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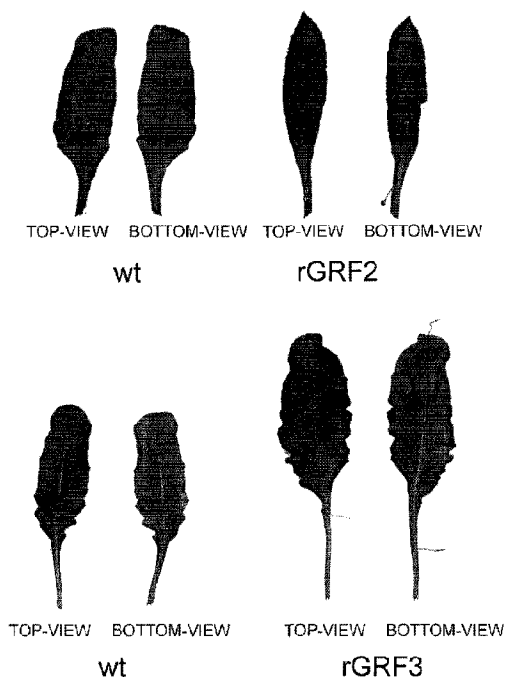
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(54) Title: GRF3 MUTANTS, METHODS AND PLANTS



(57) **Abrégé/Abstract:**

The present disclosure provides a novel modified gene, rGRF3, or an orthologue thereof, which is shown to be decoupled from control by miR396, particularly in the presence of over-expression of at least one GIF gene, such as GIF1, AtGIF 2, AtGIF 3, Os11g40100, Os12g31350, Os03g52320 or combinations thereof. When present in a plant, the rGRF3 results in a phenotype of increased productivity (e.g. increased yield, increased biomass, increased stress resistance, increased seed production, increased seed yield, increased root growth, increased root elongation speed, delayed leaf senescence or increased drought tolerance and combinations thereof).

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(54) **Title:** GRF3 MUTANTS, METHODS AND PLANTS

(57) **Abstract:** The present disclosure provides a novel modified gene, rGRF3, or an orthologue thereof, which is shown to be decoupled from control by miR396, particularly in the presence of over-expression of at least one GIF gene, such as GIF1, AtGIF 2, AtGIF 3, Os11g40100, Os12g31350, Os03g52320 or combinations thereof. When present in a plant, the rGRF3 results in a phenotype of increased productivity (e.g. increased yield, increased biomass, increased stress resistance, increased seed production, increased seed yield, increased root growth, increased root elongation speed, delayed leaf senescence or increased drought tolerance and combinations thereof).



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GRF3 MUTANTS, METHODS AND PLANTS

FIELD OF THE INVENTION

Plants exhibiting improved productivity and/or yield phenotypes and/or increased drought tolerance by introducing into such plants mutations in the *GRF3* growth factor, or in a *GRF3* orthologue, which mutants deregulate the *GRF3* or *GRF3* orthologue from miR396 control (optionally in combination with overexpression of at least one *GIF* gene).

BACKGROUND OF THE INVENTION

In contrast to animals, plants continue to produce new organs throughout their life cycle. The above-ground organs are derived from the shoot apical meristem (SAM), which includes a pool of stem cells residing at the growing tip of the plant. Proliferating SAM cells produce an excess of daughter cells that are either incorporated into the developing leaf primordia at the SAM periphery or become part of the shoot. The core machinery controlling the progression of the cell cycle in plants, as well as in other eukaryotes, relies on the activity of cyclin-dependent kinases (Inze and De Veylder, 2006). Many aspects of cell cycle regulation are highly conserved among eukaryotes. It is, however, the integration of the basic cell cycle mechanisms with the developmental program that generates the enormous phenotypic variation among multicellular organisms, a process that is much less understood (Inze and De Veylder, 2006).

In contrast to the indeterminate SAM in *Arabidopsis thaliana*, leaves are determinate organs that have a defined morphology. Leaf development involves the concerted action of various hormone signalling pathways and transcription factor networks. Some of the major transcriptional regulators involved in the control of cell proliferation in leaves include *AINTEGUMENTA* (Mizukami and Fischer, 2000), *PEAPOD* (White, 2006), *JAGGED* (Dinneny et al., 2004; Ohno et al., 2004), *BLADE ON PETIOLE* (Ha et al., 2003), *TCPs* (Nath et al., 2003) and *GROWTH-REGULATING FACTORS* (*GRFs*) (Kim et al., 2003).

To obtain their characteristic final size and shape, growth of the developing leaf needs to be tightly coordinated first through cell proliferation and then by cell

expansion (Piazza et al., 2005; Tsukaya, 2006). Initially, cell proliferation is observed throughout the developing leaf (Donnelly et al., 1999). Then, the cell cycle stops at the tip of the leaf and a mitotic arrest front moves towards the base of the organ (Donnelly et al., 1999). Once cells cease to divide, they begin to enlarge and cell growth becomes the driving force regulating organ size (Piazza et al., 2005; Tsukaya, 2006).

Currently, little is known about the molecular mechanisms that coordinate cell proliferation throughout a developing leaf. A known regulator is the *TCP* gene *CINCINNATA* (*CIN*), which controls the progression of the mitotic arrest front in snapdragon (Nath et al., 2003). Mutations such as *cin* (Nath et al., 2003) and triple knock-outs of its *Arabidopsis* homologues *tcp2/4/10* (Schommer et al., 2008) cause changes in leaf morphogenesis and uneven organ curvature due to excess cell proliferation at the leaf margins. Interestingly, five *Arabidopsis* *TCPs* (*TCP2*, 3, 4, 10 and 24), as well as *CIN*, have a target site for microRNA (miRNA) miR319 (Palatnik et al., 2003). Overexpression of miR319 causes the degradation of these *TCPs* and the generation of crinkled leaves similar to those observed in *tcp* loss-of-function mutants (Palatnik et al., 2003). Mutations in the target site of the *TCPs* that diminish the interaction with the miRNA affect leaf morphology in *Arabidopsis* (Palatnik et al., 2003; Palatnik et al., 2007) and leaf complexity in tomato (Ori et al., 2007), and are lethal in extreme cases (Palatnik et al., 2003).

The *GRF* family of transcription factors comprises nine members in *Arabidopsis* (Kim et al., 2003). Seven of them have a target site for miR396 (Jones-Rhoades and Bartel, 2004). Loss-of-function mutations in different *GRFs* or overexpression of miR396, which decreases *GRF* levels, have been shown to reduce cell number in *Arabidopsis* leaves (Horiguchi et al., 2005; Kim et al., 2003; Kim and Kende, 2004; Liu et al., 2009). The *GRFs* work together with *GRF-INTERACTING FACTORS* (*GIFs*), a small gene family encoding proteins with homology to the human SYT transcriptional co-activator (Horiguchi et al., 2005; Kim and Kende, 2004). Inactivation of *GIF1* (Kim and Kende, 2004), also known as *ANGUSTIFOLIA 3* (*AN3*) (Horiguchi et al., 2005), produces narrower leaves as a result of a reduction in cell proliferation.

It has been disclosed by Rodriguez et al., Development 137, 103-112 (2010), that a microRNA, miR396, plays a role in the coordination of cell proliferation in *Arabidopsis*

leaves. They showed that in leaf primordia, miR396 is expressed at low levels, but its expression steadily increases during organ development. They showed that miR396 antagonizes the expression pattern of its targets, the *GROWTH-REGULATING FACTOR* (*GRF*) transcription factors. miR396 was shown to accumulate preferentially in the distal part of young developing leaves, restricting the expression of *GRF2* to the proximal part of the organ. This, in turn, was shown to coincide with the activity of the cell proliferation marker *CYCLINB1;1*. miR396 was shown to attenuate cell proliferation in developing leaves through the repression of *GRF* activity and a decrease in the expression of cell cycle genes. Furthermore, they reported that over-expression of miR396 in a mutant lacking *GRF-INTERACTING FACTOR 1* (*GIF1*) severely compromised the shoot meristem. miR396 was found to be expressed at low levels throughout the meristem, overlapping with the expression of its target, *GRF2*. In addition, it was shown that overexpression of miR396 can reduce cell proliferation and the size of the meristem. *Arabidopsis* plants with an increased activity of the transcription factor TCP4, which reduces cell proliferation in leaves, were shown to have higher miR396 and lower *GRF* levels. Modified *GRF2*, which was mutated to interfere with the interaction with miR396, was shown to be independent of miR396 regulation to which the wild-type *GRF2* was subject. These plants were reported to have slightly bigger leaves than those of wild-type, however these leaves were curved downwards which could be detrimental for light capture and photosynthesis. Those results indicated that miR396 levels can significantly restrict cell proliferation in plants.

In the present disclosure, it is shown that a mutant *GRF3* (sometimes referred to herein as *rGRF3*) and mutant *GRF3* orthologues (sometimes referred to herein as *rGRF3* orthologues) are relieved of miR396 regulation, and that plants comprising the mutant *GRF3* or mutant *GRF3* orthologues have improved productivity and/or yield (including greater leaf area, greater cell numbers, increased biomass, increased stress resistance, delayed leaf senescence, increased seed production, increased seed yield, increased root growth, increased root elongation speed and greater tolerance to drought), whether compared to wild-type plants or to plants comprising a mutant *GRF2* relieved of miR396 regulation. Furthermore, the leaves from mutant *GRF3* plants or mutant *GRF3* orthologue plants were not curved downwards as those of mutant *GRF2*. The slight increase in leaf area observed in mutant *GRF2* plants were caused by increasing its level at least twenty-fold compared with the level of *GRF2* in wild-type plants; however, just three to five times more mutant

GRF3 compared with the level of *GRF3* in wild-type plants has been observed to cause a much larger impact on leaf size and plant biomass.

When the *GRF3* modification or *GRF3* orthologue modification is combined in a plant overexpressing *GIF1*, these effects are greatly enhanced.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows nucleic acid constructs and sequences of relevance to this invention; top panel shows the sequence of *GRF3* wild-type sequence in the region that is substantially complementary to miR396b, showing the binding affinity ($\Delta G = -33.9$ kcal/mole); middle panel shows the modified *GRF3* sequence (*rGRF3*), which includes five base changes from the wild-type sequence (an A→U, a G→A, a U→A, a G→A and a A→G modification), all of which retain the native amino acid sequence, but which substantially destabilizes the interaction with the miR396b microRNA, (reducing the ΔG to -14.4 kcal/mole); and the bottom panel shows a graphic of a 35S:*GIF1* expression construct.

Figure 2 shows the relative expression levels of *GRF3* and *GIF1* in transgenic Arabidopsis plants as estimated by RT-qPCR as well as in crosses between such transgenic plants, representing *GRF3* levels in wild-type plants as having a relative value of 1, it can be seen that over-expression of miR396 (under the control of the 35S promoter), reduces the *GRF3* expression, while the level of expression of *GRF3* in *rGRF3* transgenics is approximately five-fold the level of expression of *GRF3* in wild type plants. This increase of *GRF3* in transgenic plants expressing the mutant version is caused by the relief of the miRNA repression.

In the cross between *rGRF3* and 35S:*GIF1* plants, the *rGRF3* expression is slightly (but not significantly) lower than the five-fold expression level seen in the *rGRF3* plants. By comparison, the expression levels of *GIF1*, again representing levels in wild-type plants as 1, it is not significantly altered in the 35S:miR396 expressing plants, but is almost forty times the wild-type level in both *rGRF3* plants and crosses between *rGRF3*×*GIF1* plants. The measurements are triplicates $\pm$ SEM

Figure 3 shows the modification in leaf development observed in *rGRF3* plants, 35S:*GIF1* plants and *rGRF3*×35S:*GIF1* plants. In the left panel, leaf area, fresh weight and dry weight were determined for fully expanded first leaves, which show

the most easily observed changes; the right panel shows leaf phenotypes of developing plants in short days, while the bottom right panel shows plants grown in large pots in short day conditions.

Figure 4 shows, in the top panel, delayed leaf senescence of *rGRF3x35S:GIF1* crossed plants; in the bottom panel, delayed leaf senescence of an individual leaf is shown for fully expanded leaf 5, which was detached and incubated in the dark (dark induced senescence). The progression of senescence was followed by measuring chlorophyll fluorescence (Fv/Fm).

Figure 5 shows leaf area of plants transformed with the wild-type version of *GRF3* (*GRF3*) and/or with the miR396 resistant version of *GRF3* (*rGRF3*).

Figure 6 shows the fresh weight (Figure 6A) and dry weight (Figure 6B) of *rGRF3*, *GRF2* and *35S:miR396* plants, all in long day conditions, with the vertical axis being in units of grams.

Figure 7 shows a neighbour joining analysis of GRFs from *Arabidopsis thaliana* (AtGRF#), *Oryza sativa* (OsGRF#), *Zea mays* (ZmORF#), *Glycine max* (GmGRF), *Populus trichocarpa* (PtGRF), *Prunus persica* (PpGRF), *Medicago truncatula* (MtGRF) and *Carica papaya* (CpGRF) shown as an unrooted cladogram. Underlined: GRFs with a miR396 binding site: Labelled with an asterisk: GRFs with a FFD conserved motif.

Figure 8 shows the distribution of QLQ, WRC and FDD protein motifs in GRFs from *Arabidopsis thaliana* (AtGRF#), *Oryza sativa* (OsGRF#), *Zea mays* (ZmORF#), *Glycine max* (GmGRF), *Populus trichocarpa* (PtGRF), *Prunus persica* (PpGRF), *Medicago truncatula* (MtGRF), and *Carica papaya* (CpGRF)

Figure 9 shows a neighbour joining analysis of GIF from *Arabidopsis thaliana* and *Oryza sativa* shown as an unrooted cladogram: Sequences were retrieved from PlantTFDB 2.0 (<http://planttfdb.cbi.pku.edu.cn>).

Figure 10 shows the detrimental leaf-shape changes (downward "rolling") which are found with *rGRF2*, but not in *rGRF3*.

