CROATICA CHEMICA ACTA
CCACAA **81** (4) 641–646 (2008)

ISSN-0011-1643
CCA-3285
Original Scientific Paper

Effect of Indole-3-butyric Acid on Rooting of Phytoplasma-recovered and Healthy Periwinkle *Catharanthus roseus* (L.) G. Don

Mirna Ćurković-Perica

University of Zagreb, Faculty of Science, Division of Biology, Department of Botany, Marulićev trg 9a
HR-10000, Zagreb, Croatia
(E-mail: mirna@botanic.hr)

RECEIVED MAY 9, 2007; REVISED APRIL 7, 2008; ACCEPTED APRIL 18, 2008

After prolonged treatment with the auxin, indole-3-butyric acid (IBA), the rooting efficiency of healthy *C. roseus* shoots was compared with recovered shoots containing a high titer of '*Candidatus* Phytoplasma' species '*Ca*. P. ulmi' and '*Ca.* P. solani' and recovered shoots in which, upon IBA-treatment, '*Ca.* P. asteris' was undetectable or present in low titer. On a medium containing the same concentration of IBA, the number of rooted shoots decreased with statistical significance, following the trend: healthy shoots > '*Ca.* P. asteris'-recovered shoots > '*Ca*. P. ulmi' and '*Ca.* P. solani'-recovered shoots. Furthermore, after the rooting experiment, '*Ca.* P. asteris' was detected in all the tested unrooted shoots while, among the rooted periwinkles, only 20 % were positive. These results constitute the first report in which a correlation between the presence of phytoplasmas and rooting efficiency of the periwinkle host has been shown. Since auxins are phytohormones involved in the initiation and emergence of adventitious roots, reduced rooting efficiency may reflect disturbed levels or transport of auxin(s) in phytoplasma-infected periwinkles.

Keywords
bacteria
Catharanthus roseus
indole-3-butyric acid
phytoplasma
rooting
tissue culture

INTRODUCTION

Phytoplasmas are non-cultivable, endocellular, phloem-limited plant pathogenic bacteria. Plants infected by these prokaryotes exhibit an array of symptoms that suggest profound disturbances in the normal balance of plant growth regulators.[1–4] Within periwinkle plants, phytoplasmas induce symptoms such as leaf yellowing, growth aberrations including proliferations, internode shortening and stunting, flower malformations and/or decline.[5] Chang[1] showed that different levels of plant growth regulators are needed for efficient rooting of healthy, as opposed to phytoplasma-infected, periwinkles. The best medium for rooting of aster yellow phytoplasma-infected periwinkle contained 2.5 mg/L indole-3-butyric acid (IBA) in combination with 0.1 mg/L kinetin, while α-naphthaleneacetic acid (NAA) at 0.5 mg/L and kinetin at 0.1 mg/L were the best for rooting of healthy periwinkle plants. In the same publication, Chang hypothesized that phytoplasmas may block the transport of endogenous auxin and affect auxin levels in infected periwinkles. Pertot *et al.*[6] used an enzyme immunoassay to quantify the endogenous indole-3-acetic acid (IAA) in healthy and '*Ca*. P. trifolii'-infected *C. roseus* plants grown *in vitro.* This experiment showed that the IAA concentration inreased in infected plants. This increase could have been caused by the reaction of the diseased host to the presence of phytoplasmas. On medium supplemented with

high concentrations of auxins, the abundance of phytoplasmas decreased in cells of infected *C. roseus* plants (as revealed on electron micrograph of cell sections).

In *C. roseus* shoots infected with three different '*Candidatus* Phytoplasma' species, exogenous addition of IBA induced remission of symptoms, as well as better growth and photosynthesis.[7] The detection of '*Ca.* P. asteris' (phytoplasma reference strain HYDB), '*Ca*. P. ulmi' (EY-C) and '*Ca.* P. solani' (SA-I) in periwinkle shoots grown *in vitro* was done during the first four subcultures, on medium supplemented with IBA. During that period, nested PCR amplification of conserved phytoplasma 16S rDNA revealed that the titer of '*Ca.* P. asteris' gradually decreased. In the 2nd and 3rd subcultures, '*Ca.* P. asteris' was no longer detectable in half of the tested shoots grown on IBA-containing media. Approximately one-third of the shoots permanently escaped infection after being retransferred to medium supplemented with 6-benzylaminopurine instead of IBA, a system in which phytoplasmas are usually present in high titer in infected periwinkles. The titer of '*Ca*. P. ulmi' and '*Ca.* P. solani' in the shoots studied remained high during IBA treatment.

