**Aspen as a model system.** B/SE17/14020, Umeå University, Department of Plant Physiology, 2017. Stefan Jansson. Dnr 4.6.18-14201/2017

Translation of excerpts from the application

**D. Information on the genetic modified plant**

**D1. Description of the genetic modified plant**

This application comprises up- and down regulation of 21 different genes (27 constructions). 18 of the genes affect the phenology of the aspen – budding, growth cessation, hardiness, dormancy, and senescence during the autumn. We have affected the expression in different ways; sometimes by over expression and down regulation, sometimes down regulation with either antisense or RNAi. It is about partially light receptors, partly components in the circadian clock – that steers these characteristics – partly the genes downstream of the clock, that regulates autumn phenology. These genes, how they are modified as well as which phenotypical data we have for these genes are described. In summary, it can be said that either bud setting, growth cessation etc are affected by the modifications in the greenhouse or not, despite our expectations as we created these lines. Therefore, they are being tested under natural conditions. Besides these 18 genes we include 3 constructions that down regulate the subunits in the “mediator co-activator complex”, a multi-protein complex that has affected the regulation of many genes and changes in expression of these genes can be expected to have broad pleiotropic effects, and this applies to Arabidopsis. In some of these cases, the constructions are down regulated even by another very similar gene with probably the same function. All of the lines that are to be planted outside have been cultivated at least one season in the greenhouse.

**D 2. Information on the sequences that have actually been inserted or removed:**

1. **The inserted sequences size and structure as well as the methods for describing of them, including information on the parts of the vector that have been inserted in the genetically modified higher plant and possibly on the carrier – or foreign DNA that may remain in the genetically modified higher plant.**

The different parts that are inserted in the 27 different lines are described in Annex I. Control that the transformation has actually been inserted has been made with PCR with specific primers for every construction.

1. **The size and function of removed areas.**

The changes were not meant to remove fragments.

1. **The number of copies of the inserted sequence**

Normally one, sometimes a few copies are inserted with the method. The exact number of copies for each transformation event was not decided.

1. **The single or several inserted sequences’ placement in the plants cells (integrated in the chromosome, chloroplast, mitochondria or preserved in a none-integrated form and methods for determining of this.**

The core genome (otherwise the fragment would not be functional.

**D 3. Information on the inserted sequences developmental expressions during the plant’s life cycle and methods for describing this as well as the parts of the plants where the inserted sequences are expressed.** Describe the promoter and its usual function.

The 35S promoter has earlier been considered to drive the gene expression in all tissues. Even it has been shown to be a truth with modifications, it is easiest from a risk assessment point of view to assume that the potential of the genes can be expressed in all of the plant parts.

**D 4. Information on how the genetically modified plant is different from the receiving (parent) plant.**

1. **Way of reproduction or rate of reproduction.**

None-modified aspens do not bloom in greenhouse conditions, so we cannot often attain this data. Many of the aspens genes, including those we describe here, can of course affect time for flowering, development of flower, seed development, etc., so up- or down regulation of these can affect these parameters. From a risk assessment viewpoint, we assume therefore that reproduction capability can both increase or decrease.

1. **Spread**

There is no data and we really wish that we were able to study this because it would have great value for basic research and even for risk research and future risk assessments of field trials with genetical modified trees. Unfortunately, we assess that today we cannot obtain permission to let the trees bloom in the field trial, so this cannot be studied.

1. **Ability to survive**

See the reasoning under D1. As with all forms of plant breeding with or without genetic modification, an overwhelming majority of changes lead to reduced ability to survive in natural conditions. Despite this, it can of course not be excluded, and it is our hope at some of our constructions should be able to give faster growth under natural conditions. It cannot be excluded that they could give a better ability to spread and even increased fitness under natural conditions, even if the probability of this is low according to argumentation above. From a risk assessment viewpoint, we assume that survival and fitness can be affected both positively as well as negatively.

**D 5. The stability of the inserted sequences and genetically modified higher plant’s phenotypical stability.**

These trees are primary transformants, that after transformation are kept sterile in stock cultures and reproduced as sticklings before they are planted in the greenhouse. For the majority of the constructions, the greenhouse trials have been repeated in this way, sometimes with long times between the growing times to confirm a stabile phenotypical effect. We would guess that even the genotyped that have only been tested once are aso stabile, but we of course, do not know.

**D 6. Possible changes in the genetically modified higher plant’s ability to transfer genetic material to other organisms.**

There are no other wild species in Sweden that can cross with aspen, but a few cultured hybrids. The is no reason to believe that the ability to cross with these hybrids or their fertility, should change more than is the cross would occur with other aspens, but we must perhaps still assume that one could imagine that their ability could either increase or decrease.

