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Stimulation of *ex vitro* growth of *Rhododendron* hybrids 'Nova Zembla' and 'Alfred' by inoculation of roots with *Serendipita indica*

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Abstract: Experiments were carried out to study the consequences of inoculating *Rhododendron* 'Nova Zembla' and 'Alfred' microcuttings and young plants with *Serendipita indica* (formerly *Piriformospora indica*), an endophytic fungus. The inoculation at the *in vitro* rooting stage decreased the *in vitro* root and shoot quality, but after planting microplants in a greenhouse in a non-fertilised peat substrate, the plants grew 33% taller, with 10% more leaves and with more abundant roots in comparison with the non-inoculated microplants. The young plants rooted *in vitro*, acclimated in a greenhouse in a peat substrate and inoculated with *S. indica* at the time of the transplantation to the pots displayed, after 15 months, an increase in the shoot lengths by 35% and 13%, depending on the cultivar, in the leaf number by 47% and in the chlorophyll content by 31% when compared with the non-inoculated plants. The beneficial effect of *S. indica* on the young *Rhododendron* plants indicates the possibility of its practical use in the nursery production of this plant.

Keywords: endophyte; growth stimulation; micropropagation; *Rhododendron*; roots colonisation

Serendipita indica is an endophytic fungus that was isolated from the desert soil in India (Verma et al. 1998). This organism was classified to the order Sebacinales, the Basidiomycota group and initially named *Piriformospora indica* (Verma et al. 1998; Varma et al. 2013). Recently, it was reclassified to the *Serendipita* genus as *Serendipita indica* (Weiß et al. 2016). Unlike most arbuscular mycorrhizal fungi, *S. indica* can be propagated *in vitro*, both on solid and liquid media (Verma et al. 1998). The endophytes colonise the roots by penetrating the interior of the cells, as well as the intercellular spaces, producing pear-shaped chlamydospores in the tissues of the root cork, but it does not colonise the aboveground plant parts (Oelmüller et al. 2009). Numerous authors have emphasised the beneficial effects of *S. indica* on a plant's biomass increase: the length and number of the roots, the diameter and height of the shoots, the number and size of the leaves (e.g. Sahay, Varma 1999; Varma et al. 1999; Franken 2012; Dolatabadi, Goltapeh 2013; Johnson et al. 2014), and an increase in the seed production (Bagde et al. 2011) as well as the role that the endophytes play in bio-protection against abiotic (Sun et al. 2010; Hosseini et al. 2017) and biotic factors (Serfling et al. 2007; Fakhro et al. 2010; Sun et al. 2014; Bajaj et

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al. 2015). Due to the possibility to grow *S. indica* in a liquid medium which enables the easy production of a sterile inoculum and in a wide spectrum of host plants (Qiang et al. 2012), *S. indica* could be potentially useful in plant production, also for organic farming (Ansari et al. 2013; Rabiey et al. 2017). Bio-fertilisation and bio-protection are two reasons why the applications of *S. indica* make it potentially useful in the production of *Rhododendron* plants, which belong to an economically important group of ornamentals whose production develops dynamically, according to the needs of the market.

In this study, our objective was to determine whether *S. indica* can stimulate the growth of *Rhododendron* plants grown without any fertilisation in a series of experiments. Inoculation of the microshoots was carried out during the *in vitro* rooting and in a greenhouse after acclimatisation, during the transplantation of the plants into pots.

MATERIALS AND METHODS

Standard protocol of *Rhododendron in vitro* propagation and acclimatisation in a greenhouse. The unrooted or rooted *Rhododendron* microshoots cvs. Nova Zembla and Alfred belonging to the *R. catawbiense* group (Dirr 1998) were used in the experiments. The shoots were propagated on a Woody Plant Medium (WPM; Lloyd, McCown 1980) supplemented with 5 mg/L of cytokinin 2-isopentenyladnine (2iP), 80 mg/L of adenine sulphate and 1 mg/L of indole-3-acetic acid (IAA). The medium was solidified with 7 g/L of a Bacto agar. The rooting was carried out in a sterile coarse perlite saturated with a liquid WPM medium supplemented with 1 mg/L of indole-3-butric acid (IBA). Each medium contained 30 g/L of sucrose. The pH of each medium was set to 4.8. The cultures were incubated in a growth room with 16 h of light at 22 ± 2 °C. After rooting, the microplants were rinsed with water to remove the medium. They were then planted in boxes filled with a 2 : 1 peat : perlite mix with a pH of 4.5. The boxes were placed under foil tunnels in a greenhouse for acclimatisation. After about 2 weeks, the foil was gradually tilted until its complete removal. The shoots were protected against grey mould with Gwarant 500 SC (active substance chlorothalonil 40.6%, Arista, Poland) at a concentration of 0.2%. The contents of the macro-elements in the growth substrate at the beginning of the experiments (at planting) were as follows (in mg/L): N (in the nitrate form) – 5, P – 26, K – 5, Mg – 40, Ca – 524. Throughout the duration of the experiments, the plants were not fertilised.

