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Impact of barley (*Hordeum vulgare* L.) transgenic line H228.2A on substrate and rhizosphere microorganisms and the possibility of horizontal gene transfer

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Abstract

Numerous investigations have been carried out to determine the impact of genetically modified plants, such as potato, maize, alfalfa and tobacco, on soil microorganisms and the results are contradictory. We applied classical microbiology methods to study quantitative changes of bacterial and fungal abundance in substrate and rhizosphere from barley (*Hordeum vulgare* L.) transgenic line H228.2A containing *Rpg1* and *bar* genes, and its parent non-transgenic variety 'Golden Promise'. In addition, molecular biology methods were used to determine, if horizontal gene transfer from barley transgenic line to soil bacteria has occurred under experimental conditions by screening bacterial genomes for the presence of *Rpg1* gene sequences using polymerase chain reactions. Results did not show significant impact on substrate and rhizosphere microorganisms. In the second pot experiment the average number of filamentous fungi in the rhizosphere of parent line 'Golden Promise' was higher than in the rhizosphere of transgenic line, but the variation among samples was very high probably caused by the high variation of substrate moisture content. None of bacterial deoxyribonucleic acid (DNA) samples isolated from substrate of rhizosphere of barley transgenic line resulted in positive amplification of *Rpg1* gene-specific primer product.

Key words: 'Golden Promise', horizontal gene transfer, microorganisms, rhizosphere, *Rpg1* gene, barley transgenic line H228.2A.

Introduction

The interaction among crop plants and soil microorganisms in the soil and rhizosphere and its importance is well studied (Garbeva et al., 2004). The diversity of rhizosphere microbial populations is largely affected by plant species due to chemical composition of root exudates (Di Giovanni et al., 1999).

The importance of soil microbiota for soil fertility and sustainable land use is increasingly recognized. In this context, numerous investigations have been carried out to assess the impact of current agricultural practices involving extensive monoculture, reduced crop rotation, use of fertilizers, herbicides and pesticides (Giller et al., 1997), and, currently, also the increasing use of genetically modified plants (GMP) (Bruinsma et al., 2003). Potential impact of GMP on soil microorganisms is often linked to specific properties of the transgenic trait, e.g., herbicide tolerant crops will be used together with a specific herbicide, insect resistant crops will possess different variant of *Bacillus thuringensis* (*Bt*) toxins (Römbke et al., 2010), while some of GMP also possess antibiotic resistance marker genes (Demanèche et al., 2008). A number of investigations have been carried out in order to determine the impact of GMP on soil microorganisms. They are dealing with various crops such as potatoes, maize, alfalfa, tobacco, etc. and the results are contradictory. Results of the investigations until 2004 are summarized in several review articles (Bruinsma et al., 2003; Dunfield, Germida, 2004). The variety of parameters examined and the techniques employed was broad. The authors summarized that GMP have been found to affect bacteria, non-target fungi, target fungi, enzyme activity, substrate utilization, and decomposition (for review see Bruinsma et al., 2003). The impact of genetically modified cereals on environmental microorganisms was assessed only in one investigation dealing with wheat expressing anti-fungal protein KP4. Seeds expressing this protein inhibited growth of *Ustilago maydis* (for review see Bruinsma et al., 2003). Although other cereal modifications existed – barley and wheat with stilbene synthase gene for increased fungal resistance (Leckband, Lorz, 1998), barley containing *Rpg1* gene providing resistance to stem rust fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*) (Horvath et al., 2003) and wheat resistant to the powdery mildew fungus *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* (Altpeter et al., 2005), the impact of these cereals on soil microorganisms has not been investigated.

