

Promotion of root development and root growth of forest plants by rhizobacteria

Irmtraut Zaspel, Dietrich Ewald

Federal Research Centre for Forestry and Forest Products, Institute for Forest Genetics and Forest Tree Breeding, Eberswalder Chaussee 3, D-15377 Waldsiedersdorf, Germany

Abstract

The treatment of conifers and deciduous trees from vegetative propagation (*Larix*, *Taxus*, *Thuja*, *Picea*, *Quercus*) and seedlings (*Quercus*, *Fagus*) with beneficial bacteria (*Bacillus*, *Pseudomonas*, *Agrobacterium*) was used to promote the root development. Increased root growth was observed at all tested plant species in form of root induction, length growth and root architecture. The success of root promotion depends on the used strain and genotypic response of plant species. Furthermore the physiological age of plant modified the root development.

Introduction

The practical use of vegetative propagation of woody plants and the raising and cultivation of forest plants produced by plant tissue culture techniques often accompanied by problems of inadequate development of root system. The causes are structures and growth pattern of root systems of cuttings and micropropagated plants varying from the natural structures, the genetic variability of root formation, the age-dependending rooting ability controlled by the internal hormone status of genotypes, the incidence of soilborne pathogens in the artificial substrates and soils and the lack of the native rhizosphere flora at plantlets derived from tissue culture. The initial establishment of transplants under natural conditions is dependent on its ability to produce an efficient root system .

It is well known that rhizosphere of plants supports several species of bacteria including those that can affect the growth and formation of root system and their mode of action is well documented (Haansuu *et al.* 1998, Whiteman *et al.* 1987). Some genera like *Pseudomonas*, *Agrobacterium* and *Bacillus* have this potential benefits to forest plants by its ability to promote root formation and root growth (Shishido *et al.* 1996).

With regard to this traits some known bacterial strains were investigated with the aim to incorporate plant growth promoting bacteria for different methods of vegetative propagation and raising of forest plants.

Material and methods

1. Plant material

Conifers: *Taxus baccata* cuttings were harvested from the small autochthonous relic population in Chorin/Germany within the scope of a gene conservation program. The cuttings of *Larix decidua* derived from old single trees in Brandenburg/Germany with outstanding growth traits. The triploid *Thuja plicata* clone used possessed a fast growth performance as well. These three tree species were cultivated after bacterial inoculation under misted glass-house conditions at 18°C. Root development and rooting rate were evaluated and compared with untreated control (*T. baccata*) and treatment with 2000ppm IAA (*L. decidua*, *T. plicata*) respectively.

Cuttings of adult *Picea abies* trees were harvested from a clone collection located near Waldsiedersdorf/Germany. These clones go back to relics of a spruce population of Saxon Ore

Mountain/Germany possessed a tolerance against SO₂ air pollution. The cuttings of the juvenile material came from seedlings of this clone collection (Weiser et al. 1988). The treated material was grown under plastic greenhouse conditions in a sandy soil with mist. The root development and rooting rate were observed and compared with untreated control.

Deciduous tree species: Oak lines were established in vitro from buds of young *Quercus robur* and *Q. petraea* plants (Ewald et al. 1997). These clones are progenies of a very old oak population (300 – 600 years) in Brandenburg/Germany represents a valuable gene resource with high genetic diversity. The propagation in vitro is part of a long-term conservation program. The rooted plantlets were cultivated after treatment in quartz sand under misted glass-house conditions at temperature of 18°C for four months.

Testing the *Bacillus* preparation FZB24 under nursery conditions one year old *Q. robur* and *Fagus sylvatica* plants from seed orchards of indigenous provenience were used. The success of treatment was evaluated one year later. Root fresh weight, root collar diameter and the branching index of roots were determined for every treatment group. The branching index of roots were assessed in steps from 1 to 3 for each plant, where 1 is poor branching of root system and 3 is very well root formation.

Tab 1: Overview of investigated plant materials and used bacteria

Plant species	Kind of plant material	No of clones / replications	Bacteria treatment	Duration of test
<i>Taxus baccata</i>	cuttings from old trees (50 yr)	5 clones / 24 replic.	<i>B. subtilis</i> T99, Z1	5 months
<i>Larix decidua</i>	cuttings from old trees (140 yr)	3 clones / 18 replic.	<i>A. rhizogenes</i> DSM30148	3 months
<i>Thuja plicata</i>	cutting from a triploid tree (15 yr)	1 clone/ 20 replic.	<i>A. rhizogenes</i> DSM 30148	3 months
<i>Quercus robur</i>	rooted plantlets from in vitro	2 clones / 40 replic.	<i>A. rhizogenes</i> DSM30148	4 months
<i>Quercus petraea</i>	rooted plantlets from in vitro	4 clones/ 30 replic.	<i>A. rhizogenes</i> DSM 30148	4 months
<i>Picea abies</i>	cuttings, 15 yr (mature) and 8 yr (juvenile) plants	population/ 20 replic.	<i>B. subtilis</i> T99 <i>Pseudomonas</i> sp.	8 months
<i>Quercus robur</i>	1 yr plants	population / 50 replic.	<i>B. subtilis</i> FZB24	12 months
<i>Fagus sylvatica</i>	1 yr plants	population / 50 replic.	<i>B. subtilis</i> FZB24	12 months