Figure 11 shows that a mild increase in *GRF3* (3x) causes a higher increase in productivity, e.g. biomass compared with a large accumulation of *GRF2* (25x).

Figure 12 shows that *rGRF3* plants display higher rates of stem growth and stem biomass accumulation. Left: elongation of a 4.5 cm long stem segment in 10 days of wild type (wt) and *rGRF3* plants.

Figure 13 shows - rosette phenotypes of short-day grown plants. Note increased leaf size and biomass accumulation with plants according to the present invention.

Figure 14 shows drought effects in the different transgenic plants.

Figure 15 shows Arabidopsis GRFs are expressed in proliferative tissues.

Left panel: *GRF3* expression pattern during leaf development (DAS = days after sowing). Right panel: *GRF3* is coexpressed with mitosis-specific genes during Arabidopsis development.

Figure 16 shows that maize GRFs are co-expressed with mitosis-specific genes.

Figure 17 shows an increase in plant size caused by Arabidopsis miR396-resistant *GRF3*.

A) 30 days old plants corresponding to independent transgenic plant lines: empty vector (WT, left), miR396-resistant *GRF3* (*rGRF3* centre) and wild-type *GRF3* (*GRF3*, right). Note the bigger size of the rosettes transformed with the *rGRF3*.

B) Fully expanded first leaf area of the different transgenic plants depicted in (A). At least 50 independent plants were scored for each vector. Bars marked with different letters are significantly different as determined by ANOVA and Duncan's multiple range test ($P < 0.05$).

Figure 18 shows that tissue-specific expression improves *rGRF3* performance in plant productivity. Area of fully expanded first leaf of transgenic plants expressing *rGRF3* from different promoters: *GRF3*, *ASYMMETRIC LEAVES 1* (*AS1*) or *AINTEGUMENTA* (*ANT*). At least 50 plants were scored for each vector. For *AS1:rGRF3* and *ANT:rGRF3* the data represent independent primary transgenics, whereas for *GRF3:rGRF3* a representative line was used. Bars marked with different letters are significantly different as determined by Kruskal-Wallis and Dunn's multiple range test ($P < 0.05$).

Figure 19 shows an increase in stem diameter due to *rGRF3*. Stem diameter of transgenic plants expressing *rGRF3* from different promoters: *GRF3*, *ASYMMETRIC LEAVES 1 (AS1)* and *AINTEGUMENTA (ANT)*. Bars marked with different letters are significantly different as determined by Kruskal-Wallis and Dunn's multiple range test ($P < 0.05$).

Figure 20 shows uncoupling of effects on leaf size from those on timing of leaf-senescence using tissue specific promoters. As shown herein *GRF3:rGRF3* increases leaf size and delays leaf senescence. The latter effect can be decoupled from the increase in leaf size if desired. Expression of *rGRF3* from *ANT* and *AS1* promoters significantly increased leaf size with a minor effect on leaf senescence. Dark-induced senescence of fully expanded leaf #5. Pictures were taken immediately after the full expanded leaves were cut from the rosette (Day 1) and after they were incubated 6 days in darkness (Day 6). For *GRF3:rGRF3* a representative line was used, and for *AS1:rGRF3* and *ANT:rGRF3* vector the 4 primary transgenic plants with the biggest leaf area were selected.

Figure 21 shows the nucleotide sequences of the *Arabidopsis thaliana* GRFs of which there are 9, namely AtGRF1 (SEQ ID No. 40), AtGRF2 (SEQ ID No. 87), AtGRF3 (SEQ ID No. 2), AtGRF4 (SEQ ID No. 19), AtGRF5 (SEQ ID No. 41), AtGRF6 (SEQ ID No. 42), AtGRF7 (SEQ ID No. 43), AtGRF8 (SEQ ID No. 44), and AtGRF9 (SEQ ID No. 45). The underlined section of the sequences represent the portion of the nucleotide sequence encoding the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the miR396 target site, if one is present.

Figure 22 shows the amino acid sequences of the *A. thaliana* GRFs of which there are 9 (AtGRF1 (SEQ ID No. 46), AtGRF2 (SEQ ID No. 47), AtGRF3 (SEQ ID No. 20), AtGRF4 (SEQ ID No. 21), AtGRF5 (SEQ ID No. 48), AtGRF6 (SEQ ID No. 49), AtGRF7 (SEQ ID No. 50), AtGRF8 (SEQ ID No. 51), and AtGRF9 (SEQ ID No. 52), the underlined section of the sequences represent the portion of the amino acid sequence known as the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the FFD motif.

Figure 23 shows the nucleotide sequences of the *Oryza sativa* (rice) GRFs of which there are 12. (OsGRF1 (SEQ ID No. 3), OsGRF2 (SEQ ID No. 4), OsGRF3 (SEQ ID

No. 5), OsGRF4 (SEQ ID No. 6), OsGRF5 (SEQ ID No. 53), OsGRF6 (SEQ ID No. 54), OsGRF7 (SEQ ID No. 55), OsGRF8 (SEQ ID No. 56), OsGRF9 (SEQ ID No. 57), OsGRF10 (SEQ ID No. 58), OsGRF11 (SEQ ID No. 59), and OsGRF12 (SEQ ID No. 60)), The underlined and bolded section of the sequences represent the miR396 target site, if one is present.

Figure 24 shows the amino acid sequences of the *Oryza sativa* (rice) GRFs of which there are 12. (OsGRF1 (SEQ ID No. 22), OsGRF2 (SEQ ID No. 23), OsGRF3 (SEQ ID No. 24), OsGRF4 (SEQ ID No. 25), OsGRF5 (SEQ ID No. 61), OsGRF6 (SEQ ID No. 62), OsGRF7 (SEQ ID No. 63), OsGRF8 (SEQ ID No. 64), OsGRF9 (SEQ ID No. 65), OsGRF10 (SEQ ID No. 66), OsGRF11 (SEQ ID No. 67), and OsGRF12 (SEQ ID No. 68)).

Figure 25 shows the nucleotide sequences of the *Zea mays* (maize) GRFs of which there are 14) (ZmORF1 (SEQ ID No. 7), ZmORF2 (SEQ ID No. 69), ZmORF3 (SEQ ID No. 8), ZmORF4 (SEQ ID No. 70), ZmORF5 (SEQ ID No. 9), ZmORF6 (SEQ ID No. 10), ZmORF7 (SEQ ID No. 11), ZmORF8 (SEQ ID No. 71), ZmORF9 (SEQ ID No. 12), ZmORF10 (SEQ ID No. 72), ZmORF11 (SEQ ID No. 13), ZmORF12 (SEQ ID No. 73), ZmORF13 (SEQ ID No. 74), and ZmORF14 (SEQ ID No. 14)) The underlined and bolded section of the sequences represent the miR396 target site, if one is present.

Figure 26 shows the amino acid sequences of the *Zea mays* (maize) GRFs of which there are 12. (ZmORF1 (SEQ ID No. 26), ZmORF2 (SEQ ID No. 75), ZmORF3 (SEQ ID No. 27), ZmORF4 (SEQ ID No. 76), ZmORF5 (SEQ ID No. 28), ZmORF6 (SEQ ID No. 29), ZmORF7 (SEQ ID No. 30), ZmORF8 (SEQ ID No. 77), ZmORF9 (SEQ ID No. 31), ZmORF10 (SEQ ID No. 78), ZmORF11 (SEQ ID No. 32), ZmORF12 (SEQ ID No. 79), ZmORF13 (SEQ ID No. 80), and ZmORF14 (SEQ ID No. 33)).

Figure 27 shows the nucleotide sequence for a GRF with high similarity to AtGRF3, namely *Glycine max* (soybean) GRF (GmGRF) (SEQ ID No. 16). The underlined and bolded section of the sequences represent the miR396 target site, if one is present.

Figure 28 shows the nucleotide sequence for a GRF with high similarity to AtGRF3, namely *Medicago truncatula* GRF (MtGRF) (SEQ ID No. 17).

Figure 29 shows the nucleotide sequence for a GRF with high similarity to *AtGRF3*, namely *Populus trichocarpa* GRF (PtGRF) (SEQ ID No. 18).

Figure 30 shows the nucleotide sequence for a GRF with high similarity to *AtGRF3*, namely *Prunus persica* GRF (PpGRF) (SEQ ID No. 15).

Figure 31 shows the amino acid sequence for a *Medicago truncatula* GRF (MtGRF) (SEQ ID No. 36); the underlined section of the sequences represent the portion of the amino acid sequence known as the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the FFD motif.

Figure 32 shows the amino acid sequence for a *Glycine max* (soybean) GRF (GmGRF) (SEQ ID No. 35); the underlined section of the sequences represent the portion of the amino acid sequence known as the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the FFD motif.

Figure 33 shows the amino acid sequence for a *Populus trichocarpa* GRF (PtGRF) (SEQ ID No. 37); the underlined section of the sequences represent the portion of the amino acid sequence known as the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the FFD motif.

Figure 34 shows the amino acid sequence for a *Prunus persica* GRF (PpGRF) (SEQ ID No. 34); the underlined section of the sequences represent the portion of the amino acid sequence known as the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the FFD motif.

Figure 35 shows the nucleotide sequence for the *Arabidopsis GRF3* with a mutated miR396-target site (*At-rGRF3*) (SEQ ID No. 81); the shaded and underlined portion of the sequence is the mutated miR396-target site. The lower case refers to base substitutions to make the GRF resistant to miR396. For the avoidance of doubt when the mutant *AtGRF3* is referred to herein unless stated otherwise it is this sequence that is being referred to. This sequence is also referred to herein as *At-rGRF3* and *rGRF3*. This mutated *At-rGRF3* was used herein to generate *inter alia* transgenic *Arabidopsis* plants.

Figure 36 shows the nucleotide sequence for a *Glycine max* GRF with a mutated miR396-target site (*Gm-rGRF*) (SEQ ID No. 82); the shaded and underlined portion of the sequence is the mutated miR396-target site. The lower case refers to base

substitutions to make the GRF resistant to miR396. This mutated Gm-rGRF was used herein to generate transgenic *Arabidopsis* plants.

Figure 37 shows the nucleotide sequence for an *Oryza sativa* GRF4 with a mutated miR396-target site (Os-rGRF4.1) (SEQ ID No. 83); the shaded and underlined portion of the sequence is the mutated miR396-target site. The lower case refers to base substitutions to make the GRF resistant to miR396. This mutated Os-rGRF4 was used herein to generate transgenic *Arabidopsis* plants. This sequence is also referred to herein as Os-rGRF4.1 and rOsGRF4.1.

Figure 38 shows similarity tables between At-GRF3 and GRFs from other plant species based on primary amino acid sequence. The global similarity between GRF3 and every GRF from At, Os and Zm (plus other highly similar GRFs from selected species) was scored using Needle (EMBOSS: <http://www.ebi.ac.uk/Tools/psa/>). Identity relates to when an identical amino acid is in the corresponding position; whereas similarity relates to when a conservative substitution of an amino acid is found in a corresponding position.

Figure 39 shows the nucleotide sequence encoding JD16_*GIF1* (including 35S promoter (nt 427-1295) – underlined section; *GIF1* coding Sequence (nt 1310-1942) – section in italics and bold; and Terminator (nt 2106-2755) – section in bold and underline.

Figure 40 shows the nucleotide sequence encoding RER32 *GRF3* (SEQ ID No. 85) (including *GRF3* Promoter (427-1707) - underlined section; 5'UTR (1708-1913) - lower case and italics; *GRF3* Coding Sequence + introns [in lower case] (1914-4231) - italics & bold; 3'UTR (4232-4454) - lower case and italics; and Terminator (4455-5105) – section in bold and underlined.

Figure 41 shows a map of the vector 35S:*GIF1* (JD16) (SEQ ID No. 84)- Vector size: 11332 pb Digest with *bam*HI and *Sall*. Products: 10682 and 650 pb.

Figure 42 shows a map of the vector *GRF3:GRF3r* (RER32) - Vector size: 13642 pb Digest with *Xba*I and *Sall*. Products: 11962 and 1680 pb.

Figure 43 shows that overexpression of *GIF1*, *GIF2*, and *GIF3* promotes cell proliferation and leaf size and that *GIF2* and *GIF3* proteins are functional equivalents of *GIF1* (see Figure 43 in combination with Figure 9).

Figure 44 shows the maps of the two plasmids comprising *rGRF3:GIF1* in pBRACT114. pBRACT114 is available from www.bract.org. The pBRACTs are based on the pGreen/pSoup vector system and the original reference for pGreen is: Hellens *et al* 2000.

Figure 45 shows delayed leaf senescence in primary transgenic *Arabidopsis* plants by a mutated *Arabidopsis GRF* (*At-rGRF3*) and by a mutated soybean *GRF* (Gm-*rGRF*).

Figure 46 shows that expression in *Arabidopsis* of *GRF3* orthologues from soybean and from rice, when decoupled from miR396 regulation also increase plant biomass. The area of fully expanded first leaf of transgenic plants expressing *GRF* from *Arabidopsis*, soybean or rice was measured.

Figure 47 shows the nucleotide sequence for a *GRF* with high similarity to *AtGRF3*, namely *Carica papaya GRF* (CpGRF) (SEQ ID No. 88).

Figure 48 shows the amino acid sequence for a *Carica papaya GRF* (CpGRF) (SEQ ID No. 89); the underlined section of the sequences represent the portion of the amino acid sequence known as the WRC (Trp, Arg, Cys) domain; and the underlined and bolded section of the sequences represent the FFD motif.

Figure 49 shows data comparing stem width 10cm above soil level at flowering and maximum stem width at flowering in *Brassica oleracea* plants transformed with *Arabidopsis rGRF3* and control plants (without the *At rGRF3*).

Figure 50 shows expression of *rGRF3* from tissue-specific promoters.

