Reduction in Symptom Expression of Belladonna Mottle Virus Infection on Tobacco Plants by Boron Supply and the Antagonistic Action of Silicon

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Boron and silicon have a dramatic influence on the expression of symptoms caused by plant virus infections. For tobacco plants, boron decreases and silicon enhances symptom expression after belladonna mottle virus infection. Infected leaf tissues are highly enriched on both trace elements. Boron supply stimulates the silicon accumulation. In the applied concentrations the trace elements have no phytotoxic effects on the host. Except endogeneous β-interferones, this could be the first example of antiviral action without any cytotoxicity.

Introduction

In a general study of the simultaneous action of boron and silicon on plant metabolism and in the aim to search for possibilities to overcome physical, chemical and pathogenic stress factors we have examined the course of plant virus infections as a function of both elements.

The influence of trace elements on plant growth and nutrition has been carefully studied [1, 2] and several relations are well known. On the other hand plant virology is a ninety years old well established science. About 1000 plant viruses are known and some of them are subject of fundamental researches [3–6]. The physiological role of boron in higher plants has been examined [7–11] as well as the actions of silicon [8, 12].

Boron is essential for higher plants. It is *e.g.* a regulator of phytohormone level such as auxin, IAA, ABA [13, 14]. The Ca metabolism [15] as well as the metabolism of cell wall polysaccharides [16] are also depending on boron content. The physiological role of silicon is less understood. Silicon is an important constituent of cell walls. It is necessary for the mechanic stabilization of these organes. High amounts of silicon are found in *Equisetum, Urtica* and rice [1, 2].

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Simultaneous effects of boron and silicon on healthy and on infected plants seem to be unknown. In a newly published paper the effect of another trace element, manganese, on symptom expression of beet mild yellowing virus (BMYV) of sugar beet is described [17]. In this paper we report about the action of boron and silicon on the symptom expression in belladonna mottle virus (BdMV) infected tobacco plants.

Materials and Methods

Seeds of tobacco plants (variety Samsun) were sown at the beginning of June 1987 either in unfertilized peat or in peat treated with boron (1 ppm) and/or silicon (60 ppm) fertilizer solutions. In a preliminary study these concentrations of both trace elements have been shown to be favourable for tobacco plant growth.

Three months later the plants (about 20 cm long), including control plants grown in peat cultures without boron and silicon supply, were transferred to hydrocultural pots of 2 liter volume. The roots have been cleaned thoroughly before the plants were transferred into the nutrient solutions.

The following hydroponic solutions have been used

1. Hoagland A and B (control conditions: neither boron nor silicon)
2. Hoagland A and B including 1 ppm boron (without silicon)

3. Hoagland A and B including 60 ppm silicon (without boron)
4. Hoagland A and B including 60 ppm silicon and 1 ppm boron.

The solutions Hoagland A and B have been prepared as described in [18], however the Hoagland standard solution B was free of boron. Boron and silicon have been supplied individually using stock solutions. These solutions were prepared by titrating water glass (Merck) and borax (Sigma) respectively to pH 5.5 with sulfuric acid (Merck) avoiding any formation of SiO_2-gels or suspension. The hydroponic nutrient solutions were aired continuously and exchanged every 2 or 3 weeks in all cases.

The plants were inoculated with the belladonna mottle virus (BdMV) at December 3, 1987. After powdering two adult leaves of each tobacco plant with carborundum the inoculation was carried out with 0.5 ml press sap from virus infected tobacco leaves. Best evidence of infection could be seen after reducing the plant heights up to a third about 3 weeks after the inoculation. The plants were photographed on February 2, 1988 (see Fig. 1–4).

Another cultivation procedure of tobacco plants was propagation of cuttings (about 10 cm long) from healthy parent plants grown in normally fertilized peat. Their leaves were then shortened strongly to reduce transpiration and the cuttings were placed into tap water for rooting. When the first roots appeared (after approx. 1 week), the tap water was replaced by nutrient solution Hoagland A and B beginning with half concentration during a transition period of two weeks for osmotic adaption of the cutting. After rooting the cuttings were placed into the corresponding nutrient solutions on the beginning of April 1988 as described above. Two inoculations were carried out in this series (on May, 3 and June, 24 resp.) because only weak virus infection symptoms could be detected at the first inoculation. The heights of tobacco plants were reduced on the beginning of June.

Parallel to the hydrocultural attempts a study on the basis of peat cultures was started in spring of 1987. The tobacco plants grew in pots filled with peat (TKS O) during the whole period. Four pots were prepared with different contents of B / Si: 0 / 0, 0 / 1100 ppm, 1 ppm / 0 and 1 ppm / 1100 ppm. The higher concentrations of Si takes into account the soil adsorption effects. The whole tobacco plants (one plant per pot) were taken out of the pots and immersed into the corresponding nutrient solution (Hoagland A and B according to boron and silicon) twice a week for about 1 h during a period of 5 weeks. Control plants remained uninoculated and unfertilized with B and Si additionally (compare Table II).

