

Willow and poplar breeding: From ecology to molecular genetics

One-day **SAMBA** workshop

Uppsala, Sweden, November 19, 2009
Conference hall "Loftet" ("The Loft") at the SLU, Ultuna



Preface

Sweden should be able to use more than 40 percent bio-energy by 2020, and bio-energy could be more than doubled compared to 2005. Since bio-energy is a limited resource, we must do our best to use it efficiently. *Salix* is an excellent energy crop. It grows fast and has a positive energy balance: The produced biomass yields 20 times more energy compared to the energy input, a figure which is more than 3 times higher than the energy balance of annual energy crops such as wheat and rape. *Salix* plantations are often located on less productive soils, not aimed for food production. In addition, *Salix* is also used for phytoremediation, because of the plant's good ability to take up pollutants.

Breeding of *Salix* has resulted in up to 60 percent increase in biomass yield, but there is still a great potential for further improvements. New *Salix* clones, suitable for different conditions, have to be produced if Sweden will continue to lead the production and marketing of high yielding *Salix* clones in Europe.

The SAMBA (SAlix Molecular Breeding Activities) project started in 2006. Our goal is to develop breeding tools for a rapid improvement of *Salix*. The focus is on developing molecular markers, which will provide a basis for accelerated breeding through early selection and recombination of interesting genotypes. Genetic linkage maps have been developed for studying the genetics behind resistance, tolerance and growth traits.

We have arranged this workshop to get the possibility to present and discuss our SAMBA project. Internationally recognized scientists have been invited to give presentations over our key subjects: molecular markers, the genetic regulation of growth, and the functional mechanisms involved in tolerance/resistance to biotic and abiotic stresses.

Best wishes for a fruitful Session in communicating willow and poplar knowledge effectively between different research groups spanning a broad range from ecology to molecular genetics.

Sara von Arnold & Stig Larsson (project coordinators)

Ann Christin Rönnerberg-Wästljung, Sofia Berlin Kolm & Luisa Ghelardini
(Genetic background of growth and resistance)

Martin Weih & Lorenzo Bonosi (Eco-physiology: Improved growth)

Jan Stenlid & Berit Samils (Improved resistance to rust)

Christer Björkman, Johan Stenberg & Anna Lehrman (Improved resistance to insects)

Inger Åhman (Testing and application of markers in breeding)



PROGRAMME (numbers in [brackets] indicate the page number(s) for the corresponding summary)

8.30 – 9.00: Registration

9.00 – 9.15: Welcome and overview of the SAMBA project (S. v. Arnold & S. Larsson, Lantmännen Agroenergi AB, Örebro, Sweden SLU)

Chair morning session: S. v. Arnold

9.15 – 9.45: *Populus* genetics and breeding [6-7] (C. Bastien, INRA, Orléans, France), *keynote*

9.45 – 10.00: COFFEE

10.00 – 10.30: Genetic improvement of willow as a biomass crop in the UK [8] (S. Hanley, Rothamsted Research, UK), *keynote*

10.30 – 10.45: Cloning and expression analysis of cell wall biosynthesis genes in willow [9] (L.B. Smart, Cornell University, NY, USA)

10.45 – 11.15: Key traits for improved growth in *Salix* [10] (M. Weih & L. Bonosi, SLU)

11.15 – 11.45: Poplar ecology: genes to ecosystems [11] (R. Lindroth, Univ. of Wisconsin-Madison, USA), *keynote*

11.45 – 12.15: Direct and indirect resistance against insects in *Salix* [12] (C. Björkman, A. Lehrman, J. Stenberg, SLU)

12.20 – 13.30: LUNCH

Chair afternoon session: S. Larsson

13.30 – 14.00: Quantitative and comparative genomics in *Populus* [13] (P. Ingvarsson, Umeå University, Sweden), *keynote*

14.00 – 14.30: Genetic linkage maps and identification of quantitative trait loci (QTL) in *Salix* [14] (A.C. Rönnberg-Wästljung, S. Berlin & L. Ghelardini, SLU)