A) Top: Schematic representation of a construct expressing *GRF3* as a translational fusion to GFP. Bottom: Expression pattern of *GRF3*-GFP fusion protein in leaves of different ages collected from *GRF3:GRF3*-GFP and *GRF3:rGRF3*-GFP plants. B) Expression level of *GRF3* mRNA in apex and leaves of different ages. C) GUS staining of plants transformed with *ANT:GUS* and *AS1:GUS* reporters. Upper part, schematic representation of the reporters.

Figure 51 shows the expression levels of *rGRF3* under tissue-specific promoters and leaf area of transformants.

A) Expression levels of *GRF3* in transgenic seedlings expressing *GRF3* from different promoters. Determinations were carried out by RT-qPCR and normalized to wild-type plants. B) Area of fully expanded first and second leaves. C) Fully expanded first (left) and third (right) leaves.

Figure 52 shows pictures of 40 day old plants expressing *rGRF3* from their endogenous promoters and from the *ANT* and *AS1* promoters.

Figure 53 shows delayed senescence when *rGRF3* is expressed under the control of its own promoter. Senescence is evident in wild-type and when *rGRF3* is expressed under the control of *AS1* and/or *ANT*.

A) Pictures of 50 day old rosettes. Note the delayed senescence of *GRF3: rGRF3* plants and the normal development of *AS1:GRF3* and *ANT:GRF3*. B) Senescence of an individual leaf is shown for fully expanded leaf 5, which was detached and incubated in the dark (dark induced senescence). The progression of senescence was quantitated by determining Fv/Fm

Figure 54 shows leaf area plotted for independent primary transgenic plants. CHF3 is an empty vector control, *rGRF3* with the FFD motif is the *rGRF3* cDNA expressed from its own promoter. *rGRF3* AAD is the cDNA of *rGRF3* with three mutations in the FFD motif (FFDDW) that replace the two phenylalanine amino acids and the tryptophan with three alanine amino acids (AADDA).

Figure 55 shows a comparison between plants expressing *rGRF2* and *rGRF3*. As shown herein *rGRF3* expression leads to the production of bigger plants than wild-type or *rGRF2* expression. *rGRF2* also generates distorted rosettes.

Figure 56 is a table showing widest stem width at flowering and 10 cm stem weight for *Brassica oleracea* transformants expressing *rGRF3* and control plants (TC).

Figure 57 left, a graph showing root length at various days after sowing for wild-type *Brassica oleracea*, and two transgenic *Brassica oleracea* plants expressing *rGRF3*.

Right, a graph showing root elongation speed for wild-type or two transgenic plants expressing *rGRF3*.

SUMMARY OF THE INVENTION

The present invention is predicated upon the surprising finding that a novel modified *GRF3* gene, *rGRF3*, which is shown to be decoupled from control by miR396, particularly in the presence of over-expression of *GIF1*, can be used to significantly improve the biomass, improve stress resistance, improve drought tolerance, delay leaf senescence in plants. The improvement in biomass accumulation is surprisingly high and unexpectedly better than the only other reported miRNA decoupled GRF, namely *rGRF2*, while the tolerance to drought is unexpected from previously reported data.

The present inventors have also surprisingly found that orthologues of *GRF3* which are also modified to be decoupled from control by miR396 also provide these surprising and unexpected effects.

In a first aspect there is provided an isolated nucleic acid encoding a modified growth regulatory factor (GRF)-3 or an orthologue thereof which nucleic acid is decoupled from control by miR396.

In another aspect there is provided a construct comprising the nucleic acid according to the present invention operably linked with a promoter and a terminator.

The present invention further provides a vector comprising the nucleic acid of the present invention or the construct according to the present invention.

In a further aspect the present invention provides a plant, plant cell or plant tissue comprising the nucleic acid according to the present invention, the construct according to the present invention or the vector according to the present invention.

In yet another aspect there is provided a method for using the nucleic acid according to the present invention which comprises introducing said nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention into a plant.

In another aspect of the present invention there is provided nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention for use in the manufacture of a plant with increased productivity and/or yield (including for example increased biomass, increased stress resistance, increased drought tolerance, increased seed production, increased seed yield, increased root growth, increased root elongation speed, delayed leaf senescence and combinations thereof).

In another aspect of the present invention there is provided a method of producing a plant with increased productivity and/or yield (including for example one or more of increased biomass, increased stress resistance, increased drought tolerance, delayed leaf senescence, increased seed production, increased seed yield, increased root growth, increased root elongation speed and combinations thereof) comprising transforming the plant with nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention.

A further aspect provides the use of a nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention in the manufacture of a plant for increasing productivity and/or yield (for example one or more of increasing biomass, increasing stress resistance, increasing drought tolerance, delaying leaf senescence, increasing seed production, increasing seed yield, increasing root growth, increasing root elongation speed or combinations thereof).

In another aspect the present disclosure provides a novel modified gene, *rGRF3*, which is shown to be decoupled from control by miR396, particularly in the presence of over-expression of *GIF1*.

Accordingly, it is an object of this invention to provide a novel modified *GRF3* gene or a novel modified *GRF3* orthologue gene.

It is a further object of this invention to provide novel plants comprising a modified *GRF3* gene or a modified *GRF3* orthologue gene.

It is a further object of this invention to provide novel plants comprising a modified *GRF3* or a modified *GRF3* orthologue in the presence of over-expression of *GIF1*.

It is yet a further object of this invention to provide a method for using the modified *GRF3* or modified *GRF3* orthologue disclosed herein.

It is a further object of this invention to provide a method for producing plants with a phenotype of increased productivity and/or yield (for example a phenotype of delayed leaf senescence, increased biomass, increased stress response, increased drought tolerance, increased seed production, increased seed yield increased root growth, increased root elongation speed or combinations thereof), as compared with either wild-type plants or plants comprising a modified *GRF2* (*rGRF2*).

A further object of the present invention is to provide plants with a phenotype of increased productivity and/or yield (for example a phenotype of delayed leaf senescence, increased biomass, increased stress response, increased drought tolerance, increased seed production, increased seed yield increased root growth, increased root elongation speed or combinations thereof) without adverse side effects observed in plants expressing modified *GRF2* (*rGRF2*), such as detrimental leave shape changes, e.g. curved leaves or downwardly rolling leaves.

Further objects and advantages of this invention will be appreciated by referring to the entire disclosure provided herein, and the appended claims.

DETAILED DISCLOSURE OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Rodriguez et al. (2010) followed the expression pattern of miR396 directly using small RNA blots and in situ hybridization in apices, and indirectly through the differential expression of wild-type and miRNA-resistant *GRF2-GUS* reporters. miR396 was expressed at low levels in the meristem and leaf primordia, and then it steadily accumulated with the development of the leaf. In contrast, the *GRFs*, which are highly expressed in the SAM and young leaves, decreased during leaf development, in concert with the retreat of cell proliferation.

Temporal antagonistic patterns of expression have been observed for miR156 and miR172 and their targets, the SPL and AP2-like transcription factors, respectively (Chuck et al., 2007; Wu and Poethig, 2006). The heterochronic miR156 and miR172 networks correspondingly regulate juvenile to adult, and vegetative to reproductive phase transitions, which require decisions implicating the whole plant (Aukerman and Sakai, 2003; Chen, 2004; Chuck et al., 2007; Schmid et al., 2005; Wu and Poethig, 2006). The observations on miR396 indicated that this miRNA is also involved in the coordination of developmental events in plants; however, its role would be restricted to individual organs.

The *Arabidopsis* developmental program directs a basiplastic pattern, whereby leaf maturation begins at the tip and then proceeds towards the base of the organ (Donnelly et al., 1999). Cell division occurs first throughout the primordia and then a mitotic arrest front moves from the tip to the base of the leaf, so that cells in the distal part of the leaf stop cycling and begin to expand, while cells at the base continue to proliferate (Donnelly et al., 1999). Rodriguez et al.'s results showed that the distal part of the leaf accumulates more miR396 and a gradient of miRNA activity proceeds towards the base of the organ. That result was supported by small RNA blots and the observed retreat of the wild-type *GRF2-GUS* reporter, which then matched the pattern of a *CYCB1;1* reporter. Those observations prompted those authors to implicate the repression of *GRF* expression by miR396 as a component of the mitotic arrest front.

Similar spatial patterns of expression for *GRF2* mRNA and miR396 in the meristem and leaf primordial have been observed, indicating that there is co-expression of the miRNA and its target at this early stage. The situation was different, however, at later stages of leaf development. The wild-type *GRF2-GUS* reporter was active only in the proximal part of young developing leaves, whereas the *rGRF2-GUS* reporter was expressed throughout the leaf. This qualitative change in the expression of wild-type *GRF2-GUS* was paralleled by a large increase in miR396, whose levels change by up to 10-30-fold in leaves with different developmental ages. Interestingly, the decrease in *GRF* expression occurred before miR396 reached its maximum level, indicating that a partial increase in the miRNA is sufficient to repress the *GRFs* *in vivo*; however, it cannot be ruled out that additional factors that act in concert with miR396 may participate in this process.

It has been proposed that miRNAs could have both qualitative effects, leading to complete elimination of their targets, and more subtle quantitative effects (Bartel and Chen, 2004). In plants, these quantitative interactions have been proposed for miR169 (Cartolano et al., 2007) and miR156 (Wang et al., 2008), miR319 (Ori et al., 2007) and miR164 (Baker et al., 2005; Nikovics et al., 2006), and their targets. From a mechanistic point of view, it is tempting to speculate that miR396 has dual functions during leaf development: it might quantitatively regulate *GRF* expression in the SAM and leaf primordia, while causing a large qualitative effect contributing to the clearance of GRF activity from older organs. This latter functional role in clearing *GRF* transcripts might explain the continued rise in miR396 levels, even after cell proliferation has ceased. On the other hand, the potential quantitative regulation of GRF activity during early leaf development might play a relevant role in the fine-tuning of cell proliferation, it has been shown that modifications of the balance between miR396 and *GRF2* levels have important consequences for the final number of cells in the organ.

miR396 was first identified because of its conservation between *A. thaliana* and rice (Jones-Rhoades and Bartel, 2004). miR396 and *GRFs* with an miR396 target site are present in many plant species (Axtell and Bartel, 2005; Jones-Rhoades and Bartel, 2004), indicating an ancient origin for the miR396-GRF regulatory network. The function of the GRFs as regulators of cell number in leaves is well established based on the phenotypes of *grf* (Horiguchi et al., 2005; Kim et al., 2003; Kim and Lee, 2006) and *gif* (Horiguchi et al., 2005; Kim and Kende, 2004) mutants, and plants with high miR396 levels (Liu et al., 2009).

Rodriguez et al. (2010) extended these observations and found that the *GRFs* regulate cell proliferation in the SAM, which at least partially explains the lack of a functional meristem in *an3-1* mutants overexpressing miR396 (this study) and in *grf* multiple knock-outs (Kim et al., 2003; Kim and Lee, 2006). Analysis of the transcriptome of moderate miR396 overexpressers has shown that the downregulation of mitosis-specific genes is one of the main molecular effects of high miR396 levels. However, the *GRFs* themselves do not change their expression during the cell cycle (Menges et al., 2005) and future work will be required to identify the mechanisms underlying the activity of the GRFs.

Measurements of the *GRFs* by RT-qPCR indicated that miR396 targets and non-targets are turned off at similar stages of leaf development, and that they act redundantly. Previous studies in which promoters have been fused directly to a GUS reporter have shown that the transcription of the *GRF* genes can occur in different regions of the leaf (Horiguchi et al., 2005). Rodriguez et al. observed that the post-transcriptional control of *GRF2* by miR396 contributes significantly to its final expression pattern, and concluded that it is possible that the miRNA also plays a key role in adjusting the expression of other *GRFs*.

The snapdragon *TCP* gene *CIN* has been shown to be expressed in a dynamic pattern during leaf development and to regulate cyclin expression (Nath et al., 2003). *CIN*-like genes from *Arabidopsis*, which are regulated by miR319, have also been implicated in the coordination of cell proliferation and differentiation in leaves (Efroni et al., 2008; Koyama et al., 2007; Masuda et al., 2008; Palatnik et al., 2003; Schommer et al., 2008). An increase of *TCP4* levels due to mutations that impair the interaction with miR319 produces smaller leaves (Efroni et al., 2008; Palatnik et al., 2003; Schommer et al., 2008).

Rodriguez et al. observed that plants expressing miR319-resistant forms of *TCP4* induced miR396. As the quantitative balance between miR396 and the *GRFs* regulates cell number in leaves, the increase in miR396 caused by *TCP4* might be responsible for at least part of the reduction in cell number in *soj8* mutants. They observed, however, that the increase in *TCP4* levels also caused a reduction in the *GRFs* that were not regulated by miR396 and *GIF1*, indicating an effect at the transcriptional level. Regulatory circuits in which a transcription factor causes both the transcriptional repression of target genes and the induction of an miRNA that in turn post-transcriptionally inhibits the same group of genes are well described in animals, where they are referred to as coherent feed-forward loops (Hornstein and Shomron, 2006).

miR319 overexpressers (Efroni et al., 2008; Ori et al., 2007; Palatnik et al., 2003) and *tcp* knock-outs (Nath et al., 2003; Schommer et al., 2008) have large changes in leaf morphology, as well as other phenotypic defects, such as a delay in flowering time (Palatnik et al., 2003). This indicates that the *TCPs* have functions that go beyond leaf development. However, it may be possible that the miR319-regulated *TCPs* recruit the miR396 network as part of their biological function. Rodriguez et al.

proposed that the miR396 network could be a link between different developmental inputs or environmental stimuli and the components of the cell cycle machinery.

In this disclosure, the effects in plants of mutating *GRF3* (and orthologues thereof) to produce a novel molecule, *rGRF3* (or orthologues thereof), in a manner analogous to that for *GRF2* reported by Rodriguez et al. are shown. Surprisingly, however, it is reported here that the result is a plant with a pronounced increase in productivity and/or yield (for example with a pronounced increase in biomass, increased stress response, delayed leaf senescence, increased seed production, increased seed yield, increased root growth, increased root elongation speed and/or increased tolerance to drought), whether compared to plants with wild-type (e.g. non-mutated) *GRF3*, wild type *GRF2* or the mutated *GRF2* (*rGRF2*) described in Rodriguez et al.