Preparation of *S. indica* inoculum. The cultures of *S. indica* were kept on 90-mm-diameter Petri dishes containing a Käfer agar medium (Hill, Käfer 2001) at 25 °C. The cultures were co-cultivated every 6 months with roots of *Arabidopsis thaliana* (Columbia-0 ecotype) on Petri dishes according to the recommendations of Johnson et al. (2011). The mycelium of *S. indica* for the experiments was produced in 250 mL flasks containing 100 mL of the liquid Käfer medium. The medium was inoculated with eight 5-mm-diameter discs overgrown with the mycelium, which was growing on the Käfer agar medium. The cultures were incubated in the dark at 25 °C on a rotary shaker at 90 rpm. After three weeks, the mycelium was drained, washed three times with distilled water, drained again thoroughly and weighed. The mycelium was then homogenised in distilled water (50 g in 300 mL). The density of the chlamydospores in the obtained inoculum was determined using a Bürker haemocytometer counting chamber and ranged from 1.3 to 2.5 × 10^5 between the preparations. The peat substrate was inoculated with a proportion of 1 g of inoculum and 100 mL of peat (1% w/v).

Inoculation of *Rhododendron* microshoots with *S. indica* during *in vitro* rooting. The 'Nova Zembla' microshoots, approx. 2 cm long, which were obtained as described above, were rooted in jars in an autoclaved perlite, into which 30 mL of the liquid WPM medium was added. Three basic treatments were used: a – medium without auxin, b – with 1 mg/L of IBA, c – with 1 mg/L of IAA. Each treatment involved 12 jars, each with 5 microshoots. Each of the 6 jars from each treatment was supplemented with 1 mL of the inoculum prepared as above at a concentration of the chlamydospores of 2.3 × 10^5 in sterile distilled water. In the control cultures, 1 mL of sterile distilled water was added. After 9 weeks, the number of rooted shoots, the length of the shoots, the colour of the leaves, the length of the longest root and the abundance of the roots were recorded. Due to many branched roots, their number was often not quantifiable, therefore, a scale of abundance 1–3, with 3 being the most abundant was implemented. Then, the microplants were planted in boxes in the peat substrate as above and grown in the greenhouse. After 5 months, the plants were

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transplanted into pots with a diameter of 7 cm. Root fragments were collected to assess the *S. indica* colonisation. After an additional 10 months of growth in the greenhouse, the shoot length, leaf colour and number, and root system size were evaluated (on a scale of 1–3).

Inoculation of *Rhododendron* plants with *S. indica* during growth in the greenhouse. The greenhouse-acclimatised microplants of four months old of *Rhododendron* 'Nova Zembla' and 'Alfred', which were rooted *in vitro* and grown in the peat substrate in boxes, but not co-cultivated with *S. indica*, were included in the subsequent experiments. They were transplanted into 9 × 9 × 9 cm cubic pots with a peat substrate containing an inoculum of *S. indica* at a concentration of 1% (w/v). The control plants were planted into a substrate not inoculated with *S. indica*. At the time of the transplanting, at the beginning of the experiment, each plant was evaluated by measuring the length of the main shoot and determining the number of leaves in 'Nova Zembla'. The leaves of 'Alfred' were not counted due to their large number and varied sizes in this growth phase. After 3, 7 and 15 months, the length of the main shoot of both cultivars and the number of 'Nova Zembla' leaves were evaluated. After 15 months, the root system of the plants and the chlorophyll index of the 'Nova Zembla' leaves were evaluated. The chlorophyll index was measured on the tenth leaf from the apex using a CCM-200 (Chlorophyll Content Meter, Opti-Sciences, USA). The root system was evaluated as the root abundance on a scale of 1–3, with "3" as the most abundant. There were 25 plants in each treatment. The experiment was repeated the following year. At the end of the experiment, leaf samples were collected for analysis of the macro- and microelement contents.