14.30 – 15.00: COFFEE

15.00 – 15.30: Variability and population biology of *Melampsora* rusts on poplars [15] (P. Frey, INRA, Nancy, France), *keynote*

15.30 – 16.00: Genetic basis of and markers for resistance to leaf rust in *Salix* [16] (B. Samils & J. Stenlid, SLU)

16.00 – 16.30: Application of genetic markers in *Salix* breeding [17-18] (I. Åhman & A.C. Rönnberg-Wästljung, SLU)

16.30 – 17.00: Final discussion (*Moderator: C. Bastien*)

17.15 – 18.45: “Mingle” in the lunchroom of the Ecology building, Ulls väg 16

19.00 – 21.00: DINNER at the Ultuna restaurant (same building as the conference hall “Loftet”, lower floor)



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Poplar Breeding Strategies and Poplar-Rust Interactions (*Melampsora larici-populina*)

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Populus Genetics and Breeding lar breeding strategies: lessons from the past, recent advances and perspectives for the future

Poplar breeding programs around the world have achieved substantial increases in growth and yield potential through careful combination of intra/interspecific hybridization and clonal selection. Major challenges of future poplar breeding include (1) continuous genetic gains for recognized economic traits while addressing new breeding goals, (2) optimization of large-scale deployment of the selected genetic variation to limit economical and environmental risks associated to clonal forestry, (3) bringing genomic technologies to application in conventional poplar breeding.

Breeding strategies defined for the French Poplar Breeding Program (GIS Peuplier) and for other European programs will be analysed in the light of significant developments of the past ten years in the following areas:

- (1) Intra/interspecific hybridization: performances and heterosis level in different hybrid combinations including *Populus deltoides*, *P. trichocarpa* and *P. nigra*; respective levels of among- and within-family variations for a suite of traits of interest; relationships between intra and interspecific combining abilities.
- (2) Breeding for durable rust resistance. Durable resistance to *Melampsora larici-populina* (*Mlp*) leaf rust is a major challenge for poplar breeding in Northern Europe. When no rust epidemic was evident in the natural *Populus nigra* populations, cultivation of interspecific clones carrying qualitative resistances have strongly modified both qualitative and quantitative pathogenicity components of *Mlp* populations in cultivated areas (Pinon and Frey 2005). The mixed, sexual/asexual reproduction system of *Mlp* and the high ability of dispersion of its uredospores place *Mlp* in the highest position of Mc Donald and Lande (2002) scale of evolution risk. In monoclonal poplar plantations, durability of rust resistance will depend strongly on (1) resistance mechanisms associated in a given genotype, (2) genetic diversity present in *Mlp* populations submitted to these high selection pressures and (3) possibility of recombination rate associated with sexual reproduction on larch.
- (3) Development of marker/gene-based selection: a marker-based recurrent selection to balance genetic diversity and multitrait genetic gains, when non-pedigree association genetics needs to replace pedigree populations to detect QTL, genome sequence and development of a genome-wide selection approach for an optimal construction of elite genotypes.

The breeder's efforts will not stop at designing genetically improved trees, but need to define rational deployment strategies that do not neglect the potential impacts that improved material may have on the environment and for

Acknowledgements: All results and projects represent the collective efforts of a large team of collaborators from the genetic team at INRA-UAGPF Orléans (A. Dowkiw, V. Jorge, V. Guérin, F. Lefèvre, M. Villar, L. Sanchez), the forest pathology team at INRA-IAM (J. Pinon, B. Thoirain, P. Frey, C. Husson, S. Duplessis), the genomic team at INRA-URGV (P. Faivre-Rampant, A. Bresson), the entomology team at INRA-URZF (S. Augustin), the ecophysiology team of Orleans University-LBLGC (F. Brignolas, N. Marron, R. Monclus) and of INRA-EEF (E. Dreyer). The genetic material present in these experiments has been developed by the technical team from INRA-UE Orléans and is tested in collaboration with different partners of the French national breeding program (A. Berthelot-AFOCEL; P. Baldet and V. Bourlon-CEMAGREF; O. Forestier-Pépinère forestière de Guéméné-Penfao) Part of the activities and results received partial funding from the European Community's Sixth Framework Programme (TreeBreedeX) and the European Community's Seventh Framework Programme (NovelTree FP7-211868).

