

ABSTRACTS OF THE 74th SCIENTIFIC CONFERENCE OF THE UNIVERSITY OF LATVIA

January – February 2016

Balodite E., Strazdina I., Galinina N., Poole R.K., Kalnenieks U.

Zymomonas mobilis strains with modified NADH dehydrogenase activity: expression and purification of Ndh

45

Šiliņš R., Druvietis I., Kurilovica Z., Reihmanis J.

Influence of fragmentation of reed beds on the ecosystem of northern part of Lake Engure

47

Ošiņa K., Germini D., Botha S., Vassetzky Y., Sjakste T., Sjakste N.

1,4-dihydropyridine derivatives decrease Tat induced γH2AX expression in B lymphocytes

49

Poppels A.

Seasonal assessment of changes in zoobenthos structure in some small rivers in Latvia

50

Grantina-levina L., Kalniņa I.

Strawberry crown rot – a common problem in 2015

51

Krišāns O., Jansons Ā., Levinsh G.

Effect of decreased precipitation events on primary growth and photosynthesis-related characteristics of spruce (*Picea abies*) saplings grown in different substrates

53

Dokāne K., Jonkus D., Sjakste T.

Monitoring of three single nucleotide polymorphisms in *RYR1* gene of local 'Latvian White' pig breed

55

Zymomonas mobilis strains with modified NADH dehydrogenase activity: expression and purification of Ndh

Elina Balodite¹, Inese Strazdina¹, Nina Galinina¹, Robert K. Poole², Uldis Kalnenieks^{1*}

¹Laboratory of Microbial Bioenergetics; Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

²Department of Molecular Biology and Biotechnology, The University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK

*Corresponding author, E-mail: kalnen@lanet.lv

Key words: *Zymomonas mobilis*, NAD(P)H dehydrogenase, expression and purification.

Zymomonas mobilis possesses a constitutive respiratory chain which can be used in metabolic engineering for rapid NAD(P)⁺ regeneration. NAD(P)H type II dehydrogenase (Ndh) is the sole functional respiratory dehydrogenase in *Zymomonas mobilis*, able to oxidize both nicotinamide redox cofactors, although with higher specificity towards NADH.

To increase the specificity towards NADPH of the *Z. mobilis* Ndh protein glutamate in the position 219 was replaced by glutamine, as previously reported for *Agrobacterium tumefaciens* (Desplats et al. 2007). A strain with an Ndh-deficient background (strain Zm6-ndh, derived from Zm6 ATCC29191) (Kalnenieks et al. 2008), was transformed by plasmid pBBR1MCS2 containing native and mutated *ndh* under their own promoters yielding strains Zm6-ndh pBBR1MCS::ndh and Zm6-ndh pBBR1MCS::glu→gln, respectively. The mutant Ndh showed lower activity, yet a higher relative affinity towards NADPH (Balodite et al. 2014).

In the present study we aimed to overexpress the *Z. mobilis* *ndh* wild-type and mutant variants in *E. coli* and obtain purified Ndh protein for further kinetic study.

To obtain purified Ndh protein, the *Z. mobilis* *ndh* gene was inserted in the pBADHisA vector and expressed in *E. coli* strain ANNO221 (a strain deficient in both respiratory dehydrogenases). Ndh transcription was induced, by addition of 0.01% arabinose when cultures reached OD₅₅₀ = 0.6. After 4 h of further growth, cell disintegrates were made by ultrasonication. Disintegrates were ultracentrifuged at 44000 × g for 1 h, washed and ultracentrifuged again for 1.5

h to obtain membrane vesicles. Dehydrogenase activity was measured, using ubiquinone Q₁ as electron acceptor under anaerobic conditions with 20mM KCN. No *Z. mobilis* Ndh activity could be detected in *E. coli* membrane vesicles, while the dehydrogenase activity detected in *E. coli* disintegrates was considerably lower compared to the *E. coli* MG1655 native Ndh activity (Table 1). The measured Ndh activity was even lower than the activity of NADH dehydrogenase in *Z. mobilis* Zm6-ndh, bearing pBBR1MCS::ndh.

Protein gels confirmed the presence of the expected 47 kDa product in cell disintegrates and in the supernatant after ultracentrifugation, but not in membrane fraction (Fig. 1). This indicated that the *Z. mobilis* Ndh protein had not integrated in *E. coli* membrane, possibly due to differences in membrane composition and physical properties. Attempts to purify protein from disintegrate using Ni²⁺ affinity column were unsuccessful.

Western blotting was performed with disintegrate samples, one and four hours after induction. Signal was acquired only in the *ndh*-bearing strain, but the signal length did not correspond to 47kDa. The signal length tended to decrease between the both time points (not shown).

