FINAL REPORT
Tandem Forest Values:
SOMAGENO – Potentials of combining somatic embryogenesis and genomic selection in Norway spruce

1. Description of the research that has been carried out

In the present project the potential of production of more resistant forest regeneration through somatic embryogenesis was studied. The key findings were that the root rot resistance locus *PaLAR3B* is successfully delivered from elite Norway spruce parent trees to their SE-progeny and that the resistance locus does not interfere with SE-initiation or embryo production capacity. However, there was indication that the resistance allele may interfere with embling establishment in the nursery during the first growing season.

During the project, SE-plants were phenotyped for the first time for root rot resistance. The results confirmed the effect of the known resistance allele *PaLAR3* effect also in SE-plants but also gave basis for detection of the role of novel candidate genes related to root rot resistance/SE-propagation success in SE-propagated plants. Moreover, during the project, also parents of the studied SE-plants were phenotyped in seed orchards which enables to follow the inheritance of resistance traits from parents to their SE-progeny.

The work was carried out under three workpackages (WP). The detailed research carried out in these WP-s is described in detail below.

1.1 WP1 Genotyping for Heterobasidion resistance loci in elite Norway spruce materials

The aim of WP1 was to screen the presence of the resistance locus *PaLAR3* among progeny-tested Norway spruce plus-trees used for SE-plant production in Finland.

Material and methods: In total, 80 elite trees were genotyped for *PaLAR3* alleles.

The results reveal that the *PaLAR3B* allele which is related to 27% higher root rot resistance is well-presented among the tested plus-tree population as 47.5 % of the tested trees carry at least one copy of the PaLAR3B allele (45 % are heterozygous and 2.5 are homozygous for the B-allele).

1.2 WP2: SE-propagation ability

The aim of WP2 was to study whether there is trade-off between the resistance allele and SE-propagation ability of Norway spruce material i.e. whether more resistant forest regeneration material could be propagated by SE-method.

Material and methods: For this, the SE-progeny from the 80 genotyped plus-trees representing different *PaLAR3* genotypes were analyzed for SE-initiation, embryo formation, germination and SE-plant viability at the end of first growing season. SE-initiation rate was analyzed form 55 full-sib families (5-540 initiation attempts, ie explants per family, 8588 initiation attempts in total resulting in 5393 successful SE-initiations). Allele delivery, embryo formation, germination and SE-plant viability were analysed from 8 full-sib SE-families originating from the genotyped plus-tree crosses (full-sib families) representing different parent *PaLAR3* genotypes (AAxAB, ABxAA, ABxAB and ABxBB). During the analyses we found that one of pollen donor had been misidentified and therefore one family was dropped from the analyses and final analyses consisted of 7 full-sib SE-families. The *PaLAR3* resistance allele delivery was analyzed from 241 SE-lines (19 – 67 lines/family) and analyzed against Mendelian inheritance rule. Embryo production ability E/gFW was analyzed form 191 lines (3-5 plates per line). From each SE line, 5 to 81 embryos were germinated (94 SE lines from four families: 18–32 SE lines/family, total 3450 embryos) and the germination rate was measured. The number of embryos going into germination was adjusted so that they could be transplanted in full rows in Plantek 81f containers. No more than 81 embryos (one full pl 81f container) were germinated even if there were more embryos available. SE-plant viability after first growing season in the nursery (% of emblings alive in the end of first growing season from transplanted embryos) was analyzed from 49 SE lines (from four families: 3–9 SE lines/family, 6–36 emblings/SE line, total 1498 emblings).

Results: The results reveal that the resistance allele was present among the SE lines as expected based on Mendelian segregation and did not interfere with somatic embryo production capacity. All embryos from *PaLAR3* genotypes germinated well and emblings were viable in the end of first growing season. However, in three families, *PaLAR3B* homo- or heterozygotes had 23.2% to 32.1% lower viability compared to their respective hetero- or *PaLAR3A* homozygotes (Fig. 1). Therefore, it may be concluded that there is no trade-off between root rot resistance locus *PaLAR3B* and somatic embryo production ability, but the allele may interfere with Norway spruce embling establishment.