Assessment of an ability of *S. indica* for root colonization of *Rhododendron* after incubation without live plant tissues. In the laboratory experiment, 1 mL of the *P. indica* inoculum containing 1.3×10^5 chlamydospores in sterile distilled water was added to each jar with perlite and the WPM liquid medium. The *in vitro* rooted microplants of both *Rhododendron* cultivars were transplanted into the above jars after 2, 4 or 6 weeks. The root colonisation was assessed after 3–4 weeks. In the greenhouse experiment, the peat substrate containing 1% (w/v) of the *S. indica* inoculum was used. After 2, 4 or 6 weeks, the rooted *in vitro* microshoots were transplanted to the inoculated peat. Evaluation of the root colonisation was carried out after 3–4 weeks.

Ten root fragments per plant, 1.5 cm in length, were collected from five plants in total. They were thoroughly washed with distilled water, and, following the method of Johnson et al. (2011), they were sterilised with a mixture (for a 100 mL solution): 4 mL of lauroylsarcosine, 32 mL of Ace (Fater Temizlik Ürünleri Ltd. Turkey), a whitening preparation containing about 5% sodium hypochlorite, and 64 mL of sterile water. The root preparations were stained with cotton blue (Kumar et al. 2009) or assessed without staining. The presence or absence of *S. indica* chlamydospores in the 10 fields of view (magnification of 40×) in each of the extracted root fragments was assessed and expressed in %.

Analysis of macro- and microelements in the leaves. The first upper leaves per plant, which had a size typical of the cultivar, were collected for the analysis. After drying in a forced-draft oven at 60 °C and determining the dry weight, the samples for the total nitrogen content were mineralised in concentrated sulfuric acid in the presence of a copper-potassium catalyst. The N content was determined using a Kjeldahl apparatus (Vapodest, Gerhardt, Germany) according to the Official Methods of Analysis AOAC International, 16th Edition (1995). After distillation of nitrogen in the form of NH_3, the N content was determined by the titration method (AOAC International, as above). For the other elements (P, K, Mg, Na, S in the form of sulfate, Ca, Fe, Mn, Cu, Zn, B), the dried plant samples were ground in a Wiley stainless steel mill, then digested in HNO_3 in a microwave, using closed Teflon vessels, following the methods of Walinga et al. (1989). The elements were determined by an inductively coupled plasma spectrometer (ICP Model OPTIMA 2000DV, Perkin Elmer, USA).

Statistical analysis. The data related to the rooting under the *in vitro* and greenhouse conditions were analysed by means of a one-way analysis of variance (ANOVA). The growth analysis was performed within a repeated-measures ANOVA model. The sphericity assumption was assessed with Mauchly's test. If the sphericity was violated, adjustments to the freedom degree of the *F* distribution were made with the Greenhouse-Geisser correction procedure. The absence of an interaction between the treatment and replication of the greenhouse experiment caused the results to be presented as the mean of two experiment repetitions. Multiple comparisons of the means were performed using a Duncan multiple range test

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(DMRT) at $P = 0.05$. The mineral composition of the leaves was analysed using a two-tail t-student test. The analyses were performed with the DELL STATISTICA software package, Dell Inc. (2016), Version 13.

RESULTS

Ability of *S. indica* to colonize *Rhododendron* roots depending on the time of inoculum incubation in a perlite or peat substrate lacking live plant tissues. The colonisation of the roots ranged from 35 to 45%, depending on the cultivar and the experiment repetition, when the rooted shoots were planted into the perlite with the rooting medium 2, 4 or 6 weeks after the inoculation (Figure 1). In the experiment where the peat substrate was inoculated with *S. indica* and planting was undertaken after 2, 4 or 6 weeks, the chlamydospores were found in 50–55% of the assessed roots. The colonisation percentage did not depend on the time of the planting into the previously inoculated perlite or peat substrate.