Z. mobilis *ndh* gene transcription in the recombinant *E. coli* was confirmed by RT-qPCR. The highest amount

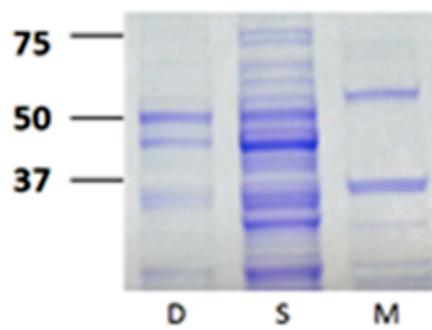


Fig. 1. Protein detection in disintegrates using 10% SDS-PAGE. D, disintegrate; S, supernatant after ultracentrifugation; M, membrane fraction.

Table 1. NADH dehydrogenase activity (U mg⁻¹ protein) in cell disintegrates, pH 7, using ubiquinone Q₁ as an electron acceptor. ND, not detectable

Zm6-ndh	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
pBBRMCS::ndh	ANNO221	ANNO221	MG1655
2.60 ± 0.13	1.80 ± 0.09	ND	30.48 ± 3.84

of *Z. mobilis* *ndh* mRNA was detected 1 h after induction (11.07 ng), as compared to the uninduced sample (0.33 ng). However, there was a dramatic fall in the amount of mRNA 4 h after induction (2.46 ng).

These results indicate that the low efficiency of Ndh synthesis probably is due to rapid mRNA degradation, in which case translation is promptly interrupted. It is yet unknown, whether mRNA and, possibly, also protein degradation (as indicated by decreasing Western blot signal length), might be caused by incapability of *Z. mobilis* Ndh protein to be correctly folded, transported and/or integrated in *E. coli* membrane. Apparently, differences between *Z. mobilis* and *E. coli* membrane structures and/or protein transport and signal systems may lead to complexities in membrane-bound protein expression.

Acknowledgements

Authors are grateful to Johan Van Beilen for providing strain ANNO221. This study was supported by the Latvian Council

of Science project No. 536/2013 and ERASMUS+ mobility 2014/2015.

References

- Auriol C., Bestel-Corre G., Claude J.B., Soucaille P., Meynial-Salles I. 2011. Stress-induced evolution of *Escherichia coli* points to original concepts in respiratory cofactor selectivity. *Proc. Natl. Acad. Sci. USA* 108: 1278–1283.
- Balodite E., Galinina N., Strazdina I., Kalnenieks U. 2014. Respiratory type II NAD(P)H dehydrogenase of *Zymomonas mobilis* with altered cofactor specificity. 18th European Bioenergetics Conference, Lisbon
- Desplats C., Beyly A., Cuiné S., Bernard L., Cournac L., Peltier G. 2007. Modification of substrate specificity in single point mutants of *Agrobacterium tumefaciens* type II NADH dehydrogenase. *FEBS Lett.* 581: 4017–4022.
- Kalnenieks U., Galinina N., Strazdina I., Kravale Z., Pickford J.L., Rutkis R., Poole R.K. 2008. NADH dehydrogenase deficiency results in low respiration rate and improved aerobic growth of *Zymomonas mobilis*, *Microbiology* 154: 989–994.

Influence of fragmentation of reed beds on the ecosystem of northern part of Lake Engure

Roberts Šiliņš^{1*}, Ivars Druvietis², Zane Kurilovica², Jānis Reihmanis³

¹Lake Engure Nature Park Foundation, Bērzcems, Engures parish LV-3113, Latvia

²Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

³Latvian Nature Foundation, Dzirnavu 73-2, Riga LV-1011, Latvia

*Corresponding author, E-mail: eedp@inbox.lv

Key words: Great bittern, reed fragmentation, phytoplankton, water vegetation.

Lake Engure is a shallow lagoon type lake situated on Western coast of the Gulf of Riga. Lake Engure is typical Charophyte lake characterised by high water transparency (till bottom), low phytoplankton biomass, and lack of summer cyanobacterial blooms (Springe et al. 2011). It is an important resting and nesting area for waterfowl and waders: 186 bird species are found there. Territory is designated as Natura 2000 site, Important Bird Area and Ramsar site (Šiliņš et al. 2013). Since 2001 permanent management works such as reed cutting, cattle grazing and restoration of coastal meadows has been started. Alongside hydrobiological monitoring management activities of the lake were performed.

Eutrophication of the lake was caused by the lowering of water level in 1842 and from influence of pollution from agriculture and households in the Soviet period. As a result large territories of the lake were overgrown with dry monotonous reed stands that are not suitable for most of the aquatic birds. In the LIFE Nature project "Coastlake" that started on 2013, 30 ha of open water areas are already created in the reed stands at the Northern part of the lake.

Outflow of Mērsrags canal, which connect lake with the Gulf of Riga, was restored. Fluctuation of water level due to windy conditions in the Gulf of Riga and in Northern part of the lake made better habitat for Great bittern (*Botaurus stellaris* L.). These actions significantly improved the water flow between different parts of the lake (Fig. 1).

