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Mechanical Transmission of Soil-borne Barley Yellow Mosaic Virus

By

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With 3 figures

The yellow mosaic disease of winter barley (*Hordeum vulgare* L.), caused by the soil-borne Barley Yellow Mosaic Virus (BaYMV) causes increasing damage in winter barley crop of northern and central West Germany, France (Huth 1981, 1982) and eastern England (Hill and Evans 1980). In some regions large areas are completely and uniformely infested with the virus and the damage in susceptible crop can cause grain yield reductions from 40 % up to complete losses ("winter killing") (Huth 1982). Leaves of infected plants have irregular chlorotic streaks of varying size running along their veins (Fig. 1), with upward rolling of leaf margins (Hill and Evans 1980). Streaks are most distinct on youngest leaves, in certain cultivars chlorotic streaks can change to orange-yellow color accompanied by rapid death of the older leaves.

The virus was for the first time reported from Japan by Ikata and Kawai (1940), where it became afterwards the most important disease of barley (Inouye and Saito 1975, Sanada 1982 pers. comm.). In the Federal Republic of Germany, the symptoms had first been observed in the early seventies and were identified as being caused by BaYMV in 1978 by Huth and Lesemann. As chemical control agents are not available, the disease can only be controlled by growing resistant cultivars. The importance of available resistant German cultivars (Barbo, Birgit, Franka and Ogra, Huth 1982) is limited due to their lack of other important agronomic characters. Therefore, new resistant lines and cultivars are urgently needed, preferably with different genetic bases of resistance. As a prerequisite for both, genetic studies as well as successful breeding, an efficient test method for BaYMV is needed. Up till now it can only be tested once a year under non-reproducible conditions in infested fields. In the present paper experiments are reported which succeeded in mechanical transmission of soil-borne BaYMV.

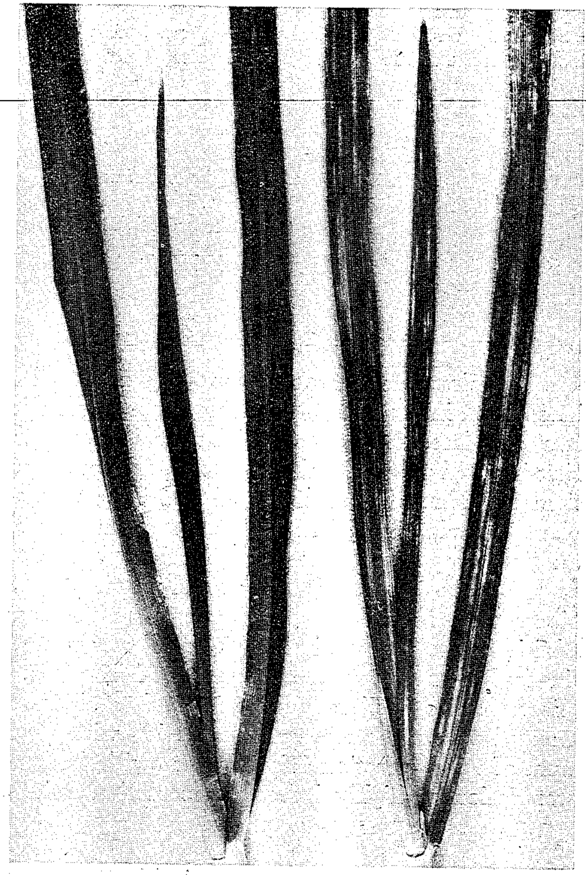

Fig. 1. BaYMV infected plant (right) and non-infected plant (left) of winter barley cv. Gerbel

Materials and Methods

As experimental material plants of the highly BaYMV-susceptible winter barley cv. Gerbel were used. Test-plants were grown in the greenhouse in 30 × 50 cm containers with 20 to 30 plants per container at 10—12 °C and 14 h daylength (natural light supplemented by Osram lamps HQI-TS 400 W/D).

For growing the virus, Gerbel plants were cultivated in heavily infested soil from a field of Sunstedt near Braunschweig (Germany F.R.) in a growth chamber. Temperatures were 6/4 °C (day/night) in the 1—3 leaves stage and were subsequently raised corresponding to the growth stage up to a maximum of 20/18 °C. Daylength was adjusted to 12 h at 16,000 lux light intensity. Plants showing typical BaYMV-symptoms (a maximum of 33 % of all plants) but otherwise vigorous were subsequently used as donors for preparation of the inoculation sap. The two or three youngest leaves of donor plants were cut into 3 mm pieces and triturated in cold water or chemical additive (3—5 °C) in a freezed porcellain mortar. Depending on the experiment 3—5 g of plant material and 5—6.5 ml of additive were used for subsequent inoculation of one container (i. e. one treatment). The additives to the inoculation sap are given in Table 1 together with their concentrations and pH-values.

