

genes contain a signal peptide coding sequence interrupted by a short intervening sequence (about 60 base pairs) at a conserved site. Conserved sequences occur in the 5' mRNA untranslated region, in the adjacent 35 base pairs of upstream flanking sequence and at -200 base pairs from the mRNA start position in each of the cuticle genes.

[0107] Standard methods of insect cell culture, cotransfection and preparation of plasmids are set forth in Summers and Smith (Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experiment Station Bulletin No. 1555, Texas A&M University (1987)). Procedures for the cultivation of viruses and cells are described in Volkman and Summers, *J. Virol* 19: 820-832 (1975); Volkman et al., *J. Virol* 19: 820-832 (1976); and O'Reilly et al., Eds., *Baculovirus Expression Vectors: A laboratory Manual*, W.H. Freeman & Co., New York, N.Y. (1994); all of which are herein incorporated by reference in their entirety.

[0108] (e) Plant Constructs and Plant Transformants

[0109] The present invention also relates to a plant recombinant vector or construct comprising a structural nucleotide sequence encoding a *D. v. virgifera* protein or fragment thereof. The present invention also relates to a transformed plant cell or plant comprising in its genome an exogenous nucleic acid molecule encoding one or more *D. v. virgifera* proteins or fragments thereof. The present invention also relates to methods for creating a transgenic plant in which one or more *D. v. virgifera* proteins or fragments thereof are overexpressed.

[0110] By "exogenous" it is meant that a nucleic acid originates from outside the plant. An exogenous nucleic acid molecule can have a naturally occurring or non-naturally occurring nucleotide sequence. One skilled in the art understands that an exogenous nucleic acid molecule can be a heterologous nucleic acid derived from a different plant species than the plant into which the nucleic acid is introduced or can be a nucleic acid derived from the same plant species as the plant into which it is introduced.

[0111] The term "overexpression" refers to the expression of a polypeptide or protein encoded by an exogenous nucleic acid introduced into a host cell, wherein said polypeptide or protein is either not normally present in the host cell, or wherein said polypeptide or protein thereof is present in said host cell at a higher level than that normally expressed from the endogenous gene encoding said polypeptide or protein. By "endogenous gene" refers to a native gene in its natural location in the genome of an organism.

[0112] The term "genome" as it applies to plant cells encompasses not only chromosomal DNA found within the nucleus, but organelle DNA found within subcellular components of the cell. DNAs of the present invention introduced into plant cells can therefore be either chromosomally integrated or organelle-localized. The term "genome" as it applies to bacteria encompasses both the chromosome and plasmids within a bacterial host cell. Encoding DNAs of the present invention introduced into bacterial host cells can therefore be either chromosomally integrated or plasmid-localized.

[0113] Method which are well known to those skilled in the art may be used to construct the plant recombinant construct or vector of the present invention. These method include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook et al., *Molecular Cloning, A Labora-*

tory Manual, Cold Spring Harbor Press, Plainview, N.Y. (1989); and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y. (1989).

[0114] A plant recombinant construct or vector of the present invention contains a structural nucleotide sequence encoding one or more *D. v. virgifera* proteins or fragments thereof and operably linked regulatory sequences or control elements.

[0115] The term "operably linked", as used in reference to a regulatory sequence and a structural nucleotide sequence, means that the regulatory sequence causes regulated expression of the operably linked structural nucleotide sequence. "Expression" refers to the transcription and stable accumulation of sense or antisense RNA derived from the nucleic acid of the present invention. Expression may also refer to translation of mRNA into a polypeptide or protein. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Pat. No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-translated sequence, introns, or the coding sequence. "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA.

[0116] "Regulatory sequences" or "control elements" refer to nucleotide sequences located upstream (5' noncoding sequences), within, or downstream (3' non-translated sequences) of a structural nucleotide sequence, and which influence the transcription, RNA processing or stability, or translation of the associated structural nucleotide sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

[0117] The promoter sequence may consist of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions.

[0118] Promoters which are known or are found to cause transcription of DNA in plant cells can be used in the present invention. Such promoters may be obtained from a variety of sources such as plants and plant viruses. A number of promoters, including constitutive promoters, inducible promoters and tissue-specific promoters, that are active in plant cells have been described in the literature. It is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of a protein to cause the desired phenotype. In addi-