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- Dowkiw A, Bastien C. 2004. Characterization of two major genetic factors controlling quantitative resistance to *Melampsora larici-populina* leaf rust in hybrid poplars: strain-specificity, field expression, combined effects, and relationship with a defeated qualitative resistance gene. *Phytopathology* 94: 1358-1367.
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Genetic improvement of willow as a biomass crop in the UK

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In the UK, willow breeding for bioenergy end-uses is the remit of the Government-funded Biomass for Energy Genetic Improvement Network (BEGIN), which is centred at Rothamsted Research (RRes). This breeding programme builds upon a long history of willow research that goes back as far as the 1920s, at which time the UK National Willow Collection was established at Long Ashton Research Station, Somerset, UK. The collection originally comprised species and hybrids that were of importance to the basketry industry but has since been supplemented with many accessions of relevance to biomass production and several species and hybrids that were previously unrepresented. At present, the collection comprises approximately 1400 accessions representing over 100 different species. Although ongoing, molecular characterisation of this fundamental resource has already shed light on the genetic diversity present and has provided unprecedented insight into species relationships. This information is now being deployed within the breeding programme to make more intelligent use of the available germplasm and to mine for useful allelic diversity.

Over the past decade, we have used molecular markers to further understanding of the genetic basis of complex traits and to provide markers for use in marker-assisted selection (MAS). To date, most of this work has focused on the large K8 willow mapping population (947 full-sibs) which has been assessed over several harvest cycles at two contrasting UK sites. Quantitative trait loci (QTL) have now been mapped in this population for biomass yield and correlated stem traits, rust resistance, phenology, and enzyme-derived glucose yields. The genetic map of this population was also used to demonstrate macrosynteny between *Salix* and *Populus* genomes. This has greatly increased the ease with which targeted markers can be developed and has aided cloning of candidate genes from several finely mapped QTL. Advanced bioinformatic data integration tools are also being developed to improve the efficiency with which candidate genes are selected for downstream functional analysis and marker development. To identify novel QTL, and for validation of known QTL in different genetic backgrounds, 11 additional large mapping populations have now been established at RRes.

More recently, projects have been initiated that aim to provide more detailed information on the genetic, biochemical and physiological components of several key traits. As one example, the coppice response of willow is being investigated using the increasing amount of information available on the genetic and hormonal control of shoot development in *Arabidopsis*. Willow orthologues of several key genes have been cloned and are undergoing detailed characterisation in *Arabidopsis* mutant rescue experiments. Bud traits are also being characterised using both laboratory bioassays and field experiments. Other current lines of investigation within the remit of willow genetic improvement include the physiology of biomass yield, nutrient-use efficiency, the chemical ecology of pest resistance and analysis of cell walls and composition. An overview of our willow genetic improvement programme will be presented in addition to some recent scientific highlights.

Further information:

Rothamsted Research :

BEGIN :

