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Researches on the Indicator Plants of Satsuma Dwarf and Hassaku Dwarf Viruses

SATSUMA DWARF and Hassaku dwarf are important virus diseases of citrus in Japan. The authors have carried out a number of inoculations to transmit these diseases to citrus and other plants for the purpose of finding indicator plants.

By the lime test, the authors (2) have already proved that Satsuma orange (*Citrus unshiu* Marcovitch) trees in Japan, except very young seedlings, always carry tristeza virus. The occurrence of Satsuma dwarf is, however, not very common but is limited to certain areas where citrus is grown. Therefore, this disease is undoubtedly caused by a virus other than tristeza virus, or by a virus complex including tristeza virus.

Since 1960, when mechanical transmission of infectious variegation virus was reported by Grant and Corbett (1), the authors have made inoculations onto 32 species of leguminous plants with sap from Satsuma orange trees infected with Satsuma dwarf virus. By repeated experiments, it was proved that Satsuma dwarf virus induces striking symptoms in Blackeye cowpea, kidney bean, and some other plants.

Subsequently, K. Kishi, one of the authors, inoculated many species of non-leguminous plants known to be susceptible to other viruses and demonstrated that only sesame, of the plants tested, is susceptible to Satsuma dwarf virus. At present the authors are of the opinion that sesame may be the most suitable indicator plant for this virus.

It has been found, on the other hand, that the virus of Hassaku dwarf does not produce symptoms in the legumes or sesame but causes striking vein corking, stem pitting, and severe stunting on Marumera (*C. obovoidea* Takahashi) seedlings inoculated by tissue-graft. Therefore, Marumera is a very good indicator plant for Hassaku dwarf virus.

Materials and Methods

Plants used for inoculation were grown in small earthen pots and kept in a screenhouse. The primary leaves of the legume seedlings were inoculated with expressed sap. The cotyledons or the first and second true leaves of sesame and other non-leguminous plants were inoculated. The expressed saps from young shoots infected with Satsuma dwarf or Hassaku dwarf were buffered with an equal volume of a 0.05-0.1 molar solution of K_2HPO_4. The plants were inoculated by the ordinary rubbing method, using Carborundum as an abrasive, and were kept in a screenhouse at temperatures lower than 35°C.

The tissue-graft inoculation experiments with Hassaku dwarf were carried out by the same method as the lime test, and about ten species and varieties of the genus *Citrus* and related plants were inoculated in order to find good indicator plants.

Results of Sap-Inoculations on Leguminous Plants

Among 32 species of leguminous plants tested, the following proved to be susceptible: *Arachis hypogaea* L. (peanut), *Astragalus sinicus* (Chinese milk vetch), *Canavalia gladiata* DC (sword bean), *Crotalaria spectabilis* Roth., *Kummerovia striata* (Thunb.) Schindler (Japan clover), *Phaseolus vulgaris* L. (Satisfaction, Top Crop, Nagauzura, and Improved Ideal Market varieties of kidney bean), *Vigna sesquipedalis* Fruwirth (Kurosanjaku and Akasanjaku varieties of asparagus bean), and *Vigna sinensis* Savi (Blackeye and Bombay varieties of cowpea).

The species of leguminous plants that did not prove to be susceptible were: *Aeschynomene indica* L., *Albizzia julibrissin* Durazz., *Amphicarpaea edgeworthii* Bench. var. *japonica* Oliver, *Phaseolus chrysanthos* Savi (Azuki bean), *Cassia nomane* (Sieb.) Honda, *C. tora* L., *Desmodium racemosum* (Thunb.) D. C., *Gleditschia japonica* Miq., *Glycine max* Merr. (soybean), *Indigofera pseudo-tinctoria* L. (indigo), *Kummerovia stipulacea* (Maxim.) Makino, *Lathyrus odoratus* L. (sweet pea), *Lespedeza buergeri* Miq., *L. cuneata* Don, *L. pilosa* Sieb. et Zucc., *Phaseolus vulgaris* L. (Mantle, Dwarf Red Cranberry, Shirokuro, Kurosando, and Kentucky Wonder varieties of kidney bean), *Pueraria lobata* (Willd.) Ohwi (Kudzu-vine), *Stizolobium hassjoo* Piper et Tracy (Yokahama bean), *Sophora japonica* L. (Japanese pagoda-tree), *Trifolium pratense* L. (red clover), *T. repens* L. (white clover), *Vicia hirsuta* (L.) S. F. Gray, *V. sativa* L. (spring vetch), and *Wisteria brachyobotrys* Sieb. et Zucc.