First year after water flow restoration works were performed we observed increasing numbers of Great bittern (*Botaurus stellaris* L.) and other waterfowl in restored open water areas. Monthly measurements of the hydrochemical parameters were performed at 10 sampling stations. Five of them were at newly made parts, and water flows revealed comparatively high values of conductivity and total dissolved substances in the Northern part of the Lake. This shows significant influence of water incomes from the Gulf of Riga. In favourable conditions saline water from the Gulf of Riga flow till to the middle part of the lake. pH values higher than 8.0 and the oversaturation with oxygen along with a low turbidity suggest that Charophyte beds are still the main contributors to the intensive primary production in sampling stations at central part of the lake.



Fig. 1. Water flows made in the Northern part of lake Engure. Photo: A. Jermuts. <http://ldf.lv/lv/article/noslegusies-niedraju-fragmentacija-engures-ezera-0>.

Low phytoplankton biomass (0.011 to 1.35 mg L⁻¹) were observed practically in all lake aquatoria of the lake except in restored open water areas where phytoplankton analyses show high biomass of potentially harmful Cyanobacteria (10.33 mg L⁻¹) that could be explained by the absence of higher vegetation due to recent digging works and high quantity of nutrients available for rapid increase of algal biomass.

Monitoring of regeneration of reed beds was also performed in 136 sampling sites nearby restored water flows. Regeneration of reeds and changes of projective cover (Braun-Blanquet method) of plant communities on the sediment layer that was taken out when water flows was observed. Fast regeneration of plant communities was observed on out taken sediment layer nearby restored

water flows. Regeneration of submerged and emergent vegetation in water flows is much slower. In total 32 higher plant species were observed. Dominating species were *Phragmites australis*, *Mentha aquatica*, *Lycopus europaeus*, *Agrostis stolonifera*, *Galium elongatum*, *Sium latifolium*, *Peucedanum palustre*, *Naumburgia thyrsiflora*.

References

- Šiliņš R., Druvietis I., Poppels A. 2013. Seasonal dynamics of benthic and planktonic communities in shallow lagoon bird lake Engure, Latvia. *Acta Biol. Univ. Daugavpiliensis* 13: 147–159.
- Springe G., Briede A., Druvietis I., Grinberga L., Konosonoka I., Parele E., Rodinovs V., Skuja A. 2011. Long-term development of the hydroecosystem of the Lake Engure and its influencing factors. *Environ. Climate Technol.* 7: 100–105.

1,4-dihydropyridine derivatives decrease Tat induced γH2AX expression in B lymphocytes

Kristīne Ošiņa^{1,2*}, Diego Germini³, Shirmoné Botha³, Yegor Vassetzky³, Tatjana Sjakste¹, Nikolajs Sjakste^{2,4}

¹Institute of Biology of the University of Latvia, Salaspils, Latvia

²Latvian Institute of Organic Synthesis, Riga, Latvia

³Institut de Cancérologie Gustave Roussy, Villejuif, France

⁴Faculty of Medicine, University of Latvia, Riga, Latvia

*Corresponding author, E-mail: kristine.osina@gmail.com

Key words: human immunodeficiency virus, transactivator protein, 1,4-dihydropyridine.

The transactivator protein (Tat) of human immunodeficiency virus (HIV) is involved in transcription of the viral and host genomes. It is excreted by HIV-infected cells and activates immature T-lymphocytes. Tat can also penetrate other cell types, including B cells, and cause oxidative stress that can lead to DNA damage. 1,4-dihydropyridine (1,4-DHP) derivatives possess antioxidative properties as free radical scavengers and promote DNA repair.

B cells were isolated from healthy donors and treated with 250 ng mL⁻¹ Tat protein for six hours. Cells were simultaneously treated with 1,4-DHP derivatives at different concentrations (5–500 nM). Level of γH2AX, a marker for DNA damage, was analyzed with immunofluorescence and confocal microscopy. Statistical analysis was performed using the Chi-square test.

Six hours post infection γH2AX level was significantly increased in Tat-treated cells compared to control (57 vs. 33%, $p < 0.001$). After treatment with AV-153-Ca and AV-

153-K, γH2AX level did not significantly differ from the controls at 50 nM concentration (42 and 38%). For AV-153-Na and AV-153-Mg, this effect was observed already at 25 nM concentration (45 and 41%). The increase of concentrations of these four compounds up to 100 nM lowered the γH2AX level even more. However, cells treated with 500 nM AV-153-Na showed significantly increased γH2AX level compared to the control (53%, $p < 0.001$). J-9-125 and PP-544-Na manifested positive effect at 100 nM concentration (36 and 28%), but metcarbatone and glutapryrone only at 500 nM concentration (39% for both).