Recovery, defined as remission of symptoms in phytoplasma-infected plants, which may or may not involve elimination of the pathogen from the host, has been previously reported for naturally-grown apple, apricot and grapevine.[8–11] However, the factors involved in the phytoplasma recovery are not completely understood. Ćurković-Perica *et al.*[7] showed that supplementation of the medium with IBA can induce the recovery of phytoplasma-infected periwinkle grown *in vitro*. IBA is a phytohormone mainly used in tissue culture for the induction of adventitious roots. However, during the 45-day culture period used in the previous experiments,[7] none of the phytoplasma-recovered periwinkle shoots rooted during the first four subcultures.

The purpose of the experiments presented here was: (1) to examine the effect of prolonged IBA treatment on the rooting of healthy, as opposed to '*Ca*. P. ulmi' – and '*Ca.* P. solani' – recovered shoots in which high titer of phytoplasma was retained during the IBA-treatment, and recovered shoots in which, upon IBA-treatment, '*Ca.* P. asteris' was undetectable or present in low titer, and (2) to correlate the efficiency of rooting with the presence of phytoplasmas in the tested samples.

EXPERIMENTAL

Material and Plant Tissue Culture Methods

Catharanthus roseus (L.) G. Don shoots infected with '*Candidatus* Phytoplasma' species: '*Ca.* P. asteris' (phytoplasma reference strain HYDB; hydrangea phyllody; aster yellows; 16SrI-B subgroup), '*Ca.* P. solani' (phytoplasma reference strain SA-I, grapevine yellows, stolbur, 16SrXII-A subgroup) and '*Ca*. P. ulmi' (phytoplasma reference strain EY-C; elm yellows; 16SrV-A subgroup), were grown *in vitro* on MS basal nutrient medium[12] supplemented with 100 mg/L myo-inositol, 1 g/L casein hydrolyzate, 30 g/L sucrose, 9 g/L agar and 0.5 mg/L 6-benzylaminopurine (BA) (= 2.2 μmol dm^{-3} BA). In such a system, the above mentioned '*Candidatus* Phytoplasma' species are present in a high titer and all infected shoots express typical symptoms of phytoplasma infection. Each population of shoots infected with one of the above mentioned '*Candidatus* Phytoplasma' species was micropropagated from one single explant. Phytoplasma-infected explants were obtained from the collection maintained at the Phytoplasmology Laboratory of the University of Bologna.[13] Healthy, symptomless periwinkle shoots grown on the same medium were included in the experiments as controls. However, the population of healthy periwinkle shoots was not micropropagated from a single explant, but from four plants grown *in vitro* from seeds.

Healthy and '*Ca.* P. asteris', '*Ca.* P. solani' – and '*Ca*. P. ulmi' – infected shoots were transferred to MS medium, which instead of BA, contained different concentrations of IBA. '*Ca.* P. asteris' – infected periwinkle shoots were transferred to MS medium containing 0.5, 1, 2 or 4 mg/L IBA (= 2.5, 4.9, 9.8 or 19.7 μmol dm^{-3} IBA); '*Ca*. P. ulmi'-infected shoots to 9.8 or 19.7 μmol dm^{-3} IBA and '*Ca.* P. solani' – infected shoots to 9.8 μmol dm^{-3} IBA. Healthy shoots were transferred to MS medium containing 9.8 μmol dm^{-3} IBA. As previously reported, phytoplasma-infected shoots transferred to media containing IBA already showed remission of symptoms in the first four subcultures.[7] Forty-eight healthy and infected periwinkle shoots per treatment and per '*Candidatus* Phytoplasma' species were subcultured in a 45-day cycle for 16 months. Explants removed for subculturing were approximately 1 cm high. After the 11th subculture, 24 shoots per '*Candidatus* Phytoplasma' species and per treatment, as well as healthy shoots, were used for rooting experiments. For this purpose, the subculturing period was prolonged and roots were counted after 7, 9 and 11 weeks. After 11 weeks, the plants were subcultured, the roots were cut off and the experiment was repeated. Results from 3 repetitions were merged and arithmetic means, analysis of variance and Duncan's test (for the number of rooted shoots per treatment) or Newman-Keul's test (for the number of adventitious roots induced per rooted shoot) were used for analysis and interpretation of the data. Throughout the experiment, each explant was subcultured in a separate tube, filled with approximately 15 mL of nutrient medium.