2. Bacterial strains and their application

The tested *Agrobacterium rhizogenes* DSM 30148 came from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. The *Pseudomonas* strain and the *Bacillus subtilis* strains were originally isolated from rhizosphere soil of agricultural and horticultural plants (Zaspel 1992, Bochow et al. 1988). The *Bacillus* preparation FZB24 was produced by FZB Biotechnik GmbH, Berlin, Germany and is admitted as a promoter of plant growth.

Inoculum was prepared by plating the bacteria on yeast extract mannitol agar containing 1% mannitol (*Agrobacterium*, *Pseudomonas*) and tryptic soy agar (*Bacillus*) and the fresh cells were diluted in sterile water immediately before treatment. The treatment of cuttings and plantlets with pure bacteria was carried out by dipping the pruning wound of shoots and small roots of plantlets resp. in an aqueous dilution of bacteria with a concentration of about 10⁷ till

10⁸ cfu/ml. The *Bacillus* preparation FZB24 was dissolved and applied according to the instructions for use. Two different treatments were tested. The rootstocks of one year old oak and beech plants were dipped in the bacterial solution for 30 min. Soil drenching was done by watering the soil surface with bacteria solution. Control plants were treated by clear water only. An overview of tested plants and bacteria is given in Tab 1.

3. Statistics

The results were computed by procedure GLM of the SAS statistical software (SAS Institute Inc. 1989).

Results

Treatment of plantlets from tissue culture

The treatment of in vitro propagated *Quercus* plantlets with *A. rhizogenes* strain DSM 30148 revealed a different response of the oak genotypes compared with the untreated control. Some clones of sessile and pedunculate oak showed distinct increase of number of main and lateral roots and the total root length but other clones were not or negatively influenced in their root development. Significance ($\alpha=5\%$) was evident at one clone of *Q. petraea* and *Q. robur* only (Fig1).

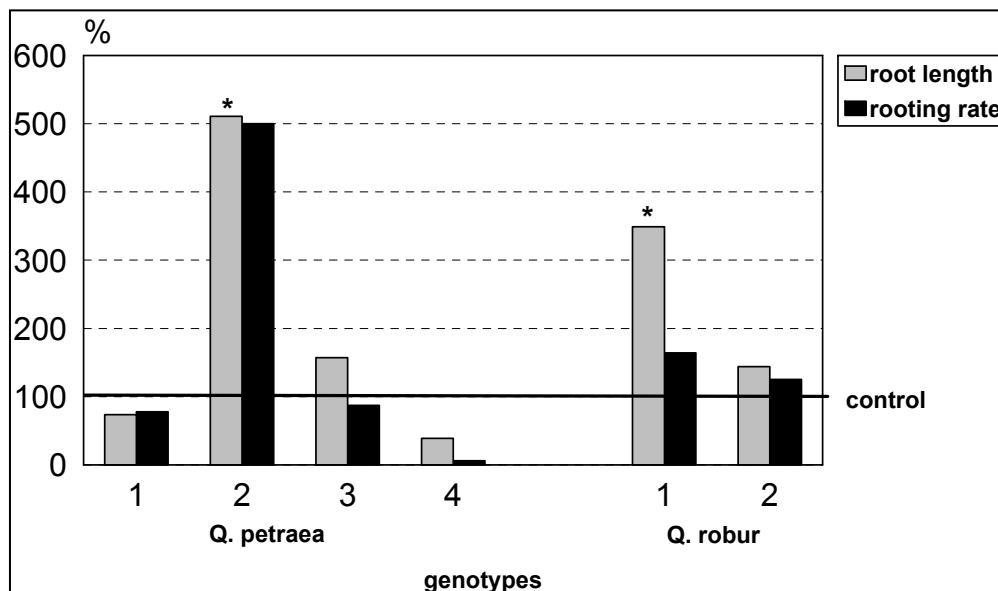


Fig 1: Influence of *A. rhizogenes* DSM 30148 on root growth of oak genotypes

2. Treatment of cuttings

L. decidua, *T. plicata*: Two of three tested clones of *L. decidua* responded to treatment with the *A. rhizogenes* strain such as the triploid *T. plicata* clone in comparison to the conventional auxin treatment with 2000 ppm of IAA. The bacteria treatment had induced the development of roots at clones LH17 and LH27 at the whole. The combination of IAA and bacteria showed no additional effect to development of root system (Fig 2). The survival rate of treated cuttings after transfer to greenhouse increased between 10 and 40% compared to control with auxin only.