In conclusion, 1,4-DHP derivatives are capable to decrease γH2AX level in Tat-treated B cells. However, different compounds require different concentrations to reach a positive effect: AV-153-Na and AV-153-Mg are active at 25 nM, AV-153-Ca and AV-153-K at 50 nM, J-9-125 and PP-544-Na at 100 nM, metcarbatone and glutapryrone at 500 nM.

Seasonal assessment of changes in zoobenthoss structure in some small rivers in Latvia

Arkādijs Poppels*

Riga National Zoological garden, Meža prospekts 1, Riga LV-1014, Latvia

*Corresponding author, E-mail: apoppels@hotmail.com

Key words: seasonal assessment, small rivers, zoobenthoss.

Seasonal assessment of zoobenthoss communities were performed in May, July and November during 2014–2015 in small Latvia's rivers Ličupe, Lobe, Mergupe, Neriņa, Abza and Ranka. The aim of the research was to detect seasonal changes in structure of zoobenthoss communities as well as obtained data use in into practice put in evaluation of fish food resources. Samples of invertebrates were collected with Petersen bottom grab sampler in the medium and littoral parts of investigated stretch of river.

Spring samples in all investigated rivers were characterised by noticeable biomass of zoobenthoss (77.9 g m^{-2} in River Neriņa) where dominated groups were Ephemeroptera, Trichoptera and Chironomidae (Fig. 1). Zoobenthoss biomass in summer period was less (11.9 g m^{-2} in River Mergupe) due to fly out species of Insecta such as Ephemeroptera, Trichoptera and Chironomidae (Fig. 1). Most insects are benthic, living on or burrowing into sediments or on macrophytic vegetation and plant detritus (Wetzel 2000). Consequently, in this period communities of macrozoobenthoss were dominated by Mollusca and Malacostraca.

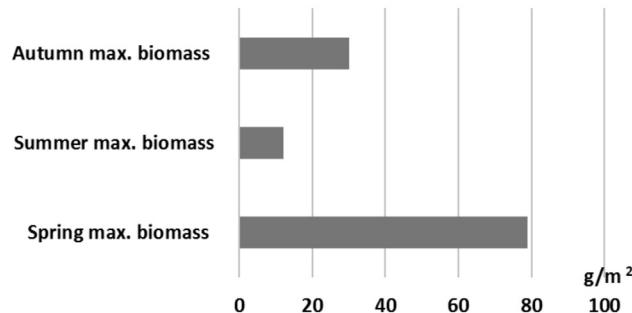


Fig. 1. Seasonal changes of zoobenthoss maximal biomass in observed rivers (g m^{-2}).

At the autumn period in all observed small rivers benthic communities were characterised by high increase of numbers of individuals (4300 individuals per m^2 in River Neriņa, 3800 individuals per m^2 in River Abza) due to juvenile specimens of Ephemeroptera, Trichoptera and Chironomidae. Autumn samples shows insignificant growth of zoobenthoss biomass (from 23.4 g m^{-2} in River Ranka till 30.2 g m^{-2} in River Lobe) (Fig. 1).

Spring samples were dominated by Ephemeroptera species as *Baetis rhodani*, *Baetis niger*, *Baetis vernus* and *Caenis horaria*; Trichoptera species as *Hydropsyche angustipennis*, and larvae of Chironomidae. Whereas in summer period after depart (fly out) of adult individuals as a leading species remain Mollusca species such as *Radix ovata*, *Bithynia tentaculata*, *Sphaerium corneum*, and *Asellus aquaticus* from Malacostraca. During this period specimens from Hirudinae (*Glossiphonia complanata*) were observed in high numbers. In autumn period zoobenthoss communities are supplemented by species of Ephemeroptera, Trichoptera, Chironomidae and larvae of another flying and drifting species of Insecta. Some taxa routinely drift in great abundance, including the Ephemeroptera, some Diptera, Plecoptera and Trichoptera (Allan 1995). However these groups do not form great biomass because their dimensions are small.

Investigated rivers were recognized with high biological diversity of benthic organisms and plenty high biomass for fish food.

References

- Allan D. 1995. *Stream Ecology. Structure and Function of Running Waters*. Chapman and Hall.
- Wetzel R.G. 2001. *Lake and River Ecosystems*. 3rd ed. Academic Press.

Strawberry crown rot – a common problem in 2015

L. Grantina-levina^{1*}, I. Kalniņa²

¹Latvian Plant Protection Research Centre, Struktoru 14A, Riga LV-1039, Latvia

²Institute of Horticulture, Graudu 1, Cericīni, Krimūnu district, Dobeles region LV-3701, Latvia

*Corresponding author, E-mail: lelde.grantina@apollo.lv

Key words: *Colletotrichum acutatum*, *Coniella fragariae*, *Fusarium* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., strawberry crown rot.