Phytoplasma Detection

In the 11th subculture, ten randomly selected shoot samples were chosen per treatment and per '*Candidatus* Phytoplasma' species. These samples were tested for the presence of phytoplasma. Furthermore, after the 3rd repetition of the rooting experiment, 10 randomly chosen rooted and 10 unrooted '*Ca.* P. asteris' – recovered periwinkle plants from media with 2.5 and 4.9 μmol dm^{-3} IBA were also checked for phytoplasma presence. The procedure, including total nucleic acid isolation, PCR amplification and product analysis was previously described.[7,14] For the amplification of high-

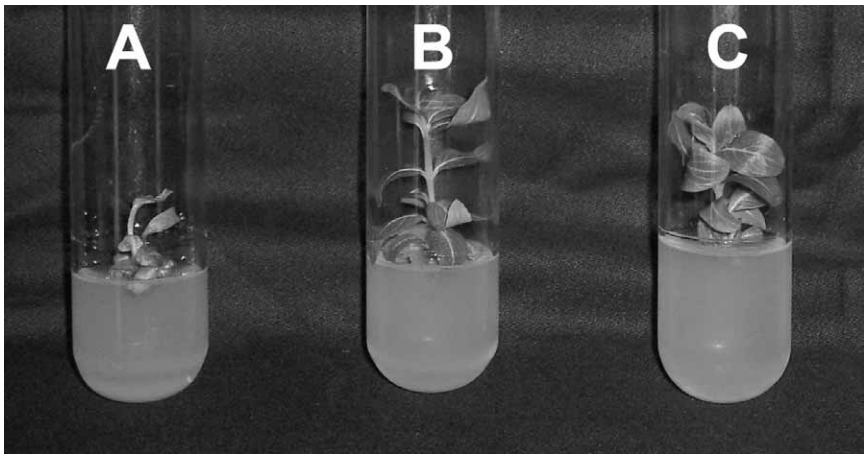

Figure 1. *C. roseus* shoots grown *in vitro* on MS (Murashige and Skoog, 1962) basal nutrient medium: '*Ca.* P. asteris' – infected shoot from the medium supplemented with 2.2 μmol dm^{-3} 6-benzylaminopurine (BA) (A); '*Ca.* P. asteris' – recovered shoots from the media supplemented with 4.9 μmol dm^{-3} indole-3-butyric acid (IBA) (B) and 9.8 μmol dm^{-3} IBA (C). Photograph was taken 3 weeks after the inoculation of explants (approximately 1 cm long) on the medium.

ly conserved phytoplasma 16S rDNA, direct PCR assays were performed using the universal primer pair R16F1/R0.[15] When periwinkle shoots infected with '*Ca.* P. asteris' were analyzed, the amplification products obtained with the R16F1/R0 primers were diluted 1:30 with sterile deionized water and reamplified in the first nested PCR with the primers R16F2n/R2.[16] Additional second nested PCRs were performed using the primer pair R16(I)F1/R1.[17]

RESULTS AND DISCUSSION

In contrast to control shoots grown on the medium supplemented with BA (Figure 1a), shoots treated with IBA were recovered (Figures 1b,c). Molecular detection of '*Ca.* P. asteris' in recovered *C. roseus* shoots in the 11th subculture, using consecutive PCR assays with the primer pairs R16F1/R0 (Figure 2a), R16F2n/R2 (Figure 2b) and R16(I)F1/R1 (Figure 2c), showed the absence of '*Ca.* P. asteris' in: 7 out of 10 tested samples from media containing 2.5 and 9.8 μmol dm^{-3} IBA; 6 out of 10 from medium containing 4.9 μmol dm^{-3} IBA; and 5 out of 10 from medium containing 19.7 μmol dm^{-3} IBA. Confirmation of '*Ca.* P. asteris' in 15 out of 40 tested samples that proved positive was possible only in the second nested PCR (Figure 2c). The presence of '*Ca.* P. ulmi' and '*Ca.* P. solani' was confirmed by direct PCR using the primer pair R16F1/R0 (Figure 2a), in all tested samples from media with IBA, although the shoots were symptomless. In the controls (symptom-expressing periwinkle shoots grown on medium supplemented with BA), all three '*Candidatus* Phytoplasma' species were present in high titer and were always detected by direct PCR (Figure 2a).

