

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978
PUBLICATION PARTICULARS AND ABSTRACT
[Section 32 (3) (a) - Regulations 22 (1) (g) and 31]

OFFICIAL APPLICATION NO		LODGING DATE		ACCEPTANCE DATE	
21	Q1 2006/10559	22	15 Dec 2006	43	7/4/2008

INTERNATIONAL CLASSIFICATION		Not for publication	
51	C12N A01H	Classified by:	

FULL NAME(S) OF APPLICANT(S)	
71	SCHMÜLLING, Thomas; WERNER, Tomas

FULL NAME(S) OF INVENTOR(S)	
72	SCHMÜLLING, Thomas; WERNER, Tomas

TITLE OF INVENTION	
54	METHOD FOR MODIFYING PLANT MORPHOLOGY, BIOCHEMISTRY AND PHYSIOLOGY COMPRISING EXPRESSION OF CYTOKININ OXYDASE IN THE SEEDS

EARLIEST PRIORITY CLAIMED	COUNTRY	NUMBER	DATE
NB - Use International abbreviation for country (See Schedule 4)	33 US	31 10/871,304	32 18 Jun 2004

57	Abstract (not more than 150 words) and figure of the drawings to which the abstract refers, are attached.	Number of sheets	115
----	---	------------------	-----

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 December 2005 (29.12.2005)

PCT

(10) International Publication Number
WO 2005/123926 A1

(51) International Patent Classification⁷: **C12N 15/82**,
15/53, 15/63, A01H 5/00, 5/10

(21) International Application Number:
PCT/EP2005/006620

(22) International Filing Date: 20 June 2005 (20.06.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/871,304 18 June 2004 (18.06.2004) US

(71) Applicants and

(72) Inventors: **SCHMULLING, Thomas** [DE/DE]; Preussenaallee 30, 14052 Berlin (DE). **WERNER, Tomas** [DE/DE]; Berlinerstrasse 118/119, 10713 Berlin (DE).

(74) Agents: **DE CLERCQ, Ann** et al.; De Clercq, Brants & Partners, E. Gevaertdreef 10a, B-9830 Sint-Martens-Latem (BE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with amended claims

Date of publication of the amended claims: 16 February 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR MODIFYING PLANT MORPHOLOGY, BIOCHEMISTRY AND PHYSIOLOGY COMPRISING EXPRESSION OF CYTOKININ OXYDASE IN THE SEEDS

(57) Abstract: The present invention relates to methods and compositions for increasing seed yield of a plant. The methods comprise expression of a cytokinin oxidase in the aleurone and/or embryo of a seed. The invention also relates to vectors comprising a nucleic acid encoding a cytokinin oxidase that is operably linked to a promoter capable of driving expression in the aleurone and/or embryo of a seed, and to host cells, transgenic cells and plants comprising such sequences. The use of these sequences for increasing yield is also provided.



WO 2005/123926 A1

METHOD FOR MODIFYING PLANT MORPHOLOGY, BIOCHEMISTRY AND PHYSIOLOGY COMPRISING
EXPRESSION OF CYTOKININ OXIDASE IN THE SEEDS

5 The present invention generally relates to methods for modifying plant morphological
properties or characteristics, such as developmental processes, and in particular seed
development and/or seed yield. The methods comprise expressing a cytokinin degradation
control protein, in particular cytokinin oxidase, in the plant, operably under the control of a
regulatable promoter sequence, wherein the regulatable promoter is an aleurone and/or
embryo-specific promoter sequence. The present invention extends to genetic constructs
10 which are useful in performing the method of the invention and to plants transformed therewith,
the plants having altered morphological properties compared to their otherwise isogenic
counterparts.

Seeds are the reproduction unit of higher plants. Seeds contain reserve compounds to
ensure nutrition of the embryo after germination. These storage compounds contribute
15 significantly to human nutrition as well as to cattle feeding. Seeds consist of three major parts,
namely the embryo, the endosperm and the seed coat. Reserve compounds are deposited in
storage tissue, which is either the endosperm (resulting from double fertilisation; e.g. in all
cereals), the so-called perisperm (derived from the nucellus tissue) or the cotyledons (e.g.
bean varieties). The endosperm is covered by a layer of dense cytoplasmic cells, known as
20 the aleurone. Storage compounds include lipids (oil seed rape), proteins (e.g. in the aleuron of
cereals) or carbohydrates (starch, oligosaccharides like raffinose).

Starch is the storage compound in the seeds of cereals. The most important cereal
species are maize (yearly production ca. 570 million tonnes), rice (540 million tonnes per
annum) and wheat (530 million tonnes per annum). Protein-rich seeds include different kinds
25 of beans (*Phaseolus spec.*, *Vicia faba*, *Vigna spec.*; ca. 20 million tonnes per annum), pea
(*Pisum sativum*; million tonnes per annum) and soybean (*Glycine max*; 136 million tonnes per
annum). Soybean seeds are also an important source of lipids, as are the seeds of different
Brassica species (app. 30 million tonnes per annum), cotton, oriental sesame, flax, poppy,
castor bean, sunflower, peanut, coconut, oilpalm and some other plants of lesser economic
30 importance.

After fertilization, the developing seed becomes a sink organ that attracts nutritional
compounds from source organs of the plant and uses them to produce the reserve compounds
in storage organs, such as seeds, bulbs or tubers. The common concept predicts that
cytokinins are a positive regulator of sink strength.

Numerous reports ascribe a stimulatory or inhibitory function to cytokinins in different developmental processes such as root growth and branching, control of apical dominance in the shoot, chloroplast development, and leaf senescence (Mok M.C. (1994) in *Cytokines: Chemistry, Activity and Function*, eds., Mok, D.W.S. & Mok, M.C. (CRC Boca Raton, FL), pp.155-166). Conclusions about the biological functions of cytokinins have mainly been derived from studies on the consequences of exogenous cytokinin application or endogenously enhanced cytokinin levels (Klee, H.J. & Lanehon, M.B. (1995) in *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, ed. Davies, P.J. (Kluwer, Dordrecht, the Netherlands), pp. 340-353, Schmülling, T., Rupp, H.M. Frank, M. & Schafer, S. (1999) in *Advances in Regulation of Plant Growth and Development*, eds. Surnad, M. Pac P. & Beck, E. (Peres, Prague), pp. 85-96).

The cloning of cytokinin oxidase allowed the study of the relevance of iP- and Z-type cytokinins during the whole life cycle of higher plants. The catabolic enzyme cytokinin oxidase (CKX) plays a principal role in controlling cytokinin levels in plant tissues. CKX activity has been found in a great number of higher plants and in different plant tissues. The enzyme is a FAD-containing oxidoreductase that catalyzes the degradation of cytokinins bearing unsaturated isoprenoid side chains and is sometimes referred to as a cytokinin dehydrogenase (Frébortova et al., *Biochem. J.* 380, 121-130, 2004). The free bases iP and Z, and their respective ribosides are the preferred substrates. The reaction products of iP catabolism are adenine and the unsaturated aldehyde 3-methyl-2-butanal (Armstrong, D.J. (1994) in *Cytokinins: Chemistry, Activity and Functions*, eds. Mok. D.W.S & Mok, M.C. (CRC Boca Raton, FL), pp. 139-154).

Cytokinin oxidase genes from various plant species have been isolated: *Zea mays* (Morris, R.O., Bilyeu, K.D., Laskey, J.G. & Cherich, N.N. (1999) *Biochem. Biophys. Res. Commun.* 255, 328-333, Houba-Herlin, N., Pethe, C., d'Alayer, J. & Laloue, M. (1999) *Plant J.* 17, 615-626), *Arabidopsis thaliana* (WO 01/96580, WO 03/050287), and *Dendrobium* sp. (Yang et al., *J. Exp. Bot.* 53, 1899-1907). The manipulation of CKX gene expression is used as a powerful tool to study the relevance of iP- and Z-type cytokinins during the life cycle of higher plants; in particular the manipulation of CKX expression allowed the influence of cytokinin on root growth and shoot development and morphology, on seed size, and on leaf senescence to be determined (WO 01/96580, WO 03/050287, Werner et al. *Plant Cell* 15, 2632-2550, 2003). Transformants showing constitutive *AtCKX* mRNA expression and increased cytokinin oxidase activity manifested enhanced formation and growth of roots. Negative effects on shoot growth were also observed.

It was postulated that cytokinins play a fundamental role in maize for establishing seed size, decreasing tip kernel abortion and increased seed setting during unfavourable environmental conditions (WO 00/63401). Smigocki et al. (Proc Natl Acad Sci USA 85, 5131-5135, 1988) observed increased shoot organogenesis upon infection of stems, leaf pieces and seedling with *Agrabacterium tumefaciens* overexpressing isopentenyltransferase (ipt). It was therefore also postulated that enhanced levels of cytokinins in the seed would provide for improved seed size and increased seed set (WO 00/63401).

It is known in the art that constitutive CKX expression in a plant leads to increased root growth but decreased shoot growth (WO 01/96580). It has furthermore been demonstrated that expression of CKX under the control of a strong constitutive promoter in a plant results in increased seed size, increased embryo size and increased cotyledon size (WO 03/050287). However, it was not known which parts of the seeds would be best suited for CKX expression so as to achieve increased seed yield. The inventors have now shown for the first time that increased seed yield may be obtained relative to control plants when CKX is overexpressed in certain parts of the seed, namely the aleurone and/or embryo. The inventors have also shown that aleurone- and/or embryo-specific overexpression of CKX leads to a larger increase in seed yield compared to endosperm-specific CKX overexpression.

In accordance with the present invention, it has been surprisingly discovered that transgenic plants overexpressing a cytokinin oxidase gene in a confined part of the seed, such confined part being the aleurone and/or embryo, have an increased seed yield compared to corresponding wild type plants. These results are surprising, as a reduced cytokinin content would have been expected to be associated with a reduced organ growth and hence with reduced yield.

The present invention provides a method for modifying plant morphological properties, in particular for increasing seed yield of a plant, comprising expressing a cytokinin oxidase under the control of a regulatable promoter capable of driving expression in the aleurone and/or embryo of a seed. The invention also provides compositions for modifying plant morphological properties; in particular for increasing seed yield of a plant.

"Plant morphology" or "plant morphological characteristic" or similar term will, when used herein, be understood by those skilled in the art to refer to the external appearance of a plant. More particularly the plant morphological characteristic that is improved by using the methods according to the present invention is seed yield.

As used herein, the term "control plant" refers to plants that are, except for the modified or improved morphological characteristics, as similar as possible or identical to the modified

plant. Such control plants are also known as "wild type plants/lines" or "otherwise isogenic plants/lines". The inventors have furthermore shown that the increase in seed yield is higher for plants wherein CKX is overexpressed in the aleurone and/or embryo, compared to plants wherein CKX is overexpressed in the endosperm. The term "control plants" therefore also
5 includes plants wherein CKX is overexpressed in the endosperm.

There are several well known parameters which may be used for measuring increased seed yield which include but are not limited to: total weight of seeds, total number of seeds, total number of filled seeds, harvest index, and Thousand Kernel Weight. As described in the examples herein, the total weight of seeds may be measured by weighing all filled seeds
10 harvested from a plant. The total number of seeds may be measured by counting the number of seeds harvested from a plant. The total number of filled seeds may be measured by counting the number of filled seeds harvested from a plant. The term "harvest index" as used herein is defined as the ratio between the total seed weight and the above ground area (mm^2), multiplied by a factor 10^6 . Thousand Kernel Weight may be derived from the number of filled
15 seeds counted and their total weight.

Advantageously, performance of the methods according to the present invention results in plants having increased yield or biomass, relative to corresponding control plants.

By "yield" is meant the amount of harvested material per area of production. The term "increased yield" encompasses an increase in biomass in one or more parts of a plant relative
20 to the biomass of corresponding control plants. Depending on the crop, the harvested part of the plant may be different, for example it may be seed (e.g. rice, sorghum or corn when grown for seed); total above-ground biomass (e.g. corn, when used as silage, sugarcane), root (e.g. sugar beet), fruit (e.g. tomato), cotton fibres, or any other part of the plant which is of economic value. For example, the methods of the present invention are used to increase seed yield in
25 rice and in corn. The increase in yield encompasses an increase in seed yield, which includes an increase in the total biomass of the seed (total seed weight), total number of seeds and/or an increase in the number of (filled) seeds. The increase in yield is also reflected as an increase in the Harvest Index, which is expressed as a ratio of the yield of harvestable parts, such as seeds, over the total biomass and is also reflected as an increased Thousand Kernel
30 Weight (derived from the number of filled seeds counted and their total weight).

Thus, there is provided a method for increasing seed yield of a plant, increasing the level and/or activity of a cytokinin oxidase (CKX) in the embryo and/or aleurone of a plant seed, wherein said embryo and/or aleurone have increased levels and/or activity of cytokinin oxidase relative to other parts of said seed, and wherein the increase of seed yield comprises
35 at least one of increased total weight of seeds, increased total number of seeds, increased

number of filled seeds, increased harvest index or increased thousand kernel weight, each relative to corresponding control plants.

Yield is by its nature a complex parameter where total yield depends on a number of yield components. The parameters for increased yield of a crop are well known by a person skilled in the art. By way of example, key yield components for corn include number of plants per hectare or acre, number of ears per plant, number of rows (of seeds) per ear, number of kernels per row, and Thousand Kernel Weight. The improvement in yield as obtained in accordance with the methods of the invention, may be obtained as a result of one or more of these yield components. By way of example, key yield components for rice include number of plants per hectare or acre, number of panicles per plant, number of spikelets per panicle, seed filling rate and thousand kernel weight. The improvement in yield as obtained in accordance with the methods of the invention may be obtained as a result in one or more of these yield components, preferentially the improvement in yield is obtained primarily on the basis of an increased number of flowers per panicle and an increased seed filling rate.

Performance of the methods according to the present invention result in plants having modified seed yield. The modified yield includes at least an increase in any one or more of total weight of seeds, total seed number, number of filled seeds, thousand kernel weight and harvest index, each relative to control plants. Therefore, according to the present invention, there is provided a method for increasing one or more of: total seed number, total weight of seeds, number of filled seeds, thousand kernel weight and harvest index of plants, which method comprises modulating expression of a nucleic acid molecule encoding a CKX protein and/or modulating activity of the CKX itself in a plant in a aleurone- and/or embryo-preferred way, preferably wherein the CKX protein is encoded by a nucleic acid sequence represented by SEQ ID NO: 26, SEQ ID NO: 42, or a portion thereof or by sequences capable of hybridising therewith or wherein the CKX is represented by SEQ ID NO: 4, SEQ ID NO: 36, or a homologue, derivative or active fragment thereof. Alternatively, the CKX may be encoded by a nucleic acid sequence represented by SEQ ID NO: 37, or by a portion thereof or by sequences capable of hybridising therewith, or wherein the CKX is represented by SEQ ID NO: 38, or a homologue, derivative or active fragment of any thereof.

The present invention is applicable to any plant, in particular a monocotyledonous plants and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp.*, *Acer spp.*, *Actinidia spp.*, *Aesculus spp.*, *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon spp.*, *Arachis spp.*, *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Avena sativa*, *Baikiaea plurijuga*, *Betula spp.*, *Brassica spp.*, *Bruguiera gymnorrhiza*, *Burkea africana*, *Butea frondosa*,

- Cadaba farinosa*, *Calliandra* spp., *Camellia sinensis*, *Canna indica*, *Capsicum* spp., *Cassia* spp., *Centroema pubescens*, *Chaenomeles* spp., *Cinnamomum cassia*, *Coffea arabica*, *Colophospermum mopane*, *Coronilla varia*, *Cotoneaster serotina*, *Crataegus* spp., *Cucumis* spp., *Cupressus* spp., *Cyathea dealbata*, *Cydonia oblonga*, *Cryptomeria japonica*,
5 *Cymbopogon* spp., *Cynthea dealbata*, *Cydonia oblonga*, *Dalbergia monetaria*, *Davallia divaricata*, *Desmodium* spp., *Dicksonia squarosa*, *Diheteropogon amplexans*, *Dioclea* spp., *Dolichos* spp., *Dorycnium rectum*, *Echinochloa pyramidalis*, *Ehrhartia* spp., *Eleusine coracana*, *Eragrestis* spp., *Erythrina* spp., *Eucalyptus* spp., *Euclea schimperi*, *Eulalia villosa*, *Fagopyrum* spp., *Feijoa sellowiana*, *Fragaria* spp., *Flemingia* spp., *Freycinetia banksii*, *Geranium*
10 *thunbergii*, *Ginkgo biloba*, *Glycine javanica*, *Gliricidia* spp., *Gossypium hirsutum*, *Grevillea* spp., *Guibourtia coleosperma*, *Hedysarum* spp., *Hemarthria altissima*, *Heteropogon contortus*, *Hordeum vulgare*, *Hyparrhenia rufa*, *Hypericum erectum*, *Hyperthelia dissoluta*, *Indigo incarnata*, *Iris* spp., *Leptarrhena pyrolifolia*, *Lespedeza* spp., *Lettuca* spp., *Leucaena leucocephala*, *Loudetia simplex*, *Lotonus bainesii*, *Lotus* spp., *Macrotyloma axillare*, *Malus*
15 spp., *Manihot esculenta*, *Medicago sativa*, *Metasequoia glyptostroboides*, *Musa sapientum*, *Nicotianum* spp., *Onobrychis* spp., *Ornithopus* spp., *Oryza* spp., *Peltophorum africanum*, *Pennisetum* spp., *Persea gratissima*, *Petunia* spp., *Phaseolus* spp., *Phoenix canariensis*, *Phormium cookianum*, *Photinia* spp., *Picea glauca*, *Pinus* spp., *Pisum sativum*, *Podocarpus totara*, *Pogonarthria fleckii*, *Pogonarthria squarrosa*, *Populus* spp., *Prosopis cineraria*,
20 *Pseudotsuga menziesii*, *Pterolobium stellatum*, *Pyrus communis*, *Quercus* spp., *Rhaphiolepis umbellata*, *Rhopalostylis sapida*, *Rhus natalensis*, *Ribes grossularia*, *Ribes* spp., *Robinia pseudoacacia*, *Rosa* spp., *Rubus* spp., *Salix* spp., *Schyzachyrium sanguineum*, *Sciadopitys verticillata*, *Sequoia sempervirens*, *Sequoiadendron giganteum*, *Sorghum bicolor*, *Spinacia* spp., *Sporobolus fimbriatus*, *Stiburus alopecuroides*, *Stylosanthos humilis*, *Tadehagi* spp.,
25 *Taxodium distichum*, *Themeda triandra*, *Trifolium* spp., *Triticum* spp., *Tsuga heterophylla*, *Vaccinium* spp., *Vicia* spp., *Vitis vinifera*, *Watsonia pyramidata*, *Zantedeschia aethiopica*, *Zea mays*, amaranth, artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugarbeet, sugar cane, sunflower, tomato, squash, and tea, amongst others,
30 or the seeds of any plant specifically named above or a tissue, cell or organ culture of any of the above species.

According to a preferred embodiment of the present invention, the plant is a crop plant such as soybean, sunflower, canola, alfalfa, rapeseed, cotton, tomato, potato or tobacco. Further preferably, the plant is a monocotyledonous plant, such as sugarcane. More
35 preferably the plant is a cereal, such as rice, maize, wheat, barley, millet, rye, sorghum or oats.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, leaves, roots (including tubers), flowers, and tissues and organs, wherein each of the aforementioned comprise the gene/nucleic acid of interest. The term "plant" also encompasses suspension cultures, callus
5 tissue, embryos, meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the aforementioned comprise the gene/nucleic acid of interest. The terms "plant" and "plant part" are used interchangeably with the terms "plants" and "plant parts".

It should be clear that, although the invention is supported in the examples section by
10 AtCKX genes and proteins, the inventive concept also relates to the use of cytokinin oxidases isolated from other plants and expressed in the aleurone and/or embryo of a seed of these other plants to obtain similar effects in plants as described in the examples section.

The cytokinin oxidase gene family contains at least six members from *Arabidopsis*. It is anticipated that functional homologs of the described *Arabidopsis* cytokinin oxidases can be
15 isolated from other organisms, given the evidence for the presence of cytokinin oxidase activity in many green plants (Hare and van Staden, *Physiol Plant* 91:128-136, 1994; Jones and Schreiber, *Plant Growth Reg* 23:123-134, 1997), as well as in other organisms (Armstrong, in *Cytokinins: Chemistry, Activity and Function*. Eds Mok and Mok, CRC Press, pp139-154, 1994). Therefore, the sequence of the cytokinin oxidase, functional in the invention, need not
20 to be identical to those described herein. This invention is particularly useful for cereal crops and monocot crops in general and cytokinin oxidase genes from for example wheat or maize may be used as well (Morris et al., 1999; Rinaldi and Comandini, 1999). It is envisaged that other genes with cytokinin oxidase activity or with any other cytokinin metabolizing activity (see
25 Zažimalová et al., *Biochemistry and Molecular Biology of Plant Hormones*, Hooykaas, Hall and Libbenga (Eds.), Elsevier Science, pp141-160, 1997) can also be used for the purpose of this invention. Similarly, genes encoding proteins that would increase endogenous cytokinin-metabolizing activity can also be used for the purpose of this invention. In principle, similar phenotypes could also be obtained by interfering with genes that function downstream of cytokinin such as receptors or proteins involved in signal transduction pathways of cytokinin.

30 Any nucleotide sequence encoding a polypeptide with cytokinin oxidase activity may be used in the methods of the invention. For example, any of the various sequences provided herein encoding a polypeptide with cytokinin oxidase activity may be used in the methods of increasing seed yield.

The terms "protein(s)", "peptide(s)", "polypeptide(s)" or "oligopeptide(s)" when used
35 herein refer to amino acids in a polymeric form of any length. These terms also include known

amino acid modifications as well as non-naturally occurring amino acid residues, L-amino acid residues and D-amino acid residues.

The CKX proteins useful in the methods according to the invention are defined herein as having cytokinin oxidase/dehydrogenase activity and comprising at least 2 sequences of 17 and 19 consecutive amino acid residues respectively with a consensus sequence as shown below:

Consensus sequence 1 (17 amino acids): hTDYLhholGGTLSSsG, (SEQ ID NO: 44)

Consensus sequence 2 (19 amino acids): cLFxushGsLGQFGllstA, (SEQ ID NO: 45)

wherein the capital letters are the standard single letter IUPAC codes for the various amino acids and the other letters symbolise the nature of the amino acids, as shown in Table 1. The right column lists for each class the particular amino acids that are allowed in the consensus sequences. This classification is based on the amino acid grouping as defined in the SMART database (Schultz et al. (1998) Proc. Natl. Acad. Sci. USA 95, 5857-5864; Letunic et al. (2002) Nucleic Acids Res 30, 242-244).

Table 1

Class	Key	Allowed amino acids
h	hydrophobic	G, H, L, R, T, W, Y
o	alcohol	S, T
l	aliphatic	V, I
s	small	A, D, G, N, T, V
c	charged	D, E, R
x	any	Y, R, N, F, D, H
u	tiny	A, G, S
t	turnlike	R, N

The crystal structure of maize CKX was elucidated by Malito et al. (J. Mol. Biol. 341, 1237-1249, 2004), which allowed a structure-function analysis and the identification of amino acids that were important for the catalytic activity of maize CKX. These results may be extrapolated to other CKX proteins by comparison of protein sequences.

Methods for measuring cytokinin oxidase/dehydrogenase activity are well known in the art. Suitable methods are based on the conversion of [2-³H]iP to adenine (Motyka et al., Plant Physiology 112, 1035-1043, 1996), on colorimetric assays (Libreros-Minotta and Tipton, Anal.

Biochem. 231, 339-341, 1995) or on the measurement of reduced electron acceptors (Bilyeu et al., Plant Physiol. 125, 378-386, 2001).

The present invention relates to methods for increasing seed yield. In particular, the methods comprise expression in the aleurone and/or embryo of a seed, of a nucleic acid encoding a cytokinin oxidase selected from the group consisting of:

- (a) nucleic acids comprising a DNA sequence as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or the complement thereof,
- (b) nucleic acids comprising the RNA sequences corresponding to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or the complement thereof,
- (c) nucleic acids specifically hybridizing to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or to the complement thereof,
- (d) nucleic acids encoding a protein comprising the amino acid sequence as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 32, 35, 36 or 38, or the complement thereof,
- (e) nucleic acids as defined in any of (a) to (d) characterized in that said nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA wherein T is replaced by U,
- (f) nucleic acid which is degenerate compared to a nucleic acid as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 31, 33 or 34, or which is degenerate compared to a nucleic acid as defined in any of (a) to (e) as a result of the genetic code,
- (g) nucleic acids which are divergent from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or which is divergent from a nucleic acid as defined in any of (a) to (e), due to the differences in codon usage between the organisms,
- (h) nucleic acids encoding a protein as given in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or nucleic acids as defined in (a) to (e) which are divergent due to the differences between alleles,
- (i) nucleic acids encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38,
- (j) functional fragments of nucleic acids as defined in any of (a) to (i) having the biological activity of a cytokinin oxidase, and
- (k) nucleic acids encoding a plant cytokinin oxidase, comprising the consensus sequence hTDYLhhoIGGTLsSsG and cLFxushGsLGQFGllstA or comprising expression in seeds of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.

The expression of a nucleic acid encoding a CKX polypeptide or a homologue thereof may also be increased by introducing a genetic modification (preferably in the locus of a CKX gene).

In addition to the cytokinin oxidase genes and corresponding proteins described above, a cytokinin oxidase 2 gene (CKX2) is particularly suited for use in increasing seed yield in a plant. In addition to the *Arabidopsis thaliana* CKX2 set forth in SEQ ID NO:36, other CKX proteins from *Arabidopsis thaliana*, such as the ones represented in GenBank Accessions NP_181682, NP_200507, NP_849470, NP_194703, NP_850863 or AAG30909 may be used. CKX proteins from other species like *Zea mays* (for example GenBank Accessions CAE55202, CAE55200 or AAC27500), *Dendrobium* (GenBank CAC17752), *Hordeum vulgare* (GenBank AAN16383, AAO50082, AAM08400), or rice (GenBank NP_913145, NP_916348, NP_922039) are also available for use in the methods and compositions of the present invention. A prokaryotic homologue of SEQ ID NO: 36 is represented by GenBank Accession P46377.

Therefore, the present invention more generally relates to method for increasing seed yield in a plant, said method comprising increasing the level and/or activity of a cytokinin oxidase (CKX) in the embryo and/or aleurone of a plant seed, wherein said embryo and/or aleurone have increased levels and/or activity of cytokinin oxidase relative to other parts of said seed. Preferred cytokinin oxidases to be used are encoded by the nucleic acids encoding the cytokinin oxidases as defined above. More preferably, the cytokinin oxidases to be used are from *Arabidopsis thaliana*, most preferably the cytokinin oxidase to be used is AtCKX2 encoded by one of SEQ ID NO: 3, 26, 37 or 42.

The terms "gene(s)", "nucleic acid(s)", "nucleic acid sequence(s)", "nucleotide sequence(s)", or "nucleic acid molecule(s)", when used herein refer to nucleotides, either ribonucleotides or deoxyribonucleotides or a combination of both, in a polymeric form of any length. The terms furthermore include double-stranded and single-stranded DNA and RNA. The terms also include known nucleotide modifications such as methylation, cyclization and 'caps' and substitution of one or more of the naturally occurring nucleotides with an analog such as inosine. The terms also encompass peptide nucleic acids (PNAs), a DNA analogue in which the backbone is a pseudopeptide consisting of N-(2-aminoethyl)-glycine units rather than a sugar.

A "coding sequence" or "open reading frame" or "ORF" is defined as a nucleotide sequence that can be transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate control sequences or regulatory sequences, i.e. when the coding sequence or ORF is present in an expressible format. This coding sequence or ORF is bound by a 5' translation start codon and a 3' translation stop codon. A coding sequence or

ORF can include, but is not limited to RNA, mRNA, cDNA, recombinant nucleotide sequences, synthetically manufactured nucleotide sequences or genomic DNA. Such coding sequence or ORF can be interrupted by intervening nucleic acid sequences.

Genes and coding sequences essentially encoding the same protein but isolated from different sources can consist of substantially divergent nucleic acid sequences. Reciprocally, substantially divergent nucleic acid sequences can be designed to effect expression of essentially the same protein. These nucleic acid sequences are the result of e.g. the existence of different alleles of a given gene, of the degeneracy of the genetic code or of differences in codon usage. Thus, amino acids such as methionine and tryptophan are encoded by a single codon whereas other amino acids such as arginine, leucine and serine can each be translated from up to six different codons. Differences in preferred codon usage are known in the art. For example, the codon GGC (for glycine) is the most frequently used codon in *Agrobacterium tumefaciens* (36.2 %), is the second most frequently used codon in *Oryza sativa* but is used at much lower frequencies in *A. thaliana* and *Medicago sativa* (9 % and 8.4 %, respectively). Of the four possible codons encoding glycine, this GGC codon is most preferably used in *A. tumefaciens* and *O. sativa*. However, in *A. thaliana* this is the GGA (and GGU) codon whereas in *M. sativa* this is the GGU (and GGA) codon.

Alleles exist in nature, and encompassed within the methods of the present invention is the use of these natural allelic variants. Allelic variants encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp. SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms.

