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Virus of garlic, allium sativum L. in japan: Identification and Characterization of Several Filamentous Viruses of Garlic.

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Viruses of garlic in Japan

Garlic (allium sativum L.) is an important crop Worldwide as a common spice and a medicinal herb. Virus diseases have caused major damages on garlic production because garlic plants are vegetatively propagated. A number of viruses and virus diseases have been reported on garlic. In many countries, mosaic diseases of garlic are mainly caused by onion yellow dwarf (OYDV) and leek yellow stripe (LYSV) potyviruses (Bos 1982; van Dijk 1993). in japan, garlic latent carlavirus (GAV) (Lee et. al. 1979) and garlic mosaic virus (GMV) (Sako 1976; Lee et. al. 1979; Abiko et. al. 1980) have been reported to infect garlic.

Van Dijk (1993) reported that GMV, reported from Japan and Korea, is Possibly a complex of at least three distinct potyviruses including OYDV, LYSV, and garlic strain of onion mite-borne latent virus (OMbLV-G). He also isolated and characterized two common latent carlavirus (GCLV-G). He also isolated and characterized from garlic: shallot latent carlavirus (SLV) and garlic common latent carlavirus (GCLV), Based on their biological and serological properties, he reported that GLV in Japan should be regarded as SLV.

Recent reports based on sequence analyses of viral RNA indicated a presence of novel filamentous viruses, including both potyviruses and carlaviruses, in garlic (Nagakubo et.al. 1994, Sumi et.al. 1993). However, these viruses were not isolated from infected garlic plants, and their biological and serological properties remained unknown. Now I present my latest results on identification and characterization of viruses from garlic including recent unpublished finding.

Leek yellow stripe potyvirus and its relationship with garlic mosaic potyvirus

In Japan, LYSV was first isolated from A. *ampeloprasum* and reported by Noda and Inouye (1989). In Aomori prefecture, most of commercial garlic (cv. Fukuchi-howaito or also called Howaito-roppen) was infected by LySV, and a garlic strain of LYSV (LYSV-G) was isolated by

single-lesion transfer to *Chenopodium quinoa* from garlic plants showing yellow stripe symptoms (Yamashita et. al. 1995). Garlic plants infected by LYSV-G had developed mosaic and yellow stripes on their leaves but high temperature conditions (ca. over 27℃) tended to mask these symptoms. LYSV-G was easily transmitted by aphids(Acyrthosiphon solani, Aphis gossypii, and Myzus persicae), and sapinoculated from infected garlic to virus-free garlic, C. amaranticolor, C.murale, and C. quinoa.

In ISEM and DIBA tests (immuno-sorbent electron microscopy and dot immunobinding assay, respectively), LYSV-G reacted with none of the antisera to potyviruses, including OYDV-Dutch (Ac41), welsh-onion yellow stripe (WYSV-Sf12I), turnip mosaic (TuMV), soybean mosaic (SoMV), and zucchinio yellow mosaic (ZYMV) potyviruses, but its own antiserum. A molecular weight of LYSV-G CP was 35 kDa estimated by SDS-PAGE. Cytoplasmic cylindrical inclusions were observed in infected leaf cells of garlic.

These properties of LYSV-G strongly resemble to some of the descriptions on GMV by Sako (1976). Further, nucleotide sequences of LYSV-G (D85436) showed 90 and 97% identities with GV-7 (D11118) and GV-2 (D28590), cDNA sequences with characteristics of potyviruses, obtained from GMV preparations from commercial garlic (Nagakubo et. al. 1994; Yamashita et. al. 1995; Sumi et. al.1996). Thus, we conclude that GMV is the same virus as LYSV-G and propose that GMV should be renamed to LYSV.

Onion yellow dwarf potyvirus and its relationship with welsh-onion yellow stripe potyvirus

OYDV is commonly occurred in garlic plants in the world. We reported an isolation of a garlic strain of OYDV (OYDV-G) from garlic cv. Ibaraki showing severe mosaic symptoms (Yamashita et.al., in press). OYDV-G was sap-transmitted to garlic and certain cultivars of onion (cv. Sapporo yellow and cv. Noordhollande strogele). OYDV-G was easily transmitted by aphids, A. *solani* and M. *persicae*. OYDV-F induced more sever nosaic symptoms on garlic leaves than LYSV-G. Cytoplasmic cylindrical inclusions were observed in infected leaf cells of garlic. OYDV-G reacted with the antiserum to OYDV-Dutch (Ac41) but not with the antisera to LYSV, WYSV, of shallot yellow stripe potyvirus. An estimated molecular weight of CP was 34 kDa for OYDV-G by SDS-PAGE, and 30, 32, and 33 kDA for OYDV-Dutch (Ac41), WYSV Kuroishi, and WYSV-Indonesian (Af12I), respectively (Yamashita and Hanada 1996).