During the first four subcultures, none of the '*Ca.* P. ulmi', '*Ca.* P. solani' – and '*Ca.* P. asteris' – recovered shoots rooted, during the 45-day subculturing period. However, in subsequent subcultures, rooting was noticed

TABLE I. Influence of different concentrations of indole-3-butyric acid on rooting of healthy and phytoplasma-recovered *C. roseus* shoots

'*Candidatus* Phytoplasma' species	IBA μmol dm^{-3}	Number of shoots[(a)] rooted after			Number of adventitious roots[(b)] per rooted periwinkle after		
		7 weeks	9 weeks	11 weeks	7 weeks	9 weeks	11 weeks
'*Ca.* P. asteris'	2.5	5,33 ± 0.47 d	9,67 ± 1,25 d	18 ± 0.82 c	5 ± 2,16 b	6,8 ± 1,55 bc	7,44 ± 2,24 c
	4.9	7,67 ± 0.47 c	12,33 ± 0.47 c	19 b	5,75 ± 2,86 b	8,92 ± 2,43 b	9,37 ± 2,68 bc
	9.8	11 ± 0.82 b	16 ± 0.82 b	24 a	8,45 ± 4,54 ab	10,19 ± 3,05 ab	12,08 ± 3,44 ab
	19.7	15,67 ± 0.47 a	24 a	24 a	13,44 ± 7,91 a	14,08 ± 5,73 a	15,42 ± 4,57 a
'*Ca.* P. ulmi'	9.8	0 e	0 f	3 ± 0.82 e	–	–	2,72 ± 0,83 d
	19.7	0 e	3,33 ± 0.47 e	4,67 ± 0.47 d	–	2,67 ± 0,94 c	3,4 ± 0,8 d
'*Ca.* P. solani'	9.8	0 e	0 f	2,67 ± 0.47 e	–	–	2,67 ± 0,47 d
healthy	9.8	16 ± 0.82 a	24 a	24 a	13,47 ± 7,13 a	14,21 ± 5,31 a	15,79 ± 4,68 a

[(a)] The number of rooted shoots is expressed as the arithmetic mean of 3 experiments ± standard deviation. In each experiment 24 shoots were tested.
[(b)] Unrooted shoots were excluded from the analysis, rather than counted as zeros.
Means labeled with identical letters are not significantly different at the 95 % level of confidence.

