



Promoting Conifer Genomics Taking research a step further

Highlights

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This project is financially supported by the European Commission under the 7th Framework Programme.

Objectives

The main goal of this project is to develop an integrative and multidisciplinary genomic research in conifers, using high-throughput platforms for sequencing, genotyping and functional analysis, to unravel genome organization and identification of genes and gene networks controlling important ecological and economic traits, such as those related to the control and the reduction of climatic change impact in relation to growth, drought and cold stress.

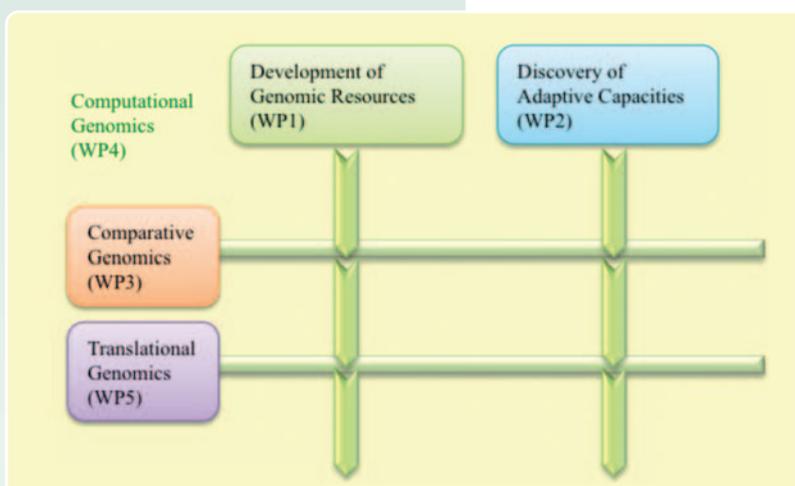
ProCoGen will make an effort to integrate and apply the outcomes of other international initiatives especially from America (*Pinus taeda*), Sweden (*Picea abies*) and Canada (*Picea glauca*, *P. sitchensis*, *P. mariana*). The research efforts will focus on economically significant model tree species namely *Pinus sylvestris*, *Pinus pinaster*, *Picea abies* and *Picea sitchensis*, representing two pine sub-families, from different European regions.

Impact

ProCoGen will contribute towards the expected impact of the call by developing an integrative research programme on conifer species combining the use of the most appropriate high-throughput (HT) technologies with advanced bioinformatics to generate new knowledge to improve forest productivity through targeted breeding programs based on better adapted material to regional climatic threats, forest stewardship in response to environmental change as well as conservation efforts. This project will build upon previous networking efforts of European initiatives on forest ecosystems and aims at integrating presently fragmented activities of European research groups. This integrative project will greatly contribute to strongly reinforce the forefront position of the European research on conifer genomics and bioinformatics.

Structure

The project is divided into seven workpackages (WP) WP 1-5 are research packages (see figure), WP6 is devoted to dissemination and integration and WP7 is management and administration.



Work Package Objectives and Progress in Brief

Development of Genomic Resources (Work Package 1)

Objectives | To develop both de novo genome sequences and extensive catalogues of genetic variation for two species of pines: Scots pine (*Pinus sylvestris*) and Maritime pine (*Pinus pinaster*). The next generation sequencing has opened new doors for tackling conifer genomes, and the availability of sequence data from *Picea abies*, *Picea glauca* and *Pinus taeda* will be a great asset for assembling and resequencing related conifer species. In addition, SNP discovery through resequencing is a promising tool in studying ecological genomics of local adaptation. This project will also greatly contribute to integrating European and Trans-Atlantic efforts, and will take conifer genomics a step further.

Main results in the first 18 months

- *P. sylvestris* genome sequencing: Haploid and diploid DNA libraries were used for whole genome sequencing. Paired end libraries with different insert sizes have been prepared and are being currently sequenced.
- *P. pinaster* genome sequencing: Haploid DNA libraries for 454 paired end sequencing have been prepared and further analysis is underway.
- For SNP discovery, seeds from different populations have been sampled for both *P. sylvestris* and *P. pinaster*. cDNAs have been used to cover the extent of natural genetic variability available in these populations, using exome capture and direct high throughput sequencing, respectively.