The impact of *S. indica* on *in vitro* rooting of *Rhododendron*. The inoculation of perlite saturated with the rooting medium with *S. indica* increased the rooting percentage of the *Rhododendron* microshoots by 24% in the combination where auxin was not added. The media containing auxin enabled the rooting of almost all the microshoots, irrespective of whether *S. indica* was applied or not (Table 1). The inoculation decreased the shoot and root length and the root abundance in relation to the microcuttings grown in the non-inoculated perlite. The fungus caused the discolouration of the leaves to a dark red colour. The percentage of microshoots with red leaves was 96% when *S. indica* was applied and 93% or 43% in which IAA or IBA were applied together with the endophytes, respectively. No discolouration appeared in the microplants rooted in the media containing only auxin. In the control microplants grown without auxin and endophytes, 20% of the leaves were coloured red (Table 1). Only 20–37% of the microshoots inoculated with *S. indica* contained leaves typical in size of the *Rhododendron* cultivar. The remaining microplants had smaller leaves (data not shown).

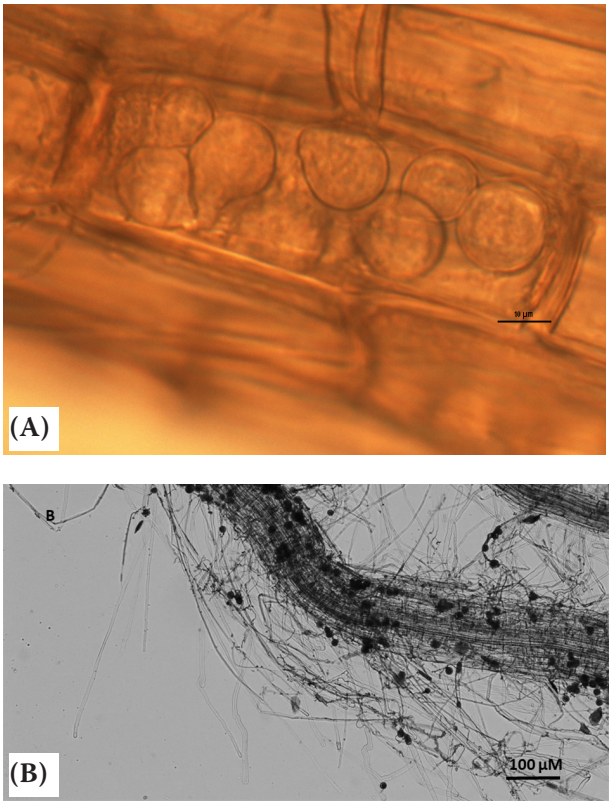

Figure 1. Colonisation of the 'Nova Zembla' *Rhododendron* roots with *Serendipita indica*: (A) 6 weeks after inoculation, and (B) 4 weeks after inoculation

Table 1. The impact of auxin and a *Serendipita indica* inoculation on the *in vitro* rooting of 'Nova Zembla' *Rhododendron* microshoots ($n = 30$), after 9 weeks

Treatment		Rooted shoots (%)	Length of rooted shoots (cm)	Roots abundance (1–3 scale, with 3 being the most abundant)	Length of roots (cm)	Shoots with red leaves (%)
Inoculation with *S. indica*	Addition of auxin (mg/L)					
–	–	67	4.4 ± 0.33[a]**	1.5 ± 0.11[b]	0.8 ± 0.09[b]	20
+*	–	83	3.4 ± 0.22[b] (23)***	1.0 ± 0.0[c] (33)	0.5 ± 0.00[d]	96
–	IBA 1	97	3.4 ± 0.18[b]	1.5 ± 0.09[b]	1.2 ± 0.08[b]	0
+	IBA 1	100	3.1 ± 0.18[b] (9)	1.3 ± 0.08[bc] (13)	0.7 ± 0.05[cd]	43
–	IAA 1	100	4.9 ± 0.19[a]	1.9 ± 0.06[a]	1.5 ± 0.08[a]	0
+	IAA 1	100	3.3 ± 0.18[b] (33)	1.2 ± 0.07[bc] (37)	0.8 ± 0.07[c]	93

*5 shoots died; ** mean values ± standard deviation followed by the same letter in the columns are not significantly different at $P = 0.05$; *** in parentheses, % decrease in comparison with the non-inoculated shoots

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Table 2. The impact of the *in vitro* rooting conditions on the growth of the 'Nova Zembla' *Rhododendron* plants after transplanting into the greenhouse (n=30), after 10 months

