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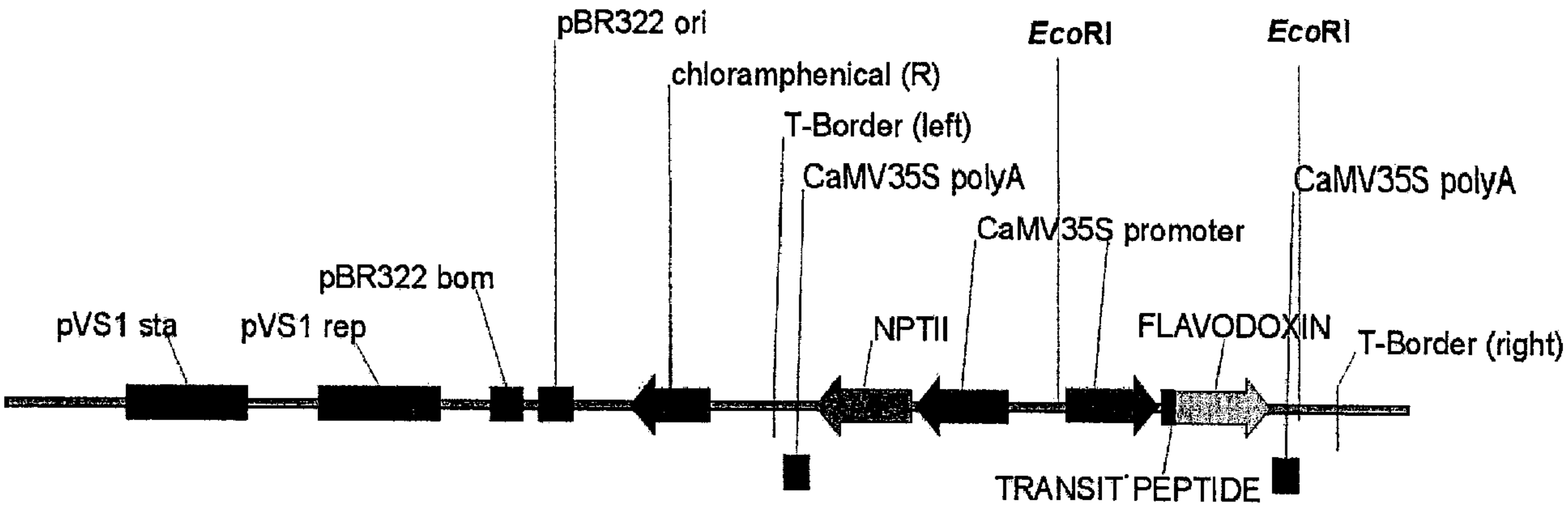

(57) Abrégé/Abstract:
The present invention relates to the finding that the accumulation of flavoproteins such as flavodoxin within plant cells, in particular within chloroplasts, provides enhanced resistance to sources of environmental stress, such as herbicide, ultraviolet AB radiation, extreme temperatures, infection and/or high doses of irradiation. Nucleic acids, cells, plants and methods pertaining thereto are described.

ABSTRACT

Stress Tolerant Plants

The present invention relates to the finding that the accumulation of flavoproteins such as flavodoxin within plant cells, in particular within chloroplasts, provides enhanced resistance to sources of environmental stress, such as herbicide, ultraviolet AB radiation, extreme temperatures, infection and/or high doses of irradiation. Nucleic acids, cells, plants and methods pertaining thereto are described.

Stress Tolerant Plants

The present invention relates to methods for improving environmental stress tolerance of plants and plants with such improved stress tolerance. More particularly, the invention relates to the finding that the expression of a flavoprotein such as flavodoxin within plant cells is beneficial to plants which are subjected to environmental stress.

Environmental stress is a major limiting factor for plant productivity and crop yield. Many of the deleterious processes undergone by plants exposed to adverse environmental conditions are mediated by reactive oxygen species (ROS) which are generated in chloroplasts through the faulty performance of the photosynthetic apparatus (Foyer, C. H. et al. (1994) Plant Cell Environ. 17,507-523, Hammond-Kosack, K. E., and Jones, J. D. G. (1996) Plant Cell 8, 1773-1791, Allen, R. (1995) Plant Physiol. 107, 1049-1054).

Auto-oxidation of components of the photosynthetic electron transport chain leads to the formation of superoxide radicals and their derivatives, hydrogen peroxide and hydroxyl radicals. These compounds react with a wide variety of biomolecules (most conspicuously, DNA), causing cell stasis and death.

To cope with the damaging effects of reactive oxygen species (ROS), aerobic organisms have evolved highly efficient antioxidant defense systems which are made up of both enzymatic and non-enzymatic constituents. In different tissues and organisms, antioxidants play different and often complementary protective functions, such as direct scavenging of ROS, replacement of damaged oxidant sensitive biomolecules and DNA repair activities (Fridovich, I. (1997). J. Biol. Chem. 272,1851-1857). At least part of the cellular response against oxidative stress is of an adaptive nature and involves *de novo* synthesis of committed members of the antioxidant barrier. Various multigenic responses have been recognized in the facultative aerobic bacterium *Escherichia coli,* including those modulated by the *soxRS* and *oxyR* regulons (Hidalgo, E., and Demple, B. (1996). In Regulation of Gene Expression in *Escherichia coli,* Molecular Biology Intelligence Unit Series (E. C. C. Lin and A. S. Lynch, eds.), pp. 434-452, Austin, TX: R. G. Landis).

The *soxRS* response appears to be specifically tailored to face the challenges imposed by exposure of the cells to superoxide radicals or to nitric oxide. Many different components of the response have been identified, including two soluble flavoproteins: FAD-containing ferredoxin-NADP+ reductase (FNR), and its electron partner substrate flavodoxin (Liochev et al. (1994) Proc. Natl Acad. Sci. USA 91,1328-1331, Zheng, M. et al (1999) J. Bacteriol. 181,4639-4643).

Flavodoxins are small monomeric proteins (Mw 18,800) containing one molecule of non-covalently bound FMN (Razquin, P. et al (1988) J. Bacteriol. 176, 7409- 7411). FNR is able to use, with roughly similar efficiencies, both flavodoxin and the iron-sulfur protein ferredoxin as substrates for its NADP(H) oxidoreductase activity. In cyanobacteria, flavodoxin expression is induced under conditions of iron deprivation, when ferredoxin cannot be synthesized.

As part of the *soxRS* response of *E. coli,* both FNR and flavodoxin levels increase over twenty times upon treatment of the bacteria with superoxide-propagating compounds such as the redox cycling herbicide methyl viologen (MV), whereas ferredoxin amounts are not affected (Rodriguez, R. E. et al (1998) Microbiology 144,2375-2376). Unlike FNR and ferredoxins, which are widely distributed among plastids, mitochondria and bacteria, flavodoxin occurrence appears to be largely restricted to bacteria. Flavodoxins have not been isolated from plant tissues, and no flavodoxin homologue has been recognized in the *Arabidopsis thaliana* genome (The *Arabidopsis* Genome Initiative (2000) Nature 408,796-815).

The present invention relates to the finding that plant lines which have been engineered to express a flavoprotein such as flavodoxin display highly enhanced tolerance compared to control, untreated plants, when exposed to a plethora of adverse environmental conditions.

In various aspects, the present invention provides nucleic acids and vectors suitable for use in methods of producing stress tolerant plants. In preferred embodiments, such nucleic acids and vectors provide for the accumulation of flavoprotein within the choloroplasts of plant cells transformed therewith. In some embodiments of the invention, accumulation within the chloroplasts is achieved by fusing the flavoprotein to a chloroplast targeting polypeptide.

A first aspect of the present invention provides an isolated nucleic acid encoding a fusion polypeptide comprising a flavoprotein polypeptide and a chloroplast targeting peptide.

A nucleic acid may encode a fusion polypeptide comprising a flavoprotein polypeptide and a chloroplast targeting peptide.

A flavodoxin polypeptide may be a bacterial flavodoxin polypeptide, for example a cyanobacterial flavodoxin polypeptide such as the flavodoxin of the cyanobacterium Anabaena PCC7119 (Fillat M. et al (1991) Biochem J. 280 187-191). Other suitable flavodoxin polypeptides include flavodoxins from photosynthetic anoxigenic bacteria, enterobacteria, diazotrophs and algae. Examples of flavodoxin polypeptides suitable for use according to the present invention are exemplified in Table 1. Whilst a wild type flavodoxin polypeptide is preferred, a flavodoxin polypeptide may also be a fragment, mutant, derivative, variant or allele of such a wild type sequence.

Suitable fragments, mutants, derivatives, variants and alleles are those which encode a protein which retain the functional characteristics of the polypeptide encoded by the wild-type flavoprotein gene, especially the ability to act as an anti-oxidant. Changes to a sequence, to produce a mutant, variant or derivative, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino acids in the encoded polypeptide. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence are included.

Flavodoxin polypeptides are monomeric hydrophillic flavoproteins of a molecular mass of less than 20 kDa, containing one mole of non covalently bound flavin mononucleotide (FMN) per molecule of apoprotein. The flavin group can be reversibly dissociated by mild acid treatment.

Flavodoxin polypeptides engage in one-electron transfer reactions with several electron partners such as FNR, pyruvate-flavodoxin reductase and photosystems, replacing ferredoxin in most of its activities. Even though flavodoxin can in principle exchange two electrons, it behaves as an obligatory one-electron carrier. Contrary to other flavoproteins, the half-reduced semiquinone and the fully reduced hydroquinone are the most stable species, and these are the forms relevant for flavodoxin functions.

A polypeptide which is a member of the Flavodoxin family or which is an amino acid sequence variant, allele, derivative or mutant thereof may comprise an amino acid sequence which shares greater than about 30% sequence identity with the sequence of Anabaena PCC7119 flavodoxin, greater than about 35%, greater than about 40%, greater than about 45%, greater than about 55%, greater than about 65%, greater than about 70%, greater than about 80%, greater than about 90% or greater than about 95%. The sequence may share greater than about 30% similarity with Anabaena PCC7119 flavodoxin, greater than about 40% similarity, greater than about 50% similarity, greater than about 60% similarity, greater than about 70%

similarity, greater than about 80% similarity or greater than about 90% similarity.