In addition, it is shown that where at least one GIF (e.g. *GIF1*) is overexpressed in the presence of the mutated *GRF3* (*rGRF3*) or an orthologue thereof, these effects are enhanced.

Furthermore, the leaves from mutant *GRF3* plants and/or mutant *GRF3* orthologue plants were not curved downwards as those of mutant *GRF2* (*rGRF2*) reported in Rodriguez et al.

A slight increase in leaf area can be observed in *rGRF2* plants if its level is increased to at least twenty times the level of *GRF2*; however, a much larger impact on productivity (for example leaf size and plant biomass) can be seen in *rGRF3* plants and *rGRF3*-orthologue plants with only three to five times more *GRF3* or *GRF3*-orthologue.

Thus, per this disclosure, as shown in detail in the examples and experimental methods provided below, *rGRF3* or orthologues thereof is/are produced comprising several synonymous mutations in the nucleic acid sequence – i.e. there is no change in the amino acid sequence of *GRF3*.

The result is a plant in which the repression otherwise achieved by miR396 is uncoupled from the *rGRF3*, and plants with increased productivity and/or yield (including with increased biomass, increased stress resistance, delayed leaf

senescence and increased drought tolerance or combinations thereof) are thereby producible.

In a first aspect there is provided an isolated nucleic acid encoding a modified growth regulatory factor (GRF)-3 or an orthologue thereof which nucleic acid is decoupled from control by miR396.

The nucleic acid may be decoupled from control by miR396 by mutating the miR396 target site.

Preferably the mutated or modified nucleic acid is only modified in the miR396 target site, e.g. with the remainder of the gene being unmodified or not being mutated.

In a preferred embodiment, the modified nucleic acid is modified in such a way as to comprise conserved nucleic acid changes. In other words, the nucleic acid is modified such that there is no change in the amino acid sequence of the *GRF3* or the *GRF3* orthologue expressed by the nucleic acid.

The modification to the nucleic acid essentially decouples the nucleic acid (e.g. gene) from control by miR396.

Preferably the nucleic acid is decoupled from control by miR396 by mutating the nucleic acid in the miR396 target site.

Preferably the nucleic acid according to the present invention encodes a protein having the FFD motif.

In some embodiments preferably the nucleic acid according to the present invention encodes a protein having the FFD(D/E)WP motif.

For the avoidance of doubt "(D/E)" means that at that position there is either a D or an E residue. In other words, FFD(D/E)WP means FFDDWP or FFDEWP.

In order to determine whether a GRF is a *GRF3*-orthologue in accordance with the present invention one may look for GRFs which encode a protein having the FFD, (e.g. FFD(D/E)WP) motif.

GRF3-orthologues in accordance with the present invention will be GRFs which at least comprise a miR396 target site.

Suitably the miR396 target site (e.g. in the nucleic acid according the present invention, such as in the *GRF3* gene or in the *GRF3*-orthologue gene) may have, comprise or consist of the following nucleotide sequence
CGTTCAAGAAAGCCTGTGGAA (SEQ ID No. 1). In some embodiments this nucleotide sequence may be considered the wild-type miR396 target site sequence.

The *GRF3*-orthologue according to the present invention is preferably one or more of the following GRFs selected from the group consisting of: *Arabidopsis thaliana* GRF4; *Oryza sativa* GRF 1, 2, 3, 4, or 5; *Zea mays* GRF 1, 3, 5, 6, 7, 9, 11 or 14; *Glycine max* GRF; *Medicago truncatula* GRF; *Populus trichocarpa* GRF, *Carica papaya* GRF and *Prunus persica* GRF which have been decoupled from control by miR396.

In one embodiment, the *GRF3*-orthologues are ones which cluster with AtGRF3 in the cladogram depicted in Figure 7. It has been found that these *GRF3*-orthologues function similarly to AtGRF3.

For the avoidance of doubt GRFs which cluster with either AtGRF2 or AtGRF9 are not of interest in the present application as it has been found that these GRFs do not function like AtGRF3.

A *GRF3*-orthologue in accordance with the present invention is one which has the same functionality as AtGRF3.

The term "orthologue" as used herein means genes of similar or same function but occurring in different species.

As shown in Figure 7 the *GRF3*-orthologue may be preferably one that comprises a miR396 target site and which encodes for a protein having the FFD (e.g. FFD(D/E)WP) motif.

The *GRF3*-orthologues in accordance with the present invention will be GRFs which at least comprise a miR396 target site.

The present invention relates to isolated nucleic acid according to any one of the preceding claims comprising i) a nucleotide sequence shown as SEQ ID No. 2 (*AtGRF3*); ii) or a nucleotide sequence which is at least 45%, preferably at least 50%, preferably at least 60%, preferably at least 65%, identical to SEQ ID No. 2; or iii) a nucleotide sequence which hybridises under stringent conditions with a nucleotide sequence of either i) or ii) wherein the nucleotide sequence comprises a modification in the miR396 target site to decouple the nucleic acid from control by miR396.

The isolated nucleic acid according to the present invention may comprise i) a nucleotide sequence shown as SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, or SEQ ID No. 19; ii) or a nucleotide sequence which is at least 45%, preferably at least 50%, preferably at least 60%, preferably at least 65%, identical to SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, or SEQ ID No. 19; or iii) a nucleotide sequence which hybridises under stringent conditions with a nucleotide sequence of either i) or ii) wherein the nucleotide sequence of i), ii) or iii) comprises a modification in the miR396 target site to decouple the nucleic acid from control by miR396.

The isolated nucleic acid according to the present invention may comprise i) a nucleotide sequence encoding a polypeptide shown herein as SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22, SEQ ID No. 23, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32, SEQ ID No. 33, SEQ ID No. 34, SEQ ID No. 35, SEQ ID No. 36 or SEQ ID No. 37; ii) or a nucleotide sequence having at least 45%, preferably at least 50%, preferably at least 60%, preferably at least 65%, identity with the nucleotide sequence of i); or iii) a nucleotide sequence which hybridises under stringent conditions with a nucleotide sequence of either i) or ii) wherein the

nucleotide sequence of i), ii) or iii) comprises a modification in the miR396 target site to decouple the nucleic acid from control by miR396.

Preferably the nucleic acid decoupled of miR396 control according to the present invention exhibits further enhancement in the presence of over-expression of at least one GIF gene (e.g. *GIF1*).

Over-expression of at least one GIF (e.g. *GIF1*) may be accomplished by transforming a plant, or a plant cell, or a plant tissue, with a construct comprising at least one GIF (e.g. *GIF1*) encoding sequence operably linked to a promoter.

In one embodiment the plant, plant cell or plant tissue comprises at least two, e.g. 2 or 3, over-expressed GIF genes.

The GIF gene in accordance with the present invention may be any suitable GIF gene, including *AtGIF1* (sometimes referred to herein as *GIF1*), *AtGIF 2*, *AtGIF 3*, *Os11g40100*, *Os12g31350*, *Os03g52320* or combinations thereof.

The GIF (e.g. *GIF1*) coding sequence may be under the control of a constitutive promoter, such as CaMV 35S promoter, or may be a tissue specific promoter.

As shown in detail in the examples and experimental methods provided below, *rGRF3* or orthologues thereof may be produced comprising several synonymous nucleic acid changes – i.e. there is no change in the amino acid sequence of *GRF3*.

In one embodiment the modified *GRF3* or orthologue thereof may comprise comprising at least one or all of the following base changes in the miR396 target site an A->U, a G->A, a U->G, a U->A, a G->C, a A->T, a G->A, a T->A, a G->A, a A->G modification. These changes may retain the native amino acid sequence, but substantially destabilize the interaction of miR396 with said *rGRF3*.

In one embodiment the modified *GRF3* or orthologue thereof may comprise comprising at least one or all of the following base changes in the miR396 target site an A->U, a G->A, a U->A, a G->A, a A->G modification. These changes may retain the native amino acid sequence, but substantially destabilize the interaction of miR396 with said *rGRF3*.

In a preferred embodiment the modified GRF 3 or orthologue thereof comprises a modified miR396 target site having the following sequence:

CGTTCxAGAAAxCCxGTxGx (SEQ ID No. 86), wherein x designates bases that have been modified (e.g. mutated) (e.g. compared with the wild-type sequence).

The modified GRF 3 or orthologue thereof comprises a modified miR396 target site having the following sequence: CGTTCtAGAAAaCCaGTaGAg (SEQ ID No. 38), wherein the lower case letters designates modified bases (e.g. compared with the wild-type sequence).

Mutant sequences can be produced by any known method and various methods are readily available to one of ordinary skill in the art. As one skilled in the art will appreciate, it is possible to produce numerous site directed or random mutations into a nucleotide sequence and to subsequently screen for improved functionality of the encoded polypeptide by various means.

Mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites.

A suitable method is disclosed in Morinaga *et al.*, (*Biotechnology* (1984) **2**, p646-649). Another method of introducing mutations in nucleotide sequences is described in Nelson and Long (*Analytical Biochemistry* (1989), **180**, p 147-151).

One method for introducing mutations into a nucleotide sequence would be to use QuikChange® Site Directed Mutagenesis Kit from Stratagene.

In some embodiments Targeted Induced Local Lesions IN Genomes (TILLING) technology described in Colbert *et al* 2001 (*Plant Physiology* June 2001, Vol. 126, pp480-484) may be used to screen for induced mutations, e.g. induced point mutations.

In another aspect there is provided a construct comprising the nucleic acid according to the present invention operably linked with a promoter and/or a terminator.

The promoter may be a constitutive promoter, such as CaMV 35S promoter, the native AtGRF3 promoter, or the native GRF3 orthologue promoter, or may be a tissue specific promoter.

In one embodiment the promoter may be a tissue specific promoter.

When it is desired to decouple the different functions of *GRF3* (such as to decouple the increased biomass from delayed leaf senescence), preferably the nucleic acid according to the present invention is operably linked with a tissue specific promoter.

In addition the use of a tissue specific promoter can improve the performance of plant production and further improve productivity.

In some embodiments the tissues specific promoter may comprise a (or may be a) promoter which is transiently expressed during early leaf development.

In one embodiment the tissue specific promoter may comprise a (or may be a) ASYMMETRIC LEAVES 1 (AS-1) promoter or a AINTEGUMENTA (ANT) promoter.

A person skilled in the art would be aware of other suitable tissue specific promoters to target expression of the nucleic acid according to the present invention in the appropriate location of the plant. Without wishing to be bound by theory, a mutant *GRF3* or mutant *GRF3* orthologue uncoupled of miR396 control with or without the co-overexpression of GIF may modify cell number in leaves or other organs when the nucleic acids are expressed specifically in those tissues. Therefore, *rGRF3* will only affect that part of the plant where expression occurs.

A person skilled in the art would also be aware that the temporal pattern and level of expression might also be modified. For example, the AS-1 promoter is active for a longer period of time than the ANT promoter, and thus generates bigger leaves when expressing the mutated *GRF3* (*rGRF3*) or mutated *GRF3* orthologue sequences.

Therefore the tissue specific promoter may be one which is spatially and/or temporally regulating expression.

The present invention further provides a vector comprising the nucleic acid of the present invention or the construct according to the present invention.

In a further aspect the present invention provides a plant, plant cell or plant tissue comprising the nucleic acid according to the present invention, the construct according to the present invention or the vector according to the present invention.

In one embodiment the plant, plant cell or plant tissue according the present invention may further comprise a modified *GRF2* (*rGRF2*) which modified *GRF2* is also decoupled from control by miR396. In other words, the *GRF2* may also be mutated in miR396 target site in accordance with the present invention. For the avoidance of doubt this embodiment only relates the combination of the *rGRF3* or *rGRF3*-orthologue in accordance with the present invention in combination with *rGRF2*.

AtGRF2 and *AtGRF9* are not *GRF3*-orthologues in accordance with the present invention.

Hence there term "*GRF3*-orthologue" as used herein does not include *AtGRF2* or *AtGRF9*.

Hence the nucleotide sequence according to the present invention does not comprise a nucleotide sequence comprising the nucleotide sequence shown herein as SEQ ID No. 87 or SEQ ID No. 45.

Likewise, the term "modified *GRF3*-orthologue" or "*rGRF3*-orthologue" as used herein does not include modified *AtGRF2* or modified *AtGRF9*.

In one embodiment of the present invention the plant, plant cell or plant tissue may in addition over-express at least one GIF (e.g. *GIF1*).

Over-expression of at least one GIF (e.g. *GIF1*) may be accomplished by transforming said plant, or a plant cell, or a plant tissue, with a construct comprising the at least one GIF (e.g. *GIF1*) encoding sequence operably linked to a promoter.

In some embodiments the plant, plant cell or plant tissue according to the present invention may comprise more than one (e.g. two, for example three) nucleic acids according to the present invention.

By way of example only, the plant, plant cell or plant tissue according to the present invention may comprise more than one (e.g. two, for example three) *rGRF3* genes and/or *rGRF3*-orthologues. For example the plant, plant cell or plant tissue according to the present invention may comprise *rGRF3* in combination with one or more *rGRF3*-orthologues.

The term "*GRF3*" as used herein means the GROWTH-REGULATING FACTOR 3 obtainable (preferably obtained) from *Arabidopsis thaliana*.

The term "*rGRF3*" as used herein means a mutated or modified GROWTH-REGULATING FACTOR 3 obtainable (preferably obtained) from *Arabidopsis thaliana*. Preferably the mutated or modified GROWTH-REGULATING FACTOR 3 has been mutated or modified to decouple it from control by miR396.

The term "*GRF3*-orthologue" as used herein may encompass one or more of the following GRFs selected from the group consisting of: *Arabidopsis thaliana* GRF4; *Oryza sativa* GRF 1, 2, 3, 4, or 5; *Zea mays* GRF 1, 3, 5, 6, 7, 9, 11 or 14; *Glycine max* GRF; *Medicago truncatula* GRF; *Populus trichocarpa* GRF; *Carica papaya* GRF and *Prunus persica* GRF.

