using the CKC protocol (Henderson and Hammond 2013). First strand cDNA was primed with either PV1 primer described by Gibbs and Mackenzie (1997) or a tagged oligo(dT) primer, NSNC-odT (Hammond et al. 2006) with MMLV Reverse transcriptase (Promega), following the manufacturer‟s instructions. Portions of the viral genomes were amplified by PCR using the Potyvirus primers CIFor/CIRev (Ha et al. 2008) and PV1 paired with WCIEN (Mota et al. 2004). For generic *Allexivirus* detection, the primers Cpallexi-senso 2/ Cpallexi-anti1 (Oliveira et al. 2013) and for generic *Carlavirus* SLV/GCLV7303/ SLV/GCLV7665 described by Mituti et al. (2015) were used. Initial cDNA synthesis and PCR amplification of the PCR-positive samples were re-amplified using specific primers for LYSV (Yoshida et al. 2012), OYDV and GarCLV (Mituti et al. 2015), SLV (Mituti et al. 2011), GarV-A, GarV-C and GarV-D (Melo Filho et al. 2004), GarV-B (GCAGAATAARCCCCCYTC / RAAGGGTTTATTCTGTTG), GarV-E (GGRTCGTCACGATTYGTTAC / YTTGAACCTCATACCYCC), GarMbFV (CCATGGACGACCCTGTTGACCCAA / CCATGGAGAACGTAATCATGGGAGG and ShV-X (GAATGCATCAGGRGAYCTC/ GCRGGRGGTTTCTTCTG) (Oliveira, unpublished). The sense primer NYSV 8373 (GC[C/A]ATGATTGAATCATGGGG) was designed based on alignment with the sequences available in GenBank in order to amplify the whole coat protein of NYSV. This primer was paired with antisense PV1. The PCR reactions were performed using Green GoTaq® Taq DNA polymerase (Promega) using either degenerate or specific upstream and downstream primers, using a total of 4 μL of Green GoTaq® Reaction Buffer 5X, 1 μL of each primer (20 μM), 1 μL of dNTPs (2.5 mM each), 0.1 μL of GoTaq® DNA Polymerase, 1 μL of cDNA and water per complete 20 μL reaction. Amplifications were performed under conditions specific to each primer pair, and products were analyzed by electrophoresis in agarose gels.

PCR product cloning and sequence analysis

At least one PCR product template of each PCR-positive species was separately cloned into a pCR2.1 TOPO vector (Invitrogen) and the cloned fragments were sequenced in both directions at Macrogen Inc. (Maryland, USA). Three clones of each fragment were sequenced in order to obtain a consensus sequence. Sequence analysis was carried out using BlastN and BlastP through NCBI, and used for confirmation of viral identification.

Enzyme-linked immunosorbent assay (ELISA) and electron microscopy

Double antibody Sandwich ELISA (DAS – ELISA) was used to detect GarCLV, OYDV, GarV-A, GarV-B, GarV-C, ShV-X and LYSV, and the Triple antibody Sandwich ELISA (TAS-ELISA) for the species SLV and SYSV (with monoclonal secondary antibody and rabbit anti-mouse alkaline phosphatase conjugate), were used following the procedure described by Clark and Adams (1977). Antisera and monoclonal antibodies were provided by Dr. Wulf Menzel from Leibniz-Institut DSMZ (Deutsche Sammlung von Mikrorganismen und Zellkulturen GmbH). For the species GarV-D, GarV-E and NYSV no specific antibody was available.

The antisera were diluted either 1:1000 or 1:500. Microtitre plates were coated with each antibody diluted in carbonate buffer (pH 9.6) with 200 μl in each well, and incubated for 2 h at 37 °C. The plates were then washed three times with phosphate buffered saline containing 0.05% (w/v) Tween 20 (PBST) and 200 μl of each plant sample (macerated in PBST + 2% [w/v] PVP (Serva-PVP 15 polyvinyl pyrrolidone) 1: 10 [w/v] dilution) was added to duplicate wells. After incubation overnight at 4 °C, the plates were washed three times with PBST and added 200 μl of antibody-alkaline phosphatase conjugate (diluted in PBST + 2% [w/v] PVP + 0.2% [w/v] egg albumin) added per well for DAS-ELISA. For TAS-ELISA, 200 μL of the diluted virus-specific monoclonal antibody was incubated for 2 h at 37 °C, followed by washing and incubation of the rabbit anti-mouse alkaline phosphatase conjugate. For both DAS-ELISA and TAS-ELISA, the plates were incubated with conjugate for 2 h at 37 °C, and washed three times with PBST. Subsequently 200 µl per well of p-nitrophenyl phosphate substrate was added at a concentration of 1 mg/mL. Absorbance at 405 nm was measured several times over 1 h at room temperature. The samples were considered positive when the average absorbance value was at least three times higher than the average absorbance value of the negative control.

Electron microscopy was performed for PCR-positive samples, and additional samples with virus-like symptoms, using either uranyl acetate (UA) or sodium phosphotungstic acid (PTA) to stain epidermal dips or leaf extracts. Samples were examined using a JEOL 100CX II electron microscope equipped with an AMT HR digital camera system.

Results

Most of the plants of *Allium* spp. tested showed mosaic or yellowing streaks. Amplification using generic primers for the genus *Allexivirus* (Cpallexi-senso 2/ Cpallexi-anti 1) yielded a product of c.240 bp. Subsequent assays with species-specific primers were utilized to identify the different species of *Allexivirus*, and virus identification confirmed by sequence analysis (data not shown), and where possible, by ELISA (Table 1). *Allium sphaerocephalon* showed mixed infection with GarV-B, GarV-C, GarV-D and GarV-E whereas *Allium caeruleum* showed mixed infection with GarV-B, GarV-C and ShV-X (Table 1). Species of the genus *Allexivirus* were the most abundant of all the three genera of the virus complex. All of the *A. sphaerocephalon* and *A. caeruleum* samples were infected by at least one species of the genus, with the most common being GarV-B and GarV-C (Table 2) generally occurring together in mixed infections, which sometimes also included GarV-D or GarV-E in plants of *A. sphaerocephalon*. Thirty percent of the *A. sphaerocephalon* tested showed mixed infection with four allexiviruses (GarV-B, GarV-C, GarV-D and GarV-E), while at triple infection of with GarV-B, GarV-C and ShV-X was detected in *A. caeruleum*. Infection with these viruses was confirmed in some plants by ELISA (Table 1) and electron microscopy (Fig 1A, B)

The generic carlavirus primers yielded a specific product of c.370 bp from *A. moly*; but for some samples (*A. ostrowkianum, A. christophii*) yielded non-specific products of similar size, but no product with SLV-specific or GarCLV-specific primer sets (data not shown). The specific primer SLV 7044/ SLV8004 (Mituti et al. 2011) yielded a product of 960 bp from three plants (30%) of *Allium moly* samples tested, which were infected with SLV as confirmed by sequencing (data not shown) , and for one plant by ELISA (Tables 1, 2). The presence of slightly flexuous carlavirus-like particles of c.650-750 nm was confirmed by electron microscopy (Fig. 2B).

All of the *Allium atropurpureum* and *Allium bulgaricum* plants tested were PCR positive using generic potyvirus primers, and were subsequently demonstrated to be infected with LYSV following PCR with CIFor/ CIRev (c.700 bp) and with LYSV-specific primers LY5P/ P1-1170 (c.420 bp), by ELISA for one plant of *A. atropurpureum* (Tables 1, 2), and by sequencing of representative PCR products (data not shown). Two of 10 *Allium flavum* plants were PCR positive using generic potyvirus primer CIFor/ CIRev and were demonstrated to be infected with LYSV by sequencing and one sample by electron microscopy (Table 1 and data not shown). LYSV induced stronger symptoms (streak mosaic and strong leaf yellowing) compared to plants infected solely with *Allexivirus* and *Carlavirus* species, (showing only mild mosaic and yellowing). No infection was detected with OYDV-specific primers in any samples tested.

One plant of *Allium carinatum* was shown to be infected with *Narcissus yellow stripe virus* (NYSV). This virus has not previously been reported in Allium species. *T. tetragonioides* showed yellowing leaves when sap inoculated with NYSV and the presence of the virus was confirmed by RT-PCR, although the isolate was not successfully transferred past the second passage in this host. Flexuous filamentous particles of 650-750 nm were observed by electron microscopy in both the original *A. carinatum* and *T. tetragonioides*. The complete sequence of the NYSV coat protein gene was determined, and showed 96% nucleotide identity with Chinese isolates (AM158908; AJ311372 and EU430294), and 98% amino acid identity with isolates from China (YP_002308453; CAC85379; YP002308564 and ACA13457).

