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(74) Agents: PABST, Patrea, L. et al.; PABST PATENT GROUP LLP, Suite 1200, 1201 Peachtree Street, Atlanta, Georgia 30361 (US).

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(71) Applicant (for all designated States except US): METABOLIX, INC. [US/US]; 21 Erie Street, Cambridge, Massachusetts 02139 (US).

(72) Inventors; and

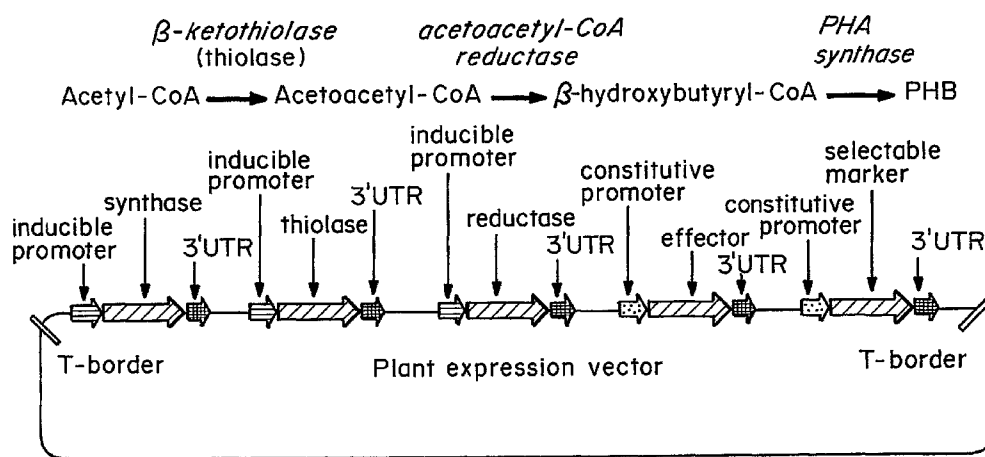
(75) Inventors/Applicants (for US only): KOURTZ, Laura-lynn [US/US]; 7 Colonial Village, #10, Arlington, Massachusetts 02476 (US). PEOPLES, Oliver, P. [GB/US]; 27 Radcliffe Road, Arlington, Massachusetts (US). SNELL, Kristi, D. [US/US]; 15 Gorham Road, Belmont, Massachusetts 02478 (US).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

[Continued on next page]

(54) Title: CHEMICALLY INDUCIBLE EXPRESSION OF BIOSYNTHETIC PATHWAYS



(57) Abstract: Methods and constructs for the introduction of multiple genes encoding enzymes in a multi-enzyme biosynthetic pathway are provided. In one embodiment, the constructs contain two or more enzyme-encoding genes, each under the control of an inducible promoter and each with a polyadenylation signal. The constructs are used to produce transgenic plants, in which the expression of the enzymes are increased when a chemical inducing agent is applied, and a biosynthetic product of the series of enzymes encoded by the transgenes is produced. Constructs may be used which contain two or more enzyme-encoding genes under the control of one or more promoters activated by activator molecules or complexes expressed from a transgene or transgenes, which are themselves under the control of one or more inducible promoters and switched on following the external application of a chemical. The transgene or transgenes expressing the activator molecules or complexes may be included in the same construct containing multiple genes encoding enzymes in a multi-enzyme biosynthetic pathway. Alternatively, the transgene or transgenes expressing the activator molecules or complexes may be on a different construct from the construct containing multiple genes encoding enzymes in a multi-enzyme biosynthetic pathway. The activator molecule can be expressed using a constitutive promoter in an inactive form which is converted to the active form following application of the chemical inducing agent.



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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER INV. C12N15/82 C12P7/62 A01H5/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C12N C12P A01H		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS, EMBASE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUGHES E H ET AL: "Metabolic engineering of the indole pathway in Catharanthus roseus hairy roots and increased accumulation of tryptamine and serpentine" METABOLIC ENGINEERING, ACADEMIC PRESS,, US, vol. 6, no. 4, October 2004 (2004-10), pages 268-276, XP004607566 ISSN: 1096-7176 page 269, right-hand column page 271	1,2,4, 19,20, 22,37, 38,40
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents :		
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed		*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family
Date of the actual completion of the international search	Date of mailing of the international search report	
24 October 2006	09/11/2006	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Bucka, Alexander	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2006/009531

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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	<p>-----</p> <p>WO 2004/071467 A2 (DU PONT [US]; KINNEY ANTHONY J [US]; CAHOON EDGAR B [US]; DAMUDE HOWAR) 26 August 2004 (2004-08-26) the whole document</p>	1-54
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No.
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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-54, all partially and as far as applicable

A recombinant vector for the expression of enzymes in a biosynthetic pathway comprising two or more elements, wherein each element comprises an inducible promoter that directs transcription of a nucleic acid sequence encoding a protein; or
a recombinant vector for the expression of enzymes in a biosynthetic pathway comprising a promoter activated by an activator molecule or complex that directs transcription of two or more nucleic acid sequence each encoding a protein;
cells comprising these vectors;
uses thereof;
wherein the two or more elements contain nucleic acid sequences encoding proteins selected from the group of enzymes involved in the synthesis of polyhydroxyalkanoates (PHA)

2. claims: 1-54, all partially and as far as applicable

A recombinant vector for the expression of enzymes in a biosynthetic pathway comprising two or more elements, wherein each element comprises an inducible promoter that directs transcription of a nucleic acid sequence encoding a protein; or
a recombinant vector for the expression of enzymes in a biosynthetic pathway comprising a promoter activated by an activator molecule or complex that directs transcription of two or more nucleic acid sequence each encoding a protein;
cells comprising these vectors;
uses thereof;
wherein the two or more elements contain nucleic acid sequences encoding proteins selected from the group of enzymes involved in the synthesis of fatty acids and/or oils

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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