

PLANTS AND METHODS OF MODIFICATION THEREOF

This invention relates to new plants and methods of modification thereof.

This invention has particular but not exclusive application to forestry and increasing the productive capacity of trees, and for illustrative purposes reference will be made to such application. However, it is to be understood that this invention could be used in other applications, such as the modification of other plants such as certain leafy food crops to increase the production of useful parts thereof.

It is well recognized that the useful structures of domestic and commercial plants are in competition in the plant with other non-essential structures for growth resources such as water, nutrients and photosynthesis products. For example, the development of reproductive structures on forest trees represents a significant burden on the resources of the trees, the reproductive effort occurring at the expense of vegetative growth. The extent of the effect of reproductive burden on vegetative growth may be estimated by measuring the proportion of photo-assimilate which is directed to flowers and fruits, or strobili and cone growth for gymnosperms. In conifers, reproductive effort is variously estimated to be up to 16% of annual photosynthate.

The formation of reproductive structures imposes a burden on the tree beyond that of carbon allocation. First, these structures have very large requirements for important nutrients at the expense of vegetative tissues so that, at least under conditions of nutrient deficiency, prevention of reproductive structure formations as proposed can lead to a greater increment in vegetative growth than the corresponding mass of reproductive structure otherwise formed.

Nutrient losses from pollen dispersal are not inconsiderable in most forest plantations, with an estimated annual production of 370 kg/ha of nutrient-rich pollen by Pinus radiata plantations. Tree crop productivity of P. radiata is as yet only about 50% of that theoretically obtainable on the basis of incident solar radiation. Therefore, in many environments, there are good prospects for

significant increase in harvest yield from trees which have been inhibited from producing reproductive structures.

It is noted that there is a deleterious effect in at least P. radiata on the value of timber caused by persistent stem cones, and research has been advocated for methods for chemically inducing cone abscission in species such as P. radiata. However, it is clear that means involving chemical treatment of forest trees will involve considerable expense in manufacture and application, this cost being exacerbated in certain circumstances where environmental factors both preclude indiscriminate broadcast of such chemicals and insist on strict control of run off.

Seeds from mature trees will produce an undergrowth of saplings which must be removed periodically to prevent competition with the tree crop for soil resources and to minimize the risk of damaging fire. This sapling removal is labour intensive, time consuming and accordingly expensive, adding considerably to the production cost of farmed forest timber.

The present invention aims to alleviate the above disadvantages and to provide plants of enhanced productive capacity and methods for the production thereof which will be reliable and efficient in use. Other objects and advantages of this invention will hereinafter become apparent.

With the foregoing and other objects in view, this invention in one aspect, resides broadly in a method of enhancing vegetative growth in a plant including the steps of:-

identifying and isolating a gene specifically expressed in the production, maintenance and/or growth of a non-essential plant structure;

modifying the isolated gene;

transforming plant cells with the modified gene such that expression of said non-essential structure is incomplete or absent in successful transformants, and

selecting and vegetatively propagating the successful transformants.

The non essential structure may be selected from any plant structure which is not essential for the survival





of the plant and being one which does not contribute to the production of commercially useful plant material. Preferably, the non essential structure is selected from the reproductive structures of plants which are economically capable of artificial vegetative propagation. Preferably, the plant selected is a tree for timber, pulp or fibre production wherein poor or absent expression of reproductive structures may result in increase in vegetative growth of the valuable material.

A large number of genes are differentially expressed between sexual and vegetative buds. Accordingly, it is preferred that the gene be selected from those specific to sexual budding, specific to the production of other sexual structures, or specifically coding for a product essential in a developmental pathway for a reproductive structure. The gene is preferably selected for its early expression, specifically in the developing reproductive tissues.

Preferably, the gene is identified in cDNA libraries prepared from mRNA isolated from reproductive tissues and selected by differential screening against mRNA of vegetative structures. Since the biochemical pathways for vegetative buds and developing reproductive structures may include common expression products and consequently similar mRNAs, it is preferred to differentially screen cDNA libraries against vegetative bud mRNA, with or without preceding enrichment of the cDNA for genes specifically expressed in reproductive tissues. The selected gene may be any which when its expression is blocked or otherwise made ineffectual, results in the failure to produce a non essential plant structure.