T. baccata: The improvement of rooting of common yew was tested by two *B. subtilis* strains. Only one strain promoted root formation in average of the tested 5 clones by 32% and 10% resp. in two independent experiments. The second *Bacillus* isolate showed no influence to rooting process of cuttings. The promotion of root growth by the promoting *B. subtilis* were

connected with light depression of shoot growth by 8% and 6% resp. in this early stage of vegetative propagation.

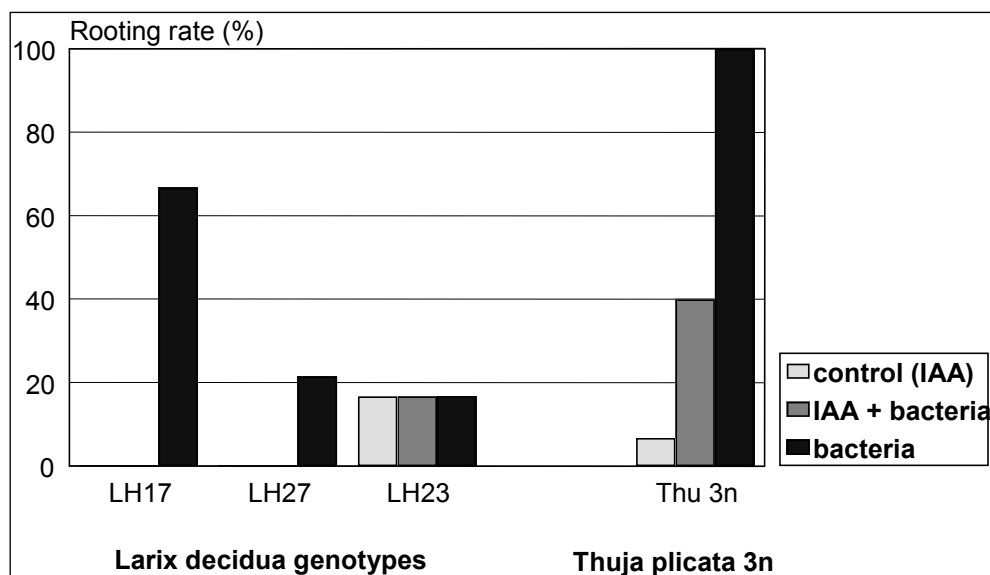


Fig 2: Rooting of cuttings of *L. decidua* genotypes and triploid *T. plicata* after treatment with *A. rhizogenes* DSM 30148

P. abies: The treatment of spruce cuttings derived from mother trees in different ages by *B. subtilis* and *Pseudomonas* sp. resulted in growth promotion of root and shoot. Whereas the cuttings from adult mother trees showed an increase of root length the juvenile material revealed a better root architecture with higher number of lateral and fine roots and more distinct increased shoot growth (Tab 2). Both strains belonging to different genera had an effect in the same direction

Tab 2: Growth of *Picea abies* cuttings derived from juvenile and adult mother trees after treatment with beneficial bacteria

Age of plant material	Treatment	Root length (cm)	No. of main roots	Root fresh weight (g)	Sprout length (cm)	Sprout fresh weight (g)
juvenile	control	25,6 a	6,9 a	1,3 a	3,7 a	1,6 a
	<i>Bacillus</i>	25,8 a	9,0 a	1,8 b	4,4 ab	1,9 b
	<i>Pseudomonas</i>	27,2 a	8,9 a	1,7 b	4,6 b	2,0 b
adult	control	24,5 a	4,0 a	1,0 a	3,0 a	2,0 a
	<i>Bacillus</i>	30,8 b	4,3 a	1,2 b	3,7 b	2,1 a
	<i>Pseudomonas</i>	27,8 ab	4,2 a	1,2 b	3,7 b	2,1 a

3. Treatment of young plants

Q. robur and *F. sylvatica*: The treatment of rootstocks of the one year old plants with a *Bacillus* preparation FZB24 demonstrated after 10 months no significant increase of measured growth parameters but an improvement of root structure especially with an increased part of fine roots at both plant species (Tab 3).

