Knot formation by *Pseudomonas syringae* subsp. *savastanoi* on the in *vitro* shoots of *Sorbus redliana*

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Summary: Two strains of Pseudomonas syringae subsp. savastanoi were isolated from Forsythia sp. and Nerium oleander in Hungary in 1997. The effects of growth regulators produced by the bacteria were studied in different experiments. The strains were co-cultured with Sorbus redliana in vitro shoots without being in contact with the plant on solid media. Further culture filtrates in different concentrations were added to the culture medium. The growth regulators presented in the agar caused knot formation on the shoots and on the leaves in both kinds of culture. There were significant differences in the cultural and physiological characters, auxin and cytokinin activity of the strains of different origin.

Introduction

The *Pseudomonas syringae* subsp. *savastanoi* causes considerable economic loss first of all in the Mediterranean countries on *Olea europaea* and *Nerium oleander*. The bacterium induces proliferation of the tissues, like callus formation and knot (gall) formation *(Holliday,* 1989). In Hungary, the bacterium was described first time by *Szatmári* et al., 1998.

The production of indoleaceticacid (IAA) and cytokinins was published in the case of some bacterial species, including the *Pseudomonas syringae subsp. savastanoi*. The effect of growth regulators was studied *in vivo* and *in vitro* as well *(Elstner,* 1983). There are hardly references concerning the identification of the growth regulators and the produced quantity.

In 1994, Iacobellis studied the different mutant strains of the *Pseudomonas syringae* subsp. *savastanoi* and he stated that the strains producing only IAA induced only plant tissue necrosis while the strains producing only cytokinins induced knot formation on the stem. The strain producing both auxin and cytokinin caused only a moderate knot formation.

The aim of our experiment was to study the effect of growth regulators produced by the bacterium on the shoots of *in vitro* cultured plants of *Sorbus redliana.* In addition, we want to deduce the type of the Hungarian isolates from the symptoms caused by this strain.

Material and method

For the experiment the bacterium strains were isolated from *Nerium oleander* (OL) and *Forsythia* sp. (FO) in 1997 at the University of Horticulture and Food Industry *(Szatmári* et al. 1998). As testplant the *in vitro* cultured shoots of *Sorbus redliana* were used *(Jámbor–Benczúr* et al., 1997). The pathogen was precultured on Nutrient agar. For the examination of the growth regulators of the bacterium the plant shoots and the bacterium were cultured together in 100 ml. Erlenmeyer flasks *(Table 1, Fig. 1).* The pathogen was inoculated in round shape on the surface of the solid medium and the shoot was placed in the middle of the round area. This way the bacteria had no contact with the shoots but the growth regulators produced by the bacteria diffunded aeross the medium to the plants. From every type of medium, bacterium metabolite free control was applied as well.

When culture filtrate was used the *Pseudomonas syringae* subsp. *savastanoi* was cultured on Nutrient broth for 5 days then filtered by cellulose acetate membrane filter (pore size 0.2 μm). Diluted culture filtrate was mixed with the SO medium (in 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32 proportion).

The cultures were illuminated by white light of 40 $\mu M/m^2/s$ using 16/8 hour light/dark cycles for 5 weeks. The temperature was 23–26 °C and 18–23 °C during the light and dark periods, respectively. The 100 ml Erlenmeyer flasks contained 30 ml of medium and were covered with three layers of 0.017 mm plastic foil.

Table 1 Media used for co-cultivation of plant and bacterium

Media	ADDITIVES		
	IBA mg/l	sucrose g/l	active carbon g/l
*MG2	0,5	30	–
MG3	0,5	30	1
MG8	1,0	30	–
MG9	1,0	30	1
**SO	–	20	–

* MG: *Murashige* and *Skoog* (1992) basic medium with half concentration of macroelements

** SO: S basic medium: BM macroelements *(Jámbor-Benczúr* and *Márta-Riffer,* 1990) + *Heller* microelements *(Heller,* 1953) + MS vitamines *(Murashige* és *Skoog,* 1992). The media were solidified with Difco-bacto agar 7 g/l, and the pH was adjusted to 5.6.

Every treatment contained 10 plants. The duration of the experiments was four weeks and the samples were taken from the plants for examinations by light and scanning electron microscopy on the 5th week.

