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(54) Title: TRANSGENIC PLANTS

(57) Abstract: The present invention relates to transgenic plants and in particular to transgenic plants having one or more modified genes resulting in phenotypic changes in leaf anatomy and/or vein spacing within the plant, from the C3 photosynthetic pathway to C4. The invention also relates to the genes which give rise to said phenotypic changes.

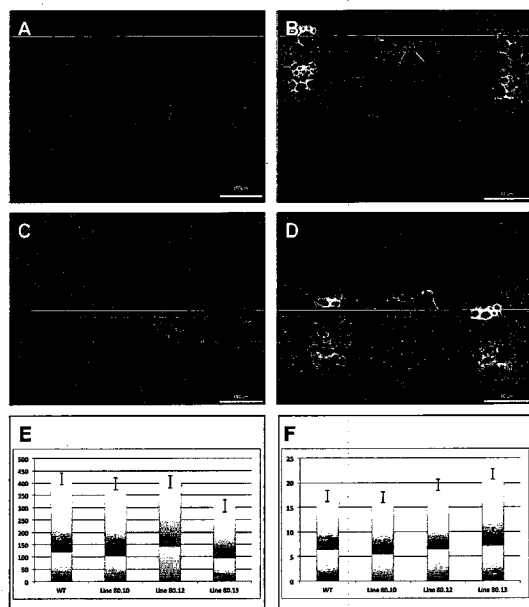


FIGURE 6



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TRANSGENIC PLANTS

FIELD OF THE INVENTION

- 5 The present invention relates to transgenic plants and in particular to transgenic plants having one or more modified genes resulting in phenotypic changes in leaf anatomy and/or vein spacing within the plant. The invention also relates to the genes which give rise to said phenotypic changes.

10 BACKGROUND TO THE INVENTION

Many commercially important plants such as the crops rice and wheat use the C₃ photosynthetic pathway. In these C₃ plants, chloroplasts accumulate Ribulose Biphosphate Carboxylase/Oxygenase (RuBisCo) and fix carbon dioxide (CO₂) in the Calvin-Benson cycle. Carbon fixation directly from the air by RuBisCo is limited
15 in its efficiency by a number of factors, most notably by a competing reaction whereby RuBisCo fixes oxygen (O₂) rather than CO₂.

The C₄ photosynthetic pathway which is found in plants such as maize is an
20 elaboration of the above-described C₃ pathway. In such C₄ plants, photosynthetic reactions are split between two morphologically distinct cell-types known as bundle sheath (BS) and mesophyll (M) cells. Specifically, CO₂ is initially fixed in the M cells into a 4-carbon compound (either malate or aspartate). The C₄ compound subsequently diffuses into the BS cells, where it is decarboxylated to release CO₂
25 that is refixed by RuBisCo to provide a substrate for carbohydrate synthesis. This two-step process maintains an elevated concentration of CO₂ within the BS cells that is able to out-compete O₂ at the active site of RuBisCo. The efficiency of the C₄ photosynthetic pathway depends on the correct organisation of BS and M cells, which are typically arranged into concentric wreaths around leaf veins to form what is
30 known as Kranz anatomy, whereby veins are separated by two BS and two M cells in a repeating pattern:

V-BS-M-M-BS-V

C₄ metabolism based on Kranz anatomy is far more productive than C₃ metabolism. It would therefore be highly advantageous if C₄ traits could be introduced into C₃ plants such as rice, oat, barley and/or wheat in order to increase productivity and thus crop yields.

5

Although Kranz anatomy apparently evolved multiple times, the underlying genetic mechanisms which pattern Kranz anatomy have not been disclosed. There is therefore a need for an understanding of the genetic process or processes which pattern Kranz anatomy in C₄ plants.

10

SUMMARY OF THE INVENTION

The present invention addresses the above need by providing one or more of the maize genes set out in Table 3. The accession numbers are provided, together with

15

the accession numbers of the Arabidopsis orthologues of these sequences. A sub-

set of these genes is provided in Table 4 (*i.e.* GRMZM2G016477,

GRMZM2G027068, GRMZM2G045883, GRMZM2G081816, GRMZM2G052102,

GRMZM2G114998, GRMZM2G171852, GRMZM2G140694, GRMZM2G028046,

GRMZM2G136494, GRMZM2G074032, GRMZM2G129261, GRMZM2G143723,

20

GRMZM2G134998, GRMZM2G150011, GRMZM2G132794, GRMZM2G172657,

GRMZM2G123900, GRMZM2G471089, GRMZM2G132794, GRMZM2G163975,

GRMZM2G151542, GRMZM2G039074, GRMZM2G178182, GRMZM2G045883,

GRMZM2G140669, GRMZM2G472945, GRMZM2G178102, GRMZM2G131516,

GRMZM2G469304, GRMZM2G172657, GRMZM2G374986, GRMZM2G002280,

25

GRMZM2G119359, GRMZM5G893117, GRMZM2G150011, GRMZM2G136494,

GRMZM2G028046, GRMZM2G098988, GRMZM2G377217, GRMZM2G148467,

GRMZM2G021573, GRMZM2G040924, GRMZM2G171365, GRMZM2G146688,

GRMZM2G425236, GRMZM2G417229, GRMZM2G069365, GRMZM5G887276,

GRMZM2G098813, GRMZM2G111045, AC215201.3_FG008, GRMZM2G399072,

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GRMZM2G318592, GRMZM2G097275, GRMZM2G095899, GRMZM2G462623,

GRMZM2G015666, GRMZM2G126018, GRMZM5G850129, GRMZM2G312419,

GRMZM2G061734, GRMZM2G082586, GRMZM2G011463, GRMZM2G480386,

GRMZM2G089819, GRMZM2G159953, GRMZM2G163724, GRMZM2G151955,

GRMZM2G039934, GRMZM2G046316, GRMZM2G034155, GRMZM2G114276,
GRMZM2G028643, GRMZM2G059117, GRMZM2G478876, GRMZM2G344857,
GRMZM2G087243, GRMZM2G133716, GRMZM2G061314, GRMZM2G112210,
GRMZM2G023051, GRMZM2G139324, GRMZM2G077219, GRMZM2G114893,
5 and GRMZM2G109480).

Over-expression of one or more of these genes or their orthologues may lead to the formation of ectopic veins and an increase in leaf vein density that is similar to Kranz anatomy. Here, a gene can be said to be “over-expressed” when the mRNA or
10 protein corresponding to that gene is expressed in a temporally, spatially or quantitatively different manner relative to the wild-type allele or in the case where an ectopic copy of that gene (either host-derived or derived from a different plant species or synthesised with or without modification of codon usage) is expressed in any host tissue.

15 Thus, in a related embodiment, the invention provides a transgenic plant which over-expresses one or more of the maize genes set out in Table 3. In a further embodiment, the invention provides a transgenic plant which over-expresses one or more of the maize genes set out in Table 4. Orthologues of these sequences from
20 other plant species may also be used. The Arabidopsis orthologue is provided. In particular, the transgenic plant may have a leaf which contains an increased number or density of veins with respect to a wild-type plant of the same species at the same stage of development as the transgenic plant. The transgenic plant can be any member of the kingdom Plantae comprising all C₃ plants including cereal crops (such
25 as rice, wheat, oat and barley); or biofuel crops.

In particular, the vein spacing within one or more leaves of the transgenic plant may be altered with respect to a wild-type plant of the same species at the same stage of development as the transgenic plant. Although vein density is increased, the vein
30 spacing within one or more leaves of the transgenic plant may not necessarily be uniform. Veins within one or more leaves of the transgenic plant may be closer together with respect to said wild-type plant. Veins within one or more leaves of the transgenic plant may be separated by fewer cells than the veins of said wild-type

plant. Vein spacing may also be decreased by reducing the volume of cells between veins.

Such morphological changes with respect to said wild-type plant can be readily
5 determined using light microscopy.

The transgenic plant may have a phenotype which is indicative of Kranz anatomy.

In one embodiment, the transgenic plant of the invention over-expresses a maize
10 LRR repeat kinase (GRMZM2G016477). Orthologues of this sequence from other plant species may also be used.

The maize LRR repeat kinase (or its orthologue) may be present in combination with one or more of the other genes (or their orthologue) from Table 3 or Table 4.

15 The present invention also provides a method of making a transgenic plant, the method comprising introducing one or more of the genes herein identified into a plant such that the gene will be over-expressed. The plant may be any member of the kingdom Plantae comprising all C₃ plants including cereal crops such as rice, wheat,
20 oat and barley. The gene to be introduced may be maize LRR repeat kinase (GRMZM2G016477). Orthologues of this sequence from other plant species may also be used. The maize LRR repeat kinase (or its orthologue) may be present in combination with one or more of the other genes (or their orthologue) from Table 3 or Table 4.

25 The one or more genes herein identified may be introduced via transformation with *Agrobacterium*, using suitable techniques known within the art (e.g. as set out in Nishimura *et al*, Nature Protocols (2006), 1(6):2796-2802, which is incorporated by reference).

30 In a further embodiment, the present invention provides a transgenic plant seed. The transgenic plant seed will comprise one or more of the genes herein identified (or their orthologues). The transgenic plant seed will be capable of producing a

transgenic plant which will over-express said one or more genes. The gene may be LRR repeat kinase (GRMZM2G016477). Orthologues of this sequence from other plant species may also be used. The maize LRR repeat kinase (or its orthologue) may be present in combination with one or more of the other genes (or their
5 orthologue) from Table 3 or Table 4.

In yet a further embodiment, the invention provides a shoot, root, cutting or seedling from the transgenic plant of the invention.

10 In a further embodiment, the invention provides the isolated maize genes (or their Arabidopsis orthologues) from Table 3 or Table 4. One or more of these genes (or their orthologues) may be present in a vector or other means suitable for causing over-expression of said gene or genes within a transgenic plant of the invention. The gene or genes (or their orthologues) may be present with a promoter. The
15 promoter may be the endogenous promoter (*i.e.* maize for the maize gene(s)). Alternatively, the gene or genes (or their orthologues) may be present with another promoter (*e.g.* the maize ubiquitin promoter).

BRIEF DESCRIPTION OF THE DRAWINGS

20

These and other aspects of the present invention will now be described by way of example only with reference to the accompanying drawings, in which:

Figure 1A shows a schematic and images of the tissues sampled for use in
25 transcriptome sequencing;

Figure 1B through to Figure 1F shows transverse sections of various samples from the leaf tissue;

30 Figure 1G through to Figure 1K shows transverse sections of various samples from the husk tissue;

Figure 2 shows a comparative profile analysis of transcriptomes in developing leaf primordia: Figure 2 shows a Table displaying descending (D1-D3), ascending (A1-A3) and neutral transcriptome profiles. The total number of genes in each foliar (F) and husk (H) profile is shown along with the number of genes encoding transcription factors (TFs). The specificity of TFs with respect to each profile is shown, along with
5 reference to the number of TFs that are shared with other profiles;

Figure 3 shows distinct transcription factor cohorts associated with early differentiation events in foliar leaf primordia. Transcription factors identified in the
10 FA2 profile that are specific to foliar leaves, and have no previously identified role in early leaf development are shown. Transcripts reported to be BS or M localized are indicated;

Figure 4A shows proposed regulators of Kranz patterning. Accession numbers and
15 gene family are indicated, along with the sub-family deduced from phylogenetic analysis. Where previously unnamed, genes have been given names on the basis of phylogenetic relationships. Presence in specific primordia profiles, and presence or absence in the list of putative positive regulators of Kranz is indicated;

20 Figure 4B shows a proposed model of the genetic pathway regulating Kranz development: it is postulated that six genes are expressed in developing procambium and/or vasculature and that their role is to regulate leaf venation patterns. The SHR1 gene is additionally expressed in BS cells, as is the SHR target gene SCR1. SCR1 is also expressed in M cells but at a lower level than in BS cells,
25 establishing a gradient of expression emanating from the vein. We propose that the SHR/SCR gradient is fixed by the cell-specific accumulation of the SHR interacting proteins RAVEN1 (BS) and JAY1 (M). BS cells are thus specified by the combined action of SHR1, SCR1, and RVN1 whereas M cells are specified by the interaction between SCR1 and JAY1. Superimposed upon this SHR module is a gradient of
30 DLK gene expression;

Figure 5 shows PCR results for a number of transgenic lines by comparison with a wild-type (negative control) and the plasmid carrying the transgene (positive control);

Figure 6: 6A, 6B, 6C and 6D show hand sections from fully expanded WT (6A, 6C) and transgenic line 80.13 (6B, 6D) leaves. Figure 6E shows inter-veinal distance in microns (μm) between two intermediate veins positioned between the closest and second closest lateral veins to the midrib, measured in three separate leaves of each of three transgenic lines (80.10, 80.12, 80.13). Figure 6F shows the number of leaf veins per mm of leaf width in the same three leaves.