In certain embodiments, a flavodoxin polypeptide may show little overall homology, say about 20%, or about 25%, or about 30%, or about 35%, or about 40% or about 45%, with the Anabaena PCC7119 flavodoxin sequence, even though it possesses the same anti-oxidation activity. However, in functionally significant domains or regions, the amino acid homology may be much higher. For example, a flavodoxin polypeptide comprises an FMN-binding domain which has high homology to the flavodoxin FMN binding domain (a flavodoxin-like domain). Putative functionally significant domains or regions can be identified using processes of bioinformatics, including comparison of the sequences of homologues.

Sequence similarity and identity is commonly defined with reference to the algorithm GAP (Genetics Computer Group, Madison, WI). GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4.

Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul *et al.* (1990) *J. Mol. Biol.* **215**: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* **85**: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) *J. Mol Biol.* ***147***: 195-197), or the TBLASTN program, of Altschul et al. (1990) supra, generally employing default parameters. In particular, the psi-Blast algorithm (Nucl. Acids Res. (1997) 25 3389-3402) may be used.

Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Particular amino acid sequence variants may differ from a known flavodoxin polypeptide sequence as described herein by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20 20-30, 30-50, or more than 50 amino acids.

Sequence comparison may be made over the full-length of the relevant sequence described herein, or may more preferably be over a contiguous sequence of about or greater than about 20, 25, 30, 33, 40, 50, 70, 120, 170 or more amino acids or nucleotide triplets, compared with the relevant amino acid sequence or nucleotide sequence as the case may be.

Other methods suitable for use in identifying flavodoxin polypeptides are well-known in the art.

In other embodiments, the isolated nucleic acid may encode a fusion polypeptide which comprises an FNR polypeptide and a heterogeneous chloroplast targeting peptide.

Many ferredoxin-NADP(+) reductase (FNR) polypeptides are known in the art and an FNR polypeptide suitable for use in accordance with embodiments of the present invention may readily identified by a skilled person. Other suitable FNR polypeptides may be found on the NCBI database, for example, FNR polypeptide sequences having database accession numbers NP 418359, NP 312876, P28861, and AAG59117. Suitable FNR polypeptides have the anti-oxidant and electron transfer activity of wild type ferredoxin-NADP(+) reductase.

A chloroplast targeting peptide suitable for use in accordance with certain embodiments of the present invention may be any peptide sequence which directs a polypeptide to the chloroplast of a plant cell. Suitable peptides may readily be identified by a skilled person and some examples are shown in Table 2. Other examples may be found on the NCBI database. In some preferred embodiments, a peptide may have the chloroplast transit polypeptide of the pea FNR, which has the sequence shown in Figure 6.

In other embodiments of the present invention, flavoprotein may accumulate within chloroplasts as a result of expression within the chloroplast of nucleic acid encoding the polypeptide following direct transformation of the chloroplast. There is no requirement for a targeting or transit peptide in such embodiments.

Particle bombardment methods (Ruf, S. et al.(2001) Nature Biotechnol. 19, 870-875) are particularly suitable for direct chloroplast transformation. With suitable plant regulatory elements, the transformed DNA may be transcribed within the plastid and translated into polypeptide in stromal ribosomes.

A nucleic acid encoding any flavoprotein polypeptide as defined above may be used in accordance with the present invention with any suitable chloroplast targeting peptide as defined above. The particular choice of flavoprotein polypeptide and targeting peptide is not critical to the practice of the present invention. Preferably, the flavoprotein polypeptide is not fused to a targeting peptide with which it is naturally associated i.e. it is fused to a heterogeneous targeting polypeptide. Flavodoxin polypeptides, which are not found in plants, are not naturally associated with chloroplast targeting signals.

In some preferred embodiments, a fusion polypeptide comprising a flavodoxin polypeptide and a chloroplast targeting peptide may have the sequence shown in Figure 4. A suitable nucleic acid molecule encoding such a fusion polypeptide may have the sequence shown in Figure 3.

The present invention also provides a nucleic acid construct or vector which comprises a nucleic acid encoding a fusion polypeptide comprising a flavodoxin polypeptide and a chloroplast targeting peptide, preferably a construct or vector from which the fusion polypeptide encoded by the nucleic acid sequence can be expressed. The construct or vector is preferably suitable for transformation into and/or expression within a plant cell.

A construct or vector comprising nucleic acid according to this aspect of the present invention need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

However, in one aspect the present invention provides a nucleic acid construct comprising a nucleic acid sequence encoding a flavodoxin polypeptide operably linked to a plant specific regulatory sequence, such as a promoter. Such constructs are particularly useful in embodiments in which chloroplasts are directly transformed with nucleic acid, which is subsequently expressed therein under the control of the plant specific regulatory element.

A plant specific regulatory sequence or element is a sequence which preferentially directs the expression (i.e. transcription) of a nucleic acid within a plant cell relative to other cell types. For example, expression from such a sequence is reduced or abolished in non-plant cells, such as bacterial or mammalian cells. A suitable regulatory sequence may for example be derived from a plant virus such as Cauliflower Mosaic Virus 35S. A regulatory sequence may be inducible, as described further below. The present invention also encompasses vectors comprising such a nucleic acid sequence.

Another aspect of the present invention provides the use of a nucleic acid as described herein in the production of a transgenic plant. Such a method may be for improving the tolerance of a plant to stress, in particular environmental stress, such as oxidative stress. Such stress may be biotic, abiotic or xenobiotic in nature and may include herbicide exposure, ultraviolet AB radiation, extreme temperatures, infection, for example with a necrotizing pathogen such as a bacterium or fungus and/or high doses of irradiation.

Nucleic acid may of course be double- or single-stranded, cDNA or genomic DNA, or RNA. The nucleic acid may be wholly or partially synthetic, depending on design. Naturally, the skilled person will understand that where the nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T. The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and methods of making the expression product by expression from encoding nucleic acid therefore under suitable conditions in suitable host cells.

Those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression, for example in a microbial or plant cell. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual:* 2nd edition, Sambrook *et al,* 1989, Cold Spring Harbor Laboratory Press.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. Specific procedures and vectors previously used with wide success upon plants are described by Bevan, Nucl. Acids Res. (1984) 12, 8711-8721), and Guerineau and Mullineaux, (1993) Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148.

A nucleic acid sequence as described herein, for example a sequence encoding a flavodoxin polypeptide, may be under operative control of a regulatory sequence active in plants for control of expression. It is indeed preferred that the coding sequence is operably linked to one or more regulatory sequences which may be heterologous or foreign to the gene (i.e. a non-bacterial sequence), for example a plant regulatory sequence. Such regulatory sequences may provide for efficient expression within a plant cell.

The term "heterologous" may be used to indicate that the gene/sequence of nucleotides in question have been introduced into said cells of the plant or an ancestor thereof, using genetic engineering or recombinant means, i.e. by human intervention. Nucleotide sequences heterologous, or exogenous or foreign, to a plant cell may be non-naturally occurring in cells of that type, variety or species. For example, there are no reports of flavodoxins in plant cells and nucleic acid encoding a flavodoxin polypeptide is therefore "heterologous" to a plant cell transformed therewith.

A nucleic acid construct which comprises a nucleic acid sequence encoding a flavoprotein such as flavodoxin, may include an inducible promoter operatively linked to the nucleic acid sequence. Such a promoter may be a stress inducible promoter. As discussed, this allows control of expression, for example, in response to an environmental stress. The invention also provides plants transformed with said gene construct and methods including introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell, e.g. by application of a suitable stimulus, which may be an environmental stress stimulus such as a change in external conditions.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus (which may be generated within a cell or provided exogenously). The nature of the stimulus varies between promoters. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon in the presence of the relevant stimulus by an amount effective to alter a phenotypic characteristic i.e. to enhance stress tolerance. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about the desired stress tolerant phenotype (and may in fact be zero). Upon application of the stimulus, which may for example, be an increase in environmental stress, expression is increased (or switched on) to a level which causes enhanced stress tolerance.

Many examples of inducible promoters will be known to those skilled in the art.

Other suitable promoters may include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level in virtually all plant tissues (Benfey et al, (1990) EMBO J 9: 1677-1684); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, e.g. inner phloem, flower primordia, branching points in root and shoot (Medford, J.I. (1992) *Plant Cell* 4, 1029-1039; Medford *et al*, (1991) *Plant Cell* 3, 359-370) and the *Arabidopsis thaliana* LEAFY promoter that is expressed very early in flower development (Weigel *et al*, (1992) *Cell* 69, 843-859).

Constructs and vectors may further comprise selectable genetic markers consisting of chimaeric genes that confer selectable phenotypes such as resistance to antibiotics such as kanamycin, hygromycin, phosphinotricin, chlorsulfuron, methotrexate, gentamycin, spectinomycin, imidazolinones and glyphosate.

When introducing a chosen gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur. Finally, as far as plants are concerned, the target cell type must be such that cells can be regenerated into whole plants.

Techniques well known to those skilled in the art may be used to introduce nucleic acid constructs and vectors into plant cells to produce transgenic plants of the appropriate stress tolerant phenotype.

Agrobacterium transformation is one method widely used by those skilled in the art to transform dicotyledonous species. Production of stable, fertile transgenic plants in almost all economically relevant monocot plants is also now routine:(Toriyama, et al. (1988) *Bio/Technology* **6,** 1072-1074; Zhang, et al. (1988) *Plant Cell Rep.* **7,** 379-384; Zhang, et al. (1988) *Theor Appl Genet* **76,** 835-840; Shimamoto, et al. (1989) *Nature* **338,** 274-276; Datta, et al. (1990) *Bio/Technology* **8,** 736-740; Christou, et al. (1991) *Bio/Technology* **9,** 957-962; Peng, et al. (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, et al. (1992) *Plant Cell Rep.* **11,** 585-591; Li, et al. (1993) *Plant Cell Rep.* **12,** 250-255; Rathore, et al. (1993) *Plant Molecular Biology* **21,** 871-884; Fromm, et al. (1990) *Bio/Technology* **8,** 833-839; Gordon-Kamm, et al. (1990) *Plant Cell* **2,** 603-618; D'Halluin, et al. (1992) *Plant Cell* **4,** 1495-1505; Walters, et al. (1992) *Plant Molecular Biology* **18,** 189-200; Koziel, et al. (1993) *Biotechnology* **11,** 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* **25,** 925-937; Weeks, et al. (1993) *Plant Physiology* **102,** 1077-1084; Somers, et al. (1992) *Bio/Technology* **10,** 1589-1594; WO92/14828). In particular, *Agrobacterium* mediated transformation is now a highly efficient alternative transformation method in monocots (Hiei et al. (1994) *The Plant Journal* **6**, 271-282).