Discovery of Adaptive Capacities (Work Package 2)

Objectives | To study the functional regulation of growth and adaptive responses to abiotic stresses via transcriptome sequencing, transcript expression profiling, small RNA (sRNA) discovery and profiling, identification of transcription regulatory networks and characterization of epigenetic dynamics in contrasting developmental and environmental conditions. The functional regulation of growth and adaptive responses will be focused on: drought stress studies in *Pinus pinaster* and cold acclimation in *Picea abies* and *Pinus sylvestris*. The molecular tools developed in earlier national and international projects will form the stepping stones for intensive studies in this project. ProCoGen will integrate the outcomes from other European initiatives like SUSTAINPINE, NovelTree, EVOLTREE and other European initiatives, creating a new dimension in conifer research.

Main results in the first 18 months

- cDNA libraries have been constructed from RNA extracted from cells and tissues using laser capture micro-dissection. Bioinformatic analyses are in progress.
- Results from microarray and metabolomics using nuclear magnetic resonance (NMR) will deepen our understanding of processes involved in nitrogen metabolism in pine.



Pinus pinaster



Pinus sylvestris



Picea abies



Picea sitchensis



Pinus sylvestris



Pinus pinaster

- A study on cuticle related gene expression suggested that cuticular wax may be involved in drought stress adaptation in maritime pine. This study also provides a set of promising candidate genes for future studies in conifers.
- Experiments have been conducted to analyze *P. pinaster* drought response using F1 individuals with contrasted functional responses. These selected clones were obtained from a controlled cross between a "drought intolerant" female parent and a "drought tolerant" male parent, from which the megagametophyte used as template for *P. pinaster* genome sequencing were harvested.
- Several sRNA extraction protocols have been tested and modified for sRNA studies.
- RNA data from somatic embryos at different stages of development and different temperature regimes will give an insight into epigenetically altered phenologies in response to cold acclimation in *Picea abies*.
- ChIP-Seq (chromatin immunoprecipitation) protocol has been established to give insight into the transcription regulatory network. Methylation Sensitive Amplified Polymorphism (M-SAP) has been optimized to analyse genome wide DNA methylation, to throw light on the epigenetic dynamics under contrasting growth conditions.

Comparative Genomics (Work Package 3)

Objectives | To integrate genomic resources developed in different European and North-American model conifer species, and to carry out comparative studies for acquiring better information about their evolution and effective transfer of information to enable the study of other conifer species. Comparative mapping and gene content and order analyses will throw light on various aspects of conifer genomics like: chromosome and genome evolution, phylogenetic relationships and provide markers for GS (genome selection) based breeding.

Main results in the first 18 months

- Segregating populations from 5 conifer species *Pinus pinaster*, *P. sylvestris*, *Picea abies*, *P. sitchensis* and *P. glauca* have been selected for comparative mapping.
- Identification of conserved orthologous sequences (COS) is in progress, these will be used for designing genotyping tools.
- An exome capture system applicable for different *Pinaceae* species is being developed.
- For the comparative genome analysis, data generated in this project and the information already available from *Pinus taeda*, *Picea glauca* and *P. abies* is being and will be applied to identify orthologous genome regions for studying micro-synteny and micro-colinearity between *Pinus* and *Picea* species.

Computational Genomics (Work Package 4)

Objectives | To perform genome assembly and structural and functional annotation, systems biology analyses and an efficient and user-friendly data management. Structural annotation will identify protein coding genes, transposable elements and non-coding genes on the assembled genome. Information from transcriptome data will help in establishing transcriptional regulatory networks using softwares to link potential regulators and targets.

Main results in the first 18 months

- Conifer genome sequence management: strategies have been developed to perform the assembly.
- State of the art genome assemblers, available globally, have been compared, reviewed and evaluated for their applicability in tackling conifer genome size.
- Development of COS markers by integrating information generated by ProCoGen partners and publically available datasets is in progress.