EXAMPLES

Example 1: Identifying regulators of Kranz anatomy

The development of Kranz anatomy has been described from a histological perspective but the mechanisms underpinning the patterning of Kranz tissues are not known.

In maize, BS cells surrounding the veins, plus the middle layer of M cells in the leaf are derived from the same cell lineage as the leaf veins. As such, genes that regulate leaf venation patterns in maize will regulate whether or not Kranz anatomy is formed. If a vein is formed, it develops with associated BS cells and if veins are formed at appropriate intervals only two M cells will separate each vein/BS unit. The timing of vein formation is such that the leaf midvein is initiated first, developing from the shoot into the leaf primordium. The time interval is measured in plastochron (P); lateral veins begin to appear at the base of Plastochron 2 (P2) primordia and develop toward the leaf tip. Intermediate veins develop during P5 and lead to the vein patterning that is characteristic of Kranz anatomy; these veins are initiated at the tip of P5 primordia and by the end of P5 they have reached the base of the leaf sheath. Anastomoses or fusions at the developing ligule result in fewer intermediate veins in the sheath than in the blade. Kranz anatomy is therefore a feature of the leaf blade but not of the leaf sheath. This is apparent in the husk leaves that surround the ear, where most or all of the tissue is sheath tissue. Veins in husk leaf

sheaths are surrounded by a wreath of BS cells but each vascular bundle is then separated by around 20 M cells rather than the two associated with Kranz anatomy.

A range of biological samples representing different developmental trajectories and anatomical traits were harvested as follows.

Plant material and growth conditions

Maize inbred line B73 was grown in soil in a greenhouse with a diurnal light regime of 16 hours of light (supplemented to $300 \mu\text{mol m}^{-2} \text{sec}^{-1}$) and 8 hours of darkness and an average daytime temperature of 28 degrees C and an average night time temperature of 20 degrees C.

Samples

Figure 1A depicts the position on the plant from which the samples were taken, together with the overall morphology of the tissue sampled.

For sample purposes, tissue was harvested as required from 2, 4 or 8 week old plants and placed directly into liquid nitrogen. In the case of husk samples, the prophyll was discarded prior to immersion in liquid nitrogen.

Foliar and husk leaf samples were both harvested at 5 stages of development. Foliar tissues were all harvested from 2 week old seedlings in which the 5th leaf was just emerging. The 5 foliar samples and the 5 husk samples covered the range from plastochron (P)1 to P9 and are set out in the table below.

FP	vegetative apical shoot meristem and P1 and P2 leaf primordia
FP3/4	pooled P3 and P4 leaf primordia
FP5	P5 leaf primordia
Fl	expanding 5th leaves at P7
FE	fully expanded 3rd leaves at P9
HP	axillary inflorescence meristem and P1 and P2 husk primordia
HP3/4	pooled P3/P4 husk primordia from ear forming nodes of 4-week

	old plants
HP5	P5 husk primordia from ear forming nodes of 4-week old plants
HI	inner husk leaf from 8 week old plants
HE	outermost husk leaf from 8 week old plants

FP5, FI and FE leaves were all harvested above the ligule to ensure that only the blade (Kranz) tissue was represented. The husk samples were harvested from the same plants as the foliar samples, from the axils of P9 and P10 leaves. The husk leaves did not form blades and thus all such samples comprised only sheath (non-Kranz) tissue.

Leaf samples were fixed overnight in FAA (4% formaldehyde, 5% acetic acid, 50% ethanol) and embedded in Paraplast Plus as described in the method of Langdale JA (1994) In situ hybridisation, The Maize Handbook (eds. Freeling and Walbot), pp. 165-179, which is hereby incorporated by reference. Sections were stained with Safranin/Fast Green and viewed with a Leica DMRB microscope.

Transverse sections of all 10 samples were examined by light microscopy. The results are shown in Figure 1B to Figure 1K. For both foliar and husk samples, P1 and P2 primordia have cells with high cytoplasm to cell volume ratios and procambium associated with the midvein is minimally visible (Figure 1B and 1G). By P4 the midvein and developing lateral veins can be readily distinguished and the distance between them is smaller in foliar (Figure 1C) than in the comparative-stage husk (Figure 1H) primordia. At P5, foliar primordia have initiated intermediate veins and each vascular centre is surrounded by a wreath of small BS cells and is separated from the next centre by 1 to 2 intervening M cells (Figure 1D). In husk samples, no intermediate veins can be seen to be formed and cell division and expansion in M cells increases the space and cell number between the veins: from 5 at P4, to around 10 at P5 (Figure 1I) and ultimately to around 15 or more in mature husk leaves (Figure 1J and 1K). Vein spacing does not change after P5 in foliar leaves and thus in FI (Figure 1E), which shows P7 foliar leaf, and FE (Figure 1F), which shows P9 foliar leaf, the classical cellular Kranz arrangement is visible (*i.e.* V-BS-M-M-BS-V). In expanded husk leaves BS cells and chloroplasts within them are

much smaller than in foliar leaves (Figure 1K). Very few chloroplasts are visible in HI leaves (Figure 1J). Table 1 shows a summary of key anatomical differences between the respective tissues. It was noted that HP samples were essentially arrested at the time of harvesting but that FP, FP3/4 and FP5 samples were
5 undergoing rapid cell division and growth.

Accordingly the present inventors have postulated that positive regulators of Kranz anatomy in maize will act between P2 and P5 in foliar leaf primordia but have reduced activity or be inactive in husk leaf primordia.

Identifying putative genes

An overview of the changes in gene activity during the patterning of Kranz anatomy was carried out using Illumina sequencing to generate transcriptomes of developing
15 foliar (Kranz) and husk sheath (non-Kranz) primordia. Transcriptome profiles were then compared with the later stages of development where initiation or maintenance of C₄ metabolism is being superimposed on pre-established anatomy. A number of genes that are active during early leaf development were identified and from these positive regulators of Kranz patterning have been isolated and tested. The results
20 reveal novel cohorts of genes that are active during early leaf development (as opposed to activity of a master regulator). From within these cohorts a regulatory network has been identified which is associated with the patterning of Kranz anatomy.

RNA extraction and cDNA synthesis

Multiple individual samples were pooled prior to RNA extractions. For each of the two sequencing replicates, N=1200 (FP); 300 (FP3/4); 100 (FP5); 12 (FI); 12 (FE); 2400 (HP); 300 (HP3/4); 100 (H5); 12 (HI) and 12(HE).

RNA was extracted using the mirVana miRNA Isolation Kit (Applied Biosystems). RNA integrity was analysed by formaldehyde gel electrophoresis. cDNA library preparation and sequencing were carried out at the Beijing Genome Centre (BGI).

Each RNA sample was treated in the same manner prior to paired end Illumina sequencing. Total RNA was first treated with DNase I and then purified over an oligo-dT column. The enriched mRNA was then sheared and converted into cDNA using standard Illumina protocols. The cDNAs were subsequently ligated to Illumina
5 adaptors and subjected to limited PCR as part of the Illumina library preparation. 72bp paired end reads were generated for all samples.

Transcript quantification and differential gene expression analysis

10 Paired end reads were subject to quality-based trimming using the FASTX toolkit described in Goecks *et al*, Genome Biol (2010), 11(8):R86, which is incorporated by reference; setting the PHRED quality threshold at 20 and discarding reads less than 21 nucleotides in length. Transcripts were quantified using RSEM as described in Li
15 *et al*, BMC Bioinformatics (2011), 12:323, which is incorporated by reference; and the predicted coding sequences from version 5b of the Maize genome. For differential gene expression analysis, expected transcript counts originating from the same gene locus were summed and all possible pairwise comparisons between replicated samples were performed using DESeq as described in Anders *et al*,
20 *Genome Biol* (2010), 11(10):R106, which is incorporated by reference. In all cases, differentially expressed genes were identified as those genes with a Benjamini-Hochberg corrected p-value of less than 0.05 as described in Benjamini *et al*,
Journal of the Royal Statistical Society Series B (Methodological) 1995, 57(1):289-
300, which is incorporated by reference.

25 Data analysis

To identify novel associations between gene cohorts and the earliest patterning processes that operate in maize leaf primordia, all genes that were detected in foliar and husk primordia samples were assigned to one of seven profiles (Figure 2). Each
30 profile was designed to identify genes associated with specific aspects of leaf development. For example, descending profiles should contain genes required for meristem function and for very early events in leaf development such as midvein formation and adaxial/abaxial axis formation. In contrast, ascending profiles should

contain genes required for the differentiation of lateral leaf veins, patterning of intermediate veins, specification of cell-types and early plastid biogenesis.

The A2 and A3 profiles were designed to identify genes required for patterning leaf
5 venation and regulating cell-type differentiation in foliar leaves. The presence of
genes in the FA2/3 profiles that are related to those that regulate xylem (e.g. XYLEM
NAC DOMAIN1) and phloem (ALTERED PHLOEM1) differentiation was confirmed.
The presence of foliar-specific AUXIN RESPONSE FACTOR (ARF) and AUX/IAA
10 homologs in the FA3 profile was confirmed. Very little is known about the patterning
of leaf venation or the regulation of BS and M differentiation, and it is apparent that
most of the genes in the FA2 profile have not been previously characterized and/or
annotated. With this in mind, there are 16 foliar-specific genes of note that fall into
four cohorts (Figure 3). First, there are three bHLH genes that may regulate cellular
differentiation given the role of other bHLH proteins in patterning processes. Notably,
15 transcripts encoded by two of these genes were detected in maize BS cells, but only
when samples also included vascular tissue. One of the genes is related to a target
of the auxin dependent transcription factor MONOPTEROS. Second, there is a novel
bZIP gene, and three DoF Zn Finger genes that share clades with Arabidopsis DoF
AFFECTING GERMINATION (DAG)-like and HIGH CAMBIAL ACTIVITY2 (HCA2)
20 genes, all of which are expressed in vascular tissue. In this case, transcripts of the
two DAG-like genes were detected both in isolated BS cells and in BS cells
associated with vasculature. Third, two GRAS family SHORTROOT (SHR)-like
genes are present, homologs of which play a role in radial patterning around
vasculature in the Arabidopsis root. Fourth and most notable are seven C2H2 Zn
25 Finger proteins. Two are related to the previously characterized Arabidopsis gene
DEFECTIVELY ORGANIZED TRIBUTARIES (DOT5) that regulates vascular
patterning, and three are related to SHOOT GRAVITROPISM 5 (SGR5) and
JACKDAW (JKD) proteins - known components of the SHR pathway in Arabidopsis.
Transcripts of one of the JKD-related genes accumulate specifically in M cells and
30 those of the second accumulate specifically in BS cells. The remaining two C2H2
ZnF proteins are related to MRP1 interacting (MRPI) proteins. MRPI proteins
interact with MRP through a C-terminal domain that is shared with SHR target
proteins. The phylogenetic relationships and expression profiles of all of these genes

thus position them as likely regulators of vascular patterning and cellular differentiation in maize foliar leaves.

In addition to the profile analysis described above, transcriptome filters were designed (based on the anatomical and developmental analyses presented in Figure 1) that would identify positive regulators of Kranz in maize tissues. For positive regulators it was postulated that genes would be expressed at higher levels in foliar leaves than in husk leaves and that patterning genes would be expressed prior to P5. It was further postulated that if genes were expressed in the expanding leaf, transcript levels would be higher in the immature basal regions of the leaf than in more distal regions. The expanding leaf gradient dataset was generated by re-mapping the read data from Li et al, Nat Genet (2010), 42:1060-1067, which is hereby incorporated by reference. The data were re-mapped to version 5b of the maize genome.