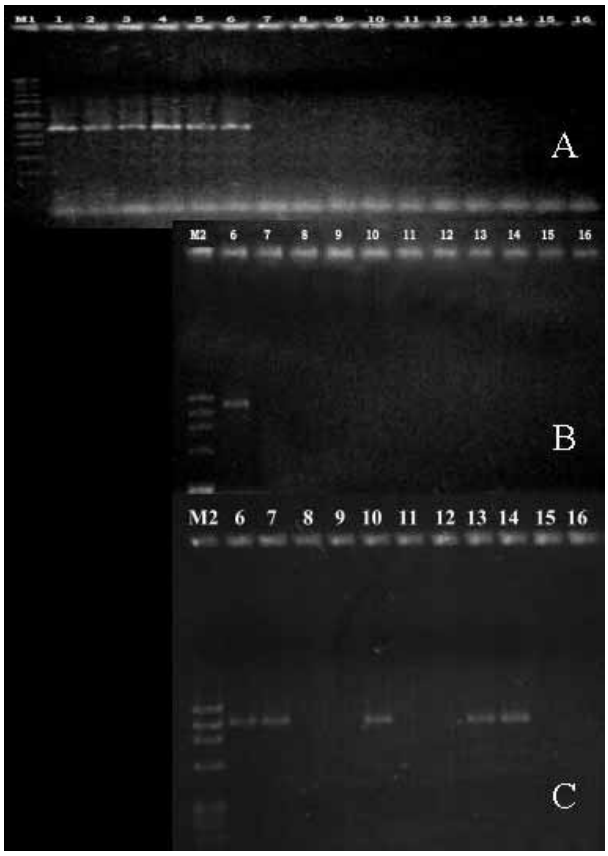

Figure 2. Agarose gel (1 %) electrophoresis of PCR amplification products of phytoplasma 16S rDNA obtained using the primer pairs R16F1/R0 (A), R16F2n/R2 (B) and R16(I)F1/R1 (C). Molecular weight marker SPP1-DNA *Eco*RI digested, fragments in base pairs from top to bottom: 8576, 7427, 6106, 4899, 3639, 2799, 1953, 1882, 1515, 1482, 1164, 992, 718, 710, 492, 359 (M1); φX174 *Hae*III digested, fragments in base pairs from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194 (M2); 'Ca. P. ulmi'-infected *C. roseus* shoot from the medium supplemented with 2.2 μmol dm^{-3} BA (1); 'Ca. P. ulmi'-recovered shoots from the media supplemented with 9.8 μmol dm^{-3} IBA (2) and 19.7 μmol dm^{-3} IBA (3); 'Ca. P. solani' – infected shoot from the medium supplemented with 2.2 μmol dm^{-3} BA (4); 'Ca. P. solani' – recovered shoot from the medium supplemented with 9.8 μmol dm^{-3} IBA (5); 'Ca. P. asteris' – infected shoot from the medium supplemented with 2.2 μmol dm^{-3} BA (6); 'Ca. P. asteris' – recovered shoots from the media supplemented with 2.5 μmol dm^{-3} IBA (7–9), 9.8 μmol dm^{-3} IBA (10,11), 19.7 μmol dm^{-3} IBA (12–14); healthy periwinkle (15); water control (16). All samples were collected after the eleventh subculture.

not only in healthy shoots, but also in '*Ca.* P. asteris' – recovered shoots. After the 11th subculture, for monitoring the rooting efficiency (1) of healthy shoots, (2) of '*Ca.* P. asteris' – recovered shoots in which phytoplasma was undetectable or detectable in the second nested PCR, and (3) of '*Ca*. P. ulmi' – and '*Ca.* P. solani' – recovered shoots in which phytoplasmas were detectable by direct PCR, the subculturing period was prolonged to 11 weeks and the rooted shoots as well as the number of roots per rooted shoot were counted after 7, 9 and 11 weeks. As expected, none of the shoots maintained on BA-containing control medium rooted during the 11-week subculturing period. However, prolonged subculturing on media supplemented with IBA induced rooting of phytoplasma-recovered periwinkle shoots. In contrast to healthy shoots, rooting of phytoplasma-recovered shoots was delayed and the rooting ability was lower (Table I). On a medium containing the same concentration of IBA, the difference between '*Ca.* P. asteris' – recovered shoots (in which '*Ca.* P. asteris' was undetectable or present in low titer) and '*Ca.* P. solani' – and '*Ca*. P. ulmi' – recovered shoots (in which phytoplasmas were present in high titer) was also obvious. After 11 weeks all healthy and '*Ca.* P. asteris' – recovered shoots grown on 9.8 and 19.7 μmol dm^{-3} IBA rooted. Through three subcultures, in each combination of '*Candidatus* Phytoplasma' species and treatment, the number of rooted shoots was quite conserved, with or without very low standard deviation (Table I). The rooting ability and the number of induced adventitious roots also depended on the concentration of IBA in the medium (Table I). Within each tested combination, including phytoplasma-recovered shoots and healthy shoots, the same shoots always rooted earlier than the others. Therefore, an additional experiment was performed to prove that the rooting ability of periwinkles is indeed dependent on the presence of phytoplasma. For that purpose 10 (5+5) unrooted and 10 (5+5) randomly chosen rooted '*Ca.* P. asteris' – recovered periwinkles, from media with 2.5 and 4.9 μmol dm^{-3} IBA, were checked for the presence of phytoplasma after the third repetition of the rooting experiment. '*Ca.* P. asteris' was detected in the second nested PCR reaction in all unrooted shoots tested (Figure 3a), while among the rooted plants, only 2 out of 10 were positive (Figure 3b).

In periwinkle shoots infected with the three studied '*Candidatus* Phytoplasma' species IBA induced remission of symptoms, better growth and photosynthesis.[7] However, even though the shoots recovered, '*Ca*. P. ulmi' and '*Ca.* P. solani' persisted in periwinkle shoots in high titer, while '*Ca.* P. asteris' was undetectable or present in low titer. These previous results from the fourth subculture were confirmed here for the 11th subculture, showing that long term IBA treatment did not have any additional effect on the tested '*Candidatus* Phytoplasma' species. Furthermore, as reported before,[7] a clear correlation between the IBA concentration and the number of samples in which '*Ca.* P. asteris' became undetectable could not be established.

