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# Isolation and identification of broad bean wilt virus in faba bean

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**Abstract**

A virus isolate (B934) from faba bean was collected in Hangzhou, Zhejiang province. The isolate could infect 13 plant species in four families. Its thermal inactivation point was 50℃~55℃, dilution end point was $10^{-3}$~$10^{-4}$ and longevity *in vitro* was 4 days. It was purified from *Chenopodium quinoa* by a method that yielded up to 128 mg/kg tissue. Purified preparation of B934 contained isometric particles ca. 25nm in diameter. The capsid protein of B934 contained two polypeptides with molecular weights of 42500 and 21200. In agarose gel diffusion tests using antisera to BBWV Ce-120, B934 gave a continuous precipitation line between B934 and Ce-120. The results indicate that B934 belongs to BBWV serotype Ⅱ group.

Key words: faba bean; broad bean wilt virus; identification

## 0 Introduction

Broad bean wilt virus (BBWV) is known to be present in various countries and has been isolated from a number of cultivated plants and weeds ( Taylor & stubbs, 1972). In China, this virus has been identified in many cultivated legumes including cowpea (*Vigna sinensis*), faba bean (*Vicia faba*), pea(*Pisum sativum*) and soybean (*Glycine max*) (Xi et al., 1982; Xu et al., 1985; Chen et al., 1985, Xia et al, 1985). It has also been identified in spinach (*Spinacia oleracea*), pepper (*Capiusum annum*), celery (*Apium graveolens*) and some weeds. The virus causes serious diseases on these crops, especially on faba bean and pea. BBWV has rather wide host range and is transmissible by sap inoculation and by several aphid species in non-persistant manner ( Stubbs,

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1949). This paper describes some of the properties of BBWV isolate collected from a faba bean field in Hangzhou in 1993.

## 1 Materials and Methods

1. Virus isolates and propagation

The isolate B934 was collected from a naturally infected faba bean plant in Hangzhou in 1993. The isolate BBWV Ce-120 was kindly presented by Professor Pu Zuqin, Nanjing Agricultural University. The virus isolates were maintained in *Vicia faba* and *Chenopodium amaranticolor* by mechanical inoculation.

2. Host range

Test plants in groups of three to five were inoculated by rubbing inoculum onto leaves previously dusted with carborundum. Symptomless plants were checked for virus by back inoculation to *C. quinoa*.

3. Stability in sap

Determinations of thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) were made in the conventional manner. *C. quinoa* was used as test plant. Three to five plants were inoculated per treatment and the experiments were repeated twicly.

4. Virus purification

Locally and systemically infected *C. quinoa* leaves 8-10 days after inoculation were ground (1g/2ml) for 1 min at room temperature in 0.5M potassium phosphate, pH7.5, containing 0.1% 2-mercaptoethanol, 25% sucrose and 0.01% Triton X-100. The homogenate was left at 4℃ for 60min and centrifuged at 6000g for 20min. The supernatant was mixed with 2.5% Triton X-100, 4% polyethylene glycol (MW 6000) and 0.1M NaCl, and stirred overnight at 4℃, then centrifuged at 8500g for 15min. The pellets were resuspended in 0.01M phosphate buffer (pH7.5) containing 0.01% Triton X-100. After low speed centrifugation, the supernatant was centrifuged in Beckman SW 55.2 Ti rotor for 2hr at 70000g and the pellets were resuspended in 0.01M phosphate buffer. The preparation was then underlaid on 8ml cushions of 20% (W/V) glycerol and centrifuged at 78000g for 2hr. The final pellets were resuspended in a small volume of 0.01M phosphate buffer. Ultraviolet absorption spectra were measured in a Shimadzu UV-2201 Recording Spectrophotometer. Virus yields were estimated assuming $A^{0.1\%}_{1cm\ 260nm} = 8.0$ (Xu et al, 1988).

5. Electron microscopy

Virus preparation was negatively stained in 2% aqueous potassium phosphotungstate (pH7.0) and examined in a JEOL JEM-1200 EX Electron Microscope.

6. Polyacrylamide gel electrophoresis of polypeptides

Virus preparation was mixed with an equal volume of electrophoresis sample buffer (0.01M Tris, 4.6% sodium dodecyl sulfate, 1% 2-mercaptoethanol and 20% sucrose) and heated for 2min in boiling water, then rapidly cooled. The samples were put on 12.5% acrylamide ( with 0.4% bisacrylamide) gel using spacers of 5% acrylamide with 0.17% bisacrylamide. Proteins were electrophoresed for a few hours at 40 mA. All the molecular weight standards came from Life Technologyes Incorporation. Gels were stained overnight in 0.25% coomassic brilliant blue and destained in methanol-acetic acid and scanned in a Shimadzu CS-930 Double Wavelength Scanner.