Table 2 shows the decision and filtration steps that were carried out to identify genes of interest involved in regulating Kranz anatomy (the p-value cut-off used was 0.05). It can be seen that following the final step 3, 283 putative positive regulators of Kranz anatomy were identified.

The putative positive regulators are presented in Table 3, together with a list of 16 transcription factors discussed further below.

In the list of 283, GO term enrichment identified a large proportion (cohort) of genes associated with e.g. the cytoskeleton and with ribosomes/protein synthesis. This was expected, since the filtration criteria selected genes that were most highly expressed in developing foliar primordia and FP3 to FP5 are actively dividing tissues. These 283 genes may play a role in regulating Kranz anatomy, but at present genes with a role in the regulation of transcription (e.g. nucleus, DNA binding) or of cell-signalling are of greater interest. 70 genes with putative regulatory roles were identified from within the list of 283.

The presence of the *ZmScarecrow 1* gene within this list is supportive of a role for this subset in vascular patterning, as *ZmScr1* is known to be expressed in early vascular development and in BS cells (as set out in Lim *et al.*, Plant Mol Biol (2005), 59:619-630, which is incorporated by reference). Similar support is demonstrated by the presence within this list of the C2H2 zinc finger encoding gene GRMZM2G150011, because it is homologous to *Defectively Organised Tributaries* (*DOT5*), which is required for appropriate patterning of veins in the Arabidopsis leaf (as set out in Petricka *et al.*, The Plant Journal (2008), 56:251-263, which is incorporated by reference).

16 transcription factors were identified through profile classification of gene expression in leaf primordia (Figure 3) and 283 potential regulators were identified through comparative analysis within a broader developmental context (Table 3). Both approaches were used to ensure that genes involved in all stages of Kranz differentiation were identified. In this regard, the development of Kranz anatomy involves three distinct but undoubtedly overlapping stages. First, procambium is initiated at regular intervals across the mediolateral leaf axis. Second, concentric wreaths of BS and M cells are specified around each vein. Finally, components of the photosynthetic machinery accumulate in a BS and M cell-specific manner. None of the genes encoding C4 photosynthetic enzymes are represented in the leaf primordia samples. As such, the data provide insight into the patterning of leaf venation and the specification of distinct BS and M cell-types, prior to the induction of a functional C4 cycle.

Of the 16 transcription factors revealed by the FA2 profile (Figure 3), six were also identified in the 283 list generated above. It is postulated that these six transcription factors play a role in patterning leaf venation. Three are homologs of Arabidopsis genes known to be involved in vascular development (*SHR*, *DOT5*), two encode proteins with a domain that is conserved in *SHR* target proteins, and the sixth is related to the bHLH protein *SPEECHLESS* which regulates stomatal spacing in the Arabidopsis epidermis. The maize bHLH gene may thus have been recruited to regulate vein spacing in sub-epidermal layers. Transcripts of all six genes

accumulate to higher levels in foliar than husk primordia, as would be expected given the higher vein density in foliar leaves.

Also present within this list is LRR repeat kinase (GRMZM2G016477). It was
5 decided that this gene would be tested within a C₃ plant.

Example 2: Over-expression of the LRR kinase

Creation of transgenic rice

10

Callus of the rice variety Kitaake was transformed with the construct MS80 using *Agrobacterium tumefaciens*. MS80 comprised a modified pVec8 binary vector that included the maize Ubiquitin promoter and intron placed upstream of the coding region of GRMZM2G016477 (a maize LRR kinase encoding gene). Putative
15 transformants were selected and regenerated on hygromycin. A number of regenerated lines were assayed by genomic PCR for presence of the transgene.

20

Figure 5 shows that the same size fragment is amplified from genomic DNA extracted from lines 80-10, 80-12 and 80-13 as with MS80 plasmid DNA (positive control). No fragments are amplified from genomic DNA extracted from the wild-type (WT) negative control. The amplified fragments from lines 80-10, 80-12 and 80-13 were sequenced and shown to correspond to regions of GRMZM2G016477. It was accordingly concluded that the three lines are transgenic and are likely to be
25 expressing the maize LRR kinase.

25

Observed phenotypic effects

30

The phenotype of each line was observed with respect to a wild-type. Samples were cut from the middle of expanded leaf blades of T0 (primary transformed) plants that had been regenerating for 6-8 weeks, at least 4 weeks of which had been on soil in a glasshouse. Transverse sections were prepared by hand sectioning under water with a razor blade. Sections were placed on a microscope slide and observed under UV light.

The results are shown in Figure 6. 6A, 6B, 6C and 6D show hand sections of leaves from fully expanded WT (6A, 6C) and transgenic 80.13 (6B, 6D) lines. Figure 6E shows the inter-veinal distance in microns (μm) between two intermediate veins positioned between the closest and second closest lateral veins to the midrib, measured in three separate leaves of three independent transgenic lines.

Figure 6F shows number of leaf veins per mm of leaf width in same three leaves as in Figure 6E.

Although none of the transgenic rice lines uniformly displays classical Kranz anatomy, it is clear that the lines 80.12 and 80.13 have veins that are more closely spaced together (*i.e.* with fewer cells between the veins) by comparison with the wild-type.

Summary

C₃ plant crops such as rice lack the Kranz anatomy and lack the C₄ photosynthetic pathway. We show here that certain genes are associated with regulation of Kranz anatomy and, when over-expressed in C₃ transgenic plants, lead to phenotypic changes that increase vascular density, modify leaf anatomy and may result in a more efficient photosynthetic pathway.

It is proposed in particular that six transcription factors play a role in patterning leaf venation (Figure 4). Three are homologs of Arabidopsis genes known to be involved in vascular development (SHR, DOT5), two encode proteins with a domain that is conserved in SHR target proteins, and the sixth is related to the bHLH protein SPEECHLESS which regulates stomatal spacing in the Arabidopsis epidermis. The maize bHLH gene may thus have been recruited to regulate vein spacing in sub-epidermal layers. Notably transcripts of all six genes accumulate to higher levels in foliar than husk primordia (Figure 4A), as would be expected given the higher vein density in foliar leaves.

Once procambial centres have been initiated in developing leaf primordia, our data indicate that a SHR-related pathway specifies BS and M cell identity. In Arabidopsis roots the SHR gene is expressed in the vasculature and SHR protein is transported to the encircling endodermal cell layer. Movement of SHR to the next cell layer (the cortex) is prevented by an interaction between SHR and its downstream target SCR in the endodermis. Boundaries between the endodermis and cortex are maintained by cell-specific compartmentalization of JKD-related proteins. This scenario is reproduced in the context of developing Kranz tissues in that transcripts of ZmScr and of two JKD-related genes (that we name ZmJay1 and ZmRaven1) accumulate differentially in BS and M cells. ZmScr1 and ZmRvn1 transcripts accumulate preferentially in BS cells whereas ZmJay1 transcripts are enriched in M cells. Genes encoding four other direct targets of SHR (two F-box proteins, a receptor like kinase and an unknown protein) were also identified in the list of putative positive regulators of Kranz anatomy. We therefore propose that Kranz anatomy in maize is patterned by a SHR-related developmental module (Figure 4B). Superimposed upon the SHR module is an expression gradient of DoF AFFECTING GERMINATION (DAG)-like genes that we have named ZmDlk1 and ZmDlk2. There is no predicted function for these genes but their classification profile and differential expression in BS and M cells warrants their inclusion in the proposed model. In summary the combinatorial action of just six transcription factors could explain the differentiation of distinct BS and M cell in C4 leaves. Importantly these six genes map directly on to the existing model of Kranz development in that there is a gradient emanating from the veins and BS and M cell-specific factors that interact with that gradient. The data herein thus suggest that a pre-existing developmental module that operates to specify cell-types in the radial axis of the root was co-opted during the evolution of Kranz anatomy to pattern BS and M cell-types around leaf veins.

Table 1. Key anatomical traits and transcriptome profiles of foliar and husk leaf samples. Signature genes are those where transcripts were detected at significantly higher levels in the named sample as compared to the other nine samples (p=0.05).

Sample	Veins initiated	No. of M cells between veins	BS cell size	BS plastid size	Growth	Photosynthesis	No. of genes represented in dataset	No. of signature genes in dataset	Metabolic pathways significantly enriched in signature gene sets
FP	Midvein	na	na	na	rapid cell division	none	27284	20	
FP3/4	Midvein Laterals	4-6	na	na	rapid cell division	none	27553	10	
FP5	Midvein Laterals Intermediates	1-2	small	na	rapid cell division	none	27431	42	Ribonucleotide biosynthesis
F1	Midvein Laterals Intermediates	2	large	small	cell division (leaf base) & expansion	active	28472	1341	Protein synthesis Lipid metabolism Tetrapyrrole biosynthesis
FE	Midvein Laterals Intermediates	2	large	large	cell expansion	very active	27431	1479	Light harvesting reactions C ₄ carbon assimilation Gluconeogenesis Photorespiration
HP	Midvein	na	na	na	arrested	none	28445	24	
HP3/4	Midvein Laterals	5	small	na	rapid cell division	none	29484	10	
HP5	Midvein Laterals	10	small	na	rapid cell division	none	29515	26	Lipid biosynthesis
H1	Midvein Laterals	~12	medium	small	cell division & expansion	none	28662	344	Cell wall biosynthesis
HE	Midvein Laterals	~15	medium	small	ceased	some around veins	28473	713	2 ^o metabolite biosynthesis Amino acid degradation Lipid degradation

Table 2. Filtration steps to identify putative regulators of Kranz anatomy. All tests were carried out at a p-value cut-off of 0.05.

Positive Regulators
All genes N = 35,770
Step 1: FP or FP3/4 or FP5 significantly > FE + HP + HI + HE Genes must pass test in 2 out of 3 FP samples. N = 918
Step 2: FP3/4 or FP5 significantly > or not different to FP FP3/4 not significantly < FP HP3/4 not significantly > FP3/4 HP5 not significantly > FP5 HP not significantly > FP HP5 significantly < FP3/4 HP significantly < FP3/4 FP5 or FP3/4 > 0 HI not significantly > FP, FP3/4, FP5, FI or FE HE not significantly > FP, FP3/4, FP5, FI or FE FE not significantly > FP, FP3/4, FP5 or FI N = 334
Step 3: If in leaf gradient dataset: Base not significantly < 1cm, 4cm or tip 1cm not significantly < 4cm, tip 4cm not significantly < tip N = 283

Table 3: putative positive regulators of Kranz anatomy

		Arabidopsis accession	Rice accession
FA2			
GRMZM2G027068	bHLH family AtTMO5 clade		
GRMZM2G081816	bHLH family Subfamily VIIIb		
GRMZM2G052102	bZIP family bZIP family		
GRMZM2G114998	C2C2-Dof family AtDAG1 clade		
GRMZM2G171852	C2C2-Dof family AtDAG1 clade		
GRMZM2G140694	C2C2-Dof family AtHCA2 clade		
GRMZM2G074032	C2H2 family AtSGR5 clade		
GRMZM2G129261	C2H2 family AtJKD clade		
GRMZM2G143723	C2H2 family AtJKD clade		
GRMZM2G134998	C2H2 family AtDOT5 clade		
Transcription Factors			
GRMZM2G123900	ZnF-Dof type	AT3G55370	Os12g38200
GRMZM2G471089	MADS box TF	AT4G18960	Os01g10504
GRMZM2G132794	GRAS TF	AT4G37650	Os03g31880
GRMZM2G163975	AP2 TF	AT1G27660	Os01g01600
GRMZM2G151542	AP2 like TF (similar to Pti6 -possible role in disease)		Os01g46870
GRMZM2G039074	Myb TF	AT5G42630	Os02g46940
GRMZM2G178182	HLH TF (similar to GBOF-1 - auxin inducible, role in steml elongation in tulips)	AT5G50915	Os09g33580
GRMZM2G045883	bHLH TF	AT1G72210	Os02g52190
GRMZM2G140669	GATA zinc finger TF	AT4G36240	Os02g43150
GRMZM2G472945	Tubby like protein (possible TF or signalling molecule)	AT1G16070	Os02g08310
GRMZM2G178102	Class III HD-ZIP TF phb phv rev like	AT1G30490	Os10g33960
GRMZM2G131516	Scarecrow	AT3G54220	Os11g03110
GRMZM2G469304	Ternary complex factor MIP1; leucine zipper (possible TF)	AT5G66600	Os03g13130
GRMZM2G172657	Short root like TF	AT4G37650	Os03g31880
GRMZM2G374986	Myb TF		Os03g03760
GRMZM2G002280	Znf_C3HC4_Ring-type	AT3G27330	Os12g16690
GRMZM2G119359	Growth regulating factor		Os06g02560