The term "*rGRF3*-orthologue" as used herein may encompass one or more of the following GRFs selected from the group consisting of *Arabidopsis thaliana* GRF4; *Oryza sativa* GRF 1, 2, 3, 4 or 5; *Zea mays* GRF 1, 3, 5, 6, 7, 9, 11 or 14; *Glycine max* GRF; *Medicago truncatula* GRF; *Populus trichocarpa* GRF; *Carica papaya* GRF and *Prunus persica* GRF which have been decoupled from control by miR396.

The nucleic acid encoding a modified GRF-3 or an orthologue thereof may comprise introns or may exclude introns.

In one embodiment the nucleic acid encoding a modified GRF-3 or an orthologue thereof comprises introns. Without wishing to be bound by theory introns may enhance the expression of the transgenes.

In yet another aspect there is provided a method for using the nucleic acid according to the present invention which comprises introducing said nucleic acid according to

the present invention or a construct according to the present invention or a vector according to the present invention into a plant.

In another aspect of the present invention there is provided nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention for use in the manufacture of a plant with increased biomass, increased stress resistance, increased drought tolerance, delayed leaf senescence and combinations thereof.

In another aspect of the present invention there is provided a method of producing a plant with increased biomass, increased stress resistance, increased drought tolerance, delayed leaf senescence and combinations thereof comprising transforming the plant with nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention.

A further aspect provides the use of a nucleic acid according to the present invention or a construct according to the present invention or a vector according to the present invention in the manufacture of a plant for increasing biomass, increasing stress resistance, increasing drought tolerance, delaying leaf senescence or combinations thereof.

Preferably plants in accordance with the present invention have increased biomass, increased stress resistance, increased drought tolerance, delayed leaf senescence or combinations thereof.

The term "increased biomass" may comprise one or more of the following selected from the group consisting of: increased overall plant biomass, increased fresh weight, increased leaf area or size, increased root length, increased dry weight, increased stem growth, increased stem biomass, increased stem diameter, and increased stem width at flowering.

A surprising technical advantage of the use of *rGRF3* or *rGRF3* orthologues (which differs from use of *rAtGRF2*) is that the increased biomass, increased drought tolerance, delayed leaf senescence or combinations thereof occurs without detrimental leaf shape changes, e.g. downward rolling.

In some embodiments it may be preferable to uncouple increased biomass from delayed leaf senescence. The inventors have surprisingly found that this can be achieved by using tissue specific promoters.

The term "increased stress resistance" as used herein means the ability of a plant to remain productive (e.g. maintain or increase biomass, etc.) even in conditions which place the plant under stress, e.g. drought etc.

The terms "increased biomass", "increased stress resistance", "increased drought tolerance", "delayed leaf senescence", "increased root growth", "increased root elongation speed" mean increased or delayed compared with either wild-type plants (e.g. plants comprising a non-modified *GRF3* or *GRF3*-orthologue) or plants comprising a modified *GRF2* (r*GRF2*).

The terms "increased overall plant biomass", "increased fresh weight", "increased leaf area or size", "increased dry weight", "increased stem growth", "increased stem biomass", "increased stem diameter", and "increased stem width at flowering" mean increased or delayed compared with either wild-type plants (e.g. plants comprising a non-modified *GRF3* or *GRF3*-orthologue) or plants comprising a modified *GRF2* (r*GRF2*).

The term "modified" as used herein may mean mutated. The term "modified" as used herein mean different from the wild-type.

The term "wild type" as used herein means a naturally-occurring nucleic acid. That is to say a nucleic acid found in an endogenous genetic code and isolated from its endogenous host organism which has not been mutated (i.e. does not contain base deletions, additions or substitutions) when compared with the genetic code of the host organism.

The vector according to the present invention may be an expression vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

Preferably, the expression vector is incorporated into the genome of a suitable host organism, e.g. plant. The term "incorporated" preferably covers stable incorporation into the genome.

The nucleotide sequence of the present invention may be present in a vector in which the nucleotide sequence is operably linked to regulatory sequences capable of providing for the expression of the nucleotide sequence by a suitable host organism, e.g. plant.

The vectors for use in the present invention may be transformed into a suitable host cell, e.g. plant cell, as described below.

The vectors for use in the present invention may contain one or more selectable marker genes such as a gene which confers antibiotic resistance e.g. ampicillin, kanamycin, chloramphenicol or tetracyclin resistance.

Vectors may be used *in vitro*, for example for the production of RNA or used to transfect, transform, transduce or infect a host cell.

Thus, in a further embodiment, the invention provides a method of making nucleotide sequences of the present invention by introducing a nucleotide sequence of the present invention into a replicable vector, introducing the vector into a compatible host (e.g. plant) cell, and growing the host (e.g. plant) under conditions which bring about replication of the vector.

The term "operably linked" as used herein refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "regulatory sequences" includes promoters and enhancers and other expression regulation signals.

The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - includes a nucleotide sequence for use according to the present invention directly or indirectly attached to a promoter.

An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the Sh1-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. In some cases, the terms do not cover the natural combination of the nucleotide sequence coding for the protein ordinarily associated with the wild type gene promoter and when they are both in their natural environment.

The construct may even contain or express a marker, which allows for the selection of the genetic construct.

For some applications, preferably the construct of the present invention comprises at least the nucleotide sequence of the present invention operably linked to a promoter.

A host organism suitable for transformation with the nucleic acid of the present invention may be a plant. In this respect, the basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. A review of the general techniques may be found in articles by Potrykus (*Annu Rev Plant Physiol Plant Mol Biol* [1991] **42**:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.*, (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208.

Other techniques for transforming plants include ballistic transformation, the silicon whisker carbide technique (see Frame BR, Drayton PR, Bagnaall SV, Lewnau CJ, Bullock WP, Wilson HM, Dunwell JM, Thompson JA & Wang K (1994) Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation, *The Plant Journal* **6**: 941-948) and viral transformation techniques (e.g. see Meyer P,

Heidmann I & Niedenhof I (1992) The use of cassava mosaic virus as a vector system for plants, *Gene* **110**: 213-217).

Further teachings on plant transformation may be found in EP-A-0449375.

Plant cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

In a further aspect, the present invention relates to a vector system which carries a nucleotide sequence or construct according to the present invention and which is capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant. The vector system may comprise one vector, but it may comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.*, (1980), Binary Vectors, *Plant Molecular Biology Manual* **A3**, 1-19.

One extensively employed system for transformation of plant cells uses the Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.*, (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.*, (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. After each introduction method of the desired promoter or construct or nucleotide sequence according to the present invention in the plants, the presence and/or insertion of further DNA sequences may be necessary. If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanthers B.B., Alblaserdam, 1985, Chapter V; Fraley, *et al.*, *Crit. Rev. Plant Sci.*, **4**:1-46; and An *et al.*, *EMBO J.* (1985) **4**:277-284.

The term GIF as used herein means *GRF-INTERACTING FACTORS* (GIFs), a small gene family encoding proteins with homology to the human SYT transcriptional co-activator (Horiguchi *et al.*, 2005; Kim and Kende, 2004).

GIF1 (Kim and Kende, 2004) is also known as *ANGUSTIFOLIA 3* (*AN3*).

In one embodiment preferably the GIF used in accordance with the present invention is *GIF1*. *GIF1* may also be referred to herein as *AtGIF1*.

In one embodiment the GIF used in accordance with the present invention may be *GIF1*, wherein *GIF1* i) comprises the amino acid shown herein as MQQHLMQMMPMAGYYPSNVTSDHIQQYLDENKSLILKIVESQNSGKLSECAENQ ARLQRNLMYLAAIADSQPQPPSVHSQYGSAGGGMIIQGEGGSHYLQQQQATQQQQ MTQQSLMAARSSMLYAQQQQQQQPYATLQHQLHHSQLGMSSSSGGGGSSGLH ILQGEAGGFHDFGRGKPEMGSGGGGEGRGSSGDGGETLYLKSSDDGN (SEQ ID No. 95) or an amino acid sequence having at least 80% identity therewith; or ii) is encoded by the nucleotide sequence:

ATGCAACAGCACCTGATGCAGATGCAGCCCATGATGGCTGGTTACTACCCAG
CAATGTTACCTCTGATCATATCCAACAGTACTTGGACGAAAACAAATCGTTGATT
CTGAAGATTGTTGAGTCTCAAACTCTGGAAAGCTTAGCGAATGCGCCGAGAAT
CAAGCAAGGCTTCAACGCAACCTAATGTACCTAGCTGCAATAGCAGATTCTCAG
CCTCAGCCACCAAGTGTGCATAGCCAGTATGGATCTGCTGGTGGTGGGATGAT
TCAGGGAGAAGGAGGGTCACACTATTTGCAGCAGCAACAAGCGACTCAACAGC
AACAGATGACTCAGCAGTCTCTAATGGCGGCTCGATCTTCAATGTTGTATGCTC
AGCAACAGCAGCAGCAGCAGCCTTACGCGACGCTTCAGCATCAGCAATTGCAC
CATAGCCAGCTTGGAATGAGCTCGAGCAGCGGAGGAGGAGGAAGCAGTGGTC
TCCATATCCTTCAGGGAGAGGCTGGTGGGTTTCATGATTTTGGCCGTGGGAAG
CCGGAATGGGAAGTGGTGGTGGCGGTGAAGGCAGAGGAGGAAGTTCAGGGG
ATGGTGGAGAAACCCTTTACTTGAAATCATCAGATGATGGGAATTGA (SEQ ID No. 39); or

iii) is encoded by a nucleotide sequence which is at least 70%, preferably 80%, more preferably 90%, even more preferably 95% identical with SEQ ID No. 39; or
iv) is encoded by a nucleotide sequence which hybridizes under stringent conditions with SEQ ID No. 39.

As can be seen from Figure 9 a number of GIF sequences from *Arabidopsis thaliana* and *Oryza sativa* cluster together. It is envisaged that any one of these GIFs may be used in accordance with the present invention. Therefore the GIF for use in accordance with the present invention may be one or more of the GIFs designated Os11g40100, Os12g31350, Os03g52320 obtainable (preferably obtained) from

Oryza sativa or may be one or more of the GIFs designated AtGIF1, AtGIF2 or AtGIF3 obtainable (preferably obtained) from *Arabidopsis thaliana*.

In one embodiment the GIF used in accordance with the present invention may be AtGIF2, wherein AtGIF2 i) comprises the amino acid shown herein as MQQQQSPQMFPMPVPSIPPANNITTEQIQKYLDENKKLIMAIMENQNLGKLAECAQY QALLQKNLMYLAADIADAQPPPTPGPSPSTAVAAQMATPHSGMQPPSYFMQHPQA SPAGIFAPRGPLQFGSPLQFQDPQQQQQIHQQAMQGHMGIRPMGMTNNGMQHA MQQPETGLGGNVGLRGGKQDGDGQGGKDDGK (SEQ ID No. 96) or an amino acid sequence having at least 80% identity therewith; or

ii) is encoded by the nucleotide sequence:

ATGCAGCAGCAGCAGTCTCCGCAAATGTTTCCGATGGTTCCGTCGATTCCCCCT
GCTAACAAACATCACTACCGAACAGATCCAAAAGTACCTTGATGAGAACAAGAAG
CTGATTATGGCCATCATGGAAAACCAGAATCTCGGTAAACTTGCTGAGTGCGCC
CAGTACCAAGCTCTTCTCCAGAAGAACTTGATGTATCTTGCTGCAATTGCTGATG
CTCAACCCCCACCACCTACGCCAGGACCTTCACCATCTACAGCTGTGCTGCC
CAGATGGCAACACCGCATTCTGGGATGCAACCACCTAGCTACTTCATGCAACAC
CCACAAGCATCCCCTGCAGGGATTTTCGCTCCAAGGGGTCCTTTACAGTTTGGT
AGCCCACTCCAGTTTCAGGATCCGCAACAGCAGCAGCAGATACATCAGCAAGC
TATGCAAGGACACATGGGGATTAGACCAATGGGTATGACCAACAACGGGGATGC
AGCATGCGATGCAACAACCAGAAACCGGTCTTGAGGAAACGTGGGGCTTAGA
GGAGGAAAGCAAGATGGAGCAGATGGACAAGGAAAAGATGATGGCAAGTGA
(SEQ ID No. 90), or

iii) is encoded by a nucleotide sequence which is at least 70%, preferably 80%, more preferably 90%, even more preferably 95% identical with SEQ ID No. 90; or

iv) is encoded by a nucleotide sequence which hybridizes under stringent conditions with SEQ ID No. 90.

In one embodiment the GIF used in accordance with the present invention may be AtGIF3 wherein AtGIF3 i) comprises the amino acid shown herein as MQQSPQMIPMVLPSFPPTNNITTEQIQKYLDENKKLIMAILENQNLGKLAECAQYQA LLQKNLMYLAADIADAQPQPPAATLTSGAMTPQAMAPNPSSMQPPSYFMQHQAV GMAQQIPPGIFPPRGPLQFGSPHQFLDPQQQLHQQAMQGHMGIRPMGLNNNNGL QHQMHHHETALAANNAGPNDASGGGKPDGTNMSQSGADGQGGSAARHGGGDA KTEGK (SEQ ID No. 97) or an amino acid sequence having at least 80% identity therewith; or

ii) is encoded by the nucleotide sequence:

ATGCAGCAATCTCCACAGATGATTCCGATGGTTCTTCCTTCATTTCCGCCCACCA
ATAATATCACCACCGAACAGATCCAAAAGTATCTTGATGAGAACAAGAAGCTGAT
AATGGCGATCTTGAAAATCAGAACCTCGGTAACTTGCAGAATGTGCTCAGTA
TCAAGCTCTTCTCCAGAAGAATTTGATGTATCTCGCTGCAATTGCGGATGCTCAA
CCTCAGCCACCAGCAGCTACACTAACATCAGGAGCCATGACTCCCCAAGCAAT
GGCTCCTAATCCGTCATCAATGCAGCCACCACCAAGCTACTTCATGCAGCAACA
TCAAGCTGTGGGAATGGCTCAACAAATACCTCCTGGGATTTTCCCTCCTAGAGG
TCCATTGCAATTTGGTAGCCCGCATCAGTTTCTGGATCCGCAGCAACAGTTACA
TCAACAAGCTATGCAAGGGCACATGGGGATTAGACCAATGGGTTTGAATAATAA
CAACGGACTGCAACATCAAATGCACCACCATGAACTGCTCTTGCCGCAAACAA
TGCGGGTCCTAACGATGCTAGTGGAGGAGGTAAACCGGATGGGACCAATATGA
GCCAGAGTGGAGCTGATGGGCAAGGTGGCTCAGCCGCTAGACATGGCGGTGG
TGATGCAAAAAGTGAAGGAAAATGA (SEQ ID No. 91), or

iii) is encoded by a nucleotide sequence which is at least 70%, preferably 80%, more preferably 90%, even more preferably 95% identical with SEQ ID No. 91; or

iv) is encoded by a nucleotide sequence which hybridizes under stringent conditions with SEQ ID No. 91.