The prolonged subculturing period induced rooting of phytoplasma-recovered shoots on media with IBA, and the rooting efficiency had the trend: healthy shoots > '*Ca.* P. asteris' – recovered shoots > '*Ca*. P. ulmi' – and '*Ca.* P. solani' – recovered shoots. These results implied that the rooting efficiency indeed correlated with the presence and the titer of phytoplasmas in the tissue. However, through each tested combination, including phytoplasma-recovered and healthy shoots, always the same

shoots rooted earlier than the others. The fact that healthy shoots were not propagated from the same mother plant implied that rooting efficiency in this case might have been dependent on the shoot genotype. In the case of infected periwinkles all the shoots were micropropagated from a single explant and therefore the influence of the genotype was negligible. Nevertheless, an additional test was performed to correlate the presence/absence of phytoplasmas with the rooting efficiency of the host. After the rooting experiment, 10 out of 10 examined unrooted '*Ca.* P. asteris' – recovered shoots were positive for phytoplasma presence, while, among the rooted plants, only 2 out of 10 were positive, implying that even a low titer of phytoplasma can cause a delay in rooting of the host.

Auxins are phytohormones involved in mediating a number of essential plant growth and developmental processes, including root development, initiation and emergence of lateral roots, patterning of the root apical meristem, gravitropism and root elongation. Auxin biosynthesis occurs in both, shoots and roots; thus, the auxin required for root development could come from either source, or from both.[18] In the stem, auxin is transported in a highly directional fashion from the top towards the base of the plant.[19] IBA, being a natural auxin, may act directly in certain processes[20] or by the conversion to indole-3-acetic acid (IAA).[21] It is mainly used in tissue culture for the induction of adventitious roots because it is much more potent than IAA or synthetic auxins.[20] The fact that '*Ca.* P. asteris' – recovered shoots rooted more efficiently with increasing IBA concentrations proved that exogenously supplemented IBA contributed to the rooting. Since the concentration of endogenous auxin(s), transported basipetally from the periwinkle shoot, is so much lower than the concentration of the IBA supplemented to the media, contribution of the endogenous auxin to the rooting of periwinkles was probably of much less importance.

The effect of exogenously supplemented auxins on healthy and phytoplasma-infected periwinkles was reported previously in two publications.[1,6] Chang[1] showed that different levels of plant growth regulators are needed for efficient rooting of healthy, as opposed to phytoplasma-infected, periwinkles. Pertot *et al.*[6] showed that concentration of IAA increased in periwinkle plants infected by '*Ca*. P. trifolii'. They hypothesized that this increase was rather an unspecific answer of the diseased host to the presence of phytoplasmas, than direct defence mechanism against the infection. While the presence of phytoplasmas was easily confirmed by transmission electron microscope in the infected plants grown on the medium without auxins, the supplement of high concentrations of IAA, IBA, NAA and 2,4-dichlorophenoxyacetic acid (2,4-D) to the medium on which infected plants were grown made this pathogen difficult to detect. In the cells of infected plants treated with 2,3,5-triiodobenzoic acid (TIBA), an inhibitor of auxin translocation, phytoplasmas were, again, numerous. These results were corroborated with the results of Ćurković-Perica *et al.*[7] and the results of long term IBA treatment presented in this paper. Although none of these results reveal the mechanism by which high concentrations of exogenously supplemented auxins affect the phytoplasmas tested, they reveal some kind of interdependence.

The fact that rooting of '*Ca*. P. ulmi' – and '*Ca.* P. solani' – recovered shoots, in which phytoplasmas are present in high titer, is delayed and not efficient, may reflect disturbed uptake of the phytohormone from the nutrient medium, its transport to, and within, the tissue layers from which adventitious roots originate. Phytoplasma infection might also affect the molecular mode of IBA-action and endogenous auxin(s) levels. Whichever mechanism might be involved, rooting efficiency having the trend: healthy shoots > '*Ca.* P. asteris' – recovered shoots > '*Ca*. P. ulmi' – and '*Ca.* P. solani' – recovered shoots,