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* **5,** 158-162.; Vasil, et al. (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* **13** (4): 653-671; Vasil, 1996, *Nature Biotechnology* **14** page 702). Wan and Lemaux (1994) *Plant Physiol.* **104**: 37-48 describe techniques for generation of large numbers of independently transformed fertile barley plants.

Other methods, such as microprojectile or particle bombardment (US 5100792, EP-A-444882, EP-A-434616), electroporation (EP 290395, WO 8706614)), microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green et al. (1987) *Plant Tissue and Cell Culture*, Academic Press) direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman et al. *Plant Cell Physiol.* **29**: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* **87**: 1228 (1990d)) may be preferred where Agrobacterium transformation is inefficient or ineffective.

Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* **9**: 1-11.

Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, e.g. bombardment with Agrobacterium coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewed in Vasil et al., *Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications*, Academic Press, 1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

A further aspect of the present invention provides a method of producing a cell which includes incorporating an isolated nucleic acid sequence encoding a flavoprotein polypeptide such as a flavodoxin polypeptide or an FNR polypeptide or a nucleic acid vector comprising such a sequence into the cell by means of transformation. Such a method of producing a cell may include recombining the nucleic acid with the cell genome nucleic acid such that it is stably incorporated therein. A plant may be regenerated from one or more cells transformed as described.

The flavoprotein polypeptide, the encoding nucleic acid, and/or the vector comprising the nucleic acid are preferably heterogeneous i.e. exogenous or foreign to the plant cell transformed therewith.

A method of producing a plant cell may include expressing the nucleic acid and causing or allowing the accumulation of flavoprotein polypeptide expressed thereby in the chloroplast of said plant cell.

A suitable flavoprotein polypeptide for use in such methods may be an FNR polypeptide or a flavodoxin polypeptide.

A method of making such a plant cell may include the introduction of such a nucleotide sequence or a suitable vector including the sequence into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the nucleic acid sequence into the genome.

A method may further include sexually or asexually propagating or growing off-spring or a descendant of the plant regenerated from said plant cell.

The invention further encompasses a host cell transformed with a nucleic acid sequence or vector as set forth above, i.e. containing a nucleic acid or vector as described above, especially a plant cell, for example a higher plant cell, or a microbial cell. Thus, a host cell, such as a plant cell, including a nucleotide sequence as herein indicated is provided. Within the cell, the nucleotide sequence may be incorporated within the chromosome or may be extra-chromosomal. There may be more than one heterologous nucleotide sequence per haploid genome. This, for example, enables increased expression of the gene product compared with endogenous levels, as discussed below. A nucleic acid sequence comprised within a plant cell may be placed under the control of an externally inducible gene promoter, either to place expression under the control of the user or to provide for expression in response to stress.

A nucleic acid which is stably incorporated into the genome of a plant is passed from generation to generation to descendants of the plant, cells of which descendants may express the encoded flavoprotein polypeptide and so may have enhanced stress or pathogen tolerance.

A plant cell may contain a nucleic acid sequence encoding a flavoprotein polypeptide as a result of the introduction of the nucleic acid sequence into an ancestor cell.

In some embodiments, the flavoprotein polypeptide may be expressed within the plant cell as part of a fusion polypeptide which also comprises a chloroplast targeting peptide.

A plant cell as described herein may be comprised in a plant, a plant part or a plant propagule, or an extract or derivative of a plant as described below.

Plants which include a plant cell as described herein are also provided, along with any part or propagule thereof, seed, selfed or hybrid progeny and descendants. Particularly provided are transgenic higher plants, especially crop plants, which have been engineered to carry genes identified as stated above. Examples of suitable plants include tobacco, cucurbits, carrot, vegetable brassica, melons, capsicums, grape vines, lettuce, strawberry, oilseed brassica, sugar beet, wheat, barley, maize, rice, soyabeans, peas, sorghum, sunflower, tomato, potato, pepper, chrysanthemum, carnation, poplar, eucalyptus and pine.

A plant according to the present invention may be one which does not breed true in one or more properties. Plant varieties may be excluded, particularly registrable plant varieties according to Plant Breeders Rights. It is noted that a plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene, introduced into a cell of the plant or an ancestor thereof.

In addition to a plant, the present invention provides any clone of such a plant, seed, selfed or hybrid progeny and descendants, and any part or propagule of any of these, such as cuttings and seed, which may be used in reproduction or propagation, sexual or asexual. Also encompassed by the invention is a plant which is a sexually or asexually propagated off-spring, clone or descendant of such a plant, or any part or propagule of said plant, off-spring, clone or descendant.

The present invention also encompasses the polypeptide expression product of a nucleic acid molecule according to the invention as disclosed herein. Such an isolated polypeptide may consist of a fusion polypeptide which comprises or consists of a flavoprotein polypeptide and a chloroplast targeting peptide, for example a fusion polypeptide which comprises or consists of a flavodoxin polypeptide and a chloroplast targeting peptide. The chloroplast targeting peptide may be heterogeneous to i.e. foreign or not normally or naturally associated with the flavoprotein polypeptide.

A preferred polypeptide includes the amino acid sequence shown in Figure 4. Such a fusion polypeptide may be encoded by a nucleic acid sequence as described herein, for example the nucleic acid sequence shown in Figure 3.

Also provided are methods of making such an expression product by expression from a nucleotide sequence encoding therefore under suitable conditions in suitable host cells e.g. *E.coli*. Those skilled in the art are well able to construct vectors and design protocols and systems for expression and recovery of products of recombinant gene expression.

The invention further provides a method of enhancing improving or increasing the stress tolerance of a plant which includes expressing a nucleic acid sequence encoding a flavoprotein polypeptide (i.e. causing or allowing transcription from a nucleic acid) within cells of the plant.

Suitable flavoprotein polypeptides include FNR polypeptides and flavodoxin polypeptides as described herein.

Improved stress tolerance may include enhanced or increased tolerance to environmental stresses such as ultraviolet UV radiation, extreme temperatures, irradiation, and/or pathogen infection, for example bacterial or fungal infection, in particular necrotizing pathogens, relative to normal, untreated plants.

The ability of a plant to tolerate stress may be increased by expression from a nucleotide sequence encoding a flavoprotein polypeptide such as a flavodoxin polypeptide within cells of a plant (thereby producing the encoded polypeptide), following an earlier step of introduction of the nucleotide sequence into a cell of the plant or an ancestor thereof. Such a method may raise the plants tolerance to stress and/or resistance to pathogen.

Preferably such a method includes causing or allowing the accumulation of the flavoprotein polypeptide within the chloroplasts of said cells, for example by expressing the nucleic acid within the chloroplasts of said cells or providing for the transport of the expressed protein into the chloroplasts. The level of flavoprotein in chloroplasts is increased or enhanced over the normal, endogenous levels of the flavoprotein as a result of such expression.

In some embodiments, chloroplast accumulation is achieved by expressing a fusion protein which comprises the flavodoxin polypeptide and a chloroplast targeting peptide.

Control experiments may be performed as appropriate in the methods described herein. The performance of suitable controls is well within the competence and ability of a skilled person in the field.

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figure described below.

Brief Description of the Figures and Tables

Figure 1 shows that flavodoxin expression increases resistance to methyl viologen toxicity in transgenic tobacco plants.

Figure 2 shows a scheme of the binary vector p*CAMBIA* 2200 containing a fragment of the in-frame fusion between the sequences encoding pea FNR transit peptide and the flavodoxin gene. The cassette inserted in the *Eco* RI site of the pCAMBIA 2200 was previously constructed in p*DH51*. This *Eco* RI fragment contained the CaMV 35S promoter, the flavodoxin chimeric gene and the CaMV35S polyadenylation signal.

Figure 3 (SEQ.ID.NO.:1) shows the nucleotide sequence of the in-frame fusions of the pea FNR transit peptide with the flavodoxin gene. The initiation codon (ATG) of the transit peptide and the initial codon of flavodoxin (ATG) are indicated in bold, and the stop codon (TAA) is underlined.

Figure 4 (SEQ.ID.NO.:2) shows the predicted protein sequence of the transit peptide and flavodoxin protein.

Figure 5 (SEQ.ID.NO.:3) shows the nucleic acid sequence of the chloroplast transit peptide of pea FNR

Figure 6 (SEQ.ID.NO.:4) shows the amino acid sequence of the chloroplast transit peptide of pea FNR.

Table 1 shows the database details of known flavodoxin genes from a variety of microbes.

Table 2 shows the database details of known chloroplast targeting peptides.

EXPERIMENTAL

Materials and Methods

Construction of Ti vectors for Flavodoxin Expression

A DNA fragment encoding *Anabaena* PCC7119 flavodoxin was obtained by PCR amplification of the cloned gene (Fillat, M. F. et al (1991) Biochem. J. 280, 187-191) from primers

5'- GACGAGCTCTCATAATGTCAAAG-3' (SEQ.ID.NO.:5) and;

5'- ACTGTCGACTTTTTACAAACCAAAT-3' (SEQ.ID.NO.:6), complementary to positions -14 to 9 and 515 to 540, respectively.