In one embodiment the GIF used in accordance with the present invention may be the GIF designated Os11g40100 wherein Os11g40100 i) comprises the amino acid shown herein as:

MQQQMAMPAGAAAAVPPAAGITTEQIQKYLDENKQLILAIENQNLGKLAECAQY
QAQLQKNLLYLAAIADAQPPQNPGRSQMMQPGATPGAGHYMSQVPMFPRTPL
TPQQMQEQQQQLQQQQAQALAFPGQMLMRPGTVNGMQSIPVADPARAADLQT
AAPGSVDGRGNKQDATSEPSGTESHKSAGADNDAGGDIAEKS (SEQ ID No. 98) or
an amino acid sequence having at least 80% identity therewith; or

ii) is encoded by the nucleotide sequence:

ATGCAGCAGCAGATGGCCATGCCGGCGGGGGCCGCCGCCGCCGCGGTGCCG
CCGGCGGCCGGCATCACCACCGAGCAGATCCAAAAGTATTTGGATGAAAATAA
ACAGCTAATTTTGGCCATCCTGGAAAATCAAAACCTAGGGAAGTTGGCTGAATG
TGCTCAGTACCAAGCTCAGCTTCAAAGAATCTCTTGTATCTGGCTGCCATTGCA
GATGCCCAACCACCTCAGAATCCAGGAAGTCGCCCTCAGATGATGCAGCCTGG
TGCTACCCCAGGTGCTGGGCATTACATGTCCCAAGTACCGATGTTCCCTCCAAG
AACTCCCTTAACCCACACAGATGCAAGAGCAGCAGCAGCAGCAACTCCAGC
AACAGCAAGCTCAGGCTCTAGCCTTCCCCGGCCAGATGCTAATGAGACCAGGT

ACTGTCAATGGCATGCAATCTATCCCAGTTGCTGACCCTGCTCGCGCAGCCGAT
 CTTCAGACGGCAGCACCGGGCTCGGTAGATGGCCGAGGAAACAAGCAGGATG
 CAACCTCGGAGCCTTCCGGGACCGAGAGCCACAAGAGTGCGGGAGCAGATAA
 CGACGCAGGCGGTGACATAGCGGAGAAGTCCTGA (SEQ ID No. 92),), or
 iii) is encoded by a nucleotide sequence which is at least 70%, preferably 80%, more
 preferably 90%, even more preferably 95% identical with SEQ ID No. 92; or
 iv) is encoded by a nucleotide sequence which hybridizes under stringent conditions
 with SEQ ID No. 92.

In one embodiment the GIF used in accordance with the present invention may be
 the GIF designated Os12g31350 wherein Os12g31350 i) comprises the amino acid
 shown herein as:

MQQQPMPMPAPPTAGITTEQIQKYLDENKQLILAIENQNLGKLAECAQYQAQL
 QKNLLYLAAIADTQPQTISRPMVPHGASPLGGQYMSQVPMFPPRTPLTPQQM
 QEQQLQQQAQLLSFGGQMMVRPGVVNGIPQLLQGEMHRGADHQNAGGATSEP
 SESHIRSTGTENDGGSDFGDQS (SEQ ID No. 99) or an amino acid sequence
 having at least 80% identity therewith; or

ii) is encoded by the nucleotide sequence:

ATGCAGCAGCAGCCGATGCCGATGCCCCGCGCAGGCGCCGCCGACGGCCGGAA
 TCACCACCGAGCAGATCCAAAAGTATCTGGATGAAAACAAGCAGCTTATTTTGG
 CTATTTTGGAAAATCAGAATCTGGGAAAAGTTGGCAGAATGTGCTCAGTATCAAG
 CGCAGCTTCAGAAGAATCTCTTGTACTTGGCTGCAATTGCTGATACTCAACCGC
 AGACCACTATAAGCCGTCCCCAGATGGTGCCGCATGGTGCATCGCCGGGGTTA
 GGGGGGCAATACATGTCGCAGGTGCCAATGTTCCCCCCCAGGACCCCTCTAAC
 GCCCCAGCAGATGCAGGAGCAGCAGCTGCAGCAACAGCAAGCCCAGCTGCTC
 TCGTTCGGCGGTCAGATGGTTATGAGGCCTGGCGTTGTGAATGGCATTCTCA
 GCTTCTGCAAGGCGAAATGCACCGCGGAGCAGATCACCAGAACGCTGGCGGG
 GCCACCTCGGAGCCTTCCGAGAGCCACAGGAGCACCGGCACCGAAAATGACG
 GTGGAAGCGACTTCGGCGATCAATCCTAA (SEQ ID No. 93), or

iii) is encoded by a nucleotide sequence which is at least 70%, preferably 80%, more
 preferably 90%, even more preferably 95% identical with SEQ ID No. 93; or
 iv) is encoded by a nucleotide sequence which hybridizes under stringent conditions
 with SEQ ID No. 93.

In one embodiment the GIF used in accordance with the present invention may be the GIF designated Os03g52320 wherein Os03g52320 i) comprises the amino acid shown herein as:

MQQQHLMQMNQGMGGYASPTTVTTDLIQQYLDENKQLILAILDNQNNNGKVEECA
RNQAKLQHNLMYLAAIADSQPPQTAAMSQYPSNLMMQSGARYMPQQSAQMMA
PQSLMAARSSMMYAQPALSPLQQQQQQQAAAAHGQLGMGSGGTTSGFSILHGEAS
MGGGGGGGGAGNSMMNAGVFSDFGRGGGGGGGKEGSTLSVDVRGANSGAQSG
DGEYLGTEEEGS (SEQ ID No. 100) or an amino acid sequence having at least 80% identity therewith; or

ii) is encoded by the nucleotide sequence:

ATGCAGCAGCAACACCTGATGCAGATGAACCAGGGCATGATGGGGGGATATGC
TTCCCCTACCACCGTCACCACTGATCTCATTGAGCAGTATCTGGATGAGAACAA
GCAGCTGATCCTGGCCATCCTTGACAACCAGAACAATGGGAAGGTGGAAGAGT
GCGCTCGGAACCAAGCTAAGCTCCAGCACAATCTCATGTACCTCGCCGCCATC
GCCGACAGCCAGCCGCCGCGAGACGGCCGCCATGTCCAGTATCCGTCTGAACC
TGATGATGCAGTCCGGGGCGAGGTACATGCCGCGAGCAGTCGGCGCAGATGAT
GGCGCCGCGAGTCGCTGATGGCGGCGAGGTCTTCGATGATGTACGCGCAGCCG
GCGCTGTGCGCGCTCCAGCAGCAGCAGCAGCAGCAGCGCGCGGCGGCGGCAC
GGGCAGCTGGGCATGGGCTCGGGGGGCGACCACCAGCGGGTTCAGCATCTCTCC
ACGGCGAGGCCAGCATGGGCGGCGGCGGCGGCGGCGGTGGCGCCGGTAACA
GCATGATGAACGCCGGCGTGTCTCCGACTTCGGACGCGGCGGCGGCGGCGG
CGGCAAGGAGGGGTCCACCTCGCTGTCCGTGACGTCCGGGGCGCCAACCTCC
GGCGCCAGAGCGGCGACGGGGAGTACCTCAAGGGCACCGAGGAGGAAGGC
AGCTAG (SEQ ID No. 94), or

iii) is encoded by a nucleotide sequence which is at least 70%, preferably 80%, more preferably 90%, even more preferably 95% identical with SEQ ID No. 94; or

iv) is encoded by a nucleotide sequence which hybridizes under stringent conditions with SEQ ID No. 94.

Furthermore, the inventors have demonstrated that overexpression of *GIF1*, *GIF2*, and *GIF3* promotes cell proliferation and leaf size and that *GIF2* and *GIF3* proteins are functional equivalents of *GIF1* (see Figure 43 in combination with Figure 9).

Previously Horiguchi et al. (2005) have shown that overexpression of the *GIF1/AN3* gene stimulates cell proliferation as well, leading to enlarged leaves by about 20%.

These results suggest that all of the GIF genes function redundantly as positive regulators of cell proliferation, thereby determining plant organ size.

Therefore the use of any GIF gene in accordance with the present invention is contemplated herein.

In addition combinations of GIF genes are also contemplated herein.

In one aspect, preferably the sequence is in an isolated form. The term "isolated" means that the sequence is at least substantially free from at least one other component with which the sequence is naturally associated in nature and as found in nature.

In one aspect, preferably the sequence is in a purified form. The term "purified" means that the sequence is in a relatively pure state – e.g. at least about 90% pure, or at least about 95% pure or at least about 98% pure.

The terms "nucleotide sequence" or "nucleic acid" as used herein refers to an oligonucleotide sequence or polynucleotide sequence, and variants, homologues, fragments and derivatives thereof (such as portions thereof). The nucleotide sequence may be of genomic or synthetic or recombinant origin, which may be double-stranded or single-stranded whether representing the sense or anti-sense strand.

The terms "nucleotide sequence" or "nucleic acid" in relation to the present invention includes genomic DNA, cDNA, synthetic DNA, and RNA. Preferably it means DNA, more preferably cDNA sequence coding for the present invention.

In a preferred embodiment, the nucleotide sequence when relating to and when encompassed by the *per se* scope of the present invention does not include the native nucleotide sequence according to the present invention when in its natural environment and when it is linked to its naturally associated sequence(s) that is/are also in its/their natural environment. For ease of reference, this preferred embodiment shall be called the "non-native nucleotide sequence" or "non-native nucleic acid"

Typically, the nucleotide sequence or nucleic acid encompassed by scope of the present invention is prepared using recombinant DNA techniques (i.e. recombinant

DNA). However, in an alternative embodiment of the invention, the nucleotide sequence or nucleic acid could be synthesised, in whole or in part, using chemical methods well known in the art (see Caruthers MH *et al.*, (1980) *Nuc Acids Res Symp Ser* 215-23 and Horn T *et al.*, (1980) *Nuc Acids Res Symp Ser* 225-232).

Due to degeneracy in the genetic code, nucleotide sequences may be readily produced in which the triplet codon usage, for some or all of the amino acids encoded by the original nucleotide sequence, has been changed thereby producing a nucleotide sequence with low homology to the original nucleotide sequence but which encodes the same, or a variant, amino acid sequence as encoded by the original nucleotide sequence. For example, for most amino acids the degeneracy of the genetic code is at the third position in the triplet codon (wobble position) (for reference see Stryer, Lubert, *Biochemistry*, Third Edition, Freeman Press, ISBN 0-7167-1920-7) therefore, a nucleotide sequence in which all triplet codons have been "wobbled" in the third position would be about 66% identical to the original nucleotide sequence. However, the amended nucleotide sequence would encode for the same, or a variant, primary amino acid sequence as the original nucleotide sequence.

Therefore, the present invention in some embodiments further relates to any nucleotide sequence that has alternative triplet codon usage for at least one amino acid encoding triplet codon, but which encodes the same, or a variant, polypeptide sequence as the polypeptide sequence encoded by the original nucleotide sequence.

Furthermore, specific organisms typically have a bias as to which triplet codons are used to encode amino acids. Preferred codon usage tables are widely available, and can be used to prepare codon optimised genes. Such codon optimisation techniques are routinely used to optimise expression of transgenes in a heterologous host.

The present invention also encompasses the use of sequences which have identity or similarity with the sequences according to the present invention.

Here, the term "identity" means an entity having a certain identity with the amino acid sequences and the nucleotide sequences. Identity means the percentage of amino acids or bases that are the same in one sequence when compared with another sequence.

Here, the term "similarity" means an entity having similar chemical properties/functions. Hence the term similarity takes into account conservative changes.

In the present context, a sequence which has a certain percentage identity or similarity is taken to include a sequence which may be at least 90% identical, preferably at least 95, 96, 97, 98 or 99% identical to a sequence of the present invention (the subject sequence). Typically, the sequences will comprise the same sequences that code for the active sites etc. as the subject sequence.

Identity or similarity comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. The available computer programs can calculate % identity and % similarity between two or more sequences.

% identity may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap.

This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % identity therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (Devereux *et al* 1984 *Nuc. Acids Research* 12 p387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 *Short Protocols in Molecular Biology*, 4th Ed – Chapter 18), FASTA (Altschul *et al.*, 1990 *J. Mol. Biol.* 403-410) and the GENWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999, *Short Protocols in Molecular Biology*, pages 7-58 to 7-60).

However, for some applications, it is preferred to use the GCG Bestfit program. A new tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see *FEMS Microbiol Lett* 1999 **174**(2): 247-50; *FEMS Microbiol Lett* 1999 **177**(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

Although the final % identity can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Alternatively, percentage identity may be calculated using the multiple alignment feature in DNASIS™ (Hitachi Software), based on an algorithm, analogous to CLUSTAL (Higgins DG & Sharp PM (1988), *Gene* **73**(1), 237-244).

