The Role of Boron, Silicon and Nucleic Bases on Pollen Tube Growth of *Lilium longiflorum* (L.)

J. Polster and M. Schwenk*

Lehrstuhl für Allgemeine Chemie und Biochemie der Technischen Universität München, D-8050 Freising-Weihenstephan, Bundesrepublik Deutschland

E. Bengsch

CNRS, Centre de Biophysique Moleculaire, F-45071 Orléans, Cedex 2, France and GSF (Gesellschaft für Strahlen- und Umweltforschung, Institut für Ökologische Chemie), Ingolstädter Landstraße 1, D-8042 München-Neuherberg, Bundesrepublik Deutschland

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Pollen Tube Growth, Boron, Silicon, *Lilium longiflorum*, Germination

It is possible to obtain pollen germination and pollen tube growth *in vitro* if boric acid is present. In this work the effect was studied using as a semiquantitative parameter the mean length ($\bar{l}$) of *Lilium longiflorum* pollen tubes. Pollen tube growth was examinated in dependence on boric acid, *ortho*-silicic acid, nucleic bases, Ca^{2+} and Zn^{2+} in 10% sucrose solution. The maximum of $\bar{l}$ is obtained for concentrations between 2–20 ppm boron. The simultaneous supply of silicon added as water glass leads to a synergistic stimulation effect on pollen tube growth and facilitates branching. The silicon action is preceded of a pollen tube growth inhibition period during 3 h. Adenine and guanosine are able to substitute partially boron as pollen germination and pollen tube growth stimulator. Concentrations of 100 ppm adenine leads to half the boron effect. The same stimulation effect is obtained by guanosine. Ca^{2+} can partially substitute boron as well. The stimulation action of boron is significantly attenuated by Zn^{2+} and by the herbicide Dicuran. These and preceding results from physiological studies indicate that boron and silicon should be essential trace elements for the regulation of molecular biological processes.

Introduction

Recent research has shown the dramatic influence which boron and silicon have on the symptoms produced by the *Belladonna Mottle Virus* in infected tobacco plants (*Nicotiana tabacum*). The preinfectional boron enrichement of the plants considerably weakens the symptom expression of the infection whilst silicon enhances them greatly [1]. It has also recently been found that boron and silicon applied in appropriate concentrations stimulate together strongly the growth of spinach (*Spinacea oleracea*) in hydrocultures. However, both elements can increase the inhibition effect of certain herbicides [2].

The most sensitive parts of plants to boron and silicon supply *in vivo* are obviously the reproductive organs. For example, boron deficiency or toxic excess leads to morphological and functional modification of pollen before other phenomena appear on the plant [3–5]. Optimal boron supply leads to strong pollen activity in plants [6–15].

Under *in vitro* conditions, boron is the most effective factor to obtain pollen germination and tube growth [16–41]. The effect of boron, however, can be influenced by other components, for example by Ca^{2+} [20–22, 25, 28, 30, 31, 42–44] and phytoregulators (IAA, gibberellic acid) [45–48].

From these and further results of literature [49–51] it became evident that boron and silicon might have central regulatory functions on vegetable systems and probably also for microorganisms and animals. Thus, the former poorly understood role which silicon plays takes on a greater importance. In an attempt to examine the influence of boron and silicon on pollen of *Lilium longiflorum, in vitro* pollen germination and pollen tube growth experiments appear to be especially indicated.

Boron and other elements are known to play an important part in the synthesis of pyrimidine bases in plant growth [52, 53]. In order to see whether a boron deficiency during germination and tube growth can be compensated for, nucleic bases and

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Reprint requests to Prof. J. Polster.

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similar compound were utilized in the presented experiments. Tests were also made to see if organic boron compounds had the same effects on pollen tube growth as boric acid. The effect of Zn^{2+} as a potential antagonist of boron in biochemical reactions and especially in germination and tube growth was also examined as well Ca^{2+}. Finally, the herbicide Dicuran was used as its toxicity can be considerable increased by supplying boron to plants.