DNA sequences as defined in the current invention can be interrupted by intervening sequences. With "intervening sequences" is meant any nucleic acid sequence which disrupts a coding sequence comprising such inventive DNA sequence or which disrupts the expressible format of a DNA sequence comprising the inventive DNA sequence. Removal of the intervening sequence restores the coding sequence or the expressible format. Examples of intervening sequences include introns and mobilizable DNA sequences such as transposons. With "mobilizable DNA sequence" is meant any DNA sequence that can be mobilized as the result of a recombination event.

The methods according to the present invention may also be practised using an alternative splice variant of a nucleic acid molecule encoding a CKX protein. The term "alternative splice variant" as used herein encompasses variants of a nucleic acid molecule in which selected introns and/or exons have been excised, replaced or added. Such variants will be ones in which the biological activity of the protein remains unaffected, which can be

achieved by selectively retaining functional segments of the protein. Such splice variants may be found in nature or can be manmade. Methods for making such splice variants are well known in the art. Therefore according to another aspect of the present invention, there is provided a method for modifying the morphological characteristics of plants, and in particular seed yield, comprising expression in the aleurone and/or embryo of a seed of an alternative splice variant of a nucleic acid molecule encoding a CKX. Preferably, the splice variant is a splice variant of a sequence represented by SEQ ID NO: 1, 3, 5, 7, 9, 11 or 33. A preferred splice variant of AtCKX2 is as represented by SEQ ID NO: 38.

Also useful in the methods of in the present invention are nucleic acids capable of hybridising under reduced stringency conditions, preferably under stringent conditions, with a CKX encoding nucleic acid. Preferred is a nucleic acid capable of hybridising to a nucleic acid represented by SEQ ID NO: 42 or 26.

"Hybridization" is the process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridization process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. Tools in molecular biology relying on such a process include PCR, subtractive hybridization and DNA sequence determination. The hybridization process can also occur with one of the complementary nucleic acids immobilized to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridization process can furthermore occur with one of the complementary nucleic acids immobilized to a solid support such as a nitrocellulose or nylon membrane or immobilized by e.g. photolithography to e.g. a silicious glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridization, plaque hybridization and microarray hybridization. In order to allow hybridization to occur, the nucleic acid molecules are generally thermally or chemically (e.g. by NaOH) denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridization is influenced by conditions such as temperature, salt concentration and hybridization buffer composition. High stringency conditions for hybridization include high temperature and/or low salt concentration (salts include NaCl and Na₃-citrate) and/or the inclusion of formamide in the hybridization buffer and/or lowering the concentration of compounds such as SDS (detergent) in the hybridization buffer and/or exclusion of compounds such as dextran sulfate or polyethylene glycol (promoting molecular crowding) from the hybridization buffer. Conventional hybridization conditions are described in e.g. Sambrook et al. (1989) but the skilled craftsman will appreciate that numerous different hybridization conditions can be designed in function of the known or

the expected homology and/or length of the nucleic acid sequence. Sufficiently low stringency hybridization conditions are particularly preferred to isolate nucleic acids heterologous to the DNA sequences of the invention defined supra. Elements contributing to heterology include allelism, degeneration of the genetic code and differences in preferred codon usage as
 5 discussed supra.

The term "specifically hybridizing" or "hybridizing specifically" refers to the binding, duplexing, or hybridizing of a molecule to a particular nucleotide sequence under medium to stringent conditions when that sequence is presented in a complex mixture e.g., total cellular DNA or RNA.

10 "Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent and are different under different environmental parameters. For example, longer sequences hybridize specifically at higher temperatures. The T_m is the temperature under defined ionic strength and pH, at which 50% of the target
 15 sequence hybridizes to a perfectly matched probe. Specificity is typically the function of post-hybridization washes. Critical factors of such washes include the ionic strength and temperature of the final wash solution.

Generally, stringent conditions are selected to be about 50°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the
 20 temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. The T_m is dependent upon the solution conditions and the base composition of the probe, and may be calculated using the following equation:

$$\begin{aligned}
 T_m = 79.8^{\circ}\text{C} &+ (18.5 \times \text{Log}[\text{Na}^+]) \\
 &+ (58.4^{\circ}\text{C} \times \%[\text{G}+\text{C}]) \\
 25 &- (820 / \# \text{ bp in duplex}) \\
 &- (0.5 \times \% \text{ formamide})
 \end{aligned}$$

More preferred stringent conditions are when the temperature is 20°C below T_m , and the most preferred stringent conditions are when the temperature is 10°C below T_m . Nonspecific binding may also be controlled using any one of a number of known techniques
 30 such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase.

Wash conditions are typically performed at or below stringency. Generally, suitable stringent conditions for nucleic acid hybridization assays or gene amplification detection procedures are as set forth above. More or less stringent conditions may also be selected.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook, J., E.F. Fritsch, et al. 1989 "Molecular Cloning: a Laboratory Manual, 2nd Edition, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press. An example of low stringency conditions is 4-6X SSC/0.1-0.5% w/v SDS at 37°-45° C for 2-3 hours. Depending on the source and concentration of the nucleic acid involved in the hybridization, alternative conditions of stringency may be employed such as medium stringent conditions. Examples of medium stringent conditions include 1-4X SSC/0.25% w/v SDS at $\geq 45^\circ$ C for 2-3 hours. An example of high stringency conditions includes 0.1-1X SSC/0.1% w/v SDS at 60 C for 1-3 hours. The skilled artisan is aware of various parameters which may be altered during hybridization and washing and which will either maintain or change the stringency conditions. For example, another stringent hybridization condition is hybridization at 4X SSC at 65° C, followed by a washing in 0.1X SSC at 65° C for about one hour. Alternatively, an exemplary stringent hybridization condition is in 50% formamide, 4XSSC, at 42° C. Still another example of stringent conditions include hybridization at 62° C in 6X SSC, .05X BLOTTO, and washing at 2X SSC, 0.1% SDS at 62° C.

Clearly, the current invention embodies the use of DNA sequences hybridising to DNA sequences encoding a cytokinin oxidase, homologue, derivative or immunologically active and/or functional fragment thereof as defined below in methods for increasing seed yield comprising expression of these DNA sequences in the aleurone and/or embryo of a seed. Preferably the cytokinin oxidase is a plant cytokinin oxidase, more specifically the *Arabidopsis thaliana* (At)CKX.

"Homologues" of a CKX polypeptide may also be useful in the present invention.

"Homologues" of a CKX useful in the methods of the invention are those peptides, oligopeptides, polypeptides, proteins and enzymes which contain amino acid substitutions, deletions and/or additions relative to the protein with respect to which they are a homologue, without altering one or more of its functional properties, in particular without reducing the activity of the resulting protein. For example, a homologue of a CKX will consist of a bioactive amino acid sequence variant of this CKX.

Two special forms of homology, orthologous and paralogous homology, are evolutionary concepts used to describe ancestral relationships of genes. The term "paralogous" relates to homologous genes that result from one or more gene duplications

within the genome of a species. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship of these genes. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins useful in the methods according to the invention. Orthologous genes can be identified by querying one or more gene databases with a query gene of interest, using for example, the BLAST program. The highest-ranking subject genes that result from the search are then again subjected to a BLAST analysis, and only those subject genes that match again with the query gene are retained as true orthologous genes. For example, to find a rice orthologue of an *Arabidopsis thaliana* gene, one may perform a BLASTN or TBLASTX analysis on a rice database (such as (but not limited to) the *Oryza sativa* Nipponbare database available at the NCBI (<http://www.ncbi.nlm.nih.gov>) or the genomic sequences of rice (cultivars indica or japonica)). In a next step, the obtained rice sequences are used in a reverse BLAST analysis using an *Arabidopsis* database. The results may be further refined when the resulting sequences are analysed with ClustalW and visualised in a neighbour joining tree. The method can be used to identify orthologues from many different species.

BLAST (Basic Local Alignment Search Tool) is a family of programs (<http://www.ncbi.nlm.nih.gov/BLAST/>) aiming to identify regions of optimal local alignment, i.e. the alignment of some portion of two nucleic acid or protein sequences, and to detect relationships among sequences which share only isolated regions of similarity (Altschul et al., Nucleic Acids Res. 25: 3389-3402 (1997)).

To produce such homologues, amino acids present in the protein can be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic moment, antigenicity, propensity to form or break α -helical structures or β -sheet structures, and so on. An overview of physical and chemical properties of amino acids is given in Table 2.

Table 2. Properties of naturally occurring amino acids.

Charge properties / hydrophobicity	Side group	Amino Acid
Nonpolar hydrophobic	Aliphatic aliphatic, S-containing aromatic imino	ala, ile, leu, val met phe, trp pro
polar uncharged	Aliphatic Amide	gly asn, gln

	Aromatic	tyr
	Hydroxyl	ser, thr
	Sulfhydryl	cys
Positively charged	Basic	arg, his, lys
Negatively charged	Acidic	asp, glu

The homologues useful in the methods according to the invention preferably have cytokinin oxidase/dehydrogenase activity and comprise at least 2 sequences of 17 and 19 consecutive amino acid residues respectively with a consensus sequence as shown below:

5 Consensus sequence 1 (17 amino acids): hTDYLhhoLGGTLSssG, (SEQ ID NO: 44)

Consensus sequence 2 (19 amino acids): cLFxushGsLGQFGllstA, (SEQ ID NO: 45)

wherein the capital letters are the standard single letter IUPAC codes for the various amino acids and the other letters symbolise the nature of the amino acids, as shown in table 2 above.

10 The right column lists for each class the particular amino acids that are allowed in the consensus sequences.

Substitutional variants of a protein of the invention are those in which at least one residue in the protein or amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will
15 usually be of the order of about 1-10 amino acid residues and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions, such as those described *supra*.

Insertional amino acid sequence variants of a protein of the invention are those in which one or more amino acid residues are introduced into a predetermined site in the protein.
20 Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in a two-hybrid
25 system, phage coat proteins, (histidine)₆-tag, glutathione S-transferase, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope (EETARFQPGYRS), c-myc epitope (EQKLISEEDL), FLAG®-epitope (DYKDDDK), lacZ, CMP (calmodulin-binding peptide), HA epitope (YPYDVPDYA), protein C epitope (EDQVDPRLIDGK) and VSV epitope (YTDIEMNRLGK).

Deletional variants of a protein of the invention are characterized by the removal of one or more amino acids from the amino acid sequence of the protein.

Amino acid variants of a protein of the invention may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known to those skilled in the art, such as by M13 mutagenesis, T7-Gen in vitro mutagenesis kit (USB, Cleveland, OH), QuickChange Site Directed mutagenesis kit (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

Derivatives of a CKX protein may also be useful in the methods of the present invention. "Derivatives" of a protein of the invention are those peptides, oligopeptides, polypeptides, proteins and enzymes which comprise at least about five contiguous amino acid residues of the polypeptide but which retain the biological activity of this protein. A "derivative" may further comprise additional naturally-occurring, altered glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. Alternatively or in addition, a derivative may comprise one or more non-amino acid substituents compared to the amino acid sequence of a naturally-occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound thereto to facilitate its detection.

With "immunologically active" is meant that a molecule or specific fragments thereof such as specific epitopes or haptens are recognized by, i.e. bind to antibodies. Specific epitopes may be determined using, for example, peptide scanning techniques as described in Geysen *et al.* (1996) (Geysen, H.M., Rodda, S.J. and Mason, T.J. (1986). *A priori* delineation of a peptide which mimics a discontinuous antigenic determinant. *Mol. Immunol.* 23, 709-715.).

The term "fragment of a sequence" or "part of a sequence" means a truncated sequence of the original sequence referred to. The truncated sequence (nucleic acid or protein sequence) can vary widely in length; the minimum size being a sequence of sufficient size to provide a sequence with at least a comparable function and/or activity or the original sequence referred to (e. g. "functional fragment"), while the maximum size is not critical. In some applications, the maximum size usually is not substantially greater than that required to provide the desired activity and/or function(s) of the original sequence. Typically, the truncated amino acid sequence will range from about 5 to about 60 amino acids in length. More

typically, however, the sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 60 amino acids. It is usually desirable to select sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids. Functional fragments comprise at least 50 amino acids, include an FAD binding domain (as defined in Pfam (version 14.0, June 2004) accession number 1565, Bateman et al., Nucleic Acids Research Database Issue 32, D138-D141, 2004) and exhibit cytokinin oxidase/dehydrogenase activity. Most preferably, functional fragments exhibit cytokinin oxidase/dehydrogenase activity and comprise an amino acid sequence corresponding to the sequence spanning from Leu 94 to Leu 461 of SEQ ID NO: 26. Functional fragments can also include those comprising an epitope which is specific for the CKX proteins.

It should thus be understood that functional fragments may also be immunologically active fragments.

In the methods of the invention use of homologues, derivatives and/or immunologically active and/or functional fragments of the cytokinin oxidases as defined *supra* is contemplated. Particularly preferred homologues, derivatives and/or immunologically active and/or functional fragments of the cytokinin oxidase proteins which are contemplated for use in the methods of the current invention are derived from plants, more specifically from *Arabidopsis thaliana*, even more specifically the cytokinin oxidases are the *Arabidopsis thaliana* (At)CKX, or are capable of being expressed in the aleurone and/or embryo of a seed of cereals, preferably of rice or corn. The present invention clearly contemplates the use of functional homologues or derivatives and/or immunologically active fragments of the AtCKX proteins and is not to be limited in application to the use of a nucleotide sequence encoding one of these AtCKX proteins in the methods of the present invention.

To effect expression of a CKX protein in a cell, tissue or organ, preferably of plant origin, either the protein may be introduced directly to the cell, such as by microinjection or ballistic means or alternatively, an isolated nucleic acid molecule encoding the CKX protein may be introduced into the cell, tissue or organ in an expressible format.

In the context of the present invention it should be understood that the term "expression" and/or 'overexpression' are used interchangeably and both relate to an "enhanced and/or ectopic expression" of a plant cytokinin oxidase. It should be clear that herewith an enhanced expression of the plant cytokinin oxidase as well as "de novo" expression of plant cytokinin oxidases is meant. Methods for increasing expression of genes or proteins are well documented in the art and include, for example, overexpression driven by appropriate promoters, the use of transcription enhancers or translation enhancers. Isolated nucleic acids which serve as promoter or enhancer elements may be introduced in an

appropriate position (typically upstream) of a non-heterologous form of a polynucleotide so as to upregulate expression of a CKX-encoding nucleic acid or variant thereof. For example, endogenous promoters may be altered in vivo by mutation, deletion, and/or substitution (see, Kmiec, U.S. Pat. No. 5,565,350; Zarling et al., PCT/US93/03868), or isolated promoters may be introduced into a plant cell in the proper orientation and distance from a gene of the present invention so as to control the expression of the gene. Methods for reducing the expression of genes or gene products are well documented in the art.

The expression of a nucleic acid encoding a CKX polypeptide or a homologue thereof may also be increased in the aleurone and/or embryo of a seed by introducing a genetic modification (preferably in the locus of a CKX gene). The locus of a gene as defined herein is taken to mean a genomic region, which includes the gene of interest and 10 kb up- or down stream of the coding region.

The genetic modification may be introduced, for example, by any one (or more) of the following methods: T-DNA activation, TILLING, site-directed mutagenesis, directed evolution and homologous recombination or by introducing and expressing in a plant a nucleic acid encoding a CKX polypeptide or a homologue thereof. Following introduction of the genetic modification, there follows a step of selecting for modified expression of a nucleic acid encoding a CKX polypeptide or a homologue thereof, which modification in expression gives plants having increased yield.

T-DNA activation tagging (Hayashi et al. Science (1992) 1350-1353) involves insertion of T-DNA, usually containing a promoter (may also be a translation enhancer or an intron), in the genomic region of the gene of interest or 10 kb up- or down stream of the coding region of a gene in a configuration such that the promoter directs expression of the targeted gene. Typically, regulation of expression of the targeted gene by its natural promoter is disrupted and the gene falls under the control of the newly introduced promoter. The promoter is typically embedded in a T-DNA. This T-DNA is randomly inserted into the plant genome, for example, through *Agrobacterium* infection and leads to overexpression of genes near the inserted T-DNA. The resulting transgenic plants show dominant phenotypes due to overexpression of genes close to the introduced promoter. According to the present invention, the promoter to be introduced is a promoter capable of driving expression in the aleurone and/or embryo of a seed.

A genetic modification may also be introduced in the locus of a CKX gene using the technique of TILLING (Targeted Induced Local Lesions In Genomes). This is a mutagenesis technology useful to generate and/or identify, and to eventually isolate mutagenised variants of a CKX nucleic acid capable of exhibiting CKX activity. TILLING also allows selection of plants

carrying such mutant variants. These mutant variants may even exhibit higher CKX activity than that exhibited by the gene in its natural form. TILLING combines high-density mutagenesis with high-throughput screening methods. The steps typically followed in TILLING are: (a) EMS mutagenesis (Redei GP and Koncz C (1992) In *Methods in Arabidopsis Research*, Koncz C, Chua NH, Schell J, eds. Singapore, World Scientific Publishing Co, pp. 16-82; Feldmann et al., (1994) In Meyerowitz EM, Somerville CR, eds, *Arabidopsis*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 137-172; Lightner J and Caspar T (1998) In J Martinez-Zapater, J Salinas, eds, *Methods on Molecular Biology*, Vol. 82. Humana Press, Totowa, NJ, pp 91-104); (b) DNA preparation and pooling of individuals; (c) PCR amplification of a region of interest; (d) denaturation and annealing to allow formation of heteroduplexes; (e) DHPLC, where the presence of a heteroduplex in a pool is detected as an extra peak in the chromatogram; (f) identification of the mutant individual; and (g) sequencing of the mutant PCR product. Methods for TILLING are well known in the art (McCallum et al., (2000) *Nat Biotechnol* 18: 455-457; reviewed by Stemple (2004) *Nat Rev Genet* 5(2): 145-50).

Site-directed mutagenesis may be used to generate variants of CKX nucleic acids. Several methods are available to achieve site-directed mutagenesis, the most common being PCR based methods (current protocols in molecular biology. Wiley Eds. <http://www.4ulr.com/products/currentprotocols/index.html>).

Directed evolution may also be used to generate variants of CKX nucleic acids. This consists of iterations of DNA shuffling followed by appropriate screening and/or selection to generate variants of CKX nucleic acids or portions thereof encoding CKX polypeptides or homologues or portions thereof having an modified biological activity (Castle et al., (2004) *Science* 304(5674): 1151-4; US patents 5,811,238 and 6,395,547).

T-DNA activation, TILLING, site-directed mutagenesis and directed evolution are examples of technologies that enable the generation of novel alleles and CKX variants.

Homologous recombination allows introduction in a genome of a selected nucleic acid at a defined selected position. Homologous recombination is a standard technology used routinely in biological sciences for lower organisms such as yeast or the moss *Physcomitrella*. Methods for performing homologous recombination in plants have been described not only for model plants (Offringa et al. (1990) *EMBO J* 9(10): 3077-84) but also for crop plants, for example rice (Terada et al. (2002) *Nat Biotech* 20(10): 1030-4; Iida and Terada (2004) *Curr Opin Biotech* 15(2):132-8). The nucleic acid to be targeted (which may be a CKX nucleic acid or variant thereof as hereinbefore defined) need not be targeted to the locus of a CKX gene, but may be introduced in, for example, regions of high expression. The nucleic acid to be

targeted may be an improved allele used to replace the endogenous gene or may be introduced in addition to the endogenous gene.

The invention also relates to a method for the production of modified plants having increased seed yield, comprising the introduction of a genetic modification, which genetic
5 modification results in increased expression of cytokinin oxidase in the aleurone and/or embryo of a seed.

The invention furthermore relates to a method for increasing seed yield in a plant comprising the introduction of a nucleic acid molecule encoding a CKX as defined above, operably linked to one or more control sequences or a vector stably integrated into the genome
10 of a plant cell capable of driving expression in the aleurone and/or embryo of a seed.

Therefore, the invention also relates to a vector comprising a nucleic acid encoding a CKX as defined above, wherein the vector is an expression vector and wherein the nucleic acid encoding a CKX is operably linked to one or more control sequences allowing the expression in the aleurone and/or embryo of a seed.

15 In the present invention, the inventors have surprisingly shown that the expression of cytokinin oxidases in the aleurone and/or embryo of a seed resulted in the above-mentioned seed-related features. Examples of seed-specific promoters include but are not limited to those listed in Table 3.

Table 3. Examples of plant-expressible promoters capable of driving expression in the seed

20

GENE SOURCE	EXPRESSION PATTERN	REFERENCE
α -amylase (<i>Amy32b</i>)	aleurone	Lanahan, M.B., <i>et al.</i> , <i>Plant Cell</i> 4:203-211, 1992; Skriver, K., <i>et al. Proc. Natl. Acad. Sci. (USA)</i> 88: 7266-7270, 1991
cathepsin β -like gene	aleurone	Cejudo, F.J., <i>et al. Plant Molecular Biology</i> 20:849-856, 1992.
seed-specific genes	seed	Simon, <i>et al.</i> , <i>Plant Mol. Biol.</i> 5: 191, 1985; Scofield, <i>et al.</i> , <i>J. Biol. Chem.</i> 262: 12202, 1987.; Baszczynski, <i>et al.</i> , <i>Plant Mol. Biol.</i> 14: 633, 1990.

Brazil Nut albumin	seed	Pearson, et al., Plant Mol. Biol. 18: 235-245, 1992.
Legumin	seed	Ellis, et al., Plant Mol. Biol. 10: 203-214, 1988.
glutelin (rice)	seed	Takaiwa, et al., Mol. Gen. Genet. 208: 15-22, 1986; Takaiwa, et al., FEBS Letts. 221: 43-47, 1987.
Zein	seed	Matzke et al Plant Mol Biol, 14(3):323-32 1990
NapA	seed	Stalberg, et al, <i>Planta</i> 199: 515-519, 1996.
wheat LMW and HMW glutenin-1	endosperm	Mol Gen Genet 216:81-90, 1989; NAR 17:461-2, 1989
wheat SPA	seed	Albani et al, Plant Cell, 9: 171-184, 1997
wheat α , β , γ -gliadins	endosperm	EMBO 3:1409-15, 1984
barley <i>ltr1</i> promoter	endosperm	
barley B1, C, D, hordein	endosperm	Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996
barley DOF	endosperm	Mena et al, The Plant Journal, 116(1): 53-62, 1998
<i>blz2</i>	endosperm	EP99106056.7
synthetic promoter	endosperm	Vicente-Carbajosa et al., Plant J. 13: 629-640, 1998.
rice prolamin NRP33	endosperm	Wu et al, Plant Cell Physiology 39(8) 885-889, 1998
rice α -globulin Glb-1	endosperm	Wu et al, Plant Cell Physiology 39(8) 885-889, 1998
rice OSH1	embryo	Sato et al, Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996
rice α -globulin REB/OHP-1	endosperm	Nakase et al. Plant Mol. Biol. 33: 513-522, 1997
rice ADP-glucose PP	endosperm	Trans Res 6:157-68, 1997

maize ESR gene family	endosperm	Plant J 12:235-46, 1997
sorgum γ -kafirin	endosperm	PMB 32:1029-35, 1996
KNOX	embryo	Postma-Haarsma <i>et al.</i> , Plant Mol. Biol. 39:257-71, 1999
rice oleosin	embryo and aleuron	Wu <i>et al.</i> , J. Biochem., 123:386, 1998
sunflower oleosin	seed (embryo and dry seed)	Cummins, <i>et al.</i> , Plant Mol. Biol. 19: 873-876, 1992

Further examples of promoters suitable for seed specific expression in a plant may be found in the examples section, Table 4.

In accordance with the present invention, there are provided methods and compositions for increasing seed yield in a plant. Seed yield may be increased by increasing the expression of a cytokinin oxidase gene in the embryo and/or aleurone of a plant seed. Thus, an embryo and/or aleurone preferred promoter may be utilized to drive expression of a cytokinin oxidase in these particular components of a seed.

In accordance with the present invention, a cytokinin oxidase gene may be placed in a genetic construct such as a vector, under control of a embryo and/or aleurone-preferred promoter. An example of such a promoter capable of driving expression in the embryo and/or aleurone is the sequence as represented in GenBank under accession number AF019212 (sequence from nucleotide 1 to 1256, hereafter named PRO0218).

For example, the AtCKX2 gene (SEQ ID NO: 42) may be placed under control of a promoter capable of driving expression in the seed, more particularly a promoter capable of driving expression in the embryo and/or aleurone, for example PRO0218.

Therefore, there is provided a vector comprising the isolated nucleic acid molecule having the sequence set forth in SEQ ID NO: 41 or 43.

These constructs may then be used to transform plants, either dicotyledonous or monocotyledonous. In accordance with the present invention, plants transformed with a vector according to the present invention have an increased seed yield, when compared to nullizygous control plants.

Preferably, the vector of the invention comprises a coding sequence or open reading frame (ORF) encoding a cytokinin oxidase protein or a homologue or derivative thereof or an immunologically active and/or functional fragment thereof as defined *supra*. Preferably the

cytokinin oxidase is a plant cytokinin oxidase and more specifically an *Arabidopsis thaliana* (At)CKX. Most preferably the CKX is as represented by one of SEQ ID NO: 4, 36 or 38.

With "vector" or "vector sequence" or "genetic construct" is meant a DNA sequence which can be introduced in an organism by transformation and can be stably maintained in this organism. Vector maintenance is possible in e.g. cultures of *Escherichia coli*, *A. tumefaciens*, *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. Other vectors such as phagemids and cosmid vectors can be maintained and multiplied in bacteria and/or viruses. Vector sequences generally comprise a set of unique sites recognized by restriction enzymes, the multiple cloning site (MCS), wherein one or more non-vector sequence(s) can be inserted. As an alternative to multiple cloning sites, the vector may also comprise recombination sites. Gene cloning through recombination is well known in the art.

With "non-vector sequence" is accordingly meant a DNA sequence which is integrated in one or more of the sites of the MCS comprised within a vector.

"Expression vectors" form a subset of vectors which, by virtue of comprising the appropriate regulatory or control sequences enable the creation of an expressible format for the inserted non-vector sequence(s), thus allowing expression of the protein encoded by this non-vector sequence(s). Expression vectors are known in the art enabling protein expression in organisms including bacteria (e.g. *E. coli*), fungi (e.g. *S. cerevisiae*, *S. pombe*, *Pichia pastoris*), insect cells (e.g. baculoviral expression vectors), animal cells (e.g. COS or CHO cells) and plant cells (e.g. potato virus X-based expression vectors).

By "expressible format" is meant that the isolated nucleic acid molecule is in a form suitable for being transcribed into mRNA and/or translated to produce a protein, either constitutively or following induction by an intracellular or extracellular signal, such as an environmental stimulus or stress (mitogens, anoxia, hypoxia, temperature, salt, light, dehydration, etc) or a chemical compound such as IPTG (isopropyl- β -D-thiogalactopyranoside) or such as an antibiotic (tetracycline, ampicillin, rifampicin, kanamycin), hormone (e.g. gibberellin, auxin, cytokinin, glucocorticoid, brassinosteroid, ethylene, abscisic acid etc), hormone analogue (indoleacetic acid (IAA), 2,4-D, etc) , metal (zinc, copper, iron, etc), or dexamethasone, amongst others. As will be known to those skilled in the art, expression of a functional protein may also require one or more post-translational modifications, such as glycosylation, phosphorylation, dephosphorylation, or one or more protein-protein interactions, amongst others. All such processes are included within the scope of the term "expressible format".

It should be understood that for expression in monocots of the cytokinin oxidase genes according to the methods of the invention, a nucleic acid sequence corresponding to the cDNA sequence should be used to avoid mis-splicing of introns in monocots. Preferred cDNA sequences to be expressed in monocots have a nucleic acid sequence as represented in any of SEQ ID NOs: 25 to 30, SEQ ID NO: 34, SEQ ID NO: 37, or SEQ ID NO: 42.

Constitutive expression of the cytokinin oxidase gene in plants resulted in increased root growth and decreased shoot growth, illustrating the importance of confined expression of the cytokinin oxidase gene for general plant growth properties. Containment of cytokinin oxidase activity can be achieved by using cell-, tissue- or organ-specific promoters, since cytokinin degradation is a process limited to the tissues or cells that express the CKX protein, this in contrast to approaches relying on hormone synthesis.

Preferably, expression of a protein in a specific cell, tissue, or organ, preferably of plant origin, is effected by introducing and expressing an isolated nucleic acid molecule encoding the protein, such as a cDNA molecule, genomic gene, synthetic oligonucleotide molecule, mRNA molecule or open reading frame, into this cell, tissue or organ, wherein the nucleic acid molecule is placed operably in connection with suitable regulatory or control sequences including a promoter, preferably a plant-expressible promoter, and a terminator sequence. In particular, and according to the methods of the present invention, a nucleic acid sequence encoding a CKX is operably linked to a promoter capable of driving expression in the aleurone and/or embryo of a seed.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences derived from a classical eukaryotic genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory or control elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner.

The term "promoter" also includes the transcriptional regulatory sequences of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or a -10 box transcriptional regulatory sequences.

The term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ.

Promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression and/or to alter the spatial expression and/or temporal expression of a nucleic acid molecule to which it is operably linked. Such regulatory elements may be placed adjacent to a heterologous promoter sequence to drive expression of a nucleic acid molecule in response to e.g. copper, glucocorticoids, dexamethasone, tetracycline, gibberellin, cAMP, abscisic acid, auxin, wounding, ethylene, jasmonate or salicylic acid or to confer expression of a nucleic acid molecule to specific cells, tissues or organs such as meristems, leaves, roots, embryo, flowers, seeds or fruits.