BBSRC Sustainable Bioenergy Centre

www.rothamsted.bbsrc.ac.uk,

www.biomass4energy.org

www.bsbec.bbsrc.ac.uk

CLONING AND EXPRESSION ANALYSIS OF CELL WALL BIOSYNTHESIS GENES IN WILLOW

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Genetic improvement of fast-growing shrub willow (*Salix* spp.), a proven perennial bioenergy crop for temperate climates, will be expedited by a thorough understanding of the molecular basis for cell wall composition and by molecular tools for early selection in breeding programs. By identifying the genes that are critical for regulating variation in biomass composition, we may be able to develop molecular markers for the early selection of genotypes with varied and improved biomass properties. Using wet chemical analysis and high-resolution thermogravimetric analysis, we have characterized significant variation in biomass composition among genotypes of shrub willow produced through breeding. To gain an understanding of the molecular basis for difference in biomass composition, we are studying genes encoding enzymes involved in lignin biosynthesis and a set of carbohydrate active enzymes selected based on their functional characterization and conservation in *Populus trichocarpa* and *Arabidopsis thaliana*. Fragments of nearly 20 genes have been cloned from cDNA made from young stems of *Salix sachalinensis* and *S. miyabeana*. Expression profiling in willow stem apical tissue and developing stem tissue was performed for each isolated gene using probe-based quantitative real-time PCR. Two willow parental genotypes and six progeny within a hybrid family were selected for analysis and significant differences in expression among the individuals and between tissue types were observed for most of the genes. Patterns of gene expression were correlated with variation in the biomass composition of those genotypes to provide insights into the genetic regulation of lignocellulosic deposition in this important bioenergy crop.

Relevant literature:

Serapiglia, M.J., Cameron, K.D., Stipanovic, A.J., Smart, L.B. (2009) Analysis of biomass composition using high-resolution thermogravimetric analysis and percent bark content for the selection of shrub willow bioenergy crop varieties. *BioEnergy Research* 2:1-9.

Smart, L.B. and Cameron, K.D. (2008) Genetic improvement of willow (*Salix* spp.) as a dedicated bioenergy crop. In Vermerris, W. E. (ed.) *Genetic Improvement of Bioenergy Crops*, Springer Science, NY, 347-376.

KEY TRAITS FOR IMPROVED GROWTH IN *SALIX*

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Breeding histories of the most promising perennial crops, among them *Salix*, are short and there is a great potential for significant crop improvements. In contrast to the breeding of an annual crop, the particular challenge in breeding of a perennial crop such as *Salix* is the identification of key plant traits indicating long-term growth performance of field-grown plants.

Research on the identification of relevant key traits for improved growth is performed within a national Swedish breeding project (SAMBA, *Salix* Molecular Breeding Activities), which was started in 2007 and in which the focus is to develop breeding tools for a rapid improvement of *Salix* grown for energy purpose. Various subprojects focus on the genetic background for important growth traits, water and nutrient use efficiency and heat tolerance, improved resistance against insects and leaf rust. The final goal is to develop molecular markers and tools to implement the marker-based information into the commercial breeding process. This will provide a basis for accelerated breeding through early selection and recombination of interesting genes.

Breeding programs to improve long-term biomass yield are often relying on the results of short-term screening studies performed on juvenile plants. A pre-requisite for successful breeding of perennial energy crops is thus the identification of relevant juvenile plant traits indicating long-term plant performance under field conditions. We identified two easy-to-measure juvenile plant traits, i.e., shoot biomass and leaf N concentration to be most powerful for the evaluation and selection of willow genotypes to be grown agriculturally for biomass production. The results could be helpful to speed up especially the later screening stages of breeding programs.

We also investigated the effects of genotype and environment on spring and autumn phenology and the relationships between phenology, shoot growth and leaf nitrogen (N) retranslocation. Delayed growth cessation and leaf abscission were generally associated with greater biomass production, but especially the relationship between growth cessation and biomass was weak. The results show that the timing of bud-burst and leaf abscission are more important for willow biomass production than growth cessation. Delayed leaf abscission has a negative effect on leaf N retranslocation and increases N losses. These results have implications for the breeding of *Salix* and other perennial energy crops.

Another aspect of our research is the relationship between water availability, leaf temperature and biomass production, which we address in both greenhouse and field studies. The generated knowledge will be used to assess the genetic basis of relevant plant traits improving heat and drought tolerance, which will facilitate marker-assisted breeding towards plant material that is more suitable to a warmer and drier climate compared to the currently available material. The research is particularly relevant with respect to the potential markets of *Salix* grown for bioenergy in southern Europe.

Relevant literature:

Weih, M. (2009) Genetic and environmental variation in spring and autumn phenology of biomass willows (*Salix* spp.): Effects on shoot growth and nitrogen economy. *Tree Physiology* (in press)
doi:10.1093/treephys/tpp081.