Materials and Methods

In the present study aqueous solutions containing 9 ppm boron as boric acid and 10% sucrose were used for the growth of *Lilium longiflorum* pollen (*cf.* Röderer and Reiss [40]). In each series of tests uniform pollen charges were used. The following compounds were applied: boric acid (Merck), water glass (Merck), adenine (Merck), guanosine (Serva), AMP (Boehringer), adenosine (Merck), deoxyadenosine (Merck), deoxycytidine (Sigma), uracil (Sigma), uridine (Sigma), thymidine (Sigma), 2-thio-5-dihydroxyboronic uracil (= BTU, gift of Prof. Dr. D. Gabel, University of Bremen), $ZnSO_4$ (EGA-Chemie), $Ca(NO_3)_2$ (Merck), Dicuran (= 3-(3-chloro-4-methylphenyl)-1,1-dimethylurea, Ciba Geigy).

For germination studies single lily anthers were dipped in 1 ml of the sugar solution (10% sucrose and 9 ppm boron or no boron at all) for about 10 min, and then removed. Each time 100 µl were taken from the pollen suspension and transferred by pipette to 900 µl of the corresponding test solution (standardized approach). Each test solution contained 10% sucrose and, according to the test method either 9 ppm boron or no boron. Each series of experiments was carried out with the pollen suspension derived from the same anther. An exception to this was the pilot test in which pollen growth was tested of a wide range of boron concentrations. Here, one anther was used for each concentration. The experiments were performed at about 22 °C.

The following stock solutions were used for the different series of tests and results were entered in aliquots: silicon (0.1, 1, 10, and 100 ppm), purine bases (20, 100, 200 and 1000 ppm), pyrimidine bases (20, 100, 200 and 1000 ppm), Zn^{2+} (2, 8 and 40 ppm), Dicuran (2 and 20 ppm), BTU (10, 30, and 50 ppm). All tests were carried out at pH 6.0 ± 0.2. Generally the pollen suspensions were tested microscopically one day after starting the test. However, some tests were stopped by adding chloroform 3.5–7 h after beginning the experiment.

For the evaluation of the experiments 0.5 ml of the upper pollen-free part of the 1 ml and 1 day old pollen suspension were carefully removed with a pipette and then discarded. (This is easy to do as, after 1 day the pollen gravitates to the bottom of the solution.) The remaining 0.5 ml were transferred to a microscope slide using a magnification of 100 × to ascertain growth lengthwise.

The lengths of the pollen tubes were assigned in four categories (for comparison: the diameter of an ungerminated pollen grain of *Lilium longiflorum* lies between 0.07–0.1 mm):

short	:	< 0.2 mm
medium	:	0.2–0.6 mm
long	:	0.6–1.6 mm
very long	:	> 1.6 mm

For the evaluation only fully germinated pollen (about 20–50% of the total number) was taken into account. The total of germinated pollen which was counted was set as 100%.

The mean length of pollen tubes ($\bar{l}$) was estimated according to the equation

$$\bar{l} = (0.1\,x_1 + 0.4\,x_2 + 1.1\,x_3 + 2.5\,x_4)\ \text{mm}/100\%.$$

The quantities x_1, x_2, x_3 and x_4 are the percentages of tube lengths obtained experimentally for the four categories (short, medium, long, very long). The values 0.1, 0.4, 1.1 and 2.5 are the mean values of the tube length in mm units referring to the four categories.

For the kinetic study of the influence of silicon on pollen germination 50 µl chloroform were added to the test solution to stop growth after 3.5 and 7 h, respectively.

Boron and silicon analysis of anthers of *Lilium longiflorum* was performed by using the pressure ashing method and the inductively coupled plasma emission spectroscopy (ICP) [1].