Alternatively, the cDNAs may be utilized as probes to select corresponding genomic clones from genomic libraries. The genomic clones may be used to isolate and identify gene promoters that specifically express genes unique to reproductive structures. Such promoters can then be combined with lethal genes which when expressed will inhibit or terminate growth of the cells within which the lethal gene is expressed. The promoter-gene fusions when stably incorporated into a plant, by any suitable known

means, will result in failure to develop reproductive tissues in whole or in part.

Candidate cDNA clones may be raised and are preferably selected for the presence of genes expressed in both male and female buds. The selection may be directed to those genes which are naturally produced or those which are induced by plant growth regulators which promote flowering, such as various gibberellins. Preferably, the screening is confirmed using an in situ RNA hybridization procedure with sexual buds.

The preferred cDNA clones are those further selected for the characteristics of early appearance and highly specific expression.

The modification of the identified gene may be by fusing the tissue-specific promoter of said gene with a structural gene for a deleterious or lethal product such that regenerated plants transformed with said gene-fusion will not form said non-essential structure. Alternatively, a critical function of said gene may be disrupted or modified by expression of the modified gene in transformed plants.

The modified gene is preferably introduced into a plant normally containing the identified gene such that a critical function of the identified gene is disrupted or modified. However, it is also envisaged that introduction of the modified gene into plants not containing the specifically identified gene may result in useful reduction or elimination of a non-essential plant structure, particularly where the gene selected has analogues represented across several species, or in closely related species where the corresponding gene is essentially homologous with the gene in question.

Preferably the plant is modified to increase the useful growth thereof.

Modification of the gene may be achieved by any suitable means, with the expression strategy desired being the primary arbiter of the modification process utilized. For example, it may be intended to constitutively express an antisense or perhaps ribozyme version of a gene which is critical to development of reproductive structures, so that



the normal gene action is disrupted and vegetative development occurs instead. The method may be to splice a promoter specific to a reproductive structure with a lethal gene which codes for an expression product which will cause abortion of the tissue in which it is produced, for example, buds that differentiate as reproductive structures.

Genes expressing antisense RNA against the mRNA coded by each of the selected target genes may be constructed. These and shorter RNA sequences that bind to the translation initiation region of the target mRNA may be used for inhibition of translation. Of course, other critical regions apart from the translation initiation site may be targeted for binding of antisense RNA.

Accordingly, in a further aspect this invention resides broadly in a modified plant wherein a gene specific for a translation product essential for the production, growth or maintenance of a non essential structure has been modified whereby said non essential structure is poorly or not expressed.

In a yet further aspect this invention resides broadly in a modified plant wherein said plant is provided with a gene expressing lethally under the control of a gene promoter specific to a non essential structure whereby said non essential structure is poorly or not expressed.

For embodiments of the present invention where abortion of the reproductive structure is desired, a genomic library may be screened with the selected cDNAs. The promoter sequence may be identified and characterized using methods such as S1 nuclease mapping and sequencing of the region upstream of the transcription initiation site. Of course, other methods of promoter identification are envisaged for application to the methods of the present invention. The promoter may then be spliced to a lethal gene. One option is to use an antisense or ribozyme version of a critical house-keeping gene, such as the actin gene or a gene coding for an enzyme of aromatic amino acid biosynthesis, for example enolpyruvyl shikimate phosphate synthase. Alternatively, a deleterious enzyme such as a protease, ribonuclease, or deoxyribonuclease may be encoded



for biosynthesis under control of a sexual promoter or any promoter specifically expressed in a non essential structure.

The invention will be further described with reference to a preferred embodiment thereof, pertaining particularly to a method of modification of Pinus radiata.

EXAMPLE 1.

1. Identification and isolation of an early expressing, reproductive-structure specific genes.

A cDNA library was prepared from mRNA isolated from reproductive buds of P. radiata in the vector gt10. Colonies containing inserts were then screened using labelled cDNA from the mRNA prepared from vegetative buds, and non-hybridising colonies were selected as candidates.