7. Serology

Antisera to BBWV Ce-120 belonging to serotype Ⅱ were obtained from a rabbit given three intramuscular (1ml) and one intravenous (0.5ml) weekly injections of purified BBWV Ce-120 emulsified with complete Freund's adjuvant but incomplete adjuvant for subsequent injections, and bled 10 days after the last injection.

Serological tests were done by double diffusion tests in 0.8% agarose dissolved in 0.85% sodium chloride (NaCl) containing 0.02% sodium azide. The pattern in the agar consisted of six peripheral wells and a central well. BBWV antiserum was placed in the central well and crude plant extracts of BBWV B934 and Ce-120 and healthy plant extracts were added in the peripheral wells.

## 2 Results

1. Host range and symptoms

Of 18 tested species, 13 plant belonging to *Leguminosae*, *Solanaceae*, *Chenopodiaceae*, and *Amarantheae* were infected by BBWV B934. On faba bean, the isolate produced vein clearing and developed severe systemic mottle and some leaflet malformation and stunting (Fig.1). On pea, a mild chlorotic mottle developed on the young leaves followed by wilting of the growing point (Fig. 2).

Responses of the different plant species to inoculation of B934 are summarized in six categories,

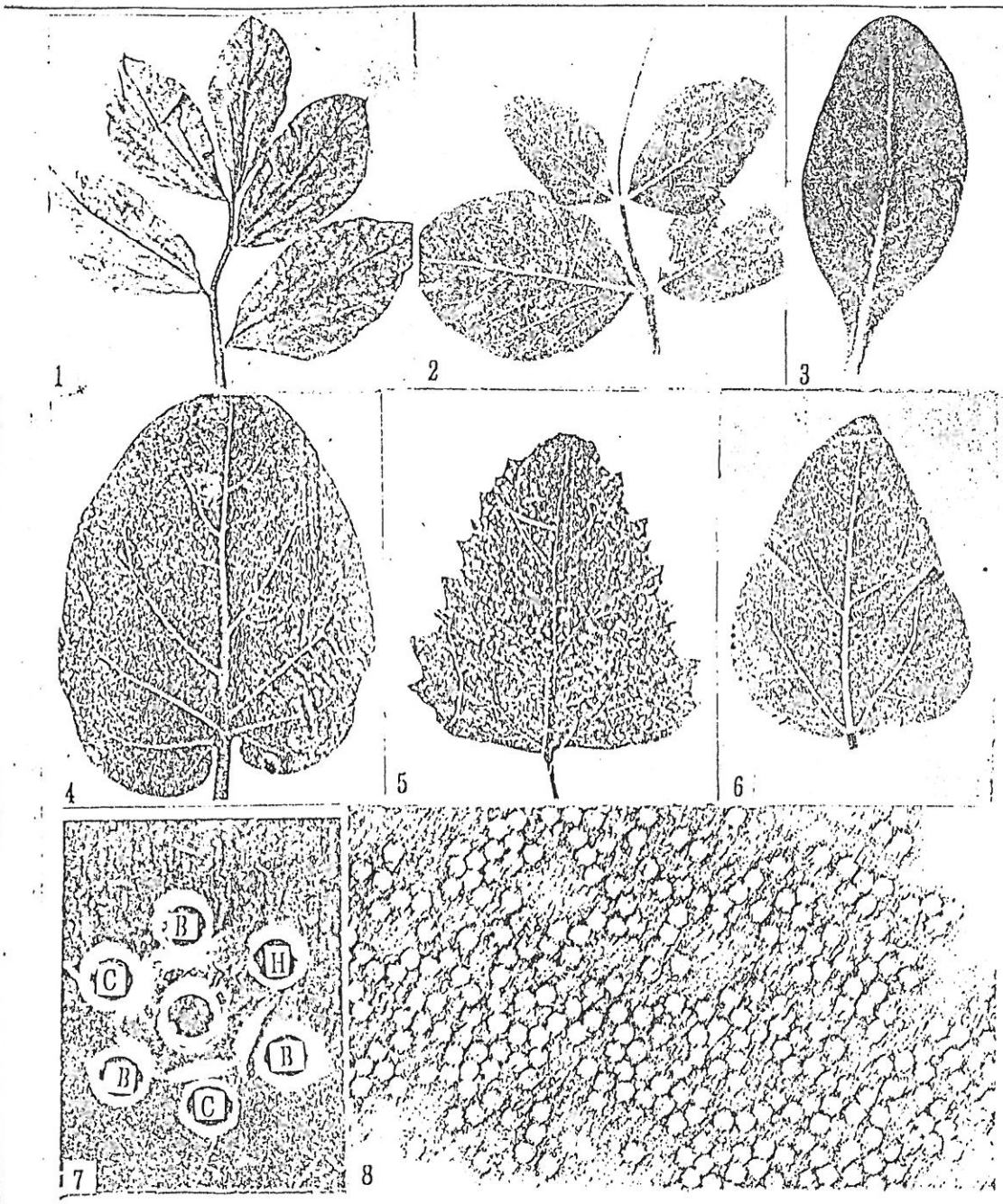


Fig.1 Systemic symptoms on *Vicia faba*.
Fig.2 Systemic symptoms on *Pisum sativum*.
Fig.3 Ringspots on *Petunia hybridum*.
Fig.4 Ringspots on *Nicotiana glutinosa*.
Fig.5 Local lesions on *Chenopodium quinoa*.
Fig.6 Local lesions on *Vigna sinensis*.
Fig.7 Immunodiffusion reactions of B934(B), BBWV Ce-120(C), and healthy extracts(H) with Ce-120 antiserum.
Fig.8 Particles of B934 negatively stained in potassium phosphotungstate (X120000).

(i) Local reaction on inoculated leaves followed by systemic symptoms: *Chenopodium album*, *C. quinoa* (Fig. 5).

(ii) Local lesions: *Vigna sinensis* (Fig.6) and *Celosia cristata*.

(iii) Systemic symptoms with local ringspots: *Petunia hybridum*(Fig. 3), *Nicotiana glutinosa* (Fig.4), *N. clevelandii*, *N. tobacum* cv., Huang Miaoyu.

(iv) Systemic symptoms without local reaction: *Datura stramonium*.

(v) Symptomless infection: *N. tobacum* cv. Samsum.

(vi) No infection: *Glycine max*, *Phaseolus vulgaris*, *P. mingo* *Dolichus lablab*, *Cucumis sativus*, *Brassica chinensis* and *Nicandra physoloolos*.

2. Stability in sap

Properties *in vitro* were determined using infective *C. quinoa* sap to inoculate *C. quinoa*. The TIP was between 50℃ and 55℃, DEP was between $10^{-3}$ and $10^{-4}$ and LIV was 4 days.

3. Virus purification and electron microscopy