Test-plants in the 3—4 leaves stage were powdered with carborundum abrasive (300 mesh) and afterwards thoroughly rubbed off with a little sponge soaked in the respective inoculum fluid. The inoculum was allowed to dry on the treated leaves without post-washing and the plants were kept in the shadow at 18—20 °C for about 24 h and were afterwards transferred to a growth room at temperatures of 10/8 °C (day/night) and 12 h daylength (16,000 lux light intensity, fluorescent tubes Philips TL 215 W/33 RS and Sylvania Gro-Lux FR 96 T 12/GRO 235). In some experiments, the inoculation procedure was repeated seven days after the first treatment. Treated plants did usually not show severe injury as a consequence of the inoculation procedure itself. However, sodium sulphite (Na_2SO_3) as an additive to the inoculum fluid regularly caused necrosis of the leaf tips a few hours after the treatment. This did however not influence the further healthy growth of these plants.

Results

The first yellow mosaic symptoms appeared three weeks after the first inoculation in the double-inoculated sodium sulphite treatment (Fig. 2). Only two days later, the treatment inoculated once with Na_2SO_3-additive showed first infected plants. At the same day, the container treated twice with potassium phosphate buffer exhibited one infected plant, whereas the group of plants inoculated only once with potassium phosphate buffered sap showed the first symptoms 25 days after the first treatment. The proportion of infected plants subsequently increased more rapidly in the sodium sulphite treated plants than in the potassium phosphate-inoculum treated ones. For both additives, double inoculation yielded faster progress of infection rates and, finally higher maximum proportions of infection. Whereas the repeated application of potassium phosphate-buffered inoculum yielded finally 100 % infected plants, the repeated inoculation with Na_2SO_3-fluid brought a maximum of 96 % infection (Fig. 2). Therewith, both additives are useful for the preparation of highly infective inoculation fluids for practical application.

Table 1
Chemical additives to the inoculation sap

Substance	Concentration	pH	Effective as
$K_2HPO_4 + KH_2PO_4$	0.04 M	7.0	buffer
Na_2HPO_4	0.10 M	9.6	buffer
Na_2SO_3	0.10 M	9.9	reducing agent
Glycocoll-buffer (Sörensen)		9.4	buffer
Na-DIECA*)	0.01 M	8.1	chelating agent
Bentonite (in 0.04 M phosphate buffer)		7.7	nuclease-adsorber

*) Sodium diethyldithiocarbamate: chelates copper and inhibits polyphenoloxidase, thus preventing the formation of virus-inactivating o-quinones (Gibbs and Harrison 1980).

Similar success is also possible with sodium phosphate additive as indicated by the results in Figure 3 (75 % infection). Other additives did however not improve the infectivity of inoculum; with glycocoll-buffer (48 %) and sodium diethyldithiocarbamate (Na-DIECA, 31%) similar infection rates were obtained as with the addition of pure water to the inoculation fluid (43 %). The addition of Bentonite in phosphate buffer prevented virus transmission completely.

In one of the experiments it was also studied if storage of virus-containing plant material under low temperatures (—22 °C) affects infectivity of plant sap. As shown in Figure 3 is the infectivity of inoculum prepared from frozen leaves about 20 % lower than that of fresh material. Nevertheless, it is demonstrated that preservation of infection material by simple freezing is possible; this is important for an economic production and storage of large quantities of unique inoculum.

Discussion

It could be demonstrated, that BaYMV-infection via mechanical transmission is feasible. The technique reported is faster and easier than the field-test, and much more reliable than a greenhouse-test in infested soil, which

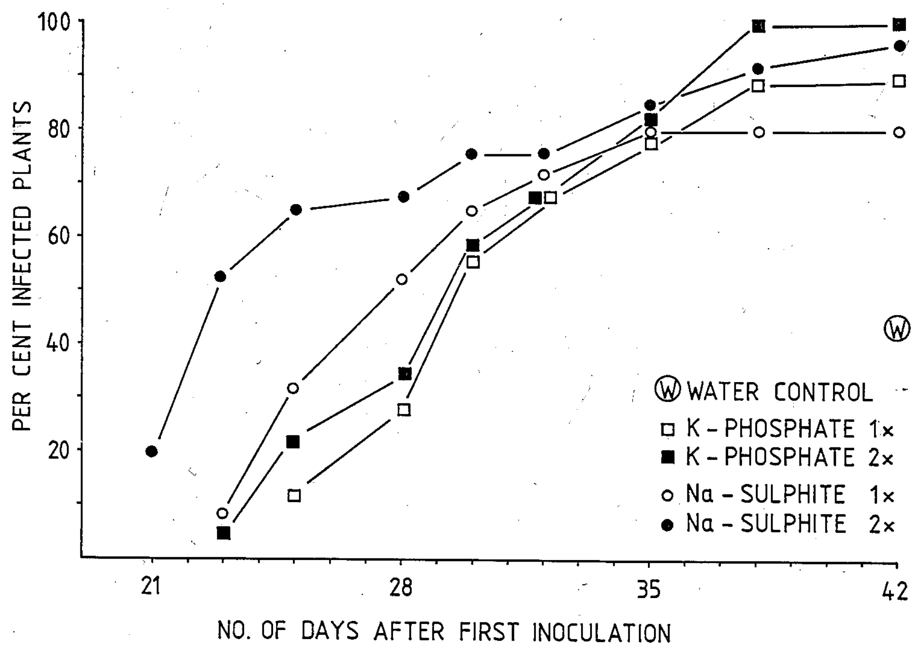

Fig. 2. Progression of the frequency of BaYMV infected plants (i. e. appearance of visible symptoms) of cv. Gerbel after single and double manual inoculation with sodium sulphite and potassium phosphate-buffered fluids, respectively

yielded only 33 % infection of the susceptible cv. Gerbel (unpublished data). The salient point is the addition of phosphate or reducing Na_2SO_3 to the inoculum. This is in full agreement with the earlier findings, that different phosphates (K_2HPO_4, Na_2HPO_4) and sodium sulphite can increase the efficiency of mechanical inoculation of many viruses spectacularly (MATTHEWS 1981, SCHMELZER 1980).