Discussion

Viruses infecting Alliaceae are widespread throughout the world and affect a great number of *Allium* species, causing significant yield losses (Lunello et al. 2005). However the information on viruses affecting ornamental *Allium* species is scarce. Potyviruses are the most prevalent virus type infecting *Allium* species (Conci et al. 2003; Lot et al. 1998) *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) have been detected affecting edible *Allium* species worldwide (Conci et al. 1992; Chen et al. 2001; Van Dijk 1993). These are the most important viruses in terms of the damage and economic losses, with garlic bulb weight reduced between 24 and 60% for OYDV and between 17 and 54% for LYSV (Canavelli et al. 1998; Lot et al. 1998); however, although LYSV and OYDV infect garlic as well as leek and onion, respectively, the isolates from garlic are reported to be serologically distinct from those from leek and onion (Barg et al. 1997), and garlic strains LYSV-G and OYDV-G can not infect leek and onion, while LYSV from leek and OYDV from onion only infect garlic with great difficulty (Van Dijk 1993; Lot et al. 1998). SYSV and *Turnip mosaic virus* (TuMV) have also been reported in *Allium* spp. with restricted distribution (Van Dijk 1993) including from *A. ampeloprasum* and *A. roseum* in the former Yugoslavia (Stefanac and Plese 1980) and from *A. ampeloprasum* in Israel (Gera et al. 1997). TuMV has occasionally been reported to infect ornamental Allium species and was detected with a low incidence in *A. sphaerocephalon, A. guttatum, A. subhirsutum,* and *A. neapolitanum* in Greece (Katis et al. 2012). Carlaviruses and allexiviruses are usually latent in *Allium* spp., however, co-infection of these viruses with potyviruses can have synergistic effects resulting in stronger symptoms (Conci et al. 2003; Lot et al. 1998). There is no previous information about allexiviruses and carlaviruses infecting ornamental Allium in the United States, and very limited information about their occurrence in ornamental species elsewhere in the world. However garlic plants infected with allexiviruses have shown severe symptoms and yield losses, mainly with multiple infections (Takaichi et al. 1998). GarV-A, GarV-B, Garv-C and GarV-D have been reported in onion, garlic and shallot in New Zealand but just GarV-A was reported infecting the ornamental species *Allium senescens* (Ward et al. 2009). GarV-C was seen to be the prevalent species in all garlic production systems in Brazil, while GarV-D was limited to the Cerrado region (Fayad-Andre et al. 2011). On the other hand, Mituti et al. (2015) found GarV-A and GarV-D to be the most frequently detected allexiviruses across all Brazilian garlic production areas. GarV-A and GarV-C have been found to be the most prevalent allexiviruses infecting garlic from Argentina, with garlic bulb yields reduced by 14% to 32% in plants infected with GarV-A (Cafrune et al., 2006). Although GarV-A and GarV-D have been reported to be the most prevalent allexiviruses in most garlic production areas, our survey of ornamental *Allium* spp. showed that GarV-B and GarV-C were the most prevalent. This presumably reflects the separate production areas for ornamental *Allium* spp. and garlic. The transmission of allexiviruses can easily occur because the mite vectors are a pest commonly found in bulbs, facilitating the transmission of viruses (Cafrune et al. 2006). Thus there is a risk of changing the distribution of allexiviruses in both kinds of crop if garlic and ornamental *Allium* spp. are grown or stored together. Miteborne spread of allexiviruses in garlic has been reported to occur primarily during bulb storage, and to be rare in the field (Lange and Mann 1960); allexivirus transmission in storage has also been noted by Barg et al. (1997).

Carlavirus infection in *Allium* species appears to be restricted to only a few regions around the world (Dovas et al. 2001). GarCLV was the most frequently detected carlavirus infecting *Allium* spp. in New Zealand (Ward et al. 2009). These authors found different commercial crops infected with GarCLV, including onion, garlic, shallot and the ornamental species *A. murrayanum, A. senescens* and *A. moly,* while SLV was detected in onion, garlic, shallot and *Allium senescens*.

NYSV has not previously been reported in *Allium* spp. The potyvirus NYSV is the most common virus in Narcissus sp. and induces stunting, mosaic, leaf distortion and chlorotic streaks early in the season, with some flower breaking which may reduce floral quality (Chen et al. 2003; Wylie and Jones 2012; Wylie et al. 2014). Our results differ from those obtained by Wylie et al. (2014) who observed chlorotic spots on *C. amaranticolor* inoculated leaves 4-6 days after inoculation with infected *Narcissus* sp. When they inoculated individual chlorotic lesions to further *C. amaranticolor* seedlings, no symptoms of infection became apparent; however, symptoms of systemic infection were observed on *N. benthamiana* plants, appearing on emerging leaves 10-15 days after inoculation as mild chlorosis and mosaic. At 30-40 days post-inoculation, the leaves of inoculated plants were pale green to yellow in color, while control plants remained green. In our experiments we were unable to establish infection in either *C. amaranticolor* or *N. benthamiana*, and infections of *T. tetragonioides* could not be maintained in this host, as reported by Wylie et al. (2014) for *C. amaranticolor*. Our results also differ from those obtained by Mowat et al. (1989), who reported difficulty in detecting potyvirus particles following aphid transmission of yellow stripe disease to virus-tested narcissus. Mowat et al. (1989) also observed lamellar fragments of cytoplasmic inclusion by electron microscopy of leaf extracts of infected *Narcissus* sp. and inoculated *N. benthamiana* and *N. clevelandii*. In contrast we were able to observe particles typical of potyviruses either directly from *A. carinatum*, or in plants of *T. tetragonioides* inoculated from *A. carinatum*.

A. sphaerocephalon, A. caeruleum and *A. moly* are reported here for the first time as hosts of allexiviruses and SLV species in United States and *A. carinatum* for the first time as a host of NYSV anywhere in the world. We can conclude that various species of *Allium* commonly available in the nursery trade are frequently virus-infected, and that mixed infections are common. However the information about viruses in these crops is scarce.

Notably, in our experiments we failed to transmit any of the viruses from ornamental *Allium* spp. to garlic, chives, onion or leek, which may indicate that the isolates are distinct from those previously identified from those edible crops, and that they show a high degree of host specificity, as previously reported for isolates of LYSV and OYDV (Van Dijk 1993; Lot et al. 1998). The differences in species and prevalence of potyviruses, allexiviruses and carlaviruses between the various species of ornamental *Allium* examined here may reflect either differences in susceptibility between the species examined, or differences in exposure due to bulb provenance of different locations.

Further studies with ornamental *Allium* spp. available in the nursery trade and cultivated in the field are necessary to enable appropriate implementation of disease control measures. Experiments to determine whether the isolates from ornamental *Allium* spp. are transmissible to edible species such as garlic, leek, and onion by the natural aphid and mite vectors would be valuable in this regard.

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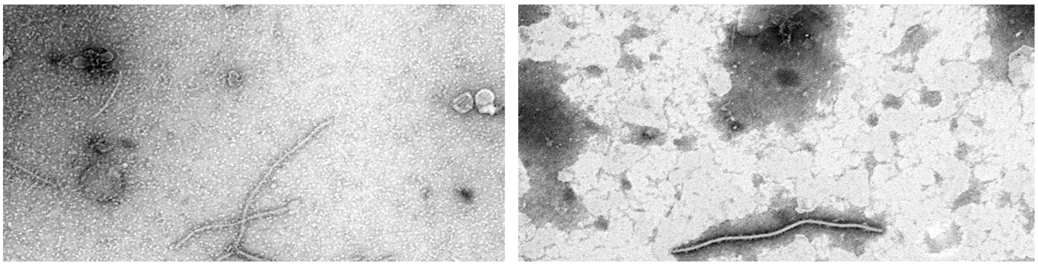
Table 1: *Allexivirus*, *Carlavirus* and *Potyvirus* detected by RT-PCR and ELISA in ornamental Allium.