In the context of the present invention, the promoter is a plant-expressible promoter sequence, capable of driving expression in the aleurone and/or embryo of a seed. By "plant-expressible" is meant that the promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a plant cell, tissue or organ, preferably a monocotyledonous or dicotyledonous plant cell, tissue, or organ, *in casu* the aleurone and/or embryo.

The terms "plant-operable" and "operable in a plant" when used herein, in respect of a promoter sequence, shall be taken to be equivalent to a plant-expressible promoter sequence.

Regulatable promoters as part of a binary viral plant expression system are also known to the skilled artisan (Yadav 1999 – WO9922003; Yadav 2000 – WO0017365).

In the present context, a "regulatable promoter sequence" is a promoter that is capable of conferring expression on a structural gene in a particular cell, tissue, or organ or group of cells, tissues or organs of a plant, optionally under specific conditions, however does generally not confer expression throughout the plant under all conditions. Accordingly, a regulatable promoter sequence may be a promoter sequence that confers expression on a gene to which it is operably linked in a particular location within the plant or alternatively, throughout the plant under a specific set of conditions, such as following induction of gene expression by a chemical compound or other elicitor.

Preferably, the regulatable promoter used in the methods of the present invention confers expression in a specific location within the plant, either constitutively or following induction, however not in the whole plant under any circumstances. In particular, the regulatable promoter for use in the present invention is a promoter capable of driving expression in the aleurone and/or embryo of a seed. Other types of such promoters are cell-specific promoter sequences, tissue-specific promoter sequences, organ-specific promoter sequences, cell cycle specific gene promoter sequences, inducible promoter sequences and constitutive promoter sequences that have been modified to confer expression in a particular

part of the plant at any one time, such as by integration of such constitutive promoter within a transposable genetic element (*Ac*, *Ds*, *Spm*, *En*, or other transposon).

The term "cell-specific" shall be taken to indicate that expression is predominantly in a particular cell or cell-type, preferably of plant origin, albeit not necessarily exclusively in this cell or cell-type. The term "tissue-specific" shall be taken to indicate that expression is predominantly in a particular tissue or tissue-type, preferably of plant origin, albeit not necessarily exclusively in this tissue or tissue-type. Similarly, the term "organ-specific" shall be taken to indicate that expression is predominantly in a particular organ, preferably of plant origin, albeit not necessarily exclusively in this organ. Similarly, the term "cell cycle specific" shall be taken to indicate that expression is predominantly cyclic and occurring in one or more, not necessarily consecutive phases of the cell cycle albeit not necessarily exclusively in cycling cells, preferably of plant origin.

Those skilled in the art will be aware that an "inducible promoter" is a promoter the transcriptional activity of which is increased or induced in response to a developmental, chemical, environmental, or physical stimulus. Similarly, the skilled craftsman will understand that a "constitutive promoter" is a promoter that is transcriptionally active throughout most, but not necessarily all parts of an organism, preferably a plant, during most, but not necessarily all phases of its growth and development.

Those skilled in the art will readily be capable of selecting appropriate promoter sequences for use according to the present invention from publicly-available or readily-available sources, without undue experimentation.

Placing a nucleic acid molecule under the regulatory control of a promoter sequence, or operably linking with a promoter sequence, means positioning the nucleic acid molecule such that expression is controlled by the promoter sequence. A promoter is usually, but not necessarily, positioned upstream, or at the 5'-end, and within 2 kb of the start site of transcription, of the nucleic acid molecule which it regulates. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting (i.e., the gene from which the promoter is derived). As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting (i.e., the gene from which it is derived). Again, as is known in the art, some variation in this distance can also occur.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in cells derived from viruses, yeasts, molds, bacteria, insects, birds, mammals and plants are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the gene constructs of the present invention include the *Agrobacterium tumefaciens* nopaline synthase (NOS) gene terminator, the *Agrobacterium tumefaciens* octopine synthase (OCS) gene terminator sequence, the Cauliflower mosaic virus (CaMV) 35S gene terminator sequence, the *Oryza sativa* ADP-glucose pyrophosphorylase terminator sequence (t3'Bt2), the *Zea mays* zein gene terminator sequence, the *rbcS-1A* gene terminator, and the *rbcS-3A* gene terminator sequences, amongst others.

Those skilled in the art will be aware of additional promoter sequences and terminator sequences which may be suitable for use in performing the invention. Such sequences may readily be used without any undue experimentation.

In the context of the current invention, the terms "ectopic expression" or "ectopic overexpression" of a gene or a protein are conferring to expression patterns and/or expression levels of this gene or protein normally not occurring under natural conditions, more specifically is meant increased expression and/or increased expression levels in one or more of: aleurone and/or embryo of a seed.

Preferably, the promoter sequence used in the context of the present invention is operably linked to a coding sequence or open reading frame (ORF) encoding a cytokinin oxidase protein or a homologue, derivative or an immunologically active and/or functional fragment thereof as defined supra.

The vector may optionally comprise a selectable marker gene. As used herein, the term "selectable marker gene" or "selectable marker" or "marker for selection" or "screenable marker" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a gene construct of the invention or a derivative thereof. Suitable selectable marker genes contemplated herein include the ampicillin resistance (Amp^r), tetracycline resistance gene (Tc^r), bacterial kanamycin resistance gene (Kan^r), phosphinothricin resistance gene, neomycin phosphotransferase gene (*nptII*), hygromycin resistance gene, β -glucuronidase (GUS) gene,

chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein (*gfp*) gene (Haseloff *et al*, 1997), and luciferase gene, amongst others.

5 The invention also relates to a host cell containing any of the nucleic acid molecules or vectors of the invention. This host cell is chosen from the group comprising bacterial, insect, fungal, plant or animal cells.

The invention further relates to a method for the production of transgenic plants, plant cells or plant tissues comprising the introduction of a nucleic acid molecule of the invention in an expressible format or a vector of the invention in this plant, plant cell or plant tissue.

10 Therefore, there is provided a method for producing a plant having increased seed yield, such method comprising:

- (a) introducing into a plant cell a genetic construct comprising an isolated nucleic acid molecule encoding a cytokinin oxidase wherein the isolated nucleic acid molecule is operably linked to a promoter capable of driving expression in the aleurone and/or embryo, endosperm of a seed;
- 15 (b) regenerating a plant therefrom;
- (c) growing the regenerated plant to seed set; and
- (d) selecting a plant with increased seed yield compared to a corresponding control plant.

20 Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well
25 known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.
30

The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells

transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

The invention also relates to a transgenic plant cell comprising a nucleic acid sequence of the invention which is operably linked to regulatory elements allowing transcription and/or expression of the nucleic acid in the aleurone and/or embryo of a seed.

According to another preferred embodiment, the invention relates to a transgenic plant cell as described hereinabove wherein the nucleic acid of the invention is stably integrated into the genome of this plant cell.

The invention further relates to a transgenic plant or plant tissue comprising plant cells as herein described and also to a harvestable part of such transgenic plant, preferably selected from the group consisting of seeds, leaves, fruits, stem cultures, roots, tubers, rhizomes and bulbs. The present invention furthermore relates to products directly derived from a harvestable part of a transgenic plant according to the invention, such as dry pellets or powders, oil, fat and fatty acids, starch, or proteins. The invention also relates to the progeny derived from any of the transgenic plants according to the invention.

Preferably, transgenic plants are produced which express in the aleurone and/or embryo of a seed a nucleic acid encoding a CKX, preferably an *Arabidopsis* CKX, most preferably a CKX encoded by a nucleic acid as set forth in any of SEQ ID NOs: 3, 26, 37, or 42 or an ortholog of such nucleic acid. Preferably, the ortholog is derived from a related species of the transgenic plant. Even more preferably, the ortholog is specific (native or endogenous) to the species of the transgenic plant.

Means for introducing recombinant DNA into plant tissue or cells include, but are not limited to, transformation using CaCl_2 and variations thereof, in particular the method described by Hanahan (1983), direct DNA uptake into protoplasts (Krens *et al.*, 1982; Paszkowski *et al.*, 1984), PEG-mediated uptake to protoplasts (Armstrong *et al.*, 1990) microparticle bombardment, electroporation (Fromm *et al.*, 1985), microinjection of DNA (Crossway *et al.*, 1986), microparticle bombardment of tissue explants or cells (Christou *et al.*, 1988; Sanford, 1988), vacuum-infiltration of tissue with nucleic acid, or in the case of plants, T-DNA-mediated transfer from *Agrobacterium* to the plant tissue as described essentially by An *et al.* (1985), Dodds *et al.*, (1985), Herrera-Estrella *et al.* (1983a, 1983b, 1985). Methods for transformation of monocotyledonous plants are well known in the art and include *Agrobacterium*-mediated transformation (Cheng *et al.*, 1997 - WO9748814; Hansen 1998 - WO9854961; Hiei *et al.*, 1994 - WO9400977; Hiei *et al.*, 1998 - WO9817813; Rikiishi *et al.*, 1999 - WO9904618; Saito *et al.*, 1995 - WO9506722), microprojectile bombardment (Adams *et al.*, 1999 - US5969213;

Bowen *et al.*, 1998 - US5736369; Chang *et al.*, 1994 - WO9413822; Lundquist *et al.*, 1999 - US5874265/US5990390; Vasil and Vasil, 1995 - US5405765. Walker *et al.*, 1999 - US5955362), DNA uptake (Eyal *et al.*, 1993 - WO9318168), microinjection of *Agrobacterium* cells (von Holt, 1994 - DE4309203) and sonication (Finer *et al.*, 1997 - US5693512).

5 A whole plant may be regenerated from the transformed or transfected cell, in accordance with procedures well known in the art. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a gene construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best
10 suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

The term "organogenesis", as used herein, means a process by which shoots and roots
15 are developed sequentially from meristematic centers.

The term "embryogenesis", as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes.

Preferably, the transgenic plant produced according to the inventive method is
20 transfected or transformed with a genetic sequence by any art-recognized means, such as microprojectile bombardment, microinjection, *Agrobacterium*-mediated transformation (including *in planta* transformation), protoplast fusion, or electroporation, amongst others. Most preferably such plant is produced by *Agrobacterium*-mediated transformation.

Agrobacterium-mediated transformation or agrolistic transformation of plants, yeast,
25 molds or filamentous fungi is based on the transfer of part of the transformation vector sequences, called the T-DNA, to the nucleus and on integration of this T-DNA in the genome of the eukaryote.

With "T-DNA", or transferred DNA, is meant that part of the transformation vector flanked by T-DNA borders which is, after activation of the *Agrobacterium* *vir* genes, nicked at
30 the T-DNA borders and is transferred as a single stranded DNA to the nucleus of an eukaryotic cell.

With "agrolistic transformation" is meant a transformation method combining features of *Agrobacterium*-mediated transformation and of biolistic DNA delivery. As such, a T-DNA

containing target plasmid is co-delivered with DNA/RNA enabling in planta production of VirD1 and VirD2 with or without VirE2 (Hansen and Chilton 1996; Hansen et al. 1997; Hansen and Chilton 1997 - WO9712046).

5 With "foreign DNA" is meant any DNA sequence that is introduced in the host's genome by recombinant techniques. The foreign DNA includes e.g. a T-DNA sequence or a part thereof such as the T-DNA sequence comprising the selectable marker in an expressible format. Foreign DNA furthermore include intervening DNA sequences as defined supra.

10 The present invention also encompasses use of CKX-encoding nucleic acids and use of CKX polypeptides operably linked to a promoter capable of driving expression in the aleurone and/or embryo of a seed. One such use relates to increasing plant, especially seed yield. The seed yield may include one or more of the following: increased number of flowers per panicle, increased total seed weight, increased number of filled seeds, increased thousand kernel weight and increased harvest index, each relative to corresponding wild type plants.

15 In particular, the present invention provides use of cytokinin oxidase-encoding nucleic acids operably linked to a promoter capable of driving overexpression in the aleurone and/or embryo relative to other parts of the seed for increasing seed yield of a plant. One such use is wherein the cytokinin oxidase-encoding nucleic acid is comprised in a genetic construct that is introduced into a plant cell. In particular, use of cytokinin oxidase-encoding nucleic acids operably linked to a promoter capable of driving overexpression in the aleurone and/or embryo
20 relative to other parts of the seed is envisaged for increasing seed yield of a plant, wherein the cytokinin oxidase-encoding nucleic acid is selected from the group consisting of:

- (a) nucleic acids comprising a DNA sequence as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
- (b) nucleic acids comprising the RNA sequences corresponding to any of SEQ ID
25 NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
- (c) nucleic acids specifically hybridizing to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or to the complement thereof,
- (d) nucleic acids encoding a protein comprising the amino acid sequence as given
30 in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or the complement thereof,
- (e) nucleic acids as defined in any of (a) to (d) characterized in that said nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA wherein T is replaced by U,

- (f) nucleic acid which is degenerate compared to a nucleic acid as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or which is degenerate compared to a nucleic acid as defined in any of (a) to (e) as a result of the genetic code,
- 5 (g) nucleic acids which are divergent from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or which is divergent from a nucleic acid as defined in any of (a) to (e), due to the differences in codon usage between the organisms,
- 10 (h) nucleic acids encoding a protein as given in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38 or nucleic acids as defined in (a) to (e) which are divergent due to the differences between alleles,
- (i) nucleic acids encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38,
- 15 (j) functional fragments of nucleic acids as defined in any of (a) to (i) having the biological activity of a cytokinin oxidase, and
- (k) nucleic acids encoding a plant cytokinin oxidase, comprising the consensus sequence hTDYLhhoIGGTLSSsG and cLFxushGsLGQFGIIstA.

Furthermore, use of SEQ ID NO: 41 and the use of a vector comprising a cytokinin oxidase as defined in (a) to (k) above for increasing seed yield in a plant is provided.

- 20 Those skilled in the art will be aware that the invention described herein is subject to variations and modifications other than those specifically described. It is to be understood that the invention described herein includes all such variations and modifications. The invention also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any or more of
- 25 these steps or features.

Throughout this specification, unless the context requires otherwise the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

As used herein, the term "derived from" shall be taken to indicate that a particular integer or group of integers has originated from the species specified, but has not necessarily been obtained directly from the specified source.

The following examples are given by means of illustration of the present invention and are in no way limiting. The contents of all references included in this application are incorporated by reference herein as if fully set forth.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic representation of plant cytokinin oxidase genes.

Shown are the structures of different cytokinin oxidase genes isolated from maize (*ZmCKX1*, accession number AF044603, Biochem. Biophys. Res. Com. 255:328-333, 1999) and *Arabidopsis* (*AtCKX1* to *AtCKX4*). Exons are denominated with 'E' and represented by shaded boxes. Introns are represented by white boxes. Further indicated are the gene sizes (in kb, on top of each structure), the gene accession numbers (under the names) and a size bar representing 0.5 kb.

15 Figure 2. Alignment of plant cytokinin oxidase amino acid sequences.

The amino acid sequences from cytokinin oxidases from maize (*ZmCKX1*) and *Arabidopsis* (*AtCKX1* to *AtCKX4*) are aligned. Identical amino acid residues are marked by a black box, similar amino acid residues are in a grey box. Amino acid similarity groups: (M,I,L,V), (F,W,Y), (G,A), (S,T), (R,K,H), (E,D), (N,Q).

20 **Figure 3:** Schematic presentation of the entry clone p41, containing CDS0427_2 within the *AttL1* and *AttL2* sites for Gateway® cloning in the pDONR201 backbone. CDS0427_2 is the internal code for the *Arabidopsis thaliana* CKX2 coding sequence (SEQ ID NO: 26). This vector contains also a bacterial kanamycin-resistance cassette and a bacterial origin of replication.

25 **Figure 4:** Binary vector p37 for the expression in *Oryza sativa* of the *Arabidopsis thaliana* CKX2 gene under the control of the PRO0218 promoter. This vector contains a T-DNA derived from the Ti plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58)). From the left border to the right border, this T-DNA contains: a selectable and a screenable marker for selection of transformed plants, each under control of a
30 constitutive promoter; the PRO0218 – CDS0427_2 -zein and *rbcS*-deltaGA double terminator cassette for expression of the *Arabidopsis thaliana* CKX2 gene. This vector also contains an origin of replication from pBR322 for bacterial replication and a selectable marker (Spe/SmeR) for bacterial selection with spectinomycin and streptomycin.

Figure 5: Binary vector p35 for the expression in *Oryza sativa* of the *Arabidopsis thaliana* CKX2 gene under the control of the PRO0090 promoter. This vector contains a T-DNA derived from the Ti plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58)). From the left border to the right border, this T-DNA contains: a selectable and a screenable marker for selection of transformed plants, each under control of a constitutive promoter; the PRO0090 – CDS0427_2 -zein and rbcS-deltaGA double terminator cassette for expression of the *Arabidopsis thaliana* CKX2 gene. This vector also contains an origin of replication from pBR322 for bacterial replication and a selectable marker (Spe/SmeR) for bacterial selection with spectinomycin and streptomycin.

10 EXAMPLES

Example 1. Brief description of the sequences of the invention and examples of seed specific promoters

SEQ ID NO:	DESCRIPTION
1	AtCKX1 genomic
2	AtCKX1 protein
3	AtCKX2 genomic
4	AtCKX2 protein
5	AtCKX3 genomic
6	AtCKX3 protein
7	AtCKX4 genomic
8	AtCKX4 protein
9	AtCKX5 genomic (short version)
10	AtCKX5 protein (short version)
11	AtCKX6 genomic
12	AtCKX6 protein

13	5'primer <i>AtCKX1</i>
14	3'primer <i>AtCKX1</i>
15	5'primer <i>AtCKX2</i>
16	3'primer <i>AtCKX2</i>
17	5'primer <i>AtCKX3</i>
18	3'primer <i>AtCKX3</i>
19	5'primer <i>AtCKX4</i>
20	3'primer <i>AtCKX4</i>
21	5'primer <i>AtCKX5</i>
22	3'primer <i>AtCKX5</i>
23	5'primer <i>AtCKX6</i>
24	3'primer <i>AtCKX6</i>
25	<i>AtCKX1</i> cDNA
26	<i>AtCKX2</i> cDNA
27	<i>AtCKX3</i> cDNA
28	<i>AtCKX4</i> cDNA
29	<i>AtCKX5</i> cDNA (short version)
30	<i>AtCKX6</i> cDNA
31	<i>AtCKX2</i> cDNA fragment
32	<i>AtCKX2</i> peptide fragment
33	<i>AtCKX5</i> genomic (long version)

34	AtCKX5 cDNA (long version)
35	AtCKX5 protein (long version)
36	AtCKX2, CDS0427_2 deduced protein sequence
37	AtCKX2 splice variant, DNA sequence
38	AtCKX2 splice variant, deduced protein sequence
39	PRM3769 (sense, start codon at positions 35 to 37)
40	PRM1526 (reverse, complementary stop codon at positions 30-32)
41	Expression cassette with PRO0218 – CDS0427_2 - zein and rbcS-deltaGA double terminator
42	AtCKX2, CDS0427_2 cDNA
43	Expression cassette with PRO0090 – CDS0427_2 - zein and rbcS-deltaGA double terminator
44	HTDYLhhoIGGTLsSsG signature
45	cLFxushGsLGQFGIstA signature

Table 4: Examples of promoters suitable for seed specific or seedling preferred expression

Gene name	Expression
Metallothionein Mte	embryo/scutellum + calli
putative beta-amylase	embryo/scutellum
unknown	scutellum
proteinase inhibitor Rgpi9	seed
structural protein	young tissues, calli, embryo/scutellum
prolamine 10 Kda	strong in endosperm
allergen RA2	seed
prolamine RP7	endosperm
Metallothioneine-like ML2	embryo/scutellum + calli

prolamine RM9	strong in endosperm
prolamine RP5	strong in endosperm
putative methionine aminopeptidase	embryo
putative 40S ribosomal protein	weak in endosperm
alpha-globulin	strong in endosperm
alanine aminotransferase	aleurone, endosperm
cyclophyllin 2	shoot, embryo/endosperm
sucrose synthase SS1 (barley)	medium constitutive, shoot endosperm/aleurone
trypsin inhibitor ITR1 (barley)	weak in endosperm
WSI18	embryo/aleurone
aquaporine	seedlings
RAB21	embryo/aleurone
OSH1	seedling
Arceline 5A	seed
Cruciferine	seed
Albumine 2S3	seed
Albumine 2S2	seed
FAE1	embryo
Phaseolin Beta subunit	seed
Lec1	embryo
Gamma zein	seed
lipid Transfer Protein	seed

Example 2. Seed-preferred or seedling-preferred expression of a CKX2 gene results in increased seed yield

5 **A) DNA Manipulation and cloning of AtCKX2**

Unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in (Sambrook & Russell (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York) or in Volumes 1 and 2 of Ausubel et al. (1994), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

The *Arabidopsis* CKX2 gene (corresponding to SEQ ID NO: 42) was amplified by PCR using as template an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK). After reverse transcription of RNA extracted from seedlings, the cDNAs were cloned into pCMV Sport 6.0. Average insert size of the bank was 1.5 kb, and original number of clones was 1.59x10⁷ cfu. The original titer was determined to be 9.6x10⁶ cfu/ml, and became after a first amplification 6x10¹¹ cfu/ml. After plasmid extraction, 200 ng of template was used in a 50µl PCR mix. Primers prm3769 (SEQ ID NO: 39) and prm1526 (SEQ ID NO: 40), which include the AttB sites for Gateway recombination, were used for PCR amplification.

PCR was performed using Hifi Taq DNA polymerase in standard conditions. A PCR fragment of 1506 bp was amplified and purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombines *in vivo* with the pDONR plasmid to produce, according to the Gateway terminology, an "entry clone", p41 (Figure 3). pDONR was purchased from Invitrogen, as part of the Gateway technology.

B) Vector construction

The entry clone p41 was subsequently used in an LR reaction with p831 or p830, both destination vectors according to the Gateway TM terminology, used for rice transformation.

p831 contains as functional elements within the T-DNA borders a plant selectable marker, a screenable marker and a Gateway cassette intended for LR *in vivo* recombination with the sequence of interest already cloned in the donor vector. The PRO0218 promoter for embryo and aleurone preferred expression is located upstream of this Gateway cassette.

Similarly, p830 contains as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker; and a Gateway cassette intended for LR *in vivo* recombination with the sequence of interest already cloned in the donor vector. The PRO0090 promoter for endosperm-preferred expression is located upstream of this Gateway cassette.

After the recombination step, the resulting expression vectors p37 (originating from p831, Figure 4) and p35 (originating from p830, Figure 5) were transformed into *Agrobacterium* strain LBA4404 and subsequently into *Oryza sativa* plants.

C) Transformation of rice

Mature dry seeds of the rice japonica cultivar Nipponbare were dehusked. Sterilization was done by incubating the seeds for one minute in 70% ethanol, followed by 30 minutes in 0.2% HgCl₂ and by 6 washes of 15 minutes with sterile distilled water. The sterile seeds were then germinated on a medium containing 2,4-D (callus induction medium). After a 4-week incubation in the dark, embryogenic, scutellum-derived calli were excised and propagated on the same medium. Two weeks later, the calli were multiplied or propagated by subculture on the same medium for another 2 weeks. 3 days before co-cultivation, embryogenic callus pieces were sub-cultured on fresh medium to boost cell division activity. The *Agrobacterium* strain LBA4404, harbouring T-DNA vectors comprising a suitable selection marker, was used for co-cultivation. *Agrobacterium* was cultured for 3 days at 28°C on AB medium with the appropriate antibiotics. The bacteria were then collected and suspended in liquid co-cultivation medium at an OD₆₀₀ of about 1. The suspension was transferred to a petri dish and the calli were immersed in the suspension during 15 minutes. Next, the callus tissues were blotted dry on a filter paper, transferred to solidified co-cultivation medium and incubated for 3 days in the dark at 25°C.

Hereafter, co-cultivated callus was grown on 2,4-D-containing medium for 4 weeks in the dark at 28°C in the presence of a selective agent at a suitable concentration. During this period, rapidly growing resistant callus islands developed. Upon transfer of this material to a regeneration medium and incubation in the light, the embryogenic potential was released and shoots developed in the next four to five weeks. Shoots were excised from the callus and incubated for 2 to 3 weeks on an auxin-containing medium from which they were transferred to soil. Hardened shoots were grown under high humidity and short days in a greenhouse. Finally seeds were harvested three to five months after transplanting. The method yielded single locus transformants at a rate of over 50 % (Aldemita and Hodges, 1996, Chan et al., 1993, Hiei et al., 1994).

D) Evaluation of transformants: vegetative growth measurements

Approximately 15 to 20 independent T0 transformants were generated. The primary transformants were transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seed. Four events (for p37 transformants, PRO0218 promoter) or five events (for p35 transformants, PRO0090 promoter) of which the T1 progeny segregated 3:1 for presence/absence of the transgene were retained. For each of these events, 10 T1 seedlings containing the transgene (hetero- and homo-zygotes), and 10 T1 seedlings lacking the transgene (nullizygotes), were selected by monitoring visual marker expression. The selected T1 plants were transferred to a greenhouse. Each plant received a unique barcode label to

link unambiguously the phenotyping data to the corresponding plant. The selected T1 plants were grown on soil in 10 cm diameter pots under the following environmental settings: photoperiod: 11.5 h, daylight intensity: 30,000 lux or more, daytime temperature: 28°C or higher, night time temperature: 22°C, relative humidity: 60-70%. Transgenic plants and the corresponding nullizygotes were grown side-by-side at random positions. From the stage of sowing until the stage of maturity the plants were passed several times through a digital imaging cabinet. At each time point digital images (2048x1536 pixels, 16 million colours) were taken of each plant from at least 6 different angles.

In a next step, the mature primary panicles were harvested, bagged, barcode-labelled and then dried for three days in the oven at 37°C. The panicles were then threshed and all the seeds were collected and counted. The filled husks were separated from the empty ones using an air-blowing device. The empty husks were discarded and the remaining fraction was counted again. The filled husks were weighed on an analytical balance and the cross-sectional area of the seeds was measured using digital imaging. This procedure allows deriving a set of seed-related parameters.

The parameters described below were derived in an automated way from the digital images using image analysis software and were analysed statistically.

A two factor ANOVA (ANalysis Of VAriance) corrected for the unbalanced design was used as statistical model for the overall evaluation of plant phenotypic characteristics. An F-test was carried out on all the parameters measured in all the plants and of all the events transformed with that gene. The F-test was carried out to check for an effect of the gene over all the transformation events and to verify for an overall effect of the gene, also named herein "global gene effect". If the value of the F test showed that the data are significant, then it was concluded that there is a "gene" effect, meaning that not only presence or the position of the gene is causing the effect. The threshold for significance for a true global gene effect was set at 5% probability level for the F test.

To check for an effect of the genes within an event, i.e., for a line-specific effect, a t-test was performed within each event using data sets from the transgenic plants and the corresponding null plants. "Null plants" or "Null segregants" or "Nullizygotes" are the plants treated in the same way as the transgenic plant, but from which the transgene has segregated. Null plants can also be described as the homozygous negative transformed plants. The threshold for significance for the t-test was set at a 10% probability level. The results for some events can be under or below this threshold. This is based on the hypothesis that a gene might only have an effect in certain positions in the genome, and that the occurrence of this position-dependent

effect is not uncommon. This kind of gene effect is also named herein a "line effect of the gene". The p value was obtained by comparing the t value to the t distribution or alternatively, by comparing the F value to the F distribution. The p value gives the probability of the null hypothesis (i.e., that there is no effect of the transgene) is correct. The threshold for
5 significance was set at a 5% p-value for the F test and a 10% p-value for the t-test.

Vegetative growth and seed yield was measured according to the methods as described above. The inventors surprisingly found that the seed yield of transgenic plants was increased for both the endosperm-preferred and embryo and/or aleurone-preferred promoter constructs
10 (expressed as total weight of seeds, number of (filled) seeds and harvest index), when compared to null plants, and that transgenic plants with the embryo and/or aleurone-preferred promoter constructs additionally had an increased Thousand Kernel Weight compared to transgenics with the endosperm-preferred promoter construct. The inventors furthermore observed that the yield increase was higher for plants transformed with the embryo and/or
15 aleurone-preferred promoter constructs than for plants with the endosperm-preferred promoter construct. Details are given in paragraphs E and F.

The data obtained in the experiment with T1 plants were then confirmed in a further experiment with T2 plants. Seed batches from the positive plants (both hetero- and
20 homozygotes) in T1, were screened by monitoring marker expression. For each chosen event, the heterozygote seed batches were then retained for T2 evaluation. Within each seed batch an equal number of positive and negative plants were grown in the greenhouse for evaluation. In particular, four events of p37 T2 transformants and three events of p35 T2 transformants were selected for further analysis. For both p37 T2 transformants and p35 T2 transformants, a
25 total of 120 plants were tested, evenly distributed over each event.