GRMZM5G893117	Growth-regulating factor		Os02g53690
GRMZM2G150011	Zinc finger C2H2 type	AT1G13290	Os09g13680
GRMZM2G136494	ZnF_C2H2 domain	AT1G75710	Os04g59380
GRMZM2G028046	Zinc finger C2H2 type	AT1G75710	Os11g06840
GRMZM2G098988	bHLH T	AT1G29950	Os03g39432
GRMZM2G377217	WRKY TF	AT2G44745	Os02g43560,Os04g46060
GRMZM2G148467	SBP protein TF	AT1G27370	Os04g46580
GRMZM2G021573	AP2 TF	AT4G37750	Os03g56050,Os07g03250
GRMZM2G040924	R2R3 Myb TF	AT3G61250	Os02g42870
GRMZM2G171365	MADS box TF	AT2G45660	Os10g39130
GRMZM2G146688	AP2 domain TF	AT4G37750	Os03g56050
GRMZM2G425236	Zinc finger homeodomain protein	AT4G24660	Os03g50920
GRMZM2G417229	Zinc finger homeodomain protein		Os02g47770
GRMZM2G069365	Zn finger homeodomain	AT4G24660	Os02g47770
GRMZM5G887276	Myb TF SHAQKYF class		Os10g39550
GRMZM2G098813	floricaula/leafy-like 1, TF	AT5G61850	Os04g51000
GRMZM2G111045	P-type R2R3 Myb protein	AT3G61250, AT1G34670	Os02g42870
AC215201.3_FG008	HLH TF	AT2G40435	Os05g27090
GRMZM2G131577	NAC domain TF	AT1G17880	Os03g01910
GRMZM2G399072	AP2 TF		Os03g12950
GRMZM2G318592	ZnF_C2H2 domain		Os06g44200
GRMZM2G097275	SBP protein TF	AT1G27370, AT5G43270	Os02g04680
GRMZM2G095899	HLH TF	AT4G37850	Os03g46860
GRMZM2G462623	DP-1 TF	AT5G03415	Os01g48700
GRMZM2G015666	bHLH TF	AT1G72210	Os09g29360
GRMZM2G126018	SBP domain TF	AT2G42200	Os09g31438
GRMZM5G850129	Growth regulating factor	AT3G13960	Os06g10310
GRMZM2G312419	R2R3 Myb TF	AT1G69560	Os01g16810
GRMZM2G061734	SBP domain TF -TGA1	AT5G50570	Os09g32944
GRMZM2G082586	bHLH TF	AT1G72210	Os09g29360
Auxin-related	6		
GRMZM2G129413	aic1 auxin import carrier 1	AT2G38120	Os03g14080
GRMZM2G098643	Auxin efflux, ZmPIN1a protein	AT1G77110	Os05g50140
GRMZM2G121309	auxin-responsive Aux/IAA family member	AT3G16500	Os02g13520
GRMZM2G053338	indole-3-acetic acid amido synthetase	AT1G23160,AT2G23170, AT1G28130	Os01g55940,Os07g47490
GRMZM2G011463	Auxin inducible - SAUR family		Os09g26590

GRMZM2G480386	Flavin-containing monooxygenase YUCCA-like - role in auxin biosynthesis	AT4G28720	Os03g06654
Kinases	13		
GRMZM2G089819	Brassinosteroid LRR receptor kinase	AT3G56100, AT3G51740	Os11g01620, Os12g01700
GRMZM2G016477	LRR repeat kinase	AT1G75640	Os04g48760
GRMZM2G159953	Lectin family receptor kinase	AT5G03140	Os06g17490
GRMZM2G163724	LRR RLK	AT4G37250	Os09g02250
GRMZM2G151955	LRR-RLK (LRR-RLKII subfamily - development and disease)	AT5G16000, AT1G71830	Os08g34380, Os06g16330
GRMZM2G039934	TDR/PXY, LRR-LRK	AT3G24240	Os05g51740
GRMZM2G046316	LRR-RLK	AT5G45800	Os02g14480, Os06g35200
GRMZM2G034155	LRR-RLK	AT4G20270	Os07g05740
GRMZM2G114276	LRR-RLK	AT1G75640	Os04g48760
GRMZM2G028643	LRR-kinase	AT3G19230	Os03g08610
Kinases		Arabidopsis accession	
GRMZM2G059117	LRR RLK	AT3G56370	Os03g21730
GRMZM2G478876	Serine/threonine kinase	AT4G34500	Os03g12680
GRMZM2G344857	Phosphatidylinositol-4-phosphate 5-kinase 4	AT2G26420	Os11g04840
Post-transcriptional regulation	6		
GRMZM2G021149	Small nuclear ribonucleoprotein-associated protein B	AT5G44500	Os07g07220
GRMZM2G065097	PolyA polymerase like (polynucleotide adenylyltransferase activity)	AT2G45620	Os02g02970
GRMZM2G132340	PolyA polymerase like (polynucleotide adenylyltransferase activity)	AT2G45620	Os02g02970
GRMZM2G152774	3-5 exonuclease/ nucleic acid binding protein		Os01g47180
GRMZM2G117642	Endoribonuclease	AT3G20390	Os07g33240
GRMZM2G149520	DEAD/DEAH box helicase family protein	AT5G62190	Os09g34910
DNA regulation	6		
GRMZM2G075505	Nucleic acid binding protein (Alba family - Alba binds DNA without sequence specificity)	AT3G07030	Os03g06980
GRMZM2G369260	delta DNA polymerase		Os08g06620
GRMZM2G133838	H/ACA ribonucleoprotein complex subunit 3-like protein (nucleor protein family required for 18s rRNA production)	AT2G20490	Os02g40514
AC196278.2_FG003	DNA polymerase subunit	AT5G63960	Os11g08330
GRMZM2G026580	DNA pol	AT4G24790	Os12g19180
AC196278.2_FG004	DNA polymerase subunit	AT5G63960	Os11g08330

Heat Shock and molecular chaperone	5		
GRMZM2G040890	Heat shock related	AT5G57710	Os02g54720
GRMZM2G032547	Heat shock protein like	AT4G29920	Os04g23220
AC216247.3_FG001	Heat shock TF (B-4d like)	AT1G46264	Os03g25120
GRMZM2G129987	chaperone protein dnaJ 6	AT3G12170	Os02g10220
GRMZM2G359779	Heat shock protein like	AT4G29920	Os04g23220
Intra and inter cellular signalling/membrane trafficking	10		
GRMZM2G363474	DEM protein like (Vacuolar import/degradation)	AT3G19240	Os02g54780
GRMZM2G143253	rac GTPase activating protein	AT4G03100	Os07g22580
GRMZM2G010306	Dynamin like (GTPases involved in endocytosis, organelle division, cytokinesis)	AT1G53140	Os01g54420
GRMZM5G819523	Glutaredoxin family	AT5G03870	Os04g33680
GRMZM2G087243	Armadillo/beta catenin like (cell-cell interactions and signalling)	AT2G45720	Os10g37850
GRMZM2G086637	Patellin 5 (carrier protein may be involved in membrane trafficking during cytokinesis)	AT3G51670	Os05g27820
GRMZM2G323936	rac GTPase activating protein	AT4G03100	Os12g34840
GRMZM2G064852	C2 domain protein (Ca2+ binding - intracellular signalling/membrane trafficking)	AT5G12970	Os05g30750
GRMZM2G133716	Forkhead domain (putative nuclear signalling domain)	AT1G34355	Os11g07050
GRMZM2G329710	Fasciclin1 domain (possible cell adhesion)	AT5G06920	Os02g28970
Ribosomal	14		
GRMZM2G166963	60S ribosomal protein	AT1G26880	Os09g24690
GRMZM2G360677	40S ribosomal protein	AT5G59240	Os02g28810, Os04g28180
GRMZM2G081102	60S ribosomal protein	AT3G49010	Os06g02510
GRMZM2G113720	60S ribosomal protein	AT2G34480	Os05g49030
GRMZM2G088060	60S ribosomal protein	AT2G19730	Os01g51020, Os02g57540
GRMZM2G336875	40S ribosomal protein	AT5G59240	Os04g28180
AC230013.2_FG007	60S ribosomal protein L18a	AT2G34480, AT1G29965	Os05g49030
GRMZM2G177720	40S ribosomal protein	AT1G07770	Os02g27760
GRMZM2G086906	40S ribosomal protein	AT3G10610	Os10g27190
GRMZM2G065868	60S ribosomal protein	AT4G18100	Os08g41300, Os09g32500
AC225147.4_FG002	40S ribosomal protein S23	AT5G02960	Os03g60400

GRMZM2G007695	60S ribosomal protein L4/L1	AT3G09630	Os07g08330
GRMZM2G027232	50S ribosomal protein L11	AT4G35490	Os10g32870
GRMZM2G325749	50S ribosomal protein	AT5G27820	Os01g15290
F-box (not TF specified) and Ubiquitin related	5		
GRMZM2G002830	ubiquitin-conjugating enzyme X	AT3G20060	Os01g16650
GRMZM2G007057	Ubiquitin carrier protein	AT3G20060	Os01g16650
GRMZM2G016323	ubiquitin-specific protease 23	AT5G57990	Os06g08530
GRMZM2G440543	F-box protein GID2	AT5G48170	Os03g10040
GRMZM2G040278	F-box cyclin like domain, similar to GID2 protein	AT5G48170	Os03g10040
Cytoskeletal	22		
GRMZM2G136838	Kinesin heavy chain	AT3G43210	Os02g43130
GRMZM2G030284	Microtubule associated protein	AT5G51600	Os05g47970
GRMZM2G034828	Kinesin heavy chain	AT3G20150	Os03g39020
GRMZM2G082384	Kinesin like	AT5G02370	Os05g38480
GRMZM2G150984	phragmoplast-associated kinesin	AT4G14330	Os02g56540
GRMZM2G325783	Xlp2 targeting domain in some isoforms (Xlp2 binds centromeres)	AT1G03780	Os07g32390
GRMZM2G157616	Kinesin like	AT1G18550	Os01g42070
GRMZM2G154904	Xlp2 targeting domain in some isoforms (Xlp2 binds centromeres)	AT1G03780	Os07g32390
GRMZM2G301194	Kinesin heavy chain	AT2G47500	Os11g44880
GRMZM2G176047	Kinsasin motor protein	AT1G72250, AT2G22610	Os03g02290, Os12g42160
GRMZM5G887077	Kinesin heavy chain	AT5G27000	Os11g44880
GRMZM2G033785	Centromeric protein E (motor protein)	AT1G59540	Os11g35090
GRMZM2G091543	Xklp2 like (MAP associated with centrosome separation)	AT5G15510	Os03g11400
GRMZM2G100473	Xlp2 targeting protein (Xlp2 binds centromeres)	AT5G15510	Os03g11400
GRMZM5G832989	Microtubule associated protein	AT1G68060	Os02g50320
GRMZM2G083475	Kinesin motor protein like	AT3G45850	Os05g02670
GRMZM2G040965	Actin binding formin-homology1 like (cytoskeletal rearrangements)	AT5G67470, AT3G25500 AT2G43800	Os09g34180
GRMZM2G141208	Microtubule associated protein	AT4G26760	Os05g33890
GRMZM2G471186	Kinesin like	AT3G23670	Os02g28850
GRMZM2G318849	Microtubule associated protein	AT2G07170	Os09g38710
GRMZM2G132486	Actin binding formin-homology1 like (cytoskeletal rearrangements)	AT5G67470	Os09g34180
GRMZM2G136219	Kinesin like		Os04g30720