The purification method described was suitable for B934. The ultraviolet absorption spectrum of the virus preparation was typical of nucleoproteins. The ratio of optical density at 260 and 280 nm was about 1.54. Assuming that the extinction coefficient for BBWV is 8.0, the yield of purified virus was calculated to be 128mg/kg infected tissue.

Purified preparation of B934 contained isometric particles ca.25nm in diameter (Fig. 8).

4. Capsid proteins

Polyacrylamide gel electrophoresis showed that BBWV B934 contained two polypeptides with molecular weights of 42500 and 21200 respectively (Fig.9).

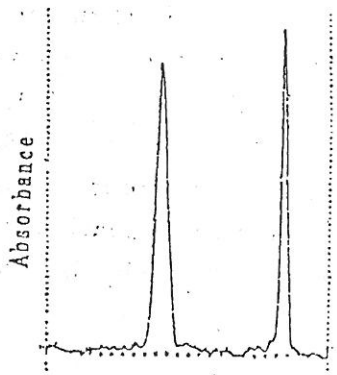


Fig.9 Densitometer scan of B934 peptides separated by electrophoresis in 12.5% polyacrylamide gel.

5. Serology

In agarose double diffusion tests, sap extracts of B934 infected leaves gave a single line of precipitaton when tested against Ce-120 antiserum. A continuous precipitation line was formed between B934 and Ce- 120 placed in adjacent wells. No precipitation spurs developed, showing the B934 isolate serologically closely related to BBWV (Fig.7).

## 3 Discussion

BBWV was first described by Stubbs (1949). Sahambi et al. (1973) stated that BBWV shared many properties with comoviruses. Matthews (1979) had assigned BBWV to the comovirus group as a possible member. In the Fifth ICTV Report (Francki et al. 1991), the virus was assigned to a new group-Fabaviruses with only two members by now.

BBWV is widespread in China. Since it was first reported in 1982 in the Bejjing area, it has been identified in crops and weeds in several provinces including Jilin in the north, Jiangsu in the east, Yunnan in the south-west and Xingjia in the north-west. The B934 isolated from faba bean in Hangzhou was idetified as BBWV based on its host range, stability in sap, particle morpholoy, molecular weight of coat protein and serology. We found this virus had very high incidence in the Hangzhou area. This is the first report of BBWV in Zhejiang province.

Based upon spur formation in agarose gel plate, Uyemoto and Provvidenti (1974) divided seven isolates of BBWV into two distinct serological type groups and named serotypes Ⅰ and Ⅱ group. Xu et al. compared some Chinese BBWV isolates and concluded that all the Chinese isolates belonged to serotype Ⅱ group. The B934 isolate we studied also belongs to serotype Ⅱ group.

The molecular weights of coat protein of B934 were different from the values reported by Doel (1975) and Lisa et al. (1982). They reported the molecular weight of the small peptide was up to 27000. The differences may result from the change in structure of coat protein between B934 isolate and others. It may also contribute to the determination method and the quality of the molecular weight standards. The molecular biology of this virus is being studied.

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