To facilitate further manipulations, a *Sac*I recognition site (GAGCTC) was introduced in the 5´ end primer and a *Sal*I site (GTCGAC) in the 3´ end primer. The PCR conditions were 30 cycles of 60 s at 94 °C, 90 s at 52 °C and 90 s at 72 °C, using 1ng of template DNA and 50 pmol of each primer in a medium containing 10 mM Tris-HCl pH 8.4, 5 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 5 units of Taq DNA polymerase. After the 30 cycles were completed, the reactions were incubated at 72 °C for 10 min. A purified PCR fragment of the predicted length (540 bp) was digested with *Sac*I and *Sal*I. The fragment was cloned into compatible sites of a pUC9-derived recombinant plasmid encoding the entire FNR precursor (Ceccarelli, E. A. et al (1991) J. Biol. Chem. 266, 14283-14287) between *Bam*HI and *Sal*I restriction sites, and from which a DNA fragment encoding the mature region of pea FNR had been removed by digestion with *Sac*I and *Sal*I. This generated an in-frame fusion of the chloroplast transit peptide derived from FNR with the flavodoxin protein.

The sequence of the chimeric gene was determined on both strands, and excised from the corresponding plasmid by digestion with *Bam*HI and *Sal*I. The 710-bp fragment was then cloned between the CaMV 35S promoter and polyadenylation regions of pDH51 (Pietrzcak, M. et al (1986) Nucleic Acids Res. 14, 5857-5868). The entire cassette was further isolated as an *Eco*RI fragment and inserted into the *Eco*RI site of the binary vector pCAMBIA 2200 (Hajdukiewiez, P. et al (1994) Plant Mol. Biol. 25, 989-994). The construct was finally mobilized into *Agrobacterium tumefasciens* strain LBA 4404 by electroporation (Ausubel, F. M. et al (1987) Current Protocols in Molecular Cloning. New York, NY: John Wiley and Sons).

Plant transformation and characterization.

Tobacco leaf disc transformation was carried out using conventional techniques (Gallois, P. and Marinho, P. (1995) Plant gene transfer and expression protocols. In Methods in Molecular Biology (H. Jones, ed.), vol. 49, pp. 39-49. Humana Press Inc., Totowa, NJ), and the progenies of kanamycin-resistant transformants were analyzed further. The presence of the flavodoxin gene in the transgenic lines was evaluated by Southern blot hybridization, using standard procedures. Primary transformants expressing high levels of bacterial flavodoxin, as evaluated by SDS-PAGE and immunoblotting (Krapp, A. R. et al (1997) Eur. J. Biochem. 249, 556-563), were self-pollinated and all subsequent experiments were carried out with the homozygous progeny.

Seeds of control and transgenic plants were germinated on Murashige-Skoog (MS) solid medium supplemented with 2 % (w/v) sucrose and, in the case of transformants, 100 μg ml^{-1} kanamycin. After two weeks at 25°C and 200 μmol quanta m^{-2} s^{-1} (14 h light/10 h dark), seedlings were transplanted to fresh MS medium in Magenta vessels. When required, four-week old plantlets were placed on soil or grown hydroponically in nutrient medium (Geiger, M. et al. (1999) Plant Cell Environ. 22, 1177-1199). MV was included in the watering solution. Leaf discs of 12 mm diameter were punched from young fully expanded leaves of two-month old tobacco plants grown on soil. Discs were weighted and floated individually, top side up, on 1 ml sterile distilled water containing the indicated amounts of MV in 24-well plates, infiltrated in vacuum, and incubated in the dark for 2 h at 25 °C to allow diffusion of the MV into the leaf. Wells were illuminated with a white light source at 500 μmol quanta m^{-2} s^{-1}. Controls were kept in the dark. Electrolyte leakage of the leaf discs during MV stress was measured as conductivity of the medium with an Horiba model B-173 conductivity meter.

To evaluate the tolerance of flavodoxin-expressing transformants to extreme temperatures, two-week old plantlets were exposed to 500 µmol quanta m^{-2} s^{-1} plantlets at either 8 °C or 40 °C for 12 h. Light treatments were carried out on four-week old plants, by focusing a light beam (8 cm diameter) of 2000 µmol quanta m^{-2} s^{-1} on the upper surface of the third or fourth fully expanded leaf with the aid of a light cannon for 18 h at at 25 °C. Tolerance to ultraviolet AB radiation was assayed by exposing four-week old plants to a combination of UV-A (315-400 nm at 2.2 W m^{-2}), UV-B (250-315 nm at 1.0 W m^{-2}) and photosynthetic active radiation (67 µmol quanta m^{-2} s^{-1}) for 24 h at 25 °C. The UV lamps were wrapped in cellulose acetate foil (0,076 mm thick) to screen out any UV-C radiation (<280 nm). UV intensities were measured with UV-AB radiometer (Macam photometrics LTD, Scotland).

Exposure to plant pathogens

X. campestris pv. *vesicatoria* cells were grown to OD_{600} ~ 1.3 in PYDAC medium (Vernière, C. et al (1991) Fruits 46, 162-170). The third and fourth leaves above hypocotyl from eight-week old control and transgenic tobacco plants were inoculated with a suspension of these bacteria in 0.85 % (w/v) NaCl.

Isolates from *Alternaria alternata* strain CEREMIC 1333 were grown for six days in potato dextrose agar. Exposure of tobacco leaves to the pathogen was carried out using two different methods. In one of the procedures, discs of 7 mm were excised from fungal cultures and layered on the surface of cut leaves placed on 1.5 % (w/v) agar in Petri dishes. Plates were incubated at 25 °C essentially as described by Deák et al. (Deák, M. et al. (1999) Nature Biotechnol. 17, 192-196). Alternatively, conidia from a saturated culture were re-suspended in 500 μl of sterile distilled water and infiltrated on the upper surface of the corresponding leaves with the aid of a needle-less syringe.

In all cases, symptoms were evaluated by estimating the diameters of necrotized leaf areas.

Analytical procedures

Carbon dioxide assimilation in response to changes in incident PPFD were measured on the second fully expanded leaf of 4 four-week old transgenic and control plants, using a Qubit Systems Inc. infra-red gas analyzer (IRGA) Package (Kingston, Canada). Chlorophyll contents in leaves and plastids were determined using standard methods (Lichtenthaler, H.K. (1987) Methods Enzymol. 148, 350-382).

Results

Expression Of *Anabaena* PCC 7119 Flavodoxin In Transgenic Tobacco Chloroplasts

To express cyanobacterial flavodoxin in tobacco plastids, a chimeric gene was prepared in which the flavodoxin coding region (Fillat, M. F. et al (1991) Biochem. J. 280, 187-191) was fused in-frame to DNA sequence encoding the chloroplast transit peptide of pea FNR (Newman, B. J., and Gray, J. C. (1988) Plant Mol. Biol. 10, 511-520) (for details, see Methods). The construct was cloned into an *Agrobacterium* binary vector under the control of the constitutive CaMV 35S gene promoter, and delivered into tobacco cells via *Agrobacterium*-mediated leaf disc transformation.

Kanamycin-resistant plants were recovered from tissue culture and evaluated for flavodoxin accumulation by immunoblotting.

Proteins extracted from sampled primary transformants (pfl5-pfl12) or from a wild-type tobacco specimen (PH) were resolved by SDS-PAGE, and either stained with Coomassie Brilliant Blue, or blotted onto nitrocellulose membranes and probed with antisera raised against *Anabaena* PCC7119 flavodoxin using standard techniques (Krapp, A. R. et al. (1997) supra). Proteins corresponding to 16 mm^2 of foliar tissue were loaded onto each lane of the gel. Typical homozygous (pfl5-8 and pfl5-6), heterozygous (pfl5-7) and segregant (pfl5-22) plants were recognised based on their flavodoxin contents.

A mature-sized reactive band could be detected at various levels in leaf extracts obtained from several transformants, suggesting plastid import and processing of the expressed flavoprotein. Immunodetection of flavodoxin in purified chloroplast fractions from transformed plants confirmed that the bacterial protein was targeted to plastids.

Primary transformants displaying high levels of flavodoxin expression, and containing a single transgene insertion *locus* per genome as assessed by Southern blot hybridization, were selfed, and homozygous lines were further selected by flavodoxin dosage.

Chloroplast-Targeted Bacterial Flavodoxin Promotes Tolerance To Methyl Viologen Toxicity In Transgenic Tobacco

Two-week old transgenic (pfl5-8) or wild-type (PH) plantlets were cultured on MS agar broth containing 15 μM or 30 μM MV and illuminated at 300 μmol quanta m^{-2} s^{-1} for 24 h at 25°C. Four-week old plants grown under hydroponic conditions were exposed to 30 μM MV in the nutrient solution, using the incubation time and light regime reported above.

Two-week old plantlets expressing flavodoxin were observed to survive treatment with 30-50 μM of the superoxide radical propagator MV in illuminated agar plates, whereas non-transformed tobacco controls were extensively bleached under the same conditions. Four week old plants were more tolerant to MV, but significant differences were still evident between transgenic plants and their wild-type siblings (Fig. 1).

Experiments were performed to show the tolerance of flavodoxin-proficient leaf discs to MV as described above. Leaf tissue bleaching was perceived visually in the control discs, reflecting increased chlorophyll degradation. Conductance values were corrected for ion leakage occurring in the dark under the same conditions. The ion leakage values of each sample were expressed as a percentage of the total ion content (maximal value obtained after autoclaving the leaf disks at the end of the MV treatment). Chlorophyll contents were expressed as the fraction of the total chlorophyll content of leaf disks incubated under the same conditions in the absence of MV. The heights of the vertical bars represent the averages of four experiments with SE lower than 15% (Fig. 1).

Over-production of flavodoxin was observed to provide protection against MV-induced ion leakage (which is indicative of cell membrane deterioration) and bleaching of leaf discs from two-month old plants. In all cases, the extent of damage decreased as the levels of chloroplastic flavodoxin were raised.

Flavodoxin Protects Tobacco Plants Against Extreme Temperatures And Irradiation.

Transformants of the F_1 generation (pfl5-8) and their wild-type siblings (PH) were exposed to 40°C, high fluence rates, or ultraviolet UV-AB radiation, as described under Methods. Four-week old plants were used in all cases, except for the heating experiments, in which two-week old specimens were employed.

To investigate differences in chilling sensitivity of wild-type and flavodoxin-expressing tobacco plants, seeds of control and transgenic plants were germinated on MS solid medium supplemented with 2 % (w/v) sucrose for two weeks at 25°C and 200 µmol quanta m^{-2} s^{-1} (14 h light/10 h dark). Seedlings were transferred to 9°C under the same light regime for other two weeks allowing plants to acclimate. Thereafter, they were continuously illuminated (500 µmol quanta m^{-2} s^{-1}) for one more week at 9°C.