	Infected plants						
	A. sphaerocephalon	*A. caeruleum*	*A. moly*	*A. bulgaricum*	*A. flavum*	*A. atropurpureum*	*A. carinatum*
Tested plants	20	6	10	3	10	4	10
GarV-B*	19	4	-	-	-	-	-
GarV-B**	19	3	-	-	-	-	-
GarV-C*	20	1	-	-	-	-	-
GarV-C**	7	0	-	-	-	-	-
GarV-D*	12	-	-	-	-	-	-
GarV-E*	7	-	-	-	-	-	-
ShV-X*	-	2	-	-	-	-	-
ShV-X**	-	1	-	-	-	-	-
SLV*	-	-	3	-	-	-	-
SLV**	-	-	1	-	-	-	-
LYSV*	-	-	-	3	2	4	-
LYSV**	-	-	-	0	0	1	-
NYSV	-	-	-	-	-	-	1

* Samples tested by PCR; ** Samples tested by ELISA.

Table 2: Percentage of each virus detected in ornamental Allium by RT-PCR

	% of detected vírus by RT-PCR						
	A. sphaerocephalon	*A. caeruleum*	*A. moly*	*A. bulgaricum*	*A. flavum*	*A. atropurpureum*	*A. carinatum*
GarV-B	95	67	-	-	-	-	-
GarV-C	100	17	-	-	-	-	-
GarV-D	60	-	-	-	-	-	-
GarV-E	35	-	-	-	-	-	-
ShV-X	-	33	-	-	-	-	-
SLV	-	-	30	-	-	-	-
LYSV	-	-	-	100	20	100	-
NYSV	-	-	-	-	-	-	10



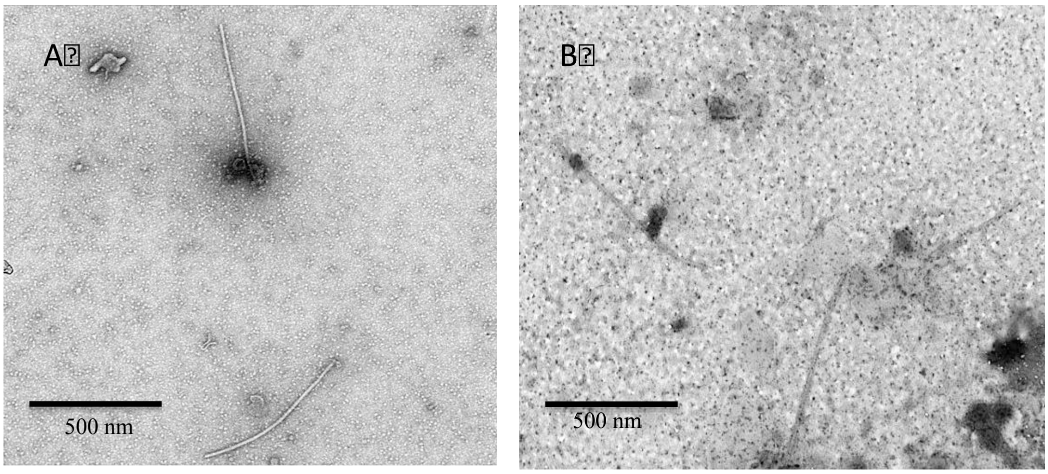


Figure 2: Particles of *Narcissus yellow stripe virus* (NYSV) in *Allium carinatum* (A) and of *Shallot latent virus* (SLV) in *Allium moly* (B). Particles stained with uranyl acetate. Bar represents 500 nm.

CONCLUSÕES GERAIS

- Análise da região codificadora para a proteína P1 de isolados brasileiros de LYSV, indicam que estes compartilham possivelmente um ancestral comum com o isolado de Okinawa e isolados de LYSV do grupo S;
- As espécies de Allium disponíveis em floriculturas na região de Beltsville, MD, nos Estados Unidos, estão frequentemente infectadas com vírus pertencentes aos gêneros *Allexivirus*, *Carlavirus* e *Potyvirus*;
- *Narcissus yellow stripe virus* foi relatado pela primeira vez em *Allium carinatum*.

APÊNDICE

Apêndice 1: Paper publicado em colaboração com Dr. John Hammond durante o doutorado sanduíche no United States Department of Agriculture. USDA. Beltsville ,MD.

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Disease Notes

First Report of *Plantago asiatica mosaic virus* in Imported Asiatic and Oriental Lilies (*Lilium* hybrids) in the United States

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Asiatic and Oriental hybrid lilies (*Lilium* sp., *Liliaceae*) are bulbous ornamentals valued for their flowers. Bulbs of several varieties of each lily type, imported from the Netherlands, were purchased in spring 2013 from retail nurseries and grown in a cool greenhouse; additional bulbs were obtained in 2014. After flowering in 2013, but prior to leaf senescence, necrotic streaking was observed in midstem leaves of several plants. RNA extracted from leaves of several individual plants was subjected to reverse-transcription–polymerase chain reaction (RT-PCR) assay using NSNC-odT primed cDNA and PCR with primers PxDeg/BNSNC or potyS/BNSNC to amplify potexvirus/carlavirus and potyvirus products respectively (2,3,4). Sequencing of a c. 1.7-kb PCR product from one lily identified *Lily symptomless virus* (LSV). Mechanical inoculation of pooled lily leaf samples to *Nicotiana benthamiana, N. glutinosa*, and *Chenopodium quinoa* (not hosts of LSV) yielded chlorotic or necrotic local lesions on *C. quinoa* and systemic mosaic with necrotic spotting, streaking, or apical necrosis on *N. benthamiana*; electron microscopy revealed potexvirus-like flexuous particles. RT-PCR from *C. quinoa* and *N. benthamiana* with PxDeg/BNSNC yielded a c. 1.3-kb product, which was cloned and sequenced; the consensus sequence (KM205357) had 98.7% nucleotide identity to a Dutch isolate of *Plantago asiatica mosaic virus* (PlAMV, KF471012; 78.5 to 87.8% to other isolates), and 99.0% coat protein amino acid identity to KF471012 (88.9 to 93.2% to other isolates). The 2013 lilies were stored overwinter at 4°C, and RNA was extracted from roots of individual bulbs. Primers PlAMV CP-F2 (TTCGTCACCCTCAGCGG) and PlAMV CP-R3 (AAACGGTAAAATACACACCGGG) were designed based on alignment of KM205357 with all PlAMV sequences available in GenBank. RT-PCR using PlAMV CP-F2/CP-R3 yielded products of the expected 511 bp from 20 bulbs and no product from a no-template control. ELISA of root and bulbscale samples using PlAMV-lily specific antibody and conjugate (a gift of R. Miglino, BKD, The Netherlands) confirmed PlAMV in seven of 20 bulbs positive by RT-PCR. Bioassay of PCR-positive lilies on *N. benthamiana, C. quinoa*, and *Tetragonia expansa* confirmed infection in three out of eight by both symptoms and ELISA. Altogether nine out of 13 Asiatic lilies (four of four cultivars: America, Connecticut King, Grand Cru, and Pink Pixie) and 11 Oriental lilies (cvs. Stargazer and Starfighter) were found to be infected with PlAMV by RT-PCR, of which seven were confirmed by bioassay and/or ELISA. Bulbs obtained in 2014 were tested only by ELISA; five of 18 Asiatic lilies (three of six cultivars: Connecticut King, Crimson Pixie, and Yellow Electric) and three of 13 Oriental lilies (three of six cultivars: Anastasia, Casa Blanca, and Garden Party) were found to be infected. PlAMV was reported in lilies in the Netherlands in 2010, with losses of up to 80% in greenhouse cut-flower production (1). The Nandina mosaic isolate (PlAMV-NMV) has been known in the United States since 1976 (5), but PlAMV infection of lily has not previously been documented in the United States. Both RT-PCR and ELISA tests also detected PlAMV-NMV. The degree of damage observed in the Netherlands suggests that growers should seek bulb stocks free of PlAMV.

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Apêndice 2: Alinhamento múltiplo utilizando o programa BiologyWorkBench (Clustal W) para a região codificadora para a proteína P1 de isolados brasileiros de LYSV e isolados da Australia (JX429965), Aomori (AB194650), China (NC_004011) e Okinawa (AB636327). Isolados da Australia e China apresentam a deleção típica de nucleotídeos na P1.