Several strawberry (*Fragaria × ananassa* Duch.) growers in Latvia have complained about dieback of recently planted strawberries in 2015. This problem has been recorded also in previous years, especially with imported plant material. In present investigation we mainly analyzed problematic strawberry plants from a trial at the Institute of Horticulture and particular plants from farm "Lazdukalns" (Mālpils district).

On diseased plants crown rot, damaged roots and wilting and dieback of plants were observed. The objective of the investigation was to determine the main causal agents of crown rot and to evaluate the impact of this disease on the yield. Several tasks were defined: to observe the vitality of the plants during the growth season, to identify the main causal agents of crown rot and to determine the impact of crown rot on the yield parameters.

The trial of strawberries at the Institute of Horticulture was established in 24th April, 2015 in a high tunnel (FVG Folien GmbH) (length 50, height 3.5 m, width 4 m). The tunnel was covered with polyethylene film before the third yield in the autumn. Strawberry A+ category cold-stored plants were obtained from Goossens Flevoplant B.V. (the Netherlands). In the trial five everbearing cultivars were planted: 'Florin', 'Florentina', 'Florina', 'Evie 2' and 'Everest'. Plants from each variety were planted in four randomized replicates of 45 plants on elevated rows at 0.25 × 0.25 m distance between plants and 1.20 m between rows. The rows were covered with black plastic film. In the spring the soil was treated with Ca(NO₃)₂, and during the development of fruits leaf fertilization with iron chelate and KNO₃ (0.01%) was performed. The soil was heavy sandy loam. According to the analyses carried out in the autumn of 2015 the soil pH was 7.3 and the content of organic matter was 2.4%. The content of nutrients was: 218 mg kg⁻¹ K₂O; 309 mg kg⁻¹ P₂O₅; 778 mg kg⁻¹ Mg; 1308 mg kg⁻¹ Ca. Fungicides and insecticides were not used. The vitality of plants was assessed visually on 17th of July according to the scale: 0, no signs of disease; 1, infected plant showing wilting symptoms; 2, dead plants (Kurze et al. 2001). Twelve plants from each cultivar with signs of wilting were taken to the laboratory of Latvian Plant Protection Research Centre. From the farm in Mālpils district several plants from the

cultivars 'Sonata' and 'Figaro' were sent to the laboratory. They were daughter plants taken by the owner from the imported plants in 2014.

In the laboratory plant roots and crown were washed with water and crown parts were surface disinfected with 1.25% sodium hypochlorite solution, rinsed with distilled water, dried under laminar airflow and inoculated on potato dextrose agar – chloramphenicol medium. Fungal isolates were identified according to the morphological characteristics and using molecular biology methods (sequencing rDNA ITS1-5.8S-ITS2 region). Fungal isolates were preserved in the collection of Latvian Plant Protection Research Centre.

The visual assessment of the plants in the trial was done in the period between the first and second yield. The proportion of the dead plants varied between 0.6 to 11 %. The proportion of infected plants showing wilting symptoms ranged from 41 to 72 %. The most heavily infected cultivars were 'Everest' (52 %) and 'Florina' (72 %). At the end of September the percentage of dead plants reached from 9 % ('Evie 2') to 50 % ('Florina'). Correlation analysis showed that low number of healthy plants in the summer resulted in lower number of living plants in autumn ($r = 0.97$, $R^2 = 0.94$). High percentage of plants showing wilting symptoms resulted in reduced amount of total yield: 72 % of damaged plants gave the reduction of the yield by 30 % ($r = -0.5$, $R^2 = 0.26$). The condition of plant health affected also the yield quality: higher percentage of plants showing wilting symptoms resulted in lower quality berries ($r = 0.99$, $R^2 = 0.99$).

Main crown rot causal agents were *Coniella fragariae* (isolated from 67 to 100% of examined plants), *Pestalotiopsis* spp. (5 to 42%), *Rhizoctonia* spp. (5 to 30%), *Colletotrichum acutatum* (25%, only on 'Florina'), *Fusarium oxysporum* and other *Fusarium* spp. (8 to 22%), *Phoma exigua* (8%, only on 'Florentina'). *C. fragariae* has been isolated as potentially pathogenic fungus from the root crown of visually healthy plants in Switzerland (Rigotti et al. 2003). It has been isolated also from other parts of strawberry plants: petioles (Rigotti et al. 2003), flowers and berries (Sesan 2006). Previously in Latvia *Coniella castaneicola* has been isolated from berries (Laugale et al. 2004). The members of

the genus *Pestalotiopsis* have been characterized as causing agents of necrotic spots on the leaves and petioles in Brazil (Teixeira et al. 2015), as root rot pathogens in United States of America (Florid) (Mertely et al. 2013), as causing agents of root and crown rot in Spain (Chamorro et al. 2016). Four sequenced isolates from present investigations showed 96 to 97% identity with *Neopestalotiopsis natalensis* (Maharachchikumbura et al. 2014).