Blackeye cowpea first showed slight mottling on systemically infected leaves or vein clearing on newly developed leaves and subsequently necrotic streaks on the petioles as well as on the stems.

Satisfaction kidney bean (Chashiro-ingen) developed chlorotic spots, followed by clear mottling and vein clearing on systemically infected leaves, which subsequently became malformed (Fig. 1, A,B). Top Crop kidney bean exhibited chlorotic spots, which turned into necrotic spots with concentric rings. Japanese clover showed terminal wilt.

Crotalaria developed no apparent local lesions on infected leaves; systemically infected leaves showed vein clearing, followed by vein necrosis and soon turned yellowish and dropped.

Among the susceptible plants listed above, Blackeye cowpea and Satisfaction kidney bean develop the most conspicuous symptoms, and they appear to be good indicator plants for Satsuma dwarf. The authors temporarily propose the name "Satsuma dwarf virus" to the legume infecting virus. Retransmission from infected legumes to Satsuma orange has not, however, been successful in the tests conducted so far.

In comparative experiments with different buffer solutions, it was found that the expressed sap from affected Satsuma orange plants was more infectious when K_2HPO_4 solution was added to it than when distilled water or other solutions containing Na_2HPO_4, Na_2SO_3, or cystein hydrochloride were added. The young shoots of affected Satsuma orange at an early stage of development, or one month after sprouting, were demonstrated to carry highly active virus in the leaves, bark, and woody portions of twigs. One-year-old shoots or relatively old current shoots, on the other hand, carried active virus only in the wood, but not in the leaves or the bark of twigs.

Tissue-graft inoculation from trees with symptoms of Satsuma dwarf to Mexican lime seedlings always gave vein clearing and stem pitting (2). Thus it appears that these trees carry tristeza virus, but indications are that it is not the tristeza virus that is transferred mechanically to herbaceous plants. Whether or not the tristeza virus plays any role in the occurrence of the symptoms of Satsuma dwarf will have to be determined by further experiments.

Results of Inoculations on Sesame

Twenty-seven species of non-leguminous herbaceous plants known to be susceptible to virus diseases were selected from 12 families including the *Cruciferae, Chenopodiaceae, Cucurbitaceae, Solanaceae, Composi-*

