Purification of Satsuma Dwarf Virus

S. TANAKA, Y. SAITO, and K. KISHI

THE AUTHORS, in 1962, carried out experiments on purification of Satsuma dwarf virus from infected kidney bean (*Phaseolus vulgaris* L.). The first experiment seemed to be successful and indicated that Satsuma dwarf virus is spherical in form (1).

Meanwhile, S. Yamada and his co-workers of Okitsu Horticultural Research Station have performed an interesting experiment. At the suggestion of J. M. Wallace, they attempted to screen out tristeza virus from Satsuma trees infected with Satsuma dwarf virus by using trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings as a screen. They believed their experiment successful because they obtained trifoliata seedlings carrying Satsuma dwarf virus apparently free from tristeza virus. However, their further experiments revealed that these Satsuma-dwarf-inoculated trifoliata plants still carried a bit of tristeza virus, although the reaction by the lime test was extremely faint.

Materials and Methods

In 1966, the authors tried to repeat the experiment and to reconfirm the result obtained by a previous experiment. They grew seedlings of kidney bean (var. Satisfaction) and inoculated them by means of expressed sap from the trifoliata seedling which was supposed to carry Satsuma dwarf virus, but not tristeza. The authors had some diffiulty in transmitting the virus to bean seedlings from the trifoliata source as compared with inoculations from affected Satsuma trees.

In spite of the authors' efforts, the sap inoculation of Satsuma dwarf virus to bean was not as successful this year as in the previous experiments reported by the author (2). This may be partly due to abnormal climatic conditions this year in Japan, and also to the existence of different strains among Satisfaction kidney beans. Bean seedlings are very easy to grow, but compared with sesame (*Sesamum indicum* L.), they seem unstable indicators.

The authors later grew white sesame seedlings and repeated the sap inoculation using trifoliata carrrying Satsuma dwarf virus and Satsuma trees carrying both tristeza and Satsuma dwarf viruses. This time the inoculation was successful, although inoculations from trifoliata were rather more difficult than those from Satsuma. Most bean and sesame

varieties are supposed to be susceptible to Satsuma dwarf virus, but not to tristeza.

Purification of the virus from infected sesame is more difficult than from infected bean because the sesame sap contains viscous or mucilaginous substances. In spite of such circumstances, the purification of Satsuma dwarf virus seems to have been successful, but its infectivity is not yet completely proved. Therefore, the results of the first experiment will be presented here. Identification of the purified substance is still under investigation.

Results and Conclusions

The affected leaves of bean with Satsuma dwarf virus were macerated in 0.01 M phosphate buffer solution which was also 0.02 M in ethylene diamine tetra-acetate. Differential centrifugation of this sample was repeated 6 times at 30,000 rpm (60,000 to 70,000 g). The final preparation obtained was highly infectious and was composed of spherical particles measuring 26 mμ in diameter.

When the homogenate sample was treated with chloroform and differentially centrifuged, the virus obtained was again as stated above, but its infectivity was lost. Therefore, this virus seems to be rather labile.

Meanwhile, three other methods were applied to the cell sap prepared from infected bean leaves. 1) The cell sap was adsorbed on a diethylamino ethyl-cellulose column and then was eluted in 0 to 1.0 N NaCl solution. In this case, most of the virus was eluted with the NaCl-free solution (buffer solution). 2) The virus was precipitated by a saturated solution of ammonium sulphate, and then desalted by Sephadex (G50, Medium). 3) The sap was agitated, mixed with fluoro-carbon (1:1 volume) for 15 min and centrifuged. The virus remained in the supernatant liquid.

However, these three methods all failed to remove completely the normal plant components.

Literature Cited

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