Generally percentage identity is calculated over at least 50, preferably at least 100, preferably at least 200 contiguous bases or residues. Preferably the percentage identity is calculated using the full length sequence.

Once the software has produced an optimal alignment, it is possible to calculate % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in amino acid properties (such as polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues) and it is therefore useful to group amino acids together in functional groups. Amino acids can be grouped together based on the properties of their side chain alone. However it is more useful to include mutation data as well. The sets of amino acids thus derived are likely to be conserved for structural reasons. These sets can be described in the form of a Venn diagram (Livingstone C.D. and Barton G.J. (1993) "Protein sequence alignments: a strategy for the hierarchical analysis of residue conservation" *Comput. Appl. Biosci.* 9: 745-756)(Taylor W.R. (1986) "The classification of amino acid conservation" *J. Theor. Biol.* **119**; 205-218). Conservative substitutions may be made, for example according to the table below which describes a generally accepted Venn diagram grouping of amino acids.

SET		SUB-SET	
Hydrophobic	F W Y H K M I L V A G C	Aromatic	F W Y H
		Aliphatic	I L V
Polar	W Y H K R E D C S T N Q	Charged	H K R E D
		Positively charged	H K R
		Negatively charged	E D

Small	V C A G S P T N D	Tiny	A G S
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The nucleotide sequences for use in the present invention may include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones and/or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the nucleotide sequences described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of nucleotide sequences of the present invention.

The present invention also encompasses the use of nucleotide sequences that are complementary to the sequences presented herein, or any derivative, fragment or derivative thereof. If the sequence is complementary to a fragment thereof then that sequence can be used as a probe to identify similar coding sequences in other organisms etc.

Polynucleotides which are not 100% identical to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing DNA libraries made from a range of individuals, for example individuals from different populations. In addition, other homologues may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other species, and probing such libraries with probes comprising all or part of any one of the sequences in the attached sequence listings under conditions of medium to high stringency. Similar considerations apply to obtaining species homologues and allelic variants of the polypeptide or nucleotide sequences of the invention.

Variants and strain/species orthologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be

performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used.

The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences.

Alternatively, such polynucleotides may be obtained by site directed mutagenesis of characterised sequences. This may be useful where for example silent codon sequence changes are required to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides.

Polynucleotides (nucleotide sequences) of the invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term polynucleotides of the invention as used herein.

Polynucleotides such as DNA polynucleotides and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a stepwise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated procedures are readily available in the art.

Longer polynucleotides will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

The present invention also encompasses sequences that are complementary to the nucleic acid sequences of the present invention or sequences that are capable of hybridising either to the sequences of the present invention or to sequences that are complementary thereto.

The term “hybridisation” as used herein shall include “the process by which a strand of nucleic acid joins with a complementary strand through base pairing” as well as the process of amplification as carried out in polymerase chain reaction (PCR) technologies.

Preferably, the hybridisation is determined under stringent conditions (e.g. 50°C and 0.2xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na₃citrate pH 7.0}).

Suitably, the hybridisation may be determined under high stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na₃citrate pH 7.0}).

The present invention also relates to nucleotide sequences that can hybridise to the nucleotide sequences of the present invention (including complementary sequences of those presented herein).

A skilled person will understand that the modified *GRF3*-orthologue may be obtainable from any plant. In a preferred embodiment the *GRF3*-orthologue is obtainable, preferably obtained, from one or more of the plants selected from the group consisting of: *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Glycine max*, *Medicago truncatula*, *Populus trichocarpa*, *Prunus persica*, *Carica papaya*, *Triticum aestivum*, *Sorghum bicolor*, *Gossypium hirsutum*, sugar cane (*Saccharum spp.*), *Panicum virgatum*, *Helianthis annuus*, *Beta vulgaris*, and *Brassica* species.

In an even more preferred embodiment the *GRF3*-orthologue is obtainable, preferably obtained, from one or more of the plants selected from the group consisting of: *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Glycine max*, *Medicago truncatula*, *Populus trichocarpa*, *Prunus persica*, *Carica papaya*,

The nucleic acid, vector or construct according to the present invention may be transformed in to any (host) plant.

The plant, plant cell or plant tissue according to the present invention may be a monocotyledonous (monocot) plant or a dicotyledonous (dicot) plant.

In one embodiment the plant, plant cell or plant tissue according to the present invention may be a dicot.

A monocot plant may, for example, be selected from the families Arecaceae, Amaryllidaceae or Poaceae. For example, the plant may be a cereal crop, such as wheat, rice, barley, maize, oat, sorghum, rye, onion, leek, millet, buckwheat, turf grass, Italian rye grass, switchgrass, Miscanthus, sugarcane grass, false oat grass, fescue, Bermuda grass, brome, heath grass, meadow grasses (e.g. naturally mixed grassland swards, orchard grass, rye grass, Timothy-grass) or Festuca species

A dicot plant which may be selected from the families including, but not limited to Asteraceae, Brassicaceae (e.g. *Brassica napus*), Chenopodiaceae, Cucurbitaceae, Leguminosae (Caesalpiniaceae, Aesalpiniaceae Mimosaceae, Papilionaceae or Fabaceae), Malvaceae, Rosaceae or Solanaceae. For example, the plant may be selected from lettuce, sunflower, Arabidopsis, spinach, water melon, squash, oilseed rapeseed (including canola), cabbage, broccoli, kale, turnip, rutabaga (swede), tomato, potato, capsicum, tobacco, cotton, legumes sugar beet, okra, apple, rose, strawberry, alfalfa (lucerne), birdsfoot trefoil, bean, soybean, field (fava) bean, pea, lentil, peanut, chickpea, coffee, cocoa, apricots, apples, pears, peach, grape vine or citrus species.

Also included are biofuel and bioenergy crops such as sugar cane, oilseed rape/oilseed rape, linseed, jatropha, oil-palm, copra and willow, eucalyptus, poplar, poplar hybrids. Miscanthus or gymnosperms, such as loblolly pine. Also included are crops for silage (e.g. forage grass species or forage maize), grazing or fodder (pasture grasses, clover, alsike clover, red clover, subterranean clover, white clover, sanfoin, alfalfa), fibres (e.g. cotton, flax), building materials (e.g. pine, oak), pulping (e.g. poplar), feeder stocks for the chemical industry (e.g. high erucic acid oil seed rape, linseed), rubber plants, and crops for amenity purposes (e.g. turf grasses for sports and amenity surfaces), ornamentals for public and private gardens (e.g. species of Angelonia, Begonia, Catharanthus, Euphorbia, Gazania, Impatiens, Nicotiana, Pelargonium, Petunia, Rosa, Verbena, and Viola) and flowers of any plants for the cut-flower market (such as tulips, roses, daffodils, lilies, stallions, gerbera, carnations,

chrysanthemums, irises, gladioli, alstromerias, marigold, sweet pea, freesia, anemone poppy).

Preferably, the plant, plant cell or plant tissue, or host plant is a crop plant. By crop plant is meant any plant which is grown on a commercial scale for human or animal consumption or use for other non-food/feed use. Preferred plants are corn (maize), millet, wheat, Durum wheat, rice, oilseed rape (or canola), sorghum, sugar cane, soybean, sunflower, potato, tomato, barley, rye, oats, pea, bean, field bean, sugar beet, oil-palm, groundnut, peanut, cassava, alfalfa, clover, copra, raisin, coffee, cotton, lettuce, banana, broccoli or other vegetable brassicas.

In one embodiment the plant, plant cell or plant tissue, or host plant is *Brassica*, suitably *Brassica oleracea* (e.g. broccoli or other vegetable *Brassicas*).

The plant may be a tree such as eucalyptus, poplar, or conifer such as *Picea* species (e.g. spruce) or *Pinus* species (pines), a hardwood tree species such as teak, a plantation tree such as rubber (*Hevea*), palm tree (date- or oil-palm) or *jatropha* or an orchard fruit tree (such as plum, peach, apple and pear).

EXAMPLES

While the invention disclosed herein is described in general above, and those skilled in the art based on that disclosure would be enabled to practice this invention, including its best mode, the following examples are provided to further support this written description and enabling disclosure. The details of these examples are, however, non-limiting. For an understanding of the scope of this invention, reference should be had to the appended claims and their equivalents.

Transgenes

See Table 1 for a list of binary plasmids used.

Table 1

Vector	Construct	Arabidopsis chromosome: start-end ^a	Purpose
pJP123	35S: <i>miR396b</i>	5: 13628907 – 13629319	Overexpression of <i>miR396b</i> stem-loop
pRER31	<i>GRF3</i>	2: 15274101 – 15270081	Genomic <i>GRF3</i>

		  CGT TCA AGA AAG CCT GTG GAA 	
pRER32	rGRF3	2: 15274101 – 15270081	Genomic mutant <i>GRF3</i>
		  CGT TCT AGA AAA CCA GTA GAG 	
pRER35	rGRF2	4: 17729683 – 17725302	Genomic mutant <i>GRF2</i>
		  CGT TCT AGA AAA CCG GTC GAA 	
pJD16	35S: <i>GIF1</i>	5: 10647830 – 10649620	Overexpression of <i>AtGIF1</i>

* Highlighted in bold (yellow), nucleotides annealing with miR396. Underlined, mutagenized residues. Highlighted in italics (red), upstream and downstream codons.

Expression analysis

First, 0.5-1.0 µg of total RNA was treated with RQ1 RNase-free DNase (Promega). Then, first-strand cDNA synthesis was carried out using SuperScript III reverse transcriptase (Invitrogen). PCR reactions were performed in a Mastercycler ep *realplex* thermal cycler (Eppendorf) using SYBR Green I (Roche) to monitor double-stranded (ds)DNA synthesis. Quantitative (q)PCR of each gene was carried out for at least three biological replicates, with technical duplicates for each biological replicate. The relative transcript level was determined for each sample, normalized using *PROTEIN PHOSPHATASE 2A* cDNA level (Czechowski et al., 2005). Primer sequences are given in Table 2:

Table 2. Relevant *Locus* IDs and oligonucleotide primers used in RT-qPCR .

Gene	Locus ID	Forward primer	Reverse primer
<i>AtGRF3</i>	AT2G36400	GTCTTCGCTGGCCACAAGTATT	TGTTGCTGTTGTAGTGGTGGCT
<i>AtGRF2</i>	AT4G37740	CACATCAACAGAGGCCGTCATcg	AACCGGAGATTCCTTGGGTTGTAAG
<i>AtGIF1</i>	AT5G28640	TTGGACGAAAACAAATCGTTGA	CTGTTGCTGTTGAGTCGCTTGT

Small RNA analysis

RNA was extracted using TRIzol reagent (Invitrogen). Total RNA was resolved on 17% polyacrylamide gels under denaturing conditions (7 M urea). Blots were hybridized using either radioactively labelled or digoxigenin end-labelled locked nucleic acid (LNA) oligonucleotide probes designed against miR396 (Exiqon, Denmark).

Alternatively, miR396 levels were determined by stem-loop RT-qPCR, as described previously (Chen et al., 2005). The sequences of the oligonucleotides used were: retrotranscription stem-loop oligo, 5'GTCTCCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGGAGACMAGTTC3'; PCR forward primer, 5'GGCGGTTCCACAGCTTTCTT3'; and PCR reverse primer, 5'TGGTGCAGGGTCCGAGGTATT3'.

Microarray analyses

Total RNA was extracted from the aerial part of seedlings grown on plates for 10 days using the RNeasy plant mini kit (QIAGEN). Microarray analyses using the Affymetrix ATH1 platform were performed on two biological replicates as described (Schmid et al., 2005). Differentially expressed genes were identified using per-gene variance, calculated using logit-T (Lemon et al., 2003). The corresponding fold change of the transcripts was obtained by expression estimates using gcRMA (www.bioconductor.org), a modification of the robust multi-array analysis (RMA) algorithm (Irizarry et al., 2003). The expression of gene groups was assessed by gene set enrichment analysis using GSEA-P 2.0 (Subramanian et al., 2007; Subramanian et al., 2005).

Microscopic observations

Tissue was fixed in FAA and embedded in paraffin. Sections 10 µm thick were stained with Toluidine Blue.

To obtain paradermal views of palisade cells, leaves were fixed with FAA and cleared with chloral hydrate solution as described (Horiguchi et al., 2005). Palisade leaf cells were observed using differential interference contrast (DIC) microscopy.

In situ hybridization

DIG-labelled sense and antisense probes were synthesized by transcription with T7 or SP6 RNA polymerase with the DIG RNA labelling kit (SP6/T7) (Roche) using cloned cDNAs of *GRF2* and *HISTONE H4* as templates. For the miR396 probe, LNA oligonucleotides (Exiqon) were end labelled with the DIG oligonucleotide 3'-end labelling kit (Roche). Shoot apices from 15-day-old plants grown in short photoperiods were dissected and fixed in FAA. Paraffin-embedded material was sectioned to 8 µm thickness. Hybridization and detection were performed as previously described (Palatnik et al., 2003).

GUS assays

To visualize the activity of the reporters, transgenic plants were subjected to GUS staining, according to Donnelly et al. (Donnelly et al., 1999). Stained tissue was paraffin embedded, sectioned and mounted in Canada balsam.

Accession numbers

A list of relevant AGI locus identifiers is provided in Table 2. The accession number for the microarray experiments is GSE11250.

Table 3. *GRF* expression in 35S:*miR396b* plants compared to that in wild type, as estimated by Affymetrix microarray

Description	Relative expression*
<i>GRF1</i>	0.81
<i>GRF2</i>	0.58
<i>GRF3</i>	0.73
<i>GRF4</i>	WA
<i>GRF5</i>	0.89
<i>GRF6</i>	A
<i>GRF7</i>	0.57
<i>GRF8</i>	A
<i>GRF9</i>	NP

*Fold change relative to wild type, normalized with gcRMA. The average of two biological replicates for each genotype is shown.