To investigate water-deficit tolerance of transgenic tobacco expressing flavodoxin, plants were grown for two weeks in MS as described above and then transferred to soil and daily irrigated with nutrient solution. The water-deficit stress was applied to two month-old plants by withholding water for up to 3 days. All other growing conditions were the same as described in Methods

Transformants exhibited increased tolerance to this drought regime, whereas damage in control plants was reflected by extensive leaf withering and/or bleaching, with concomitant decreases in chlorophyll contents and photosynthetic capacities.

Young tobacco plantlets accumulating flavodoxin were found to survive prolonged illumination (500 µmol quanta m^{-2} s^{-1} for 12 h) at 40°C and exhibited increased tolerance to chilling. Under similar conditions, wild-type control seedlings were severely damaged.

Exposure of the plants to ultraviolet AB (UV-AB) radiation, extremely high light intensities or water deficiency yielded essentially the same results as above.

The damage caused by these treatments in control tobacco plants was reflected by extensive bleaching of the leaf tissue, with concomitant decreases in chlorophyll contents and photosynthetic capacities.

Reduced Damage In Tobacco Transformants Expressing Flavodoxin Exposed To Necrotrophic Pathogens.

Control and flavodoxin-expressing tobacco plants, grown in the greenhouse for eight weeks were inoculated with a suspension of the pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria*, known to induce the hypersensitive response in tobacco (Baker, C. J., and Orlandi, E. W. (1995) Ann. Rev. Phytopathol. 33, 299-321). The number of necrotic symptoms was drastically reduced in transformants expressing the cyanobacterial protein, with a negative correlation between the extent of damage and the flavodoxin levels accumulated in the corresponding tissue.

Fungal infection with *A. alternata* was done using two different procedures. Firstly, discs of 7 mm excised from fungal cultures were layered on the surface of cut leaves placed on 1.5 % (w/v) agar in Petri dishes. Plates were incubated at 25 °C essentially as described by Deák et al (Deák et al (1999) supra) for 10 days. Secondly, a disc of filter paper imbibed in conidia from a saturated culture (around 20,000 conidia) was placed on the upper surface of the corresponding leaves. Symptoms on the leaves were observed after 18 days of fungal infection.

Transgenic plants were observed to be more tolerant than their wild-type siblings to necrosis induced by infection with the pathogenic fungus *Alternaria alternata*.

To investigate necrotization of tobacco wild-type and transgenic leaves after infection by tobacco necrotic virus, plants were planted in soil and grown for three weeks at 25°C and 300 µmol quanta m^{-2} s^{-1} with 14 h light/10 h dark as described in Methods. The third leaves position above hypocotyl were inoculated with a suspension of the tobacco necrotic virus, with an abrasive and kept under the same growing conditions. Lesions were examined after one week of inoculation.

Transgenic plants were observed to be more tolerant than their wild-type siblings to necrosis induced by infection with the tobacco necrotic virus.

Expression of flavodoxin in plant cells was thus observed to protect transgenic plants against pathogens that cause necrotic lesions.

Table 1:

Accession No		**Gene**	**Species**
NP_358768	gi|15903218	Flavodoxin	Streptococcus pneumoniae R6
NP_345761	gi|15901157	Flavodoxin	Streptococcus pneumoniae TIGR4
NP_311794	gi|15833021	flavodoxin 2	Escherichia coli O157:H7]
NP_311593	gi|15832820	putative flavodoxin	Escherichia coli O157:H7
NP_308742	gi|15829969	flavodoxin 1	Escherichia coli O157:H7
CAC92877	gi|15980620	flavodoxin 1	Yersinia pestis
CAC89737	gi|15978964	flavodoxin 2	Yersinia pestis
NP_350007	gi|15896658	Flavodoxin	Clostridium acetobutylicum
NP_349066	gi|15895717	Flavodoxin	Clostridium acetobutylicum
NP_347225	gi|15893876	Flavodoxin	Clostridium acetobutylicum
NP_346845	gi|15893496	Flavodoxin	Clostridium acetobutylicum
NP_348645	gi|15895296	Predicted flavodoxin	Clostridium acetobutylicum
NP_347225	gi|15893876	Flavodoxin	Clostridium acetobutylicum
NP_346845	gi|15893496	Flavodoxin	Clostridium acetobutylicum
NP_282528	gi|15792705	Flavodoxin	Campylobacter jejuni
AAK28628	gi|13507531	Flavodoxin	Aeromonas hydrophila
NP_268951	gi|15674777	putative flavodoxin	Streptococcus pyogenes
NP_266764	gi|15672590	Flavodoxin	Lactococcus lactis

			subsp. lactis
NP_207952	gi|15645775	flavodoxin (fldA)	Helicobacter pylori 26695
NP_232050	gi|15642417	flavodoxin 2	Vibrio cholerae
NP_231731	gi|15642099	flavodoxin 1	Vibrio cholerae
NP_219360	gi|15639910	Flavodoxin	Treponema pallidum
NP_24012	gi|15616909	flavodoxin 1	Buchnera sp. APS
NP_214435	gi|15607053	Flavodoxin	Aquifex aeolicus
FXAVEP	gi|625194	flavodoxin	Azotobacter vinelandii
S38632	gi|481443	flavodoxin	-Synechocystis sp. (strain PCC 6803)
FXDV	gi|476442	flavodoxin	Desulfovibrio vulgaris
A34640	gi|97369	flavodoxin	Desulfovibrio salexigens
S24311	gi|97368	flavodoxin	Desulfovibrio gigas (ATCC 19364)
A37319	gi|95841	flavodoxin A	Escherichia coli
S06648	gi|81145	flavodoxin	red alga (Chondrus crispus)
S04600	gi|79771	flavodoxin	Anabaena variabilis
A28670	gi|79632	flavodoxin	Synechococcus sp
S02511	gi|78953	flavodoxin	Klebsiella pneumoniae
FXDVD	gi|65884	flavodoxin	Desulfovibrio desulfuricans (ATCC 29577)
FXCLEX	gi|65882	flavodoxin	Clostridium sp
FXME	gi|65881	flavodoxin	Megasphaera elsdenii
NP_071157	gi|11499913	flavodoxin, putative	Archaeoglobus fulgidus
BAA17947	gi|1653030	flavodoxin	Synechocystis sp. PCC 6803
BAB61723	gi|14587807	flavodoxin 2	Vibrio fischeri
BAB61721	gi|14587804	flavodoxin 1	Vibrio fischeri
AAK66769	gi|14538018	flavodoxin	Histophilus ovis
P57385	gi|11132294	FLAVODOXIN	

AAC7593	gi|1789262	flavodoxin 2	Escherichia coli K12
AAC73778	gi|1786900	flavodoxin 1	Escherichia coli K12
AAC75752	gi|1789064	putative flavodoxin	Escherichia coli K12
F69821	gi|7429905	flavodoxin homolog yhcB	Bacillus subtilis
QQKBFP	gi|2144338	pyruvate (flavodoxin) dehydrogenase nifJ	Klebsiella pneumoniae
S16929	gi|95027	flavodoxin A	Azotobacter chroococcum
F71263	gi|7430914	probable flavodoxin	Syphilis spirochete
A64665	gi|7430911	flavodoxin	Helicobacter pylori(strain 26695
JE0109	gi|7430907	flavodoxin	Desulfovibrio vulgaris
S42570	gi|628879	flavodoxin	Desulfovibrio desulfuricans (ATCC 27774)
BAB13365	gi|10047146	flavodoxin	Alteromonas sp. O-7
AAF34250	gi|6978032	flavodoxin	Desulfovibrio gigas
CAB73809	gi|6968816	flavodoxin	Campylobacter jejuni
D69541	gi|7483302	flavodoxin homolog	Archaeoglobus fulgidus
F70479	gi|7445354	flavodoxin	Aquifex aeolicus
S55234	gi|1084290	flavodoxin isoform I	Chlorella fusca
S18374	gi|2117434	flavodoxin	Anabaena sp. (PCC 7119)
S55235	gi|1084291	flavodoxin isoform II	Chlorella fusca
C64053	gi|1074088	flavodoxin A	Haemophilus influenzae (strain Rd KW20)
A61338	gi|625362	flavodoxin	Clostridium

			pasteurianum
A39414	gi|95560	flavodoxin	Enterobacter agglomerans plasmid pEA3
AAD08207	gi|2314319	flavodoxin (fldA)	Helicobacter pylori 26695
CAB37851	gi|4467982	flavodoxin	Rhodobacter capsulatus
AAC65882	gi|3323245	flavodoxin	Treponema pallidum
AAB88920	gi|2648181	flavodoxin, putative	Archaeoglobus fulgidus
AAB65080	gi|2289914	flavodoxin	Klebsiella pneumoniae
AAB53659	gi|710356	flavoprotein	Methanothermobacter thermautotrophicus
AAB51076	gi|1914879	flavodoxin	Klebsiella pneumoniae
AAB36613	gi|398014	flavodoxin	Azotobacter chroococcum
AAB20462	gi|239748	flavodoxin	Anabaena
AAA64735	gi|142370	flavodoxin(nifF)	Azotobacter vinelandii
BAA35341	gi|1651296	Flavodoxin	Escherichia coli
BAA35333	gi|1651291	Flavodoxin	Escherichia coli
AAA27288	gi|415254	flavodoxin	Synechocystis sp.
AAA27318	gi|154528	Flavodoxin	Synechococcus sp.
AAC45773	gi|1916334	putative flavodoxin	Salmonella typhimurium
AAC07825	gi|2984302	flavodoxin	Aquifex aeolicus
AAC02683	gi|2865512	flavodoxin	Trichodesmium erythraeum

Table 2:

Accession No		**Gene**	**Species**
P32260	gi|12644209	CYSTEINE SYNTHASE, CHLOROPLAST PRECURSOR	Spinacia oleracea
AAG59996	gi|12658639	ferredoxin:sulfite reductase precursor	Glycine max
S10200	gi|100078	carbonate dehydratase precursor	Pisum sativum
CAB89287	gi|7672161	chloroplast FtsZ-like protein	Nicotiana tabacum
P17067	gi|115471	CARBONIC ANHYDRASE, CHLOROPLAST PRECURSOR (CARBONATE DEHYDRATASE)	Pisum sativum
AAD22109	gi|4530595	heme oxygenase 2	Arabidopsis thaliana
AAD22108	gi|4530593	heme oxygenase 1	Arabidopsis thaliana
AAC50035	gi|450235	APS kinase	Arabidopsis thaliana
AAC12846	gi|1051180	phytoene desaturase	Zea mays
AAB87573	gi|2645999	chlorophyll a/b binding protein of LHCII type I precursor	Panax ginseng
CAA47329	gi|312944	cysteine synthase	Spinacia oleracea
CAA31137	gi|41201	O-acetylserine sulfhydrylase	Escherichia coli
AAA82068	gi|1079732	cpFtsZ	Arabidopsis thaliana
T06368	gi|7489040	photosystem II oxygen-evolving complex protein 1 precursor	Lycopersicon esculentum
S71750	gi|7488813	import intermediate-associated 100K protein precursor	Pisum sativum
S71749	gi|7459239	DCL protein precursor, chloroplast	Lycopersicon esculentum
15825883	gi|15825883	Chain B, Structure Of Threonine Synthase	Arabidopsis thaliana

15825882	gi|15825882	Chain A, Structure Of Threonine Synthase	Arabidopsis thaliana
T09543	gi|7488970	deoxyxylulose synthase TKT2 precursor	Capsicum annuum
JC5876	gi|7447856	early light-inducible protein precursor	Glycine max
P24493	gi|1170215	DELTA-AMINOLEVULINIC ACID DEHYDRATASE PRECURSOR	Spinacia oleracea
S47966	gi|1076532	probable lipid transfer protein M30 precursor	Pisum sativum
A44121	gi|322404	ribosomal protein S1 precursor	Spinacia oleracea
S01056	gi|81896	early light-induced protein precursor	Pisum sativum
O22773	gi|7388292	THYLAKOID LUMENAL 16.5 KDA PROTEIN, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P80470	gi|6093830	PHOTOSYSTEM II CORE COMPLEX PROTEINS PSBY PRECURSOR	Spinacia oleracea
P55195	gi|1709930	PHOSPHORIBOSYLAMINOIMIDAZOLE CARBOXYLASE, CHLOROPLAST PRECURSOR	Vigna aconitifolia
P11970	gi|1709654	PLASTOCYANIN B, CHLOROPLAST PRECURSOR	Populus nigra
P00299	gi|1709651	PLASTOCYANIN A, CHLOROPLAST PRECURSOR	Populus nigra
P80484	gi|1709608	PERIDININ-CHLOROPHYLL A PROTEIN 1 PRECURSOR	Amphidinium carterae
P08823	gi|134102	RUBISCO SUBUNIT BINDING-PROTEIN ALPHA SUBUNIT PRECURSOR	Triticum aestivum
P04045	gi|130173	ALPHA-1,4 GLUCAN PHOSPHORYLASE, L-1 ISOZYME, CHLOROPLAST PRECURSOR	Solanum tuberosum
S30897	gi|7427677	3-isopropylmalate dehydrogenase precursor	Solanum tuberosum
TXSPM	gi|7427615	thioredoxin m precursor	Spinacia oleracea

FEKM	gi|7427604	ferredoxin [2Fe-2S] precursor	Chlamydomonas reinhardtii
CCKM6R	gi|2144284	cytochrome c6 precursor	Chlamydomonas reinhardtii
S30145	gi|419757	ketol-acid reductoisomerase precursor	Arabidopsis thaliana
DEMZMC	gi|319840	malate dehydrogenase (NADP+) precursor	Zea mays
S20510	gi|81676	3-isopropylmalate dehydrogenase precursor	Brassica napus
S17180	gi|81509	ketol-acid reductoisomerase precursor	Spinacia oleracea
Q9SEL7	gi|15214049	PROTEASE HHOA, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
O23403	gi|13959580	THYLAKOID LUMENAL 21.5 KDA PROTEIN, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P82281	gi|12644689	PUTATIVE L-ASCORBATE PEROXIDASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
O22609	gi|9910645	PROTEASE DO-LIKE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P48417	gi|1352186	ALLENE OXIDE SYNTHASE, CHLOROPLAST PRECURSOR	Linum usitatissimum
P49080	gi|1351905	BIFUNCTIONAL ASPARTOKINASE/HOMOSERINE DEHYDROGENASE 2, CHLOROPLAST PRECURSOR	Zea mays
P31853	gi|461595	ATP SYNTHASE B' CHAIN, CHLOROPLAST PRECURSOR	Spinacia oleracea
P10933	gi|119905	FERREDOXIN--NADP REDUCTASE, LEAF ISOZYME PRECURSOR	Pisum sativum
P52422	gi|14917033	PHOSPHORIBOSYLGLYCINAMIDE FORMYLTRANSFERASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P49077	gi|14917032	ASPARTATE	Arabidopsis

		CARBAMOYLTRANSFERASE PRECURSOR	thaliana
O50039	gi|14917022	ORNITHINE CARBAMOYLTRANSFERASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P55229	gi|14916987	GLUCOSE-1-PHOSPHATE ADENYLYLTRANSFERASE LARGE SUBUNIT 1, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q96291	gi|14916972	2-CYS PEROXIREDOXIN BAS1, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q9ZT00	gi|14916690	RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE ACTIVASE, CHLOROPLAST PRECURSOR	Zea mays
Q9LZX6	gi|14547977	DIHYDRODIPICOLINATE SYNTHASE 1, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
O64903	gi|12644076	NUCLEOSIDE DIPHOSPHATE KINASE II, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
O04130	gi|3122858	D-3-PHOSPHOGLYCERATE DEHYDROGENASE PRECURSOR	Arabidopsis thaliana
O24364	gi|3121825	2-CYS PEROXIREDOXIN BAS1, CHLOROPLAST PRECURSOR	Spinacia oleracea
P49107	gi|1709825	PHOTOSYSTEM I REACTION CENTRE SUBUNIT N PRECURSOR	Arabidopsis thaliana
P49132	gi|1352199	TRIOSE PHOSPHATE/PHOSPHATE TRANSLOCATOR, CHLOROPLAST PRECURSOR	Flaveria trinervia
P37107	gi|586038	SIGNAL RECOGNITION PARTICLE 54 KDA PROTEIN, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q04836	gi|464662	31 KDA RIBONUCLEOPROTEIN,	Arabidopsis thaliana

		CHLOROPLAST PRECURSOR	
Q01909	gi|461551	ATP SYNTHASE GAMMA CHAIN 2, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P14671	gi|136251	TRYPTOPHAN SYNTHASE BETA CHAIN 1 PRECURSOR	Arabidopsis thaliana
P07089	gi|132144	RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN PRECURSOR	Flaveria trinervia
P22221	gi|130384	PYRUVATE,PHOSPHATE DIKINASE PRECURSOR	Flaveria trinervia
P22178	gi|126736	NADP-DEPENDENT MALIC ENZYME, CHLOROPLAST PRECURSOR	Flaveria trinervia
P26259	gi|118241	DIHYDRODIPICOLINATE SYNTHASE, CHLOROPLAST PRECURSOR	Zea mays
P23577	gi|118044	APOCYTOCHROME F PRECURSOR	Chlamydomonas reinhardtii
Q42522	gi|14195661	GLUTAMATE-1-SEMIALDEHYDE 2,1-AMINOMUTASE 2 PRECURSOR	Arabidopsis thaliana
Q96242	gi|13878924	ALLENE OXIDE SYNTHASE PRECURSOR	Arabidopsis thaliana
P46312	gi|13432148	OMEGA-6 FATTY ACID DESATURASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P34802	gi|13432144 |	GERANYLGERANYL PYROPHOSPHATE SYNTHETASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P50318	gi|12644295	PHOSPHOGLYCERATE KINASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P46309	gi|12644273	GLUTAMATE--CYSTEINE LIGASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P21276	gi|12644157	SUPEROXIDE DISMUTASE [FE], CHLOROPLAST PRECURSOR	Arabidopsis thaliana

O23787	gi|6094476	THIAZOLE BIOSYNTHETIC ENZYME, CHLOROPLAST PRECURSOR	Citrus sinensis
P93407	gi|3915008	SUPEROXIDE DISMUTASE [CU-ZN], CHLOROPLAST PRECURSOR	Oryza sativa
Q96255	gi|3914996	PHOSPHOSERINE AMINOTRANSFERASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
O24600	gi|3914826	DNA-DIRECTED RNA POLYMERASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
O49937	gi|3914665	50S RIBOSOMAL PROTEIN L4, CHLOROPLAST PRECURSOR	Spinacia oleracea
Q42915	gi|3914608	RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN PRECURSOR	Manihot esculenta
Q39199	gi|2500098	DNA REPAIR PROTEIN RECA, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q96529	gi|2500026	ADENYLOSUCCINATE SYNTHETASE PRECURSOR	Arabidopsis thaliana
P55826	gi|2495184	PROTOPORPHYRINOGEN OXIDASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q42496	gi|2493687	CYTOCHROME B6-F COMPLEX 4 KDA SUBUNIT, CHLOROPLAST PRECURSOR	Chlamydomonas reinhardtii
P52424	gi|1709925	PHOSPHORIBOSYLFORMYLGLYCINAMIDINE CYCLO-LIGASE, CHLOROPLAST PRECURSOR	Vigna unguiculata
P49572	gi|1351303	INDOLE-3-GLYCEROL PHOSPHATE SYNTHASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P48496	gi|1351271	TRIOSEPHOSPHATE ISOMERASE, CHLOROPLAST PRECURSOR	Spinacia oleracea
P25269	gi|1174779	TRYPTOPHAN SYNTHASE	Arabidopsis