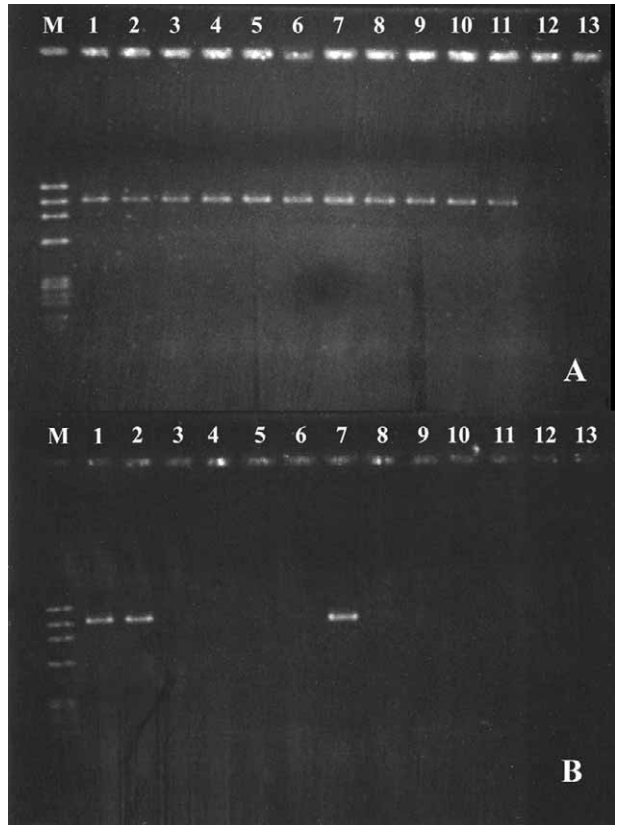

Figure 3. Agarose gel (1 %) electrophoresis of nested PCR amplification products of phytoplasma 16S rDNA, obtained using the primer pair R16(I)F1/R1, of 10 unrooted, 'Ca. P. asteris' – recovered periwinkle shoots (A); and of 10 randomly chosen rooted shoots (B), from media supplemented with 2.5 and 4.9 μmol dm^{-3} IBA. Marker φX174 *Hae*III digested, fragments' sizes same as in Figure 2. (M); 'Ca. P. asteris' – infected shoot from medium supplemented with 2.2 μmol dm^{-3} BA (1); recovered shoots from the medium supplemented with 2.5 μmol dm^{-3} IBA (2–6); shoots from the medium supplemented with 4.9 μmol dm^{-3} IBA (7–11); healthy C. *roseus* plant (12); water control (13). Samples were collected after the third repetition of the rooting experiment.

on medium with the same concentration of IBA, reflects the influence of phytoplasma presence on rooting efficiency of the host.

On the basis of the expressed symptoms, disturbances in the normal balance of plant growth regulators, as a consequence of phytoplasma infection, were hypothesized by several authors,[1–4,6,7] but the results presented in this paper correlate, for the first time, the presence and titer of phytoplasmas with the rooting efficiency of periwinkle shoots.

Acknowledgements. – This research was supported by the Croatian Ministry of Science, Education and Sports (project no. 119-1191192-1215). I am also grateful to Prof. Dr. Assunta Bertaccini for providing the '*Candidatus* Phytoplasma' species.