There are several investigations that have proved some impact of GMP on soil microbial populations. For example, in the investigation of Di Giovanni et al. (1999)

it was detected that growth of transgenic alfalfa expressing either bacterial (*Bacillus licheniformis*) genes for alpha-amylase or fungal (*Phanerochaete chrysosporium* Burds.) genes for manganese-dependent lignin peroxidase (MnP) caused differences in bacterial populations in the rhizosphere in comparison with parental line especially in the case of plants expressing lignin peroxidase (Di Giovanni et al., 1999). In two other investigations of Raubuch et al. (2007; 2010), where the degradation of stem and leaves of *Bt*-maize and near-isogenic varieties in the soil was assessed, the degradation process of *Bt*-maize residues was characterized by increased CO_2 production rates and specific respiration rates (CO_2-C/microbial biomass, CO_2-C/adenosine triphosphate (ATP)) and decreased fungal colonization. In a long term field study in the USA, growing glyphosate-resistant soybean and maize treated with glyphosate have shown increased root colonization with *Fusarium* spp. and decreased numbers of fluorescent pseudomonads in comparison to nonresistant plants or to glyphosate-resistant plants not treated with glyphosate, and low ratio of Mn reducers to Mn oxidizers in the rhizosphere of glyphosate-resistant soybean (Kremer, Means, 2009).

Several investigations have proved that GMP do not have significant impact on rhizosphere or bulk soil microbial populations (Gschwendtner et al., 2010; Hannula et al., 2010; Miethling-Graff et al., 2010; Tan et al., 2010; Weinert et al., 2010). Another concern regarding environmental biosafety of GMP is possibility of horizontal gene transfer (HGT) from transgenic plants to soil bacteria that could theoretically facilitate spread of antibiotic resistance genes in soil. The HGT is integration of extracellular DNA in bacterial genome and its expression due to naturally occurring bacterial transformation. In the nature this is the main mechanism ensuring the bacterial evolution and adaptation to changing environmental conditions. In the case of transgenic plants there are some concerns that this unique capability of bacteria to incorporate foreign genes in their genomes could lead to uncontrolled dispersal of plant transgenes (Monier et al., 2007).

In order for HGT to occur in natural environments several conditions must be fulfilled. First of all, the recipient bacterial cell must be in close contact with the extracellular DNA. Second, the bacteria must be naturally or electrically transformable (e.g., lightning-mediated gene transfer – electrotransformation), they should be capable of uptaking foreign DNA, integrating it in the genome and expressing the new genes. Third, it has been proved that the exogenous DNA must be homologous to the DNA in the recipient cell at least over a few nucleotides long region (Pontiroli et al., 2007). Recent investigations have proved that significant part of plant DNA remains in the soil during the degradation process of plant residues (Monier et al., 2007). In the case of transplastomic tobacco leaves, the transgenic DNA sequences were still detectable in the soil microcosms after four years of incubation (Pontiroli et al., 2010). Under laboratory conditions the transformation frequency (transformants per recipient cell) can vary from 1.4×10^{-4} (de Vries, Wackernagel, 2004) to 3.6×10^{-9} in the case of *Acinetobacter* sp. (Gebhard, Smalla, 1998).

The aim of the current study was to determine the impact of barley transgenic line H228.2A on substrate and rhizosphere microorganisms and the possibility of horizontal gene transfer. Classical microbiology methods were used in this research to study quantitative changes of bacterial and fungal colony forming units (CFU) numbers in substrate and rhizosphere from model organism barley line H228.2A containing *Rpg1* and *bar* genes. *Rpg1* gene provides resistance to stem rust fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*) (Brueggeman et al., 2002; Horvath et al., 2003), while *bar* gene encodes phosphinotricin acetyltransferase conferring tolerance to glufosinate-ammonium containing herbicides (Center for Environmental Risk Assessment, 2011). *P. graminis* is a common rust in Latvia with sporadic occurrence (Bankina, Priekule, 2005). Samples from substrate and rhizosphere of parent barley 'Golden Promise' were used as a control. In the second part of research, molecular methods were used to determine if horizontal gene transfer occurs from barley line H228.2A to soil bacteria screening bacterial genomic deoxyribonucleic acid (DNA) for the sequences of *Rpg1* gene.