Tab 3: Influence of *Bacillus* preparation FZB24 to root development of *Q. robur* and *F. sylvatica*

Tree species	Treatment	Root collar diameter (mm)	Root fresh weight (g)	Root branching index
<i>Q. robur</i>	control	8,6 (1,7) ¹	19,2 (8,8)	1,6
	soil drenching	9,5 (2,3)	24,9 (13,7)	2,1
	root treatment	9,4 (2,0)	21,8 (11,3)	1,9
<i>F. sylvatica</i>	control	7,4 (1,4)	13,8 (6,4)	1,9
	soil drenching	7,3 (1,4)	14,6 (6,4)	2,2
	root treatment	7,6 (1,5)	15,4 (6,6)	2,2

¹ values in paranthesis are standard deviation

Discussion

Vegetative propagation of selected forest plants is an important contribution under the scope of gene conservation as well breeding and selection works. This concerns single trees with valuable phenotypic traits, relic trees, relic populations and genotypes which cannot be reproduced by the generative way. The rooting process of vegetative parts of woody plants varies from the development of root system of seedlings of forest plants considerably. Especially those stadia like root induction, root growth and modification of root system (relation of main and lateral roots and their distribution type) have been expressed in case of vegetative propagation much poorer.

Some members of bacteria species like *A. rhizogenes*, *B. subtilis* and *Pseudomonas sp.* have been shown capable of stimulating growth of conifers but reports about promoting effects in deciduous trees are rare (Shishido et al.1996).

The tests carried out with several conifer and deciduous tree species reveal the possibility of root growth promotion by beneficial bacteria. This were root initiation e. g. higher rooting rates of unrooted cuttings as well stimulation of root growth at plants from vegetative and generative propagation especially the development of lateral and fine roots. In recent years, research had focussed on the analyses of morphological, cellular and molecular level characterising a number of mechanisms involved in this beneficial effects (O'Gara 1994). In this work the most important factors which influenced the development of root system were the genotype of plant as well the choice of bacteria strain. It is known that adventitious rooting is regulated by a large number of different genes but about the genetic regulation in this process no knowledge exists. Furthermore the physiological age of trees plays a role (Hammett et al.1993). The different reactions of *Picea* cuttings from juvenile and mature trees demonstrated this. Of great importance seems to be the complex relationship between plant root and specific microorganisms basing on an exchange of signals. This lead to an activation of gene expression and explain the different reaction of clones of one plant species as well one genotype to bacteria inoculation.

Additionally the environmental conditions may contribute to the ecology of bacteria influencing the rooting process. Under controlled conditions of greenhouse stimulation of root growth of cuttings and plantlets was evident if a of positive clone-bacteria interaction was given.

The success of a treatment of populations which raised under natural conditions was visible only poorly by quantitative traits. But the improvement of root architecture observed as the increase of fine roots is a precondition for higher stability of the plants against stress factors. The search for specific and useful micro-organisms on the basis of their complementary beneficial effects and on their compatibility for colonising plant genotypes are a possibility to define a strategy for promotion of root development of forest plants from vegetative propagation on a large scale.

References

- Bochow, H., Hentschel, K.H. & Jacob, M. (1988): Möglichkeiten und Wege zu biologischen Bekämpfung phytopathogener Bodenpilze durch Nutzung mikrobieller Antagonisten. *Wiss. Zeitschr. Humboldt Univ. Berlin* 37. 168 – 176
- Ewald, D. & Naujoks, G. (1997): Sustainability of rejuvenation in larch and oak clones. in: Development of integrated systems for large-scale propagation of elite plants using in vitro techniques. *Europ. Com. Brussels, COST 822, Report of activities, 1997*, 105-107
- Haansuu, P.; Elo, S.; Maunuksela, N.; Sarmia, N.; Vuirela, P. & Haahtela, K. (1998): Antagonistic soil and rhizosphere bacteria as potential agents for biological control of plant diseases. *Med. Fac. Landbouww. Univ. Gent* 63/4b: 1678 – 1684
- Hammett, N. & Grant, N. J. (1993): Apparent rejuvenation of mature wild cherry (*Prunus avium* L.) during micropropagation. *Journ. Plant Physiol.* 141, 341-346
- Shishido, M.; Massicotte, H. B., & Chanway, C. P. (1996): Effect of Plant Growth Promoting *Bacillus* strains on Pine and Spruce Seedling Growth and Mycorrhizal Infection. *Ann. Bot.* 77:433-441
- O`Gara, F. (1994): *Molecular Ecology of Rhizosphere Microorganisms*. VCH Weinheim
- Weiser, F. & Schachler, G. (1988): Aufbau sowie erste Ergebnisse zur Entwicklung und Nutzung eines Stecklings-Mutterquartiers mit Fichtenklonen verminderter Anfälligkeit gegenüber SO₂. *Beiträge für die Forstwirtschaft Berlin* 22, 55-61
- Whiteman, R. N., Scheffer, R. J. & Strobel, G. A. (1987): Factors influencing root formation in dicots by *Agrobacterium rhizogenes*. *Can. J. Bot.* 66: 642 – 644
- Zaspel, I. (1992): Einfluß einer Saatgutbehandlung mit bakteriellen Antagonisten auf den Ertrag und den Befallsverlauf von *Gaeumannomyces graminis* an Weizen. *Zentralbl. Mikrobiol. Jena*, 147: 173-181