Results and Discussion

Boron

Boron is an important factor of the nutrient solution for the *in vitro* pollen tube growth of *Lilium longiflorum* [16–41]. Such a boron activated pollen is shown in Fig. 1 a. In order to study the influence

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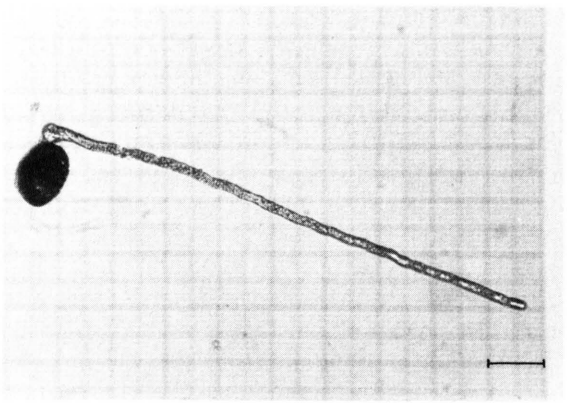

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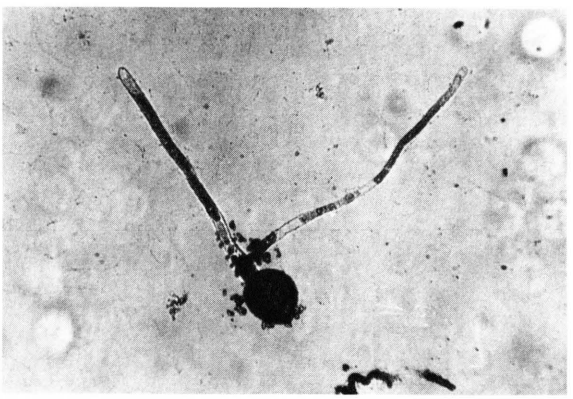

Fig. 1. a. *In vitro* germinated pollen of *Lilium longiflorum* with long pollen tube (9 ppm B, 10% sucrose) |—| ≙ 0.1 mm; b. item showing branched pollen tube (10 ppm Si in solution).

of boron on the pollen growth the boron content was varied at a constant sugar level. As can be seen from Fig. 2, pollen tubes grew easily between 2 and 20 ppm of boron. The optimum boron level is at 10 ppm with a large plateau. This agrees with results obtained using pollen of other plants [16, 52]. In all germination tests in the absence of boron it was found that *Lilium longiflorum* produced only short starting pollen tubes without any development. Already minimal traces of boron showed an effect. Thus, 0.02 ppm boron in the solution are sufficient to obtain a pronounced formation of medium-long pollen tubes.

The basic role of boron for activation of pollen seeds is confirmed by *in vivo* experiments: boron

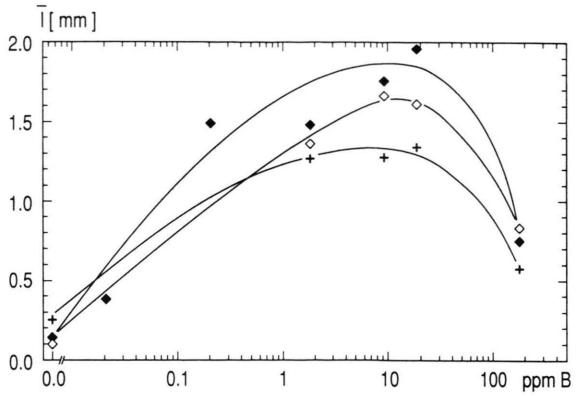

Fig. 2. Dependence of mean length $\bar{l}$ of pollen tubes on boron concentration.

treatment of plants leads to significantly increased vitality of pollen [6, 8–10, 12, 15].

Silicon

Boron and silicon can dramatically influence plant processes [54–57]. Therefore, it was of special interest whether silicon can influence the elongation of pollen tubes. Silicic acid does not stimulate pollen activity if boron is absent. In boron containing solution silicon contribute an additional effect to pollen growth, as it is shown in Fig. 3. A small growth stimulation is already seen at 0.1 ppm Si. Silicon concentrations above 10 ppm do not lead to further changes in germination behavior. High silicon concentrations (more than 100 ppm) show an inhibiting effect.