Results

During the experiments knot formation was observed at the basis of the shoots, on the upper part of shoots and on the leaves that were in contact with the medium *(Fig. 1).*

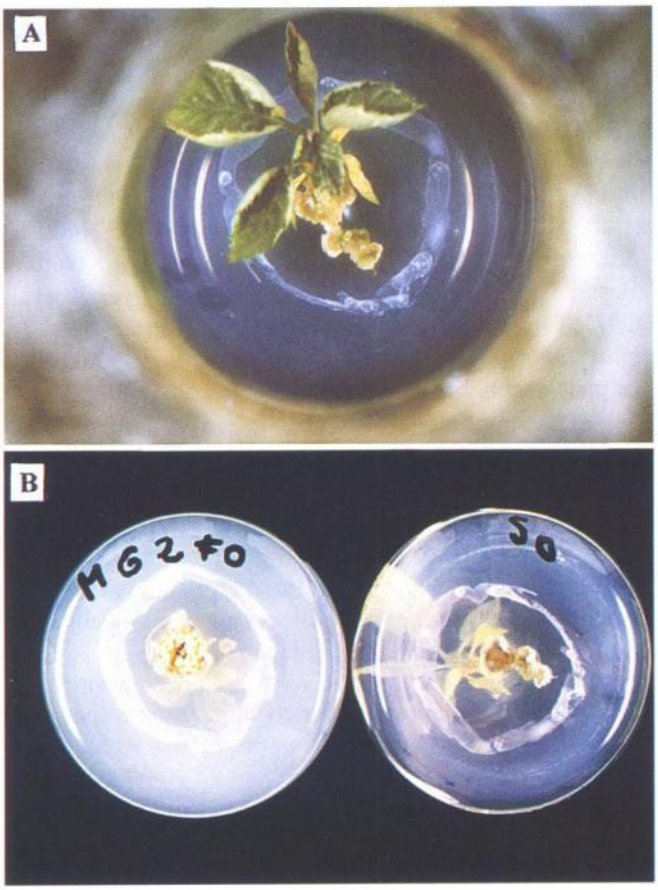

Figure 1 Knot (callus) formation on the basis of Sorbus redliana in vitro shoot and leaf induced by Pseudomonas syringae subsp. savastanoi. **A.** Knot formation on SO medium induced by the FO strain. **B.** Comparison of knot formation on MG2 and SO medium.

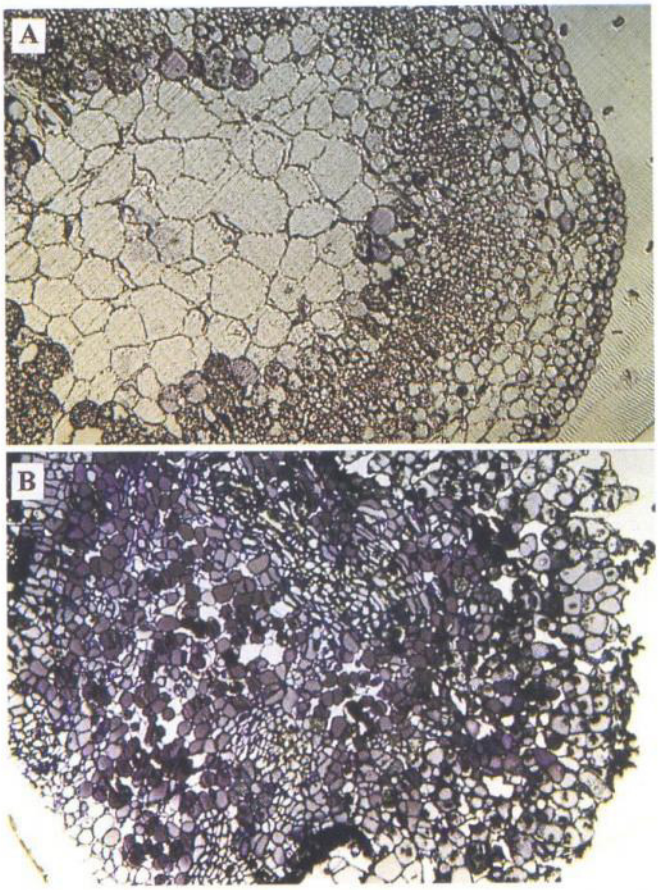

Figure 2 Comparison of the anatomical structure of Sorbus redliana in vitro shoots by light microscope, X 40. **A.** Cross-section of a healthy shoot. **B.** Cross-section of the shoot with knot formation on the upper part of the shoot.

The greatest knot was formed on the SO medium which did not contain growth regulators at all compared with the other media containing different concentrations of auxin. *(Fig. 1).* During the knot growing period the part of the shoot above the knot became necrotic and then died. By electron microscopy it was observed that the surface of the knots was very similar to that of the callus and this phenomenon was characteristic both on the leaves and on the stems *(Fig. 3).*

By light microscopy it was observed that the knot started to grow from the primary cortex and then the callus-like cells appeared in the phloem and the xylem as well. In the end only the callus cells can be found in the cross-section. These cells were uniform showing no differentiation. This resulted in the loss of the tissue structure and stem necrosis above the knot. Similar process happened with the leaves, too *(Fig. 2, 3).*

The size of the knots was much greater in the case of FO strain than in the OL one. The FO strains showed much intensive growth on all kinds of media compared with the OL originating strains.

Similarly to the results achieved in the first experiment, the culture filtrate in 1:2 proportion caused knot induction and stem necrosis on the plants.

