US006753462B2

(12) **United States Patent**
Wyatt et al.

(10) **Patent No.: US 6,753,462 B2**
(45) **Date of Patent: Jun. 22, 2004**

(54) **TRANSGENIC PLANTS WITH INCREASED CALCIUM STORES**

(75) Inventors: **Sarah Wyatt**, Raleigh, NC (US); **Pei-Lan Tsou**, Raleigh, NC (US); **Dominique Robertson**, Cary, NC (US); **Wendy Boss**, Raleigh, NC (US)

(73) Assignee: **North Carolina State University**, Raleigh, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 168 days.

(21) Appl. No.: **09/844,006**

(22) Filed: **Apr. 27, 2001**

(65) **Prior Publication Data**

US 2002/0083496 A1 Jun. 27, 2002

Related U.S. Application Data

(60) Provisional application No. 60/200,233, filed on Apr. 28, 2000.

(51) **Int. Cl.**[7] **C12N 15/82**; A01H 5/00; A01H 5/08; A01H 5/10

(52) **U.S. Cl.** **800/289**; 800/298; 800/288; 800/300; 435/419; 435/69.8

(58) **Field of Search** 800/298, 289, 800/300, 288; 435/418, 69.8

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO WO 98/36084 8/1998

OTHER PUBLICATIONS

Rhoads et al. Regulation of the cyanide–resistant alternative oxidase of plant mitochondria. J. Biol. Chem., Nov. 1998, Vo 273, No. 46, pp. 30750–30756.*

Haseloff, Jim, et al., *Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic Arabidopsis plants brightly, Proc. Natl. Acad. Sci. USA*, vol. 94, pp. 2122–2127 (Mar. 1997).

Abstract, Wyatt, W. E., et al., *Effects of altered expression of the calcium–binding protein calreticulin in Arabidopsis thaliana*, Abstract XP–002185046 (Oct. 7, 1999).

International Search Report, International Application No. PCT/US01/13563 dated Dec. 28, 2001.

* cited by examiner

Primary Examiner—David T. Fox
Assistant Examiner—Cynthia Collins
(74) *Attorney, Agent, or Firm*—Myers Bigel Sibley & Sajovec, P.A.

(57) **ABSTRACT**

The present invention provides transgenic plants over-expressing a transgene encoding a calcium-binding protein or peptide (CaBP). Preferably, the CaBP is a calcium storage protein and over-expression thereof does not have undue adverse effects on calcium homeostasis or biochemical pathways that are regulated by calcium. In preferred embodiments, the CaBP is calreticulin (CRT) or calsequestrin. In more preferred embodiments, the CaBP is the C-domain of CRT, a fragment of the C-domain, or multimers of the foregoing. In other preferred embodiments, the CaBP is localized to the endoplasmic reticulum by operatively associating the transgene encoding the CaBP with an endoplasmic reticulum localization peptide. Alternatively, the CaBP is targeted to any other sub-cellular compartment that permits the calcium to be stored in a form that is biologically available to the plant. Also provided are methods of producing plants with desirable phenotypic traits by transformation of the plant with a transgene encoding a CaBP. Such phenotypic traits include increased calcium storage, enhanced resistance to calcium-limiting conditions, enhanced growth and viability, increased disease and stress resistance, enhanced flower and fruit production, reduced senescence, and a decreased need for fertilizer production. Further provided are plants with enhanced nutritional value as human food or animal feed.

22 Claims, 14 Drawing Sheets

(6 of 14 Drawing Sheet(s) Filed in Color)