Weih, M. and Bonosi, L. (2009) Assessment of genotype ranking in long-term biomass production of *Salix* based on juvenile plant traits: Breeding implications. *Bioenergy Research* 2: 29-36.

POPLAR ECOLOGY: GENES TO ECOSYSTEMS

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The genus *Populus* comprises high-value commercial species/hybrids as well as quintessential “foundation species” in managed and natural forest ecosystems. The evolutionary and ecological success of this genus is intimately linked to its secondary chemistry, principally salicylate phenolic glycosides and condensed tannins. These compounds influence plant growth, mediate trophic interactions, influence herbivore and soil microbial community composition, and govern ecosystem dynamics.

Poplar species exhibit striking variation in secondary chemistry, due to genetic, ontogenetic, and environmental factors, and interactions among them. Genetic variation in chemical defense is related to a strong trade-off between growth and defense, and expresses itself as differential resistance to herbivores (invertebrate and vertebrate) and pathogens among *Populus* genotypes. Multiple genotypes of *Populus tremuloides* grown for seven years in a common garden in southern Wisconsin (U.S.A.) vary in size by 6-fold, and 52% of that variation is explained by differential allocation to foliar phenolic glycosides. This tradeoff has significant implications for the selection of poplar genetic stock for production as a bioenergy feedstock.

Marked developmental changes in defense strategies have established *Populus* as a model experimental system for addressing “evo-eco-devo” questions in woody plants. Emerging patterns for *Populus tremuloides* indicate that as trees mature, salicylate-based defenses decline, tannin levels increase, and trees shift their herbivore defense strategy to tolerance rather than resistance. Tannins become important in governing litter decomposition, soil microbial activity, and nutrient cycling. Clonal reproduction of some *Populus* species, coupled with genetic differences in secondary chemistry, contribute to a geographic mosaic of community assemblage and ecosystem function at the landscape level.

Global environmental changes (e.g., enriched atmospheric CO₂ and O₃) predicted for the future will likely alter *Populus* phytochemistry and thus influence key ecological interactions. Research at the Aspen FACE site in Wisconsin, U.S.A., has demonstrated CO₂- and O₃-mediated changes in the chemistry of *P. tremuloides*, with consequences for herbivores, natural enemies, and leaf litter decomposition.

Finally, *Populus* has emerged as a major woody biofuel feedstock. Numerous efforts worldwide are investigating how to alter, via genetic engineering and conventional means, poplar lignin production. Due to pleiotropic effects, such efforts will likely also alter the expression of other phenolic-based constituents in poplar, with consequences for pest resistance, growth, and long-term sustainable production.

Relevant literature:

- Osier, T.L., and R.L. Lindroth. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. *Oecologia* 148:293-303.
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Direct and indirect resistance against insects in *Salix*

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Salix is frequently subjected to high levels of herbivore damage by insects in short rotation coppices. One can divide different mechanisms of resistance against insects into two categories: direct and indirect defence. The direct defence is pointed directly at the herbivores, reducing their reproduction and overall performance; the indirect defence aims at attracting and supporting natural enemies (bodyguards) of the herbivores, thus facilitating top down regulation by the pest's natural enemies. A sustainable resistance, resulting in a more reliable biomass production, could be achieved by integrating the knowledge about these two defences in the development of new *Salix* varieties. We have investigated several different mechanisms of direct and indirect defence, and elaborated efficient methods to measure them quantitatively.

Here we show that *Salix* clones vary significantly in both direct and indirect resistance against the most severe pest leaf beetle. A reliable measure of the two defences seems to be herbivore and bodyguard reproduction (numbers of offspring), which for each clone can be estimated over a two-week period. Herbivore reproduction correlates with offspring development time, survival, and preference; bodyguard reproduction seems to correlate with plant-odour attractivity, and offspring survival.

The strong indications of two important resistance mechanisms in *Salix* open up the possibility that these may interact in desired or undesired ways. Further research remains to be done in order to anticipate optimal/suboptimal combinations of the different defences. The potential to detect QTLs for the different defences is arguably promising, however, variations in the direct and indirect defences have so far only been found in hexaploid clones, and thus a different method that enables analyses of polyploid plants has to be used.