Materials and methods

Experimental design. Barley (*Hordeum vulgare* L.) line H228.2A containing *Rpg1* gene was used in all experiments as a model. *Rpg1* gene provides race-specific resistance to several pathotypes of stem rust fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*) (Horvath et al., 2003). Samples from substrate and rhizosphere of parent barley 'Golden Promise' was used as a control. The transfer deoxyribonucleic acid (T-DNA) of the plasmid pNRG040 contains *bar* gene that encodes phosphinotricin transacetylase which provides tolerance against glufosinate-ammonium (phosphinotricin) and which was used for selection of transgenic plants, as well as *Rpg1* gene, which encodes receptor-like serine/threonine-specific protein kinase conferring resistance to several races of *Pgt*. The expression of the *Rpg1* gene occurs in all plant organs, including roots, and developmental stages with the exception of immature inflorescence (Rostoks et al., 2004). Transgenic line H228.2A contains one copy of T-DNA containing *Rpg1* gene (Horvath et al., 2003).

Three experiments were carried out. In the first experiment, six pots with three plants of H228.2A each and six pots with three plants of 'Golden Promise' were grown in multipurpose compost ("Greenworld", UK), pH 6, for 48 days in plant growth chamber, 16 h light, 8 h dark, 22/18ºC temperature. The substrate of each pot and joint rhizosphere (roots with adhered pieces of substrate) of all three plants were analyzed as one sample (n = 6). Since in the first experiment some differences were observed between the tested microbial variables in the substrate and rhizosphere of H228.2A in comparison to 'Golden Promise', but they were not statistically significant, it was decided to repeat the experiment by increasing the number of pots and subsequently the number of plants.

In the second experiment, 3–4 barley plants of H228.2A in 10 pots and 3–4 plants of 'Golden Promise' in 10 pots with lawn soil ("Biolan", Finland), pH 6, were grown for 60 days on plant growth shelves, 16 h light, 8 h dark, 22ºC temperature. Each plant rhizosphere was analyzed as one sample. In the case of 'Golden Promise', 32 barley plants were obtained, but in the case of H228.2A line – 28 plants. In the third experiment, shoots of the barley plants grown in flower boxes until full maturity and from barley grown for the second experiment were air-dried, cut in pieces and mixed in substrate Biolan Aiamaa Mustmuld ("Biolan", Finland), pH 6. Sixteen black plastic bags with barley leaves (H228.2A and 'Golden Promise') were prepared containing 800 g of substrate, 11 g of barley leaves and 250 ml water, and incubated for 36 days on plant growth shelves at 22ºC temperature. Each bag was analyzed twice (n = 8). Experimental work was carried out in accordance with Cabinet of Ministers of Republic of Latvia regulation No. 784 of 22 September, 2008 on contained use of genetically modified organisms.

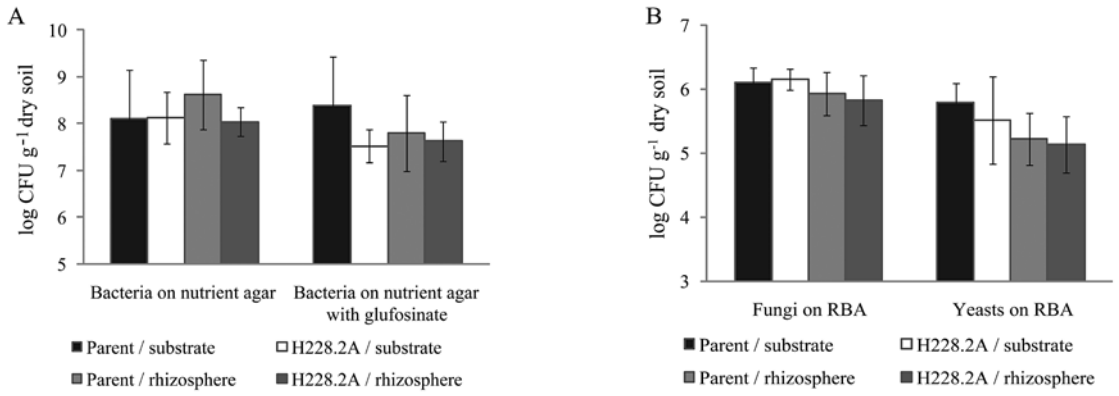
Quantification of cultivable microorganisms. In the first experiment, numbers of colony forming units (CFU) of rhizospheric and soil bacteria were determined on nutrient agar ("BioLife", Italy) without and with 1 mM glufosinate-ammonium ("Sigma-Aldrich", Switzerland). The concentration of glufosinate-ammonium has been estimated as minimum inhibitory for bacteria sensitive to it (Tothova et al., 2010). Since in the first experiment it was estimated that the sensitivity of bacteria to the 1 mM glufosinate-ammonium is low, it was not added to the growing media in the next experiments. Fungi were determined on rose Bengal agar (RBA) with chloramphenicol ("BioLife", Italy). 10 g of substrate or barley roots with rhizospheric soil (rhizosphere soil/root complex) were added to the Erlenmeyer flask with 90 ml of sterile distilled water and shaken for one hour on automatic shaker. After that decimal dilutions were prepared and 100 µl of each dilution (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} for fungi and 10^{-5} and 10^{-6} for bacteria) were inoculated on agar plates. Incubation time for bacteria was three days but for fungi five days in darkness at the temperature 20 ± 2ºC. In the second experiment, CFU counts of rhizospheric bacteria and fungi were done but in the third experiment numbers of bacterial and fungal CFU were determined in the substrate with degraded barley leaves. Since substrate used in the second and third experiment contained negligible numbers of yeasts only colonies of filamentous fungi were counted. In order to estimate the CFU counts per gram of dry substrate and rhizosphere, the substrate moisture content of each pot or bag was determined at the end of incubation using ISO 11465 method.