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Aomori         TATTCAAATCTGTCAATTACGTTGAAATTGGACACGGTTACGGA---------------
SP - 07        TACTATCATCCATTCAATACGTTGAATACGGACACAATAGAGCAACAACACTGAATGAC
SGO- 07        TACTATCATCCATTCAATACGTTGAATACGGACACAATAGAGCAACAACACTGAATGAC
LYSV-MG        TACTATCATCCATTCAATACGTTGAATACGGACACAATAGAGCAACAACACTGAATGAC
Okinawa        TATTCAGGTTTACTAAAGATGAAAAGTATGGACAAGCCAAGGCACACACATC------T
Australia      TACTAGCATCCGTTAATTACGTCGAATATGGACAAGTCAGGGCAGCAGCAGTGGGGCGT
China          CAACATTTTTCGTTAGAAACGTCAACTATGGGAATGCCAGAGCATCGGCATTGAGCGAG
                *      *         * *   *    **  *       * *
Aomori         GAGGACATGGAAGTGCGGCGTCGTCGCATGCTGAGCAATGTACTTGAACGCCTACAACAT
SP - 07        AAGGATGTAATTGCGCGGCGGCGTGAAATACTAAGCGGTATACTCAACCGCACGCAAACT
SGO- 07        AAGGATGTAATTGCGCGGCGGCGTGAAATACTAAGCGGTATACTCAACCGCACGCAAACT
LYSV-MG        AAGGATGTAATCGCGCGGCGGCGTGAAATACTAAGCGGTATACTCAACCGCACGCAAACT
Okinawa        GAGGACATGGTTGCGAAGCAGCGGAAAATGCTTAGTGGTATACTAGAGCGCTTACAAGCT
Australia      GAAGAAATAATTGCGCAGAGGCGCGCAATACTGAGTAACATATGCAACCGGGTTCAAACC
China          GAAGATAAGATCGCGCAGCGGCGTGAAATACTTGGCAACATACTTGATCGGTTGCAGACT
                * **       * *  *   **    ** **  *     **    * **    **
Aomori         AACACCCAAAAATCCTCATACGAGTTTGGGGCCTCTGGATATGGGAAGGTCCAGAACAGC
SP - 07        AATGGTCAGACTCCTGCATATGTCTTTGGAGCTACAGGCTACGGTAAGGTCCAGAATAGC
SGO- 07        AATGGTCAGACTCCTGCATATGTCTTTGGAGCTACAGGCTACGGTAAGGTCCAGAATAGC
LYSV-MG        AATGGTCAAACTCCTGCATATGTCTTTGGAGCTACAGGCTACGGGAAGGTCCAGAATAGC
Okinawa        AGTGATCAAACCTCTTCATATATCTTTGGAGCCACTGGTTATGGGAAAGTTCAGAATAGT
Australia      AACAATCAAACTCCATCATACGTTTTTGGAGCAACTGGATATGGCAAGGTTCAGAATAGT
China          AATAATCAGGCTCCATCATATGTTTTTGGGGCAACTGGTTACGGGAAAGTCCAAAATAGC
               *     **     *  ****    ***** **  * ** ** ** ** ** ** ** **

Aomori         AAAGCTCTATGTGCGCAACGAATGGGGTGTCAATATGATCGCGATGACGACTGCTACGTG
SP - 07        AAGTCTTTGTGTGCAAGGCGCATGGGAGCTCGCTATAATCGTGATGATGACGTCTATGAA
SGO- 07        AAGTCTTTATGTGCAAGGCGCATGGGAGCTCGCTATAATCGTGATGATGACGTCTATGAA
LYSV-MG        AAGTCTTTGTGTGCAAGGCGCATGGGAGCTCGCTATAATCGTGATGATGACGTCTATGAA
Okinawa        AAATCTTTGTGCGCGAGGCGTATGGGAGCTCGTTACAATCGAGAAGATGATGTGTATGAA
Australia      AAAGCCTTGTGTGCACAACGTATGGGGGCTAGATACGATTGGGAAGATGATGTTTACGAA
China          AAAGCGTTATGTGCAAAGCGCATGGGAGCCCGGTATAACTGGGACGATGATGTATATGAA
               **  *  * ** **    ** *****       **  *  * ** ** **    ** *
Aomori         TGCAGCACGTGCAAGGCTGGATTTGGCACGAAGGAGGATTATAAGGACCATGAGTGTGAG
SP_- 07        TGTACCACTTGCAATGGTGCATTCCAAACTAAGGAGACGTTCAAGGAACACGACTGCGAC
SGO _07        TGTACCACTTGCAATGGTGCATTCCAAACTAAAGAGACATTCAAGGAACACGACTGCGAC
LYSV-MG        TGTACCACTTGCAATGGTGCATTCCAAACTAAAGAGACATTCAAGGAACACGACTGCGAC
Okinawa        TGCATTACTTGCAATGGTGCATTCGGAACGAAAGAGGACTATAAGGAACATGACTGCGAT
Australia      TGCACTACTTGCAATGGTGCTTTTCAAACTAAGATAGACTTCAAGGAGCATGACTGTGAT
China          TGCACTACCTGTAGTGGTGCATTTCAGACAAAACTGGACTTTAAGGAACATGACTGCGAT
               ** *  ** ** *  * **  **    ** **       *  ***** ** ** ** **

Aomori         GAAGAGATGGAGGATGTGAATCTGTATCCAATAAATTTCTCTCTTCAGGGCGAGGAGTTC
SP - 07        GAGGAGAATGAGGACATTGACATGCCCCTTCCAAACTTCACTCTCAACGTTGAGGAATTC
SGO- 07        GAGGAGAATGAGGATATTGACATGCCCCTTCCAAACTTCACTCTCAGCGTTGAGGAATTT
LYSV-MG        GAGGAGAATGAGGATATTGACATGCCTCTTCCAAACTTCACTCTCAACGTTGAGGAATTT
Okinawa        GAGGAGAATGAGGATATTGAGATGGTCTCTGAAGTGTTCCATCCTGAAGCTAAAGCATGT
Australia      GAAGAAAATGAAGACTTTGAAGTGATCCCTGAAGATTTCTCTCTTAAACTTGAGGAATTC
China          GAGGAGAATGAAGACATTGACATGACTCCTGAAAATTTCTCTCTCAAAGCAGAGGAGTTT
               ** ** *  ** **  *  *  **        *   ***  **         * *  *

Aomori         CCTACTCTGCAAGAAAGTGCCACATGTGAGGCCAGAGCTGAGAGTGACACTATTTGTTTT
SP - 07        CCAACCCTGCTAAACAACCCCGTGCAAGCACCCAAAAGTGGGGTTGACGGCATCCACTTT
SGO- 07        CCAACCCTGCTAAACAACCCCGTACAAGCACCCAAAAGTGGGGTTGACGGCATCCACTTT
LYSV-MG        CCAACCCTGCTAAACAACCCCGTGCAAGCACCCAAAAGTGGGGTTGACGGCATCCACTTT
Okinawa        CACACTTTATTGGATGATACCGTGCATGGTCAAGAGAATGAAAGTCAAAGCATTCATTTT
Australia      CCCACGATCCAAGAGGAACTTAC-------------------------------------
China          CCCAATCTGCAAGCAGACATCAT-------------------------------------
               *  *   *
```

```
Aomori          GGTGATTTTGGGACACCCACACTACTTGAAGGTGATGTAGGATCAGAAACACCAAACAAC
SP 07           GGGAGCTTCAACACACCTGCCTCACCTGGAGGCAACCTTCAACCCGATTTGGCGGACACT
SGO- 07         GGTAGCTTCAACACACCTGCCTCACCTGGAGGCATCCTTCAACCCGATGTGGCGGACACT
LYSV-MG         GGGAGCTTCAACACACCTGCCTCACCTGGAGGCAACCTTCAACCCGATGTGGCGGACACT
Okinawa         GGTAGTTTCAATGAATCTGCGCTAGCTGGAGGCAATATTGAATCTAACACGTTGGATAAA
Australia       ------------------------------------------------------------
China           ------------------------------------------------------------


Aomori          TCTGTACATAAATCTCATAGAGATGAGGGTGTTATTTACTTTGGAAGCTTCGAAACACCC
SP - 07         CCTACGCTCGAATCCGAGAGCAAAGACAATGTCATCTACTTTGGGAGTTTCGAAACACCG
SGO- 07         CCTACGCTCGAATCCGAGAGCAAAGACAATGTCATCTACTTTGGGAGTTTCGAAACACCG
LYSV-MG         CCTACGCTCGAATCCGAGAGCAAAGACAATGTCATCTACTTTGGGAGTTTCGAAACACCG
Okinawa         CCCCTGAGTACATTGAATGACAAGGACAATGTCATCTACTTCGGAAGCTTTGACACGCCC
Australia       ------------------------------------------------------------
China           ------------------------------------------------------------