		BETA CHAIN 2 PRECURSOR	thaliana
P46225	gi|1174745	TRIOSEPHOSPHATE ISOMERASE, CHLOROPLAST PRECURSOR	Secale cereale
P46283	gi|1173345	SEDOHEPTULOSE-1,7-BISPHOSPHATASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
P32069	gi|418134	ANTHRANILATE SYNTHASE COMPONENT I-2 PRECURSOR	Arabidopsis thaliana
P29450	gi|267120	THIOREDOXIN F-TYPE, CHLOROPLAST PRECURSOR	Pisum sativum
Q9ZTN9	gi|13878459	PHYTOENE DEHYDROGENASE PRECURSOR	Oryza sativa
Q9SHI1	gi|13627881	TRANSLATION INITIATION FACTOR IF-2, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q9LR75	gi|13431553	COPROPORPHYRINOGEN III OXIDASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q9ZNZ7	gi|12643970	FERREDOXIN-DEPENDENT GLUTAMATE SYNTHASE 1, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q9SZ30	gi|12643854	BIFUNCTIONAL HISTIDINE BIOSYNTHESIS PROTEIN HISHF, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q9SJE1	gi|12643848	MAGNESIUM-CHELATASE SUBUNIT CHLD PRECURSOR	Arabidopsis thaliana
Q42624	gi|12643761	GLUTAMINE SYNTHETASE, CHLOROPLAST PRECURSOR	Brassica napus
Q38933	gi|12643749	LYCOPENE BETA CYCLASE, CHLOROPLAST PRECURSOR	Arabidopsis thaliana
Q42435	gi|12643508	CAPSANTHIN/CAPSORUBIN SYNTHASE, CHLOROPLAST PRECURSOR	Capsicum annuum

5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Plant Bioscience Limited
(B) STREET: Norwich Science Park, Colney Lane
(C) CITY: Norwich
(D) STATE: Norfolk
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): NR4 7UH

(ii) TITLE OF INVENTION: Stress Tolerant Plants

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
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(D) STATE: Ontario
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): M5H 3Y2

(v) COMPUTER READABLE FORM:
(A) COMPUTER: IBM PC compatible
(B) OPERATING SYSTEM: PC-DOS/MS-DOS
(C) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO); and Microsoft Word.

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: CA 2,360,107
(B) FILING DATE: 24-OCT-2001

(viii) PATENT AGENT INFORMATION:
(A) NAME: David W.R. Langton
(B) REGISTRATION NUMBER: 2800
(B) REFERENCE NUMBER: 420-398/DL

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 706 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGATCCATCA TCAACAACAA CAACAAACAT GGCTGCTGCA GTAACAGCCG CAGTCTCCTT 60