Methodology for detection of horizontal gene transfer. It is known that the exogenous DNA must be homologous to the DNA in the recipient cell to enhance the possibility of HGT. Therefore homology search with the *Rpg1* gene sequence as query was done against the National Centre for Biotechnology Information (NCBI) GenBank nucleotide database of bacterial sequences using the basic local alignment search tool. Pure cultures of bacteria were obtained from the agar plates used for enumeration of bacterial CFU. They were subcultured on nutrient agar. In the first experiment, 180 bacterial pure cultures were isolated (105 from 'Golden Promise' and 75 from H228.2A). In the second experiment, 300 bacterial cultures were isolated from H228.2A pots and 61 from 'Golden Promise' pots. In the third experiment, 160 bacterial cultures were isolated from the bags with H228.2A degradation products and 40 from 'Golden Promise' degradation products. Part of the bacteria lost their viability during storage at +4ºC, and, in total, the genomic DNA was successfully extracted and amplified in polymerase chain reaction (PCR) from 412 bacterial cultures – 142 pure cultures isolated from 'Golden Promise' samples and 270 from H228.2A samples. Genomic DNA in the first experiment was extracted using the *PowerSoil*™ DNA isolation kit (MO BIO Laboratories Inc., US) following the manufacturer's protocol. In the second and third experiments, the genomic DNA was extracted using the method developed by Cenis (1992). For both methods bacterial biomass was homogenized using horizontal mixer mill MM 301 ("Retsch", Germany) at a maximal speed of 30 Hz (1800 oscillations/minute) for 3 minutes. Extracted DNA was amplified by PCR with primers *REVB* 5'-GGTTACCTTGTACGACTT-3' and *FORB* 5'-AGAGTTTGATCCTGGCTCAG-3' specific for prokaryotic 16S ribosomal ribonucleic acid (rRNA) (Yeates et al., 1998) serving as internal PCR amplification control, and *Rpg1 Ex3cw2* 5'-GCCGGTGTACTATCCCTTTC-3' and *Rpg1 Ex4ccw2* 5'-TGTCGGACCCTCATAAGATT-3' specific to third and fourth exon of *Rpg1* gene (Horvath et al., 2003). The expected size of PCR products was 1500 and 500 bp, respectively. The PCR reactions in Eppendorf Mastercycler Personal ("Eppendorf", Germany) were carried out in 25 µl volume. The mixture contained 0.125 µl hot start *Taq* DNA polymerase, 2.5 µl 10 × hot start PCR buffer, 2.5 µl dNTP mix, 2 mM each, 2 µl 25 mM $MgCl_2$, 0.375 µl bovine serum albumin 20 mg ml^{-1} (all reagents from "Thermo Scientific", Lithuania), 0.5 µl of each 25 µM primer ("Operon Biotechnologies", Germany), 14.5 µl sterile distilled water and 1 µl of DNA template. The PCR conditions were as follows: the initial denaturation step of 4 min at 95°C, 40 s of denaturation at 95°C, 40 s of annealing at 56°C, 1 min of primer extension at 72°C (30 cycles) and final extension 10 min at 72°C.