A potyvirus of Negi-Isyuku-byo in Japan, means welsh-onion dwarf disease, had been considered as a strain of OYDV because symptoms of this disease on welsh-onion plants were similar to those of onion yellow dwarf disease caused by OYDV in other countries. However, van Kijk (1993) described that Japanese "OYDV" isolates from welsh-onion were distinct from OYDV-Dutch (Ac41) or OYDV-French (isolated from onion), based on biological and immunochemical characterizations. Thus, he designated these "OYDV" isolates from welsh-onion

as welsh-onion yellow stripe potyvirus (WYSV). Further, amino acid sequence of the WYSV-welsh onion CP (Yamashita and Hanada 1996) showed 100% identity with those of WYSV-Indonesian (Af12I) and WYSV-wakegi (D73378, Tsuneyoshi et.al. 1996), but 74 and 76% with those of OYDV isolates from garlic (X89402) and onion (X95874, OYDV-French) Kobayashi et.al. 1997).

Mite-borne filamentous viruses form a new virus group: garlic virus (GV)-A, -B, -D, and garlic mite-borne mosaic virus (GMbMV)

A novel filamentous virus was isolated from garlic plants infected with a mosaic disease and an eriophyid mite, *Aceria tulipae* Keifer. The mosaic disease was easily transmitted by the mite but not by aphids, *A. solani* or *M. persicae*. The virus was tentatively named garlic mite-borne mosaic virus (GMbMV: Yamashita 1992). GMbMV induced a systemic mild mosaic on garlic but local lesions on C. murale and Gomphrena globosa. GMbMV particles and antigens were easily detected in the vector mite by ISEM and the modified DIBA on slideglass (Yamashita 1992); Yamashita unpublished). Cytoplasmic cylindrical inclusion body, commonly observed with viruses in the family Potyviridae, was not detected in GMbMV-infected plants. GMbMV exhibited no serological relationship with LYSV, OYDV-Dutch, WYSV, SLV or GLV.

Van Kijk et.al. (1991) had isolated a mite-borne virus from garlic (OMbLV-G) and classified this new virus into rymovirus in family Potyviridae. Although GMbMV resembled OMbLV-G in its host range, vector relationships, and particle morphology, GMbMV, unlike OMbLV-G, did not induce cylindrical inclusions in the cytoplasm of infected leaf cells. Two major polypeptide bands with estimated molecular weights of 30 and 28.5 kDa were detected from the purified GMbMV using SDS-PAGE. GMbMV displayed a genomic organization of viral RNA in the 3'-terminal region very simila to shallot virus X, GV-A (D11157), -B (D11158), -C (D11159) , and -D (D11160) (Kanyuka et.al. 1992; Sumi et.al. 1993). The putative CPs of GMbMV and GV-C are the same virus (Yamashita et.al. 1996). We synthesized a multiple antigen peptide (MAP) for each of the N-terminal regions of putative CPs of GV-A, -B, -D and GMbMV. The antisera to these peptides were highly specific to their homologous antigens, i.e., individual antiserum reacted only to its own antigen, thus useful for the distinction of these mite-borne viruses (Yamashita unpublished).

Carlaviruses of garlic and other Allium species.

Carlaviruses from Allium plants are likely to be classified into at least three viruses based on the host range tests and their serological characteristics (Yamashita unpublished). Carlaviruses from four plant species were tested against five antisera to GLV-G (Lee et.al. 1979), GLV-S (Sako et.al. 1988), GLV-Af (Fukami et.al. 1987), SLV (Ac3B) (Bos et.al. 1978), and multi-antigen peptides (MAP) synthesized (Yamashita unpublished) based on the N-terminal

amino acid sequence of putative CP of GV-1 a garlic carlavirus (D28591) (Nagakubo et.al. 1994).

A carlavirus (CarlaV-G) from garlic developed large necrotic local lesions on C. *quinoa* and strongly reacted with the antiserum to the MAP of GV-1 but did react with other antisera tested. A carlavirus (Carla-R) from rakkyo (A. chinesis) developed chlorotic local lesions on C. quinoa and strongly reacted with the antisera to GLV-Af or the GV-1 MAP. A carlavirus (CarlaV-W) from wakegi (A. wakegi) developed small necrotic local lesions on C. quinoa and reacted with anti-GLV-Af serum but not with other antisera. Further, SLV (Ac3B) showed the same result with CarlaV-R. These results indicates that CarlaV-G differs from GLV and SLV, and that CarlaV-R is identical with GLV and SLV to be classified into the same virus. We need further characterizations of Carla-G and -W for their classification.