**Burkholderia phytofirmans** PsJN Promotes In Vitro Rooting and Acclimatization of *Helleborus*

T. Orlikowska, K. Nowak, L. Ogórek

Research Institute of Horticulture, 96-100 Skierniewice, ul. Konstytucji 3 Maja 1/3, Poland

**Abstract**

*Helleborus* is a valuable ornamental plant, flowering in early spring or winter. Its hybrid cultivars can be propagated vegetatively, including in vitro via axillary shoots. One of the obstacles preventing effective micropropagation is the necessity for culture to be kept at a temperature of around 15°C, which additionally increases costs. Knowledge obtained up to date shows that inoculating plants with bacteria *Burkholderia phytofirmans* PsJN can increase their tolerance to the non-optimal growth temperature. In this study, we tested whether the bacterium, which is known to produce a relatively high amount of IAA and demonstrates ACC deaminase activity, was able to stimulate *Helleborus* root formation at a temperature of 23°C.

In our experiment, the microshoots cultured without auxin and not inoculated with PsJN rooted in 83% with 1.7 roots of 0.6 cm long. The microshoots, which were induced to root with auxins IBA 3 mg/L and NAA 1 mg/L but not inoculated, were rooted in 94% with 2.0 roots of 1.1 cm long. A similar result was obtained for the microshoots not rooted on auxin containing medium but inoculated with PsJN – 95% rooted shoots with 2.3 roots of 1.2 mm long. The microshoots that were induced to root on the auxins containing medium and then inoculated with PsJN were rooted in 100% with 6.9 roots of 2.1 cm long. Almost all microplants from this treatment acclimatized to greenhouse conditions and grew vigorously, then flowered after winter precooling in a mildly heated greenhouse.

**Keywords:** beneficial bacteria, non-optimal temperature

**INTRODUCTION**

*Helleborus* spp. is a valuable ornamental plant, which also has a large potential as a source of numerous bioactive compounds for medical therapy (Maior and Dobrotă, 2013). Plants bloom at a low temperature in early spring or even in winter. In a nursery environment, the chilling of young plants stimulated growth (Lowder et al., 2010) and accelerated flowering time (Christiaens et al., 2012). As it turned out, microshoot multiplication and rooting in vitro is more effective at a temperature of around 15°C (Seyring, 2002; Poupet et al., 2006; Dhooghe and van Labeke, 2007; Beruto and Curir, 2009; Gabryszewska, 2014). Establishing of this condition can be a problem due to the cost of cooling. Knowledge obtained to date shows that inoculating plants with bacteria *Burkholderia phytofirmans* PsJN can help in achieving greater tolerance to the non-optimal growth temperature, both higher (Bensalim et al., 1998) and lower (Ait Barka et al., 2006), and it was for this reason we wanted to test *Burkholderia phytofirmans* PsJN to increase the tolerance of *Helleborus* to non-optimal temperature. Firstly, we would like to prove whether the bacterium, which is known to produce a relatively high amount of IAA and demonstrates high ACC deaminase activity (Sessitsch et al., 2005; Weilharter et al., 2011) would be able to

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* e-mail: Teresa.Orlikowska@inhort.pl
stimulate *Helleborus* rooting at typical growth room temperatures of around 23°C, not optimal for rooting.

**MATERIALS AND METHODS**

Cultures from the plant of an unknown genotype, probably hybrid cultivar, purchased in a pot, from a market, were initiated first from axillary buds, but due to a heavy bacterial contamination, adventitious shoots were initiated and chosen for further propagation in vitro. Adventitious shoots were obtained from the youngest leaves, placed on the medium containing ½ MS mineral salts (Murashige and Skoog, 1962) and WPM vitamins (Lloyd and McCown, 1981), supplemented with TDZ 1 mg L⁻¹, NAA 0.5 mg L⁻¹ 20 g L⁻¹ sucrose and 7.5 g L⁻¹ Bacto agar. Single buds arose directly without callus induction after several months. They were transferred to the MS medium, containing BAP 5 mg L⁻¹, 2iP 2 mg L⁻¹, NOA 0.1 mg L⁻¹, riboflavine 2.5 mg L⁻¹, sucrose 30 g L⁻¹ and Plant agar (Duchefa) 6 g L⁻¹. The explants were cultured in Petri plates and incubated at a temperature 23±3°C and 16/8 photoperiod provided with 40 umol m⁻² s⁻¹ emitted by fluorescent daylight lamps. Auxillary shoot propagation was very slow irrespective of different combinations of growth regulators, and in addition, black coloration of medium occurred. After two years from initiation, the cultures were transferred to a growth room with a temperature of 15°C±2°C, where production of axillary shoots accelerated instantly.