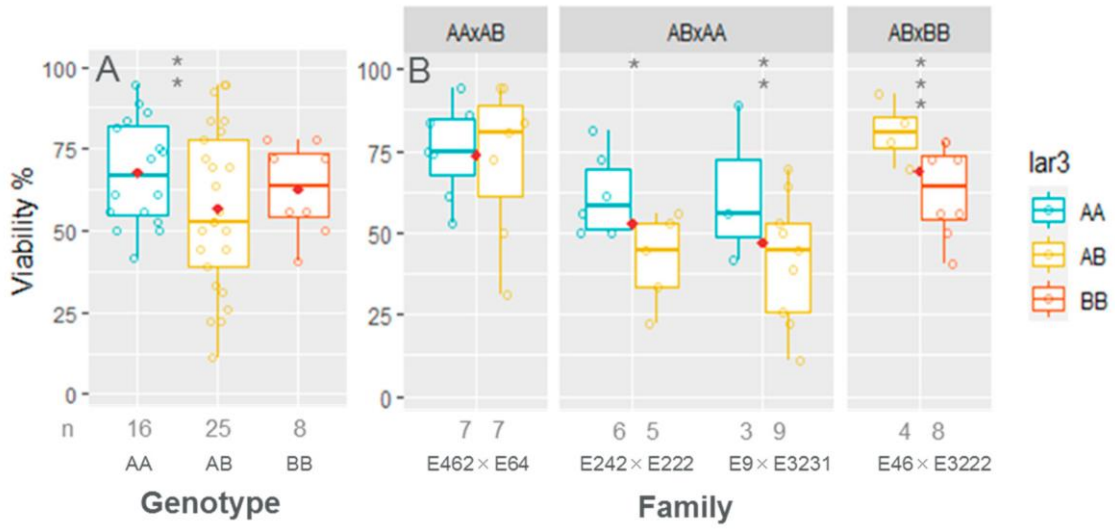

Figure 1. Embling viability (%) of different *PaLAR3* genotypes (A) of four Norway spruce full-sib SE-families (B) in the end of first growing season with the number of SE lines given for each allelic combination (n).

1.3 WP3: MAS for pathogen resistance in SE-propagation pipeline

The aim of this WP was to study the root rot resistance phenotype of SE-plants with different resistance allele *PaLAR3* genotypes and furthermore with other novel resistance-related candidate molecular markers.

1.3.1 Phenotyping SE-plants

Material and methods: In this WP, emblings with known genotypes from WP2 were studied for their fungal resistance. For the phenotyping experiment, 296 two-year old SE-plants from five families representing different *PaLAR3* genotypes (total 37 lines (8 plants/ line) were transported to SLU in Uppsala for the phenotyping experiment in greenhouse conditions. The emblings were inoculated by *Heterobasidion*-infected wood dowels (Fig. 2A, B). After three weeks of the inoculation the growth parametres (Fig. 2C) and lesion length (Fig. 2D) of the emblings was recorded and the stems were cut into 0.5 cm disks (Fig. 2E) and placed on moist Petri dish for 1 week. Thereafter the discs were scored for fungal growth in sapwood (FGS) (Fig. 2F).