REFERENCES

1. C. J. Chang. *Phytopathology* **88** (1998) 1347–1350.
2. C. J. Chang and I.-M. Lee, *Pathogenesis of diseases associated with mycoplasma-like organisms*, in: U. S. Singh, R. P. Singh, and K. Kohmoto (Eds.), *Pathogenesis and host specificity in plant diseases*, Elsevier, New York, 1995, pp. 237–246.
3. I.-M. Lee, R. E. Davis, and D. E. Gundersen-Rindal, *Annu. Rev. Microbiol*. **54** (2000) 221–254.
4. R. E. McCoy, A. Caudwell, C. J. Chang, T. A. Chen, L. N. Chiykowski, M. T. Cousin, J. L. Dale, G. T. N. de Leeuw, D. A. Golino, K. J. Hackett, B. C. Kirkpatrick, R. Marwitz, H. Petzold, R. C. Sinha, M. Sugiura, R. F. Whitcomb, I. L. Yang, B. M. Zhu, and E. Seemüller, *Plant diseases associated with mycoplasma-like organisms*, in: R. F. Whitcomb and J. G. Tully (Eds.), *The Mycoplasmas*, Vol. 5, Academic Press, New York, 1989, pp. 545–640.
5. S. Jagoueix-Eveillard, F. Tarendeau, K. Guolter, J.-L. Danet, J. M. Bove, and M. Garnier, *Mol. Plant-Microbe Interact.* **14** (2001) 225–233.
6. I. Pertot, R. Musetti, L. Pressacco, and R. Osler. *Cytobios* **95** (1998) 13–23.
7. M. Ćurković-Perica, H. Lepeduš, and M. Š. Musić. *FEMS Microbiol. Lett.* **268** (2007) 171–177.
8. R. Musetti, L. Sanità di Toppi, P. Ermacora, and M. A. Favali. *Phytopathology* **94** (2004) 203–208.
9. R. Musetti, L. Sanità di Toppi, M. Martini, F. Ferrini, A. Loschi, M. A. Favali, and R. Osler. *Eur. J. Plant Pathol*. **112** (2005) 53–61.
10. R. Osler, L. Carraro, P. Ermacora, F. Ferrini, N. Loi, A. Loschi, M. Martini, P. B. Mutton, and E. Refatti, *Roguing: a controversial practice to eredicate grape yellows caused by phytoplasmas*, in: Scientific committee of the conference (Eds.), *Proceedings of the 14th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine*, Locorotondo, Italy, 2003, p. 68.
11. R. Osler, L. Carraro, N. Loi, and E. Refatti. *Plant Disease* **77** (1993) 496–498.
12. T. Murashige and F. Skoog. *Physiol. Plant.* **15** (1962) 473–497.
13. IRPCM Phytoplasma/Spiroplasma Working Team-Phytoplasma taxonomy group. *Int. J. Syst. Evol. Microbiol.* **54** (2004) 1243–1255.
14. M. Šeruga, D. Škorić, D. Botti, S. Paltrinieri, N. Juretić, and A. F. Bertaccini, *Forest Pathol.* **33** (2003) 113–125.
15. I.-M. Lee, A. Bertaccini, M. Vibio, and D. E. Gundersen. *Phytopathology* **85** (1995) 728–735.
16. D. E. Gundersen and I.-M. Lee. *Phytopathol. Mediterr.* **35** (1996) 144–151.
17. I.-M. Lee, D. E. Gundersen, R. W. Hammond, and R. E. Davis. *Phytopathology* **84** (1994) 559–566.
18. K. Ljung, A. K. Hull, J. Celenza, M. Yamada, M. Estelle, J. Normanly, and G. Sandberg. *Plant Cell* **17** (2005) 1090–1104.
19. M. Estelle, *Nature* **413** (2001) 374–375.
20. J. Ludwig-Mûller, *Plant Growth Regul.* **32** (2000) 219–230.
21. B. K. Zolman, A. Yoder, and B. Bartel. *Genetics* **156** (2000) 1323–1337.

SAŽETAK

Učinak indol-3-maslačne kiseline na zakorjenjivanje zdravog i od fitoplazmoze oporavljenog madagaskarskog zimzelena *Catharanthus roseus* (L.) G. Don

Mirna Ćurković-Perica

Nakon dugog izlaganja djelovanju auksina indol-3-maslačne kiseline (IBA), uspoređen je učinak ovog biljnog regulatora rasta na zakorjenjivanje zdravih izdanaka vrste *C. roseus*, oporavljenih izdanaka u kojima su u visokom titru bile prisutne '*Candidatus* Phytoplasma' vrste '*Ca*. P. ulmi' i '*Ca.* P. solani' i oporavljenih izdanaka u kojima je nakon djelovanja IBA-e, '*Ca.* P. asteris' bila prisutna u niskom titru ili se njena prisutnost uopće nije mogla dokazati. Na hranidbenoj podlozi s istom koncentracijom IBA broj zakorjenjenih izdanaka smanjivao se slijedom: zdravi izdanci > izdanci oporavljeni od zaraze s '*Ca.* P. asteris' > izdanci oporavljeni od zaraze s '*Ca*. P. ulmi' i '*Ca.* P. solani'. Nakon pokusa zakorjenjivanja prisutnost '*Ca.* P. asteris' dokazana je u svim istraženim nezakorijenjenim izdancima, dok je u zakorijenjenim fitoplazma bila prisutna u samo 20 % istraženih uzoraka. Ovi rezultati povezuju po prvi put prisutnost fitoplazmi i uspješnost zakorjenjivanja biljke domaćina. Budući da su auksini biljni hormoni uključeni u poticanje rasta adventivnog korjenja, smanjena sposobnost zakorjenjivanja mogla bi odražavati poremećenu razinu ili transport auksina u biljkama zaraženim fitoplazmama.