We conclude that the prospect to reach a lucid apprehension of the resistance in *Salix* is within reach, but that the complexity should not be underestimated.

Quantitative and comparative genomics in *Populus*

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The rapid development in genomics, like high-throughput DNA sequencing and genotyping technologies, have enabled a new generation of research in plant genetics where combined quantitative and population genetic approaches can be used to better understand the relationship between naturally occurring genotypic and phenotypic diversity. Species of the genus *Populus* are highly amenable to such studies because of the availability of the complete genome sequence from one species (*Populus trichocarpa*) and the soon-to-be ready genome sequences from an additional five species from the genus. This, in parallel with large-scale transcriptome profiling using digital expression profiling, provides a unique toolbox for population and comparative genetics and functional genomics studies. For instance, association genetics can now readily be used to dissect complex adaptive traits and discover the underlying genes in *Populus*. In parallel, re-sequencing of candidate genes and modern population genetics methods are being used to discover genes under natural selection. This combined approach is very powerful in identifying the most important genes that determine patterns of complex trait adaptation observed in natural populations of *Populus*.

GENETIC LINKAGE MAPS AND IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) IN *SALIX*

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The increasing interest in bioenergy crops has put the potential of *Salix* biomass production into focus. Breeding *Salix* varieties, adapted to different environments and resistant to pathogens, for high and sustainable biomass production is of main concern nowadays. The use of easily detectable genetic markers linked to genes underlying economically important phenotypic traits would increase the efficiency of *Salix* selection and breeding. To understand the genetic basis of phenotypic variation in *Salix*, which is needed to develop markers applicable to breeding, we have constructed two genetic linkage maps of willow and used these maps in QTL analysis.

The first more detailed map (S_1) is based on 463 individuals of a *S. viminalis* × (*S. viminalis* × *S. schwerinii*) cross using genotypic information from SNP, microsatellite and AFLP markers. The consensus S_1 map with 527 linked markers formed 19 linkage groups and was aligned to the poplar physical map. The map spanned 2.479 cM with an average interval between the markers of 5.0 cM. The second map (S_3) is based on 282 individuals of a *S. viminalis* × *S. viminalis* cross and genotypic information from SNP and microsatellite markers. The S_3 consensus map with 231 markers spanned 1.793 cM with an average interval between the markers of 7.8 cM. We compared the willow and poplar genomes by aligning the S_1 map to the poplar physical map. Generally, gene order within syntenic groups is very well conserved in the willow – poplar comparison, we however found two major interchromosomal rearrangements distinguishing the two genomes, involving poplar LG I and XVI and willow LG Ia and Ib.

For the QTL analysis we are using the S_1 and S_3 maps and phenotypic data from all individuals in the mapping populations. We are conducting field, greenhouse and lab experiments to quantify the variation in different traits as resource economy in relation to growth (water and nutrient use efficiency), tolerance to heat, resistance to rust (*Melampsora* spp.), to gall midges (*Dasineura* spp.), and phenology traits such as timing of budburst and growth cessation. All these traits are important determinants of biomass production and promising targets for the breeding of biomass willows. The individuals of the mapping populations are vegetatively propagated and used in replicates to obtain accurate estimates of the phenotypes.

Our current focus is on phenology traits that we study both in field conditions and in a phytotron, where temperature and day length can be controlled. We have identified QTL for bud burst and cessation of elongation growth. We are quantifying the effect of QTL and their stability with plant age and under different environmental conditions. Since our genetic maps are largely based on SNP markers with known position in the poplar genome, we are also able to check for presence of candidate genes in the poplar chromosomal regions corresponding to willow QTL.

Relevant literature:

Berlin, S., Lagercrantz, U., von Arnold, S., Öst, T., Rönnberg-Wästljung, A.C. High density linkage mapping and evolution of paralogs and orthologs in *Salix* and *Populus* (Submitted).