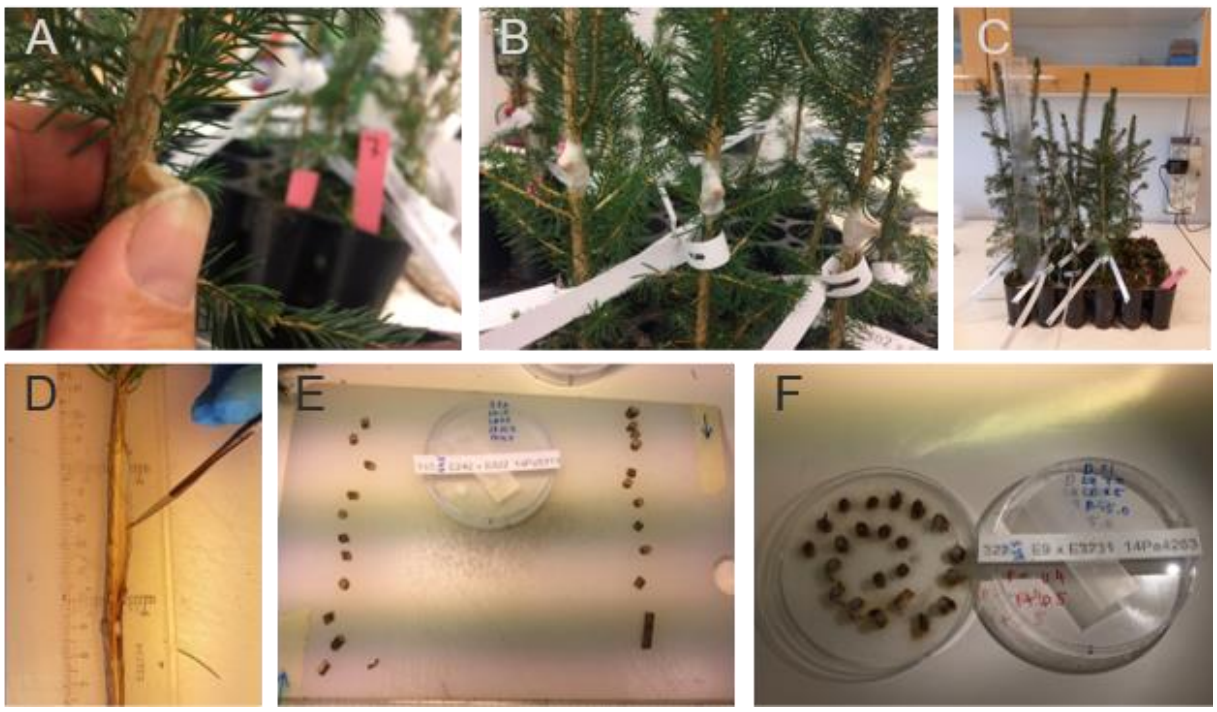

Figure 2. Root rot resistance phenotyping of Norway spruce emblings. Inoculation with Heterobasidion-infected dowel (A, B), growth measurements 3 weeks after inoculation (C), Lesion measurement (D), Preparation for fungal growth measurements (E), Scoring of fungal growth in sapwood (FGS) (F).

Results: From the total number of the plants in the experiment 82.7 %were successfully inoculated and 14.3 % were failed due to unsuccessful inoculation and were removed from the analyses (Heterobasidion conidiophores were missing from the dowel, inoculation site and no sapwood growth was detected). 3.2 % of plants were able to prevent fungal growth as conidiophores were observed either on dowel or infection site, but no FGS was detected. Final analysis included four families because according to results of WP1, one pollen donor misidentification had happened, and this pollen donor was removed from analyses both in WP1 and WP2.

Fungal Lesion

Total lesion length (Ltot) was significantly lower in *PaLAR3B* homozygotes compared to *PaLAR3A* homozygotes (Fig. 3A). However, total lesion length of different *PaLAR3* genotypes did not differ in most families except E242xE222 where *PaLAR3* heterozygotes had significantly shorter total lesion compared to

the *PaLAR3A* homozygotes (Fig. 3B). Similarly, the *PaLAR3B* homozygotes had significantly lower lesion downward from the inoculation site compared to *PaLAR3A* homozygotes (Fig. 3E). In half of the families, *PaLAR3* heterozygotes had shorter downward lesion compared to *PaLAR3A* homozygotes (Fig 3F). Upward Lesion was shorter without any differences in different *PaLAR3* genotypes (Fig. 3C, D)

