QLK5-CT-2000-00349

Importance of regulation mechanisms for the climatic adaptation of tree species (An example *of Picea abies*)

Annual Progress Report for the period

from 01-12-2000 to 01-12-2001

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Scientific Synthesis

1. Introduction

Norway spruce is the most widely planted conifer tree species in Europe. Adaptation of Norway spruce to extreme climatic conditions is generally believed to develop in the course of many generations due to the interaction of several evolutionary processes (mostly selection). Recent results with Norway spruce, have indicated that climatic adaptation can occur in only one generation in response to the climatic (temperature/photoperiode) conditions during the sexual reproduction.

These so called aftereffects seem to determine, besides natural selection during various ontogenetic stages, the environmental-specific performance of many adaptive traits in Norway spruce (and other conifer species). Therefore, it is urgently necessary to investigate the physiological, biochemical and molecular genetic mechanisms, underlying these aftereffects in order to discover the basis of adaptation processes of Norway spruce populations to their climatic conditions.

In order to establish the mechanisms underlying this short-term adaptation and to utilise the respective results for practical forestry purposes, several integrated investigations using the same progenies from controlled crosses in different environments are planned. Possible rapid selection processes during reproduction will be analysed by allozymes and DNA markers in female gametophytes and embryos of the different progenies. Possible gene regulations will be analysed by means of new innovative molecular genetic methods aiming at the detection of the mechanisms of potential differential gene expression in different progenies. Both main investigations will be supplemented by phenological and physiological studies on the same Norway spruce progenies.

1.1. General objectives

The main objectives are to analyse how the environment during sexual reproduction affects the climatic adaptation of Norway spruce , with respect to:

- Selection processes, acting on gametophytes during male and female meiosis (meiotic drive), pollen tube growth, megaspore degeneration, fertilisation and embryo competition.

- Environmentally induced gene expression (genomic imprinting) during the reproductive process specifies the production of particular gene products (proteins, enzymes or regulatory molecules) shaping the expression of adaptive traits in the progenies.

1.2. Specific objectives:

1. Crossing experiments with the same genotypes under different environments (climates) in a phytotron. Seedlings from these seeds will be tested to separate the impacts of photoperiod and temperature given during sexual reproduction of the mother clones on the progeny performance in adaptive traits.

2. Characterisation of cold resistance and bud-set phenology differences between full-sib families from crossings under different environments to test the hypothesis that either photoperiod or temperature or a combination of the two received by mother clones during sexual reproduction affect the progeny performance in adaptive traits, measured by terminal bud-set and cold hardiness.

3. A short term field trial to investigate how fast Norway spruce can adapt to different climatic conditions, to test how fast landraces develop and to describe the effects of early selection for cold hardiness in the autumn on traits related to the synchronisation of growth and dormancy with seasonal changes in temperature.

4. Characterisation of drought resistance differences between full-sib families from crossings under different environments to study to what extent adaptability to drought-stress conditions in *Picea abies* is influenced by the environment in which the seed is produced and matured.

5. Investigations of physiological traits of metabolic pathways, which are involved in drought and frost tolerance to characterise stress levels by determining the water potentials, electrolyte leakage and total osmolyte concentrations, to identify progenies with high antioxidative capacities and responsiveness to drought stress, provide functional molecular and physiological markers and characterisation of antioxidative protection in progenies after freezing stress and supply functional markers.

6. Investigations on the basis of alloenzyme markers. Analyses of seed samples (megagametophyte/embryo). To test the hypothesis that the very rapid adaptation to the climatic conditions during sexual reproduction is based on genetic selection processes occuring before or after the fertilisation.

7. Investigations on the basis of highly variable DNA-markers (microsatellites). Analyses of seed samples (megagametophyt/embryo). Inheritance analyses of the investigated markers.
To compare the potential of different types of molecular markers for evaluating adaptive properties of the progenies

8. Investigations on the basis of EST-markers.
Full-sib families which were produced from the same set of parents in different crossing environments will be characterized by means of stress related EST markers in order to study the existence of genetic selection and „after effects“.