Statistical analysis. The *F*-test and *t*-test (α = 0.05) were done with *Excel* (Microsoft, USA).

Results and discussion

Results of the quantification of cultivable microorganisms. Results of the first experiment are summarized in Figure 1 A and B. There were no statistically significant differences between the numbers of microbial CFU in substrate and rhizosphere of barley transgenic line H228.2A and parent non-transgenic variety 'Golden Promise', and CFU numbers were not significantly affected by addition of 1 mM glufosinate-ammonium in the growth media. Nevertheless, the tendency was observed that the average log CFU number of bacteria in 'Golden Promise'



Note. Microbial numbers in substrate and rhizosphere of barley transgenic line H228.2A and parent line 'Golden Promise' are shown (error bars indicate standard deviations (±S.D.), n = 6).

Figure 1. Log numbers of colony forming units (CFU) of bacteria on nutrient agar and nutrient agar with 1 mM glufosinate-ammonium (A) and microscopic filamentous fungi and yeasts on rose Bengal agar (RBA) (B)

rhizosphere was higher than in H228.2A rhizosphere – log 8.63 ± 0.74 CFU g^{-1} dry soil *vs*. log 8.04 ± 0.31 CFU g^{-1} dry soil. On the growth media with glufosinate-ammonium addition the tendency was observed that average log CFU number of bacteria in 'Golden Promise' substrate was higher than in H228.2A substrate – log 8.39 ± 1.04 CFU g^{-1} dry soil *vs*. log 7.52 ± 0.35 CFU g^{-1} dry soil.

Results of the second and third experiment are given in Figure 2 A and B. Statistically significant differences were found only in the second experiment between the number of CFU of fungi in rhizosphere of barley transgenic line H228.2A and parent line 'Golden Promise' (F = 1.26, p = 0.02). The average number of fungi in the rhizosphere of 'Golden Promise' was higher 1.52×10^6 CFU g^{-1} *vs*. 7.35×10^5 CFU g^{-1} but the variation among samples was very high. Besides that the variation of substrate moisture was also high – 37.5% to 68.1% although the average moisture of substrates in 'Golden Promise' and H228.2A pots was similar (54.7 ± 8.4% and 55.3 ± 8.4%). It is possible that in spite of the fact that soil moisture values were used to recalculate data of CFU per gram of dry soil or rhizosphere, the variation in substrate moisture could cause additional impact on substrate and rhizosphere microorganisms. Uniform moisture content was applied in the third experiment and it resulted in statistically non-significant differences between CFU numbers of bacteria and fungi in the substrate with degraded leaves of barley transgenic line H228.2A and parent line 'Golden Promise' (Fig. 2 B).

In general, our results are in line with several investigations proving that GMP do not have significant impact on rhizosphere or bulk soil microbial populations (Gschwendtner et al., 2010; Hannula et al., 2010; Miethling-Graff et al., 2010; Tan et al., 2010; Weinert et al., 2010), although in these investigations potatoes and maize have been used. The impact of genetically modified potatoes on soil and rhizosphere microorganisms has been studied in several investigations. In the investigation of Weinert and colleagues (2010), it was detected that significant differences in the CFU values of rhizospheric bacteria were detected in particular plant developmental stages, but they were not stable throughout the whole season, when comparing parental line with two genetically modified potato lines with increased zeaxanthin content. Another three-year field study has been conducted in the Netherlands to assess the impact of genetically modified potato variety with increased amylopectin content on community composition of the main phyla of fungi in