**D 7. Information on toxic, allergy causing or other harmful effects on human health or environment, which may arise as a result of the genetic modification.**

Aspen leaves are not good but are not either poisonous for humans. There are documented cases in which a researcher of wild animals that handled leaves from aspen and other kinds of trees were affected with contact eczema. An element that is found in aspen bark and in other species of trees was the cause of this (Aalto-Korte K, Valimaa J, Henriks-Eckerman ML, et al. Allergic contact dermatitis from salicyl alcohol and salicylaldehyde in aspen bark (*Populus tremula*). Contact Dermatitis 2005;52(2):93-95). More information is found for instance on <http://www.akins.com/ns/DisplayMonograph.asp?storeID=A59A6B1C10E44C9E9420A7A75B27460A&DocID=populus>. The substances that this concerns, salicyl alcohol and salicylaldehyde are found in high amounts in aspen because salicylates are the most common and important defense substances in aspen. Aspen leaves can contain up to 20 % dry weight of phenolic glucosides, that are salicylate – derivatives. We are carrying out a large research project, finances of SSF, where one of the goals is to understand just what genes steer the formation of the different salicylate-derivatives in aspen. Because these are found naturally in so great amounts in aspen, it is very improbable that – even if the “critical gene” that regulates their building is up- or down regulation – that is could affect the allergenicity significantly because “the baseline is so high”. This is not about moving a gene from one species with allergenic characteristics to aspen, but about modifying the expression of its own genes.

**D 8. Information on the genetically modified higher plant’s safety regarding animal health, in those cases a genetically modified higher plant is mean to be used as animal fodder.**

Not relevant

**D 9. The course of events for interaction between the genetically modified plant and the target organisms ( i relevant cases).**

Are all natural herbivores and pathogens of aspen considered target organisms or not? Most of the modifications will not have as a target to change biotic interactions and if there is no target, there are no target organisms – but it is a part of the purpose of the trial to study if the presence of herbivores and pathogens is changed. In most cases this will probably not be affected but if there will be an effect, we will hopefully be able to register this. In certain cases, up and down regulation of certain genes will lead to increased or decreased sensitivity to certain herbivores. The interplay here is complex, because we are leading one of the world´s largest research projects on insect herbivory on aspen, we know that there are great differences in the preferences of insects for different aspens, but the pattern is generally enormously shifting. There are absolutely no aspens that “nothing” eats or that “all” eat. It is probable that different insect species reacts differently to different defensive substances and that it is an evolutionary interplay where variations in the local insect fauna can give different selection pressure. It is probably adaptive for an individual aspen to have a different genetic composition and therefore chemistry than its “neighbors” because the insects probably develop a local adaptation for the “chemotypes” of aspen that are most common in a locality. This is in opposition to the genes that steer, for example phenology, where the selection pressure leads to decreased variation in characteristics between aspen individuals.

**D 10. Potential changes in the genetically modified plant’s interaction with none-target organisms as a result of the genetic modification.**

See D9 above.

**D 11. Potential changed interaction with the abiotic environment**.

Because growth can be altered, it is possible that for example nutrition uptake may be changed.

**D 12. Description of the methods for detection and identification of the genetically modified plant.**

We routinely use PCR on leaf DNA to detect the different plants.

**D 13. Information on earlier exposure to the environment of the genetically modified plant.**

This is an extension of a previous field trial, so they are already in the environment.

F. Information on the exposure to the environment

**F 1. The purpose of the field trial**

The purpose of the trial is to understand the phenology if the aspens, especially in the autumn. Most of the lines we planted in 2013 have been produced because we believe that the genes in question affect autumn phenology. In addition to that we want to even include lined where the gene expression of subunits in the mediator complex are affected, that will besides the effect on phenology also may have other pleiotropic effects.

The purpose with all of the trials is in a broad context to understand the functions of all of aspen’s genes. Aspen is the most developed model system for basic research of tress and Umeå Plant Science Centre is world leading in the areas. So the purpose in a broader perspective is to make it possible to understand the function of all of the tree’s genes and therefore make it possible for the world’s tree researchers to be able to provide the world with forest raw products in a sustainable and environmentally friendly way, for energy production and to use wood as raw products for building materials, paper, composite material and a number of other uses. To develop aspen as a model system is thus important for the world’s future use of forest raw products. Both through traditional forest tree breeding, where marker can thus be chosen from genes that steer important characteristics and in a longer perspective make it possible make it possible for transgenic trees to be planted for bioenergy production.