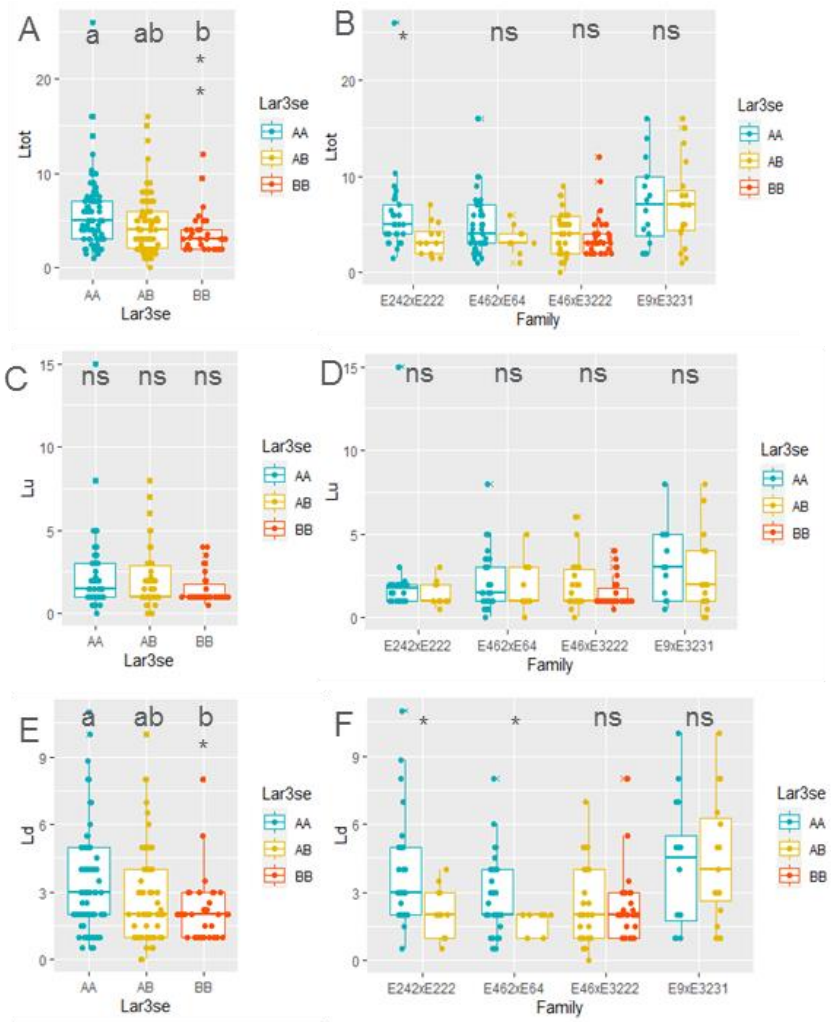

Figure 3. Total lesion length (Ltot) (mm) (A), Lesion up (Lu) (C) and Lesion down Ld (E) from the inoculation site of different *PaLAR3* genotypes combined and in different families (B, D, F).

Fungal growth in sapwood FGS

Total fungal growth was significantly lower in *PaLAR3B* hetero and homozygotes when the data from all families was pooled (Fig. 4A) but no differences were observed between different *PaLAR3* genotypes within families (Fig. 4B). Upwards from the inoculation site *PaLAR3B* allele resulted in shorter growth compared to *PaLAR3A* homozygotes (Fig 4C). The effect was present also within 3 families (Fig. 4D). Downward inoculation site the fungal growth was shorter and there were no differences between different *PaLAR3* genotypes in most families (Fig 4E, F).