Treatment		Acclimatized plants (%)	Length of shoots (cm)	Leaf number	Roots abundance (1–3 a scale, with 3 being the most abundant)
Inoculation with *S. indica*	Addition of auxin (mg/l)				
–	–	93	7.5 ± 0.51^{d}*	13.5 ± 1.01^{bc}	2.8 ± 0.09^{b}
+	–	100	9.7 ± 0.65^{c} (33)**	15.0 ± 0.81^{b} (10)	3.0 ± 0.04^{a}
–	IBA 1	73	7.5 ± 0.26^{d}	11.7 ± 0.39^{c}	3.0 ± 0.01^{a}
+	IBA 1	100	12.0 ± 0.59^{b} (47)	16.1 ± 0.72^{a} (27)	3.0 ± 0.03^{a}
–	IAA 1	80	9.8 ± 0.48^{c}	13.7 ± 0.79^{bc}	2.9 ± 0.08^{ab}
+	IAA 1	93	13.5 ± 0.52^{a} (28)	16.3 ± 0.73^{a} (18)	3.0 ± 0.05^{ab}

*Mean values ± standard deviation followed by the same letter in the columns are not significantly different at $P = 0.05$; **in parentheses, % increase in comparison with the non-inoculated plants

The impact of *S. indica* on growth in the greenhouse *Rhododendron* microplants inoculated during *in vitro* rooting. The adverse effect of *S. indica* subsided after the microplants were transferred and established in the greenhouse. All the plants inoculated with the endophytes acclimatised better than the non-inoculated ones. The inoculated plants were taller and had more leaves than their non-inoculated counterparts (Table 2). There was no obvious effect on the colour of the leaves or on the root system abundance in the plants depending on the conditions during the *in vitro* rooting.

The impact of *S. indica* inoculated in the greenhouse on *Rhododendron* plants. The inoculation of the plants in the greenhouse at transplantation had a positive effect on the plant growth of both cultivars (Table 3). The aerial parts of 'Nova Zembla' were 22.3, 32.7 and 34.5% taller than the non-inoculated ones at the measurement times, i.e., after 3, 7, and 15 months of the experiment, respectively (Figure 2, Table 3). Similarly, the aerial parts of 'Alfred' were 7.2, 9.6, and 12.7% taller than the non-inoculated ones after 3, 7, and 15 months of the experiment, respectively. The 'Nova Zembla' plants also had 20–47.3% more leaves and 31.1% more chlorophyll than the non-inoculated plants. The non-inoculated 'Nova Zembla' plants grew slower and also had fewer leaves after 3, 7 and 15 months in comparison to the number at the beginning of the experiment (Table 3) which was caused by the dropping of the lower leaves.

Table 3. The impact of the inoculation of 'Nova Zembla' (n = 50) and 'Alfred' (n = 60) *Rhododendrons* with *Serendipita indica* at re-planting on the growth parameters (the means from 2 repetitions of the experiment)

Treatment	'Nova Zembla'		'Alfred'	
	without *S. indica*	with *S. indica*	without *S. indica*	with *S. indica*
Shoot length				
at experiment beginning	4.04 ± 0.98^{a}*	4.33 ± 1.10^{a}	4.61 ± 1.13^{a}	4.52±1.00^{b}
after 3 months	7.72 ± 1.45^{a}	9.94 ± 1.86^{b} (22.3)**	6.83 ± 1.30^{a}	7.42 ± 1.09^{b} (7.2)
after 7 months	9.48 ± 1.57^{a}	14.09 ± 2.44^{b} (32.7)	10.20 ± 1.55^{a}	11.28 ± 1.21^{b} (9.6)
after 15 months	12.37 ± 2.03^{a}	18.88 ± 2.45^{b} (34.5)	13.19 ± 1.63^{a}	15.85 ± 1.03^{b} (12.7)
Leaf number			not counted	not counted
at experiment beginning	10.94 ± 1.98^{a}	11.12 ± 2.19^{a}		
after 3 months	12.06 ± 2.27^{a}	15.08 ± 2.80^{b} (20.0)		
after 7 months	9.60 ± 2.20^{a}	16.98 ± 3.05^{b} (43.5)		
after 15 months	9.60 ± 2.37^{a}	18.20 ± 2.89^{b} (47.3)		
Chlorophyll content index	38.87 ± 11.17^{a}	56.39 ± 12.07^{b} (31.1)		

*Mean values ± standard deviation followed by the same letter are not significantly different at $P = 0.05$; the comparisons were made separately for the cultivars and the terms of the records; **in parentheses, % increase in comparison with the non-inoculated control

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Figure 2. The 'Nova Zembla' *Rhododendron* 7 months after planting: in the peat substrate inoculated with *Serendipita indica* at a concentration of 1% (w/v, left side), in the peat substrate without *S. indica* (right side)

The impact of *S. indica* on content of macro- and microelements in leaves of *Rhododendron*. The analysis of the micro- and macro-elements showed significant differences in the K, Ca, Mg and Mn (Table 4) concentrations. In the plants inoculated with *S. indica*, the Ca and Mg contents were significantly higher, but the K and Mn were significantly lower than in the leaves of the non-inoculated plants. The proportion of dry matter and N content were not dependent on the type of treatment.