E) Evaluation of p37 transformants: measurement of seed-related parameters

Upon analysis of the seeds as described above, the inventors found that plants transformed with the *AtCKX2* gene under control of the embryo and/or aleurone-preferred promoter had a
30 higher total weight of seeds, a higher number of filled seeds, a higher harvest index and a higher Thousand Kernel Weight than plants lacking the *CKX2* transgene. These findings were consistent over 2 independent experiments with T1 plants as well as in an experiment with T2 plants, as shown in table 5. In addition to these yield parameters, 3 lines in T1 scored also positive for the total number of seeds. This increase in total seed number was confirmed in
35 T2, where the effect was shown to be a significant global gene effect (mean increase +24%, p-value from the F-test 0.0032).

Table 5: Analysis of seed related parameters for p37 transformants

Parameter	T1 generation, 1 st experiment	T1 generation, 2 nd experiment	T2 generation	
	Difference over null plants	Difference over null plants	Difference over null plants	p-value
Total weight of seeds	+22%	+22%	+51%	0.0000
Number of filled seeds	+22%	+17%	+46	0.0000
Harvest Index	+25%	+20%	+37%	0.0025
Thousand Kernel Weight	+1%	+5%	+3%	0.0000

5 The total seed weight was measured by weighing all filled seeds harvested from a transformed rice plant. The number of filled seeds was determined by counting the number of filled seeds harvested from a transformed rice plant. The total seed number was determined by counting the number of seeds harvested from a plant. The harvest index is defined as the ratio between the total seed weight and the above ground area (mm²), multiplied by a factor 10⁶. Thousand Kernel Weight (TKW) was derived from the number of filled seeds that were counted, and their
 10 total weight. The figures gave the mean increase (in %) of each parameter calculated from transgenes versus corresponding nullizygotes of 4 independent events in T1 generation, each event comprising 10 plants carrying the transgene and 10 nullizygotes, and of 4 independent events in the T2 generation, each event comprising 20 plants carrying the transgene and 20 nullizygotes. The p-values of the F-test listed for the data of the T2 generation demonstrate
 15 that the obtained increases for the various seed yield parameters are all significant and that there is clearly an overall gene effect.

F) Evaluation of p35 transformants: measurement of seed-related parameters

20 Plants transformed with the AtCKX2 gene under control of the endosperm-preferred promoter also had a better yield compared to the control nullizygous plants, in particular for total seed weight, number of filled seeds and harvest index. The total seed weight, number of filled seeds and harvest index are defined as above.

25 In a first experiment, plants of the T1 generation of five independent events were compared, for each event 10 T1 plants carrying the transgene versus 10 corresponding control T1 plants. For the parameter "total seed weight", two out of the five events had a significant increase

- (58% and 67%, with a p-value of the t-test of 0.0551 and 0.0211 respectively). Similar results were obtained for the number of filled seeds, for which these two lines showed an increase of 47% and 68% with a p-value of respectively 0.0846 and 0.0166. The two lines also scored positive for Harvest Index (increases of 41% (p-value of 0.0223) and 31% respectively).
- 5 Besides these two lines, a third line also scored significantly higher than the corresponding nullizygous control plants (+41%, p-value of 0.035).

The positive data for seed yield observed in the T1 generation were confirmed in the T2 generation. Data are given in Table 6.

10

Table 6: Analysis of seed related parameters for p37 transformants

Parameter	T2 generation	
	Difference over null plants	p-value
Total seed weight	+24%	0.0484
Number of filled seeds	+26%	0.0254
Harvest index	+19%	0.0277

- The figures give the mean increase (in %) of each parameter calculated from transgenes versus corresponding nullizygotes of 3 independent events in the T2 generation, each event comprising 20 plants carrying the transgene and 20 nullizygotes. The p-values of the F-test listed for the data of the T2 generation demonstrate that the obtained increases for the various seed yield parameters are all significant and that there is clearly an overall gene effect.

20 REFERENCES

- Aldemita, R.R. and Hodges, T.K. (1996) *Agrobacterium tumefaciens*-mediated transformation of japonica and indica rice varieties. *Planta* **199**, 612-617.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." *Nucl. Acids Res.* **25**, 3389-3402.
- 25 Armstrong, D.J. (1994) in *Cytokinins: Chemistry, Activity and Functions*, eds. Mok, D.W.S & Mok, M.C. (CRC Boca Raton, FL), pp. 139-154.
- An, G., Watson, B. D., Stachel, S., Gordon, M. P., and Nester, E. W. (1985). New cloning vehicles for transformation of higher plants. *EMBO J.* **4**, 277-284.

- Armstrong, C. L., Petersen, W. P., Buchholz, W. G., Bowen, B. A., and Sulc, S. L. (1990). Factors affecting PEG-mediated stable transformation of maize protoplasts. *Plant Cell Reports* **9**, 335-339.
- 5 Chan, M.T., Chang, H.H., Ho, S.L., Tong, W.F., and Yu, S.M. (1993) *Agrobacterium* mediated production of transgenic rice plants expressing a chimeric alpha-amylase promoter/beta-glucuronidase gene. *Plant Mol. Biol.* **22**, 491-506.
- Christou, P., McCabe, D. E., and Swain, W. F. (1988). Stable transformation of soybean callus by DNA-coated gold particles. *Plant Physiol.* **87**, 671-674.
- 10 Crossway, A., Oakes, J. V., Irvine, J. M., Ward, B., Knauf, V. C., and Shewmaker, C. K. (1986). Integration of foreign DNA following microinjection of tobacco mesophyll protoplasts. *Mol.Gen.Genet.* **202**, 179-185.
- Dodds, J. H. (1985). "Plant genetic engineering." Cambridge University Press.
- Ellis, J. G., Llewellyn, D. J., Dennis, E. S., and Peacock, W. J. (1987). Maize Adh-1 promoter sequences control anaerobic regulation: addition of upstream promoter elements from
15 constitutive genes is necessary for expression in tobacco. *EMBO J.* **6**, 11-16.
- Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *J.Mol.Biol* **166**, 557-580.
- Hansen, G. and Chilton, M. D. (1996). "Agrolistic" transformation of plant cells: integration of T-strands generated in planta. *Proc.Natl.Acad.Sci.U.S.A* **93**, 14978-14983.
- 20 Hansen, G., Shillito, R. D., and Chilton, M. D. (1997). T-strand integration in maize protoplasts after codelivery of a T-DNA substrate and virulence genes. *Proc.Natl.Acad.Sci.U.S.A* **94**, 11726-11730.
- Herrera-Estrella, L., De Block, M., Messens, E. H. J. P., Van Montagu, M., and Schell, J. (1983). Chimeric genes as dominant selectable markers in plant cells. *EMBO J.* **2**, 987-995.
- 25 Hiei,Y.; Ohta,S.; Komari,T.; and Kumashiro,T. (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J.* **6**, 271-282.
- Houba-Herin, N., Pethe, C., d'Alayer, J & Laloue, M. (1999) *Plant J.* **17**:615-626.
- Klee, H.J. & Lanehon, M.B. (1995) in *Plant Hormones:Physiology, Biochemisry and Molecular*
30 *Biology*, ed. Davies, P.J. (Kluwer, Dordrdrecht, the Netherlands), pp. 340-353.

Krens, F.A., Molendijk, L., Wullems, G. J., and Schilperoort, R. A. (1982). *In vitro* transformation of plant protoplasts with Ti-plasmid DNA, *Nature* **296**, 72-74.

Mok M.C. (1994) in *Cytokines: Chemistry, Activity and Function*, eds., Mok, D.W.S. & Mok, M.C. (CRC Boca Raton, FL), pp. 155-166.

Morris, R.O. et al. (1999). Isolation of a gene encoding a glycosylated cytokinin oxidase from maize. *Biochem. Biophys. Res. Commun.* **255**, 328-333

Motyka, V., Faiss, M., Strnad, M., Kaminek, M. and Schmuelling, T. (1996). Changes in cytokinin content and cytokinin oxidase activity in response to derepression of *ipt* gene transcription in transgenic tobacco calli and plants. *Plant Physiol.* **112**, 1035-1043.

Paszkowski, J., Shillito, R. D., Saul, M., Mandak, V., and Hohn, T. H. B. P. I. (1984). Direct gene transfer to plants, *EMBO J.* **3**, 2717-2722.

Rinaldi, A.C. and Comandini, O. (1999). Cytokinin oxidase strikes again. *Trends in Plant Sc.* **4**, 300.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory Press.

Schmülling, T., Rupp, H.M. Frank, M. & Schafer, S., (1999) in *Advances in Regulation of Plant Growth and Development*, eds. Strnad, M. Pac P. & Beck, E. (Peres, Prague), pp. 85-96.

"Comprises/comprising" when used in this specification is taken to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps or components or groups thereof.

The sequence listings attached hereto are to be considered as an integral part of the present specification.

Claims:

1. A method for increasing seed yield in a plant, said method comprising specifically increasing the level and/or activity of a cytokinin oxidase (CKX) in the embryo and/or aleurone of a plant seed, wherein said embryo and/or aleurone have increased levels and/or activity of cytokinin oxidase relative to other parts of said seed.
2. Method according to claim 1, wherein said increased expression in the embryo and/or aleurone is effected by introducing a genetic modification.
3. Method of claim 1, wherein the genetic modification is introduced in the locus of the cytokinin oxidase-encoding genes.
4. Method according to claim 2 or 3, wherein said genetic modification is effected by one of: T-DNA activation, TILLING, site-directed mutagenesis or directed evolution.
5. A method for increasing seed yield in a plant, said method comprising introducing into a plant cell a genetic construct comprising an isolated nucleic acid molecule encoding a cytokinin oxidase wherein said isolated nucleic acid molecule is operably linked to a promoter capable of specifically driving expression in the embryo and/or aleurone of a seed.
6. A method for producing a plant having increased seed yield and increased expression of a cytokinin oxidase in the embryo and/or aleurone of a seed, said method comprising introducing into a plant cell a genetic construct comprising an isolated nucleic acid molecule encoding a cytokinin oxidase wherein said isolated nucleic acid molecule is operably linked to a promoter capable of specifically driving expression in the embryo and/or aleurone of a seed.
7. A method for producing a plant having increased seed yield and increased expression of a cytokinin oxidase in the embryo and/or aleurone of a seed, said method comprising:
 - (a) introducing into a plant cell a genetic construct comprising an isolated nucleic acid molecule encoding a cytokinin oxidase, wherein said isolated nucleic acid molecule is operably linked to a promoter capable of specifically driving expression in the embryo and/or aleurone of a seed;
 - (b) regenerating a plant therefrom;
 - (c) growing the regenerated plant to seed set; and

- (d) selecting a plant with increased seed yield compared to a corresponding wild type plant.
8. The method of any one of claims 5 to 7 wherein the promoter capable of driving expression in the embryo and/or aleurone comprises nucleotides 1-1256 of SEQ ID NO: 41.
9. The method of any one of claims 2 to 8 wherein the nucleic acid molecule encoding a cytokinin oxidase is selected from the group consisting of:
- (a) nucleic acids comprising a DNA sequence as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
 - (b) nucleic acids comprising the RNA sequences corresponding to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
 - (c) nucleic acids specifically hybridizing to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or to the complement thereof,
 - (d) nucleic acids encoding a protein comprising the amino acid sequence as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or the complement thereof,
 - (e) nucleic acids as defined in any of (a) to (d) characterized in that said nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA wherein T is replaced by U,
 - (f) nucleic acid which is degenerate compared to a nucleic acid as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or which is degenerate compared to a nucleic acid as defined in any of (a) to (e) as a result of the genetic code,
 - (g) nucleic acids which are divergent from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or which is divergent from a nucleic acid as defined in any of (a) to (e), due to the differences in codon usage between the organisms,
 - (h) nucleic acids encoding a protein as given in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or nucleic acids as defined in (a) to (e) which are divergent due to the differences between alleles,
 - (i) nucleic acids encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38,
 - (j) functional fragments of nucleic acids as defined in any of (a) to (i) having the biological activity of a cytokinin oxidase, and
 - (k) nucleic acids encoding a plant cytokinin oxidase, comprising the consensus sequence HTDYLhholGGTLSssG and cLFxushGsLGQFGllstA or comprising

expression in seeds of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.

10. The method of any one of claims 5 to 9, wherein the isolated nucleic acid molecule operably linked to a promoter capable of driving expression in the embryo and/or aleurone comprises the nucleotide sequence as set forth in SEQ ID NO: 41.
11. The method of any one of claims 1 to 10 wherein the increase in seed yield comprises at least one of: total weight of seeds, total number of seeds, total number of filled seeds, harvest index, and thousand kernel weight.
12. An isolated nucleic acid molecule encoding a cytokinin oxidase operably linked to a promoter capable of driving overexpression of said nucleic acid molecule in the embryo and/or aleurone of a seed relative to other parts of said seed.
13. The isolated nucleic acid molecule of claim 12 wherein the nucleic acid molecule encoding a cytokinin oxidase is selected from the group consisting of:
 - (a) nucleic acids comprising a DNA sequence as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
 - (b) nucleic acids comprising the RNA sequences corresponding to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
 - (c) nucleic acids specifically hybridizing to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or to the complement thereof,
 - (d) nucleic acids encoding a protein comprising the amino acid sequence as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or the complement thereof,
 - (e) nucleic acids as defined in any of (a) to (d) wherein said nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA wherein T is replaced by U,
 - (f) nucleic acid which is degenerate compared to a nucleic acid as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or which is degenerate compared to a nucleic acid as defined in any of (a) to (e) as a result of the genetic code,
 - (g) nucleic acids which are divergent from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or which is divergent from a nucleic acid as defined in any of (a) to (e), due to the differences in codon usage between the organisms,

- (h) nucleic acids encoding a protein as given in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38 or nucleic acids as defined in (a) to (e) which are divergent due to the differences between alleles,
 - (i) nucleic acids encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38,
 - (j) functional fragments of nucleic acids as defined in any of (a) to (i) having the biological activity of a cytokinin oxidase, and
 - (k) nucleic acids encoding a plant cytokinin oxidase, comprising the consensus sequence hTDYLhholGGTLSssG and cLFxushGsLGQFGIlstA or comprising expression, of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.
- 14. The isolated nucleic acid molecule as claimed in claim 13, wherein the expression of a nucleic acid in (k) is the expression of seeds.
 - 15. A vector comprising the isolated nucleic acid molecule of any one of claims 12 to 14.
 - 16. A vector comprising the isolated nucleic acid molecule having the sequence set forth in SEQ ID NO: 41.
 - 17. A host cell comprising the isolated nucleic acid molecule of any one of claims 12 to 14.
 - 18. A host cell comprising a vector of claims 15 or 16.
 - 19. The host cell of claim 18, which is a bacterial, fungal, or plant cell.
 - 20. Plant having increased seed yield, comprising a genetic modification, which genetic modification results in increased expression of cytokinin oxidase in the aleurone and/or embryo of a seed, relative to other parts of said seed.
 - 21. A transgenic plant, plant part, or plant tissue comprising plant cells of claim 19.
 - 22. The transgenic plant of claim 21 which is a dicotyledonous or monocotyledonous plant.
 - 23. Transgenic plant according to claim 21 or 22, wherein said monocotyledonous plant is a crop plant such as sugarcane, or wherein said monocotyledonous plant is a cereal, such as rice maize, wheat, barley, millet, rye, oats or sorghum.

24. A transgenic harvestable part, or a product directly derived from a transgenic harvestable part of the transgenic plant of any one of claims 21 to 23.
25. A transgenic harvestable part of the transgenic plant of any one of claims 21 to 23 selected from the group consisting of seeds, leaves, fruits, stem cultures, rhizomes, roots, tubers and bulbs.
26. Transgenic progeny of the plant or plant part of any one of claims 21 to 23.
27. Harvestable parts of a plant according to claim 20, or products directly derived of said harvestable parts.
28. Use of cytokinin oxidase-encoding nucleic acids operably linked to a promoter capable of driving overexpression in the aleurone and/or embryo relative to other parts of the seed for increasing seed yield.
29. Use according to claim 28, wherein said cytokinin oxidase-encoding nucleic acid is comprised in a genetic construct that is introduced into a plant cell.
30. Use according to claim 28 or 29, wherein said cytokinin oxidase-encoding nucleic acid is selected from the group consisting of:
 - (a) nucleic acids comprising a DNA sequence as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
 - (b) nucleic acids comprising the RNA sequences corresponding to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or the complement thereof,
 - (c) nucleic acids specifically hybridizing to any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or to the complement thereof,
 - (d) nucleic acids encoding a protein comprising the amino acid sequence as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or the complement thereof,
 - (e) nucleic acids as defined in any of (a) to (d) wherein said nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA wherein T is replaced by U,
 - (f) nucleic acid which is degenerate compared to a nucleic acid as given in any of SEQ ID NOs: 42, 37, 27, 1, 3, 5, 7, 9, 11, 25, 26, 28 to 30, 33 or 34, or which is degenerate compared to a nucleic acid as defined in any of (a) to (e) as a result of the genetic code,

- (g) nucleic acids which are divergent from a nucleic acid encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38, or which is divergent from a nucleic acid as defined in any of (a) to (e), due to the differences in codon usage between the organisms,
 - (h) nucleic acids encoding a protein as given in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38 or nucleic acids as defined in (a) to (e) which are divergent due to the differences between alleles,
 - (i) nucleic acids encoding a protein as given in any of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 35, 36 or 38,
 - (j) functional fragments of nucleic acids as defined in any of (a) to (i) having the biological activity of a cytokinin oxidase, and
 - (k) nucleic acids encoding a plant cytokinin oxidase, comprising the consensus sequence hTDYLhholGGTLSssG and cLFxushGsLGQFGIlstA or comprising expression, of a nucleic acid encoding a protein that reduces the level of active cytokinins in plants or plant parts.
31. Use as claimed in claim 30, wherein the expression of a nucleic acid in (k) is the expression of seeds.
32. Use of SEQ ID NO: 41 for increasing seed yield.
33. Use of a vector according to claim 15 or 16 for increasing seed yield.
34. Method according to the invention for increasing seed yield in a plant, substantially as hereinbefore described or exemplified.
35. Method for increasing seed yield in a plant including any new and inventive integer or combination of integers, substantially as herein described.
36. Method according to the invention for producing a plant, substantially as hereinbefore described or exemplified.
37. Method for producing a plant including any new and inventive integer or combination of integers, substantially as herein described.
38. The isolated nucleic acid molecule as claimed in any one of claims 12 to 14, substantially as hereinbefore described or exemplified.

39. The isolated nucleic acid molecule including any new and inventive integer or combination of integers, substantially as herein described.
40. The vector as claimed in claim 15 or 16, substantially as hereinbefore described or exemplified.
41. The vector according to the invention including any new and inventive integer or combination of integers, substantially as herein described.
42. The host cell as claimed in any one of claims 17 to 19, substantially as hereinbefore described or exemplified.
43. The host cell according to the invention including any new and inventive integer or combination of integers, substantially as herein described.
44. Plant, transgenic plant, transgenic harvestable part, a product derived from the transgenic harvestable part, or transgenic progeny as claimed in any one of claims 20 to 27, substantially as hereinbefore described or exemplified.
45. Plant, transgenic plant, transgenic harvestable part, a product derived from the transgenic harvestable part, or transgenic progeny including any new and inventive integer or combination of integers, substantially as herein described.
46. Use as claimed in any one of claims 28 to 33, substantially as hereinbefore described or exemplified.
47. Use according to the invention including any new and inventive integer or combination of integers, substantially as herein described.



FIGURE 1

2/4

1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
ZmCKX1	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX1	1	MGLTSSLR	FRQNNKTF	LGIFMILV	SCIPORTN	LCNHSYS	TPKELPSS	NSDSDIR	SSLSVSD	LEGYS	IFSD	DMNVAK	DFGRSV	QOLPE	ALH	HER	SVF	-----	-----	-----
AtCKX2	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX3	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX4	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
101	110	120	130	140	150	160	170	180	190	200										
ZmCKX1	82	VALL	SAANSTPG	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX1	101	SSR	KKHIVHLG	STSN	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX2	70	SR	QOYAANG	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX3	83	TD	KL	SFDSQLS	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX4	77	AR	ER	FANGGFS	YNKGST	SPASTE	KVA	RGQHS	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
201	210	220	230	240	250	260	270	280	290	300										
ZmCKX1	173	LT	VGGT	LSN	AGIS	GOA	FR	HG	POIS	NVL	END	VIT	G	EN	WTC	-----	-----	-----	-----	-----
AtCKX1	190	LT	VGGT	LSN	AGIS	GOA	FR	HG	POIS	NVL	END	VIT	G	EN	WTC	-----	-----	-----	-----	-----
AtCKX2	154	ET	VGGT	LSN	AGIS	GOA	FR	HG	POIS	NVL	END	VIT	G	EN	WTC	-----	-----	-----	-----	-----
AtCKX3	171	LT	VGGT	LSN	AGIS	GOA	FR	HG	POIS	NVL	END	VIT	G	EN	WTC	-----	-----	-----	-----	-----
AtCKX4	177	LS	VGGT	LSN	AGIS	GOA	FR	HG	POIS	NVL	END	VIT	G	EN	WTC	-----	-----	-----	-----	-----
301	310	320	330	340	350	360	370	380	390	400										
ZmCKX1	273	RP	GC	GA	SFGP	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX1	290	EK	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX2	254	AND	IS	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX3	271	T	--	DS	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
AtCKX4	277	TND	IS	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
401	410	420	430	440	450	460	470	480	490	500										
ZmCKX1	373	AF	LD	VR	GE	EA	LNK	GLNR	VR	PH	ELN	NE	VP	SR	IA	DR	EN	FK	IG	LOQ
AtCKX1	377	EFL	DR	VI	AE	AK	EA	LNK	GLNR	VR	PH	ELN	NE	VP	SR	IA	DR	EN	FK	IG
AtCKX2	340	DEL	NR	VE	EN	KL	SL	GLNE	PH	ELN	NE	VP	SR	IA	DR	EN	FK	IG	LOQ	-----
AtCKX3	358	DEL	NR	VE	EN	KL	SL	GLNE	PH	ELN	NE	VP	SR	IA	DR	EN	FK	IG	LOQ	-----
AtCKX4	363	DEL	NR	VE	EN	KL	SL	GLNE	PH	ELN	NE	VP	SR	IA	DR	EN	FK	IG	LOQ	-----
501	510	520	530	540	550	560	570	580	590	600										
ZmCKX1	466	DL	AR	LO	EN	RI	LR	FO	DL	AG	IO	YK	YL	AR	PH	DR	SR	IA	DR	EN
AtCKX1	473	DLE	Y	DL	KON	Q	RV	NE	FO	DL	AG	IO	YK	YL	AR	PH	DR	SR	IA	DR
AtCKX2	435	DL	PE	SV	NE	KL	LR	FO	DL	AG	IO	YK	YL	AR	PH	DR	SR	IA	DR	EN
AtCKX3	451	NWE	AF	DO	EN	ME	IT	KE	CE	DAN	EN	LO	YK	YL	AR	PH	DR	SR	IA	DR
AtCKX4	457	NW	Q	B	EN	LD	KE	YK	YL	AR	PH	DR	SR	IA	DR	EN	FK	IG	LOQ	-----

FIGURE 2

3/4

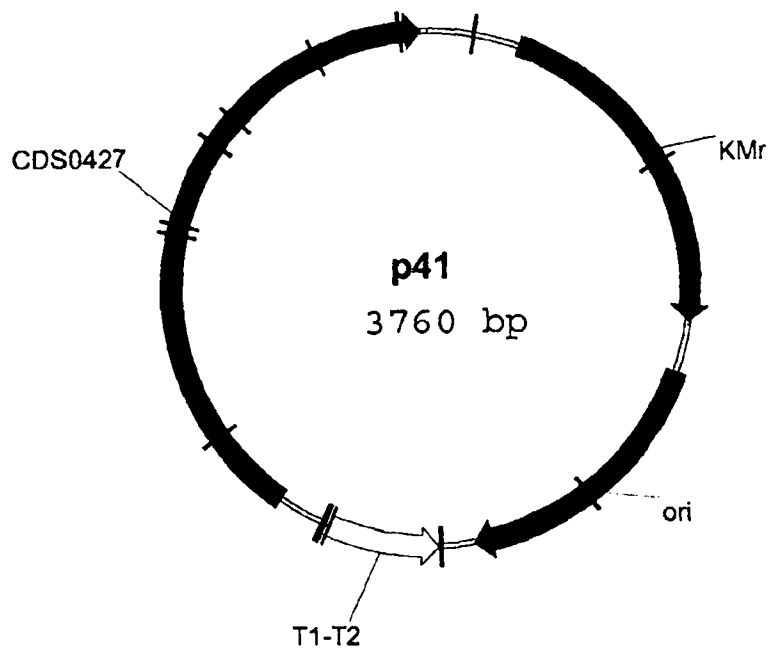


FIGURE 3

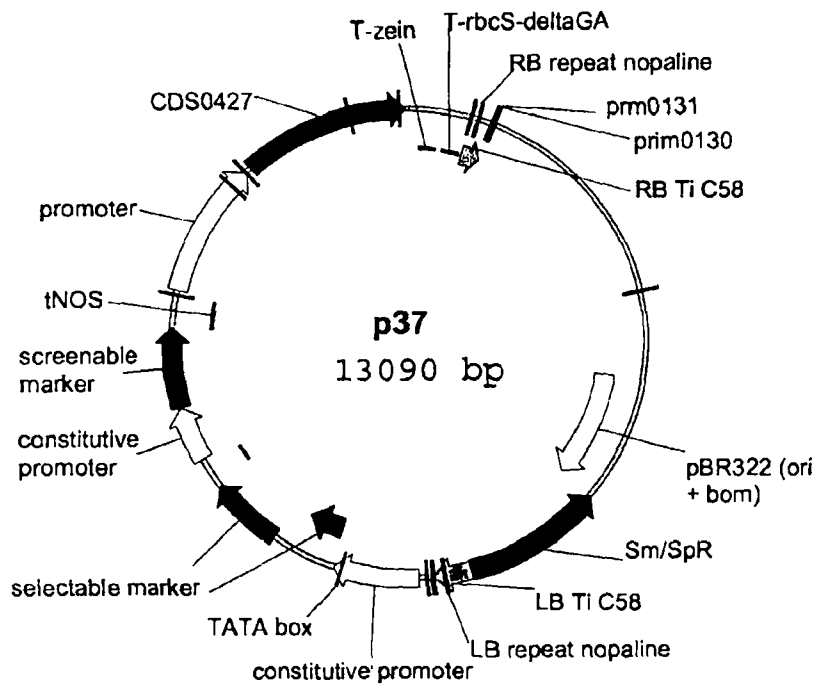


FIGURE 4

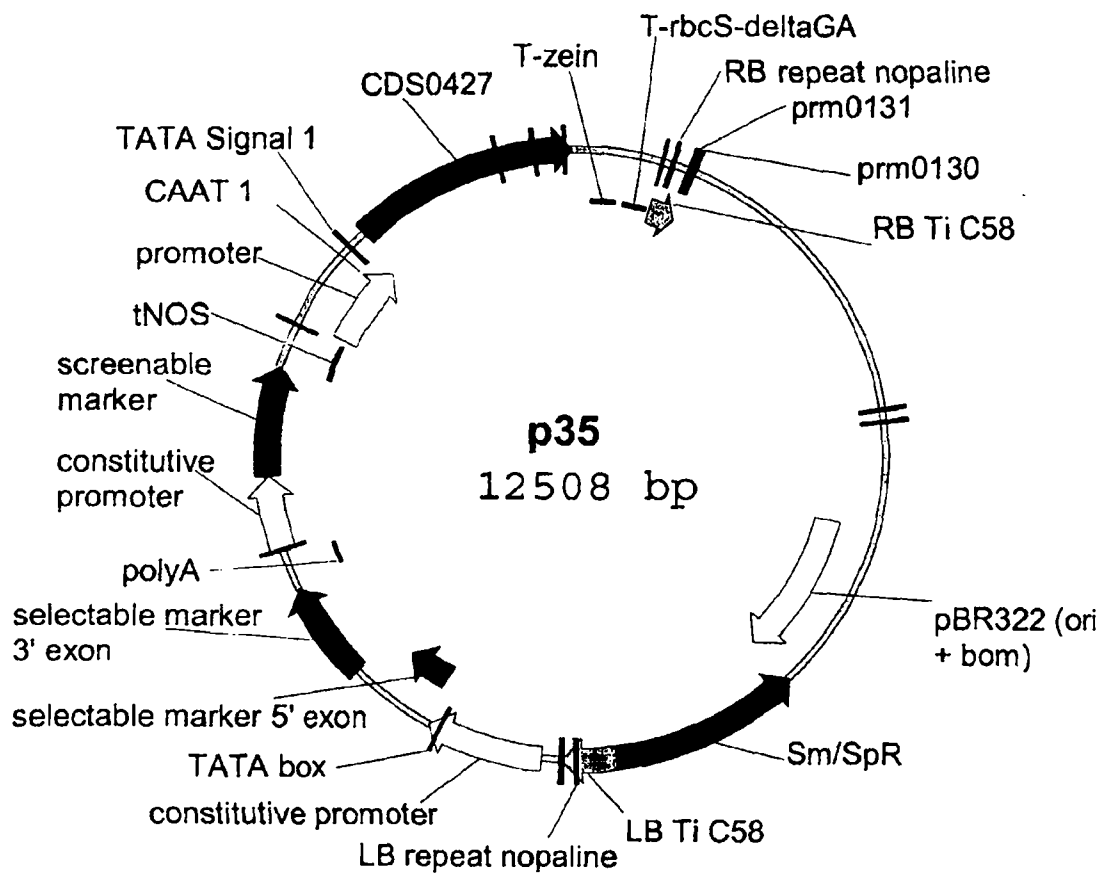


FIGURE 5

CROP033pct.ST25.txt
SEQUENCE LISTING

<110> Schmülling, Thomas
Werner, Tomás

<120> Method for modifying plant morphology, biochemistry and
physiology

<130> CROP-033-PCT

<150> US 10/871,304

<151> 2004-06-18

<160> 45

<170> PatentIn version 3.3

<210> 1

<211> 2236

<212> DNA

<213> Arabidopsis thaliana

<400> 1
atgggattga cctcatcctt acggttccat agacaaaaca acaagacttt cctcggaatc 60
ttcatgatct tagttctaag ctgtatacca ggtagaacca atctttgttc caatcattct 120
gttagtacct caaaagaatt accttcttca aatccttcag atattcgttc ctcatagtt 180
tcactagatt tggaggggta tataagcttc gacgatgtcc acaatgtggc caaggacttt 240
ggcaacagat accagttacc acctttggca attctacatc caaggtcagt ttttgatatt 300
tcatcgatga tgaagcatat agtacatctg ggctccacct caaatcttac agtagcagct 360
agaggccatg gtcactcgtc tcaaggacaa gctctagctc atcaagggtg tgtcatcaaa 420
atggagtcac ttcgaagtcc tgatatcagg atttataagg ggaagcaacc atatgttgat 480
gtctcagggtg gtgaaatatg gataaacatt ctacgcgaga ctctaaaata cggctcttca 540
ccaaagtcct ggacagacta ccttcatttg accgttggag gtacactatc taatgctgga 600
atcagcggtc aagcattcaa gcatggaccc caaatcaaca acgtctacca gctagagatt 660
gttacaggta tttcattcat gctttatctc tgcggtagtc tcaaaaaaat atgcacctgt 720
aaagaatatc catctcttca tgagcaaaaa cactgacgac tttaaataat ttttgactat 780
aaaacaagag tgcataggca caaatgtgaa atatgcaaca cacaattgta acttgcacca 840
agaaaaaagt tataaaaaca aacaactgat aagcaatata tttccaatat ttaatcaggg 900
aaaggagaag tcgtaacctg ttctgagaag cggaattctg aacttttctt cagtgttctt 960
ggcgggcttg gacagtttgg cataatcacc cgggcacgga tctctcttga accagcaccg 1020
catatggtaa agttctatct tgaacaaagt tcaacaata tacgctatga ttctaagaac 1080
cattttcctg acacagtcaa ataactttta ataggttaaa tggatcaggg tactctactc 1140
tgacttttct gcattttcaa gggaccaaga atatctgatt tcgaaggaga aaacttttga 1200
ttacgttgaa ggatttgtga taatcaatag aacagacctt ctcaataatt ggcgatcgtc 1260
attcagtcct aacgattcca cacaggcaag cagattcaag tcagatggga aaactcttta 1320
ttgcctagaa gtggtcaa atttcaaccc agaagaagct agctctatgg atcaggtaag 1380
atgtgaaagc aatatataac tagacttagt ttccacagag agctccaaat caaccgttgg 1440
ctactagcct actaacataa tgaatggttg ccgtgcagga aactggcaag ttactttcag 1500
agttaaatra tattccatcc actttgtttt catctgaagt gccatatatc gagtttctgg 1560
atcgcgtgca tatcgcagag agaaaactaa gagcaaaagg tttatgggag gtccacatc 1620