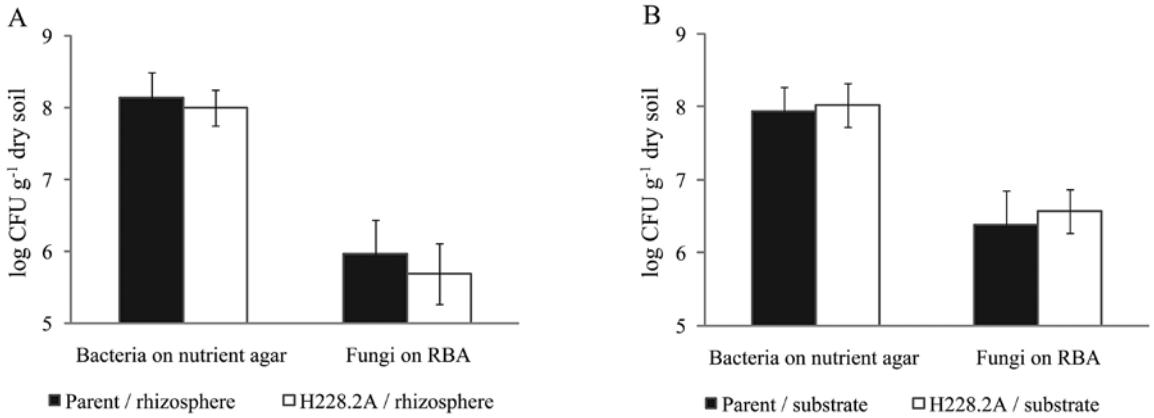


Figure 2. Log numbers of colony forming units (CFU) of bacteria on nutrient agar and microscopic filamentous fungi on rose Bengal agar (RBA) in the rhizosphere of barley in the second experiment (n = 32 for 'Golden Promise' and 28 for H228.2A) (A) and in the substrate with degraded barley leaves in the third experiment, n = 8 (±SD) (B)

soils. No detectable differences between the genetically modified cultivar and its parental line were detected (Hannula et al., 2010; 2012). Similarly, no differences between genetically modified potato and parental line were observed by quantifying with quantitative real-time PCR the abundance of particular bacterial and fungal species or genera – *Pseudomonas* spp., *Clavibacter michiganensis* ssp., *Trichoderma* spp. and *Phytophthora infestans* (Mont.) de Bary (Gschwendtner et al., 2010). The two studies with a *Bt*-maize expressing either Cry3Bb1 or Cry1A and Cry1Ab reported no significant differences between the rhizosphere bacterial community structure of *Bt*-maize and conventional cultivars using cultivation independent profiling of 16S rRNA genes (Miethling-Graff et al., 2010) and 16S and 18S rRNA genes (Tan et al., 2010).

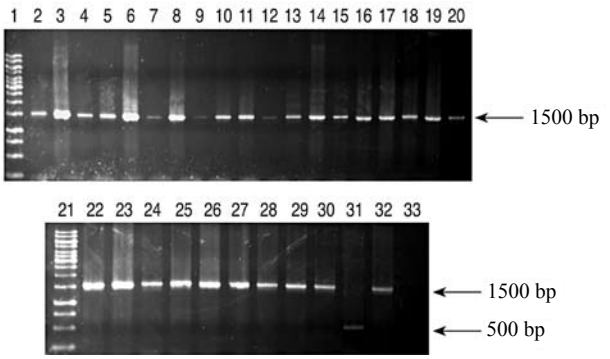
Detection of horizontal gene transfer (HGT). Homology search with the *Rpg1* gene sequence as a query against the NCBI GenBank nucleotide database identified more than 70 homologue bacterial sequences with 30–50 nucleotides long homologous regions. Several examples are given in the Table. Most of these bacteria are common soil bacteria or actinobacteria.

