

ARBEITSBERICHT

EU-Projekt “Construction and application of a multifunctional
and saturated genetic map for coniferous species”,
- Summary -

Arbeitsbericht des Instituts für Forstgenetik und Forstpflanzenzüchtung

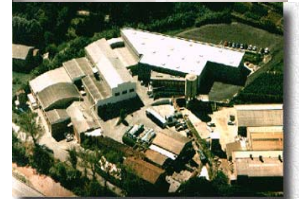
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**Bundesforschungsanstalt
für Forst- und Holzwirtschaft**

QLK5-1999-01159

**Construction and application of a
multifunctional and saturated genetic map
for coniferous species**



Final Report

Project Summary

Section 1: PROJECT IDENTIFICATION	NOT CONFIDENTIAL
Title of the project: Construction and application of a multifunctional and saturated genetic map for coniferous species	
Acronym of the project: UHD map of coniferous species	
Type of contract Cost Shared R & D	Total project cost (in euro) 1083674 €
Contract number QLK5-1999-01159	Duration (in months) 36 months + 12 months extension
	EU contribution (in euro) 899639 €
Commencement date 1 February 2000	Period covered by the progress report 1 February 2003 – 31 January 2004
PROJECT COORDINATOR	
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Key words AFLP, SSR, Linkage Map, QTL, CAP marker	
World wide web address: www.neiker.net/UHDfor	
List of participants	
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<p>P3: Université Henri Poincaré – Nancy I (UHP) (Contractor) Laboratoire de Biologie Forestière Faculté des sciences BP 239 F-54506 Vandoeuvre lès Nancy Cedex, France Responsible scientist: Prof. Dr. Jean Michel Favre Tel : 0033-3883684229 Fax :0033-383903277 E-mail: Jean-Michel.Favre@scbiol.u-nancy.fr</p>	
<p>Subcontracted Collaborator P3.1 AFOCEL Subcontracted Collaborator P3.2 INRA – Orleans</p>	
<p>(P4:) Istituto Miglioramento Genetico Piante Forestali (IMGPF) Consiglio Nazionale Delle Ricerche Via Atto Vannucci 13; 50134 Firenze, Italy, Tel : +39 055 461071, Fax : +39 055 486604 E-mail: vendramin@imgpf.fi.cnr.it <i>Participation of Partner P4 was terminated by the Commission on April 29, 2003 due to non-fulfilment of contract obligations.</i></p>	

Objectives:

- 1) To construct an ultra-high density AFLP linkage map (UHD map) of *Pinus pinaster* comprising up to 12.000 markers.
- 2) To include in this reference map the positions of published microsatellites
- 3) To exploit this UHD map to full extent for comparative genome analyses and QTL analysis.
 - 3 a) To align other linkage maps in forest species with the reference map
 - 3 b) To perform comparative genome analysis in different pine species and related gymnosperms
 - 3 c) To perform QTL analysis for important characters in different forest species
 - 3 d) To analyse variability of allele-specific markers for important QTLs in local germplasm and breeding material

Results and Milestones:

During the lifetime of the project over 400 AFLP primer combinations and nearly 300 SSR or EST primer combinations have been evaluated for segregating polymorphisms in the *Pinus pinaster* reference population descending from the cross 0024 Landes x C803 Corsica. Despite distant parental provenances, the observed number of segregating AFLP fragments was lower than expected due to a large degree of homozygosity in the parental trees. A low degree of segregating polymorphisms was observed also for SSR and EST markers which descended from different conifer and particular pine species. Moreover, not even amplification products were obtained in many cases, particular for markers descending from distant conifer species. In general, transferability between species was better for EST than for SSR markers. Therefore only a total of 2717 segregating fragments derived from 401 AFLP primer combinations (PCs) and 48 EST or SSR primer pairs were available for linkage mapping in the *Pinus pinaster* reference population. Two parental linkage maps have been constructed based on these markers. 12 linkage groups with 30 to 96 markers each were obtained for each parent. The availability of 79 anchor points based on fragments common to both parents and based on codominant SSR and EST markers allowed to determine homologous chromosomes for both maps and to construct one integrated map. Total genome length of the integrated map is around 2000 cM including 1697 markers (141 markers per linkage group). Our linkage map represents actually the map with the highest marker density in forest species.