Secure infection not only allows testing of pure breeding material such as cultivars or breeders' strains, but also the rapid and large-scale screening of segregating new breeding material. For practical application of the technique, the production of large and unique quantities of inoculum is still a major problem. Besides increasing the virus titer in the source plants, optimum conditions for low-temperature storage of infected plant material, plant sap or inoculation fluid, respectively, have to be elaborated.

According to limited published evidence, soil-borne BaYMV is most likely transmitted by at least one fungus, *Polymyxa graminis* Ledingham, into the barley roots (HILL and EVANS 1980, ZERLIK 1982 pers. comm.). In this

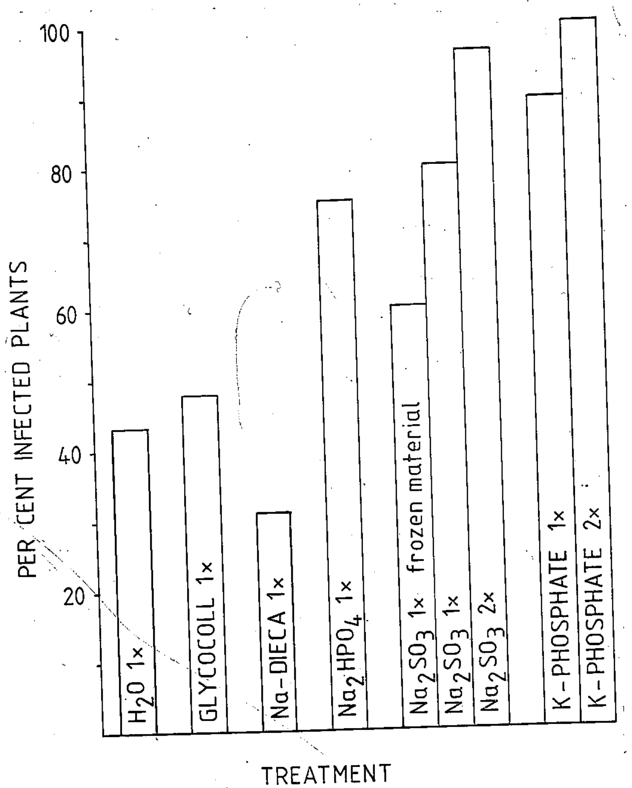

Fig. 3. Final frequencies of infected plants of cv. Gerbel six weeks after inoculation with fluid containing the various additives indicated

case, cultivar resistance might not only mean resistance to the virus but also resistance to the transmitting fungal vector, or both. At the present time, the real mechanisms of resistance in cultivars like Birgit and Franka are not understood. Consequently, it is still possible that a variety is susceptible to the virus in the mechanical inoculation test, although it exhibits field-resistance to the *Polymyxa*/BaYMV-complex. Such questions can be answered when results of comparative experiments of mechanical and natural transmission are available.

Summary

Leaves of winter barley (*Hordeum vulgare* L.) cv. Gerbel were mechanically sap-inoculated with Barley Yellow Mosaic Virus (BaYMV). Different additives to the inoculation fluid were tested. Whereas the dilution of plant sap by water alone resulted in an infection rate of 43 %, the addition of sodium sulphite (80 %) and potassium phosphate-buffer (89 %) increased the proportion of infected plants substantially. Infectivity was further increased by repeated inoculation, when sodium sulphite yielded 96 % and potassium phosphate 100 % of infection. The usefulness of the technique in further research and application is discussed.

Zusammenfassung

Mechanische Übertragung des bodenbürtigen Gelbmosaikvirus der Wintergerste

Blätter der Wintergerstensorte Gerbel wurden mit dem Preßsaft virusbefallener Pflanzen abgerieben. Es wurden verschiedene Zusätze zum Inokulum verwendet. Während mit Wasser verdünnter Saft eine Infektionsrate von 43 % hervorrief, wurde die Häufigkeit infizierter Pflanzen durch Zusatz von Natriumsulfit (80 %) oder Kaliumphosphatpuffer (89 %) erheblich gesteigert. Eine weitere Verbesserung des Infektionserfolges wurde durch eine wiederholte Abreibung mit Na_2SO_3 (96 %) und Kaliumphosphat (100 %) erreicht. Die Bedeutung dieser Infektionsmethode für die weitere Erforschung der Gelbmosaikvirusresistenz sowie für die praktische Resistenzprüfung und Züchtung wird diskutiert.

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