The main conclusions are: fungal pathogens isolated from the root crown of diseased plants in our study have been described as strawberry pathogens also in other investigations; the most common species found was *C. fragariae*. This species was isolated in lower amount from the cultivar 'Florin' which is suitable for relatively heavy soil according to the information given by the plant producer, but this cultivar had higher abundance of other pathogens. In general it was observed that plant vitality decreased during the season, as well reduced yield was obtained, and root crown rot decreased also fruit quality.

References

- Chamorro M., Aguado A., De los Santos B. 2016. First report of root and crown rot caused by *Pestalotiopsis clavigpora* (*Neopestalotiopsis clavigpora*) on strawberry in Spain. *Plant Dis.* DOI 10.1094/PDIS-11-15-1308-PDN
- Kurze S., Bahl H., Dahl R., Berg G. 2001. Biological control of fungal strawberry diseases by *Serratia plymuthica* HRO-C48. *Plant Dis.* 85: 529–534.
- Laugale V., Lepse L., Vilka L., Rancāne R. 2009. Incidence of fruit rot on strawberries in Latvia, resistance of cultivars and impact of cultural systems. *Sodininkystė ir Daržininkystė* 28: 125–134.
- Maharachchikumbura S.S., Hyde K.D., Groenewald J.Z., Xu J., Crous P.W. 2014. *Pestalotiopsis* revisited. *Studies Mycol.* 79: 121–86.
- Mertely J.C., Chamorro M., Tompkins D., Mertely J.A., Peres N.A. 2013. Fungi associated with diseased roots of strawberry runner plants after transplanting. APS and MSA Joint Meeting, August 10–14, Austin, Texas.
- Rigotti S., Viret O., Gindrat D. 2003. Fungi from symptomless strawberry plants in Switzerland. *Phytopathol. Mediterranea* 42: 85–88.
- Sesan T.E. 2006. Integrated control of strawberry diseases. *Phytopathol. Pol.* 39: 133–148.
- Teixeira M.A., Martins R.M.S., Vieira R.F., Vildoso C.I.A., Adami A.A.V., Ferreira, A.C. 2015. *In vitro* identification and control of *Pestalotiopsis longisetula* fungus, pathogens strawberry crop. *Rev. Agrogeoambiental* 7: 59–65.

Effect of decreased precipitation events on primary growth and photosynthesis-related characteristics of spruce (*Picea abies*) saplings grown in different substrates

Oskars Krišāns¹, Āris Jansons¹, Gederts levinsh^{2*}

¹Latvian State Forest Research Institute "Silava", Riga 111, Salaspils LV-2169, Latvia

²Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

*Corresponding author, E-mail: gederts.ievins@lu.lv

Key words: chlorophyll, chlorophyll *a* fluorescence, growth, *Picea abies*, water limitation.

Increasing of annual mean air temperature and prolonging of summer drought periods is forecasted for Baltic Sea region till the end of this century (IPCC 2013). The predicted changes will eventually affect the success of forest restoration. Previous studies have shown that altered distribution of precipitation without any changes in the total yearly amount does not affect growth of saplings if ground-water is available (Krišāns et al. 2015).

The aim of the present study was to test the effect of decreased amount of summer precipitation in combination with excluded ground-water on primary growth of spruce saplings. It was specifically asked if photosynthesis-related parameters [needle chlorophyll concentration, photochemical activity of photosystem II (PS II)] can be used as early indicators of water shortage effects.

In mid-April, two-year-old spruce (*Picea abies* [L.] Karst.) saplings, obtained from commercial nursery, were planted in 1 L plastic containers using three different soil types: peat soil from *Myrtillosa turf. mel*, poor sandy soil from *Cladinoso-callunosa* and fertile mineral soil from *Hylocomiosoa* forest stands. Containerized saplings from all soil types were divided into four treatment groups, receiving the amount of 0, 25, 50 and 75% from the natural precipitation. Control plants of all three soil types were subjected to natural regime of precipitation. For ground-water-affected control, saplings were planted in beds with fertile mineral soil, these plants received natural precipitation as well.

Rain sensor-based automated shelter was used to intercept natural precipitation for plants within treatments. Saplings were watered with appropriately decreased amount of water according to each treatment once per three days. Water shortage experiment started at June 22 during the period of active primary growth and was terminated on August 15, followed by a two-week recovery under natural precipitation.

Height increment of the saplings from all groups was measured weekly. During both water shortage and

recovery periods, both needle chlorophyll concentration and chlorophyll *a* fluorescence were analyzed once per week. Chlorophyll concentration was assessed using a CCM-300 chlorophyll meter (Opti-Sciences, Hudson, New Hampshire, USA). Chlorophyll *a* fluorescence was measured using fast continuous measurement method with a Handy-PEA system (Hansatech Instruments, King's Lynn, UK).