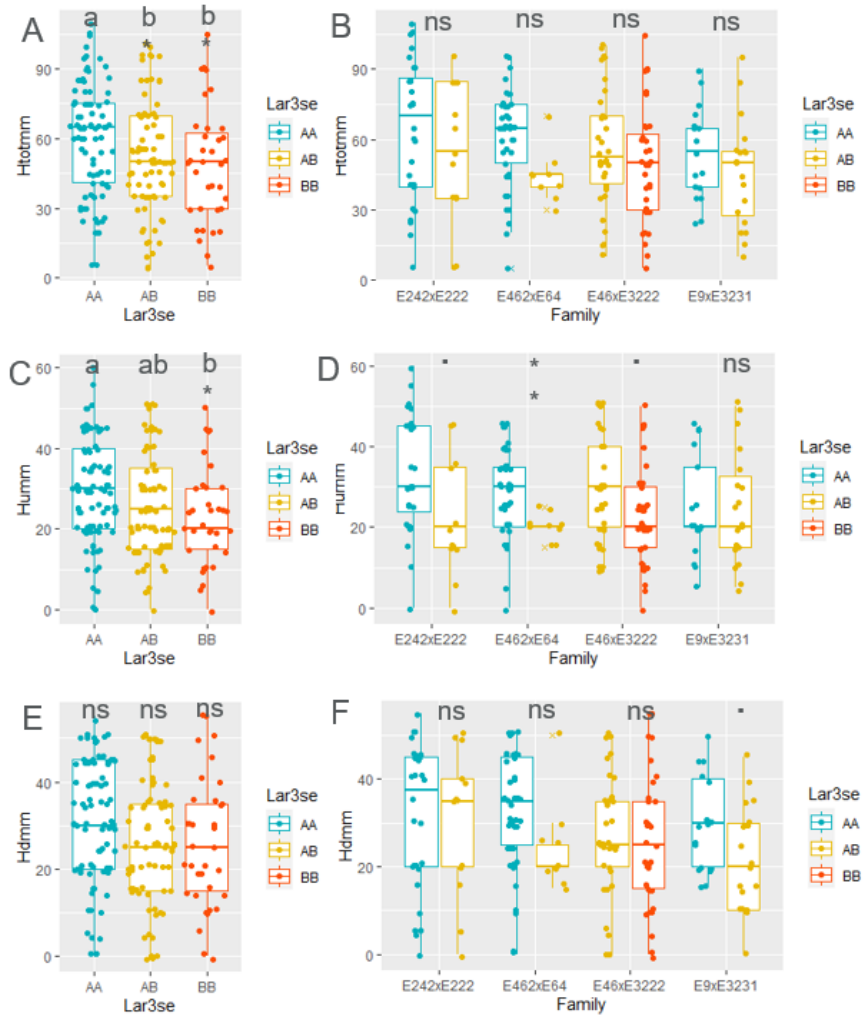

Figure 4. Total fungal growth in sapwood FGS (Htotmm) (mm) (A), FGS up (Humm) (C) and FGS down (Hdmm) (E) from the inoculation site of different PaLAR3 genotypes combined and in different families (B, D, F).

Changes in plan: The SE-progeny phenotyping experiment was done 1 year earlier (in 2019 summer) than in original plan because of the availability of suitable plant material. The change in timetable was extremely favorable for the experiment because next year (2020) when the experiment was actually planned it would'nt have been possible due to ongoing pandemic related travel restrictions. The plan was to phenotype one more family in 2020 but the plants did not grow sufficiently probably due to extraordinary winter conditions in 2020 so the plants remained too small for phenotyping. However, instead of that, we performed SE-plant parent phenotyping for 11 parent genotypes in seed orchards.

1.3.1 Parent phenotyping

Material and methods: 11 parents were phenotyped in commercial seed orchards (with the permission from the seed orchard owners) during summer-autumn 2020. From each parent genotype, 3 clones with 9 branches (3 branches/clone) were inoculated and total 3 branches were treated as controls (wounded without inoculum). After three weeks of inoculation, the branches were collected so that the inoculation

place was cut ca 1 m from the tree trunk to avoid contamination of the test tree. Total number of valid observations in the experiment was 132. From these 132 were inoculated and 33 were treated as un-inoculated controls.

Results: 95 % of the inoculations were successful, 4 % prevented the inoculation and 1 % failed inoculation. In the combined data based on *PaLAR3* genotype no differences in Lesion length and SWG are observed (Fig 5). In comparison to SE-plant phenotyping, parents had less fungal sapwood growth (parents: mean 20.4 mm, max 65 mm, SE: mean 55.0 mm, max 110 mm) but larger total lesion length (parents: mean 9.4 mm, max 46, SE: mean 4.9 mm, max 26 mm). There was lot of variation between different genotypes and even within clones of the same genotypes which is probably due to small number of clones of each parent genotype and the fact that the parents were situated in different seed orchards.

