

[0143] The DNA constructs of the present invention may be introduced into the genome of a desired plant host by a variety of conventional transformation techniques, which are well known to those skilled in the art. Preferred methods of transformation of plant cells or tissues are the *Agrobacterium* mediated transformation method and the biolistics or particle-gun mediated transformation method. Suitable plant transformation vectors for the purpose of *Agrobacterium* mediated transformation include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, e.g., by Herrera-Estrella et al., *Nature* 303:209 (1983); Bevan, *Nucleic Acids Res.* 12: 8711-8721 (1984); Klee et al., *Bio-Technology* 3(7): 637-642 (1985); and EPO publication 120,516. In addition to plant transformation vectors derived from the Ti or root-inducing (R1) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, but are not limited to, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery via microprojectile bombardment, and transformation using viruses or pollen.

[0144] A plasmid expression vector suitable for the introduction of a nucleic acid encoding a polypeptide or protein of the present invention in monocots using electroporation or particle-gun mediated transformation is composed of the following: a promoter that is constitutive or tissue-specific; an intron that provides a splice site to facilitate expression of the gene, such as the Hsp70 intron (PCT Publication WO93/19189); and a 3' polyadenylation sequence such as the nopaline synthase 3' sequence (NOS 3'; Fraley et al., *Proc. Natl. Acad. Sci. USA* 80: 4803-4807 (1983)). This expression cassette may be assembled on high copy replicons suitable for the production of large quantities of DNA.

[0145] An example of a useful Ti plasmid cassette vector for plant transformation is pMON17227. This vector is described in PCT Publication WO 92/04449 and contains a gene encoding an enzyme conferring glyphosate resistance (denominated CP4), which is an excellent selection marker gene for many plants. The gene is fused to the *Arabidopsis* EPSPS chloroplast transit peptide (CTP2) and expressed from the FMV promoter as described therein.

[0146] When adequate numbers of cells (or protoplasts) containing the exogenous nucleic acid encoding a polypeptide or protein of the present invention are obtained, the cells (or protoplasts) are regenerated into whole plants. Choice of methodology for the regeneration step is not critical, with suitable protocols being available for hosts from Leguminosae (alfalfa, soybean, clover, etc.), Umbelliferae (carrot, celery, parsnip), Cruciferae (cabbage, radish, canola/rapeseed, etc.), Cucurbitaceae (melons and cucumber), Gramineae (wheat, barley, rice, maize, etc.), Solanaceae (potato, tobacco, tomato, peppers), various floral crops, such as sunflower, and nut-bearing trees, such as almonds, cashews, walnuts, and pecans. See, for example, Ammirato et al., *Handbook of Plant Cell Culture—Crop Species*. Macmillan Publ. Co. (1984); Shimamoto et al., *Nature* 338:274-276 (1989); Fromm, *UCLA Symposium on Molecular Strategies for Crop Improvement*, Apr. 16-22, 1990. *Keystone*, Colo. (1990); Vasil et al., *Bio/Technology* 8:429-434 (1990); Vasil et al., *Bio/Technology* 10:667-674 (1992); Hayashimoto, *Plant Physiol.* 93:857-863 (1990); and Datta et al., *Bio-technology* 8:736-740 (1990). Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regenera-

tion techniques are described generally in Klee et al., *Ann. Rev. Plant Phys.* 38:467-486 (1987).

[0147] A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single exogenous gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added exogenous gene. More preferred is a transgenic plant that is homozygous for the added exogenous gene; i.e., a transgenic plant that contains two added exogenous genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single exogenous gene, germinating some of the seed produced and analyzing the resulting plants produced for the exogenous gene of interest.

[0148] The development or regeneration of transgenic plants containing the exogenous nucleic acid that encodes a polypeptide or protein of interest is well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants, as discussed above. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide or protein of the present invention is cultivated using methods well known to one skilled in the art.

[0149] Transgenic plants, that can be generated by practice of the present invention, include but are not limited to *Acacia*, alfalfa, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, broccoli, brussels sprouts, cabbage, canola, cantaloupe, carrot, cassava, cauliflower, celery, cherry, cilantro, citrus, clementines, coffee, corn, cotton, cucumber, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, figs, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, mango, melon, mushroom, nut, oat, okra, onion, orange, an ornamental plant, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, raspberry, rice, rye, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweet-gum, tangerine, tea, tobacco, tomato, turf, a vine, watermelon, wheat, yams, and zucchini.

[0150] The present invention also provides parts of the transgenic plants of present invention. Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred embodiment of the present invention, the plant part is a seed.

[0151] The present invention also further provides method for generating a transgenic plant comprising the steps of: a) introducing into the genome of the plant an exogenous nucleic acid, wherein the exogenous nucleic acid comprises in the 5' to 3' direction i) a promoter that functions in the cells of said plant, said promoter operably linked to; ii) a structural nucleic acid sequence encoding a *D. v. virgifera* protein or fragment thereof, said structural nucleic acid sequence operably linked to; iii) a 3' non-translated nucleic acid sequence that functions in said cells of said plant to cause transcriptional termination; b) obtaining transformed plant cells containing the nucleic acid sequence of step (a); and c) regenerating from said transformed plant cells a transformed plant in which said polypeptide or protein is overexpressed.