Different degrees of limitation of precipitation led to notable differences in soil water potential within containers (Fig. 1). As it was initially expected, most drastic decrease

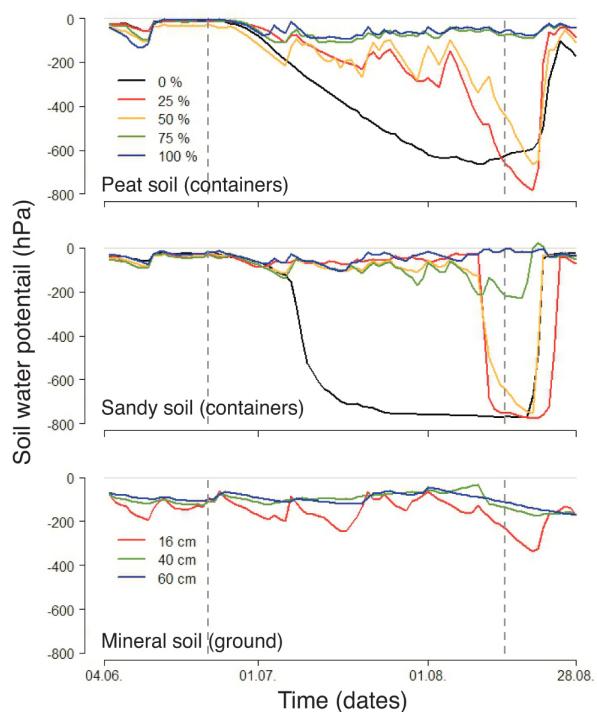


Fig. 1. Time course of substrate water potential in containers with peat soil and mineral soil with different degree of water supply, as well as in mineral soil in ground at different depths.

was in sandy soil for 0% treatment. Similar effect was seen in mineral soil, while in peat soil, decrease of water potential was less pronounced.

The start of water limitation coincided with a phase of decreased growth intensity. Within the first week of experiment most pronounced decrease in growth rate was evident for plants grown in containers with poor sandy and fertile mineral soil in 0% treatment (Fig. 2A).

First notable decrease of chlorophyll *a* fluorescence was seen for 0% treatment plants only on the 4th week after the start of the experiment (Fig. 2B). Most pronounced changes and differences among the treatments were seen for chlorophyll fluorescence parameter Performance Index. Needle chlorophyll concentration significantly decreased only after six weeks since the start of shortage of water (Fig. 2C) both in poor sandy and fertile mineral soils, while full needle dieback for 0% treatment saplings occurred a week later.

Extreme water limitation resulted in relatively fast decrease of soil water potential, however the effect was related to the particular soil type used in experiment. In peat soil, even complete water limitation led only to continuously diminishing water potential, but saplings did not have symptoms of needle dieback.

It appears that primary growth of bare-rooted spruce saplings is more drought-sensitive than photosynthesis-related characteristics in needles, e.a. photochemistry of PS II and chlorophyll concentration. Consequently, downregulation of growth is one of the first physiological events after sensing of decreased soil water potential followed by inhibition of photochemical activity in needles, while diminishing of needle chlorophyll concentration might be related to drought-associated senescence and needle death.

References

- IPCC. 2013. *Climate Change 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Stocker T.F. et al. (eds). Cambridge University Press, Cambridge & New York.
 Krišāns O., Kalniņš J., Puriņš M., Jansons Ā. 2015. Influence of various precipitation regime on growth of Norway spruce plants. *Mežzinātne* 29: 84–98. /in Latvian/