CROP033pct.ST25.txt

```

cctggctgaa tctcctgatt cctaagagca gcatatacca atttgctaca gaagttttca 1680
acaacattct cacaagcaac aacaacggtc ctatccttat ttatccagtc aatcaatcca 1740
agtaagtgag caaaatgcc aagcaaatg cgtccagtga ttctgaaaca taaattacta 1800
accatatcca acattttgtg gtttcagggtg gaagaaacat acatctttga taactccaaa 1860
tgaagatata ttctatctcg tagcctttct cccctctgca gtgccaaatt cctcagggaa 1920
aaacgatcta gagtaccttt tgaacaaaa ccaaagagtt atgaacttct gcgcagcagc 1980
aaacctcaac gtgaagcagt atttgcccca ttatgaaact caaaaagagt ggaaatcaca 2040
ctttggcaaa agatgggaaa catttgcaca gaggaacaaa gcctacgacc ctctagcgat 2100
tctagcacct ggccaaagaa tattccaaaa gacaacagga aaattatctc ccatccaact 2160
cgcaaagtca aaggcaacag gaagtcctca aaggtaccat tacgcatcaa tactgccgaa 2220
acctagaact gtataa 2236

```

<210> 2
 <211> 575
 <212> PRT
 <213> Arabidopsis thaliana

<400> 2

```

Met Gly Leu Thr Ser Ser Leu Arg Phe His Arg Gln Asn Asn Lys Thr
1      5      10      15
Phe Leu Gly Ile Phe Met Ile Leu Val Leu Ser Cys Ile Pro Gly Arg
20      25      30
Thr Asn Leu Cys Ser Asn His Ser Val Ser Thr Pro Lys Glu Leu Pro
35      40      45
Ser Ser Asn Pro Ser Asp Ile Arg Ser Ser Leu Val Ser Leu Asp Leu
50      55      60
Glu Gly Tyr Ile Ser Phe Asp Asp Val His Asn Val Ala Lys Asp Phe
65      70      75      80
Gly Asn Arg Tyr Gln Leu Pro Pro Leu Ala Ile Leu His Pro Arg Ser
85      90      95
Val Phe Asp Ile Ser Ser Met Met Lys His Ile Val His Leu Gly Ser
100     105     110
Thr Ser Asn Leu Thr Val Ala Ala Arg Gly His Gly His Ser Leu Gln
115     120     125
Gly Gln Ala Leu Ala His Gln Gly Val Val Ile Lys Met Glu Ser Leu
130     135     140
Arg Ser Pro Asp Ile Arg Ile Tyr Lys Gly Lys Gln Pro Tyr Val Asp
145     150     155     160
Val Ser Gly Gly Glu Ile Trp Ile Asn Ile Leu Arg Glu Thr Leu Lys
165     170     175
Tyr Gly Leu Ser Pro Lys Ser Trp Thr Asp Tyr Leu His Leu Thr Val

```

180 185 CROP033pct.ST25.txt 190
 Gly Gly Thr Leu Ser Asn Ala Gly Ile Ser Gly Gln Ala Phe Lys His
 195 200 205
 Gly Pro Gln Ile Asn Asn Val Tyr Gln Leu Glu Ile Val Thr Gly Lys
 210 215 220
 Gly Glu Val Val Thr Cys Ser Glu Lys Arg Asn Ser Glu Leu Phe Phe
 225 230 235 240
 Ser Val Leu Gly Gly Leu Gly Gln Phe Gly Ile Ile Thr Arg Ala Arg
 245 250 255
 Ile Ser Leu Glu Pro Ala Pro His Met Val Lys Trp Ile Arg Val Leu
 260 265 270
 Tyr Ser Asp Phe Ser Ala Phe Ser Arg Asp Gln Glu Tyr Leu Ile Ser
 275 280 285
 Lys Glu Lys Thr Phe Asp Tyr Val Glu Gly Phe Val Ile Ile Asn Arg
 290 295 300
 Thr Asp Leu Leu Asn Asn Trp Arg Ser Ser Phe Ser Pro Asn Asp Ser
 305 310 315 320
 Thr Gln Ala Ser Arg Phe Lys Ser Asp Gly Lys Thr Leu Tyr Cys Leu
 325 330 335
 Glu Val Val Lys Tyr Phe Asn Pro Glu Glu Ala Ser Ser Met Asp Gln
 340 345 350
 Glu Thr Gly Lys Leu Leu Ser Glu Leu Asn Tyr Ile Pro Ser Thr Leu
 355 360 365
 Phe Ser Ser Glu Val Pro Tyr Ile Glu Phe Leu Asp Arg Val His Ile
 370 375 380
 Ala Glu Arg Lys Leu Arg Ala Lys Gly Leu Trp Glu Val Pro His Pro
 385 390 395 400
 Trp Leu Asn Leu Leu Ile Pro Lys Ser Ser Ile Tyr Gln Phe Ala Thr
 405 410 415
 Glu Val Phe Asn Asn Ile Leu Thr Ser Asn Asn Asn Gly Pro Ile Leu
 420 425 430
 Ile Tyr Pro Val Asn Gln Ser Lys Trp Lys Lys His Thr Ser Leu Ile
 435 440 445
 Thr Pro Asn Glu Asp Ile Phe Tyr Leu Val Ala Phe Leu Pro Ser Ala
 450 455 460
 Val Pro Asn Ser Ser Gly Lys Asn Asp Leu Glu Tyr Leu Leu Lys Gln
 465 470 475 480
 Asn Gln Arg Val Met Asn Phe Cys Ala Ala Ala Asn Leu Asn Val Lys

485

CROP033pct.ST25.txt
490

495

Gln Tyr Leu Pro His Tyr Glu Thr Gln Lys Glu Trp Lys Ser His Phe
500 505 510

Gly Lys Arg Trp Glu Thr Phe Ala Gln Arg Lys Gln Ala Tyr Asp Pro
515 520 525

Leu Ala Ile Leu Ala Pro Gly Gln Arg Ile Phe Gln Lys Thr Thr Gly
530 535 540

Lys Leu Ser Pro Ile Gln Leu Ala Lys Ser Lys Ala Thr Gly Ser Pro
545 550 555 560

Gln Arg Tyr His Tyr Ala Ser Ile Leu Pro Lys Pro Arg Thr Val
565 570 575

<210> 3
<211> 2991
<212> DNA
<213> Arabidopsis thaliana

<400> 3
atggctaatac ttcgtttaat gatcacttta atcacggttt taatgatcac caaatcatca 60
aacqgtatta aaattgattt acctaaatcc cttaacctca ccctctctac cgatccttcc 120
atcatctccg cagcctctca tgacttcgga aacataacca ccgtgacccc cggcggcgta 180
atctgccccct cctccaccgc tgatatctct cgtctcctcc aatacgccgc aaacggaaaa 240
agtacattcc aagtagcggc tcgtggccaa ggcactcct taaacggcca agcctcggtc 300
tccggcggag taatcgtcaa catgacgtgt atcactgacg tgggtggttt aaaagacaag 360
aagtacgtg acgtggcggc cgggacgtta tgggtggatg tgcttaagaa gacggcggag 420
aaaggggtgt cgccggtttc ttggacggat tatttgcata taaccgtcgg aggaacgttg 480
tcgaatggtg gaattggtgg tcaagtgtt cgaaacggtc ctcttgtag taacgtcctt 540
gaattggacg ttattactgg tacgcatctt ctaaactttg atgtacatac aacaacaaaa 600
actgtttttg ttttatagta ttttctatt tttgtacat aggttttatg ttttatagtt 660
gtgctaaact tcttgacca cagtaagtc ttcgaaacac aaaatgcgta acgcatctat 720
atgttttttg tacatattga atgttggtca tgagaaataa agtaattaca tatacacaca 780
tttattgtcg tacatatata aataattaaa gacaaatttt cacaattggg agcgtgttaa 840
tttgggattt ttgtaatgta catgcatgac gcatgcatat ggagcttttc ggttttctta 900
gatttgtgta gtatttcaaa tatatcattt attttctttc gaataaagag gtggtatatt 960
tttaaaatag caacatttca gaatttttct ttgaatttac actttttaaa ttgttattgt 1020
taatatggat ttgaataaaa taatttcagg gaaagggtgaa atgttgacat gctcgcgaca 1080
gctaaacca gaattgttct atggagtgtt aggagggttg ggtcaatttg gaattataac 1140
gagagccaga attgttttgg accatgcacc taaacgggta cgtatcatca tattttacca 1200
tttgttttag tcagcattca tttttcatta gtaattccgt ttcaatttct aaattttttt 1260
agtcaataga aaatgattct tatgtcagag cttgattatt tagtgatttt tattgagata 1320
aaataaata taacctaacg gaaataatta ttttactaat cggataatgt ctgattaaaa 1380
cattttatga tattacacta agagagttag agacgtatgg atcacaaaac atgaagcttt 1440

CROP033pct.ST25.txt

```

cttagatggt atccraaaac taaagttagg tacaagtttg gaatttaggt caaatgctta 1500
agttgcatta atttgaacaa aatctatgca ttgaataaaa aaaagatatg gattatttta 1560
taaagtatat tccttgtaat cctaggactt gttgtctaat cttgtcttat gcgtgcaaat 1620
ctttttgatg tcaatatata atccttgttt attagagtca agctctttca ttagtcaact 1680
actcaaatat actccaaagt ttagaatata gtcttctgac taattagaat cttacaaccg 1740
ataaacgtta caatttggtt atcattttta aaaacagatt tggtcataat atacgatgac 1800
gttctgtttt agtttcalct attcacaat tttatataat tttttcaag aaaatattga 1860
aatactatac tgtaatatgg tttctttata tatgtgtgta taaattaaat gggattgttt 1920
tctctaaatg aaattgtgra ggccaaatgg tttcggatgc tctacagtga tttcacaact 1980
tttacaagg accaagaacg tttgatatca atggcaaacg atattggagt cgactattta 2040
gaagggtcaa ttttctatc aaacggtgtc gttgacacct cttttttccc accttcagat 2100
caatctaaag tcgctgatct agtcaagcaa cacggtatca tctatgttct tgaagtggc 2160
aagtattatg atgatcccaa tctcccatc atcagcaagg tactacacat ttacattttc 2220
atcatcgttt ttatcatacc ataagatatt taaatgattc atcattgcac cacattaaga 2280
tattcatcat catcatcggt acattttttt ttgcatctta tgcttctcat aatctactat 2340
tgtgtagggt attgacacat taacgaaaac attagttac ttgcccgggt tcatatcaat 2400
gcacgacgtg gcctacttcg atttcttgaa ccgtgtacat gtcgaagaaa ataaactcag 2460
atctttggga ttatgggaac ttctcatcc ttggcttaac ctctacgttc ctaaattctg 2520
gattctcgat tttcataacg gtgttgtaa agacattctt cttagcaaa aatcagcttc 2580
gggactcgct cttctctatc caacaaaccg gaataagtac atacttctct tcattcatat 2640
ttatcttcaa gaaccaaagt aaataaattt ctatgaactg attatgctgt tattgttaga 2700
tgggacaatc gtatgtcggc gatgatacca gagatcgatg aagatgttat atatattatc 2760
ggactactac aatccgctac cccaaaggat cttccagaag tggagagcgt taacgagaag 2820
ataattaggt ttgcaagga ttcaggtatt aagattaagc aatatcta atgcattatact 2880
agtaaagaag attggattga gcattttgga tcaaaatggg atgatttttc gaagaggaaa 2940
gatctatttg atccaagaa actgttatct ccagggaag acatcttttg a 2991

```

<210> 4
 <211> 501
 <212> PRT
 <213> Arabidopsis thaliana

<400> 4

Met Ala Asn Leu Arg Leu Met Ile Thr Leu Ile Thr Val Leu Met Ile
1 5 10 15

Thr Lys Ser Ser Asn Gly Ile Lys Ile Asp Leu Pro Lys Ser Leu Asn
20 25 30

Leu Thr Leu Ser Thr Asp Pro Ser Ile Ile Ser Ala Ala Ser His Asp
35 40 45

Phe Gly Asn Ile Thr Thr Val Thr Pro Gly Gly Val Ile Cys Pro Ser
50 55 60

CROP033pct.ST25.txt

Ser Thr Ala Asp Ile Ser Arg Leu Leu Gln Tyr Ala Ala Asn Gly Lys
 65 70 75 80
 Ser Thr Phe Gln Val Ala Ala Arg Gly Gln Gly His Ser Leu Asn Gly
 85 90 95
 Gln Ala Ser Val Ser Gly Gly Val Ile Val Asn Met Thr Cys Ile Thr
 100 105 110
 Asp Val Val Val Ser Lys Asp Lys Lys Tyr Ala Asp Val Ala Ala Gly
 115 120 125
 Thr Leu Trp Val Asp Val Leu Lys Lys Thr Ala Glu Lys Gly Val Ser
 130 135 140
 Pro Val Ser Trp Thr Asp Tyr Leu His Ile Thr Val Gly Gly Thr Leu
 145 150 155 160
 Ser Asn Gly Gly Ile Gly Gly Gln Val Phe Arg Asn Gly Pro Leu Val
 165 170 175
 Ser Asn Val Leu Glu Leu Asp Val Ile Thr Gly Lys Gly Glu Met Leu
 180 185 190
 Thr Cys Ser Arg Gln Leu Asn Pro Glu Leu Phe Tyr Gly Val Leu Gly
 195 200 205
 Gly Leu Gly Gln Phe Gly Ile Ile Thr Arg Ala Arg Ile Val Leu Asp
 210 215 220
 His Ala Pro Lys Arg Ala Lys Trp Phe Arg Met Leu Tyr Ser Asp Phe
 225 230 235 240
 Thr Thr Phe Thr Lys Asp Gln Glu Arg Leu Ile Ser Met Ala Asn Asp
 245 250 255
 Ile Gly Val Asp Tyr Leu Glu Gly Gln Ile Phe Leu Ser Asn Gly Val
 260 265 270
 Val Asp Thr Ser Phe Phe Pro Pro Ser Asp Gln Ser Lys Val Ala Asp
 275 280 285
 Leu Val Lys Gln His Gly Ile Ile Tyr Val Leu Glu Val Ala Lys Tyr
 290 295 300
 Tyr Asp Asp Pro Asn Leu Pro Ile Ile Ser Lys Val Ile Asp Thr Leu
 305 310 315 320
 Thr Lys Thr Leu Ser Tyr Leu Pro Gly Phe Ile Ser Met His Asp Val
 325 330 335
 Ala Tyr Phe Asp Phe Leu Asn Arg Val His Val Glu Glu Asn Lys Leu
 340 345 350
 Arg Ser Leu Gly Leu Trp Glu Leu Pro His Pro Trp Leu Asn Leu Tyr
 355 360 365

CROP033pct.ST25.txt

Val Pro Lys Ser Arg Ile Leu Asp Phe His Asn Gly val val Lys Asp
 370 375 380

Ile Leu Leu Lys Gln Lys Ser Ala Ser Gly Leu Ala Leu Leu Tyr Pro
 385 390 395 400

Thr Asn Arg Asn Lys Trp Asp Asn Arg Met Ser Ala Met Ile Pro Glu
 405 410 415

Ile Asp Glu Asp val Ile Tyr Ile Ile Gly Leu Leu Gln Ser Ala Thr
 420 425 430

Pro Lys Asp Leu Pro Glu val Glu Ser val Asn Glu Lys Ile Ile Arg
 435 440 445

Phe Cys Lys Asp Ser Gly Ile Lys Ile Lys Gln Tyr Leu Met His Tyr
 450 455 460

Thr Ser Lys Glu Asp Trp Ile Glu His Phe Gly Ser Lys Trp Asp Asp
 465 470 475 480

Phe Ser Lys Arg Lys Asp Leu Phe Asp Pro Lys Lys Leu Leu Ser Pro
 485 490 495

Gly Gln Asp Ile Phe
 500

<210> 5
 <211> 3302
 <212> DNA
 <213> Arabidopsis thaliana

<400> 5
 atggcgagtt ataattcttcg ttcacaagtt cgtcttatag caataacaat agtaatcatc 60
 attactctct caactccgat cacaaccaac acatcaccac aaccatggaa taccctttca 120
 cacaacgaat tcgccggaaa actcacctcc tctctctcct ccgtcgaatc agccgccaca 180
 gatttcggcc acgtcaccaa aatcttccct tccgccgtct taatcccttc ctccgttgaa 240
 gacatcacag atctcataaa actctctttt gactctcaac tgtcttttcc tttagccgct 300
 cgtggtcacg gacacagcca ccgtggccaa gcctcggcta aagacggagt tgtggtcaac 360
 atgcggtcca tggtaaaccg ggatcgaggt atcaagggtg ctaggacctg tttatatggt 420
 gacgtggacg ctgctgggct atggattgag gtgttgaata aaactttgga gttagggtta 480
 acgccggttt cttggacgga ttatttgtat ttaacagtcg gtgggacgtt atcaaacggc 540
 ggaattagtg gacaaacgtt tcggtacggt ccacagatca ctaatgttct agagatggat 600
 gttattactg gtacgtacca cgatcttttt cacacagaga ttaaaaaaaaa cagtaatagt 660
 gattttaact tcgtacgttt ctgatagaca acaagaact tcgtacgttt ttcgaagttt 720
 tttcgtcttt ttcatttttag atctgcgcgg ccatttttgg ttatgctatt gtttgtttgt 780
 attgtttgtc tctgtttatt ttttctcga acttggtgat agcttttctt cttttcacac 840
 atcaatctaa tcaccttttt tggctttaag attagaaaga agatacggac taggtaaaaa 900
 tagtggttg taaacgtaga cgcattaaaa aatattggt ttttttattt tttgataagc 960
 aaaattggtg gttggtctaa gattataaac ttgatattaa tgcaaaggct gatctagcaa 1020

CROP033pct.ST25.txt

tagaagatta atcaatattc ttggtgtttt aacaacagat tatttcatca ttaaaatcgt	1080
gaaacaaaga aattttggta gtatacatta cgtgtagttt tgtagttta ttaaaaaaa	1140
tagtatatag ttttggttaa acgcgattta tttagtaaca cattagtata ttacacgttt	1200
aaccaactaa actttttttt ttgaataatt atgttctata tttcttactc aaattatgca	1260
aatttcgtgg attcgaagtc aaatttctgc gaaatttaca tggatcatata ttataaaact	1320
gttcatataa cccggtgaac aaacagacaa ttaagggttt gaatggttac ggcggttggg	1380
gcggacacaa cctcgaatag atcagaccgt tttttattta ccattcatca atttatattc	1440
gcagtgggtt ggggtaaaaa aaatagaaga aaaccgcagc ggaccaattc cataccgttt	1500
ttacatacaa ataaacatgg tgcgcaacgg tttattgtcc gcctcaaaaa tgaaatggac	1560
taaaccgcag ataaattaga ccgctttgtc cgctgcctcc attcatagac taaaaaaaaa	1620
caacaaaaaa aaaaatggtc ccacgcccgt gattttacac gaggtttctt gtggcgtaag	1680
gacaaaactc aaaagtccat aacgtttggc cctaaccagg tgtaatggat taagtaacag	1740
tcaattttct tattatagct gtatccatta tgtccacata tgcattcata tacattacac	1800
tgttggcttc aagtgtagtt agattacgaa gactttcaag ttccattttt tggtaggag	1860
ataaacataa ttaaatgata ccgactttag cactctaggc tcaaaacaag tacagaagag	1920
aatagtttta tttcaaaactc gttgcattgt tgtatcaatt aattgtgtta gtctttgtat	1980
attctttacat aacggtccaa gtttgttgaa atagtttact tactaaactt ttcctaag	2040
ggcctaaattt tttttatag gaaaaggaga gattgcaact tgttccaagg acatgaactc	2100
ggatcttttc ttcgcggtgt taggaggttt gggcgaattc ggcattataa caagagccag	2160
aattaaactt gaagttagtc cgaagggtt atgttaaatt tgtaaatat gcaactacag	2220
aaaattctat gaaatttatg aatgaacata tatgcatttt tggatttttg taggccaagt	2280
ggtaagggtt tctatacata gattttctccg aattcacaag agatcaagaa cgagtgat	2340
cgaaaacgga cgggtgtagat ttcttagaag gttccattat ggtggaccat ggccaccg	2400
ataactggag atccacgtat tatccaccgt ccgatcactt gaggatcgcc tcaatggcca	2460
aacgacatcg tgtcatctac tgccttgaag tcgtcaagta ttacgacgaa acttctcaat	2520
acacagtcaa cgaggctcgt acatacatc aatcataaat catacatgta taattgggag	2580
atctttatgc attattcaat tatattaatt tacttttagtt atttaactta tgcaggaaat	2640
ggaggagtta agcgatagtt taaaccatgt aagagggttt atgtacgaga aagatgtgac	2700
gtatatggat ttcctaaacc gagttcgaac cggagagcta aacctgaaat ccaaaggcca	2760
atgggatggt ccacatccat ggcttaattc ctctgtacca aaaactcaaa tctccaaatt	2820
tgatgatggt gtttttaagg gtattatcct aagaaataac atcactagcg gtctgttct	2880
tgtttatcct atgaatcgca acaagtaagt ttaactcgat attgcaaaat ttactatcta	2940
cattttcgtt ttggaatccg aaatattctt acaagctaatt tttatgcggc gtttttaggt	3000
ggaatgatcg gatgtctgcc gclatacccg aggaagatgt attttatgcg gtagggtttt	3060
taagatccgc gggttttgac aattgggagg cttttgatca agaaaacatg gaaatactga	3120
agttttgtga ggatgctaatt atgggggtta tacaatatct tccttatcat tcatcacaag	3180
aaggatgggt tagacatttt ggtccgaggt ggaatatttt cgtagagaga aaatataaat	3240
atgatcccaa aatgatatta tcaccgggac aaaatatatt tcaaaaaata aactcgagtt	3300

ag

<210> 6
 <211> 523
 <212> PRT
 <213> Arabidopsis thaliana

<400> 6

Met Ala Ser Tyr Asn Leu Arg Ser Gln Val Arg Leu Ile Ala Ile Thr
 1 5 10 15
 Ile Val Ile Ile Ile Thr Leu Ser Thr Pro Ile Thr Thr Asn Thr Ser
 20 25 30
 Pro Gln Pro Trp Asn Ile Leu Ser His Asn Glu Phe Ala Gly Lys Leu
 35 40 45
 Thr Ser Ser Ser Ser Ser Val Glu Ser Ala Ala Thr Asp Phe Gly His
 50 55 60
 Val Thr Lys Ile Phe Pro Ser Ala Val Leu Ile Pro Ser Ser Val Glu
 65 70 75 80
 Asp Ile Thr Asp Leu Ile Lys Leu Ser Phe Asp Ser Gln Leu Ser Phe
 85 90 95
 Pro Leu Ala Ala Arg Gly His Gly His Ser His Arg Gly Gln Ala Ser
 100 105 110
 Ala Lys Asp Gly Val Val Val Asn Met Arg Ser Met Val Asn Arg Asp
 115 120 125
 Arg Gly Ile Lys Val Ser Arg Thr Cys Leu Tyr Val Asp Val Asp Ala
 130 135 140
 Ala Trp Leu Trp Ile Glu Val Leu Asn Lys Thr Leu Glu Leu Gly Leu
 145 150 155 160
 Thr Pro Val Ser Trp Thr Asp Tyr Leu Tyr Leu Thr Val Gly Gly Thr
 165 170 175
 Leu Ser Asn Gly Gly Ile Ser Gly Gln Thr Phe Arg Tyr Gly Pro Gln
 180 185 190
 Ile Thr Asn Val Leu Glu Met Asp Val Ile Thr Gly Lys Gly Glu Ile
 195 200 205
 Ala Thr Cys Ser Lys Asp Met Asn Ser Asp Leu Phe Phe Ala Val Leu
 210 215 220
 Gly Gly Leu Gly Gln Phe Gly Ile Ile Thr Arg Ala Arg Ile Lys Leu
 225 230 235 240
 Glu Val Ala Pro Lys Arg Ala Lys Trp Leu Arg Phe Leu Tyr Ile Asp
 245 250 255
 Phe Ser Glu Phe Thr Arg Asp Gln Glu Arg Val Ile Ser Lys Thr Asp

