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United States Patent [19][11] **Patent Number:** **6,040,498****Stomp et al.**[45] **Date of Patent:** **Mar. 21, 2000**[54] **GENETICALLY ENGINEERED DUCKWEED**[75] Inventors: **Anne-Marie Stomp; Nirmala Rajbhandari**, both of Raleigh, N.C.[73] Assignee: **North Carolina State University**, Raleigh, N.C.[21] Appl. No.: **09/132,536**[22] Filed: **Aug. 11, 1998****Related U.S. Application Data**

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[51] **Int. Cl.**⁷ **C12N 5/04**; C12N 15/82; C12N 15/84; A01H 1/00[52] **U.S. Cl.** **800/294**; 435/419; 435/469; 435/69.4; 435/69.51; 435/69.6; 435/70.21; 800/295; 800/300[58] **Field of Search** 435/69.1, 320.1, 435/410, 413, 418, 419, 430, 430.1, 469, 69.4, 69.51, 69.6, 70.21; 536/23.6, 23.51, 23.52; 800/278, 288, 294, 295, 298, 300[56] **References Cited****U.S. PATENT DOCUMENTS**

4,693,976	9/1987	Schilperoort et al.	435/469
4,956,282	9/1990	Goodman et al.	435/69.51
5,102,796	4/1992	Hall et al.	435/468
5,149,645	9/1992	Hoekema et al.	435/468
5,501,967	3/1996	Offringa et al.	435/468
5,504,200	4/1996	Hall et al.	536/24.1
5,550,038	8/1996	Goodman et al.	435/70.1
5,550,318	8/1996	Adams et al.	800/278
5,591,605	1/1997	Hall et al.	435/70.1
5,591,616	1/1997	Hiei et al.	435/468
5,629,175	5/1997	Goodman et al.	435/69.1
5,641,664	6/1997	D'Halluin et al.	435/468
5,650,307	7/1997	Sijmons et al.	435/468
5,712,135	1/1998	D'Halluin et al.	435/468
5,716,802	2/1998	Sijmons et al.	435/69.1
5,723,755	3/1998	Fortin	800/278
5,731,719	3/1998	Komari et al.	435/468
5,792,935	8/1998	Arntzen et al.	800/294

FOREIGN PATENT DOCUMENTS

WO 86/03776	7/1986	WIPO	C12N 15/00
WO 95/06722	3/1995	WIPO	C12N 15/00
WO 98/37212	8/1998	WIPO	C12N 15/82

OTHER PUBLICATIONSViyayachandra et al, *Plant Mol.Biol.*, vol. 29, pp. 125–133, 1995.Hansen et al, *Proc. Natl. Acad. Sci., USA*, vol. 91, pp. 7603–7607, 1994.Chang et al, *Z. Pflanzenphysiol.*, vol. 89, pp. 91–94, 1978.Hei et al, *Plant J.*, vol. 6, pp. 271–282, 1994.Sabelli et al, *Meth. Plant Biochem.*, vol. 10, pp. 79–100, 1993.Flavell, R.B., *Proc. Natl. Acad. Sci., USA*, vol. 91, pp. 3490–3496, 1994.Gray et al, *Proc. Natl. Acad. Sci., USA*, vol. 80, pp. 5842–5846, 1983.Ma et al, *Science*, vol. 268, pp. 716–719, 1995.Hillman, W.S. and Culley, Jr., D.D.; *The Uses of Duckweed*, *American Scientist*, vol. 66, pp. 442–451.

PCT International Search Report, Oct. 30, 1998, PCT/US98/16683.

Smith, R.H. and Hood, EE; *Agrobacterium tumefaciens* Transformation of Monocotyledons, *Crop Science* 35:301–309 (1995).Schäfer, W. et al.; T-DNA integration and expression in a monocot crop plant after induction of *Agrobacterium*, *Nature*, vol. 327, Jun. 11, 1987, pp. 529–532.Boulton, M.I. et al.; Specificity of *Agrobacterium*-mediated delivery of maize streak virus DNA to members of the Gramineae, *Plant Molecular Biology* 12: 31–40 (1989).Jach, G et al.; Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco, *Plant Journal* (1995) 8(1), 97–109.Komari, T. et al.; Vectors carrying two separate T-DNAs for co-transformation of higher plants mediated by *Agrobacterium tumefaciens* and segregation of transformants free from selection markers, *The Plant Journal* (1996) 10(1), 165–174.Jones, J.T. et al.; Isolation and characterization of a putative collagen gene from the potato cyst nematode *Globodera pallida*, *Parasitology*, 1996, vol. 113, pp. 581–588.Hoever, M. et al.; Overexpression of wild-type p53 interferes with normal development in *Zenopus laevis* embryos, *Oncogene* (1994), 9, 109–120.Bates, G.W.; Electroporation of Plant Protoplasts and Tissues, *Methods in Cell Biology*, vol. 50, 1995, pp. 363–373.Okubara, P.A. et al.; Analysis of Genes Negatively Regulated by Phytochrome Action in *Lemna gibba* and Identification of a Promoter Region Required for Phytochrome Responsiveness, *Plant Physical* (1993) 101: 915–924.Moon, H.K. and Stomp, A.M.; Effects of Medium Components and Light on Callus Induction, Growth, and Frond Regeneration in *Lemna Gibba* (Duckweed), *In Vitro Cell Dev. Biol-Plant*. 33:20–25, Jan. 1997.Slovin, J.P. and Cohen, J.D.; Levels of Indole-3-Acetic Acid in *Lemna gibba* G-3 and in a Large Lemna Mutant Regenerated from Tissue Culture, *Plant Physical* (1988) 86: 522–526.Sanford, J.C. et al.; Optimizing the Biolistic Process for Different Biological Applications, *Methods in Enzymology*, vol. 217, 1993, pp. 483–509.Rolfe et al.; Deletion Analysis of a Phytochrome-regulated Monocot *rbcS* Promoter in a Transient Assay System; *Proc. Nat'l. Acad. Sci. USA*, 88 (Apr. 1991), pp. 2683–2686.*Primary Examiner*—Gary Benzion*Assistant Examiner*—Ashwin D. Mehta*Attorney, Agent, or Firm*—Myers Bigel Sibley & Sajovec, P.A.

[57]

ABSTRACT

Methods and compositions for the efficient transformation of duckweed are provided. Preferably, the methods involve transformation by either ballistic bombardment or *Agrobacterium*. In this manner, any gene or nucleic acid of interest can be introduced and expressed in duckweed plants. Transformed duckweed plants, cells, tissues are also provided. Transformed duckweed plant tissue culture and methods of producing recombinant proteins and peptides from transformed duckweed plants are also disclosed.

65 Claims, 1 Drawing Sheet