The multi-microshoots were transferred for 4 weeks for elongation to the MS medium containing BAP 1 mg L⁻¹ as the only growth regulator. The microshoots from above medium were rooted using a 2 stage procedure: first, induction of roots in the dark and then stimulation of root growth in the light. A batch of microshoots 2-3 cm long including leaf length, was divided into two parts and one half was induced to root on the MS agar medium without any auxin, and the second part on the medium containing IBA 3 mg L⁻¹ and NAA 1 mg L⁻¹. After 5 days all microshoots were transferred to sterile perlite soaked with MS liquid medium without growth regulators and each half of the former two treatments were inoculated, or not, with bacterium PsJN, obtained from a 24 h culture in the liquid KING B medium, and adjusted to the concentration 10⁸ L⁻¹. The rooting experiment was performed in a growth room at a temperature of 23±3°C and 16/8 photoperiod provided with 40 umol m⁻² s⁻¹ emitted by fluorescent daylight lamps.

After 4 weeks the result of rooting was assessed and the root number and length were evaluated. In each of four treatments 60 microshoots were used (6 vessels with 5 microshoots in each and two replications of the experiment). Data was subjected to an analysis of variance and means compared with Duncan’s multiple range test at p 0.05.

All rooted shoots were planted in a substrate of peat and perlite (3:1) then grown in the greenhouse, at a temperature of 15-30°C.

**RESULTS AND DISCUSSION**

From the shoots that were induced to root without auxin and not inoculated with PsJN 83% formed a small quantity of very short roots – 0.6 cm (Fig. 1, Table 1). Very similar rooting results were obtained from two treatments, where the shoots were induced to root on auxins containing medium but not inoculated with PsJN and where the shoots were not induced to root with auxins but inoculated with PsJN, which rooted in 94 and 95% respectively. The number of roots in these treatments was 2.0 and 2.3 and the roots were also short, 1.1 and 1.2 cm, respectively (Fig. 1). All the microshoots that received auxin induction and PsJN inoculation rooted, forming 6.9 roots per shoot, with a length of 2.1 cm (Fig. 1, Table 1). The most developed and the greenest leaves were grown on microplants from the treatment rooted without auxin nor PsJN. Nevertheless, these shoots were not able to acclimatize in the given greenhouse condition, which can probably be attributed to their very short roots. Contrary to this, almost 100% of the microplants rooted, survived acclimatization and grew very intensively when potted, in the group treated with auxin and inoculated with PsJN. After spending winter in a mildly heated greenhouse at 5-10°C, they flowered in the spring.
In our experiment, the main obstacle for effective micropropagation of *Helleborus* was too high temperature in the growth room. The cultures began to proliferate shoots when transferred to a growth room with a temperature of 15°C. The beneficial effect of such a temperature for *Helleborus* micropropagation is known from the report of Seyring (2002). For rooting, Dhooghe and Van Labeke (2007), Beruto and Curir (2009) and Beruto et al. (2013) applied pulse, 7 day-long chilling at 5-7°C, together with drenching in auxin solution. The chilling enhanced rooting efficiency by 5% (Beruto et al. 2013).

The subsidarity role of endophytic bacteria in both plant growth and increasing defense against abiotic stress has been reported in numerous publications (rev. Gaiero et al, 2013). Some beneficial bacteria can also play a useful role in plant tissue cultures, especially influencing rooting and acclimatization (Poupin et al, 2013). At this stage, an abiotic stress is a main factor decreasing acclimatization effectiveness (Chandra et al., 2010). Incorporation of a commercial preparate containing *Agrobacterium radiobacter* K1026, used for crown gall protection, to the substrate in which micropropagated *H. niger* was planted resulted in an increased chance of survival and more intensive growth of microplants (Susek et al., 2010).

The discovery of the role of *Burkholderia phytofirmans* PsJN in plant tissue cultures (Nowak, 1998) gave impetus to further study and application of this bacterium. Poupin et al. (2013) found that it activates 408 genes when inoculated in *Arabidopsis thaliana*, including those connected with auxin and gibberellin metabolism and with stress response. Among other things, its role in supporting rooting and acclimatization has been reported (Nowak, 1998). In our experiment, we assumed that both IAA production and ACC deaminase could help in rooting in a not optimal temperature without applying cold pulse treatment. In fact, it increased rooting effectivity and, due to the high quality of microplants, also enabled efficient acclimatization. The action of bacteria seems to partly replace auxin but also influences the rooting and acclimatization processes in other ways.

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**Literature Cited**


### Tables

Table 1. Influence of auxins and inoculation with *Burkholderia phytofirmans* PsJN on rooting of *Helleborus* sp. in vitro

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Medium for root induction (5 days)</th>
<th>Medium for root growth (4 weeks)</th>
<th>Results</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<td>% of rooted shoots</td>
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<tr>
<td>Auxins -</td>
<td>PsJN -</td>
<td></td>
<td>83</td>
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<tr>
<td>Auxins +</td>
<td>PsJN -</td>
<td></td>
<td>94</td>
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<tr>
<td>Auxins +</td>
<td>PsJN +</td>
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### Figures

Fig. 1. Influence of auxins and inoculation with *Burkholderia phytofirmans* PsJN on rooting of *Helleborus* sp. in vitro