EPIDEMIOLOGY AND POPULATION GENETICS OF *MELAMPSORA* RUSTS ON POPLARS

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Poplar cultivation in Europe is highly intensive and there have been many efforts by breeders during the past decades for developing cultivars with a good level of resistance to diseases, especially to rust caused by several *Melampsora* species. However, the Eurasian poplar rust fungus, *Melampsora larici-populina*, successively broke down all the complete resistances released so far in commercial poplar cultivation, causing severe economic losses. Alternative strategies based on spatial management of available resistances and on breeding for partial resistance are on-going, but they need a good knowledge of the adaptation potential of the pathogen.

Biological characteristics, such as the dispersal distances or the relative importance of sexual vs. asexual reproduction, are often difficult to measure for such plant pathogens. A way to infer these characteristics is the use of molecular markers. Completion of the *M. larici-populina* genome sequence by the US-DoE Joint Genome Institute provided an opportunity for developing a large number of microsatellite markers, useful for population genetics studies of *M. larici-populina* at different spatial scales.

At a worldwide scale, the long distance migration capacity of *M. larici-populina* was assessed by studying eight European populations and two populations recently founded in Iceland and Canada. European populations exhibited an isolation by distance pattern, suggesting a gradual dispersal at the continental scale, whereas non-European populations appeared to result from strong founder effects with a long distance dispersal of a limited number of individuals.

At a regional scale, we focused on the spread of a rust epidemic in a natural system. Every year we monitored the epidemic of *M. larici-populina* in the Durance River valley, in the French Alps. This site is particularly well suited for the study of recurrent epidemics: the need of the alternate host plant (larch) to perform its sexual reproduction restricts the resident pathogen population upstream the valley, in a poplar-larch sympatry area. Then a clonal epidemic phase spreads downstream the valley during summer along a 200 km natural riparian stand of black poplar, *Populus nigra*. This region also includes a few cultivated stands with poplars carrying a qualitative resistance gene, thus exerting a selection pressure on pathogen populations. In this study we used epidemiology and population genetics tools to (i) sort *M. larici-populina* individuals according to their wild or cultivated origin, (ii) to describe the spread of the epidemic on the wild stands, and (iii) to assess the evolution of the genetic composition of the pathogen populations along the epidemic wave.

Relevant literature:

Barrès B., Halkett F., Dutech C., Andrieux A., Pinon J., Frey P. 2008. Genetic structure of the poplar rust fungus *Melampsora larici-populina*: evidence for isolation by distance in Europe and recent founder effects overseas. *Infection, Genetics and Evolution* 8: 577-587.

Xhaard C., Andrieux A., Halkett F., Frey P. 2009. Characterization of 41 microsatellite loci developed from the genome sequence of the poplar rust fungus, *Melampsora larici-populina*. *Conservation Genetics Resources* (in press) doi: 10.1007/s12686-009-9005-z

GENETIC BASIS OF AND MARKERS FOR RESISTANCE TO LEAF RUST IN *SALIX*

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The leaf rust fungus (*Melampsora larici-epitea*) can cause great damage in biomass willow plantations. Production losses of up to 40% have been estimated in susceptible varieties. A high rust resistance is therefore one of the most important breeding goals, and improvements of resistance has also been achieved in the breeding programs during the years. A lasting problem is that the willow leaf rust fungus, like many other rust fungi, has a high capacity to change and overcome resistance of the plant. This is especially true for the kind of resistance that is governed by a single major gene, which is common in many crops. It is therefore preferable to utilize also other types of resistances that are based on several minor genes (i.e. quantitative resistance) and hence more complicated for the fungus to adapt to. The aim of this work is to i) characterize resistance genes in the current breeding material, and ii) develop markers for a variety of resistance genes to be used in marker-assisted breeding.