Chromatin structure	6		
GRMZM2G164020	Histone H1	AT2G30620	
GRMZM5G866734	RCC	AT1G19880	Os04g56720
GRMZM2G060253	High motility group (non-histone chromatin component)	AT4G23800	Os02g15810
GRMZM2G081474	Histone deacetylase	AT4G38130	Os06g38470
GRMZM2G140799	NHP2-like protein 1 (Ribosomal family; functionally similar to HMG proteins)	AT5G20160	Os03g13800
GRMZM2G170289	Regulator of Chromatin Condensation1 and Znf-FYVE domains (thought to have a role in membrane trafficking)	AT1G65920	Os02g45980
Cell cycle	15		
GRMZM2G073003	Cyclin laZm	AT5G06150	Os01g59120
GRMZM2G047143	mitotic spindle checkpoint component	AT3G25980	Os04g40940
GRMZM2G009913	mitotic spindle checkpoint component mad3	AT2G33560	Os02g10020
GRMZM2G096184	chromosome condensation protein -like	AT5G37630	Os06g14460
GRMZM2G034647	Cyclin1	AT5G06150	Os05g41390
GRMZM5G821639	Fizzy related WD-repeat cell cycle regulatory protein	AT5G13840	Os03g03150,Os01g74146
GRMZM2G131525	syntaxin-related protein KNOLLE (role in cell plate formation during cytokinesis)	AT1G08560	Os03g52650
GRMZM2G073671	Cyclin B	AT1G20610	Os06g51110
GRMZM2G020974	Shugoshin-1 (centromeric cohesion in Pro - Met transition)		Os10g31930
GRMZM2G145879	CDK-activating kinase	AT1G73690	Os05g32600
GRMZM2G363437	Cyclin A2	AT1G47230	Os03g41100
GRMZM2G140633	D-type cyclin	AT5G65420, AT5G10440	Os09g29100
GRMZM2G079290	Phytosulfokines 2 (role in cell division)		Os12g05260
AC235534.1_FG001	replication factor C 40kDa subunit (AAA ATPase family)	AT1G63160	Os04g48060
GRMZM2G178215	Cyclin A like	AT1G47210, AT5G43080	Os03g11040,Os12g39210,Os03g41100
Cell wall related	4		
GRMZM2G168588	Peptidoglycan binding lysin motif, erwinia induced protein 1	AT2G17120	Os09g37600
GRMZM2G154301	Peptidoglycan binding lysin motif, erwinia induced protein 1	AT1G21880	Os06g10660
GRMZM2G004955	Expansin like family (cell wall extension)	AT3G45970	Os06g50960
GRMZM2G040517	Extensin like		Os05g45460
Heavy metal binding	3		

GRMZM2G049693	multicopper oxidase	AT4G12420	Os08g05820
GRMZM2G155281	Metal ion binding	AT5G50740	Os04g32030, Os02g30650
GRMZM2G113863	Copper chaperone (heavy metal transport)		Os07g47480
Carbohydrate metabolism	12		
GRMZM2G015886	Cellulose synthase	AT5G64740	Os08g06380
GRMZM2G009282	Glucosidase	AT3G45940	Os06g46340
GRMZM2G127309	Glucanase	AT1G09460	Os03g61780
GRMZM2G455642	endo-1,4-beta-glucanase	AT1G23210	Os02g50040
GRMZM2G076049	endo-1,4-beta-glucanase	AT1G70710, AT1G23210	Os06g14540
GRMZM5G824920	Glucan endo-1,3-beta-glucosidase 3	AT1G66250	Os03g12140
GRMZM2G097207	glucan endo-1,3-beta-glucosidase 6	AT4G31140	Os08g12800
GRMZM2G095725	beta-fructofuranosidase	AT3G52600, AT2G36190	Os02g33110, Os04g33740, Os04g33720
GRMZM2G081151	beta-amyrin synthase	AT2G07050, AT5G36150	Os11g35710, Os11g18194
GRMZM2G123714	glucan endo-1,3-beta-glucosidase	AT1G18650	Os03g54910
GRMZM2G162333	Pectinesterase	AT5G47500	Os04g46740
GRMZM2G071339	Pectinesterase	AT5G19730	Os01g53990
Other metabolic enzymes	18		
GRMZM2G120085	Serine protease	AT5G67360, AT2G04160	Os03g04950, Os09g26920
GRMZM2G052507	serine carboxypeptidase	AT1G28110	Os11g31980, Os04g32540
GRMZM5G879749	serine carboxypeptidase	AT3G02110, AT4G30610	Os02g55130
GRMZM2G176270	Zf-DHHC protein (palmitoyltransferase - anchors proteins in membranes)	AT4G01730	Os12g16210
GRMZM5G870067	cytokinin-O-glucosyltransferase 2	AT1G22360	Os02g36830
GRMZM2G090441	Glycosyl hydrolase/chitinase	AT4G19810	Os04g30770
GRMZM2G431504	Leucoanthocyanidin reductase	AT1G61720	Os04g53920
GRMZM2G143625	stearoyl-ACP desaturase	AT3G02610	Os04g31070
AC209819.3_FG005	Methyltransferase	AT4G35160	Os12g25450
GRMZM2G013448	Ethylene synthesis (ACC oxidase, catalyses ACC to ethylene)	AT2G19590	Os01g39860
GRMZM2G084547	peptidyl-prolyl cis-trans isomerase 1	AT2G18040	Os04g56800
GRMZM2G064701	Omega-6 fatty acid desaturase	AT3G12120	Os07g23410
GRMZM2G114140	Glycosyl hydrolase	AT5G55180	Os02g53200, Os07g35480
GRMZM2G132154	Serine carboxypeptidase	AT1G43780	Os11g10750

GRMZM2G091916	SET domain (methyltransferases, often of histones)	AT1G26760	Os03g07260
GRMZM2G063909	Citrate synthase 4	AT2G44350	Os11g33240
GRMZM2G108032	Glycosyl hydrolase family 10 protein	AT4G38650	Os03g14010
GRMZM2G122172	aldehyde dehydrogenase	AT3G48000	Os01g40860
Nucleotide metabolism	3		
GRMZM2G144504	Nucleotide exchange factor	AT5G05940	Os01g62990
GRMZM2G155546	Ribonucleotide reductase (formation of deoxyribonucleotides from ribonucleotides)	AT3G27060	Os06g03720
GRMZM2G038032	Guanine nucleotide-binding protein beta subunit-like protein	AT1G18080	Os05g47890
Transporters and ion channels	4		
GRMZM2G146670	Voltage dependent anion channel (possibly mitochondrial)	AT3G01280	Os09g19734
GRMZM2G115635	Uracil permease (membrane transport protein)	AT2G34190	Os01g55500
GRMZM2G470075	MATE family efflux protein (transparent testa like)	AT3G21690, AT1G61890	Os03g37490, Os03g37640
GRMZM2G303244	Mechanosensitive ion channel	AT1G53470	Os04g47320
Misc	8		
GRMZM2G042604	Proteasome subunit	AT5G66140	Os09g36710
GRMZM2G061314	LRR protein binding protein	AT1G78230	Os03g09070
GRMZM2G465333	Nascent polypeptide-associated complex subunit	AT3G12390	Os05g31000
GRMZM2G094639	Lipid transfer domain, Cortical cell delineating protein	AT4G12520	Os04g46810
GRMZM2G112795	Wound inducible protein (very short)	AT3G07230	
GRMZM2G014499	Xylem serine proteinase 1 precursor	AT5G51750	Os09g36110
GRMZM2G168807	Major ampullate spidroin	AT2G33510	Os02g31140
GRMZM2G164090	Gibberellin regulated protein 2	AT1G75750	Os05g35690
Misc domains	35		
GRMZM2G153488	Defensin precursor		Os02g41904
GRMZM2G112210	Dirigent-like protein		Os01g24960
GRMZM2G077227	IQ domain protein	AT2G26180	Os01g09790
GRMZM2G138840	WD repeat domain	AT2G38630	Os01g60200
GRMZM2G000608	Armadillo repeat superfamily	AT1G54385	Os11g37100
GRMZM2G050268	Tetratricopeptide repeat protein (similar to male sterility5 family protein)	AT3G51280	Os09g36740
GRMZM2G416677	12.5% similarity to Medtr2g080180.1: Legume lectin, beta domain Replication factor-A	AT1G23790	Os01g01890

GRMZM2G000825	Tetratricopeptide repeat protein (similar to male sterility5 family protein)	AT3G51280	Os09g36740
GRMZM2G115499	19.0% similarity to Medtr2g080180.1: Legume lectin, beta domain Replication factor-A	AT4G13370	Os12g02710
GRMZM2G109159	Reticulon domain (unknown function, associates with ER)	AT2G20590	Os02g19990
GRMZM2G477139	Thaumatococcus protein	AT4G36010	Os10g05660
GRMZM2G376282	GO: Biological process: cytokinesis by cell plate formation(RCA), microtubule cytoskeleton organization(RCA), pollen tube growth(RCA); Molecular function: structural constituent of cell wall(RCA)		Os03g07810
GRMZM2G000254	Query vinculin family domain (PM actin microfilament attachment)		Os05g25620
GRMZM2G078164	DVL domain - suggested role in plant development	AT2G39705	Os03g16600
GRMZM2G465208	nuclear localisation signal domains; weakly similar to a homeodomain TF 51.8% similarity to LOC_Os04g44640.1: ulp1 protease family, C-terminal catalytic dom	AT5G37010	Os04g44640
GRMZM2G129540	Transposon protein (also annotated as an asparaginase)	AT5G08100	Os03g40070
GRMZM2G040359	anther-specific proline-rich protein APG	AT5G41890	Os04g48800
GRMZM2G070553	Kelch domain protein (often associate with actin)	AT5G18590	Os11g43590
GRMZM2G074377	Similar to putative bZIP (DUF630, DUF632)	AT1G52320	Os05g32760
GRMZM2G464011	signal-peptide; transmembrane_regions		
GRMZM2G162020	IQ domain protein	AT4G00820	Os08g02250
GRMZM2G002147	Armadillo repeat superfamily	AT4G15830	Os01g70560
GRMZM2G114113	calreticulin superfamily 25.5% similarity to Sb10g008510.1: weakly similar to Putative pollen-specific LIM dom; 15.9% similarity to LOC_Os06g13030.1: OsLIM - LIM domain protein, putative actin-bin	AT5G17160	Os02g47130
GRMZM2G324467	FH2 superfamily 40.3% similarity to POPTR_0009s05710.1: similar to myosin-related; similar to low simi; 34.7% similarity to Medtr4g104600.1: Gonadotropin, beta chain; 34.5% similarity to AT1G51405.1: myosin-related	AT1G51405	Os03g11770
GRMZM2G051738	Similar to DNA binding AT hook protein	AT4G12080	Os11g05160
GRMZM2G012224	62.6% similarity to Medtr1g013340.1: Galactose-binding like	AT3G08030	Os03g59300
GRMZM2G476523	thaumatin-like protein	AT1G73620	Os06g47600
GRMZM2G127656	Pfam:10497 Zinc-finger domain of monoamine-oxidase A repressor R1; 39.2% similarity to Sb05g021980.1: similar to AT hook motif family protein, expressed; 23.3% similarity to 30205.m001596: ubiquitin-protein ligase, putative		Os03g50420
GRMZM2G160466	Proline rich extensin domain - no BLAST function		Os03g20350
GRMZM2G124744	Pyrophosphatase domain		
GRMZM2G008764	IQ domain protein	AT2G26180	Os01g51230