CROP033pct.ST25.txt

260

265

270

Gly Val Asp Phe Leu Glu Gly Ser Ile Met Val Asp His Gly Pro Pro
 275 280 285

Asp Asn Trp Arg Ser Thr Tyr Tyr Pro Pro Ser Asp His Leu Arg Ile
 290 295 300

Ala Ser Met Val Lys Arg His Arg Val Ile Tyr Cys Leu Glu Val Val
 305 310 315 320

Lys Tyr Tyr Asp Glu Thr Ser Gln Tyr Thr Val Asn Glu Glu Met Glu
 325 330 335

Glu Leu Ser Asp Ser Leu Asn His Val Arg Gly Phe Met Tyr Glu Lys
 340 345 350

Asp Val Thr Tyr Met Asp Phe Leu Asn Arg Val Arg Thr Gly Glu Leu
 355 360 365

Asn Leu Lys Ser Lys Gly Gln Trp Asp Val Pro His Pro Trp Leu Asn
 370 375 380

Leu Phe Val Pro Lys Thr Gln Ile Ser Lys Phe Asp Asp Gly Val Phe
 385 390 395 400

Lys Gly Ile Ile Leu Arg Asn Asn Ile Thr Ser Gly Pro Val Leu Val
 405 410 415

Tyr Pro Met Asn Arg Asn Lys Trp Asn Asp Arg Met Ser Ala Ala Ile
 420 425 430

Pro Glu Glu Asp Val Phe Tyr Ala Val Gly Phe Leu Arg Ser Ala Gly
 435 440 445

Phe Asp Asn Trp Glu Ala Phe Asp Gln Glu Asn Met Glu Ile Leu Lys
 450 455 460

Phe Cys Glu Asp Ala Asn Met Gly Val Ile Gln Tyr Leu Pro Tyr His
 465 470 475 480

Ser Ser Gln Glu Gly Trp Val Arg His Phe Gly Pro Arg Trp Asn Ile
 485 490 495

Phe Val Glu Arg Lys Tyr Lys Tyr Asp Pro Lys Met Ile Leu Ser Pro
 500 505 510

Gly Gln Asn Ile Phe Gln Lys Ile Asn Ser Ser
 515 520

<210> 7

<211> 2782

<212> DNA

<213> Arabidopsis thaliana

<400> 7

atgactaata ctctctgttt aagcctcatc accctaataa cgctttttat aagtttaacc 60

ccaaccttaa tcaaatcaga tgagggcatt gatgttttct taccatatac actcaacctt 120

CROP033pct.ST25.txt

acggtcctaa ccgatccctt ctccatctct gccgttctc acgacttcgg taacataacc	180
gacgaaaatc ccggcgccgt cctctgccct tcctccacca cggagggtggc tcgtctcttc	240
cgtttcgcta acggaggatt ctcttacaat aaaggctcaa ccagccccgc gtctactttc	300
aaagtggctg ctcgaggcca aggccactcc ctccgtggcc aagcctctgc acccgagggt	360
gtcgtcgtga acatgacgtg tctcgccatg gcggctaaac cagcggcggt tgttatctcg	420
gcagacggga ctacgctga cgtggctgcc gggacgatgt gggaggatgt tctgaaggcg	480
gcgggtggata gaggcgtctc gccggttaca tggacggatt atttgtatct cagcgtcggc	540
gggacgttgt cgaacgctgg aatcgggtgg cagacgttta gacacggccc tcagattagt	600
aacgttcatg agcttgacgt tattaccggg acgtaaatac caaaacttca ctaatctcgt	660
tacaattttt taattttttg gtaatatata ttttgtacgg ctcaactctt aattaagaat	720
gaaacagtat ctatgatctt ctatagcttc ttttttgtc tgcaagcttt aattgtagta	780
acatcagcga tatatatatc acatgcatgt gtattattga tgataarata taatgtttta	840
gttacaaatt tgattctcaa ggtaaaactc acacgccata accagtataa aactccaaaa	900
atcacgtttt ggtcagaaat acatatcctt cattaacagt agttatgcta taatttgtga	960
ttataaataa ctccggagtt tgttcacaat actaaatttc aggaaaagggt gaaatgatga	1020
cttgctcttc aaagttaaac cctgaattgt tctatggagt tttaggaggt ttgggtcaat	1080
tcggtattat aacgagggcc aggattgcgt tggatcatgc acccacaagg gtatgtatca	1140
tgcatctata gtgtaatcaa ttataattt taatgtagtg gtcctaaatc caaaatttga	1200
tttgatttgg ttggaacgta cgtatatata ataagtcaaa aggtgatgtt tgaagacgaa	1260
tttatatact tttgttgaat taaatctgat tttgcttacg ttttattaga ttctgcgtaa	1320
taaatcctag gacttgctcg agtgtaatct tgtcttatgc ttgcaaactc tgttgatgtc	1380
aatatctaact cttttttatt atatttccct acgtaagtgt tagatatagt tattttaaac	1440
tgctataaat tgtgtacgta tagactttag ataaaaagtt gtgggtcgctt gcacctattt	1500
gtttatcgct atagtgatcc aaaggctctat atatgattct tgggttttct ttttgaaaaa	1560
aatagaccat acaatccaag gaagatgatc ttaaattggac taatttatgg atataaattg	1620
atatacaaat ctgcaggiga aatggctctc catactctac agtgacttct cggcttttaa	1680
aagagaccaa gagcgtttta tarcaatgac caatgatctc ggagttgact ttttgaagg	1740
tcaacttatg atgtcaaatg gcttcgtaga cacctcttct tcccaactct ccgatcaaac	1800
aagagtcgca tctcttgtga atgaccaccg gatcatctat gttctcgaag tagccaagta	1860
ttatgacaga accacccttc ccattattga ccaggtaact aaatccatta ttcatgatga	1920
ttatcttcac acaatcagta tcatcaccaa ttaccatcat cacttgatcat atatgatcca	1980
aagtaaatat atcacatgat ataaataaat cgttcaaact ttttttttta aagaataaaa	2040
gaatcatttt caagcattac tcatacacat ctacgaatca ccgtgaccat atataaccat	2100
acgcttatta aataatcatt tttgtttgta ggtgattgac acgttaagta gaactctagg	2160
tttcgctcca gggtttatgt tcgtacaaga tgttccgtat ttcgatttct tgaaccgtgt	2220
ccgaaacgaa gaagataaac tcagatcttt aggactatgg gaagtccctc atccatggct	2280
taacatcttt gtcccggggt ctgcaatcca agattttcat gatgggtgta ttaatggcct	2340
tcttctaaac caaacctcaa cttctgggtgt tactctcttc tatccacaaa accgaaacaa	2400

CROP033pct.ST25.txt

gtaaataatit actttttgat ttgttttat ttgaaagtat atcccaataa tgrtatgttaa 2460
 attgttaaca agaatttatt ttattaatag atggaacaac cgcattgtcaa cgatgacacc 2520
 ggacgaagat gttttttatg tgatcggatt actgcaatca gctgggtggat ctcaaaattg 2580
 gcaagaactt gaaaatctca acgacaaggt tattcagttt tgtgaaaact cgggaattaa 2640
 gattaaggaa tatttgatgc actatacaag aaaagaagat tgggttaaac attttggacc 2700
 aaaatgggat gattttttta gaaagaaaat tatgtttgat cccaaaagac tattgtctcc 2760
 aggacaagac atatttaatt aa 2782

<210> 8
 <211> 524
 <212> PRT
 <213> Arabidopsis thaliana

<400> 8

Met Thr Asn Thr Leu Cys Leu Ser Leu Ile Thr Leu Ile Thr Leu Phe
1 5 10 15

Ile Ser Leu Thr Pro Thr Leu Ile Lys Ser Asp Glu Gly Ile Asp Val
20 25 30

Phe Leu Pro Ile Ser Leu Asn Leu Thr Val Leu Thr Asp Pro Phe Ser
35 40 45

Ile Ser Ala Ala Ser His Asp Phe Gly Asn Ile Thr Asp Glu Asn Pro
50 55 60

Gly Ala Val Leu Cys Pro Ser Ser Thr Thr Glu Val Ala Arg Leu Leu
65 70 75 80

Arg Phe Ala Asn Gly Gly Phe Ser Tyr Asn Lys Gly Ser Thr Ser Pro
85 90 95

Ala Ser Thr Phe Lys Val Ala Ala Arg Gly Gln Gly His Ser Leu Arg
100 105 110

Gly Gln Ala Ser Ala Pro Gly Gly Val Val Val Asn Met Thr Cys Leu
115 120 125

Ala Met Ala Ala Lys Pro Ala Ala Val Val Ile Ser Ala Asp Gly Thr
130 135 140

Tyr Ala Asp Val Ala Ala Gly Thr Met Trp Val Asp Val Leu Lys Ala
145 150 155 160

Ala Val Asp Arg Gly Val Ser Pro Val Thr Trp Thr Asp Tyr Leu Tyr
165 170 175

Leu Ser Val Gly Gly Thr Leu Ser Asn Ala Gly Ile Gly Gly Gln Thr
180 185 190

Phe Arg His Gly Pro Gln Ile Ser Asn Val His Glu Leu Asp Val Ile
195 200 205

Thr Gly Lys Gly Glu Met Met Thr Cys Ser Pro Lys Leu Asn Pro Glu

210

215

Leu Phe Tyr Gly Val Leu Gly Gly Leu Gly Gln Phe Gly Ile Ile Thr
225 230 235 240

Arg Ala Arg Ile Ala Leu Asp His Ala Pro Thr Arg Val Lys Trp Ser
245 250 255

Arg Ile Leu Tyr Ser Asp Phe Ser Ala Phe Lys Arg Asp Gln Glu Arg
260 265 270

Leu Ile Ser Met Thr Asn Asp Leu Gly Val Asp Phe Leu Glu Gly Gln
275 280 285

Leu Met Met Ser Asn Gly Phe Val Asp Thr Ser Phe Phe Pro Leu Ser
290 295 300

Asp Gln Thr Arg Val Ala Ser Leu Val Asn Asp His Arg Ile Ile Tyr
305 310 315 320

Val Leu Glu Val Ala Lys Tyr Tyr Asp Arg Thr Thr Leu Pro Ile Ile
325 330 335

Asp Gln Val Ile Asp Thr Leu Ser Arg Thr Leu Gly Phe Ala Pro Gly
340 345 350

Phe Met Phe Val Gln Asp Val Pro Tyr Phe Asp Phe Leu Asn Arg Val
355 360 365

Arg Asn Glu Glu Asp Lys Leu Arg Ser Leu Gly Leu Trp Glu Val Pro
370 375 380

His Pro Trp Leu Asn Ile Phe Val Pro Gly Ser Arg Ile Gln Asp Phe
385 390 395 400

His Asp Gly Val Ile Asn Gly Leu Leu Leu Asn Gln Thr Ser Thr Ser
405 410 415

Gly Val Thr Leu Phe Tyr Pro Thr Asn Arg Asn Lys Trp Asn Asn Arg
420 425 430

Met Ser Thr Met Thr Pro Asp Glu Asp Val Phe Tyr Val Ile Gly Leu
435 440 445

Leu Gln Ser Ala Gly Gly Ser Gln Asn Trp Gln Glu Leu Glu Asn Leu
450 455 460

Asn Asp Lys Val Ile Gln Phe Cys Glu Asn Ser Gly Ile Lys Ile Lys
465 470 475 480

Glu Tyr Leu Met His Tyr Thr Arg Lys Glu Asp Trp Val Lys His Phe
485 490 495

Gly Pro Lys Trp Asp Asp Phe Leu Arg Lys Lys Ile Met Phe Asp Pro
500 505 510

Lys Arg Leu Leu Ser Pro Gly Gln Asp Ile Phe Asn

<210> 9
<211> 2805
<212> DNA
<213> Arabidopsis thaliana

<400> 9
atgacgtcaa gctttcttct cctgacgttc gccatatgta aactgatcat agccgtgggt 60
ctaaacgtgg gccccagtga gctcctccgc atcggagcca tagatgtcga cggccacttc 120
accgtccacc ctccgactt agcctccgtc tcttcagact tcggtatgct gaagtcacct 180
gaagagccat tggccgtgct tcatccatca tcggccgaag acgtggcacg actcgtcaga 240
acagcttacg gttcagccac ggcgtttccg gtctcagccc gaggccacgg ccattccata 300
aacggacaag ccgcggcggg gaggaacggg gtggtggttg aaatgaacca cggcgttaacc 360
gggacgcccc agccactcgt ccgaccggat gaaatgtatg tggatgtatg ggggtggagag 420
ttatgggtcg atgtgttgaa gaaaacgttg gagcatggct tagcaccaaa atcatggacg 480
gattacttgt atctaaccgt tggaggtaca ctctccaatg caggaatcag tggtaagct 540
tttcaccatg gtctcaaat tagtaacgtc cttgagctcg acgttgtaac tggtagtat 600
taaaacattc aagttcatat attttaaatg cttttgtctg aagttttact aataacaaga 660
aattgatacc aaaaagtagg gaaaggagag gtgatgagat gctcagaaga agagaacaca 720
aggctattcc atggagttct tgggtgatta ggtcaatttg ggatcatcac tcgagcacga 780
atctctctcg aaccagctcc ccaaagggtg atattttttt aatgactagc tatcaaaaat 840
ccctggcggg tccatacgtt gtaatctttt tagtttttac tgttgatggt atttttata 900
tattttggat aataaaaccc taaaatggta tattgtgatg acaggtgaga tggatacggg 960
tattgtattc gagcttcaaa gtgtttacgg aggaccaaga gtacttaatc tcaatgcatg 1020
gtcaattaaa gtttgattac gtggaagggt ttgtgattgt ggacgaagga ctcgtcaaca 1080
attggagatc ttctttcttc tctccacgta acccgtcaa gatctcctct gttagtcca 1140
acggctctgt tttgtattgc cttgagatca ccaagaacta ccacgactcc gactccgaaa 1200
tcgttgatca ggtcactttc attattcact tagaaaaaag cgatattttc atttttata 1260
ttgatgaata tctggaagga tttaacgcta tgcgactatt gggaaatcat tatgaaaaa 1320
tatttagttt atatgattga aagtgggtct catagtattt ttgttggtgc gactttatta 1380
taacttaaat ttggaagagg acatgaagaa gaagccagag aggatctaca gagatctagc 1440
ttttccacct gaacttaata atgcacattt atataattat ttttcttctt ctaaagtta 1500
gtttatcact agcgaattaa tcatgggtac taattaagta gtggacaggg tcatggacca 1560
ctcactcacc aaataatgat tctcttttac tcttaagttt aattttaata aaaccaactc 1620
tactggaatc ttaacttatc ctgtgttttg gtaggctttt atagcaacac ggtttttta 1680
attttcctat tccagatttt gtatattaaa tgcgattttt ttttctttt gtttcaggaa 1740
gttgagattc tgatgaagaa attgaatttc ataccgacat cggcttttac aacggattta 1800
caatatgtgg actttctcga ccgggtacac aaggccgaat tgaagctccg gtccaagaat 1860
ttatgggagg ttccacaccc atggctcaac ctcttcgtgc caaaatcaag aatctctgac 1920
ttcgataaag gcgttttcaa gggcattttg ggaaataaaa caagtggccc tattcttatc 1980
taccatga acaaagacaa gtaagtcttg acattaccat tgattactac ttctaaattt 2040

CROP033pct.ST25.txt

```

cttctctaga aaaaagaata aaacgagttt tgcattgcat gcatgcaaag ttacacttgt 2100
ggggattaat tagtgggtcca agaaaaaaag tttgtcaaaa ttgaaaaaaa ctagacacgt 2160
ggtacatggg attgtccgaa aaacgttgct cacatgtgca tcgaaccagc taagattgac 2220
aacaacactt cgtcggctcg tatttctctt tttgttttgt gaccaaattcc gatgggtccag 2280
attgggttta tttgttttta agttcctaga actcatggtg ggtgggtccc aatcagattc 2340
tcctagacca aaccgatctc aacgaaccct ccgcacatca ttgattatta cattaatata 2400
gatattgtcg ttgctgacgt gtcgtaattt gatgttattg tcagatggga cgagaggagc 2460
tcagccgtga cgccggatga ggaagttttc tatctggtgg ctctattgag atcagcttta 2520
acggacggtg aagagacaca gaagctagag tatctgaaag atcagaaccg tcggatcttg 2580
gagttctgtg aacaagccaa gatcaatgtg aagcagtatc ttcctcacca cgcaacacag 2640
gaagagtggg tggctcattt tggggacaag tgggatcggg tcagaagctt aaaggctgag 2700
tttgatccgc gacacatact cgctactggt cagagaatct ttcaaaaccc atctttgtct 2760
ttgtttcttc cgtcgtcgtc ttcttcgtca gcggcttcat ggtga 2805

```

<210> 10
 <211> 536
 <212> PRT
 <213> Arabidopsis thaliana

<400> 10

Met Thr Ser Ser Phe Leu Leu Leu Thr Phe Ala Ile Cys Lys Leu Ile
 1 5 10 15

Ile Ala Val Gly Leu Asn Val Gly Pro Ser Glu Leu Leu Arg Ile Gly
 20 25 30

Ala Ile Asp Val Asp Gly His Phe Thr Val His Pro Ser Asp Leu Ala
 35 40 45

Ser Val Ser Ser Asp Phe Gly Met Leu Lys Ser Pro Glu Glu Pro Leu
 50 55 60

Ala Val Leu His Pro Ser Ser Ala Glu Asp Val Ala Arg Leu Val Arg
 65 70 75 80

Thr Ala Tyr Gly Ser Ala Thr Ala Phe Pro Val Ser Ala Arg Gly His
 85 90 95

Gly His Ser Ile Asn Gly Gln Ala Ala Ala Gly Arg Asn Gly Val Val
 100 105 110

Val Glu Met Asn His Gly Val Thr Gly Thr Pro Lys Pro Leu Val Arg
 115 120 125

Pro Asp Glu Met Tyr Val Asp Val Trp Gly Gly Glu Leu Trp Val Asp
 130 135 140

Val Leu Lys Lys Thr Leu Glu His Gly Leu Ala Pro Lys Ser Trp Thr
 145 150 155 160

Asp Tyr Leu Tyr Leu Thr Val Gly Gly Thr Leu Ser Asn Ala Gly Ile

CROP033pct.ST25.txt
170 175

165

Ser Gly Gln Ala Phe His His Gly Pro Gln Ile Ser Asn Val Leu Glu
180 185 190

Leu Asp Val Val Thr Gly Lys Gly Glu Val Met Arg Cys Ser Glu Glu
195 200 205

Glu Asn Thr Arg Leu Phe His Gly Val Leu Gly Gly Leu Gly Gln Phe
210 215 220

Gly Ile Ile Thr Arg Ala Arg Ile Ser Leu Glu Pro Ala Pro Gln Arg
225 230 235 240

Val Arg Trp Ile Arg Val Leu Tyr Ser Ser Phe Lys Val Phe Thr Glu
245 250 255

Asp Gln Glu Tyr Leu Ile Ser Met His Gly Gln Leu Lys Phe Asp Tyr
260 265 270

Val Glu Gly Phe Val Ile Val Asp Glu Gly Leu Val Asn Asn Trp Arg
275 280 285

Ser Ser Phe Phe Ser Pro Arg Asn Pro Val Lys Ile Ser Ser Val Ser
290 295 300

Ser Asn Gly Ser Val Leu Tyr Cys Leu Glu Ile Thr Lys Asn Tyr His
305 310 315 320

Asp Ser Asp Ser Glu Ile Val Asp Gln Glu Val Glu Ile Leu Met Lys
325 330 335

Lys Leu Asn Phe Ile Pro Thr Ser Val Phe Thr Thr Asp Leu Gln Tyr
340 345 350

Val Asp Phe Leu Asp Arg Val His Lys Ala Glu Leu Lys Leu Arg Ser
355 360 365

Lys Asn Leu Trp Glu Val Pro His Pro Trp Leu Asn Leu Phe Val Pro
370 375 380

Lys Ser Arg Ile Ser Asp Phe Asp Lys Gly Val Phe Lys Gly Ile Leu
385 390 395 400

Gly Asn Lys Thr Ser Gly Pro Ile Leu Ile Tyr Pro Met Asn Lys Asp
405 410 415

Lys Trp Asp Glu Arg Ser Ser Ala Val Thr Pro Asp Glu Glu Val Phe
420 425 430

Tyr Leu Val Ala Leu Leu Arg Ser Ala Leu Thr Asp Gly Glu Glu Thr
435 440 445

Gln Lys Leu Glu Tyr Leu Lys Asp Gln Asn Arg Arg Ile Leu Glu Phe
450 455 460

Cys Glu Gln Ala Lys Ile Asn Val Lys Gln Tyr Leu Pro His His Ala

CROP033pct.ST25.txt
475

465

470

480

Thr Gln Glu Glu Trp Val Ala His Phe Gly Asp Lys Trp Asp Arg Phe
485 490 495

Arg Ser Leu Lys Ala Glu Phe Asp Pro Arg His Ile Leu Ala Thr Gly
500 505 510

Gln Arg Ile Phe Gln Asn Pro Ser Leu Ser Leu Phe Pro Pro Ser Ser
515 520 525

Ser Ser Ser Ser Ala Ala Ser Trp
530 535

<210> 11
<211> 1936
<212> DNA
<213> Arabidopsis thaliana

<400> 11
atgcttatag taagaagttt caccatcttg ctctcagct gcatagcctt taagttggct 60
tgctgcttct ctagcagcat ttcttctttg aaggcgcttc ccctagtagg ccatttggag 120
tttgaacatg tccatcacgc ctccaaagat tttggaaatc gataccagtt gatcccttg 180
gccgtcttac atcccaaatc ggtaagcgac atcgctcaa cgatacgaca catctggatg 240
atgggcactc attcacagct tacagtggca gcgagaggtc gtggacattc actccaaggc 300
caagctcaaa caagacatgg aattgttata cacatggaat cactccatcc ccagaagctg 360
caggtctaca gtgtggattc ccttgcctca tatgttgatg tgtctggtgg tgagctgtgg 420
ataaacattt tgcattgagac cctcaagtac gggcttgac caaatcatg gacggattac 480
ctgcatttaa ctgtagggtg tactctgtcc aatgctggaa taagcgcca ggcatccga 540
catggaccac agatcagcaa tgtcatcaa ctggagattg tcacagggtta gttcagagtt 600
gcagtattcg tgttttgaaa gcatagactc tatatggttg gtgactatta acaacatgaa 660
gagattcccg agaatagcta cccactaatg tcatgcctat ttattgactg caggaaaagg 720
cgagatccta aactgtacaa agaggcagaa cagcgactta ttaaatggtg ttcttgggtg 780
tttaggtcag ttggcatca taacgcgggc aagaatagca ttggaaccag caccaaccat 840
ggtaaacaaat aaataaataa aaaacttaaa aactgaacac gcgtgtgtcc tcctaactct 900
gtataatgga caggtaaaat ggataagagt gttatacctg gatattgagc cttttgcaa 960
ggaccaagag caactaatat ctgccaggg ccacaaattc gattacatag aagggtttgt 1020
gataataaac aggacaggcc tcctgaacag ctggagggtg tctttcaccg cagaagagcc 1080
tttagaagca agccaattca agtttgatgg aaggactctg tattgtctgg agctagccaa 1140
gtatttgaag caagataaca aagacgtaat caaccagggtg agaaaacaga gtagaagcaa 1200
tcggtagaat cttctttggt agatgacatt cattggaact gaaaatatat atatatttgt 1260
ccaatccagg aagtgaagaa aacattatca gagctaagct acgtgacgtc gacactgttt 1320
acaacggagg tagcatatga agcattcttg gacaggggtac atgtgtctga ggtaaaactc 1380
cgatcgaaag ggcagtggga ggtgccacat ccatggctga acctcctggt accaagaagc 1440
aaaatcaatg aatttgcaag aggtgtattt ggaaacatac taacggatac aagcaacggc 1500
ccagtcacgt tctaccaggt gaacaaatca aagtaagaaa gaaagaaaga aagagctagt 1560

CROP033pct.ST25.txt

```

catgattttg tttcttttca cttgttgaca aaacaaaagc atgttggtga gcaggtggga 1620
caatcaaaca tcagcagtaa caccggagga agaggtattc tacctggtgg cgatcctaac 1680
atcggcatct ccagggtcgg caggaaagga tggagtagaa gagatcttga ggcggaacag 1740
aagaatactg gaattcagtg aagaagcagg gataggggtg aagcagtatc tgccacatta 1800
cacgacaaga gaagagtgga gatcccatct cggggacaag tggggagaat ttgtgaggag 1860
gaaatccaga tatgatccat tggcaattct tgcgcctggc caccgaattt ttcaaaaggc 1920
agtttcatac tcatga 1936

```

<210> 12
 <211> 504
 <212> PRT
 <213> Arabidopsis thaliana

<400> 12

```

Met Leu Ile Val Arg Ser Phe Thr Ile Leu Leu Leu Ser Cys Ile Ala
1      5      10      15

Phe Lys Leu Ala Cys Cys Phe Ser Ser Ser Ile Ser Ser Leu Lys Ala
20     25     30

Leu Pro Leu Val Gly His Leu Glu Phe Glu His Val His His Ala Ser
35     40     45

Lys Asp Phe Gly Asn Arg Tyr Gln Leu Ile Pro Leu Ala Val Leu His
50     55     60

Pro Lys Ser Val Ser Asp Ile Ala Ser Thr Ile Arg His Ile Trp Met
65     70     75     80

Met Gly Thr His Ser Gln Leu Thr Val Ala Ala Arg Gly Arg Gly His
85     90     95

Ser Leu Gln Gly Gln Ala Gln Thr Arg His Gly Ile Val Ile His Met
100    105    110

Glu Ser Leu His Pro Gln Lys Leu Gln Val Tyr Ser Val Asp Ser Pro
115    120    125

Ala Pro Tyr Val Asp Val Ser Gly Gly Glu Leu Trp Ile Asn Ile Leu
130    135    140

His Glu Thr Leu Lys Tyr Gly Leu Ala Pro Lys Ser Trp Thr Asp Tyr
145    150    155    160

Leu His Leu Thr Val Gly Gly Thr Leu Ser Asn Ala Gly Ile Ser Gly
165    170    175

Gln Ala Phe Arg His Gly Pro Gln Ile Ser Asn Val His Gln Leu Glu
180    185    190

Ile Val Thr Gly Lys Gly Glu Ile Leu Asn Cys Thr Lys Arg Gln Asn
195    200    205

Ser Asp Leu Phe Asn Gly Val Leu Gly Gly Leu Gly Gln Phe Gly Ile

```

CROP033pct.ST25.txt
220

210

215

Ile Thr Arg Ala Arg Ile Ala Leu Glu Pro Ala Pro Thr Met Asp Gln
 225 230 235 240
 Glu Gln Leu Ile Ser Ala Gln Gly His Lys Phe Asp Tyr Ile Glu Gly
 245 250 255
 Phe Val Ile Ile Asn Arg Thr Gly Leu Leu Asn Ser Trp Arg Leu Ser
 260 265 270
 Phe Thr Ala Glu Glu Pro Leu Glu Ala Ser Gln Phe Lys Phe Asp Gly
 275 280 285
 Arg Thr Leu Tyr Cys Leu Glu Leu Ala Lys Tyr Leu Lys Gln Asp Asn
 290 295 300
 Lys Asp Val Ile Asn Gln Glu Val Lys Glu Thr Leu Ser Glu Leu Ser
 305 310 315 320
 Tyr Val Thr Ser Thr Leu Phe Thr Thr Glu Val Ala Tyr Glu Ala Phe
 325 330 335
 Leu Asp Arg Val His Val Ser Glu Val Lys Leu Arg Ser Lys Gly Gln
 340 345 350
 Trp Glu Val Pro His Pro Trp Leu Asn Leu Leu Val Pro Arg Ser Lys
 355 360 365
 Ile Asn Glu Phe Ala Arg Gly Val Phe Gly Asn Ile Leu Thr Asp Thr
 370 375 380
 Ser Asn Gly Pro Val Ile Val Tyr Pro Val Asn Lys Ser Lys Trp Asp
 385 390 395 400
 Asn Gln Thr Ser Ala Val Thr Pro Glu Glu Glu Val Phe Tyr Leu Val
 405 410 415
 Ala Ile Leu Thr Ser Ala Ser Pro Gly Ser Ala Gly Lys Asp Gly Val
 420 425 430
 Glu Glu Ile Leu Arg Arg Asn Arg Arg Ile Leu Glu Phe Ser Glu Glu
 435 440 445
 Ala Gly Ile Gly Leu Lys Gln Tyr Leu Pro His Tyr Thr Thr Arg Glu
 450 455 460
 Glu Trp Arg Ser His Phe Gly Asp Lys Trp Gly Glu Phe Val Arg Arg
 465 470 475 480
 Lys Ser Arg Tyr Asp Pro Leu Ala Ile Leu Ala Pro Gly His Arg Ile
 485 490 495
 Phe Gln Lys Ala Val Ser Tyr Ser
 500

<210> 13

<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 13
cggtcgacat gggattgacc tcatccttac g

31

<210> 14
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 14
gcgtcgactt atacagttct aggtttcggc agtat

35

<210> 15
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 15
gcggtaccag agagagaaac ataaacaaat ggc

33

<210> 16
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 16
gcggtaccca attttacttc caccaaatg c

31

<210> 17
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 17
gcggtacctt cattgataag aatcaagcta ttca

34

<210> 18
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 18
gcggtaccca aagtggtag aacgactaac a

31

<210> 19
<211> 28
<212> DNA
<213> Artificial Sequence

CROP033pct.ST25.txt

```

<220>
<223> oligonucleotide: primer or probe

<400> 19
gcgggtacccc cattaacctt cccgtttg
28

<210> 20
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 20
gcgggtaccag acgatgaacg tacttgcctg ta
32

<210> 21
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 21
gggggtacctt gatgaatcgt gaaatgac
28

<210> 22
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 22
gggggtaccct ttcctcttgg ttttgcctg t
31

<210> 23
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 23
gctcttagatc aggaaaagaa ccatgcctat ag
32

<210> 24
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide: primer or probe

<400> 24
gctcttagatc atgagtatga gactgccttt tg
32

<210> 25
<211> 1728
<212> DNA
<213> Arabidopsis thaliana

<400> 25
atgggattga cctcatcctt acggttccat agacaaaaca acaagacttt cctcggaatc
60
ttcatgatct tagttctaag ctgtatacca ggtagaacca atctttgttc caatcattct
120