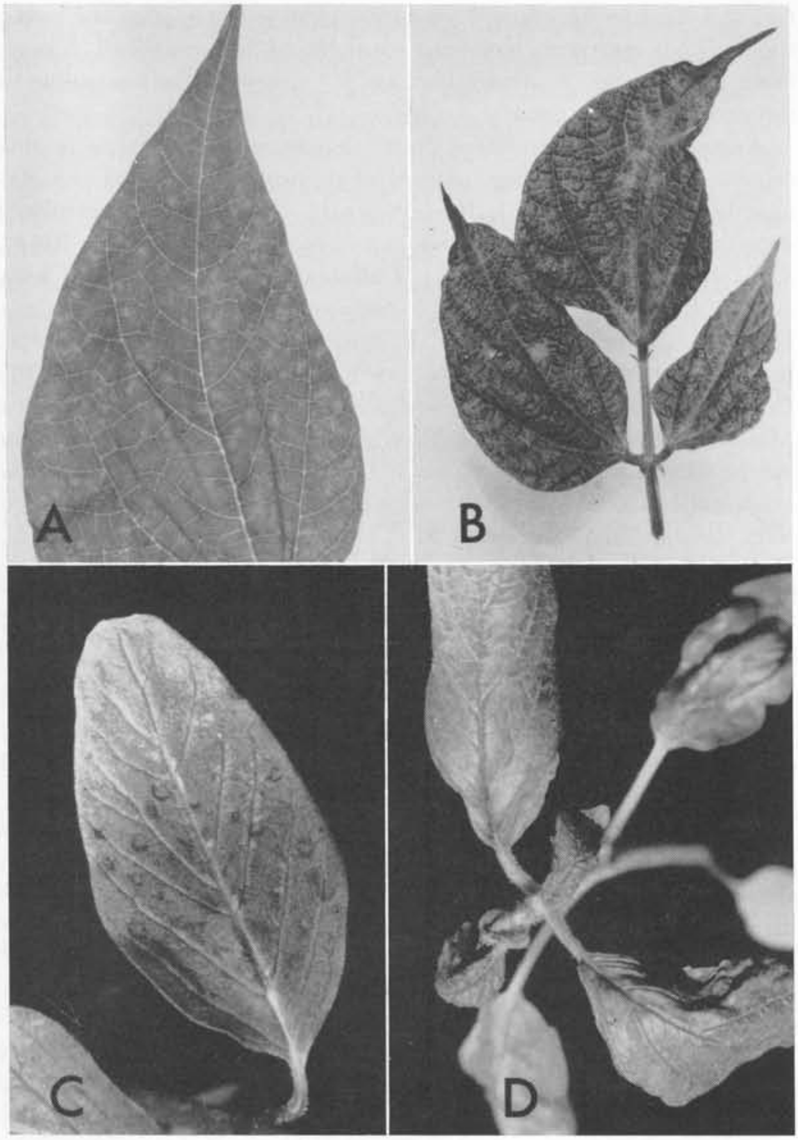

FIGURE 1. *A. Chlorotic spots on a leaf of Satisfaction kidney bean two weeks after mechanical inoculation with Satsuma dwarf virus. B. Mottling, vein clearing, and malformation on systemically infected young leaves of Satisfaction kidney bean. C. Local lesions on a leaf of white sesame one week after inoculation with Satsuma dwarf virus. D. Vein clearing, stunting, curling, and malformation on young leaves of white sesame.*

tae, and *Amaranthaceae* and were inoculated with sap from the young shoots of Satsuma orange showing symptoms of Satsuma dwarf. Among them only sesame (*Sesamum indicum* L.) proved to be susceptible to the virus of Satsuma dwarf.

Among the non-leguminous plants that were not proved to be susceptible were the following: *Amaranthus tricolor* L., *Gomphrena globosa* L. (globe amaranth), *Vinca rosea* L. (Madagascar periwinkle), *Beta vulgaris* L. (beet), *Chenopodium amaranticolor* Coste & Reyn., *Spinaceae oleracea* L. (spinach), *Callistephus chinensis* Nees (China aster), *Helianthus annuus* L. (sunflower), *Brassica juncea* Coss. (mustard), *B. napus* L. (rape), *B. rapa* L. (turnip), *Capsella bursa-pastoris* (L.) Medic. (shepherd's purse), *Raphanus sativus* L. (radish), *Citrullus vulgaris* Schrad. (watermelon), *Cucumis melo* L. var. *makuwa* Makino (melon), *C. sativus* L. (Chicago Pickling cucumber), *Cucurbita maxima* Duchesne (winter squash), *C. pepo* L. (pumpkin), *Digitaria sanguinalia* (L.) Scopoli., *Polygonum longisetum* DeBruyn, *Datura alba* Nees, *Lycopersicon esculentum* Mill. (tomato), *Nicotiana glutinosa* L., *N. rustica* L., and *N. tabacum* L. (Xanthi, Bright Yellow, and White Burley tobacco).

Several days after inoculation, the leaves of Sesame developed local lesions, which were chlorotic spots at first and afterwards became necrotic with a yellowish halo (Fig. 1,C). The systemically infected leaves first showed vein clearing and later vein necrosis. Leaves that developed after the infection showed mottling, stunting, curling, and malformation (Fig. 1,D).

Cross inoculation between the infected sesame and Blackeye cowpea or Satisfaction kidney bean always gave positive results. Therefore, the virus in the infected sesame plant was apparently the so-called Satsuma dwarf virus, which was transmitted by sap-inoculation to leguminous plants as stated above.

Among three sesame varieties tested, white sesame was the most susceptible; brown and black sesame were less readily attacked.

No significant difference was found in the susceptibility of individual sesame plants to the Satsuma dwarf virus or in that of plants at various stages of growth. Plants inoculated at the one- or two-leaf stage developed, however, the most distinctive symptoms; and such young plants are considered to be the most suitable indicator plants for Satsuma dwarf virus. Furthermore, sesame plants are much more tolerant to high temperatures than most leguminous plants; and they are useful as indicators for the virus even in midsummer.