Candidate clones were then selected for the presence of genes expressed in both male and female buds. In the present case, genes were selected which expressed on induction by the plant growth regulator which promotes flowering in this species, gibberellin A4/7. The screening was confirmed using, with sexual buds, a known in situ RNA hybridization procedure. It was determined that one clone selected contained a cDNA corresponding to a gene having the characteristic of early appearance and highly specific expression in male and female reproductive bud development.

This CDNA sequence was used as a probe to further screen a genomic library to obtain the corresponding genomic DNA sequence from which the tissue specific promoter of the gene was identified and characterised.

2. Modification of the gene

The gene was modified by fusing a portion of the gene including the tissue-specific promoter of the gene with a structural gene for a ribonuclease, and with other control and marker sequences. Candidate gene constructs were used to transform Pinus radiata tissues by microprojectile bombardment of shoot-forming tissues. Explants from successful transformants were screened for expression of the ribonuclease gene upon induction by gibberellin A4/7. Positively responding transformants were vegetatively propagated to a stage suitable for transplantation.

Modified trees in accordance with the present



invention will avoid growth limitation by the diversion of plant resources into reproductive structures. Thus, where tree growth is nutrient or water limited, modified trees may in fact exhibit a growth increase above that expected on the basis of carbon reallocation alone. The commensurate increase in leaf index will lead to increased light harvesting and hence some compounding of the increase in standing biomass over time.

Genetically modified trees will obviously not shed pollen or produce seed, thereby providing an inherent mechanism for containment of the valuable gene itself. This will provide commercial protection and will allay public concerns about environmental biohazards. The availability of such a gene containment method may well generate a requirement that trees genetically modified in other ways should also employ this or some other strategy to prevent environmental release. It is also of potential significance that the inherent genetic protection should assist greater commercial exploitation of elite germplasm that has been obtained at significant expense over a long time period through classical breeding programs.

The prevention of pollen dispersal beyond the confines of plantations will be of benefit where there is public disapproval of visible deposition, or of perceived allergic responses.

It will of course be realised that while the above has been given by way of illustrative example of this invention, all such and other modifications and variations thereto as would be apparent to persons skilled in the art are deemed to fall within the broad scope and ambit of this invention as defined in the following claims.

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CLAIMS

1. A method of enhancing vegetative growth in a plant including the steps of:-

identifying and isolating a gene specifically expressed in the production, maintenance and/or growth of a non-essential plant structure;

modifying the isolated gene;

transforming plant cells with the modified gene such that expression of said non-essential structure is incomplete or absent in successful transformants, and

selecting and vegetatively propagating the successful transformants.

2. A method in accordance with Claim 1, wherein said non essential structure is selected from the reproductive structures of the plant.

3. A method in accordance with Claim 2, wherein said plant is Pinus radiata.

4. A method in accordance with Claim 3, wherein said gene is selected from those specific to sexual budding and exhibiting the characteristics of early appearance and highly specific expression.

5. A method in accordance with any one of the preceding Claims, wherein candidate said genes are identified in cDNA libraries prepared from mRNA isolated from reproductive tissues and selected by differential screening against mRNA of vegetative structures.

6. A method in accordance with Claim 5, wherein candidate cDNAs are selected for the presence of genes expressed in both male and female buds.

7. A method in accordance with Claim 6, wherein the selected cDNAs containing genes expressed in both male and female buds are further screened to select those containing genes the expression of which is induced by plant growth

regulators which promote sexual budding.



8. A method in accordance with any one of the preceding Claims, wherein said modification of the identified gene comprises fusing a tissue-specific promoter of said gene with a structural gene for a deleterious or lethal product such that plants transformed with said gene fusion will not form said non-essential structure. -

9. A method in accordance with Claim 8, wherein said promoter is fused with a lethal gene which codes for an expression product which will cause abortion of buds that differentiate as reproductive structures. -

10. A method substantially as hereinbefore defined, with reference to the Example.

11. A modified plant wherein a gene specific for a translation product essential for the production, growth or maintenance of a non essential structure has been modified whereby said non essential structure is poorly produced or absent.

12. A modified plant wherein said plant is provided with a gene expressing lethally under the control of a gene promoter specific to a non essential structure whereby said non essential structure is poorly produced or absent.

13. Pinus radiata modified in accordance with the method of any one of Claims 1 to 10.

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