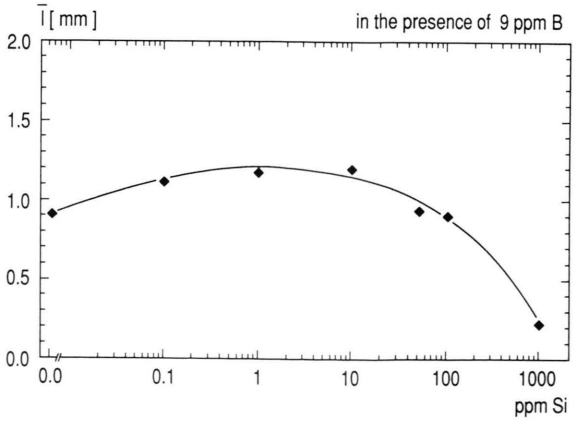

Fig. 3. Dependence of mean length $\bar{l}$ of pollen tubes on silicon concentration.

In order to reach the conclusion as to whether silicon stimulates at an early or later stage in the elongation process, pollen growth was stopped after 3.5 and 7 h, respectively, by using chloroform. As demonstrated in Fig. 4 silicon acts in the initial pollen tube elongation stade as an inhibitor. However, after the initial inhibition phase a distinct stimulation of elongation takes place. Thus, the silicon stimulation appears to occur only at a relatively late time.

The majority of germinated pollen only form a single tube. Occasionally however, pollen with branched tubes appear. The number of branched tubes (*i.e.* pollen with two tubes issuing from one initial germinated tube, *cf.* Fig. 1 b) seems to be significantly higher in solutions containing silicon than in silicon-free sucrose/boron solutions: for example, in one experiment 30 branched pollen tubes (= 4%) were found among 700 germinated grains (10 ppm Si in the nutrient solution). Without silicon, the number of branched pollen tubes ranged between 0 and 4 (0–0.6%, based on 700 germinated pollen grains suspended in the pure sucrose/boron solution), although in most cases no branching was found. It appears that silicon does not only stimulate the cell growth but also promote other cellular hyperactivity phenomena.

In vivo experiments confirm these experiments: In fact, silicon enrichment of whole plants rise significantly the pollen fertility [58–60]. This phenomenon is coupled with a significant silicon content of pollen: The anthers of *Lilium longiflorum* used in this study contain 40–100 ppm Si.

Nucleic bases and derivatives

In experiments with tobacco where plants were grown in the presence of boron and/or silicon, and where the leaves were subsequently infected with *Belladonna Mottle Virus* [1] and from other results [37, 61], there were indications that boron and silicon can directly affect the nucleotide metabolism. As the pollen of *Lilium longiflorum* does not germinate efficiently in pure sucrose solution it was of interest to study the pollen growth pattern in the presence of nucleic bases and some derivatives.

As shown in Fig. 5 adenine can partially replace boron under *in vitro* conditions for pollen germination and tube elongation. The mean length $\bar{l}$ reaches about half the value referring to the control test (control/B) where 9 ppm boron are added to the sucrose solution. The maximum stimulation occurs at 100 ppm. At 1000 ppm adenine the pollen tube growth is totally suppressed. In the case of guanosine a stimulation effect is found, too, but higher concentrations are not toxic. No effective stimulation takes place in the absence of boron using adenosine, AMP, d-AMP, uracil, uridine or thymidine in a range of 20–1000 ppm.