For the purpose of determining the thermal relations of infection, sap inoculations were repeated at various temperatures in the screenhouse. When sesame plants were exposed to a higher temperature than 34°C just after inoculation, they usually developed no symptoms; but sometimes systemic infection occurred ten days after inoculation. On the other hand, when the inoculated plants were kept at 25°C for more than eight hours, they showed striking symptoms even if they were later exposed to temperatures higher than 36°C. In midsummer, sesame plants inoculated in the evening exhibited 100 per cent infection, but inoculations made at midday resulted in a very low rate of infection.

Infectivity Tests with Other Citrus Viruses on Legumes and Sesame

Other citrus viruses, including those of tristeza, vein enation, and Hassaku dwarf, were repeatedly inoculated to legumes and sesame plants; but no marked symptoms appeared on these plants. Therefore, the symptoms described above are likely to be characteristic of Satsuma dwarf virus, and it is probable that Satsuma dwarf virus is a special strain of psorosis virus or a new and distinct citrus virus.

The Indicator Plants for Hassaku Dwarf

As previously reported (2), Mexican lime [*C. aurantifolia* (Christm.) Swing.] plants inoculated with Hassaku dwarf virus developed typical vein clearing, vein corking, stem pitting, and stunting, but neither the leaf flecking nor zonation symptoms of psorosis. Inoculated standard sour orange (*C. aurantium* L.) seedlings showed vein clearing, stem pitting, and sometimes stunting but no vein corking.

Tissue-graft transmissions of Hassaku dwarf virus were made to Tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco), rough lemon (*C. jambhiri* Lushington), Marumera (*C. obovoidea* Takahashi), and King mandarin (*C. nobilis* Loureiro). These inoculations led to stem pitting and stunting in both Thornton and Orlando tangelo, but no characteristic symptoms of xyloporosis on these plants. Hassaku dwarf virus, however, caused severe stunting in rough lemon and also striking vein clearing, vein corking, stem pitting, and severe stunting in Marumera (Fig. 2), which does not develop such conspicuous symptoms when infected with Satsuma dwarf virus. Moreover, this virus caused vein clear-

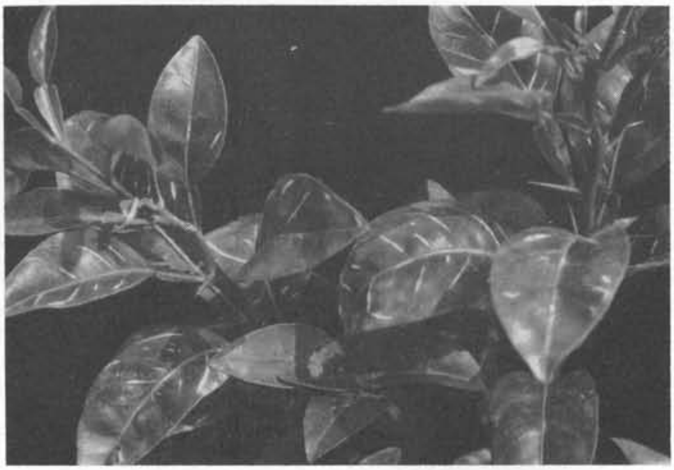

FIGURE 2. *Vein corking induced in Marumera by Hassaku dwarf virus after transmission by tissue grafting.*

ing in King mandarin, which was similar to that caused by Satsuma dwarf virus.

The results of the experiments reported above suggest that the cause of Hassaku dwarf may be a severe strain of tristeza virus, but the possibility that a mixture of two or more viruses is involved must be recognized.

Discussion

Grant and Corbett first reported mechanical transmission of infectious variegation virus to leguminous plants. The symptoms produced on *Vigna sinensis* 'Ramshorn Blackeye' shown by them considerably resemble those caused by Satsuma dwarf virus. They were successful in retransmission of this virus from infected legumes to citrus seedlings. Wallace and Desjardins (3) succeeded in transmitting mechanically a strain of the same virus to Chicago Pickling cucumber. They were also successful in retransmission of the virus from infected cucumber to lemon and *Crotalaria spectabilis.*

Repeated attempts to retransmit the Satsuma dwarf virus from legumes or sesame plants to Satsuma seedlings by means of the ordinary Carborundum method were not successful. Furthermore, the authors employed dodder plants (*Cuscuta japonica* Choisy) for retransmission, but the results have been negative in the tests so far conducted. Dr. T.

J. Grant kindly suggested that we use frozen infected tissue of legumes or sesame as inoculum. Dr. J. M. Wallace also extended an excellent idea to separate the legume infecting virus from tristeza virus. Work along these lines is in progress in our laboratory.

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