GRMZM2G459645	G1 (long sterile lemma1)/Light sensitive hypocotyls like, DUF640	AT1G07090	Os04g43580
GRMZM2G173280	Remorin, C terminal region (PM protein)	AT1G30320	Os12g41940
GRMZM2G146809	Defensin precursor	AT2G02120	Os02g41904
GRMZM2G170969	Protease inhibitor/seed storage/LTP family protein		Os01g68580
Other	41		
GRMZM2G017536	Panther:21726 PHOSPHATIDYLINOSITOL N-ACETYLGLUCOSAMINYLTRANSFERASE SUBUNIT P (DOWN SYNDROME CRITICAL REGION PROTEIN 5)-RELATED	AT3G58650	Os03g19080
GRMZM2G081521	Unknown 345aa predicted to be chloroplast localization, middle reliability	AT5G13100	Os01g01295
GRMZM2G107737	GO. Biological process: chromatin silencing(RCA), histone H3K9 methylation(RCA), regulation of DNA replication(RCA), regulation of G2/M transition of mitotic cell cycle(RCA); Cellular component: plasma membrane(IDA)	AT5G48310	Os02g10490,Os02g52280
GRMZM2G115825	Panther:21726 PHOSPHATIDYLINOSITOL N-ACETYLGLUCOSAMINYLTRANSFERASE SUBUNIT P (DOWN SYNDROME CRITICAL REGION PROTEIN 5)-RELATED	AT3G58650	Os03g19080
GRMZM2G067298	16.3% similarity to AT5G66230.1: Chalcone-flavanone isomerase family protein; 28.6% similarity to Medtr8g090020.1: Sugar transporter superfamily	AT5G66230	Os10g42660
GRMZM2G150408	GO. Cellular component: chromatin(IEA), nucleus(IEA); Molecular function: nucleosomal DNA binding(IEA)		Os03g04550
GRMZM2G057690	37.9% similarity to AT5G66230.1: Chalcone-flavanone isomerase family protein; 54.4% similarity to Medtr8g090020.1: Sugar transporter superfamily	AT5G66230, AT3G51230	Os10g42660
GRMZM2G041310	56.9% similarity to Sb04g004570.1: similar to Dentin sialophosphoprotein-like		Os02g07180
GRMZM2G029314	69.8% similarity to Bradi1g07270.1: protein kinase activity (Blast2GO)		Os03g56070
GRMZM2G023051	17.5% similarity to AT1G58220.1: Homeodomain-like superfamily protein		Os06g46990
GRMZM2G120440	GO. Biological process: histone phosphorylation(RCA), cell proliferation(RCA), DNA dependent DNA replication initiation(RCA), regulation of cell cycle(RCA), regulation of DNA replication(RCA)		Os02g47130
GRMZM2G177596	GO. Biological process: cytokinesis by cell plate formation(RCA), histone phosphorylation(RCA), microtubule cytoskeleton organization(RCA), regulation of cell proliferation(RCA)	AT4G02800	Os04g15800
GRMZM2G172758	GO. Biological process: Molecular		Os03g04550
GRMZM2G061655	Alpha-crystallin B chain		Os12g39100

GRMZM2G142609	GO. Biological process: cytokinesis by cell plate formation(RCA), microtubule cytoskeleton organization(RCA)	AT3G52110	Os03g07810
GRMZM2G050741	67.0% similarity to Bradi1g07270.1: protein kinase activity (Blast2GO)	AT4G17000	Os03g56070
GRMZM5G896728	Unknown 183aa No localization prediction	AT5G49170	Os05g29080
GRMZM2G052412	GO. Molecular function: calcium ion binding(IEA)		
GRMZM2G397927	GO. Biological process: cellular membrane fusion(RCA), endoplasmic reticulum unfolded protein(RCA), heat acclimation(RCA), negative regulation of programmed cell death(RCA), protein targeting to membrane(RCA), regulation of planttype hypersensitive response(RCA), cellular response to retinoic acid(IEP), negative regulation of transcription from RNA polymerase II promoter(IMP), negative regulation of transcription, DNA dependent(IMP), pronephros development(IMP); Cellular component: intracellular(IEA); Molecular function: nucleic acid binding(IEA), zinc ion binding(IEA)	AT5G57123	Os06g44980
GRMZM2G146143	mucin 13, cell surface associated	AT1G16520	Os03g45760
GRMZM2G139324	GO. Biological process: response to brassinosteroid stimulus(RCA)		Os12g39100
GRMZM2G019280	Unknown (very short) 54aa predicted to be mitochondrion localization, middle reliability		
GRMZM2G155340	X-RAY INDUCED TRANSCRIPT 1	AT1G14630	Os08g36380
GRMZM2G026558	49.8% similarity to LOC_Os04g44640.1: ulp1 protease family, C-terminal catalytic domain containing protein, expressed	AT5G37010	Os04g44640
GRMZM2G088543	signal-peptide; transmembrane_regions		Os02g33790
GRMZM2G077219	28.7% similarity to Medtr3g101900.1: Zinc finger, C2H2-type; 35.7% similarity to AT2G30370.1: allergen-related	AT3G13898	Os02g51950
GRMZM2G062424	Unknown 222aa predicted to be mitochondrion localization, but with low reliability	AT1G14680	Os02g58470
GRMZM2G150859	signal-peptide; transmembrane_regions	AT1G22030	Os01g65420
GRMZM2G024927	Unknown 153aa No localization prediction		
GRMZM2G114893	52.4% similarity to LOC_Os10g40584.1: zinc finger family protein, putative, expressed; 24.4% similarity to Sb05g004260.1: similar to Zinc finger, C2H2 type, putative	AT1G62520	Os10g40584
GRMZM2G089517	43.8% similarity to AT3G57860.1: UV-B-insensitive 4-like	AT3G57860	Os02g37850
GRMZM2G070515	36.5% similarity to 29917.m001997: Microtubule-associated protein futsch, putative; 27.9% similarity to Bradi3g02910.1: protein serine/threonine kinase activity (Blast2GO)		Os09g32540
GRMZM2G109480	Pfam:02362 B3 DNA binding domain; GO:0006355 regulation of transcription, DNA-dependent; GO:0003677 DNA binding	AT5G42700, AT3G19184	Os03g08620

GRMZM2G300788	76.6% similarity to Bradi4g01260.1: zinc ion binding (Blast2GO); Panther:12972 DOWNSTREAM NEIGHBOR OF SON	AT3G54750	Os12g42700
GRMZM5G849149	vacuolar protein sorting 72 homolog; putative glycoside hydrolase; Mucin related 29B	AT1G16520	Os03g45760
GRMZM2G496319	transmembrane_regions		
GRMZM2G466394	72.7% similarity to LOC_Os03g40780.1: transport protein-related, putative, expressed	AT1G49870	Os03g40780
GRMZM2G527028	Unknown 99aa predicted to be mitochondrion localization, middle reliability		
GRMZM2G300786	25.0% similarity to Bradi1g13530.1: microtubule plus-end binding (Blast2GO); 23.7% similarity to LOC_Os03g44760.5: SWITCH1, putative, expressed		Os12g42830
GRMZM5G862623	Unknown 99aa predicted to be mitochondrion localization, but with VERY low reliability		
GRMZM2G011010	Unknown 252aa predicted to be mitochondrion localization, but with VERY low reliability		

Table 4: genes of particular interest		
GRMZM2G123900		GRMZM2G399072
GRMZM2G471089		GRMZM2G318592
GRMZM2G132794		GRMZM2G097275
GRMZM2G163975		GRMZM2G095899
GRMZM2G151542		GRMZM2G462623
GRMZM2G039074		GRMZM2G015666
GRMZM2G178182		GRMZM2G126018
GRMZM2G045883		GRMZM5G850129
GRMZM2G140669		GRMZM2G312419
GRMZM2G472945		GRMZM2G061734
GRMZM2G178102		GRMZM2G082586
GRMZM2G131516		GRMZM2G011463
GRMZM2G469304		GRMZM2G480386
GRMZM2G172657		GRMZM2G089819
GRMZM2G374986		GRMZM2G016477
GRMZM2G002280		GRMZM2G159953
GRMZM2G119359		GRMZM2G163724
GRMZM5G893117		GRMZM2G151955
GRMZM2G150011		GRMZM2G039934
GRMZM2G136494		GRMZM2G046316
GRMZM2G028046		GRMZM2G034155
GRMZM2G098988		GRMZM2G114276
GRMZM2G377217		GRMZM2G028643
GRMZM2G148467		GRMZM2G059117
GRMZM2G021573		GRMZM2G478876
GRMZM2G040924		GRMZM2G344857
GRMZM2G171365		GRMZM2G087243
GRMZM2G146688		GRMZM2G133716
GRMZM2G425236		GRMZM2G061314
GRMZM2G417229		GRMZM2G112210
GRMZM2G069365		GRMZM2G023051
GRMZM5G887276		GRMZM2G139324
GRMZM2G098813		GRMZM2G077219
GRMZM2G111045		GRMZM2G114893
AC215201.3_FG008		GRMZM2G109480
GRMZM2G027068		
GRMZM2G081816		

GRMZM2G052102
GRMZM2G114998
GRMZM2G171852
GRMZM2G140694
GRMZM2G074032
GRMZM2G129261
GRMZM2G143723
GRMZM2G134998

CLAIMS

1. A transgenic plant which over-expresses one or more maize genes (or their orthologues in any other plant species) selected from the list consisting of:
 - 5 GRMZM2G016477, GRMZM2G027068, GRMZM2G045883, GRMZM2G081816, GRMZM2G052102, GRMZM2G114998, GRMZM2G171852, GRMZM2G140694, GRMZM2G028046, GRMZM2G136494, GRMZM2G074032, GRMZM2G129261, GRMZM2G143723, GRMZM2G134998, GRMZM2G150011, GRMZM2G132794, GRMZM2G172657, GRMZM2G123900, GRMZM2G471089, GRMZM2G132794,
 - 10 GRMZM2G163975, GRMZM2G151542, GRMZM2G039074, GRMZM2G178182, GRMZM2G045883, GRMZM2G140669, GRMZM2G472945, GRMZM2G178102, GRMZM2G131516, GRMZM2G469304, GRMZM2G172657, GRMZM2G374986, GRMZM2G002280, GRMZM2G119359, GRMZM5G893117, GRMZM2G150011, GRMZM2G136494, GRMZM2G028046, GRMZM2G098988, GRMZM2G377217,
 - 15 GRMZM2G148467, GRMZM2G021573, GRMZM2G040924, GRMZM2G171365, GRMZM2G146688, GRMZM2G425236, GRMZM2G417229, GRMZM2G069365, GRMZM5G887276, GRMZM2G098813, GRMZM2G111045, AC215201.3_FG008, GRMZM2G399072, GRMZM2G318592, GRMZM2G097275, GRMZM2G095899, GRMZM2G462623, GRMZM2G015666, GRMZM2G126018, GRMZM5G850129,
 - 20 GRMZM2G312419, GRMZM2G061734, GRMZM2G082586, GRMZM2G011463, GRMZM2G480386, GRMZM2G089819, GRMZM2G159953, GRMZM2G163724, GRMZM2G151955, GRMZM2G039934, GRMZM2G046316, GRMZM2G034155, GRMZM2G114276, GRMZM2G028643, GRMZM2G059117, GRMZM2G478876, GRMZM2G344857, GRMZM2G087243, GRMZM2G133716, GRMZM2G061314,
 - 25 GRMZM2G112210, GRMZM2G023051, GRMZM2G139324, GRMZM2G077219, GRMZM2G114893, and GRMZM2G109480.
2. The transgenic plant of claim 1, which is a C₃ plant.
- 30 3. The transgenic plant of claim 2, which is a cereal crop such as rice, wheat, oat, or barley; or a biofuel crop.

4. The transgenic plant of any one of the preceding claims, wherein the transgenic plant comprises at least one leaf which comprises one or more ectopic veins or increased vascular density compared to a wild-type plant.

5. The transgenic plant of any one of the preceding claims, wherein the vein spacing or number within one or more leaves of the transgenic plant is altered with respect to a wild-type plant.

6. The transgenic plant of claim 5, wherein the altered vein spacing comprises any one or more of the following:

- (i) uneven vein spacing with respect to said wild-type plant;
- (ii) veins closer together with respect to said wild-type plant; and/or
- (iii) veins separated by fewer or smaller cells than the veins of said wild-type plant.

7. The transgenic plant of any one of the preceding claims, wherein the transgenic plant has a phenotype which is indicative of Kranz anatomy.

8. The transgenic plant of any one of the preceding claims, wherein the transgenic plant of the invention over-expresses LRR repeat kinase (GRMZM2G016477) or its orthologue in any other plant species; optionally in combination with one or more of the other genes recited in claim 1.