Table. Examples of genes from various bacteria homologous to *Rpg1* gene

Bacterial species, GenBank accession number	Gene and encoded protein	Characterization of the homologous part of the gene, homologous nucleotides/total length (similarity)
Sorangium cellulosum AM746676.1	hypothetical protein predicted by Glimmer/Critica	33/36 bp (91%)
Sorangium cellulosum AM746676.1	murein transglycosylase	44/58 (75%)
Starkeya novella CP002026.1	phosphoglucomutase	38/46 (82%)
Kineococcus radiotolerans CP000750.2	α-L-fucosidase	29/31 (93%)
Kineococcus radiotolerans CP000750.2	myo-inositol-1-phosphate synthase	46/58 (79%)
Pseudomonas mendocina CP000680.1	radical SAM N-terminal domain protein	51/65 (78%)
Pseudomonas mendocina CP000680.1	conserved hypothetical protein 95	29/31 (93%)
Streptomyces ambofaciens AM238663.1	putative FtsK/SpoIIIE family protein	28/29 (96%)
Herbaspirillum seropedicae CP002039.1	tolA-related transport transmembrane protein	37/45 (82%)
Rhodococcus opacus AP011116.1	putative ABC transporter ATP-binding protein	41/51 (80%)
Rhodococcus opacus AP011115.1	putative esterase	25/25 (100%)
Rhodococcus opacus AP011115.1	extradiol dioxygenase	47/61 (77%)
Pseudomonas aeruginosa FM209186.1	hypothetical protein	37/45 (82%)
Streptomyces coelicolor AL939124.1	guanosine pentaphosphate synthetase	28/30 (93%)
Streptomyces scabiei FN554889.1	conserved hypothetical protein	32/37 (86%)

Examples of PCR results with primers *REVB*, *FORB*, *Ex3cw2* and *Ex4ccw2* are given in Figure 3. The lane 32 ('Golden Promise') showed an amplification product of the same size as from bacterial 16S rRNA, which may have occurred because barley leaves contained some endophytic bacteria, or because leaves were not sterilized before the DNA extraction. In total, genomic DNA was successfully extracted and amplified in PCR from 412 bacterial cultures – 142 cultures isolated from 'Golden Promise' samples and 270 from H228.2A samples. In spite of the fact that theoretically soil bacteria with homologous gene sequences to *Rpg1* gene exist, none of the bacterial DNA samples resulted in positive amplification of *Rpg1* gene specific primer product. For the first and second experiments it was not tested if the barley plants released DNA in the substrate, as there is no reliable way to separate barley roots from the substrate to guarantee that soil DNA extracts do not contain barley DNA extracted from small pieces of barley roots. This technical issue was solved in the third experiment where barley leaves were 90% degraded in the substrate during incubation due to decomposition processes carried out by saprophytic microorganisms in the substrate. However, the third experiment did not result in HGT of the *Rpg1* gene in tested culturable bacterial isolates.

Our results are in line with several other investigations that have not proved that HGT events



Notes. Bacterial isolates were obtained from the second experiment. Lanes: 1 and 21 – 1 kb ladder Gene Ruler ("Thermo Scientific", Lithuania); 2–6 and 12–16 – PCR products of bacterial DNA isolated from bacteria of the 'Golden Promise' rhizosphere; 7–11, 17–20 and 22–30 – PCR products of bacterial DNA isolated from bacteria of the H228.2A rhizosphere; 31 – DNA from barley line H228.2A; 32 – DNA from 'Golden Promise'; 33 – negative control (sterile H_2O instead of DNA). Amplification was done with primers *REVB* and *FORB* specific for prokaryotic 16S ribosomal ribonucleic acid serving as internal PCR amplification control (1500 bp), and with primers *Rpg1 Ex3cw2* and *Rpg1 Ex4ccw2* specific to third and fourth exon of *Rpg1* gene (500 bp).

Figure 3. Visualisation of the amplification products of bacterial deoxyribonucleic acid (DNA) in polymerase chain reactions (PCR) in 1% agarose gel