Information from US, Canadian and European initiatives are highly relevant and are being integrated in ProCoGen sequence assembling and annotation.

Translational Genomics (Work Package 5)

Objectives | To identify and quantify associations between variation at the genotypic and the phenotypic levels to enable genome assisted breeding and natural resource management. Thus, translating basic scientific discoveries into applications would result in a more accurate and efficient breeding.

Main results in the first 18 months

- State of the art approaches available for statistical analyses of genotype-phenotype relationships best suited for conifers have been reviewed and compiled.
- Phenotypic and genotypic data already available, applicable to the three case studies & biological material: *Pinus pinaster*, *P. sylvestris* and *Picea sitchensis*, has also been compiled.
- Genome based simulation & modelling tools have been reviewed & data from public sources from other species has been compiled for training the models.



Pinus taeda



Picea abies



Picea sitchensis



Integration & Dissemination (Work Package 6)

Objectives | To integrate efforts in this project with similar large-scale initiatives e.g. in North America and Canada and to ensure efficient transfer of knowledge and applied outcomes of the project to other research groups as well as to organisations involved in conifer breeding and management.

Main results in the first 18 months

- The ProCoGen partners are working closely with US, Canadian and European initiatives. There has been transfer of knowhow and interactions at training workshops, meetings, lab visits and through mailings.
- The first training workshop and staff exchange programme have been accomplished. The first dissemination workshop is planned for November in Latvia, in collaboration with Dr. Daniel Rungis.
- Public relations material, including a **website (www.procogen.eu)** have been set up for use by all partners and designed to provide updated information to stakeholders. A **facebook** page has also been set up for dissemination purposes.
- The public has also shown keen interest in the project blog (**www.procogen.wordpress.com**) - as stated by the fact that it has been viewed 1080 times in over 40 countries in less than a year.

Suggested reading and references

- Barski A. & Zhao K. (2009) Genomic location analysis by ChIP-Seq. *Journal of Cellular Biochemistry*, 107: 11–18. DOI: 10.1002/jcb.22077.
- Birol I. et al. (2013) Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics*, 29(12): 1492–1497. DOI: 10.1093/bioinformatics/btt178.
- Bräutigam K. et al. (2013) Epigenetic regulation of adaptive responses of forest tree species to the environment. *Ecology and Evolution*, 3(2): 399–415. DOI: 10.1002/ece3.461.
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- Le Provost G. et al. (2013) Soil water stress affects both cuticular wax content and cuticle-related gene expression in young saplings of maritime pine (*Pinus pinaster* Ait). *Bio-MedCentral Plant Biology*, 13: 95. DOI: 10.1186/1471-2229-13-95.

Loblolly pine project: <http://pinegenome.org/pinerefseq/>

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Savolainen O. et al. (2013) Ecological genomics of local adaptation. *Nature Reviews Genetics*, 14: 807–820. DOI: 10.1038/nrg3522.

Whole Genome Sequencing: Roche 454 sequencing (<http://454.com/applications>)

Whole RNA sequencing: Illumina (<http://www.illumina.com/applications>)

Yakovlev I. A. et al. (2010) MicroRNAs the epigenetic memory and climatic adaptation in Norway spruce. *New Phytologist*, 187: 1154–1169. DOI: 10.1111/j.1469-8137.2010.03341.

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Yakovlev I. A. et al. (2012) An adaptive epigenetic memory in conifers with important implications for seed production. *Seed Science Research*, 22: 63–76. DOI: 10.1017/S0960258511000535.

Events

Training Workshop | At the venue of the first annual meeting in Umea, Sweden, a two day training workshop was held this year to mark the first hand-on-initiative of this project. A total of nine invited experts, two from the project consortium, two from Spruce Genome initiative in Sweden and five scientists from trans-Atlantic institutions shared their experiences and views on **"Genome Sequencing and Gene Discovery"** in 50 minute presentations followed by a 10 minute discussion. The workshop was attended on an average by 45-50 people. The topics of the presentations extended from sequencing, gene annotation, genome evolution, and comparative genomics to population genetics (the lectures are available on the project website).