DISCUSSION

Along with the first description of the fungus (Verma et al. 1998), information about its ability to colonise a wide spectrum of plant species has been reported. Jacobs et al. (2011) suggested that this effective colonisation is based on the specific suppression of the natural plant immunity by *S. indica*. Varma et al. (1999) were the first to point out that both the co-cultivation of young plant roots with the fungus as well as with the fungal culture filtrate resulted in an increase in the fresh weight of maize, tobacco, poplar and other plant species. Over the years, the list of plant species responding to colonisation with enhanced productivity has increased to more than 20 (Johnson et al. 2014). *S. indica* is now considered a beneficial microorganism that can be used as a biofertiliser and bio-protector (Oelmüller et al. 2009; Varma et al. 2012). Its special role is related to the impact on young plants – cuttings (Druege et al. 2007), microplants obtained *in vitro* (Sahay, Varma 1999; Das et al. 2017), and seedlings (Dolatabadi, Goltapeh 2013; Gupta et al. 2017). *S. indica* provides plants the opportunity for rapid growth, which leads to the production of a larger biomass and the faster development of generative organs, and, as a consequence, a higher seed yield (Achatz et al. 2010; Badge et al. 2011). Despite a number of studies, the way in which the fungus affects plants has not been completely defined. Certainly, the fungus facilitates access to phosphates by the activity of acid phosphatase (Malla et al. 2004), to nitrogen through the stimulation of nitrate reductase plant genes, to carbon through the activity of starch degrading enzymes (Sherameti et al. 2005), and to sulphur, due to the increased expression of the fungal genes related to the sulphate reduction. According to Sirrenberg (2007), *S. indica* affects the growth of plants and, above all, the development of the roots, through the production and sharing of IAA. In biotests with *Arabidopsis*, IAA from liquid fungal cultures had an effect on the root growth comparable to that obtained with a synthetic IAA. Lee et al. (2011) stated, however, that, at least, in Chinese cabbage, the fungus affects the activation of the auxin synthesis in the plant's roots.

Table 4. The effect of the inoculation of the 'Nova Zembla' *Rhododendron* with *Serendipita indica* on the mineral composition of the leaves after 15 months of cultivation in a greenhouse (n = 4)

S. indica	Dry weight	N	P	K	Ca	Mg	Na	S**	Fe	Mn	Cu	Zn	B
	(%)	(mg/kg d.w.)											
–	34.7	1.0	358	8 610	7 882	3 498	310	818	102	238	2.35	38.2	58.9
+	35.0	0.94	429	6 368*	10 083*	4 149*	380	778	85.5	181*	2.28	34.6	52.7

*The means significantly different from the control at P = 0.05, **in the form of sulphate; d.w. – dry weight

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In our experiment on the *in vitro Rhododendron* rooting, the mycelium suspension added to the liquid WPM medium with perlite led to contradictory results. The microshoots after the *in vitro* co-cultivation with *S. indica* were shorter and the roots were shorter and less abundant. Moreover, there was a significant reddening of the leaves and leaf miniaturisation in the treatments with the fungal inoculum; whereas the IAA or IBA addition to the medium did not change the colour and more shoots had leaves of normal size. A similar negative influence of *S. indica* has not yet been reported, but no investigation was made using perlite as a support in the plant tissue culture. On the other hand, the inoculation caused an increase in the proportion of the rooted shoots by 24% compared to the control, which may be associated with the presence of the auxin either produced by the fungus or by the modified plant auxin metabolism. The negative effect of *S. indica* on the microshoots, especially on the shoot and root length, colour and size of leaves could be explained by the nutritional stress, especially the lack of a sufficient amount of nitrogen in the nutrient solution due to its possible depletion by the mycelium. Moreover, a red leaf colour is considered a symptom of nutritional stress. Transplanting the rooted microplants to the substrate in the greenhouse caused the opposite effect. The plants that were rooted in the *S. indica* inoculated medium grew taller, with more leaves and roots. In addition, more plants successfully acclimated. Similar results have been described by Vyas et al. (2008). When *S. indica* was inoculated at the *in vitro* stage, 30% of the *Feronia limonia* micro-cuttings rooted, but the non-inoculated cuttings were rooted twice as much. In our experiment, the inoculation at the time of transplanting into the peat substrate resulted in an increase in the plant's height, the shoot's diameter, larger leaves, and the number of leaves relative to the plants growing in the non-inoculated substrate.