GCCATACTCC AACTCCACTT CCCTTCCGAT CAGAACATCT ATTGTTGCAC CAGAGAGACT 120

TGTCTTCAAA AAGGTTTCAT TGAACAATGT TTCTATAAGT GGAAGGGTAG GCACCATCAG 180

AGCTCTCATA ATGTCAAAGA AAATTGGTTT ATTCTACGGT ACTCAAACTG GTAAAACTGA 240

ATCAGTAGCA GAAATCATTC GAGACGAGTT TGGTAATGAT GTGGTGACAT TACACGATGT 300

TTCCCAGGCA GAAGTAACTG ACTTGAATGA TTATCAATAT TTGATTATTG GCTGTCCTAC 360

TTGGAATATT GGCGAACTGC AAAGCGATTG GGAAGGACTC TATTCAGAAC TGGATGATGT 420

AGATTTTAAT GGTAAATTGG TTGCCTACTT TGGGACTGGT GACCAAATAG GTTACGCAGA 480

```
TAATTTTCAG GATGCGATCG GTATTTTGGA AGAAAAAATT TCTCAACGTG GTGGTAAAAC      540

TGTCGGCTAT TGGTCAACTG ATGGATATGA TTTTAATGAT TCCAAGGCAC TAAGAAATGG      600

CAAGTTTGTA GGACTAGCTC TTGATGAAGA TAATCAATCT GACTTAACAG ACGATCGCAT      660

CAAAAGTTGG GTTGCTCAAT TAAAGTCTGA ATTTGGTTTG TAAAAA                     706
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Asp Pro Ser Ser Thr Thr Thr Thr Asn Met Ala Ala Ala Val Thr Ala
1               5                   10                  15

Ala Val Ser Leu Pro Tyr Ser Asn Ser Thr Ser Leu Pro Ile Arg Thr
            20                  25                  30

Ser Ile Val Ala Pro Glu Arg Leu Val Phe Lys Lys Val Ser Leu Asn
        35                  40                  45

Asn Val Ser Ile Ser Gly Arg Val Gly Thr Ile Arg Ala Leu Ile Met
    50                  55                  60

Ser Lys Lys Ile Gly Leu Phe Tyr Gly Thr Gln Thr Gly Leu Thr Glu
65                  70                  75                  80

Ser Val Ala Glu Ile Ile Arg Asp Glu Phe Gly Asn Asp Val Val Thr
                85                  90                  95

Leu His Asp Val Ser Gln Ala Glu Val Thr Asp Leu Asn Asp Tyr Gln
            100                 105                 110

Tyr Leu Ile Ile Gly Cys Pro Thr Trp Asn Ile Gly Glu Leu Gln Ser
        115                 120                 125

Asp Trp Glu Gly Leu Tyr Ser Glu Leu Asp Asp Val Asp Phe Asn Gly
    130                 135                 140

Lys Leu Val Ala Tyr Phe Gly Thr Gly Asp Gln Ile Gly Tyr Ala Asp
145                 150                 155                 160

Asn Phe Gln Asp Ala Ile Gly Ile Leu Glu Glu Lys Ile Ser Gln Arg
                165                 170                 175

Gly Gly Lys Thr Val Gly Tyr Trp Ser Thr Asp Gly Tyr Asp Phe Asn
            180                 185                 190

Asp Ser Lys Ala Leu Arg Asn Gly Lys Phe Val Gly Leu Ala Leu Asp
        195                 200                 205

Glu Asp Asn Gln Ser Asp Leu Thr Asp Asp Arg Ile Lys Ser Trp Val
    210                 215                 220

Ala Gln Leu Lys Ser Glu Phe Gly Leu
225                 230
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 162 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGCTGCTG CAGTAACAGC CGCAGTCTCC TTGCCATACT CCAACTCCAC TTCCCTTCCG 60

ATCAGAACAT CTATTGTTGC ACCAGAGAGA CTTGTCTTCA AAAAGGTTTC ATTGAACAAT 120

GTTTCTATAA GTGGAAGGGT AGGCACCATC AGAGCTCTCA TA 162

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ala Ala Ala Val Thr Ala Ala Val Ser Leu Pro Tyr Ser Asn Ser
1               5                   10                  15

Thr Ser Leu Pro Ile Arg Thr Ser Ile Val Ala Pro Glu Arg Leu Val
            20                  25                  30

Phe Lys Lys Val Ser Leu Asn Asn Val Ser Ile Ser Gly Arg Val Gly
        35                  40                  45

Thr Ile Arg Ala Leu Ile
    50
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GACGAGCTCT CATAATGTCA AAG 23

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACTGTCGACT TTTTACAAAC CAAAT 25

CLAIMS:

1. A nucleic acid encoding a fusion polypeptide comprising a flavodoxin polypeptide and a chloroplast targeting peptide.

2. A nucleic acid according to claim 1 wherein the flavodoxin polypeptide is a bacterial flavodoxin.

3. A nucleic acid according to claim 1 or claim 2 wherein the flavodoxin polypeptide is a cyanobacterial flavodoxin.

4. A nucleic acid according to claim 3 wherein the flavodoxin polypeptide is the Anabaena PC7119 flavodoxin polypeptide.

5. A nucleic acid according to any one of claims 1 to 4 wherein the chloroplast targeting signal is the chloroplast transit polypeptide of the pea ferredoxin-NADP reductase (FNR).

6. A nucleic acid according to any one of claims 1 to 5 encoding a fusion polypeptide having the sequence of SEQ ID No. 2.

7. A nucleic acid according to claim 6 having the nucleotide sequence of SEQ ID No. 1.

8. A nucleic acid vector suitable for transformation of a plant cell comprising a nucleic acid according to any one of claims 1 to 7.

9. A host cell containing a nucleic acid or nucleic acid vector according to any one of claims 1 to 8.

10. A host cell according to claim 9 which is microbial.

11. A host cell according to claim 9 which is a plant cell.

12. A plant cell comprising a heterologous nucleic acid encoding a flavodoxin polypeptide.

13. A plant cell according to claim 11 or claim 12 having said nucleic acid within its chromosome.

14. A plant cell according to any one of claims 11 to 13 having more than one said nucleic acid per haploid genome.

15. An isolated polypeptide encoded by a nucleic acid according to any of claims 1 to 7.

16. A method of producing a cell, the cell being a cell according to any of claims 9 to 14, the method including incorporating said nucleic acid or nucleic acid vector into the cell by means of transformation.

17. A method according to claim 16 which includes recombining the nucleic acid with the cell genome nucleic acid such that it is stably incorporated therein.

18. A method according to claim 16 or claim 17 which includes regenerating a plant from one or more transformed cells.

19. A method of producing a plant, the method including incorporating a nucleic acid encoding a flavodoxin polypeptide into a plant cell and regenerating a plant from said plant cell.

20. A method according to claim 19 wherein the nucleic acid is a nucleic acid or nucleic acid vector according to any one of claims 1 to 8.

21. A method according to claim 19 or claim 20 including sexually or asexually propagating or growing off-spring or a descendant of the plant regenerated from said plant cell.

22. A method of improving the stress tolerance of a plant, the method including causing or allowing transcription from a heterologous nucleic acid encoding a flavodoxin polypeptide within cells of the plant.

23. A method according to claim 22 comprising allowing the accumulation of the flavodoxin polypeptide in the chloroplasts of said cells.

24. A method according to any one of claims 20 to 23 wherein nucleic acid is a nucleic acid according to any one of claims 1 to 7.

25. A method according to any one of claims 22 to 24 wherein said stress is selected from the group consisting of ultraviolet UV radiation, extreme temperatures, irradiation, and pathogen infection.

26. Use of a nucleic acid according to any of claims 1 to 7 in the production of a transgenic plant.

27. Use of a nucleic acid according to any one of claims 1 to 7 in a method of increasing the tolerance of a plant to stress.

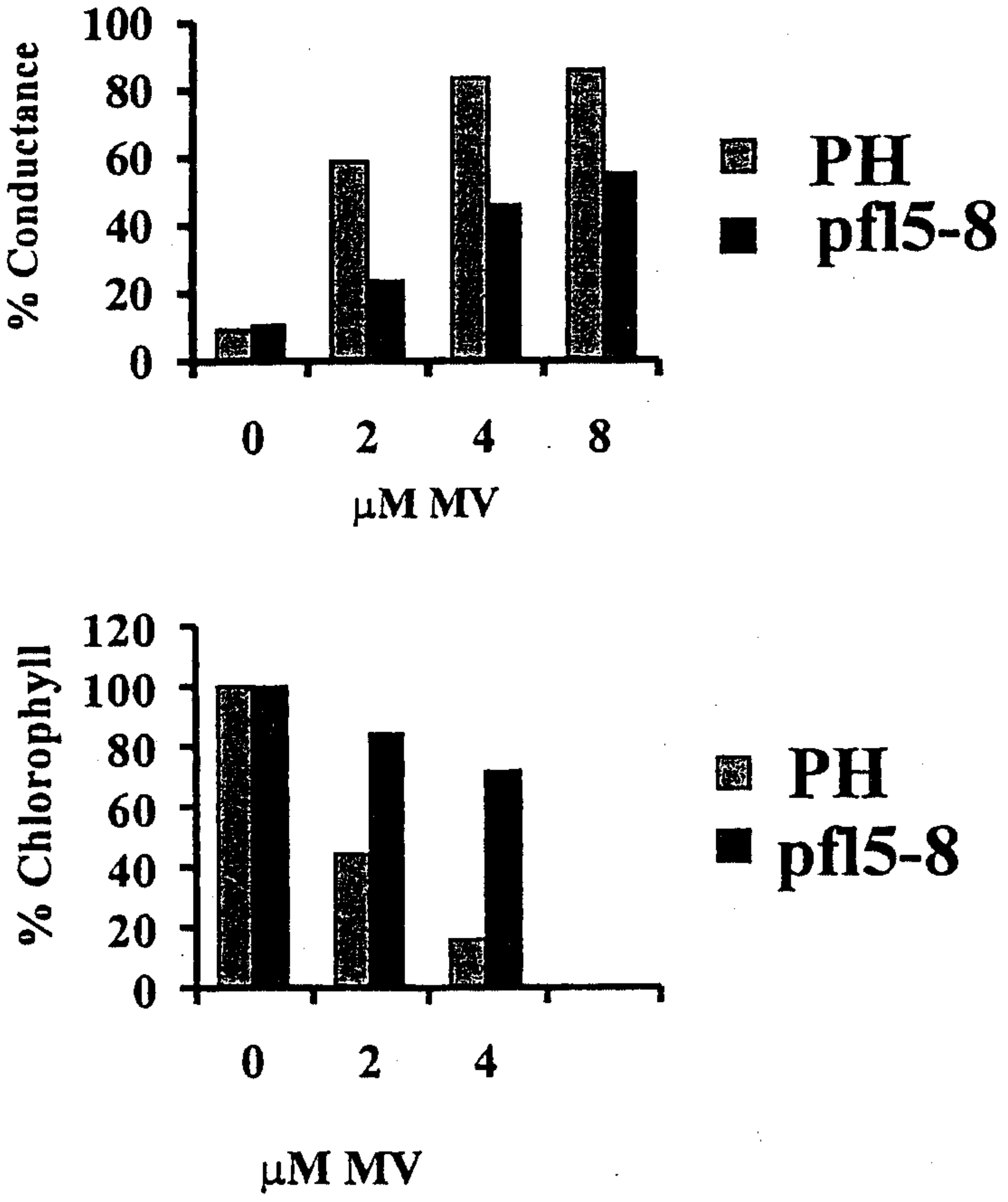
100
80
60
40
20
0
% Conductance
0
2
4
8
µM MV
PH
pfl5-8
120
100
80
60
40
20
0
% Chlorophyll
0
2
4
µM MV
PH
pfl5-8

Figure 1

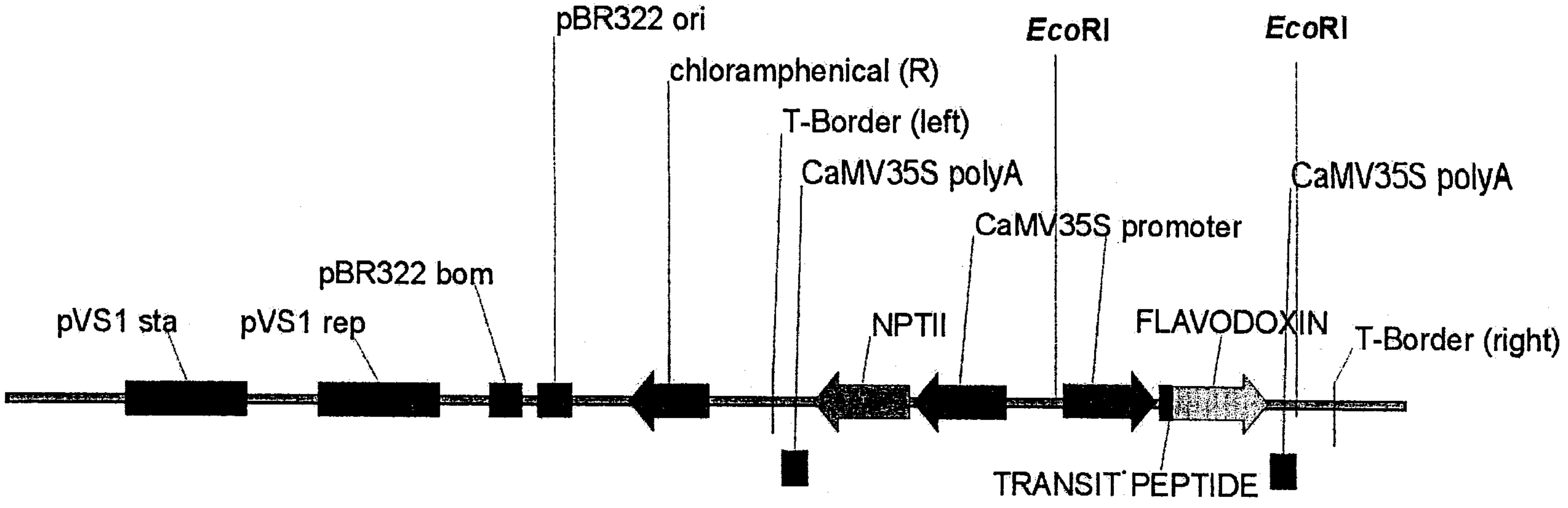
pBR322 ori
chloramphenical (R)
T-Border (left)
CaMV35S polyA
EcoRI
EcoRI
CaMV35S polyA
CaMV35S promoter
pBR322 bom
pVS1 sta
pVS1 rep
NPTII
FLAVODOXIN
T-Border (right)
TRANSIT PEPTIDE

Figure 2

GGATCCATCATCAACAACAACAACAAAC**ATG**GCTGCTGCAGTAACAGCCGCAGTCTC
CTTGCCATACTCCAACTCCACTTCCCTTCCGATCAGAACATCTATTGTTGCACCAGA
GAGACTTGTCTTCAAAAAGGTTTCATTGAACAATGTTTCTATAAGTGGAAGGGTAGG
CACCATCAGAGCTCTCATA**ATG**TCAAAGAAAATTGGTTTATTCTACGGTACTCAAAC
TGGTAAAACTGAATCAGTAGCAGAAATCATTCGAGACGAGTTTGGTAATGATGTGGT
GACATTACACGATGTTTCCCAGGCAGAAGTAACTGACTTGAATGATTATCAATATTT
GATTATTGGCTGTCCTACTTGGAATATTGGCGAACTGCAAAGCGATTGGGAAGGACT
CTATTCAGAACTGGATGATGTAGATTTTAATGGTAAATTGGTTGCCTACTTTGGGAC
TGGTGACCAAATAGGTTACGCAGATAATTTTCAGGATGCGATCGGTATTTTGGAAGA
AAAAATTTCTCAACGTGGTGGTAAAACTGTCGGCTATTGGTCAACTGATGGATATGA
TTTTAATGATTCCAAGGCACTAAGAAATGGCAAGTTTGTAGGACTAGCTCTTGATGA
AGATAATCAATCTGACTTAACAGACGATCGCATCAAAAGTTGGGTTGCTCAATTAAA
GTCTGAATTTGGTTTG**TAA**AAA

Figure 3

D P S S T T T T N M A A A V T A A V S L P Y
S N S T S L P I R T S I V A P E R L V F K K
V S L N N V S I S G R V G T I R A L I M S K
K I G L F Y G T Q T G L T E S V A E I I R D
E F G N D V V T L H D V S Q A E V T D L N D
Y Q Y L I I G C P T W N I G E L Q S D W E G
L Y S E L D D V D F N G K L V A Y F G T G D
Q I G Y A D N F Q D A I G I L E E K I S Q R
G G K T V G Y W S T D G Y D F N D S K A L R
N G K F V G L A L D E D N Q S D L T D D R I
K S W V A Q L K S E F G L

Figure 4

ATGGCTGCTGCAGTAACAGCCGCAGTCTCCTTGCCATACTCCAACTCCACTTCCCTT
CCGATCAGAACATCTATTGTTGCACCAGAGAGACTTGTCTTCAAAAAGGTTTCATTG
AACAATGTTTCTATAAGTGGAAGGGTAGGCACCATCAGAGCTCTCATA

Figure 5

M A A A V T A A V S L P Y S N S T S L P I R T S

I V A P E R L V F K K V S L N N V S I S G R V G

T I R A L I

Figure 6


pBR322 ori
*Eco*RI
*Eco*RI
chloramphenical (R)
T-Border (left)
CaMV35S polyA
CaMV35S polyA
CaMV35S promoter
pBR322 bom
pVS1 sta
pVS1 rep
NPTII
FLAVODOXIN
T-Border (right)
TRANSIT PEPTIDE