To identify various resistance genes we are utilizing two linkage maps of *Salix*, one based on a hybrid backcross between *S. viminalis* and *S. schwerinii* (population I), and the other based on a pure *S. viminalis* family (population III). By doing controlled infection experiments in growth chambers we were able to characterize various resistance reactions in these families. Examples of measurements are latent period and number and size of the rust pustules. We have also done comparative assessments of rust resistance in the field on the same individuals. By doing QTL mapping (mapping of quantitative trait loci) a number of genomic regions that are important for rust resistance have been identified in the two populations. In two cases, QTLs were located close to markers for annotated rust resistance genes from the *Populus* genome. The next step will be to evaluate the resistance markers identified in the QTL mapping on unrelated willow clones of various genetic backgrounds.

Relevant literature:

Rönnerberg-Wästljung AR, Samils B, Tsarouhas V and Gullberg U (2008) Resistance to *Melampsora larici-epitea* leaf rust in *Salix*: analyses of quantitative trait loci. *Journal of Applied Genetics* 49: 321-331.

APPLICATION OF GENETIC MARKERS IN *SALIX* BREEDING

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That *Salix* has a great potential as an energy crop was discovered during the 1970ies, and research on *Salix* breeding for further improved biomass yield and resistance to pests and diseases was initiated at SLU in Uppsala. For this purpose, an extensive collection of *Salix* from northern Europe and North America was established at SLU. Commercial breeding of *Salix* started in 1987 by Svalöf Weibull AB, a program that progressed rapidly and led to the release of the first dedicated biomass varieties in the early 1990ies. Willow breeding in Sweden is currently performed by Lantmännen Agroenergi AB. At present, more than 10 bred varieties are available for use in biomass plantations. It is noteworthy that most of these varieties are species hybrids with some Russian ancestry.

Thus, domestication of *Salix* for bioenergy purposes has been possible using traditional breeding with field selections for the characteristics mentioned above, including selection for a growth form adapted for mechanised cutting production, planting and harvesting. Yields have been raised by up to 60% and the most severe biotic threat to the plantations, *Melampsora* leaf rust, has been reduced to very low levels. In some of the varieties frost tolerance is high enough for cultivation in the coastal regions of northern Sweden. A recent breeding goal is to extend the geographic range of *Salix* biomass cultivation southwards in Europe, down to the latitude of northern Portugal. For this reason selection methods for improved drought and heat tolerance are requested. Also, it will be more and more difficult to make further progress for the other desired traits without more sophisticated breeding methods.

A way forward for more efficient breeding of *Salix* is the use of marker assisted selection (MAS). MAS enables selection of breeding material by the use of easily detectable genetic markers linked to QTL or genes controlling phenotypic traits. MAS is particularly useful for traits that are difficult to measure, exhibit low heritability, and/or are expressed late in development, such as productivity, disease resistance, drought and heat tolerance. MAS is also useful in backcrossing programs for introgression of valuable alleles into varieties as well as in pyramiding of multiple genes in development of, for example, durable resistance. To develop efficient MAS in willow, we have within the SAMBA project identified QTL and genes responsible for certain phenotypic traits. For the significant QTL, we develop genotyping methods that are easy and cheap to score in breeding materials. We are currently working with a system based on a PCR-RFLP method (restriction fragment length polymorphism in PCR products), where sequence differences between alleles are located in the recognition site of restriction enzymes. One of the sequence variants causes a loss of the recognition site and a digest of the fragment leads to a different pattern of bands on an agarose gel.

The first markers expected to come out of the SAMBA project are markers for rust resistance genes. Primarily these will be used for characterisations of parents and offspring in order to track which and how many resistance alleles they carry. DNA sampling in the offspring is primarily planned to take place in the outdoor seedling nursery during July-August, after which seedlings with favourable marker combinations are kept whereas most of the others are removed. The following years the selected plants are cloned and tested in successive field trials where selections are mainly based on visual scorings and eventually biomass yield quantifications. Depending on the size of the breeding program and specific

breeding goals, DNA screens may also be used during other phases of selection, e.g. in larger sib groups in the greenhouse before planting in the nursery, and at successive selections for the advanced observation trial and the yield trial. The more diagnostic markers there are the better, since it is relatively easy to screen for several DNA markers at a time. New varieties should always be characterised for rust resistance markers and also other available DNA markers.

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