9. Environmental effects on methylation of DNA and transcription and translation of phytochrome genes, cycline and other identified genes. To determine the general methylation pattern of DNA in vegetative buds, the levels of mRNA encoding different phytochromes during the process of cold acclimatisation and the localisation pattern and pattern of accumulation in vegetative buds of the two known phytochromes.

10. Differential display of newly identified expressed genes.
Identification of common genes involved in general stress response, of differentially expressed mRNAs derived from identical genotypes exposed to different stress treatments and of newly synthesized proteins involved in stress response.

11. Development of practical recommendations for seed producers, seed centers and tree nurseries. Development of practical recommendations for tree breeding, seed transfer, silviculture and gene resource conservation based on the project results. Establishment of a web site which provides the project results.

The results of this proposal will significantly contribute to our understanding of the naturally occurring adaptation processes in Norway spruce (and other tree species) and will have far-reaching consequences for all practical aspects of tree breeding, seed transfer, silviculture and gene resource conservation within the Community. Furthermore the results of this project might have a bearing on the Council Directive on the marketing of forest reproductive Material.

Synthesis of the results during the first reporting period

WP 1.1 Crossing experiments with the same genotypes under different environments in a phytotron.

Progress in the first year:

Grafts from five Norway spruce clones growing in large buckets were induced to produce female flowers. Male flowers were induced on another set of five clones, and pollen was produced from each of these clones. Grafts with female flowers were transported to a phytotron where they were placed in eight rooms.Controlled crosses were made to produce identical full-sib families. Four climatic treatments were given during fertilisation, embryo development and seed ripening, combining short and long days and low and high temperatures.

WP 1.3 How fast can Norway spruce adapt to different climatic conditions?

Progress in the first year:
A field trial was established at the experimental farm Hoxmark with seedlings from seed harvested in Norwegian spruce plantations planted with materials from Central European and local provenances.
In three field trials comprising spruce provenances, families and clones measurements were made of frost hardiness in artificial freezing tests conducted in spring and autumn. Measurements were also made of chlorophyll fluorescence and quantification of soluble carbohydrates and starch during spring, summer and autumn.
In one of the trials the effect of directional selection was tested at the age of two years on the performance of surviving plants after freezing tests in the early autumn. Only minor differences between control and surviving plants were found on frost tolerance in the autumn after 9 years in the field trial. No differences could be found in hardiness and flushing in spring and cessation of leader shoot extension in the summer, that could be related to selection at two years old. However, the plants that survived the lowest temperatures at young age were shorter than the control plants after 8 years in the field trial, which probably was due to the severe set back suffered by these plants after partial injury during the freezing tests. For the provenances included in the trial a systematic increase was found in hardiness, which was positively related to their elevation above see level. The differences were rather small, however, and far less than the variation among full-sib families in one seed orchard that covers a plantation zone of 400–500 meters above sea level.

WP 1.4 Morphological characterisation of drought resistance differences between full-sib-families from crossings under different environments.

Progress in the first year:
Seeds from 30 *Picea abies* families, produced in indoor as well as in outdoor seed orchards, were in early 2001 sown in tubular plastic containers, diameter 30 mm and length 150 mm, filled with granulated pumice. After rooting, the seedlings were randomized in six combinations of temperature and drought stress treatments: normal watering and drought treatment in 18 degrees centigrade, 21 degrees centrigrade and 24 degrees centigrade, respectively. In total, 5 400 seedlings were grown in these experimental conditions, beginning in March 2001. This program, intended to be repeated with an additional 5400 seedlings in 2002, is designed to offer genetic variance components based upon 30 seedlings per family and treatment.
The climate chambers were programmed to induce bud-set in the plants after five months and to induce a second bud-flush after an additional month of dormancy. The plants were intended to be harvested after a second bud-set, after a total of 11 months of growth in the climate chambers.

WP 1.5 Investigations of physiological traits of metabolic pathways, which are involved in drought and frost tolerance.

Progress in the first year:
Methods for the characterisation of antioxidative systems, stress responses and stress-status, have been established from March to June and adapted to Norway spruce.
1. Determination of thiol compounds via HPLC
2. Enzyme-assays (for example:guaiacol- peroxidase, superoxide dismutases.)