Large differences were found when nucleic bases and their derivatives were added to sucrose solutions in the presence of boron (Fig. 6). In addition to the boron effect small amounts of adenine (<20 ppm) stimulate the growth of pollen tubes whereas large amounts act as an inhibitor. A similar behavior was found in the absence of boron (Fig. 5). The differences between the adenine

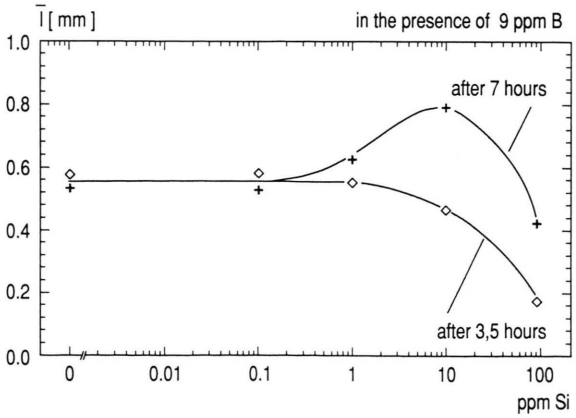

Fig. 4. Mean length $\bar{l}$ of pollen tubes in dependence on silicon concentration and time.

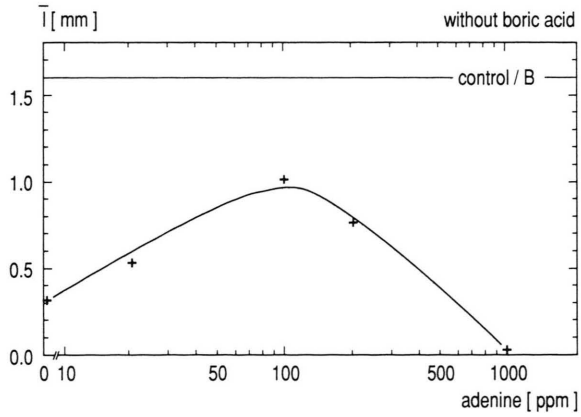

Fig. 5. Dependence of mean length $\bar{l}$ of pollen tubes on adenine concentration (no boron present). Control/B: control test in the presence of boron (9 ppm B).

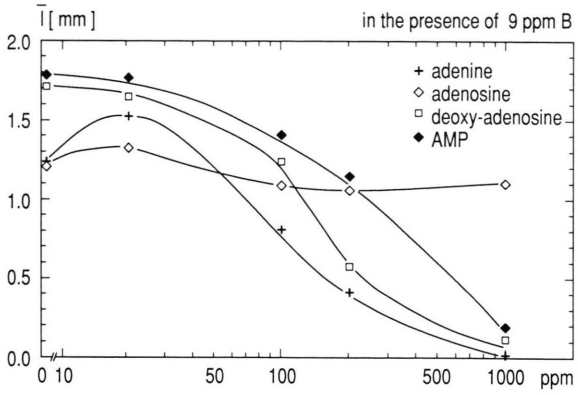

Fig. 6. Dependence of $\bar{l}$ on adenine, adenosine, deoxyadenosine and AMP in the presence of boron (9 ppm B).

curves from Fig. 5 and Fig. 6 reveal that i) the adenine stimulation is additive to the boron effect at low concentrations, and ii) the toxic effect of adenine begins at lower concentrations if boron is present.

Adenosine has no pronounced influence on the boron effect (Fig. 6). Deoxyadenosine and AMP inhibited continuously the growth of pollen tubes when their concentrations were increased.

In order to test whether nucleic bases containing boron also influence pollen elongation, 2-thio-5-dihydroxyboronicuracil (BTU, used in supported neutron therapy) was used instead of boric acid. In fact, BTU can partially replace boron. 20 and 50 ppm BTU enhance distinctly the mean length of pollen tubes (a value up to about ⅓ is obtained in comparison to the control test when 9 ppm boron is used).

Zn^{2+}, Ca^{2+} and Dicuran

In vivo experiments show that zinc [62–64] participates like boron [29, 36, 37, 65] in RNA, DNA and protein synthesis in germinating pollen. Synergistic and antagonistic interactions of both trace elements are possible. In order to examine the effect of zinc, $ZnSO_4$ was added to the sucrose/boron solution. It was found that even 2 ppm Zn^{2+} strongly inhibited the formation of tubes (the very long tubes were reduced by up to 90%) and 8 ppm virtually suppressed the formation of tubes completely.