9. An isolated maize gene (or its orthologue in any other plant species) selected from the list consisting of: GRMZM2G016477, GRMZM2G027068, GRMZM2G045883, GRMZM2G081816, GRMZM2G052102, GRMZM2G114998, GRMZM2G171852, GRMZM2G140694, GRMZM2G028046, GRMZM2G136494, GRMZM2G074032, GRMZM2G129261, GRMZM2G143723, GRMZM2G134998, GRMZM2G150011, GRMZM2G132794, GRMZM2G172657, GRMZM2G123900, GRMZM2G471089, GRMZM2G132794, GRMZM2G163975, GRMZM2G151542, GRMZM2G039074, GRMZM2G178182, GRMZM2G045883, GRMZM2G140669, GRMZM2G472945, GRMZM2G178102, GRMZM2G131516, GRMZM2G469304, GRMZM2G172657, GRMZM2G374986, GRMZM2G002280, GRMZM2G119359,

GRMZM5G893117, GRMZM2G150011, GRMZM2G136494, GRMZM2G028046,
 GRMZM2G098988, GRMZM2G377217, GRMZM2G148467, GRMZM2G021573,
 GRMZM2G040924, GRMZM2G171365, GRMZM2G146688, GRMZM2G425236,
 GRMZM2G417229, GRMZM2G069365, GRMZM5G887276, GRMZM2G098813,
 5 GRMZM2G111045, AC215201.3_FG008, GRMZM2G399072, GRMZM2G318592,
 GRMZM2G097275, GRMZM2G095899, GRMZM2G462623, GRMZM2G015666,
 GRMZM2G126018, GRMZM5G850129, GRMZM2G312419, GRMZM2G061734,
 GRMZM2G082586, GRMZM2G011463, GRMZM2G480386, GRMZM2G089819,
 GRMZM2G159953, GRMZM2G163724, GRMZM2G151955, GRMZM2G039934,
 10 GRMZM2G046316, GRMZM2G034155, GRMZM2G114276, GRMZM2G028643,
 GRMZM2G059117, GRMZM2G478876, GRMZM2G344857, GRMZM2G087243,
 GRMZM2G133716, GRMZM2G061314, GRMZM2G112210, GRMZM2G023051,
 GRMZM2G139324, GRMZM2G077219, GRMZM2G114893, and
 GRMZM2G109480.

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10. A vector comprising one or more of the isolated genes of claim 9.

11. A method of making a transgenic plant, the method comprising introducing

- 20 (i) one or more of the genes of claim 9; or
 (ii) the vector of claim 10;
 into a plant such that the gene will be over-expressed.

12. The method of claim 11, wherein the transgenic plant is a C₃ plant.

25 13. The method of claim 12, wherein the transgenic plant is a cereal crop such as
 rice, wheat, oat, or barley; or a biofuel crop.

14. The method of any one of claims 11 to 13, wherein the gene to be introduced
 is maize LRR repeat kinase (GRMZM2G016477) or its orthologue in any other plant
 30 species; optionally in combination with one or more of the other genes recited in
 claim 9.

15. A transgenic plant seed comprising one or more of the maize genes (or their orthologues in any other plant species) selected from the list consisting of:

GRMZM2G016477, GRMZM2G027068, GRMZM2G045883, GRMZM2G081816,
 GRMZM2G052102, GRMZM2G114998, GRMZM2G171852, GRMZM2G140694,
 5 GRMZM2G028046, GRMZM2G136494, GRMZM2G074032, GRMZM2G129261,
 GRMZM2G143723, GRMZM2G134998, GRMZM2G150011, GRMZM2G132794,
 GRMZM2G172657, GRMZM2G123900, GRMZM2G471089, GRMZM2G132794,
 GRMZM2G163975, GRMZM2G151542, GRMZM2G039074, GRMZM2G178182,
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 15 GRMZM5G887276, GRMZM2G098813, GRMZM2G111045, AC215201.3_FG008,
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 GRMZM2G112210, GRMZM2G023051, GRMZM2G139324, GRMZM2G077219,
 GRMZM2G114893, and GRMZM2G109480.

25

16. The transgenic seed of claim 15, wherein the transgenic seed is from a C₃ plant.

17. The transgenic seed of claim 16, wherein the transgenic seed is from a cereal
 30 crop such as rice, wheat, oat, or barley; or a biofuel crop.

18. The transgenic seed of any one of claims 15 to 17, wherein the gene is maize LRR repeat kinase (GRMZM2G016477) or its orthologue in any other plant species; optionally in combination with one or more of the other genes recited in claim 15.

5 19. The transgenic seed of any one of claims 15 to 18, wherein the transgenic plant seed will be capable of producing a transgenic plant which will over-express said one or more genes.

10 20. A shoot, root, cutting or seedling from the transgenic plant of any one of claims 1 to 8 or the seed of any one of claims 15 to 19.

21. The transgenic plant of any one of claims 1 to 8, or the isolated gene of claim 9, or the vector of claim 10, or the seed of any one of claims 15 to 19, or the shoot, root cutting or seedling of claim 20, wherein the orthologue is Arabidopsis.

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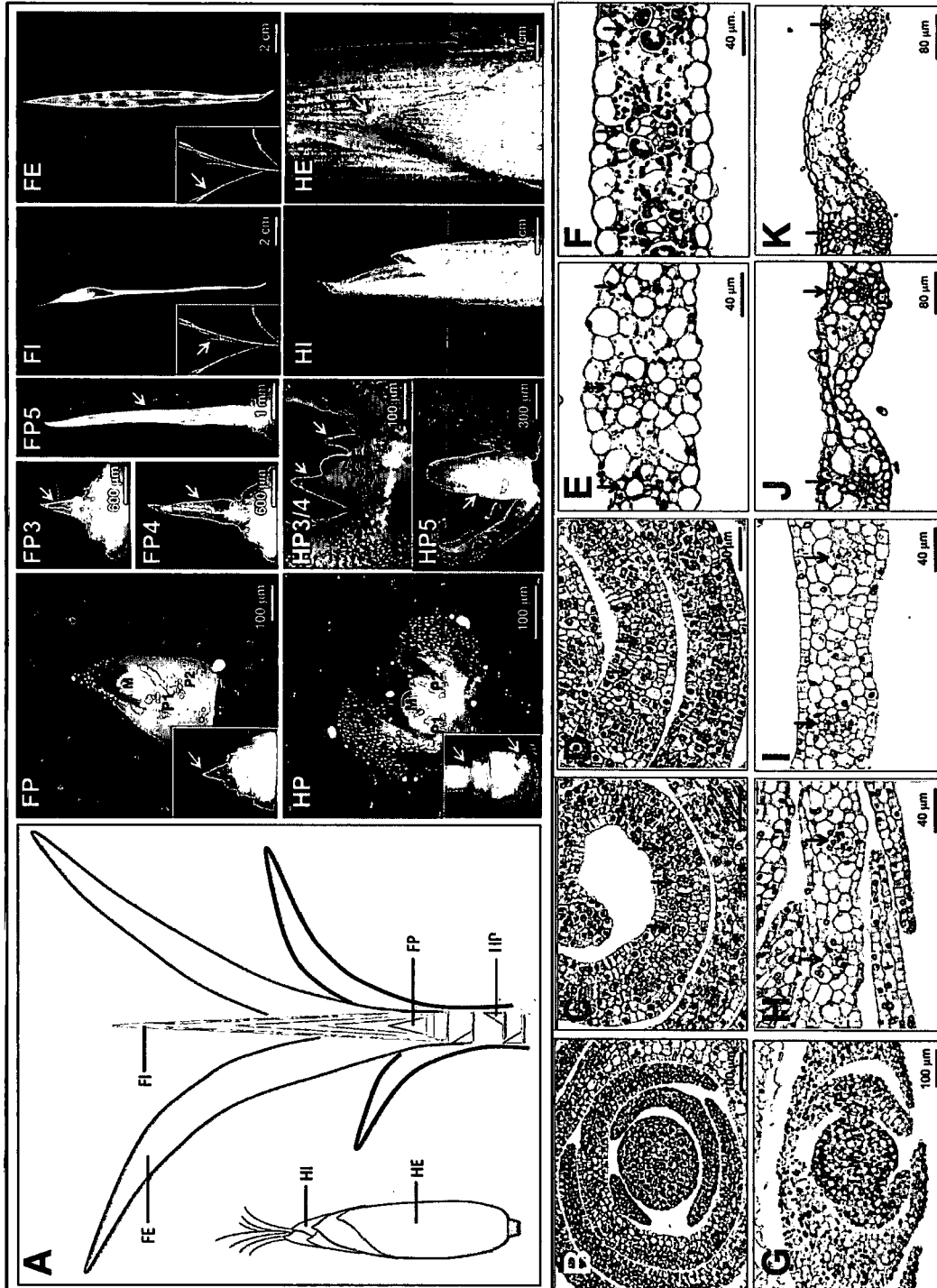
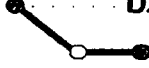
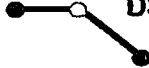
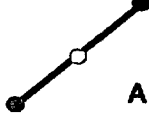
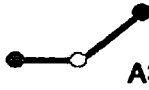
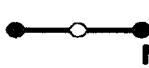


FIGURE 1

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Figure 2

	No. of genes/ TFs in F cluster	TF groupings	No. of genes/ TFs in H cluster	TF groupings
 D1	56/12	2 FD1 only 6 FD1/HD1 4 FD1/HD2	13/7	6 HD1/FD1 1 HD1/FD3
 D2	178/20	12 FD2 only 8 FD2/HD2	175/28	12 HD2 only 4 HD2/FD1 8 HD2/FD2 4 HD2/FD3
 D3	413/50	42 FD3 only 1 FD3/HD1 4 FD3/HD2 3 FD3/HD3	157/22	18 HD3 only 3 HD3/FD3 1 HD3/FA3
 A1	171/25	13 FA1 only 1 FA1/HA1 8 FA1/HA2 3 FA1/HA3	60/4	1 HA1/FA1 3 HA1/FA3
 A2	463/59	37 FA2 only 21 FA2/HA2 1 FA2/HA3	573/64	31 HA2 only 8 HA2/FA1 21 HA2/FA2 4 HA2/FA3
 A3	580/59	41 FA3 only 3 FA3/HA1 4 FA3/HA2 10 FA3/HA3 1 FA3/HD3	469/33	19 HA3 only 3 HA3/FA1 1 HA3/FA2 10 HA3/FA3
 N	26160/ 1263	Not determined	28467/ 1457	Not determined

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Figure 3

FA2		
GRMZM2G027068 ^{BS}	bHLH family	AtTMO5 clade
GRMZM2G045883 ^{BS}	bHLH family	Subfamily Ia (SPCH-like)
GRMZM2G081816	bHLH family	Subfamily VIIIb
GRMZM2G052102	bZIP family	bZIP family
GRMZM2G114998 ^{BS}	C2C2-Dof family	AtDAG1 clade
GRMZM2G171852 ^{BS}	C2C2-Dof family	AtDAG1 clade
GRMZM2G140694	C2C2-Dof family	AtHCA2 clade
GRMZM2G028046	C2H2 family	ZmMRPI clade
GRMZM2G136494	C2H2 family	ZmMRPI clade
GRMZM2G074032	C2H2 family	AtSGR5 clade
GRMZM2G129261 ^M	C2H2 family	AtJKD clade
GRMZM2G143723 ^{BS}	C2H2 family	AtJKD clade
GRMZM2G134998	C2H2 family	AtDOT5 clade
GRMZM2G150011	C2H2 family	AtDOT5 clade
GRMZM2G132794 ^{BS}	GRAS family	AtSHR clade
GRMZM2G172657	GRAS family	AtSHR clade