```

CROP033pct.ST25.txt

```

gtagtagacc caaaagaatt acctcttca aatccttcag atattcggtc ctcatragtt 180
tcactagatt tggagggtta tataagcttc gacgatgtcc acaatgtggc caaggacttt 240
ggcaacagat accagttacc acctttggca attctacatc caaggtcagt ttttgatatt 300
tcacgatga tgaagcatat agtacatctg ggctccacct caaatcttac agtagcagct 360
agaggccatg gtcactcgct tcaaggacaa gctctagctc atcaagggtg tgtcatcaaa 420
atggagtcac ttcgaagtcc tgatatcagg atttataagg ggaagcaacc atatgttgat 480
gtctcagggtg gtgaaatatg gataaacatt ctacgcgaga ctctaaaata cggctcttca 540
ccaaagtcct ggacagacta ccttcatttg accgttggag gtacactatc taatgctgga 600
atcagcgggtc aagcattcaa gcatggaccc caaatcaaca acgtctacca gctagagatt 660
gttacagggg aaggagaagt cgtaacctgt tctgagaagc ggaattctga acttttcttc 720
agtgttcttg gcgggcttgg acagtgtggc ataatacccc gggcacggat ctctcttgaa 780
ccagcaccgc atatggttaa atggatcagg gtactctact ctgacttttc tgcattttca 840
agggaccaag aatatctgat ttcgaaggag aaaacttttg attacgttga aggatttgtg 900
ataatcaata gaacagacct tctcaataat tggcgatcgt cattcagtc caacgattcc 960
acacaggcaa gcagattcaa gtcagatggg aaaactcttt attgcctaga agtgggtcaaa 1020
tatttcaacc cagaagaagc tagctctatg gatcaggaaa ctggcaagtt actttcagag 1080
ttaaattata ttccatccac tttgttttca tctgaagtgc catatatcga gtttctggat 1140
cgcgtgcata tcgcagagag aaaactaaga gcaaagggtt tatgggaggt tccacatccc 1200
tggctgaatc tcctgattcc taagagcagc atataccaat ttgctacaga agttttcaac 1260
aacatttctc caagcaaaa caacggctct atccttattt atccagtcaa tcaatccaag 1320
tggaagaaac atacatcttt gataactcca aatgaagata tattctatct cgtagccttt 1380
ctccccctg cagtgcctaaa ttcctcaggg aaaaacgatc tagagtacct tttgaaacaa 1440
aaccaaagag ttatgaactt ctgcgcagca gcaaacctca acgtgaagca gtatttgccc 1500
cattatgaaa ctcaaaaaga gtggaaatca cactttggca aaagatggga aacatttgca 1560
cagaggaaac aagcctacga cctctagcg attctagcac ctggccaaag aatattccaa 1620
aagacaacag gaaaattatc tcccatccaa ctgcgaaagt caaaggcaac aggaagtcct 1680
caaaggtagc attacgcac aatactgccg aaacctagaa ctgtataa 1728

```

<210> 26
 <211> 1506
 <212> DNA
 <213> Arabidopsis thaliana

```

<400> 26
atggctaata ttcgtttaat gatcacttta atcacgggtt taatgatcac caaatcatca 60
aacgggtatta aaattgattt acctaaatcc cttaacctca cctctctac cgatccttcc 120
atcatctccg cagcctctca tgacttcgga aacataacca ccgtgacccc cggcggcgta 180
atctgccccct cctccaccgc tgatatctct cgtctcttcc aatacgccgc aaacggaaaa 240
agtacattcc aagtagcggc tcgtggccaa ggccactcct taaacggcca agcctcgggtc 300
tccggcggag taatcgtcaa catgacgtgt atcactgacg tgggtggttc aaaagacaag 360
aagtacgctg acgtggcggc cgggacgtta tgggtggatg tgcttaagaa gacggcggag 420

```

CROP033pct.ST25.txt

```

aaaggggtgt cgccgggttc ttggacggat tatttgata taaccgtcgg aggaacgtg 480
tcgaatggtg gaattggtgg tcaagtgtt cgaacggtc ctcttgtag taacgtcctt 540
gaattggacg ttattactgg gaaaggtgaa atgttgacat gctcgcgaca gctaaaccca 600
gaattgttct atggagtgtt aggaggtttg ggtcaatttg gaattataac gagagccaga 660
attgttttgg accatgcacc taaacgggcc aaatggtttc ggatgctcta cagtgatttc 720
acaactttta caaaggacca agaacgtttg atatcaatgg caaacgatat tggagtcgac 780
tatttagaag gtcaaatatt tctatcaaac ggtgtcgttg acacctcttt tttccacct 840
tcagatcaat ctaaagtcgc tgatctagtc aagcaacacg gtatcatcta tgttcttgaa 900
gtagccaagt attatgatga tcccaatctc cccatcatca gcaaggttat tgacacatta 960
acgaaaacat taagtacttt gcccggttc atatcaatgc acgacgtggc ctacttcgat 1020
ttcttgaacc gtgtacatgt cgaagaaaat aaactcagat ctttgggatt atgggaactt 1080
cctcatcctt ggcttaacct ctacgttcct aaatctcgga ttctcgattt tcataacggt 1140
gttgtcaaag acattcttct taagcaaaaa tcagcttcgg gactcgtctt tctctatcca 1200
acaaaccgga ataaatggga caatcgtatg tcggcgatga taccagagat cgatgaagat 1260
gttatatata ttatcggact actacaatcc gctaccccaa aggatcttcc agaagtggag 1320
agcgttaacg agaagataat taggttttgc aaggattcag gtattaagat taagcaatat 1380
ctaattgcatt atactagtaa agaagattgg attgagcatt ttggatcaaa atgggatgat 1440
ttttcgaaga ggaaagatct atttgatccc aagaaactgt tatctccagg gcaagacatc 1500
ttttga 1506

```

<210> 27
 <211> 1572
 <212> DNA
 <213> Arabidopsis thaliana

```

<400> 27
atggcgagtt ataactttcg ttcacaagtt cgtcttatag caataacaat agtaatcatc 60
attactctct caactccgat cacaaccaac acatcaccac aaccatggaa tatectttca 120
cacaacgaat tcgccggaaa actcacctcc tctctctcct ccgtcgaatc agccgccaca 180
gatttcggcc acgtcaccaa aatcttccct tccgcgtct taatcccttc ctccgttgaa 240
gacatcacag atctcataaa actctctttt gactctcaac tgtcttttcc tttagccgct 300
cgtggtcacg gacacagcca ccgtggccaa gcctcggcta aagacggagt tgtggtcaac 360
atgcggtcca tggtaaaccg ggatcgaggt atcaagggtg ctaggacctg tttatatgtt 420
gacgtggacg ctgctgggct atggattgag gtgttgaata aaactttgga gttagggtta 480
acgccggttt ctgggacgga ttatttgat ttaacagtcg gtgggacgtt atcaaacggc 540
ggaattagtg gacaaacgtt tcggtacggt ccacagatca ctaatgttct agagatggat 600
gttattactg gaaaaggaga gattgcaact tgttccaagg acatgaactc ggaatctttc 660
ttcgcggtgt taggaggttt gggtaattc ggcattataa caagagccag aattaaactt 720
gaagtagctc cgaaaagggc caagtgggta aggtttctat acatagattt ctccgaattc 780
acaagagatc aagaacgagt gatatcgaaa acggacggtg tagatttctt agaaggttcc 840
attatgggtg accatggccc accggataac tggagatcca cgtattatcc accgtccgat 900
cacttgagga tcgcctcaat ggtcaaacga catcgtgtca tctactgcct tgaagtcgtc 960

```

CROP033pct.ST25.txt

```

aagtattacg acgaaacttc tcaatacaca gtcaacgagg aaatggagga gtraagcgat 1020
agtttaaac accatgaagagg gtttatgtac gagaaagatg tgacgtatat ggatttccta 1080
aaccgagttc gaaccggaga gctaaacctg aaatccaaag gccaatggga tggtccacat 1140
ccatggctta atctcttcgt accaaaaact caaatctcca aatttgatga tgggtgtttt 1200
aagggtatta tcctaagaaa taacatcact agcggctctg ttcttgttta tcctatgaat 1260
cgacaacaagt ggaatgatcg gatgtctgcc gctatacccg aggaagatgt attttatgcg 1320
gtagggtrtt taagatccgc gggttttgac aattgggagg cttttgatca agaaaacatg 1380
gaaatactga agttttgtga ggatgcta atgggggtta tacaatatct tccttatcat 1440
tcatacacaag aaggatgggt tagacatttt ggtccgaggt ggaatatatt cgtagagaga 1500
aaatataaat atgatcccaa aatgatatta tcaccgggac aaaatatatt tcaaaaaata 1560
aactcgagtt ag 1572

```

```

<210> 28
<211> 1575
<212> DNA
<213> Arabidopsis thaliana

```

```

<400> 28
atgactaata ctctctgttt aagcctcatt accctaataa cgctttttat aagtttaacc 60
ccaaccttaa tcaaatcaga tgagggcatt gatgttttct taccatatac actcaacctt 120
acggctcctaa ccgatccctt ctccatctct gccgcttctc acgacttcgg taacataacc 180
gacgaaaatc ccggcgccgt cctctgccct tcctccacca cggagggtggc tcgtctcttc 240
cgtttcgcta acggaggatt ctcttacaat aaagggtcaa ccagccccgc gtctactttc 300
aaagtggctg ctcgaggcca aggccactcc ctccgtggcc aagcctctgc acccgagggt 360
gtcgtcgtga acatgacgtg tctcgccatg gcggctaaac cagcggcggg tgttatctcg 420
gcagacggga cttacgctga cgtggctgcc gggacgatgt ggggtggatgt tctgaaggcg 480
gcgggtggata gaggcgtctc gccggttaca tggacggatt atttgtatct cagcgtcggc 540
gggacgttgt cgaacgctgg aatcggtggt cagacgttta gacacggccc tcagattagt 600
aacgttcatt agcttgacgt tattaccgga aaagggtgaaa tgatgacttg ctctccaaag 660
ttaaaccctg aattgttcta tggagtttta ggaggtttgg gtcaattcgg tattataacg 720
agggccagga ttgcgttga tcatgcaccc acaagggtga aatgggtctc catactctac 780
agtgacttct cggcttttaa aagagaccaa gagcgtttaa tatcaatgac caatgatctc 840
ggagttgact ttttgaagg tcaacttatg atgtcaaag gcttcgtaga cacctctttc 900
ttcccaactc ccgatcaaac aagagtcgca tctcttgatga atgaccaccg gatcatctat 960
gttctcgaag tagccaagta ttatgacaga accacccttc ccattattga ccagggtgatt 1020
gacacgttaa gtagaactct aggtttcgtt ccagggttta tgttcgtaca agatgttccg 1080
tatttcgatt tcttgaaccg tgtccgaaac gaagaagata aactcagatc tttaggacta 1140
tggaaggttc ctcatccatg gcttaacatc tttgtcccg ggtctcgaat ccaagatttt 1200
catgatgggt ttattaatgg cttcttcta aaccaaact caacttctgg tgttactctc 1260
ttctatccca caaaccgaaa caaatggaac aaccgatgt caacgatgac accggacgaa 1320
gatgtttttt atgtgatcgg attactgcaa tcagctggtg gatctcaaaa ttggcaagaa 1380

```

CROP033pct.ST25.txt

```

cttgaaaatc tcaacgacaa ggtrattcag tttgtgaaa actcgggaat taagattaag 1440
gaatatttga tgcactatac aagaaaagaa gattgggtta aacatttttg accaaaatgg 1500
gatgattttt taagaaagaa aattatgttt gatcccaaaa gactattgtc tccaggacaa 1560
gacatattta attaa 1575

```

<210> 29
 <211> 1611
 <212> DNA
 <213> Arabidopsis thaliana

```

<400> 29
atgacgtcaa gctttcttct cctgacgttc gccatatgta aactgatcat agccgtgggt 60
ctaaacgtgg gccccagtga gctcctccgc atcggagcca tagatgtcga cggccacttc 120
accgtccacc cttccgactt agcctccgtc tcctcagact tcggtatgct gaagtcacct 180
gaagagccat tggccgtgct tcatccatca tcggccgaag acgtggcacg actcgtcaga 240
acagcttacg gttcagccac ggcgtttccg gtctcagccc gaggccacgg ccattccata 300
aacggacaag ccgcggcggg gaggaacggg gtggtgggtg aaatgaacca cggcgtaacc 360
gggacgcccc agccactcgt ccgaccgat gaaatgtatg tggatgtatg ggggtggagag 420
ttatgggtcg atgtgttgaa gaaaacgttg gagcatggct tagcaccaaa atcatggacg 480
gattacttgt atctaaccgt tggaggtaca ctctccaatg caggaatcag tggtaagct 540
tttcaccatg gtcctcaaat tagtaacgtc cttgagctcg acgttgtaac tgggaaagga 600
gaggtgatga gatgtcaga agaagagaac acaaggctat tccatggagt tcttgggtga 660
ttaggtcaat ttgggatcat cactcgagca cgaatctctc tcgaaccagc tccccaaagg 720
gtgagatgga tacgggtatt gtattcgagc ttcaaagtgt ttacggagga ccaagagtac 780
ttaatctcaa tgcattgtca attaaagttt gattacgtgg aaggttttgt gattgtggac 840
gaaggactcg tcaacaattg gagatcttct ttcttctctc cacgtaacct cgtaagatc 900
tcctctgtta gttccaacgg ctctgttttg tattgccttg agatcaccaa gaactaccac 960
gactccgact ccgaaatcgt tgatcaggaa gttgagattc tgatgaagaa attgaatttc 1020
ataccgacat cgggtctttac aacggattta caatatgtgg actttctcga ccgggtacac 1080
aaggccgaat tgaagctccg gtccaagaat ttatgggagg ttccacaccc atggctcaac 1140
ctcttcgtgc caaaatcaag aatctctgac ttcgataaag gcgttttcaa gggcattttg 1200
ggaaataaaa caagtggccc tattcttatt taccccatga acaaagacaa atgggacgag 1260
aggagctcag ccgtgacgcc ggatgaggaa gttttctatc tgggtggctct attgagatca 1320
gctttaacgg acggtgaaga gacacagaag cttagagtatc tgaaagatca gaaccgtcgg 1380
atcttgagat tctgtgaaca agccaagatc aatgtgaagc agtatcttcc tcaccacgca 1440
acacaggaag agtgggtggc tcattttggg gacaagtggg atcgggttcag aagcttaaa 1500
gctgagtttg atccgcgaca catactcgct actggtcaga gaatctttca aaacccatct 1560
ttgtctttgt ttcctccgtc gtcgtcttct tcgtcagcgg cttcatgggtg a 1611

```

<210> 30
 <211> 1515
 <212> DNA
 <213> Arabidopsis thaliana

<400> 30

CROP033pct.ST25.txt

```

atgcttatag taagaagttt caccatcttg cttctcagct gcatagcctt taagttggct 60
tgctgcttct ctagcagcat ttcttcttg aaggcgcttc ccctagtagg ccatttgag 120
tttgaacatg tccatcacgc ctccaaagat tttggaaatc gataccagtt gatcccttg 180
gcggtcttac atcccaaatc ggtaagcgac atcgctcaa cgatacgaca catctggatg 240
atgggcactc attcacagct tacagtggca gcgagaggtc gtggacattc actccaaggc 300
caagctcaaa caagacatgg aattgttata cacatggaat cactccatcc ccagaagctg 360
caggtctaca gtgtggattc cctgctcca tatgtgatg tgtctggtg tgagctgtgg 420
ataaacattt tgcattgagac cctcaagtac gggcttgac caaatcatg gacggattac 480
ctgcatttaa ctgtagggtg tactctgtcc aatgctggaa taagcgcca ggcattccga 540
catggaccac agatcagcaa tgttcacaa ctggagattg tcacaggaaa aggcgagatc 600
ctaaactgta caaagaggca gaacagcgac ttatttaatg gtgttcttg tggtttagg 660
cagtttgga tcataacgcg ggcaagaata gcattggaac cagcaccaac catggacca 720
gagcaactaa tatctgcca gggccacaaa ttcgattaca tagaagggtt tgtgataata 780
aacaggacag gcctcctgaa cagctggagg ttgtcttca ccgcagaaga gcctttagaa 840
gcaagccaat tcaagtttga tggaaggact ctgtattgtc tggagctagc caagtattg 900
aagcaagata acaaagacgt aatcaaccag gaagtgaag aaacattatc agagctaagc 960
tacgtgacgt cgacactgtt tacaacggag gtagcatatg aagcattctt ggacagggta 1020
catgtgtctg aggtaaaact ccgatcgaaa gggcagtggt aggtgccaca tccatggctg 1080
aacctcctgg taccaagaag caaatcaat gaatttgcaa gaggtgtatt tggaaacata 1140
ctaacggata caagcaacgg ccagtcacg gtctaccag tgaacaaatc aaagtgggac 1200
aatcaaacat cagcagtaac accggaggaa gaggtattct acctggtggc gatcctaaca 1260
tcggcatctc cagggtcggc aggaaggat ggagtagaag agatcttgag gcggaacaga 1320
agaatactgg aattcagtga agaagcagg ataggggtga agcagtatct gccacattac 1380
acgacaagag aagagtggag atccatttc ggggacaagt ggggagaatt tgtgaggagg 1440
aaatccagat atgatccatt ggcaattctt gcgcctggcc accgaatttt tcaaaaggca 1500
gtctcatact catga 1515

```

<210> 31
 <211> 84
 <212> DNA
 <213> Arabidopsis thaliana

```

<400> 31
tcagcttcgg gactcgctct tctctatcca acaaaccgga ataatggga caatcgtatg 60
tcggcgatga taccagagat cgat 84

```

<210> 32
 <211> 28
 <212> PRT
 <213> Arabidopsis thaliana

```

<400> 32
Ser Ala Ser Gly Leu Ala Leu Leu Tyr Pro Thr Asn Arg Asn Lys Trp
1      5      10      15

```

Asp Asn Arg Met Ser Ala Met Ile Pro Glu Ile Asp

<210> 33
 <211> 2814
 <212> DNA
 <213> *Arabidopsis thaliana*

```

<400> 33
atgaatcgta tgacgtcaag ctttcttctc ctgacgttcg ccatatgtaa actgatcata    60
gccgtgggtc taaacgtggg cccagtgag ctctccgca tcggagccat agatgtcgac    120
ggccacttca ccgtccaccc ttccgactta gcctccgtct cctcagactt cggtatgctg    180
aagtcacctg aagagccatt ggccgtgctt catccatcat cggccgaaga cgtggcacga    240
ctcgtcagaa cagcttacgg ttcagccacg gcgtttccgg tctcagcccg aggccacggc    300
cattccataa acggacaagc cgcggcgggg aggaacggtg tgggtggtga aatgaaccac    360
ggcgtaaccc ggacgcccac gccactcgtc cgaccggatg aaatgtatgt ggatgtatgg    420
ggtggagagt tatgggtcga tgtgtgaag aaaacgttgg agcatggctt agcaccaaaa    480
tcatggacgg attacttgta tctaaccgtt ggaggtagac tctccaatgc aggaatcagt    540
ggtcaagctt ttcacatggt tctcaaat agtaacgtcc ttgagctcga cgttgtaact    600
ggttagtatt aaaacattca agttcatata ttttaaatgc ttttgtctga agttttacta    660
ataacaagaa attgatacca aaaagtaggg aaaggagagg tgatgagatg ctcaagaaga    720
gagaacacaa ggctattcca tggagtctt ggtggattag gtcaatttgg gatcatcact    780
cgagcacgaa tctctctcga accagctccc caaagggtaa tttttttta atgactagct    840
atcaaaaatc cctggcgggt ccatacgttg taatcttttt agtttttact gttgatggta    900
ttttttatat attttgata ataaaacctt aaaaaggat attgtgatga caggtagat    960
ggatacgggt attgtattcg agcttcaaag tgtttacgga ggaccaagag tacttaatct   1020
caatgcatgg tcaattaaag ttgattacg tggagggtt tgtgatttg gacgaaggac   1080
tcgtcaacaa ttggagatct tcttcttctc ctccacgtaa ccccgtaag atctcctctg   1140
ttagtccaa cggctctggt ttgtattgcc ttgagatcac caagaactac cacgactccg   1200
actccgaaat cgttgatcag gtcactttca ttattcactt agaaaaaagc gatattttca   1260
ttttttatat tgatgaatat ctggaaggat ttaacgctat gcgactatg ggaaatcatt   1320
atgaaaaaat atttagtta tatgattgaa agtgggtctc atagtatttt tgttggtctg   1380
actttattat aacttaaat tggagagga catgaagaag aagccagaga ggatctacag   1440
agatctagct tttccacctg aacttaataa tgcacattta tataattatt tttcttcttc   1500
taaagtttag tttatcacta gcgaattaat catggttact aattaagtag tggacagggt   1560
catggaccac tcactacca aataatgatt cctctttact cttaagtta attttaataa   1620
aaccaactct actggaatct taacttatcc ttggttttgg taggctttta tagcaacacg   1680
gtttttttaa ttttctatt ccagatttg tatattaaat gtcgattttt tttctttttg   1740
tttcaggaag ttgagattct gatgaagaaa ttgaatttca taccgacatc ggtctttaca   1800
acggatttac aatatgtgga ctttctcgac cgggtacaca aggccgaatt gaagctccgg   1860
tccaagaatt tatgggaggt tccacacca tggctcaacc tcttcgtgcc aaaatcaaga   1920
atctctgact tcgataaagg cgttttcaag ggcattttgg gaaataaaac aagtggccct   1980
attcttatct accccatgaa caagacaag taagtcttga cattaccatt gattactact   2040

```

CROP033pct.ST25.txt

tctaaatttc ttctctagaa aaaagaataa aacgagtttt gcattgcatg catgcaaagt 2100
 tacacttgtg gggattaatt agtgggtccaa gaaaaaaagt ttgtcaaaat tgaaaaaac 2160
 tagacacgtg gtacatggga ttgtccgaaa aacgttggtc acatgtgcat cgaaccagct 2220
 aagattgaca acaacacttc gtcggctcgt atttctcttt ttgttttgtg accaaatccg 2280
 atgggtccaga ttgggtttat ttgtttttaa gttcctagaa ctcatgggtg gtgggtccca 2340
 atcagattct cctagaccaa accgatctca acgaaccctc cgcacatcat tgattattac 2400
 attaatatag atattgtcgt tgctgacgtg tcgtaatttg atgttattgt cagatgggac 2460
 gagaggagct cagccgtgac gccggatgag gaagttttct atctgggtggc tctattgaga 2520
 tcagctttta cggacgggtga agagacacag aagctagagt atctgaaaga tcagaaccgt 2580
 cggatcttgg agttctgtga acaagccaag atcaatgtga agcagtatct tcctcaccac 2640
 gcaacacagg aagagtgggt ggctcatttt ggggacaagt gggatcgggt cagaagctta 2700
 aaggtcaggt ttgatccgcg acacatactc gctactggtc agagaatctt tcaaaaccca 2760
 tctttgtctt tgtttctctc gtcgtcgtct tcttcgtcag cggcttcagt gtga 2814

<210> 34
 <211> 1620
 <212> DNA
 <213> *Arabidopsis thaliana*

<400> 34
 atgaatcgta tgacgtcaag ctttcttctc ctgacgttcg ccatatgtaa actgatcata 60
 gccgtgggtc taaacgtggg ccccgatgag ctctctcgca tcggagccat agatgtcgac 120
 ggccacttca ccgiccacc cttccgactta gcctccgtct cctcagactt cggatatgtg 180
 aagtcacctg aagagccatt ggccgtgctt catccatcat cggccgaaga cgtggcacga 240
 ctctgcagaa cagcttacgg ttcagccacg gcgtttccgg tctcagcccg aggccacggc 300
 cattccataa acggacaagc cgcggcgggg aggaacgggt tgggtggtga aatgaaccac 360
 ggcgttaaccg ggacgcccaa gccactcgtc gcaccggatg aaatgtatgt ggatgtatgg 420
 ggtggagagt tatgggtcga tgtgttgaag aaaacgttgg agcatggctt agcaccaaaa 480
 tcatggacgg attacttgta tctaaccgtt ggagggtacac tctccaatgc aggaatcagt 540
 ggtcaagctt ttcacatggt tcttcaaatt agtaacgtcc ttgagctcga cgttgtaact 600
 gggaaaggag aggtgatgag atgctcagaa gaagagaaca caaggctatt ccatggagtt 660
 cttggtggat taggtcaatt tgggatcatc actcgagcac gaatctctct cgaaccagct 720
 ccccaaaggg tgagatggat acgggtattg tattcgagct tcaaagtgtt tacggaggac 780
 caagagtact taatctcaat gcattggtcaa ttaaagtgtt attacgtgga aggttttgtg 840
 attgtggacg aaggactcgt caacaattgg agatcttctt tcttctctcc acgtaacccc 900
 gtcaagatct cctctgttag ttccaacggc tctgttttgt attgccttga gatcaccaag 960
 aactaccacg actccgactc cgaaatcgtt gatcaggaag ttgagattct gatgaagaaa 1020
 ttgaatttca taccgacatc ggtctttaca acggatttac aatatgtgga ctttctcgac 1080
 cgggtacaca aggccgaatt gaagctccgg tccaagaatt tatgggaggt tccacaccca 1140
 tggctcaacc tcttcgtgcc aaaatcaaga atctctgact tcgataaagg cgttttcaag 1200
 ggcatttttg gaaataaaac aagtggccct attcttatct accccatgaa caaagacaaa 1260

CROP033pct.ST25.txt

tgggacgaga ggagctcagc cgtgacgccg gatgaggaag ttttctatct ggtggctcta	1320
ttgagatcag ctttaacgga cggatgaagag acacagaagc tagagtatct gaaagatcag	1380
aaccgctcga tcttgagatt ctgtgaacaa gccaagatca atgtgaagca gttcttctt	1440
caccacgcaa cacaggaaga gtgggtggct catcttgggg acaagtggga tcggttcaga	1500
agcttaaagg ctgagtttga tccgcgacac atactcgcta ctggtcagag aatctttcaa	1560
aaccctatctt tgtctttggt tcctccgtcg tcgtcttctt cgtcagcggc ttcattggtga	1620