Transplanting the acclimatised microplants into the *S. indica*-inoculated peat substrate improved the plant growth of both *Rhododendron* cultivars compared to the control plants. The difference in plant height, which was 22% for 'Nova Zembla' and 7%, for the 'Alfred' plants after 3 months of growth, increased after 15 months to 35% and 13%, respectively. The number of leaves and the chlorophyll content increased by 47% and 31%, respectively, after 15 months. The leaves of the inoculated plants were greener and much larger than that of the non-inoculated plants. It is worth noting that these plants were intentionally grown in very poor conditions. Their pots were relatively small and fertilisation was not applied. Indeed, they had exhausted almost all of the substrate by the end of the experiment. The non-inoculated 'Nova Zembla' plants lost their lower leaves probably due to the malnutrition. In the experiment that is currently being carried out, a positive effect of the inoculation with endophytes on the growth of rhododendron plants is obvious, despite the fertilisation with a complete fertiliser recommended for Ericaceae plants. Our experiments confirmed earlier findings regarding the beneficial effect of *S. indica* on the growth of different plants, e.g., tobacco (Baranzani et al. 2005), soybean (Bajaj et al. 2015), *Spilanthes* and *Withania* (Rai et al. 2001), corn, bacopa, artemisia, parsley and poplar (Varma et al. 1999). The reaction of tomato to the inoculation with this endophyte was different in that the fungus had little effect on the vegetative growth, but it promoted an increase in the fresh and dry weight of the fruit (Fakhro et al. 2010).

In our experiment, which was conducted under nutrient poor conditions, the inoculation with *S. indica* caused an increase in the leaf-level content of Ca and Mg, and a reduction in K and Mn relative to the non-inoculated control plants. The P and Fe contents were higher in the *S. indica* inoculated plants, but the differences were not significant. The inoculation did not change the dry matter content of the leaves and the content of the other elements. Apparently, the specificity of the plants, as well as the growth conditions, are important factors given that Tuladhar et al. (2017) observed an increase in both the dry matter and nitrogen content in tomato plants. In contrast, Achatz et al. (2010) did not find a higher nitrogen and phosphorus content in barley plants as a result of the *S. indica* colonisation. Similar results were obtained in relation to *Nicotiana attenuata* by Barazani et al. (2005).

Our study has shown that in extremely poor conditions, *S. indica* stimulates growth of rhododendron plants. It has also been shown to play another and even more important role in stimulating the resistance of *Rhododendron* to pathogens of the *Phytophthora* spp. (Trzewik et al. 2020). Nevertheless, to develop a functional nursery technology enabling use of *S. indica*, further experiments aimed at the optimal fertilisation should be conducted.

It was a valuable confirmation from a practical point of view that *S. indica* can persist in the state enabling the root colonisation in an environment lacking live plant roots and plant exudates. Indeed, Rabiey et al. (2017) reported that *S. indica* can persist in the soil

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for 15 months despite the absence of plant hosts, but our results were obtained in different conditions. In our experiment, the endophyte inoculum that was incubated in a sterile medium/perlite substrate and in a virgin peat substrate did not lose its colonisation capacity for 6 weeks. This means that this fungus could survive without the presence of host roots for at least 6 weeks, which allows for the use of an inoculated substrate for non-rooted cuttings.

In conclusion, we have found, for the first time, that the *S. indica* endophyte stimulated growth of *Rhododendron* plants grown in a greenhouse. The inoculation of the microshoots at the *in vitro* rooting stage was not beneficial and did not fully replace the auxin, while the inoculation of the microplants at the time of transplantation into pots enhanced the plant height, the number of leaves, and increased the chlorophyll content in comparison with the control plants. The beneficial influence of the *P. indica* inoculation continued up to 15 months after planting. These results indicate the advantage of using *S. indica* in the production of a *Rhododendron* planting material.

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