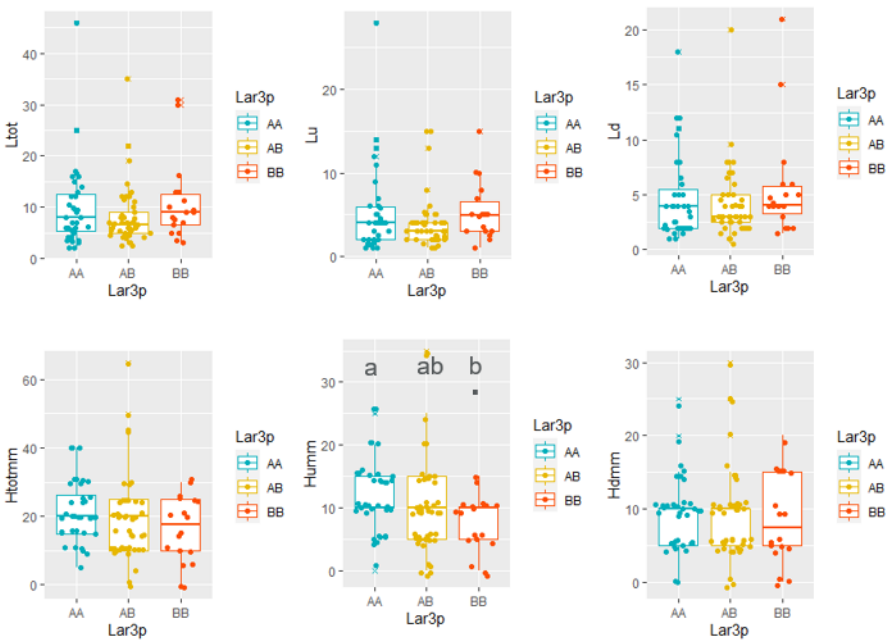

Fig. 5 Total lesion length (Ltot), Lesion up (Lu) and Lesion down (Ld), total fungal growth in sapwood (Htotmm), up (Humm) and down (Hdmm) from the inoculation site.

1.3.3 Combining parent and progeny phenotype and genotype information

The aim: The SE-progeny as well as parent phenotype data will be further studied for other genes than PaLAR3 associated with fungal resistance/SE-propagation ability.

Material and methods: During the SOMAGENO project, 164 genotypes (153 SE lines from 6 families as well as 11 parents) were genotyped with 181 genes (86 genes related to resistance/propagation ability) in highly multiplexed genotyping approach. The amplicons were pooled into 2 libraries which were sequenced at NGI Uppsala – SNP&SEQ Technology Platform (SciLifelab). After initial quality check with FastQC, the forward and reverse fastq files were combined, filtered, aligned to heel sequences and finally detagged to represent individual samples. Thereafter, the detagged samples were analyzed by following Founders workflow which consisted of creating index for genome, alignment each sample to the Norway spruce genome and finally processing the aligned reads by Samtools, Picardtools and GATK haplotype caller.

Results: The sequencing was successful, and the two sequenced libraries resulted in 4.6 and 5.3 million high-quality sequences, respectively. After filtering and detagging, 8.7 and 17.6 % of the total reads were included in Founders workflow and variant analyses.

Changes in project plan: In the original plan, the bioinformatics analyses were planned to be carried out in SLU Uppsala on the bioinformatics grid of SLU by using the well-established pipeline and bioinformatics support. However, due to pandemic travel restrictions, the remote period in SLU was not possible. Therefore, the bioinformatics analyses were carried out on Finnish High Performance Computing Platform CSC (https://research.csc.fi/-/puhti). Setting up the bioinformatics analysis environment and learning curve of standalone analyses affected the timetable of the analyses. However, with the remote support from the group in SLU and participation in CSC courses and training, it is seen as a valuable experience for the post-doc period. Standalone analyses enabled to learn more and dive deeper in bioinformatic analyses which will surely help during the continuation of the research topic in the group in LUKE and in collaboration with SLU.

2. If the work has resulted in publications – attach a reference list.

WP1 and WP2 resulted in the publication below. The results from WP3 studies will be published in international peer-reviewed series.

- Edesi, J.; Tikkinen, M.; Elfstrand, M.; Olson, Å.; Varis, S.; Egertsdotter, U.; Aronen, T. Root Rot Resistance Locus *PaLAR3* Is Delivered by Somatic Embryogenesis (SE) Pipeline in Norway Spruce (*Picea abies* (L.) Karst.). *Forests* **2021**, *12*, 193. https://doi.org/10.3390/f12020193

3. Description of how the grant has contributed to competence building that will facilitate and strengthen long term collaboration between Finland and Sweden.

The project initiated long-term collaboration with Forest Mycology and Pathology Department as SLU as the forest breeding group leader Tuija Aronen in LUKE was recently named one of the long-term collaborators between the group in SLU and LUKE. FIN-SWE collaboration started in SOMAGENO continues in other research projects such as Public Private Partnership (PPP) described below (in section 4), as well as in joint applications for research financing, such as the one made to FORMAS by A. Puentes, SLU, Sweden with T. Aronen, Luke, Finland; the topic related to pest resistance of Norway spruce SE plants.

The project enabled the post-doc to develop competence areas that are valued in current LUKE research strategy e.g. genomic analyses related to production of more resistant forest regeneration material. The need for genomic analysis competence is increasingly needed already at present as these experiences have led to employment of the post-doc for extra 3 years to apply the competence obtained during SOMAGENO project.