Dissemination Workshop | The first dissemination workshop will be held in Nov. 2013 in Riga on **"Conifer sequencing: basic concepts in conifer genomics"** for transfer of knowledge and know how and to interact with the scientific community in the non participating EU and EU associated countries. The lectures will be made available on the website for download.



Photo sources page 1 (wiki commons): Bugaboo forest fire: Mark Wolfe/FEMA; Unter der Rinde: Smial; Dry earth on Sonora desert: Tomas Castelazo; Logging in Finnish Lapland: Greenpeace Finland

Photo sources page 3: *Pinus pinaster*-male cones: Meneerke Bloem; *Pinus sylvestris* var. *hamata* foliage and cones: Bulgaria; *Picea abies*: Leo Michels; *Picea sitchensis*: Gerald and Buff Corsi@California Academy of Sciences.

Photo sources page 4 (wiki commons): *Pinus sylvestris*: Jonathan Kington; *Pinus pinaster*: William Scot;

Photo sources page 5 (wiki commons): *Pinus taeda*: US NPS photo; *Picea abies*: MPF; *Picea sitchensis*: Roland Tanglao;

Photo sources page 6: *Pinus pinaster* plantation, Didier Bert, INRA, France;

Photo sources page 5: Polytent nursery_pnie breeding: Didier Bert.tif

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Publications

Brütigam K. et al. (2013) Epigenetic regulation of adaptive responses of forest tree species to the environment. *Ecology and Evolution*, 3(2): 399–415. DOI: 10.1002/ece3.461.

Canovas F. et al. (2013) Transcriptome analysis in maritime pine using laser capture microdissection and 454 pyrosequencing. *Tree Physiology* (accepted).

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Ruby M. (2012) SNP-markers paternity analysis in maritime pine progeny trial and consequences on breeding value estimates. M. Sc. thesis, University Rennes1, Rennes, France.

Vidal et al. (2013) Paternity recovery in a maritime pine polycross trial. Poster presentation at IUFRO Forest Genetic meeting, 22nd - 25th July 2013, Whistlers, British Columbia, Canada.

Yakovlev I. A. et al. (2012) An adaptive epigenetic memory in conifers with important implications for seed production. *Seed Science Research*, 22: 63–76. DOI: 10.1017/S0960258511000535.

Project Partners

- Spain:** Universidad de Alcalá (UAH)
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Universidad de Málaga (UMA)
Centro Nacional de Supercomputación (BSC)
Universidad de Valencia (UVEG)
Centre de Regulacio Genomica(CRG)
- France:** Institut National de la Recherche Agronomique Orleans (INRA)
Institut Technologique- Foretcellulose Bois-Construction Ameublement (FCBA)
- Sweden:** Umea Universitet (UMU)
Sveriges lantbruksuniversitet (SLU)
- Belgium:** Vlaams Instituut voor Biotechnologie (VIB)
- Austria:** Bundesforschungs-und Ausbildungszentrum für Wald, Naturgefahren und Landschaft (BFW)
- Finland:** Oulun Yliopisto (UOULU)
- Italy:** Consiglio Nazionale Delle Ricerche- Istituto di Genetica Vegetale (CNR-IGV)
- Portugal:** Instituto de Biologia Experimental e Tecnologica (IBET)
- UK:** The University of Edinburgh (UEDIN), Forestry Commission Research Agency (FR)
- The Netherlands:** Stichting Dienst Landbouwkundig Onderzoek (DLO)
Wageningen University & Research Centre (WU)
- Norway:** Norsk institutt for skog og landskap (NFLI)
- Canada:** Université Laval (Ulaval)

For further information
please visit:

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BLOG:
www.procoGen.wordpress.com

FACEBOOK:
<http://www.facebook.com/pages/ProCoGen/112980742191691>