Aomori          GTTTCACTTGGTGGTATCTTGAAACCAAATGTGCAAACTAACTCTGTGTGCGAGCCTAAG
SP - 07         GTCTCAGTTAGGAGCGACTCCAAACTAAATATATCAGACAGTTCCGCACATGAGTCTAGA
SGO- 07         GTCTCAGTTAGGAGCGACTCCAAACTAAATATATTATACAGTTCCGCACATGAGCCTGGA
LYSV-MG         GTCTCAGTTAGGAGCGACTCCAAGCTAAATATATTAGACAGTTCCGCACATGAATCTAGA
Okinawa         ATATCACTTGGAGACACTCAGAGGAAAAATGCACTGGAGGACTCCACACATGACTCTAAG
Australia       -----------------------------------------------AACTGAATCTAAG
China           -----------------------------------------------TAGTGAGCCGAAG
                                                               **  *

Aomori          AGTGGGGACAACACGATACGTTTTGGAAGTTTTGAAACACCCATACCTCTTACAGTCGTC
SP - 07         ACTGAACAGGATGTTATATACTTTGGGAGCTTCGATACACCCGCACCACTTGTAAGCGAC
SGO- 07         ACTGAACAGGATGTTATATACTTTGGGAGCTTCGAGACACCCGCACCACTTGTAAGCGAC
LYSV-MG         ATTGAACAGGATGTTATATACTTTGGGAGCTTCGAGACACCCGCACCACTTGTAAGCGAC
Okinawa         GGTGAAGATGCAGTTATTTACTTCGGAAGTTTTGAGACACCCATAACTCTCGGAAATGGT
Australia       AGTGAAGACGCAGTAGTTTACTTTGGGAACTTTGAAACACCCGTACAACTTAAAAGTGTT
China           AATGAAGATGCCGTTGTCTTCTTTGGAAGCTTTGAAACACCTGTGCCACTTGTGAGTGCA
                  **   *        *    ** ** *  ** ** *****       **       *

Aomori          CCAAAGCTAATTGAAATATCTAGCGTAGACGTACACCAGCAGGAGAGTGAGAAGAACTTA
SP - 07         CCTGAACTCATCATAACATCTAATATGAACATGTGCCAACTAGAGCGCGAAAGTGCCGCA
SGO- 07         CCTGAACTCATCATAACATCTAATATGAACATGTGCCAACTAGAGCACGAAAGTACCGCA
LYSV-MG         CCTGAACTCATCATAACATCTAATGTAAACATGTGCCAACTAGAGCGCGAAAGTACCGCA
Okinawa         CCTGCTTTTAACAAGGCACCTGCAATGGACACGCATAAACTAGGACATGAGCAGAAAGTA
Australia       CCTACGTGCGGTGTCGTGTTTAGTGTAGACGCGCGCCAACAGGAACAGTTAGGCAATGTG
China           ACCAAACTTAACGAAGCACCTATTATACATGAGTGTCAACAGGAGCTTATTAACGTGTCA
                 *                  *    *  *        * *  *

Aomori          GATAGTAAAATAGAGTCAAACATCATTTGCACACCC---GATGGTGCAGGTGAACAAAAC
SP - 07         AATCCAGAATTAGATCAAGTTCTAATTCATACACCCATAACTAACGCCGAAACCCAAACC
SGO- 07         AATCCAGAATTAGATCAAGTTCTAATTCATACACCCATTACTAACGCCGAAACCCAAACC
LYSV-MG         AATCCAGAATTAGATCAAGTTCTAATTCATACACCCATTACTAACGCCGAAACCCAAACC
Okinawa         GGTGAACAAGTAGAACAAGCTATAGTTAATGTAATTAGCAGTGAGACAAAAGAACAAGTC
Australia       GATGGAAAGTTGGAACAAAATGCAGTATGCACACTTGACAATAACAACGAAACCCCTATT
China           AACGATAAACTAGAGCAAGGCGAAGTTAGCGTATCAAAAAGCATAGAGGAAACCCATGTC
                       *  * **   *       *      *                     *

Aomori          ACGAAGCACATTAAACCAGATCCGTCTTTGGTCAGGCTACAGAGCAGGCAACGTACT
SP - 07         ACACAGCATACTACACCAGATCCGTCTTTCGTCAAGCCACAGAGCAGGCAACGTACT
SGO- 07         ACACAGCATACTACACCAGATCCGTCTTTCGTCAAGCCACAGAGCAGGCAACGTACT
LYSV-MG         ACACAGCATACTACACCAGATCCGTCTTTCGTCAAGCCACAGAGCAGGCAACGTACT
Okinawa         ACAAAGCACGCCACACCAGATCCGTCTTTGGTCAGGCTACAGAGCAGGCAACGTACT
Australia       ACGAAACACACAATACCAGATCCGTCTTTGGTCAGTCTACAGAGCAGGCAACGTACT
China           GCGGAACACAAGAAACCAGATCCGTCTTTGGTCAGCCTACAGAGCAGGCAACGTACT
                 *  * **    * *************** ****  * *******************
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Apêndice 3: Sequência completa do isolado LYSV – MG traduzida com o códon de iniciação e finalização para a poliproteína e os sítios de clivagem destacados em negrito e indicados pelo símbolo / .

1 taatctcaaaacccacttatgcaaaaccaaaacaactcaattacaatcaagcaaacaaca
M A K I L S S I Q Y V E Y
61 aacaaaagcaccaaccataac**atg**gctaaaatactatcatccattcaatacgttgaatac
G H N R A T T L N D K D V I A R R R E I
122 ggacacaatagagcaacaacactgaatgacaaggatgtaattgcgcggcggcgtgaaata
L S G I L N R T Q T N G Q T P A Y V F G
182 ctaagcggtatactcaaccgcacgcaaactaatggtcagactcctgcatatgtctttgga
A T G Y G K V Q N S K S L C A R R M G A
242 gctacaggctacggtaaggtccagaatagcaagtctttgtgtgcaaggcgcatgggagct
R Y N R D D D V Y E C T T C N G A F Q T
302 cgctataatcgtgatgatgacgtctatgaatgtaccacttgcaatggtgcattccaaact
K E T F K E H D C D E E N E D I D M P L
362 aaagaaacattcaaggaacacgactgcgacgaggagaatgaggatattgacatgcccctt
P N F T L N V E E F P T L L N N P V Q A
422 ccaaacttcactctcaacgttgaggaatttccaaccctgctaaacaaccccgtgcaagca
P K S G V D G I H F G S F N T P A S P G
482 cccaaaagtggggttgacggcatccactttgggagcttcaacacacctgcctcacctgga
G N L Q P D V V D T P T L E S E S K D N
542 ggcaaccttcaacccgatgtggtggacactcctacgctcgaatccgagagcaaagacaat
V I Y F G S F E T P V S V R S D S K L N
602 gtcatctactttgggagtttcgaaacaccggtctcagttaggagcgactccaaactaaat
I L D S S A H E S R T E Q D V I Y F G S
662 atattagacagttccgcacatgagtctagaactgaacaggatgttatatactttgggagc
F E T P A P L V S D P E L I I T S N M N
722 ttcgagacacccgcaccacttgtaagcgaccctgaactcatcataacatctaatatgaac
M C Q L E R E S T T N P E L D Q V L I H
782 atgtgccaactagagcgcgagagtaccacaaatccagaattagatcaagttctaattcat
T P I T N A E T Q T T Q H T T P D P S F
842 acacccattactaacgccgaaacccaaaccacacagcatactacaccagatccgtctttc
V K P Q S R Q R T T H V T S G V S G Q H
902 gtcaagccacagagcaggcaacgtactacgcatgtgactagtggagtgagtggacagcac
F S E L I R N L A Q I V H D R Q L D V H
962 ttttctgagctgatccgcaacctcgcacaaatagtgcatgatagacagctcgatgttcat
F V G K R R L D G R C V R S D G K S Y L
1022 tttgtaggcaagaggagattggacggtcgttgcgtgcgaagtgatggaaaatcatatcta
Q L Q T K H A L G L K N R V D C H I P K
1082 cagctacagactaaacacgctcttggtttaaagaatagggtggattgtcacattcctaag
G L E H I V H E L A K G S G Y K S R T R
1142 ggcttagaacacattgtacatgaactcgcaaaaggtagtggatacaagtcacgtacacgc
V R D I K K G W S G F V L Q P R N I I G
1202 gtgcgtgacattaagaagggatggagtgggtttgttttgcaaccaagaaatatcatcggg
L S G K S K H D F F V V R G S L K G K L
1262 ttatctgggaagagcaaacatgatttctttgttgtgcgtggctcattaaaaggaaagcta
I D A C T C I N P N A S G **L E H Y/ S** A G
1322 attgacgcatgtacgtgtatcaatccgaatgcttcgggacttgaacattatagcgctggg
Q I I W K N F E E E F I K Q R P D I K H
1382 cagatcatttggaaaaacttcgaggaagaatttataaagcagcggccagatataaagcac
K C M S D I E A S K C G I L A A T L I Q
1442 aaatgtatgtctgatattgaggcttcaaagtgtggcatactggcagcaacgttaatccag
L F H P C G K I T C D E C I A N T T S K
1502 ttatttcatccatgcggcaagataacatgcgacgagtgcattgcgaacacaactagtaaa
S P M E Y T T D L L T R L P S A Q K R I
1562 tcacccatggaatacaccacagatttactcactcggttaccatcagcacagaagagaata