Using a common set of 25 AFLP-PCs as well as additional PCs and numerous EST and SSR markers, linkage maps in six different conifer populations were obtained (2 populations of *Pinus radiata*, 2 populations of *P. sylvestris*, one population of *Pseudotsuga menziesii* and *Picea abies*).

The individual parental and integrated linkage maps showed a varying degree of saturation. They contained between 11 and 17 linkage groups and between 54 (*Pseudotsuga*, parental map) and over 750 markers (*Picea*, integrated map). In accordance the map lengths varied between 500 and 2000 cM.

Based on co-dominant EST and SSR markers which have been mapped in different populations comparative genome analyses were performed. Partial alignments of linkage groups of our reference population with those of other published maps have been obtained. Co-migrating AFLP markers seem not to be useful for map alignments, at least in conifer species. We have evaluated numerous SSR and EST markers in our study but the low level of polymorphism of EST and SSR markers observed in our reference population did not allow complete alignments between different linkage maps. Moreover, SSR and EST locations were not always consistent across different maps. A "majority principle" (i.e. co-locations of most of the markers) could be applied to detect correct associations. Nevertheless 9 of the 12 LGs of the reference map could be assigned to *P. taeda* linkage groups as well as to 7 *Pinus elliottii*, 7 *P. pinaster* (INRA) and 6 *Pinus radiata* (New Zealand) linkage groups. Moreover, several linkage groups of the *P. radiata* partner populations could be associated with linkage groups of the reference map and other maps.

QTL analysis for several characters related to wood quality (alpha cellulose, lignin content, pulp yield, extractives content, CIE, wood densities), growth characteristics (height, diameter, spring growth, flushing, branching) and physiological traits (vegetative and generative bud formation, flowering) were performed in reference and partner populations and QTLs were detected for most characters.

In the reference population between 3 and 8 QTLs were detected for the characters under study. Individual QTLs explained between 5 and 19% of the total variation and their cumulative effects up to 58% (diameter). In several cases QTLs were detected in both parents in equivalent homologous regions, indicating probably segregating QTL alleles in both parents. In several occasions pleiotropic effects of probably identical QTLs influencing different characters were observed (alpha cellulose, lignin and total extracts or height and diameter). This is also reflected in the partial trait correlations which were observed. Some QTLs for height and diameter measured in two different years remained stable over years.

In the partner populations QTLs were detected for height in *P. radiata*, *P. sylvestris* and *Pseudotsuga*, for diameter and spring growth in *P. radiata* and for flushing in *Pseudotsuga*. Some traits segregated as controlled by major genes and could be mapped. These include vegetative and generative bud formation and tree form in *P. radiata*, cone production in *Picea* and polycyclism in *Pseudotsuga*.

The analysis of several common characters allowed also to perform comparative QTL studies in reference, partner and other published mapping populations. The lack of crossreferenced markers hampered the comparison of QTLs. Nevertheless, certain common structures were detected for height QTLs in different conifer species.

In addition, several CAP markers for QTLs for wood quality and growth characteristics have been developed by the consortium. In several cases specific allele markers were significantly informative for height, diameter, rectitude and for lignin, cellulose or pulp contents of *P. pinaster* breeding material. Such markers can be applied conveniently for marker assisted selection of unknown breeding material in this species. These promising results will be further analysed within the frame of national projects related to forest genetics.

Benefits and Beneficiaries:

The project will provide tremendous amounts of markers which are useful for a broad range of forest species. On one hand they serve for possible isolations of genes in the future. On the other hand it provides markers for important characters which can be applied in early selection tests. This will accelerate breeding of forest species and lead to increased productivity of forests and improved quality of wood products, which in its turn will make forests more sustainable.

The UHD map will serve as a reference map for all mapping experiments of forest species in the future. In addition, the collaborative research will provide some guidelines for researchers and policy makers in the European Community for the future availability of important genes (located at QTLs) for e.g. genetic engineering.

Increased productivity of forests will increase the availability of forest products which must be processed. In its turn this will improve the employment situation in the European Community.

Increased production of quality wood will have positive impacts on the environment due to CO₂-fixation through increased photosynthetic activities.

If sufficient industrial wood products can be produced in reduced forest areas, then more space will be left to be occupied by autochthonous forests with increased biodiversity and for the maintenance of important forest genetic resources. Considering the social and cultural functions of forests this will promote the quality of life of European citizens.

Future Actions :

All tasks have been completed successfully. Project results will be exploited within other national and international R&D projects.