Boron and silicon analysis of leaves dry matter: for all plant material, the boron and silicon content was determined. 100 to 500 mg of dry matter was treated by pressure ashing method with 1–3 ml conc. HNO_3, according to [19]. The analysis were performed by inductively coupled plasma emission spectroscopy (ICP) using the boron emission frequency of 49.68 nm and the silicon frequency of 251.66 nm [20]. The silicon solutions obtained by the ashing method were totally clear indicating the absence of suspended silicate. The spectrometer was a JY 38^+ from Instruments SA (France).

Results and Discussion

Six weeks after inoculation with BdMV tobacco plants which were cultivated in hydrocultures in the second half of 1987 showed the first symptoms of virus infection. After reducing the plant heights up to a third and changing the nutrient solutions as described above the virus symptoms could be recognized particularly distinct on the new leaves. Typical symptoms on the four tobacco plant series are shown in the Fig. 1 to 4 and listed in Table I.

Fig. 1 shows the control experiment realized in boron and silicon free hydroponic solution. The virus infection symptoms are expressed as described earlier [21, 22]. The ring-like chlorotic mottling predominates here. About 10% of the leaf area is covered by pale yellow necrotic spots.

In contrast to the normal development of the virus infection the boron fertilized tobacco plants are free from necrosis (Fig. 2). However, the chlorotic mottle symptoms are expressed distinctly. This indicates that the plant is influenced by virus infection to a lesser extent when boron is present.

The most pronounced virus symptoms can be observed in the hydrocultural pot with a Si content of 60 ppm (Fig. 3). About 40% of the leaf area consists of brown-yellow necrotic spots surrounded by chlorotic halos. Furthermore, chlorosis can be observed in the intercostal fields.

Intermediate symptoms are found in the culture with simultaneous supply of B and Si (1 ppm and

Fig. 1. Tobacco leaf nine weeks after inoculation with BdMV (nutrient solution: Hoagland A and B (without boron); control).

Fig. 2. Tobacco leaf nine weeks after inoculation with BdMV (nutrient solution: Hoagland A and B including 1 ppm boron).

Fig. 3. Tobacco leaf nine weeks after inoculation with BdMV (nutrient solution: Hoagland A and B (without boron) including 60 ppm silicon).

Fig. 4. Tobacco leaf nine weeks after inoculation with BdMV (nutrient solution: Hoagland A and B including 1 ppm boron and 60 ppm silicon).

60 ppm, resp.). There are only a few necrotic spots (<5% of the leaf area). The original green colour of the leaves is observed only adjacent to the veins. The intercostal fields are chlorotic to a certain extent. The symptom expression is clearly situated between plants supplied with B and Si (Fig. 4).

The repetitive hydrocultural attempts in spring and summer of 1988 based on cuttings led to similar results. Generally, it was striking that the symptoms were weaker compared to hydrocultural attempts carried out in winter of 1987/1988. Furthermore, the virus symptoms could be noticed later than those ob-

Table I. Summary of observed symptom expressions in BdMV infected tobacco plants.

Experiment	Fig.	Chlorosis	Necrosis			
			Main coloration	Size [mm]	Density of necrotic spots	Approx. part of leaf area
control	1	+	white to yellow	1–5	medium	10%
boron	2	+	–	0	0	0%
silicon	3	+	brown	1–10	high	40%
boron + silicon	4	+	yellow to brown	1–2	small	5%

served in winter period. This may be an indication for the influence of light activity on symptom expression by BdMV.

The study carried out in spring and summer of 1987 with peat as a substrate led also to similar results again. In this case a relative high silicon content was chosen because of the extended adsorption properties of peat. After inoculation of tobacco plants with BdMV the Si treated tobacco (without B) showed extended necrosis as it was expected. The tobacco treated simultaneously with Si and B showed a weak necrosis only. No necrosis could be noticed on the tobacco leaves treated with B (without Si). These results are in total agreement with those of the hydrocultures.

Table II. Boron and silicon content in dry matter of BdMV infected tobacco leaves.

Experiment	Solution	Content [ppm] Boron	Silicon	$\frac{B}{Si}$
control	1	15	140	0.1
boron	2	450	170	3
silicon	3	20	400	0.05
boron + silicon	4	400	1000	0.4
control (virus free)		25	110	0.2

Table II shows that supply of boron and/or silicon leads to high enrichments of these elements in the infected tissue of the cuttings. Boron supply stimulate the silicon accumulation but silicon does not influence the boron enrichment. The severity of the symptoms correlates with the boron-silicon ratio determined in the infected leaves. The control plants (virus infected and virus free) showed similar B and Si contents.

Conclusions

These results demonstrate that boron significantly attenuates the symptom expression of BdMV in tobacco plants. In contrast, a Si supply enhances dramatically the severity of disease. This is expressed distinctly by a total necrosis. The effects of B and Si which are enriched in the infected tissues are antagonistic.

Thus, the disease severity can be classified as follows:

	B-plant	<	B/Si-plant	<	control plant	<	Si-plant
B/Si	3		0.4		0.1		0.05

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