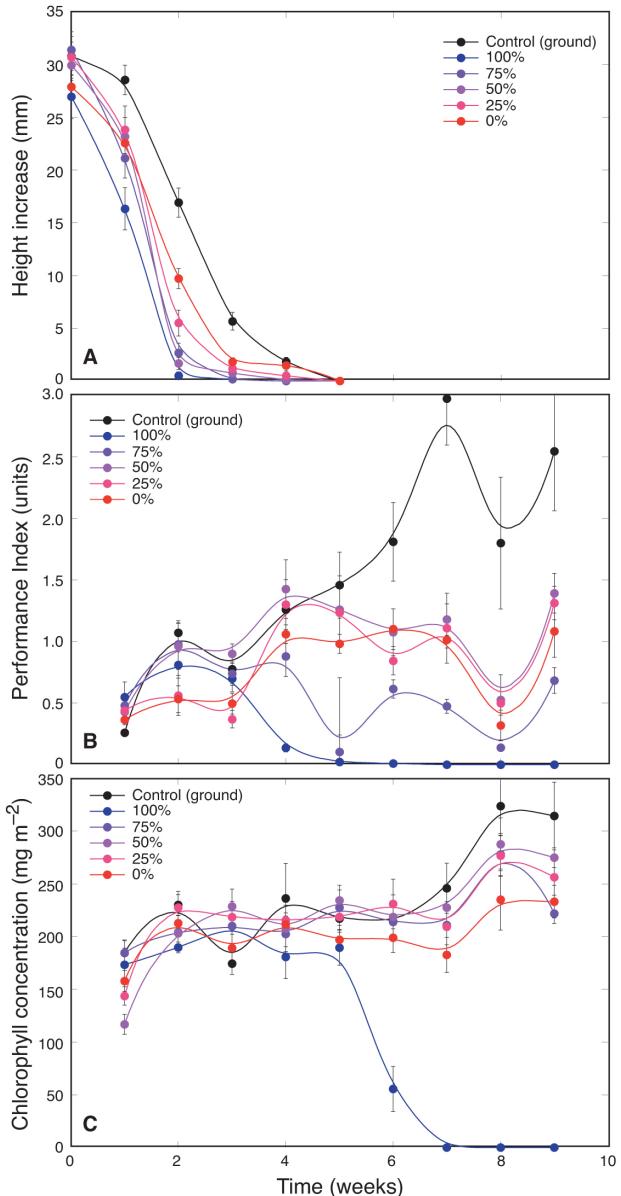


Fig. 2. Height increase (A), Performance Index (B) and needle chlorophyll concentration (C) of spruce saplings during water shortage experiment started at week 0. Saplings were grown in containers with mineral soil except ground control.

Monitoring of three single nucleotide polymorphisms in *RYR1* gene of local 'Latvian White' pig breed

Kristīne Dokāne^{1*}, Daina Jonkus², Tatjana Sjakste¹

¹Genomics and Bioinformatics, Institute of Biology, University of Latvia, Salaspils, Latvia

²Institute of Agrobiotechnology, Latvia University of Agriculture, Jelgava, Latvia

*Corresponding author, E-mail: kristine.dokane@lu.lv

Key words: *RYR1*, polymorphism, pigs.

RYR1 encodes the ryanodine receptor 1, which is a calcium ion channel in the sarcoplasmic reticulum of skeletal muscle. Multiple single nucleotide polymorphisms (SNPs) in coding gene region (cSNPs) in human are associated with malignant hyperthermia susceptibility, central core disease, and myopathy. Remarkably, that homologous in human and pig missense mutation Arg614Cys and Arg615Cys resulted from C>T substitution at the rs118192172 and rs344435545 locirespectively, is associated in both species with malignant hyperthermia. Presence of the T allele at the rs344435545is associated in pig also with porcine stress (PSS, halothane susceptibility) and pale, soft and exudative meat (PSE) syndromes. It is suggested, however, that heterozygote carriers in some breeds may have some adaptive advantage. Up today about 2000 SNPs and 150 cSNPs have been reported for pig *RYR1* gene. However, there is still no data on their genetic diversity and possible association with phenotype.

The aim of current study was to genotype the rs344435545 (C1912T, Arg615Cys), rs196953058 (T8434C, Phe2769Leu) and rs323041392 (G12484A, Asp4119Asn) in Latvian local pig breed 'Latvian White'.

Sample collections include the Primary (PR) collection of eight samples of original 'Latvian White' randomly collected from Latvia University of Agriculture (LLU) herd in 2006 within Latvian Ministry of Agriculture supported project and Representative (RP) collection of 103 samples collected in 2015 in three geographically distant private farms in frame of VPP 2014-2017 "AgroBioRes". DNA was isolated from 500 mL of blood of each animal by using a K0512 Genomic DNA Purification Kit (Fermentas). Sequence information for swine *RYR1* gene available in

GenBank was used for genomic region reconstruction and PCR primer design. All three polymorphisms were genotyped by restriction fragment length polymorphism (RFLP) technology using restriction enzymes *Hha*I, *Alw*21I, *Apa*I and *Bcl*I. The study was approved by the LLU review board.

Locus rs344435545 was found to be monomorphic in both the PR and RP collections. This result was expected as unfavorable 1912T (615Cys) allele has not been introduced in 'Latvian White' and related phenotype had not been reported for breed until now. Similarly, locus rs196953058 was observed as monomorphic in both PR and RP collections.

Instead, the rs323041392 showed in PR high level of polymorphism with GG/GA/AA genotype correlation equal to 3/4/1. As the LLU herd had been under strong selection control focusing on 'Latvian White' breed maintenance and conservation, polymorphism at the rs323041392 observed in PR collection could be considered as breed specific character. However, in RP collection this locus was found to be monomorphic. So, full loss of the rs323041392 variability in 'Latvian White' happened in nine years (from 2006 till 2015) of private farming. Our results may signal to Latvian livestock industry that breeding intensification and over introgression of favorable alleles may lead to loss of local breeds' specific features and decrease of selective facilities and adaptability to environmental challenges in local breeds of small populations.

Acknowledgements

The study was supported by the National Research Programme VPP 2014-2017 "AgroBioRes".