H. Risk assessment

We will here assess the risks from a ”worst case scenario” that is to say, what is the worst that could happen if these trees or their offspring began to freely spread in nature.

We consider that the DNA fragment – the selection markers – that are inserted from the cloning vector do not influence the plants characteristics under natural conditions where the tree is not affected by large amounts of antibiotics, so there is no increased risk for human health or the environment. There is not either a risk for transmission of the selection markers to for example, humans or tame cattle (livestock).

The genes that we have altered are the aspens own genes. We have sequenced the genome of the aspen. Our main result is that the genetic variation between aspen individuals is enormous. It seems as if the two parents to a randomly chosen aspen individual is as differently genetically as a human and a chimpanzee, and that there are a large number of genes that are only found in certain aspen individuals and not in others. Our aspen can be the organism – all categories that until now has been genome sequenced where the “haplotypes” differ most from each other – an astonishing result. There are more than 5 billion aspens in Sweden. These flower far from every year, but if only 0.1 % of them do, there would be 5 million flowering aspens per year. A flowering female aspen can spread up to 4 million seeds per year, if we again count low, perhaps the average would be around 1 % of these, 40, 000 seeds/aspen, probably these have been pollinated by thousands of different male aspens. Each of these approximately 200 billion aspen seeds that are spread over Sweden every year, have a unique genetic composition, where the different alleles/haplotypes that are in the population are randomly mixed. In our project where we study the natural variation in the Swedish aspen population, the most significant result is that we can carry out “association mapping” with higher resolution than in any other plant or animal species that has been studied up to now because there is such a large variation between individuals and that the alleles are mixed so randomly because of aspens pollination biology. Our data indicates that aspen can be the species – all categories – that until now have been studied that best corresponds to what is called “the ideal population”, that is to say that is infinitely large and where all individuals have the same probability to cross with each other. It is therefore probable that every year large amounts of “wild” aspen seeds that contain alleles that give a powerfully changed expression of most – perhaps all – genes are spread. If we assume that one gene of 10 000 in a haplotype have a mutation in the promoter region that makes that it is not expressed – probably an underestimate based on what is known about genetic variation of gene expression levels, the number of lethal mutations in populations etc. – one aspen seed in 100 million that arise with random crossing of random aspens in the forest would entirely lack expression of a random gene (as compared with the hundreds of billion aspen seeds that are produced every year). In other words, all of the up- and down regulations that this application contains would spontaneously arise in nature, although exposed of course, to natural selection and a overwhelming majority would not survive.

Despite this, we must in the risk assessment that was presented above (DI) for safety’s sake assume that among the gene constructions in this application, are those that lead to the tress survives, grows, survives better against insect attacks/infestations and more, better than worse, and that these because of a somewhat undefined genetic mechanism do not arise naturally. Should the end result of free spread of these aspens or their offspring, be aspens that survive worse than “wild” aspens, these will of course be selected away, in the same way as all aspen seeds with “worse genetic makeup” are. Therefore, there is no risk that these will spread.

What is interesting for the risk assessment is that the aspens that should have better growth, resistance to biotic or abiotic stress or ability to spread. The goal for Swedish forest improvement by breeding is (according to Skogsforsk) to “supply Swedish forestry with seeds and plants of high quality, vitality, production and timber quality as well as to create preparedness for future climate- and environmental changes. The Swedish Parliament has decided on four goals for forestry’s environmental goals:

* Long term protection of valuable forest land
* Strengthen biological diversity
* Protection of cultural environments
* Action plans for threatened species

Aspen is probably the most important variety of tree for biological diversity and threatened species in Swedish forests; to preserve deciduous trees and in particular aspen is an important measure in the environmentally adapted forestry. What would be the consequences if aspens with increases growth slowly spread in our forest as a result of this field trial? If they really grew better under “normal” forestry conditions, that is to say without fertilizing, herbicides and pesticides etc. and did this as well in “future climate and environmental changes”, would this with time lead to a greater amount of aspen in our forest and that forestry would take advantage of this by adapting timber extraction and use of aspen wood. Thus Sweden would without active efforts of forest refinement, attain forests that both grow better with a greater amount of aspen and therefore are better for diversity and threatened species. The probability that this will happen is low because the probability that a line that is in this application should have an increased “Darwin fitness” is low. But if a “worst case scenario” of the spread of trees, seeds or pollen from this trial would be that production goals and environmental goals in Swedish forestry would easier and without costs and risks for human health, be achieved, we can as a matter of fact not see any risks with the field trial. Therefore, no measures to prevent the spread are needed.