4. Description of research areas being started or strengthened at the departments in Finland and Sweden.

In Finland, the project initiated research related to combining usage of genomic information for production of more resistant forest regeneration material. Forest companies have been interested in the research outcome and the work initiated in SOMAGENO project will be continued as a part of a bigger three-year Public Private Partnership (PPP) project in spruce somatic embryogenesis started in the beginning of 2021. This PPP project involves Luke and six other partners representing private Finnish-Swedish forestry companies and plant producers, and it is pre-competative public research project, Luke owning all the IPR. There are three work packages in the PPP project, of which one is direct continuation of the SOMAGENO

research. The post-doc will continue the work in collaboration with SLU to finish the WP3 work and to further screen Finnish SE materials and donor trees in order to produce more resistant forest regeneration material.
In Sweden, the results from SOMAGENO, showing that the expression of root rot resistance traits may vary between individual lines in an SE-family has raised interest from Swedish forest industry to combine SE with genomic selection for root rot resistance in Swedish elite material. Several major forest companies have financed a pilot project at the Forest Mycology and Pathology Department as SLU (ca 250 KSEK through 2021 but with possibilities for extension) through the Swedish forest biotechnology company Swetree technologies.

5. Description of how the grant has contributed to strengthening the forest sector in Finland and in Sweden.

Awareness of forest sector stakeholders of possibilities of resistance breeding and MAS in combination of SE propagation pipeline have increased through the project presentations (see below) and personal contacts, and their interest awakened. This interest in novel opportunities has also already resulted in participation and financing the PPP project in which the SOMAGENO research will be continued towards practical applications. The project has also led to an interest from Swedish forest industry to combine SE with genomic selection for root rot resistance in Swedish material. Several major forest companies have financed a pilot project through the Swedish forest biotechnology company Swetree technologies.

6. Description of communication with relevant stakeholders and end users.

Presentations:

- Tikkinen, M and Edesi, J: Kasvullisen lisäyksen nykyaihe: Metsätaimitarhapäivät 2021, 20.01.2021

- Edesi J: Potentials of somatic embryogenesis and genomic selection in Norway spruce (SOMAGENO). Forest Mycology and Pathology seminar, SLU. 10.12.2020.

- Edesi J: SOMAGENO: Potentials of combining somatic embryogenesis and genomic selection in Norway spruce. Early Career Researchers (ECR) research seminar in LUKE. 4.11.2020

- Edesi J, Elfstrand Malin, Tikkinen M, Varis S, Egertsdotter U and Aronen T: Potential of marker assisted selection (MAS) and somatic embryogenesis (SE) in production of more root rot resistant Norway spruce material. Forest Science Day 20.10.2020.

- Edesi J: Heterobasidion case study: potentials of combining somatic embryogenesis and genomic selection. Bioacademy Finland: Indian forest probationer's education: special exposure to overseas forestry practices. 04.03.2020

Upcoming presentations:

- Presentation entitled: Combining DNA-based selection for Heterobasidion resistance and SE on Workshop on Vegetative Propagation: Multiplied conifer seed – prospects of somatic embryogenesis – A Webinar on 26-27 May 2021
- Abstract entitled: Root rot resistance locus *PaLAR3* is delivered by somatic embryogenesis (SE) pipeline in Norway spruce (Picea abies (L.) Karst.) submitted to 2021 Forest Gentetics CFGA/WFGA Student and PostDoc Symposium which will take place on 19-20 May 2021

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Other communications:

- Aronen, T, Varis S, Edesi J: Several meetings with forestry companies in 2020, negotiations on PPP project and its content

7. Financial accounting

Somageno costs at LUKE:

Personnel costs	160 704,90
Overheads	152 669,65
Other costs	12 733,69
TOTAL	326 108,24
Post-doc/Jaanika Edesi	
Salary, including indirect personnel costs	125 431,26
Overhead 119159,70€, applied from Tandem Forest Values	83 411,79
TOTAL	208 843,05

Somageno costs at SLU:

Post	**Cost (Euro)**
Salary Malin Elfstrand	13000
Running costs	
PCR costs	3000
MiSeq sequencing at Scilife	1744
Material for phenotyping	500
Plant cultivation facilities	800
Total running costs	**6044**
Total costs	**19044**