<210> 35
 <211> 539
 <212> PRT
 <213> Arabidopsis thaliana

<400> 35

Met Asn Arg Met Thr Ser Ser Phe Leu Leu Leu Thr Phe Ala Ile Cys
 1 5 10 15

Lys Leu Ile Ile Ala Val Gly Leu Asn Val Gly Pro Ser Glu Leu Leu
 20 25 30

Arg Ile Gly Ala Ile Asp Val Asp Gly His Phe Thr Val His Pro Ser
 35 40 45

Asp Leu Ala Ser Val Ser Ser Asp Phe Gly Met Leu Lys Ser Pro Glu
 50 55 60

Glu Pro Leu Ala Val Leu His Pro Ser Ser Ala Glu Asp Val Ala Arg
 65 70 75 80

Leu Val Arg Thr Ala Tyr Gly Ser Ala Thr Ala Phe Pro Val Ser Ala
 85 90 95

Arg Gly His Gly His Ser Ile Asn Gly Gln Ala Ala Ala Gly Arg Asn
 100 105 110

Gly Val Val Val Glu Met Asn His Gly Val Thr Gly Thr Pro Lys Pro
 115 120 125

Leu Val Arg Pro Asp Glu Met Tyr Val Asp Val Trp Gly Gly Glu Leu
 130 135 140

Trp Val Asp Val Leu Lys Lys Thr Leu Glu His Gly Leu Ala Pro Lys
 145 150 155 160

Ser Trp Thr Asp Tyr Leu Tyr Leu Thr Val Gly Gly Thr Leu Ser Asn
 165 170 175

Ala Gly Ile Ser Gly Gln Ala Phe His His Gly Pro Gln Ile Ser Asn
 180 185 190

Val Leu Glu Leu Asp Val Val Thr Gly Lys Gly Glu Val Met Arg Cys
 195 200 205

Ser Glu Glu Glu Asn Thr Arg Leu Phe His Gly Val Leu Gly Gly Leu
 210 215 220

CROP033pct.ST25.txt

Gly Gln Phe Gly Ile Ile Thr Arg Ala Arg Ile Ser Leu Glu Pro Ala
 225 230 235 240

Pro Gln Arg Val Arg Trp Ile Arg Val Leu Tyr Ser Ser Phe Lys Val
 245 250 255

Phe Thr Glu Asp Gln Glu Tyr Leu Ile Ser Met His Gly Gln Leu Lys
 260 265 270

Phe Asp Tyr Val Glu Gly Phe Val Ile Val Asp Glu Gly Leu Val Asn
 275 280 285

Asn Trp Arg Ser Ser Phe Phe Ser Pro Arg Asn Pro Val Lys Ile Ser
 290 295 300

Ser Val Ser Ser Asn Gly Ser Val Leu Tyr Cys Leu Glu Ile Thr Lys
 305 310 315 320

Asn Tyr His Asp Ser Asp Ser Glu Ile Val Asp Gln Glu Val Glu Ile
 325 330 335

Leu Met Lys Lys Leu Asn Phe Ile Pro Thr Ser Val Phe Thr Thr Asp
 340 345 350

Leu Gln Tyr Val Asp Phe Leu Asp Arg Val His Lys Ala Glu Leu Lys
 355 360 365

Leu Arg Ser Lys Asn Leu Trp Glu Val Pro His Pro Trp Leu Asn Leu
 370 375 380

Phe Val Pro Lys Ser Arg Ile Ser Asp Phe Asp Lys Gly Val Phe Lys
 385 390 395 400

Gly Ile Leu Gly Asn Lys Thr Ser Gly Pro Ile Leu Ile Tyr Pro Met
 405 410 415

Asn Lys Asp Lys Trp Asp Glu Arg Ser Ser Ala Val Thr Pro Asp Glu
 420 425 430

Glu Val Phe Tyr Leu Val Ala Leu Leu Arg Ser Ala Leu Thr Asp Gly
 435 440 445

Glu Glu Thr Gln Lys Leu Glu Tyr Leu Lys Asp Gln Asn Arg Arg Ile
 450 455 460

Leu Glu Phe Cys Glu Gln Ala Lys Ile Asn Val Lys Gln Tyr Leu Pro
 465 470 475 480

His His Ala Thr Gln Glu Glu Trp Val Ala His Phe Gly Asp Lys Trp
 485 490 495

Asp Arg Phe Arg Ser Leu Lys Ala Glu Phe Asp Pro Arg His Ile Leu
 500 505 510

Ala Thr Gly Gln Arg Ile Phe Gln Asn Pro Ser Leu Ser Leu Phe Pro
 515 520 525

CROP033pct.ST25.txt
 Pro Ser Ser Ser Ser Ser Ser Ser Ala Ala Ser Trp
 530 535

<210> 36
 <211> 501
 <212> PRT
 <213> Arabidopsis thaliana

<400> 36

Met Ala Asn Leu Arg Leu Met Ile Thr Leu Ile Thr Val Leu Met Ile
 1 5 10 15

Thr Lys Ser Ser Asn Gly Ile Lys Ile Asp Leu Pro Lys Ser Leu Asn
 20 25 30

Leu Thr Leu Ser Thr Asp Pro Ser Ile Ile Ser Ala Ala Ser His Asp
 35 40 45

Phe Gly Asn Ile Thr Thr Val Thr Pro Gly Gly Val Ile Cys Pro Ser
 50 55 60

Ser Thr Ala Asp Ile Ser Arg Leu Leu Gln Tyr Ala Ala Asn Gly Lys
 65 70 75 80

Ser Thr Phe Gln Val Ala Ala Arg Gly Gln Gly His Ser Leu Asn Gly
 85 90 95

Gln Ala Ser Val Ser Gly Gly Val Ile Val Asn Met Thr Cys Ile Thr
 100 105 110

Asp Val Val Val Ser Lys Asp Lys Lys Tyr Ala Asp Val Ala Ala Gly
 115 120 125

Thr Leu Trp Val Asp Val Leu Lys Lys Thr Ala Glu Lys Gly Val Ser
 130 135 140

Pro Val Ser Trp Thr Asp Tyr Leu His Ile Thr Val Arg Gly Thr Leu
 145 150 155 160

Ser Asn Gly Gly Ile Gly Gly Gln Val Phe Arg Asn Gly Pro Leu Val
 165 170 175

Ser Asn Val Leu Glu Leu Asp Val Ile Thr Gly Lys Gly Glu Met Leu
 180 185 190

Thr Cys Ser Arg Gln Leu Asn Pro Glu Leu Phe Tyr Gly Val Leu Gly
 195 200 205

Gly Leu Gly Gln Phe Gly Ile Ile Thr Arg Ala Arg Ile Val Leu Asp
 210 215 220

His Ala Pro Lys Arg Ala Lys Trp Phe Arg Met Leu Tyr Ser Asp Phe
 225 230 235 240

Thr Thr Phe Thr Lys Asp Gln Glu Arg Leu Ile Ser Met Ala Asn Asp
 245 250 255

Ile Gly Val Asp Tyr Leu Glu Gly Gln Ile Phe Leu Ser Asn Gly Val

CROP033pct.ST25.txt

260

265

270

Val Asp Thr Ser Phe Phe Pro Pro Ser Asp Gln Ser Lys Val Ala Asp
275 280 285

Leu Val Lys Gln His Gly Ile Ile Tyr Val Leu Glu Val Ala Lys Tyr
290 295 300

Tyr Asp Asp Pro Asn Leu Pro Ile Ile Ser Lys Val Ile Asp Thr Leu
305 310 315 320

Thr Lys Thr Leu Ser Tyr Leu Pro Gly Phe Ile Ser Met His Asp Val
325 330 335

Ala Tyr Phe Asp Phe Leu Asn Arg Val His Val Glu Glu Asn Lys Leu
340 345 350

Arg Ser Leu Gly Leu Trp Glu Leu Pro His Pro Trp Leu Asn Leu Tyr
355 360 365

Val Pro Lys Ser Arg Ile Leu Asp Phe His Asn Gly Val Val Lys Asp
370 375 380

Ile Leu Leu Lys Gln Lys Ser Ala Ser Gly Leu Ala Leu Leu Tyr Pro
385 390 395 400

Thr Asn Arg Asn Lys Trp Asp Asn Arg Met Ser Ala Met Ile Pro Glu
405 410 415

Ile Asp Glu Asp Val Ile Tyr Ile Ile Gly Leu Leu Gln Ser Ala Thr
420 425 430

Pro Lys Asp Leu Pro Glu Val Glu Ser Val Asn Glu Lys Ile Ile Arg
435 440 445

Phe Cys Lys Asp Ser Gly Ile Lys Ile Lys Gln Tyr Leu Met His Tyr
450 455 460

Thr Ser Lys Glu Asp Trp Ile Glu His Phe Gly Ser Lys Trp Asp Asp
465 470 475 480

Phe Ser Lys Arg Lys Asp Leu Phe Asp Pro Lys Lys Leu Leu Ser Pro
485 490 495

Gly Gln Asp Ile Phe
500

<210> 37
<211> 1455
<212> DNA
<213> Arabidopsis thaliana

<400> 37
atggctaattc ttcgtttaat gatcacttta atcacgggttt taatgatcac caaatcatca 60
aacgggtatta aaattgattt acctaaatcc cttaacctca ccctctctac cgatccttcc 120
atcatctccg cagcctctca tgacttcgga aacataacca ccgtgacccc cggcggcgta 180
atctgccccct cctccaccgc tgatatctct cgtctcctcc aatacgccgc aaacggaaaa 240

CROP033pct.ST25.txt

```

agtacattcc aagtagcggc tcgtggccaa ggccactcct taaacggcca agcctcggtc 300
tccggcggag taatcgtcaa catgacgtgt atcactgacg tggtaggttc aaaagacaag 360
aagtacgctg acgtggcggc cgggacgtta tgggtggatg tgcttaagaa gacggcggag 420
aaaggggtgt cggcggtttc ttggacggat tatttgcata taaccgtccg aggaacgttg 480
tcgaatggtg gaattgggtg tcaagtgttt cgaaacggtc ctcttgtag taacgtcctt 540
gaattggacg ttattactgg gaaagggtgaa atgttgacat gctcgcgaca gctaaacca 600
gaattgttct atggagtgtt aggagggttg ggtcaatttg gaattataac gagagccaga 660
attgttttgg accatgcacc taaacggcaa gaacgtttga tatcaatggc aaacgatatt 720
ggagtcgact atttagaagg tcaaatattt ctatcaaacg gtgtcgtga cacctctttt 780
ttcccacctt cagatcaatc taaagtcgt gatctagtca agcaacacgg tatcatctat 840
gttcttgaag tagccaagta ttatgatgat cccaatctcc ccatcatcag caaggttatt 900
gacacattaa cgaaaacatt aagttacttg cccgggttca tatcaatgca cgacgtggcc 960
tacttcgatt tcttgaaccg tgtacatgtc gaagaaaata aactcagatc tttgggatta 1020
tgggaacttc ctcatccttg gcttaacctc tacgttccta aatctcggat tctcgatttt 1080
cataacggtg ttgtcaaaga cattcttctt aagcaaaaat cagcttcggg actcgtctt 1140
ctctatccaa caaacgggaa taaatgggac aatcgtatgt cggcgatgat accagagatc 1200
gatgaagatg ttatatatat tatcggacta ctacaatccg ctaccccaaa ggatcttcca 1260
gaagtggaga gcgttaacga gaagataatt aggttttgca aggattcagg tattaagatt 1320
aagcaatatc taatgcatta tactagtaaa gaagattgga ttgagcattt tggatcaaaa 1380
tgggatgatt ttctgaagag gaaagatcta ttgatccca agaaactgtt atctccaggg 1440
caagacatct tttga 1455

```

<210> 38
 <211> 484
 <212> PRT
 <213> Arabidopsis thaliana

<400> 38

Met Ala Asn Leu Arg Leu Met Ile Thr Leu Ile Thr Val Leu Met Ile
 1 5 10 15

Thr Lys Ser Ser Asn Gly Ile Lys Ile Asp Leu Pro Lys Ser Leu Asn
 20 25 30

Leu Thr Leu Ser Thr Asp Pro Ser Ile Ile Ser Ala Ala Ser His Asp
 35 40 45

Phe Gly Asn Ile Thr Thr Val Thr Pro Gly Gly Val Ile Cys Pro Ser
 50 55 60

Ser Thr Ala Asp Ile Ser Arg Leu Leu Gln Tyr Ala Ala Asn Gly Lys
 65 70 75 80

Ser Thr Phe Gln Val Ala Ala Arg Gly Gln Gly His Ser Leu Asn Gly
 85 90 95

Gln Ala Ser Val Ser Gly Gly Val Ile Val Asn Met Thr Cys Ile Thr

CROP033pct.ST25.txt

100 105 110

Asp Val Val Val Ser Lys Asp Lys Lys Tyr Ala Asp Val Ala Ala Gly
 115 120 125

Thr Leu Trp Val Asp Val Leu Lys Lys Thr Ala Glu Lys Gly Val Ser
 130 135 140

Pro Val Ser Trp Thr Asp Tyr Leu His Ile Thr Val Arg Gly Thr Leu
 145 150 155 160

Ser Asn Gly Gly Ile Gly Gly Gln Val Phe Arg Asn Gly Pro Leu Val
 165 170 175

Ser Asn Val Leu Glu Leu Asp Val Ile Thr Gly Lys Gly Glu Met Leu
 180 185 190

Thr Cys Ser Arg Gln Leu Asn Pro Glu Leu Phe Tyr Gly Val Leu Gly
 195 200 205

Gly Leu Gly Gln Phe Gly Ile Ile Thr Arg Ala Arg Ile Val Leu Asp
 210 215 220

His Ala Pro Lys Arg Gln Glu Arg Leu Ile Ser Met Ala Asn Asp Ile
 225 230 235 240

Gly Val Asp Tyr Leu Glu Gly Gln Ile Phe Leu Ser Asn Gly Val Val
 245 250 255

Asp Thr Ser Phe Phe Pro Pro Ser Asp Gln Ser Lys Val Ala Asp Leu
 260 265 270

Val Lys Gln His Gly Ile Ile Tyr Val Leu Glu Val Ala Lys Tyr Tyr
 275 280 285

Asp Asp Pro Asn Leu Pro Ile Ile Ser Lys Val Ile Asp Thr Leu Thr
 290 295 300

Lys Thr Leu Ser Tyr Leu Pro Gly Phe Ile Ser Met His Asp Val Ala
 305 310 315 320

Tyr Phe Asp Phe Leu Asn Arg Val His Val Glu Glu Asn Lys Leu Arg
 325 330 335

Ser Leu Gly Leu Trp Glu Leu Pro His Pro Trp Leu Asn Leu Tyr Val
 340 345 350

Pro Lys Ser Arg Ile Leu Asp Phe His Asn Gly Val Val Lys Asp Ile
 355 360 365

Leu Leu Lys Gln Lys Ser Ala Ser Gly Leu Ala Leu Leu Tyr Pro Thr
 370 375 380

Asn Arg Asn Lys Trp Asp Asn Arg Met Ser Ala Met Ile Pro Glu Ile
 385 390 395 400

Asp Glu Asp Val Ile Tyr Ile Ile Gly Leu Leu Gln Ser Ala Thr Pro

405

CROP033pct.ST25.txt
410 415

Lys Asp Leu Pro Glu Val Glu Ser Val Asn Glu Lys Ile Ile Arg Phe
420 425 430

Cys Lys Asp Ser Gly Ile Lys Ile Lys Gln Tyr Leu Met His Tyr Thr
435 440 445

Ser Lys Glu Asp Trp Ile Glu His Phe Gly Ser Lys Trp Asp Asp Phe
450 455 460

Ser Lys Arg Lys Asp Leu Phe Asp Pro Lys Lys Leu Leu Ser Pro Gly
465 470 475 480

Gln Asp Ile Phe

<210> 39
<211> 60
<212> DNA
<213> Artificial sequence

<220>
<223> oligonucleotide: primer or probe

<400> 39
ggggacaagt ttgtacaaaa aagcaggctt cacaatggct aatcttcggt taatgatcac 60

<210> 40
<211> 49
<212> DNA
<213> Artificial sequence

<220>
<223> oligonucleotide: primer or probe

<400> 40
ggggaccact ttgtacaaga aagctgggtt caaaagatgt cttgccctg 49

<210> 41
<211> 3328
<212> DNA
<213> Artificial sequence

<220>
<223> expression cassette

<220>
<221> misc_feature
<222> (2495)..(2496)
<223> n is a, c, g, or t

<400> 41
ggtcagccaa tacattgatc cgttgccaat catgcaaagt attttggtg tggccgagtg 60
ccggaattga taattgtgtt ctgactaaat taaatgacca gaagtcgcta tcttccaatg 120
tatccgaaac ctggattaaa caatcctgtt ctgttctcta gccctcctg catggccgga 180
ttgttttttt gacatgtttt cttgactgag gcctgtttgt tctaaacttt ttcttcaaac 240
ttttaacttt ttcatcacat cagaactttt ctacacatat aaacttttaa cttttccgtc 300
acatcgttcc aatttcaatc aaactttcaa ttttggcgtg aactaaacac accctgagtc 360
ttttattgct cctccgtacg ggttggtggt ttgagaatag gtattttcag agagaaaatc 420

CROP033pct.ST25.txt

tagatattgg gaggaacttg gcatgaatgg ccactatatt tagagcaatt ctacgggtcct	480
tgaggaggta ccatgaggta ccaaaatatt agtgtaaatt ttagtatctc attataacta	540
ggtattatga ggtaccaaatt ttacaataga aaaaatagta ctatcatgga cttcttaag	600
taccgtaaaa ttgtcctat atttaagggg atgtttatat ctatccatat ccataatttg	660
attttgataa gaaaaaatgt gagcacacca agcatgtcca tgaccttgca ctcttggtc	720
actcgtcaac tgtgaagaac ctcaaaaatg ctcaatatag ctacagggtgc ctgaaaaaat	780
aactttaaag ttttgaacat cgatttact aaacaacaat tattatctcc ctctgaaaga	840
tgatagtta gaactctaga atcattgtcg gcggagaaag taaattatct tccccaaatt	900
tccagctatg aaaaaacctt caccaaacac catcaaacaa gagttcacca aaccgccc	960
gcggccatgc tgtcacgcaa cgcaccgat tgcctgatgg ccgctcgatg catgcatgct	1020
tccccgtgca catatccgac agacgcgccc tgtcagcgag ctctcgcacc gacctgtgta	1080
gcccattcaa gcatccaccc ccgccaagta cccccctcc tctcctac gtgtcaccgc	1140
tctctccacc tatatatgcc cactggccc ctctcctccc atctccactt caccgcatg	1200
cttctcttc tcttcgttg cattcatctt gctagcattt aaatcaacta gggatatcac	1260
aagttgtac aaaaaagcag gcttcacaat ggctaattct cgtttaatga tcactttaat	1320
cacggttta atgacacca aatcatcaaa cggattaaa attgatttac ctaaaacctt	1380
taacctcacc ctctctaccg atctctccat catctccgca gctctcatg acttcggaaa	1440
cataaccacc gtgacccccg gcggcgtaat ctgccccctc tccaccgctg atatctctcg	1500
tctctccaa tacgccgcaa acggaaaaag tacattccaa gtacgggtc gtggccaagg	1560
ccactccta aacggccaag cctcgggtc cggcgagta atcgtcaaca tgacgtgat	1620
cactgacgtg gtggtttcaa aagacaagaa gtacgtgac gtggcggccg ggacgttatg	1680
gggtggatgtg cttaagaaga cggcgagaa aggggtgtcg ccggtttctt ggacggatta	1740
tttgcatata accgtcggag gaacgtgtg gaatgggtga attggtgtc aagtgttcg	1800
aaacggtcct ctgttagta acgtccttga attggacgtt attactggga aaggtgaaat	1860
gttgacatgc tcgcgacagc taaaccaga attgttctat ggagtgttag gaggtttggg	1920
tcaatttgga attataacga gagccagaat tgttttgac catgcaccta aacggcaaga	1980
acgtttgata tcaatggcaa acgatattgg agtcgactat ttagaaggtc aaatatttct	2040
atcaaacggg gtcgttgaca cctctttttt cccaccttca gatcaatcta aagtcgctga	2100
tctagtcaag caacacggt tcatctatgt tcttgaagta gccagttat atgatgatcc	2160
caatctcccc atcatcagca aggttattga cacattaacg aaaacattaa gttacttgcc	2220
cgggttcata tcaatgcacg acgtggccta cttcgatttc ttgaaccgtg tacatgtcga	2280
agaaaataaa ctcatgctt tgggattatg ggaacttct catccttggc ttaacctcta	2340
cgttcctaaa tctcggattc tcgattttca taacggtgtt gtcaaagaca ttcttcttaa	2400
gcaaaaatca gcttcgggac tcgctcttct ctatccaaca aaccggaata agtacatact	2460
tctcttcatt catatttatc ttcaagaacc aaagnnatgg gacaatcgta tgtcggcgat	2520
gataccagag atcgatgaag atgttatata tattatcgga ctactacaat ccgtacccc	2580
aaaggatctt ccagaagtgg agagcgttaa cgagaagata attaggtttt gcaaggattc	2640
aggtattaag attaaagcaat atctaattgca ttatactagt aaagaagatt ggattgagca	2700

CROP033pct.ST25.txt

```

ttttggatca aaatgggatg atttttcgaa gaggaagat ctatttgatc ccaagaaact 2760
gttatctcca gggcaagaca tcttttgaac ccagctttct tgtacaaagt ggtgatatca 2820
caagccccggg cggctcttcta gggataacag ggttaattata tccctctaga tcacaagccc 2880
gggcgggtctt ctacgatgat tgagtaataa tgtgtcacgc atcaccatgg gtggcagtgt 2940
cagtgtgagc aatgacctga atgaacaatt gaaatgaaaa gaaaaaaagt actccatctg 3000
ttccaaatta aaattcattt taacctttta ataggtttat acaataattg atatatgttt 3060
tctgtatatg tctaatttgt tatcatccgg gcgggtcttct agggataaca gggttaattat 3120
atccctctag acaacacaca acaataaga gaaaaaaca ataataataa ttgagaatg 3180
aacaaaagga ccatatcatt cattaactct tctccatcca tttccatttc acagttcgat 3240
agcgaacc gaataaaaaa cacagtaa atacaagcaca acaaatggta caagaaaaac 3300
agttttccca atgccataat actcgaac 3328

```

<210> 42
 <211> 1506
 <212> DNA
 <213> *Arabidopsis thaliana*

```

<400> 42
atggctaate ttcgtttaat gatcacttta atcacggttt taatgatcac caaatcatca 60
aacggtatta aaattgattt acctaaatcc cttaacctca cctctctac cgatccttcc 120
atcatctccg cagcctctca tgacttcgga aacataacca ccgtgacccc cggcggcgta 180
atctgccccct cctccaccgc tgatatctct cgtctctctc aatacgccgc aaacggaaaa 240
agtacattcc aagtagcggc tcgtggccaa ggccactcct taaacggcca agcctcggtc 300
tcgggcggag taatcgtcaa catgacgtgt atcactgacg tgggtggttc aaaagacaag 360
aagtacgctg acgtggcggc cgggacgtta tgggtggatg tgcttaagaa gacggcggag 420
aaaggggtgt cgccggtttc ttggacggat tatttgcata taaccgtccg aggaacgttg 480
tcgaatggtg gaattggtgg tcaagtgttt cgaaacggtc ctcttggttag taacgtcctt 540
gaattggacg ttattactgg gaaagggtgaa atgttgacat gctcgcgaca gctaaacca 600
gaattgttct atggagtgtt aggaggtttg ggtcaatttg gaattataac gagagccaga 660
attgttttgg accatgcacc taaacgggcc aaatgggttc ggatgctcta cagtgatctc 720
acaactttta caaaggacca agaacgtttg atatcaatgg caaacgatat tggagtcgac 780
tatttagaag gtcaaatatt tctatcaaac ggtgtcgttg acacctctt tttccacct 840
tcagatcaat ctaaagtcgc tgatctagtc aagcaacacg gtatcatcta tgttcttgaa 900
gtagccaagt attatgatga tcccaatctc cccatcatca gcaagggtat tgacacatta 960
acgaaaacat taagttactt gcccgggttc atatcaatgc acgacgtggc ctacttcgat 1020
ttcttgaacc gtgtacatgt cgaagaaaat aaactcagat ctttgggatt atgggaactt 1080
cctcatcctt ggcttaacct ctacgttcct aaatctcgga ttctcgattt tcataacggt 1140
gttgtcaaag acattcttct taagcaaaaa tcagcttcgg gactcgtct tctctatcca 1200
acaaaccgga ataaatggga caatcgtatg tcggcgatga taccagagat cgatgaagat 1260
gttatatata ttatcggact actacaatcc gctaccccaa aggatcttcc agaagtggag 1320
agcgttaacg agaagataat taggttttgc aaggattcag gtattaagat taagcaatat 1380
ctaattgcatt atactagtaa agaagattgg attgagcatt ttggatcaaa atgggatgat 1440

```

CROP033pct.ST25.txt

ttttcgaaga ggaaagatct atttgatccc aagaaactgt tatctccagg gcaagacatc 1500
ttttga 1506

<210> 43
<211> 2746
<212> DNA
<213> Artificial sequence

<220>
<223> expression cassette

<220>
<221> misc_feature
<222> (1913)..(1914)
<223> n is a, c, g, or t

<400> 43
cttctacatc ggcttaggtg tagcaacacg actttattat tattattatt attattatta 60
ttattttaca aaaatataaa atagatcagt cctcaccac aagtagagca agttggtgag 120
ttattgtaaa gtctacaaa gctaatttaa agttattgac attaacttat ttcattattac 180
aaacaagagt gtcaatggaa caatgaaaac catatgacat actataattt tgtttttatt 240
attgaaatta tataattcaa agagaataaa tccacatagc cgtaaagttc tacatgtggt 300
gcattaccaa aatatatata gcttacaaaa catgacaagc ttagtttgaa aaattgcaat 360
ccttatcaca ttgacacata aagtgagtga tgagtcataa tattattttc ttgctaccc 420
atcatgtata tatgatagcc acaaagttac ttgatgatg atatcaaaga acatttttag 480
gtgcacctaa cagaatatcc aaataatatg actcacttag atcataatag agcatcaagt 540
aaaactaaca ctctaaagca accgatggga aagcatctat aaatagacaa gcacaatgaa 600
aatcctcatc atccttcacc acaattcaaa tattatagtt gaagcatagt agtaatttaa 660
atcaactagg gatatcaca gttgtacaa aaaagcaggc ttcacaatgg ctaatcttcg 720
tttaatgatc actttaatca cggttttaat gatcaccaa tcatcaaagc gtattaaaat 780
tgatttacct aaatccctta acctcaccct ctctaccgat ccttccatca tctccgcagc 840
ctctcatgac ttcggaaaca taaccaccgt gacccccggc ggcgtaatct gcccctctc 900
caccgctgat atctctcgtc tcttccaata cgccgcaaac ggaaaaagta cattccaagt 960
agcggctcgt ggccaaggcc actccttaaa cggccaagcc tcggtctccg gcggagtaat 1020
cgtcaacatg acgtgtatca ctgacgtggt ggtttcaaaa gacaagaagt acgtgacgt 1080
ggcggccggg acgttatggg tggatgtgct taagaagacg gcggagaaag ggggtgcgcc 1140
ggtttcttgg acggattatt tgcatataac cgtcggagga acgttgtcga atgggtggaat 1200
tggtgggcaa gtgtttcgaa acggtcctct tgttagtaac gtccttgaat tggacgttat 1260
tactgggaaa ggtgaaatgt tgacatgctc gcgacagcta aaccagaat tgttctatgg 1320
agtgttagga ggtttgggtc aatttgggaat tataacgaga gccagaattg ttttggacca 1380
tgcacctaaa cggcaagaac gtttgatata aatggcaaac gatattggag tcgactattt 1440
agaaggtaaa atatttctat caaacgggtg cgttgacacc tcttttttcc cacttcaga 1500
tcaatctaaa gtcgctgac tagtcaagca acacggtatc atctatgttc ttgaagtagc 1560
caagtattat gatgatccca atctcccat catcagcaag gttattgaca catcaacgaa 1620
aacattaagt tacttgcccg ggttcataatc aatgcacgac gtggcctact tcgatttctt 1680

CROP033pct.ST25.txt

```

gaaccgtgta catgtcgaag aaaataaact cagatctttg ggattatggg aacttcctca 1740
tccttggett aacctctacg ttcctaaatc tcggattctc gattttcata acggtgttgt 1800
caaagacatt cttcttaagc aaaaatcagc ttcgggactc gctcttctct atccaacaaa 1860
ccggaataag tacatacttc tcttcattca ttttatctt caagaaccaa agnnatggga 1920
caatcgtatg tcggcgatga taccagagat cgatgaagat gttatatata ttatcggact 1980
actacaatcc gctaccccaa aggatcttcc agaagtggag agcgttaacg agaagataat 2040
taggttttgc aaggattcag gtattaagat taagcaatat ctaatgcatt atactagtaa 2100
agaagattgg attgagcatt ttggatcaaa atgggatgat ttttcgaaga ggaaagatct 2160
atttgatccc aagaaactgt tatctccagg gcaagacatc ttttgaaccc agctttcttg 2220
tacaaagtgg tgatatcaca agcccgggcg gtcttctagg gataacaggg taattatata 2280
cctctagatc acaagcccgg gcggtcttct acgatgattg agtaataatg tgtcacgcat 2340
caccatgggt ggcagtgtca gtgtgagcaa tgacctgaat gaacaattga aatgaaaaga 2400
aaaaaagtac tccatctgtt ccaaattaaa attcatttta accctttaat aggtttatac 2460
aataattgat atatgttttc tgtatatgtc taatttgta tcatccgggc ggtcttctag 2520
ggataacagg gtaattatat ccctctagac aacacacaac aaataagaga aaaaacaaat 2580
aatattaatt tgagaatgaa caaaaggacc atatcattca ttaactcttc tccatccatt 2640
tccatttcac agttcgatag cgaaaaccga ataaaaaca cagtaaatta caagcacaac 2700
aaatgggtaca agaaaaacag ttttccaat gccataatac tcgaac 2746

```

<210> 44
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> protein signature

<220>
 <221> MISC_FEATURE
 <222> (6)..(7)
 <223> Xaa can be any hydrophobic amino acid of the list: Gly, His, Leu, Arg, Thr, Trp or Tyr

<220>
 <221> MISC_FEATURE
 <222> (8)..(8)
 <223> Xaa can be any alcohol of the list: Ser or Thr

<220>
 <221> MISC_FEATURE
 <222> (9)..(9)
 <223> Xaa can be any aliphatic amino acid of the list: Val or Ile

<220>
 <221> MISC_FEATURE
 <222> (15)..(16)
 <223> Xaa can be any small amino acid of the list: Ala, Asp, Gly, Asn, Thr or Val

<400> 44

His Thr Asp Tyr Leu Xaa Xaa Xaa Xaa Gly Gly Thr Leu Ser Xaa Xaa
 1 5 10 15

Gly

<210> 45
 <211> 19
 <212> PRT
 <213> Artificial sequence

<220>
 <223> protein signature

<220>
 <221> MISC_FEATURE
 <222> (1)..(1)
 <223> Xaa can be any charged amino acid of the list: Asp, Glu or Arg

<220>
 <221> MISC_FEATURE
 <222> (4)..(4)
 <223> Xaa can be any amino acid, preferably of the list: Tyr, Arg, Asn, Phe, Asp or His

<220>
 <221> MISC_FEATURE
 <222> (5)..(5)
 <223> Xaa can be any tiny amino acid of the list: Ala, Gly or Ser

<220>
 <221> MISC_FEATURE
 <222> (6)..(6)
 <223> Xaa can be any small amino acid of the list: Ala, Asp, Gly, Asn, Thr or Val

<220>
 <221> MISC_FEATURE
 <222> (7)..(7)
 <223> Xaa can be any hydrophobic amino acid of the list: Gly, His, Leu, Arg, Thr, Trp or Tyr

<220>
 <221> MISC_FEATURE
 <222> (9)..(9)
 <223> Xaa can be any small amino acid of the list: Ala, Asp, Gly, Asn, Thr or Val

<220>
 <221> MISC_FEATURE
 <222> (15)..(16)
 <223> Xaa can be any aliphatic amino acid of the list: Val or Ile

<220>
 <221> MISC_FEATURE
 <222> (17)..(17)
 <223> Xaa can be any small amino acid of the list: Ala, Asp, Gly, Asn, Thr or Val

<220>
 <221> MISC_FEATURE
 <222> (18)..(18)
 <223> Xaa can be any turnlike amino acid of the list: Arg or Asn

<400> 45

Xaa Leu Phe Xaa Xaa Xaa Gly Xaa Leu Gly Gln Phe Gly Xaa Xaa
 1 5 10 15

Xaa Xaa Ala