E E L D A F P Q V L D L L S L I E R K F
1622 gaagaacttgatgcattcccacaagtcttagacctattatcacttatagagagaaaattt
E E P T Y N S E I Y A Q I L H S I G H I
1682 gaagaaccaacatataatagtgaaatatacgcacagatactacatagtattgggcatatc
K T Q P F D K L N N M N A L L A N H Y G
1742 aaaacacagccatttgacaagttgaacaacatgaatgcgcttcttgcaaatcactatggg
S L P N P I I Q K L Q E D L L E V V R Y
1802 tcacttcctaaccctatcatccagaaactgcaagaggatctacttgaagtcgttcgatac
T K K R T D S I N R G D L Q H Y R N K I
1862 acaaagaaacgaacagattcgattaatcgtggcgatttgcaacattacaggaacaagata
A S K A H F N L D L M C D N Q L D K N G
1922 gcttcaaaagcgcacttcaacttagatttaatgtgcgataatcagctagacaagaatggt
N F M W G E R G Y H A K R F F S N Y F D
1982 aacttcatgtggggagagagaggttatcatgctaagcggttcttctcgaattactttgat
L I D P T A N Y A S H A I R H N P N G S
2042 ttgatagatccaacagcgaactatgctagtcacgcaataaggcataatccaaacggttcg
R K L A T G R L I V S T N F E T Y R E N
2102 agaaagttggcgaccggacgactgatagtgtcaacaaactttgaaacatatagggagaat
L K G D K V H A H Q I T E E C V S R D G
2162 ttaaagggcgataaagtgcacgcacaccagatcacagaagaatgtgtgagccgtgatggg
K N F V Y S C S C V T H E D G S A M E S
2222 aagaattttgtttacagttgttcatgtgttacacatgaggatgggagtgctatggaatct
R V I L P T K N H L V I G N S G D P K Y
2282 cgtgttatactaccaacgaaaaatcatctcgtcattggtaattctggtgatccgaaatac
I D L P T E S E L T L Y I A K E G Y C Y
2342 atcgacttaccgactgaatctgagttgacattgtacatagctaaagagggatattgttac
I N I F L A M L V N V R E S Q A K T F T
2402 atcaacatttcttggcaatgttagttaatgtacgtgagtcgcaggcgaagacatttaca
K M V R D I L V D K L G K W P T M M D V
2462 aaaatggttcgcgatatattggtggataaacttgggaaatggccaactatgatggatgtc
A T A C S L L T V F Y P D T I T A E L P
2522 gcaacagcgtgcagtttgttaaccgttttctatccggatacgataacagcggaattgcct
R I L V D H N C K S M H V I D S Y G S L
2582 cgaatcttagtggatcacaactgcaagagtatgcatgtcattgattcatacggttcgcta
D T G F H I L K A N T V N Q I L H F A S
2642 gacacagggtttcacatattgaaggcaaatacagtcaatcagatattacattttgcgagc
T D L K S D L K H **Y R V G/ G** E T N D I L
2702 acggatctgaaatctgatttaaagcactatcgcgttggaggcgaaacgaatgacattcta
Y V D V A P K L D M T T S T L Y M L I T
2762 tacgttgatgtagccccaaaacttgatatgacaactagcacactctacatgctaattacg
S I Y K P K R F R Q I V E H E P Y T L V
2822 agtatctataaaccaaagcgattccgccaaatagtggagcatgaaccgtacaccctagtg
L S V L S P A I M L A M L N N E S F E K
2882 ttatcagtgttatcaccagctataatgctcgccatgctgaacaatgaatcattcgaaaag
S I S Y W I H R D R D I T Q I M A L L K
2942 agcatttcatattggattcatcgagatagggacattacgcaaattatggcattactgaaa
T L A Q R I P I S N S L Q D Q M Q F L E
3002 accttagcacaaagaattccaatctccaatagtctacaagaccaaatgcaattcctagaa
S N V E T L H R L L E K T S H A M H S R
3062 agcaacgtggaaacattgcatagattgcttgagaagactagtcatgctatgcactcaagg
A L A S K V I S S V Y N K S L T D K S L
3122 gctttagccagtaaggtcatatcttctgtatataacaagagcctaactgacaagtcattg
L E E G F I N A T D L S R E V Y E K N Y
3182 ctagaggaagggttcattaatgcgactgatctatcacgagaggtatatgaaaaaaattat
Q E H L Q V Q W R E Q P L S Q R L S S I
3242 caggagcatttacaggtgcagtggcgagagcaacctttgtctcaaagattatcttcaatc
I A T T K S Y L R N V G P T K F S A P D
3302 attgctaccacaaaatcttacttgcgaaatgttggaccaaccaaattcagcgctccagat

```
      L  K  G  K  A  H  V  Y  T  T  Q  S  L  G  V  I  A  K  T  G
3362  ttgaaaggcaaagcacatgtatatacaacgcaatcacttggtgtgattgcaaaaacgggc
      S  V  V  K  E  N  A  Q  R  C  C  S  T  W  Y  Y  S  I  I  R
3422  agtgtagtaaaagagaacgcgcaaagatgttgtagcacgtggtattatagtattattagg
      H  V  F  F  Y  S  L  R  S  V  K  R  L  C  P  D  L  L  T  Y
3482  catgtattcttttactctttacgtagtgtgaaacgattgtgccctgatctactcacatat
      C  S  I  V  G  V  F  Y  Y  M  V  S  I  F  V  K  V  K  N  Y
3542  tgtagtatagtgggcgttttctattatatggtgagtatatttgtaaaggttaaaaattat
      L  D  V  H  R  S  L  K  E  F  K  V  I  S  D  Y  N  K  T  V
3602  ctagatgttcataggtctttgaaggaatttaaagtaattagtgattataataagacagtg
      K  H  L  D  Y  L  Y  G  K  L  C  V  K  L  N  G  L  P  S  E
3662  aaacatttagactacctgtatggcaagctgtgtgtgaagctaaatgggttacctagtgaa
      S  E  F  L  A  Y  I  D  R  K  Q  P  S  L  N  Q  I  A  A  L
3722  agtgaattttagcgtatattgataggaaacagcctagtcttaatcagatagcagcactc
      E  T  S  L  C  A  P  N  M  E  F  Q /A  K  G  D  F  Q  H  L
3782  gaaacaagcctgtgcgcaccaaatatggaattccaagcaaaaggggattttcagcacctt
      E  K  M  I  A  L  L  V  L  L  T  M  L  F  D  A  N  R  S  D
3842  gaaaagatgatcgctttgctagttctacttactatgctatttgatgctaatcgaagcgat
      A  V  Y  K  I  L  N  K  F  K  G  V  M  S  S  I  D  K  E  P
3902  gcagtgtacaaaattctgaataaatttaagggtgtcatgagttccattgataaagaacca
      M  L  H  Q/ S  L  D  D  I  Q  D  I  I  E  E  K  N  L  T  V
3962  atgttgcaccaaagcttagacgatatacaggacataattgaggagaaaaatctcacagtt
      D  F  S  I  T  D  A  D  P  T  I  N  R  I  S  G  A  T  F  S
4022  gatttagcataacagatgctgatcccaccattaaccgtatatccggcgctacattctca
      Q  W  W  K  N  Q  I  E  R  N  R  M  L  P  H  Y  R  S  G  G
4082  cagtggtggaagaatcaaatagagcgcaatcgaatgctaccacactacaggtcaggggc
      H  F  L  E  F  T  R  A  N  A  G  S  L  A  S  I  I  A  H  E
4142  catttctggagtttacgagagcaaatgctggtagcttggcatcgataatcgcacatgaa
      E  H  K  E  I  L  I  R  G  A  V  G  S  G  K  S  T  S  L  P
4202  gaacacaaggaaatttgattagaggggctgtaggttctggaaagtcaacgtcgttaccc
      F  H  L  H  T  K  G  S  I  L  L  L  E  P  T  R  P  L  A  E
4262  tttcacttacacacgaaaggctccatttgttattggaaccaactaggccactggctgag
      N  V  Y  K  Q  L  K  G  A  P  F  F  T  N  P  T  F  K  M  R
4322  aatgtgtataaacaactcaagggtgcacccttctttactaatccaactttcaaaatgagg
      G  L  T  Q  F  G  S  S  P  I  T  V  M  T  S  G  F  A  F  Q
4382  ggcttaacgcaatttggatcatcaccaataacagtaatgactagtggcttcgcttttcaa
      F  Y  A  N  N  V  N  Q  L  K  D  F  D  F  I  M  F  D  E  C
4442  ttctatgccaacaatgtcaaccagctgaaagattttgatttcataatgtttgatgaatgt
      H  C  F  D  A  Q  A  M  A  F  F  C  L  L  K  E  H  E  F  E
4502  cactgtttcgacgcacaggcgatggctttcttttgtttgttaaaagaacacgaattcgaa
      G  K  I  L  K  V  S  A  T  P  P  G  R  E  V  E  F  T  T  Q
4562  gggaagattcttaaagtctcagcgacgccaccgggacgtgaggtagaattcacaacgcaa
      F  P  V  D  V  R  I  E  G  H  L  S  H  Q  Q  F  V  S  N  L
4622  tttcctgtcgacgtgcgtattgaaggccaccttagtcaccagcagtttgtgagcaattta
      G  T  G  A  N  S  D  V  L  P  C  G  D  N  I  L  I  Y  V  A
4682  ggtacaggtgcaaatagcgacgttcttccttgtggagataacattcttatttacgtggct
      S  Y  N  E  V  D  Q  L  S  K  L  L  M  D  Q  K  F  K  V  S
4742  agttacaatgaggttgatcagctgagcaaactactgatggatcaaaagttcaaagtttca
      K  V  D  G  R  T  M  K  S  G  K  I  E  I  V  T  E  G  T  P
4802  aaggttgacgggcgaaccatgaagagtggtaagattgaaatagttacagagggcacaccc
      T  K  K  H  F  I  V  A  T  N  I  I  E  N  G  V  T  L  D  V
4862  accaaaaagcattttatagtggccacgaacataatcgaaaatggagtaacacttgatgtg
      D  V  V  V  D  F  A  T  K  V  V  P  E  L  D  E  E  A  H  M
4922  gatgttgtagtggattttgcgacgaaagttgtaccagagctcgatgaggaagcacatatg
      I  R  Y  N  K  K  S  I  S  Y  G  E  R  I  Q  R  M  G  R  V
4982  attcggtacaataaaaagagcatatcatacggtgagcgtatacagcggatggggcgtgtc
      G  R  H  K  R  G  T  V  L  K  I  G  E  T  E  K  A  S  W  R
5042  ggacgacacaagcgcgggacggtcttgaagattggtgaaacagaaaaagctagctggcgc
```