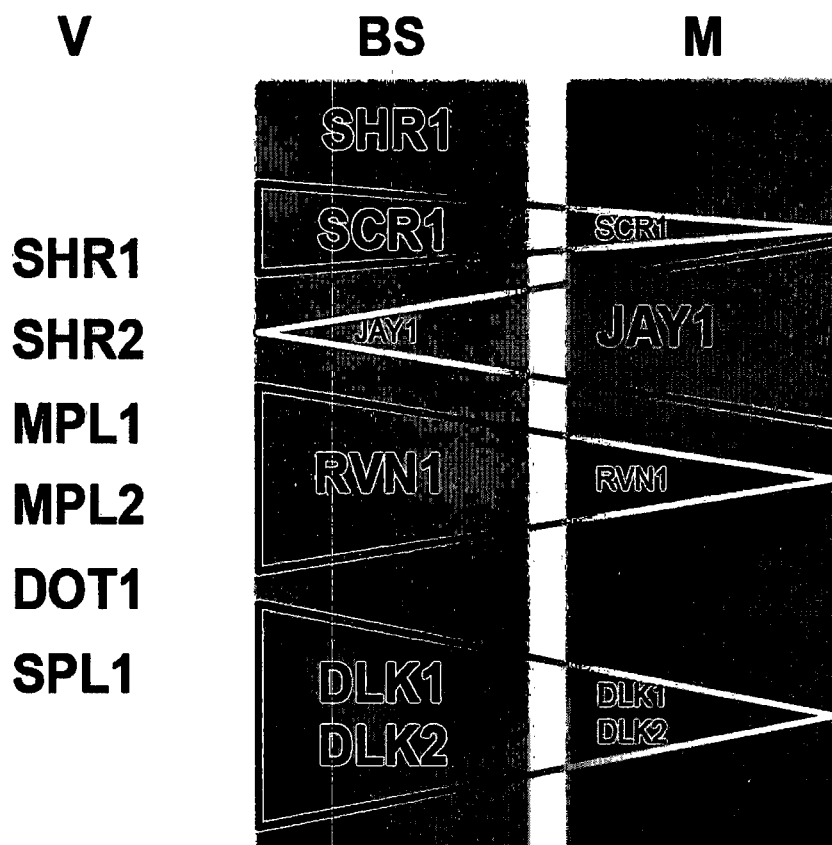
Figure 4

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a

Accession	Gene family	Gene subfamily	Gene name	F profile	H profile	positive regulator list	Ratio BS:M	Ratio FP3/4 : HP3/4
GRMZM2G045883	bHLH	SPCH clade	<i>ZmSpch-like 1 (ZmSpl1)</i>	FA2	HN	Yes	0	3:1
GRMZM2G132794	GRAS	SHR clade	<i>ZmShr1</i>	FA2	HN	Yes	10:0	4:1
GRMZM2G172657	GRAS	SHR clade	<i>ZmShr2</i>	FA2	HN	Yes	0	3:1
GRMZM2G028046	C2H2	MRPI clade	<i>ZmMrpi-like1 (ZmMpl1)</i>	FA2	HN	Yes	0	3:1
GRMZM2G136494	C2H2	MRPI clade	<i>ZmMpl2</i>	FA2	HN	Yes	0	4:1
GRMZM2G150011	C2H2	DOT5 clade	<i>ZmDot1</i>	FA2	HN	Yes	0	10:1
GRMZM2G114998	C2C2-Dof	DAG1 clade	<i>ZmDag-like 1 (ZmDlk1)</i>	FA2	HN	No	10:1	2:1
GRMZM2G171852	C2C2-Dof	DAG1 clade	<i>ZmDlk2</i>	FA2	HN	No	15:1	2:1
GRMZM2G129261	C2H2	ID1 clade	<i>ZmJay1</i>	FA2	HN	No	1:6	2:1
GRMZM2G143723	C2H2	ID1 clade	<i>ZmRaven1 (ZmRvn1)</i>	FA2	HN	No	4:1	5:1
GRMZM2G131516	GRAS	SCR	<i>ZmScr1</i>	FN	HN	Yes	2:1	2:1

b



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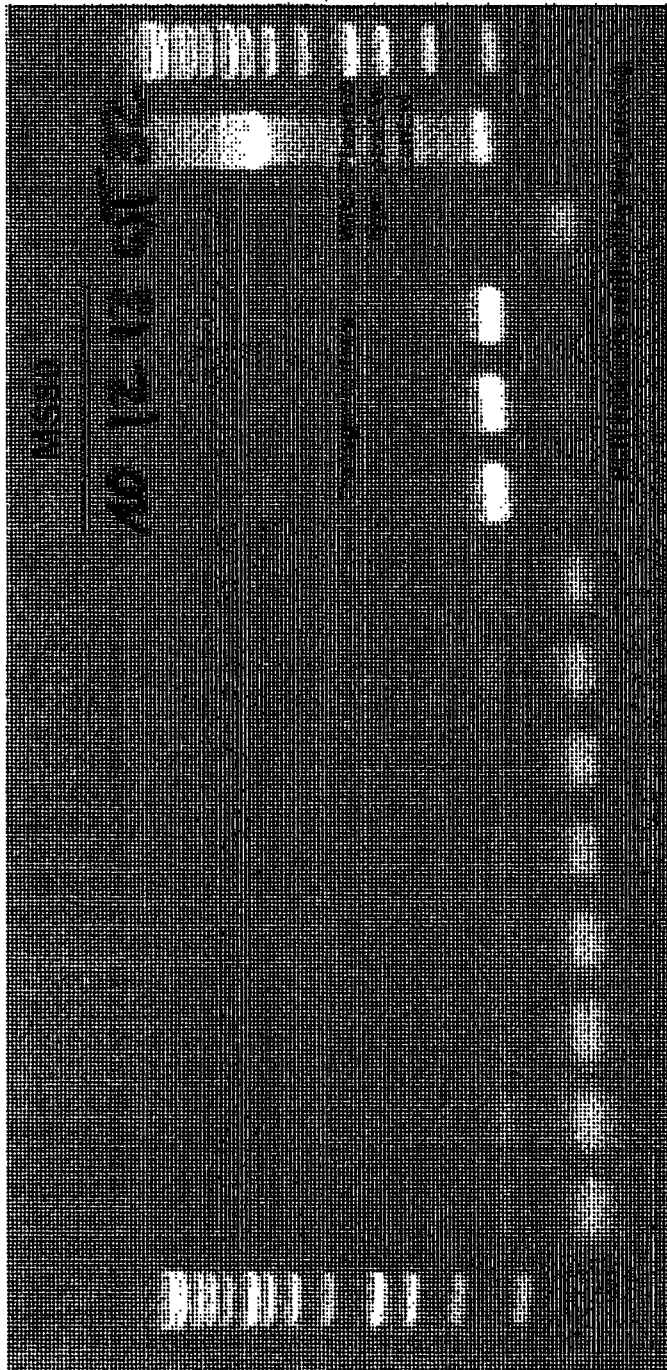


FIGURE 5

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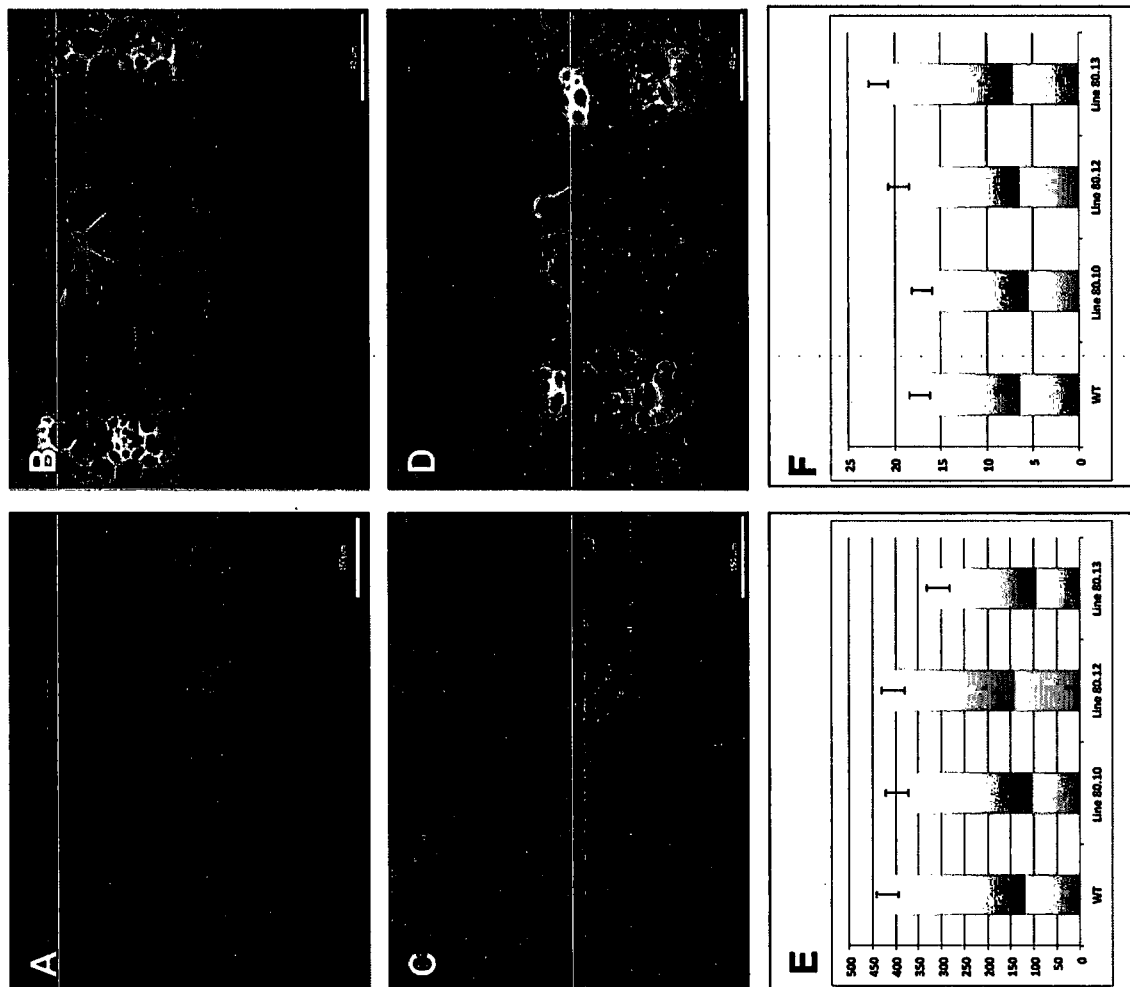


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/053312

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C12N9/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/039067 A1 (FELDMANN KENNETH A [US] ET AL) 15 February 2007 (2007-02-15) sequence 29965 claims 1-15	1-21
X	----- DATABASE EMBL [Online] 27 July 2006 (2006-07-27), "Arabidopsis thaliana mRNA for putative receptor-like protein kinase, complete cds, clone: RAFL07-93-A13.", XP002721372, retrieved from EBI accession no. EM STD:AK226809 Database accession no. AK226809 sequence ----- -/--	9,10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 March 2014

Date of mailing of the international search report

03/06/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Authorized officer

Blanco Urgoiti, B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2013/053312

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

8, 14, 18(completely); 1-7, 9-13, 15-17, 19-21(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 8, 14, 18(completely); 1-7, 9-13, 15-17, 19-21(partially)

Transgenic plant that overexpresses maize gene GRMZM2G016477 or an ortologue thereof; isolated maize gene or ortologue thereof; vectors, methods for making the transgenic plant thereof; transgenic plant seed , shoot, root cutting and seedling thereof.

- 2-86. claims: 1-7, 9-13, 15-17, 19-21(all partially)

Invention 2 being idem to invention 1, but related to GRMZM2G027068; and invention 86 being idem to invention 1, but related to GRMZM2G109480

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2013/053312

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	PENG WANG ET AL: "Genome-wide transcript analysis of early maize leaf development reveals gene cohorts associated with the differentiation of C 4 Kranz anatomy", THE PLANT JOURNAL, vol. 75, no. 4, 8 August 2013 (2013-08-08), pages 656-670, XP055106111, ISSN: 0960-7412, DOI: 10.1111/tpj.12229 the whole document	1-21
A	----- PINGHUA LI ET AL: "The developmental dynamics of the maize leaf transcriptome", NATURE GENETICS, vol. 42, no. 12, 31 October 2010 (2010-10-31), pages 1060-1067, XP055105793, ISSN: 1061-4036, DOI: 10.1038/ng.703 cited in the application the whole document	1-21
A	----- HIBBERD JULIAN M ET AL: "The regulation of gene expression required for C4 photosynthesis.", ANNUAL REVIEW OF PLANT BIOLOGY 2010, vol. 61, 2010, pages 181-207, XP002721373, ISSN: 1545-2123 the whole document	1-21
A	----- T. L. SLEWINSKI ET AL: "Scarecrow Plays a Role in Establishing Kranz Anatomy in Maize Leaves", PLANT AND CELL PHYSIOLOGY, vol. 53, no. 12, 1 December 2012 (2012-12-01), pages 2030-2037, XP055106118, ISSN: 0032-0781, DOI: 10.1093/pcp/pcs147 the whole document	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2013/053312

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007039067 A1	15-02-2007	US 2007039067 A1	15-02-2007
		US 2010083407 A1	01-04-2010