from GMP to soil or rhizosphere microorganisms could happen in natural or semi natural conditions. The longest period of GMP cultivation (10 years) and subsequent HGT detection experiments have been carried out in France with transgenic *Bt*-176 corn, which has a *bla*TEM marker gene. The authors found four bacterial isolates originating from the soils cultivated with the transgene which had *bla*TEM-116 allele that is also present in the genetically modified corn event *Bt*-176. However, it was concluded that the resistance gene was already prevalent in soil and that there was no evidence for HGT from the genetically modified corn variety over the 10-year period (Demanèche et al., 2008). HGT has been proved to occur in an investigation *in situ* under optimized conditions and with homology between plant and bacterial sequences. In this investigation transplastomic tobacco, containing up to 10 000 copies of transgene per cell, was co-infected with a mix of *Ralstonia solanacearum* (Smith) Yabuuchi et al. and *Acinetobacter baylyi*. *A. baylyi* was able to develop competence in infected tissues, and to acquire and express the transgene (Pontiroli et al., 2007). Theoretical models say that in large bacterial populations like soil bacterial community under non-optimized conditions at the most only 1% of the bacterial cells are exposed to the DNA. The theoretical time interval from the DNA exposure until HGT detection is possible in the soil is 10^5 bacterial generations or 3 000 years assuming that the average generation time of soil bacteria in natural conditions is two weeks (Townsend et al., 2012). After analyses of previously published GMP field monitoring studies Townsend and colleagues (2012) concluded that HGT events could only be achieved under conditions of strong positive selection of the hypothesized transformants. Positive selection means that transformants have obtained new advantageous properties in comparison to non-transformed cells. The integration of *Rpg1* gene in microbial genome would not give any obvious advantages for transformed bacteria. In the future experiments it would be useful to screen bacteria for HGT of *bar* gene from the barley transgenic line H228.2A, although in the case of positive results in PCR amplification it would be difficult to prove that the *bar* gene sequence in the bacteria originated from the genetically modified barley plants and not from naturally resistant bacteria like *Streptomyces hygroscopicus* from which the *bar* gene was initially cloned (Thompson et al., 1987).

Conclusions

1. Two out of the three independent experiments with barley transgenic line H228.2A did not show statistically significant impact on abundance of substrate and rhizosphere microorganisms compared to its non-transgenic parental variety 'Golden Promise'. In the second pot experiment, the average number of filamentous fungi in the rhizosphere of parent line 'Golden Promise' was higher than in the rhizosphere of transgenic line, but the variation among samples was very high probably caused by the high variation of substrate moisture content.

2. None of the 270 bacterial deoxyribonucleic acid (DNA) samples extracted from bacterial cultures isolated from substrate or rhizosphere of barley transgenic line H228.2A resulted in positive amplification of *Rpg1* gene-specific primer product indicating that no plant-to-microorganism DNA transfer had occurred under described experimental conditions.

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Paprastojo miežio (*Hordeum vulgare* L.) transgeninės linijos H228.2A įtaka substrato bei rizosferos mikroorganizmams ir genų horizontalaus perkėlimo galimybė

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Santrauka

Siekiant nustatyti genetiškai modifikuotų augalų – bulvių, kukurūzų, liucernų ir tabako – įtaką dirvožemio mikroorganizmams, atlikta daug tyrimų, tačiau jų rezultatai yra prieštaringi. Tiriant kiekybinius bakterijų bei grybų gausumo pokyčius substrate ir paprastojo miežio (*Hordeum vulgare* L.) transgeninės linijos H228.2A, turinčios *Rpg1* bei *bar* genus, ir jos tėvinės netransgeninės veislės 'Golden Promise' rizosferoje, taikyti klasikiniai mikrobiologiniai metodai. Molekulinės biologijos metodai taikyti siekiant nustatyti, ar įvyko horizontalus genų perkėlimas iš paprastojo miežio transgeninės linijos į dirvožemio bakterijas. Tai tirta bandymų metu, bakterijų genome ieškant *Rpg1* geno sekos polimerazės grandininėmis reakcijomis. Tyrimo rezultatai neparodė substrato ir rizosferos mikroorganizmų pokyčių. Atliekant antrą vegetacinį tyrimą vidutinis siūlinių grybų kiekis tėvinės linijos 'Golden Promise' rizosferoje buvo didesnis nei transgeninės linijos rizosferoje, tačiau variacija tarp mėginių buvo labai didelė, galbūt dėl didelės substrato drėgmės kiekio variacijos. Nė vienas bakterijų DNR mėginių, išskirtų iš paprastojo miežio transgeninės linijos rizosferos substrato, neparodė *Rpg1* genui specifinių pradmenų produkto teigiamos amplifikacijos.

Reikšminiai žodžiai: 'Golden Promise', horizontalus genų perkėlimas, mikroorganizmai, rizosfera, *Rpg1* genas, paprastojo miežio transgeninė linija H228.2A.