V P P C I A T E A A F Y C F A Y G L P V
5102 gttccaccatgcattgcaacggaagcagcattctattgctttgcttatggactacctgtg
I S D G V S T S V L E N C T V P Q A R T
5162 atttctgatggagtgtctacaagcgtgcttgaaaactgcacagtaccacaagcgagaaca
M M Q F E L S I F F M F H F V K H D G S
5222 atgatgcaatttgaattaagtatattcttcatgtttcatttcgtgaaacatgatggtagt
M H P A I H D R L K Q Y K L R D S E I I
5282 atgcatccagctattcacgatcgtttaaagcagtataaattgcgtgactcggaaatcatt
L N K T A I P H R G L T T W P T V E E M
5342 ttgaacaaaacagccattccacataggggattaaccacttggcctacagttgaagaaatg
K R H G C S I N Q P D E V R L P F F I K
5402 aaacggcatggttgctctataaatcaaccagacgaggtgagactgcccttttttcatcaag
D V P D K L Y G D L H E V L K K H S G D
5462 gatgtacctgataaattatatggagatctccatgaagtgttaaagaagcatagtggagat
A C F G R I R G I S A S K I A Y T L Q T
5522 gcttgctttggaaggattcgagggataagcgctagcaaaatcgcatacacgttacaaact
D P A S I Q R T I K I L D K L Y E S E L
5582 gatccagcgtctatccagcgaacaattaagatacttgataagttatatgagagtgagtta
Q K K A Y F A N V T S S S C S T F N Y A
5642 cagaagaaagcatatttgcaaatgtgacatctagttcttgttcaaccttcaactatgct
L T T I T N A I R A R H M Q D Y T T E N
5702 ctcactacaataacgaatgccattcgcgctaggcatatgcaagactacacaaccgagaat
L S V I Q N A K S Q L L E F N N I R T P
5762 ttgagcgttatccaaaatgctaaaagccagctacttgaattcaacaatataagaacaccg
K M T E D A L V D Y G A L E **C M M Y Q/ S**
5822 aaaatgactgaggatgcgttggtcgattatggggccttggaatgtatgatgtaccagtct
E N E M S R H L G L K G R W N K S I I I
5882 gaaaatgagatgagcaggcatcttggattaaaaggccgttggaataaaagtatcatcatc
K D V V L A G V T V I G S I L M M Y E Y
5942 aaagatgttgtactcgcaggagtcacagtcattggcagcatcttaatgatgtacgaatat
V K G K L E E P **M D Y Q /G** K N Y R Q R Q
6002 gttaaaggaaagttggaggaacccatggattaccaaggaaagaattataggcaaagacag
K L R F R E A H D S K H A Y E I H G D D
6062 aaacttcgattccgtgaggcacacgattcaaaacacgcctatgaaatacatggtgatgat
A Q L Q T Y F G N A Y T K K G K K S G N
6122 gctcaattacaaacatatttcggcaatgcgtacacaaagaaaggaaagaagtccggaaac
T T G A G K K M H R F Y N V Y G F E P T
6182 acgactggagctggtaagaaaatgcatcgattttataatgtttatggatttgaacccact
D Y S F A R Y V D P L T G A T L D E S T
6242 gactattcatttgcacgctatgttgacccactcacaggcgccactttagacgaaagcacc
V T D L S L V Q D H F G T I R N Q M R Q
6302 gtgacagatttaagtttggtgcaagatcactttggaacaattcgaaatcaaatgaggcaa
S G D L E P E R I S R N T N I E C Y Y V
6362 agtggagatctagagccggaacggatatcgagaaacacaaacatcgaatgctactacgtc
N D L A K K V L K I D L T P H N P L R V
6422 aacgatttggcaaagaaagtgctgaaaattgacttaactccacataacccactgcgtgtg
S G K S N N V M G F P E R S L D L R Q T
6482 agcggaaaatcaaacaatgtcatgggctttccggaacgttcgttagaccttagacagact
G A P V T V S Y N Q L P P S N R D A G A
6542 ggagccccagtcacagtatcatacaatcaattaccaccttcgaatcgggatgctggtgcg
F E F E/ G K S L L S G L R D Y N P I A A
6602 tttgagttcgagggtaagtcgctattgagtggactccgcgattacaatcccatagcggcg
C V C K I T N E S D G V I T H I F G I G
6662 tgtgtatgcaaaataacgaacgaatcagacggtgtcatcacacacatctttggcatcgga
Y G P Y V I T N Q H L F R R N N G I L R
6722 tatggtccatatgttattactaatcaacacctatttcgtagaaataacggaatactacga
I Y T H V G E Y L I N N A C T L K M H P
6782 atatacacacacgtgggtgaatatctgatcaataacgcgtgcacacttaaaatgcatccc