As Ca^{2+} can increase the boron effect on pollen germination and tube growth [20–22, 28, 30, 31, 42–44] the influence of Ca^{2+} was tested in the absence of boron. As shown in Fig. 7 Ca^{2+} can partially compensate boron deficiency in the nutrient solution.

A vigorous inhibition was found when the herbicide Dicuran was added to the sucrose/boron solution. For example, only short pollen tubes occurred in optimized nutrient solutions (9 ppm B) containing additionally 20 ppm Dicuran. This is in agreement with analogous *in vivo* studies showing that herbicide treatment damages firstly and most severely the male reproductive system of plants [66–73]. Even pollen activity loss can be used as an indicator for pesticide pollution [74].

The activity of pollen

The type of storage and the age of the pollen have an important influence on the activity of the pollen [18]. Within a few weeks pollen stored in the desiccator over silica gel at 4 °C can suffer total loss of activity as well as being affected by mould. It was also discovered that there was a strong variation in pollen activity between different samples.

The number of pollen per unit volume used in the experiment had, in itself, an influence on the results. Like other biological processes (virus intrusions in host cells) pollen seeds mutually stimulate each other during growth when the number of pollen seeds in the suspension medium increases. This observation agrees with published findings [18, 75]. For this reason usually experiments within

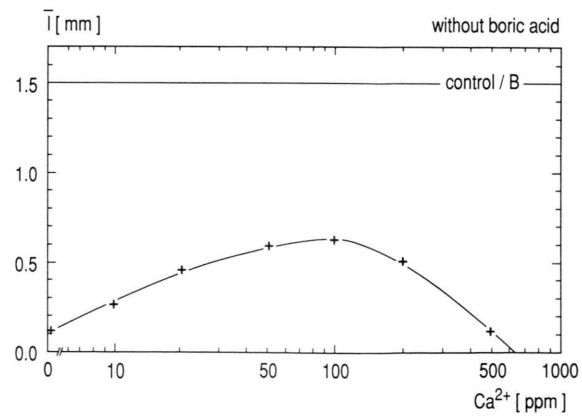

Fig. 7. Dependence of $\bar{l}$ on Ca^{2+} (no boron present). Control/B: control test in the presence of boron (9 ppm B).

a series were conducted with the same pollen count.

Conclusion

This study demonstrates that boron is essential to promote vigorous pollen germination and tube growth *in vitro* and *in vivo*. The hypothesis of the biological functions of boron in the reproductive activity of plants is also supported by the fact that this element is naturally enriched in pollen and stigma materia in respect to the global content of the vegetable. In the pollen of *L. longiflorum* used in this study 20–30 ppm boron were found.

Silicon leads to an additional activation of pollen of *L. longiflorum* under *in vitro* conditions. Like in the case of boron silicon enrichment of the whole plant produces the same effect.

The synthesis of the results obtained or referred to in this work leads to the following conclusions: Boron and silicon play a definitive role in 3 highly dynamic phases of the life cycle of plants.

i) In the reproductive phase both elements stimulate the intensified physiological processes. Boron availability is a *sine qua* non-condition for pollen seed activation *in vitro*. Silicon has a synergistic effect.

ii) During the seed germination and the first growth boron supply is essential. Boron and silicon stimulate growth of higher plants [2, 53, 54, 57] and algae [76].

iii) After infestation by virus [1] boron and silicon can produce dramatical effects on the host plant. In function of the parasite/plant cell system, the action of boron and silicon can be protective or destructive, synergistic or antagonistic.

The actions of boron and silicon are obviously coupled. The small concentration range between boron deficiency and toxic excess seems to become considerable larger if silicon is present.

Further investigations are necessary to elucidate the role of boron and silicon in molecular biological processes to identify active boron and silicon compounds and complexes in plant physiology.

Finally, the *in vitro* pollen experiments may be used for simple, rapid and inexpensive test systems to control environmental pollution.

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