Conclusions

The bacterial strains originating from different host plants caused knot formation on the *in vitro* plants of *Sorbus redliana*.

It was justified that the growth regulators produced by the strains elicited knot formation on the *in vitro* plants without being in contact with the plants. *Surico* (1993) stated that for the elicitation of the symptoms it was needed to wound the plant *in vivo*. As a consequence of our experiments it was concluded that the disease symptoms can be induced without wounding the host plant *in vitro*. On the culture filtrate containing media the symptoms appeared in the case of the highest concentrations. The further dilutions did not induce knot formation.

Iacobellis et al. (1994) described that the different bacterium strains had different capability in induction of different symptoms. Our FO strain caused knot formation and necrosis as well. We suppose that this strain produces both IAA and cytokinin in greater quantity. In the case of our OL strain we had neither necrosis nor knot formation on the upper part of the shoot. Consequently this strain produced less amount of IAA. Because the knots were smaller, it was supposed that the cytokinin production was also reduced.

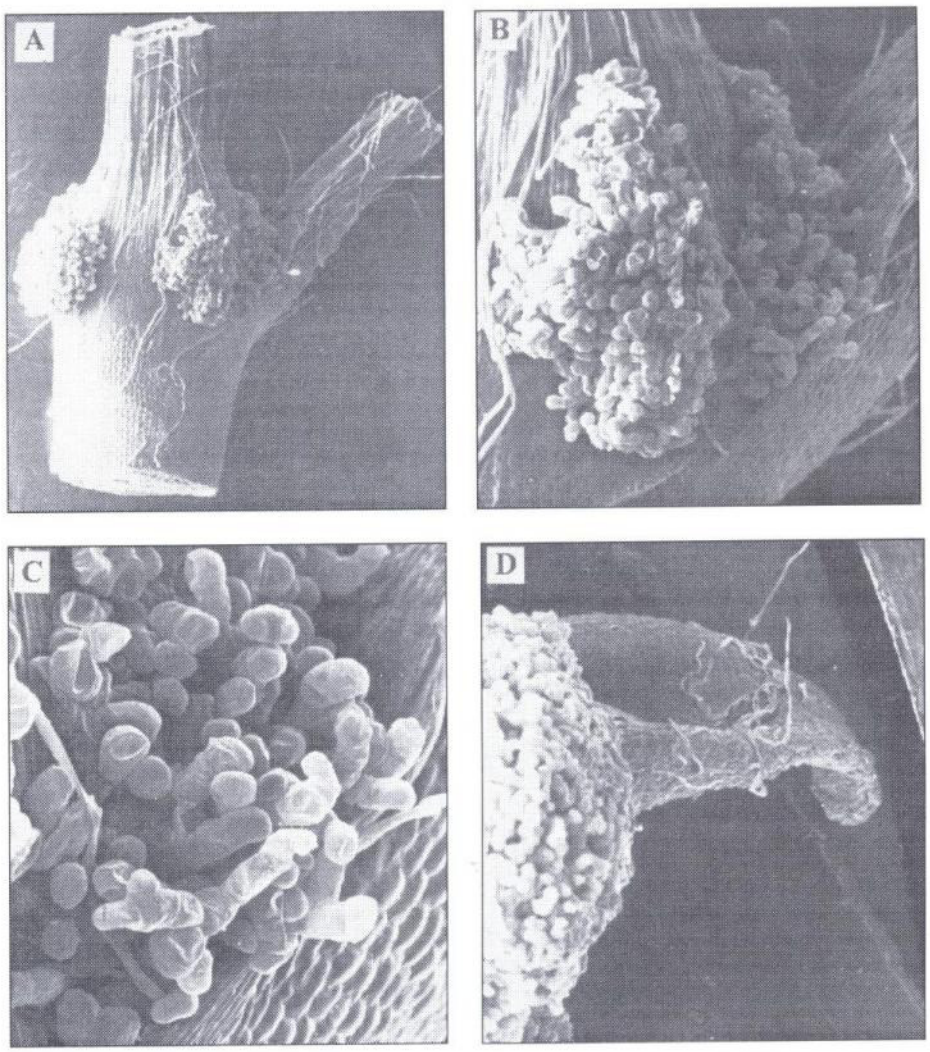

Figure 3 Surface of *Sorbus redliana* shoot and leaf with knot formation by the use of electron microscope. **A.** Knot formation on the node, with the dead part of the shoot above the knot, X 25. **B.** The knot, X 75. **C.** The callus-like cells of the knot, X 200. **D.** The knot growing on the leaf, X 70.

Strenghtening our results, *Gardini* et al. (1992) examined 131 bacterium strains and he stated that there were differences in the ability of the auxin production of the strains depending on the host plant. Strains were found without auxin production at all.

Because of the different cultural and physiological characters and biological activity of the strains we suppose that our strains were not identical or they had different virulence.

Acknowledgement

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