I P E R D I V I I R L P K D F T P F P Q
6842 attcctgaacgcgacattgtcataatacgcttaccgaaagatttcacgccatttccacaa
R L K F R P T R V G E H V C L V S S N F
6902 agactcaaatttcggccaactcgtgttggtgaacatgtatgcctagtgagctcaaatttc
Q T K S I S S V I S E T S A T A G T A N
6962 caaacaaagagcatctcgagtgtcatttcggaaacaagtgcgacagctgggaccgcaaat
K S F F K H W I T T K H G Q C G N P L V
7022 aaaagtttctttaaacattggataaccacaaagcatgggcagtgtggtaacccgttggtt
S V T D G C I V G I H S M T S T V S S M
7082 tctgtgacggacggttgtattgttggtattcacagcatgacgagtacagttagctcaatg
N M Y A G F P D N F V S D Y L S N D L L
7142 aatatgtatgctggtttccctgacaactttgtgtccgattacctcagcaatgatctattg
E W T K G W K L S A D R S C W D G I T M
7202 gagtggacaaagggatggaaactgagcgcagacaggagttgctgggatggaatcacaatg
V D N K A E G L F K L T K E I F T L D D
7262 gtagataacaaggccgagggtttattcaaactcactaaagaaatattcactcttgacgac
G K **W E F Q/ S** G H K D W M Y N K L E G N
7322 gggaagtgggaattccagtcaggccacaaggactggatgtataacaagttagagggaaat
L K A V G R T S G N L V T K H S V K G K
7382 ttgaaggctgttggacgcacgagcgggaatctcgtcaccaaacactcagtaaaaggaaag
C M L F Q T Y L S V E P E E A A Y F T P
7442 tgcatgctctttcaaacttatttgtcagttgagccagaggaagcagcatacttcacacct
L M G A Y A K S A L N K E A Y I K D L S
7502 cttatgggtgcctatgctaaaagtgctcttaataaggaggcgtacattaaagatctgagc
K Y S G E I S V G N V D C D V F E R A F
7562 aaatactctggtgaaatcagcgttggaaatgtagactgtgatgtgtttgagcgagctttt
E K V V T L M E S K G F H E C A Y I T N
7622 gaaaaggttgtaactttaatggagagtaaggggtttcatgagtgtgcatacattacaaat
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7682 gagcacgaaatcttagcagcactaaacatgaaagcagccgtcggggcactgtactcagga
K K R E Y F A D F S D Q D K Y D I V K E
7742 aagaagcgggaatatttgcagacttctctgaccaagacaagtatgacatcgttaaagaa
S C E R L F L G K M G V W N G S L K A E
7802 agttgcgaaagactatttcttgggaaaatgggcgtgtggaatgggtccttgaaggctgaa
L R P I E K V M A N K T R S F T A A P L
7862 cttcgcccaattgagaaagtcatggcaaataaaacccgttcattcacggcagcaccgtta
D T L L G G K V C V D D F N N Q F Y S R
7922 gatacacttcttggtggaaaggtttgtgtggacgacttcaacaaccaattttacagtcga
H F D L P W T V G M S K F R K G W D T L
7982 cacttcgacctaccttggacagttggtatgagcaaatttcgcaaagggtgggacacttta
L R K L P D N W V Y C D A D G S Q F D S
8042 ttgagaaagctgccagataattgggtctattgtgacgcggatggctctcaatttgatagc
S L S P Y L I N A V L K L R L H F M E S
8102 tcgttatcaccatatttgataaatgccgtcttaaagctacggttacacttcatggaaagt
W D V G E T M L K N L Y T E I V Y T P I
8162 tgggacgttggtgagaccatgttaaagaacttatatacagagatcgtctacacaccgata
A T P D G T I V K K F K G N N S G Q P S
8222 gcaacgccagatggcacaatagtgaagaaattcaagggtaataatagtgggcagccatcc
T V V D N S L M V V F S M Y Y A M E M S
8282 accgtggttgacaactcactaatggttgtttctctatgtattacgctatggagatgagc
D I K N I H D K C V F F V N G D D L I I
8342 gatataaaaaatatacatgataaatgcgttttcttcgtgaatggtgatgatttaattata
A V E P G S E A F L D S L Q N L F Q Q L
8402 gcagttgaaccaggctcggaagcttttctggatagtttacaaaatctatttcagcagctc
G L N Y N F N S R T R D K E K L C F M S
8462 ggcttgaactacaactttaacagtcgaacacgcgacaaagagaagttatgcttcatgtca
H M G V L Q D G I Y I P K L D K E R I V
8522 cacatgggtgtcttacaagatggcatttacataccgaaactggacaaggagcgtatagtg

S I L E W D R A Q Q P E H R L E A I C A
8582 tcgatattggaatgggatcgtgctcaacaacctgagcacaggttagaagccatttgtgca
A M I E A W G Y P D L L Q R I R K F Y C
8642 gcaatgatcgaggcatggggttacccggacctcttacaacgaatccgaaaattctattgt
W I L E Q A P Y N E L S T L G K A P F I
8702 tggattctcgaacaagcgccatacaatgaattaagcacattaggtaaagcaccattcatt
S E A A L R N L Y T D C E A T E A E L A
8762 tctgaggcagctctacgtaatttgtatactgattgcgaggccacggaggcagagttggca
R Y L E L Y D S D T P T E E V **F E Y Q/ A**
8822 aggtaccttgagctttacgatagtgacacacccacagaagaagtatttgaatatcaagcc
G D E L D A G T Q A S K N Q R N N A D K
8882 ggcgacgaactagatgcagggacacaagcaagcaagaatcaaaggaataacgcagacaaa
S I E Q R S P L V S Q T N L N E A K G I
8942 tctattgagcaacgaagcccactagtgtcacaaacaaatttaaacgaggcgaagggcatt
G S S S G Q N V N R D R D V N V G T T G
9002 gggagtagttcgggtcaaaacgtgaacagagacagagatgtaaatgtcggcaccacagga
T F S V P R I K Q I P Q K G I V I P M D
9062 actttagtgtaccacggataaagcaaatcccacaaaaaggcatagtaattccaatggat
G G K S I L N L D H L L Q Y K P S Q L C
9122 ggagggaaatcaatacttaacttagaccatctactacaatacaagccaagtcaattatgc
I S N T R A T K A Q F M T W K A R L Q E
9182 atatcaaacactagagccacgaaggcacaatttatgacttggaaggcgaggctgcaagag
E Y G V T E S E M S I I L N G L M V W C
9242 gaatatggcgtcactgagagtgagatgagcatcattctaaatggcttaatggtgtggtgc
I E N G T S P N I N G V W P M M D G E E
9302 attgagaacgggacttcacccaatataaacggcgtttggccaatgatggatggcgaggag
Q V E F P L R P V V E H A Q P T L R Q I
9362 caagtcgaatttcctttacgccctgttgttgagcacgcacaaccaacactacgtcagata
M A H F S A L A E A Y I E M R N S E Q A
9422 atggcgcacttctcagcattagcagaagcctacattgagatgaggaactcagagcaggct
Y M P R Y G L Q R N L T D M G L A R Y A
9482 tacatgccgcggtatggattacaaagaaatcttacagatatgggtctcgcacggtatgca
F D F Y E I T S R T P V R A R E A H A Q
9542 tttgacttctatgaaatcacatcaagaacaccagttagagcgcgcgaggctcatgcacaa
M K A A A L R N S R P K L F G L D G N V
9602 atgaaagcagctgccttacgtaattcaaggccaaagctgtttggattagacggcaacgtc
T T T D E D T E R H T A H D V N A R M H
9662 acaaccacggatgaggacacggagaggcacacggcacatgacgtgaatgcacggatgcac
H L D G A H M Q
9722 catcttgatggtgcgcatatgcag**tga**tgtttcggttagcaaccggttatgggcttccac
9782 ctaaaaagtcccgagtgccaaactcgtgttttggctagtgtagtgaagctatgattcact
9842 cgttcaaggcagtttctactttaaggatgacttgggaattattagctcccaatgccggtc
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9962 ggagtacggttttgtaaagaaggtggatttaattttaaggatagtaagtctgaaaccat
10022 aatggtgtacaagcagcctgaatactaataccccacctttaccctacaatggagtgctac
10082 tttaaagactagctaggattgagaccgaaatctcacgtgctggcatggccagcagttgat
10142 cacttaacgatcaggtgaccactttacagtcaagttggaacccgtagtatcctatcctta
10202 cctaatatcgtaagttttatttactggtgtagcgtggtttaaccaccttataaacacttt
10262 gaatattaggatcgagctgttcgtctgaaggcactaagagtggttgaccatgtgtgcgtt
10322 cttgcgttgcagtaagagac

Apêncide 4: Mapa do experimento a campo com Allium Ornamental, mostrando o número de plantas avaliadas para cada espécie.