A, a gene termed 'absent' by MAS 5.0 software (Affymetrix); NP, not present in ATH1 arrays; WA, wrongly annotated in ATH1 arrays.

EXAMPLE #1

A *miR396* resistant version of *GRF3* increases plant size and biomass accumulation

The GRF family of transcription factors comprises nine members in Arabidopsis (Kim et al., 2003). Seven of them, including *GRF3*, have a target site for *miR396* (Jones-Rhoades and Bartel, 2004). *GRF* loss-of-function or overexpression of *miR396* have been shown to reduce cell number in Arabidopsis leaves (Horiguchi et al., 2005) (Kim et al., 2003) (Kim and Kende, 2004; Liu et al., 2009).

To study the importance of miR396 in restricting *GRF3* expression, two *GRF3* genomic fragments were introduced into *Arabidopsis thaliana* plants. One of them contained the wild-type *GRF3* gene (Figure 1, top panel), while the second harbored a modified *GRF3* sequence in which the miRNA-targeting motif was altered through synonymous mutations that prevent miR396 targeting (named *rGRF3*, Figure 1, middle panel).

The wild-type sequence of *GRF3* contains a region complementary to miR396 with a high interaction energy ($\Delta G = -33.9$ kcal/mol). In contrast, the modified *GRF3* sequence (*rGRF3*), which includes five changes from the wild-type sequence (A→U, G→A, U→A, a G→A and a A→G modifications) does not have a clear miR396 interacting site, as the interaction energy is reduced from -33.9 kcal/mol to -14.4 kcal/mol. The complete sequence of *GRF3* is detailed in Figures 21 and 22. The complete sequence of *rGRF3* is detailed in Figure 35. The full sequence and a map of the binary vector used (named RER32, see Table 1) can be found in Figures 40 and 42, respectively.

Transgenic *Arabidopsis* plants expressing *rGRF3* had bigger leaves and rosettes than wild-type or transgenic plants expressing the miR396-regulated *GRF3* sequence (Figures 3, 5, 13 and 17). They also accumulate more biomass, as judged by the fresh and dry weight of leaves and rosettes (Figure 6). In general, it was observed that *rGRF3* nearly doubled the size and weight of the first leaf with respect to wild-type plants (Figure 3). The FFD domain of *rGRF3* increased the activity of the protein (Figure 54).

Plants expressing *rGRF3* also had a thicker stem with higher dry weight and growth speed than wild-type plants (Figure 12). It was observed that the stem diameter increased 20% in *rGRF3* plants with respect to wild type (Figure 12).

Materials and methods

The *Arabidopsis thaliana* Columbia (Col-0) accession was used as a wild type. All transgenics are in the Col-0 background. Plants were grown in long photoperiods (16 hr light/8 hr dark) or in short photoperiods (8 hr light/16 hr dark) at 23 °C. See Table 1 for a list of binary plasmids generated and details on how transgenic plants were prepared. The miRNA target motif in *AtGRF3* was altered introducing synonymous

mutations in a cloned *AtGRF3* wild type genomic fragment using the QuikChange® Site Directed Mutagenesis Kit (Stratagene).

All constructs were cloned in the binary vector pCHF3 (Jarvis et al., 1998). T-DNA constructs were introduced into *Agrobacterium tumefaciens* strain ASE and *Arabidopsis* transgenic plants were obtained by floral-dip.

Leaf area was measured by first taking a photograph of detached fully-expanded leaves, and then measuring the foliar area with the NIH software ImageJ.

To determine biomass accumulation, complete rosettes or individual leafs were weighed to measure fresh weight. Then, tissue was dried at 60°C during 2 days and dry weight was measured. To determine stem growth, elongation was measured starting with 5 cm long stems during 10 days until full extension was reached. Maximum elongation speed was calculated from the elongation plot. Stem biomass accumulation was estimated by measuring the dry weight of 10 cm long fully elongated stem segments. Finally stem diameter was measured in the lower part of the stem, 0.5 cm above the rosette.

The FFD motif in *AtGRF3* was altered introducing mutations in a cloned *AtGRF3* cDNA using the QuikChange® Site Directed Mutagenesis Kit (Stratagene). The *rGRF3* cDNA native sequence "TTC TTT GAC GAT TGG" coding for FFDDW was mutagenized to "GCT GCT GAC GAT GCT" coding for **AADDA**, replacing all aromatic amino acids for alanines in the FFD motif. The wt (*rGRF3* (FFD)) and mutagenized (*rGRF3*(AAD)) genes were placed under the *AtGRF3* promoter.

Conclusions

- Transgenic *Arabidopsis* plants transformed with the miR396-resistant version of *GRF3* (named *rGRF3*) show a striking increase in leaf size and biomass accumulation in comparison to wild-type plants or transgenic plants expressing a *GRF3* sequence with a miR396 binding site.
- *rGRF3* promotes growth of other tissues as well, such as the stems.
- The FFD domain increases the activity of *rGRF3*.

EXAMPLE #2**Overexpression of *GIF1* enhances the effect of *rGRF3***

The *GRF* family of transcription factors comprises nine members in *Arabidopsis* (Kim et al., 2003). Seven of them have a target site for miR396 (Jones-Rhoades and Bartel, 2004). Mutations in different *GRFs* or overexpression of miR396 have been shown to reduce cell number in *Arabidopsis* leaves (Horiguchi et al., 2005; Kim and Kende, 2004; Rodriguez et al., 2010). The *GRFs* interact with *GRF-INTERACTING FACTORS* (*GIFs*), a small gene family composed by three members (*GIF1*, *GIF2* and *GIF3*) encoding proteins with homology to the human SYT transcriptional co-activator (Kim and Kende, 2004). Inactivation of *GIF1*, also known as *ANGUSTIFOLIA 3* (*AN3*), produces narrower leaves as a result of a reduction in cell proliferation in a similar way to *GRF*-deficient plants (Horiguchi et al., 2005).

Transgenic plants overexpressing *GIF1* (Figures 1 and 2) from the 35S viral promoter (named 35S:*GIF1*) were prepared. The full sequence and a map of the binary vector used (named JD16, see Table 1) can be found in Figures 39 and 41, respectively. These plants were similar to wild-type plants. Later, 35S:*GIF1* was crossed to plants expressing *rGRF3* (*GRF3* insensitive to miR396, described in example #1). The resulting plants co-overexpressing *rGRF3* and *GIF1* (named *rGRF3* x 35S:*GIF1*) were analyzed in more detail (Figures 1 and 2).

Upon analysing the biomass productivity of these plants it was found that *rGRF3* in combination with *GIF1* overexpression produce plants with larger leaves and accumulate more than double fresh and dry weight than wild-type plants (Figures 3 and 13). The performance of *rGRF3* x *GIF* was better than *rGRF3* alone.

Materials and methods

The *Arabidopsis thaliana* Columbia (Col-0) accession was used as a wild type. All transgenics are in the Col-0 background. Plants were grown in long photoperiods (16 hr light/8 hr dark) or in short photoperiods (8 hr light/16 hr dark) at 23 °C. See Table 1 for a list of binary plasmids generated and details on how transgenic plants were prepared. The miRNA target motif in *AtGRF3* was altered introducing synonymous mutations in a cloned *AtGRF3* wild type genomic fragment using the QuikChange® Site Directed Mutagenesis Kit (Stratagene). All constructs were cloned in the binary

vector pCHF3 (Jarvis et al., 1998). T-DNA constructs were introduced into *Agrobacterium tumefaciens* strain ASE and Arabidopsis transgenic plants were obtained by floral-dip.

For expression analysis by RT-PCR, RNA was prepared from apices of 20-day-old plants grown in short photoperiods, including developing leaves smaller than 3 mm. 0.5 to 1.0 µg of total RNA was treated with RQ1 RNase- free Dnase (Promega). Then, first-strand cDNA synthesis was carried out using SuperScript™ III Reverse Transcriptase (Invitrogen). PCR reactions were performed in a Mastercycler® ep realplex thermal cycler (Eppendorf) using SYBRGreen I (Roche) to monitor dsDNA synthesis. qPCR for each gene was done on at least 3 biological replicates with technical duplicates for each biological replicate. The relative transcript level was determined for each sample, normalized using PROTEIN PHOSPHATASE 2A cDNA level (Czechowski et al., 2005).

Leaf area and fresh and dry weight measurements were made as in Example#1.

Conclusions

-The *rGRF3* performance in plant productivity can be enhanced by co-overexpression of *GIF1*.

EXAMPLE #3

Delayed leaf senescence and increased drought resistance of *rGRF3* plants

As shown in Example #1, *rGRF3* plants produces bigger leaves than wild-type plants, accumulating more biomass. This effect is enhanced by co-overexpression of *rGRF3* and *GIF1* (*rGRF3* x 35S:*GIF1*) (see example #2 for further details). In addition, the inventors observed that *rGRF3* and *rGRF3/35S:GIF1* stay green for a longer period of time than wild-type plants (Figure 4).

To test whether there this delay in leaf senescence in *rGRF3* and *rGRF3* x 35S:*GIF1* transgenic plants, a dark-induced senescence experiment was performed. Incubation of detached leaves in the dark induces senescence and this process can be followed by measuring the decrease in the maximum efficiency of photosystem II (PSII) photochemistry (Fv/Fm) as described previously (Baker, 2008; Schommer et al., 2008). To do this, the fifth leaf of wild-type, *rGRF3*, 35S:*GIF1* and *rGRF3* x 35S:*GIF1*

were collected and kept in the dark, and Fv/Fm was measured every day. As detailed in Figure 4, there is no difference between wild-type and 35S:*GIF1* plants. However, senescence in *rGRF3* leaves starts 2 days after the wild-type leaves. Interestingly, leaves that co-overexpress high levels of both *rGRF3* and *GIF1* showed an even larger delay in Fv/Fm decay, indicating that overexpression of *GIF1* enhances even further the senescence delay of *rGRF3* plants.

Furthermore, the performance of the transgenic under water deprivation (Figure 14) was assayed. 25 days-old plant of 35S:*miR396*, wild-type, *rGRF3*, 35S:*GIF1* and *rGRF3* x 35S:*GIF1* were deprived of water for 2 weeks. Then, the plants were irrigated once a week. *MiR396* over-expressers, wild-type and 35S:*GIF1* were severely affected in their growth by the end of the water deprivation and subsequently to it (Figure 14). In contrast, both *rGRF3* and *rGRF3* x 35S:*GIF1* lines recovered and developed well following the water deprivation (Figure 14).

Materials and methods

To study leaf senescence, fifth-fully expanded leaves were detached and stored in darkness. Dark-induced senescence was followed by measuring Maximal Photochemical Efficiency (Fv/Fm) of Photosystem II, as described (Baker, 2008). In the water deprivation assays, plants were grown in long photoperiods (16 hour light/8 hour dark) at 23°C. When the plants were 25 day-old, they were deprived of water for two weeks. After that, the plants were irrigated once a week. Pictures were taken when the plants were 50 day-old.

Conclusions

-*rGRF3* plants have a delay in leaf senescence. This effect is further enhanced by the co-overexpression of *GIF1*.

-*rGRF3* plants are more tolerant to water deprivation.

EXAMPLE #4

Expression from tissue specific promoters improves *rGRF3* performance in plant productivity

The *GRF* family of transcription factors comprises nine members in Arabidopsis (Kim et al., 2003). Seven of them, including *GRF3*, have a target site for *miR396* (Jones-Rhoades and Bartel, 2004). *MiR396* is expressed at low levels in the meristem and

leaf primordia, and then it steadily accumulates with the development of the leaf, in concert with the retreat of cell proliferation (Rodriguez et al., 2010). It is shown in Examples #1 and #2 that the abolishment of miR396-repression of *GRF3* in Arabidopsis generates plants with a significant increase in biomass accumulation and a delay in senescence.

To study if it is possible to improve further performance of *rGRF3* this miR396 resistant version of *GRF3* was expressed from tissue specific promoters. The promoters of *AS1* (*ASYMMETRIC LEAVES 1*) and *ANT* (*AINTEGUMENTA*), which are known to be specifically expressed in the proliferative stages of leaf development, were selected (Figure 50).

Transgenic Arabidopsis plants transformed with the vectors *AS1:rGRF3* and *ANT:rGRF3* had bigger leaves than wild-type plants and even than plants expressing the *rGRF3* from the native *GRF3* promoter (Figures 18 and 51). These plants also had thicker stems (Figure 19).

Interestingly, the expression of *rGRF3* from *ANT* and *AS1* promoters had only a minor effect on leaf senescence, and less than that observed in *rGRF3* plants expressing plants from the endogenous promoter (Figures 20 and 54).

Expression of *rGRF3* from the *ANT* and *AS1* promoters shows similar apical dominance (Figure 52) to wild-type plants.

Materials and methods

The Arabidopsis thaliana Columbia (Col-0) accession was used as a wild type. All transgenics are in the Col-0 background. Plants were grown in long photoperiods (16 hr light/8 hr dark) or in short photoperiods (8 hr light/16 hr dark) at 23 °C. See Table 1 for a list of binary plasmids generated and details on how transgenics plants were prepared. The miRNA target motif in *AtGRF3* was altered introducing synonymous mutations in a cloned *AtGRF3* wild type genomic fragment using the QuikChange® Site Directed Mutagenesis Kit (Stratagene).

Leaf area was measured by first taking a photograph of detached fully expanded leaves, and then measuring the foliar area with the NIH software ImageJ. Finally stem diameter was measured in the lower part of the stem, 0.5 cm above the rosette.

Senescence phenotype was analyzed by dark-induced senescence experiments on fully expanded leaves #5. Pictures were taken just after the full expanded leaves were detached from the rosette (Day 1) and after they were incubated 6 days in darkness (Day 6). Chlorophyll degradation is an indicator of senescence (Schommer et al., 2008).

Conclusions

- Expression of *rGRF3* from tissue specific promoters can improve its performance in plant productivity.
- Expression of *rGRF3* from tissue specific promoters can uncouple the different functions of *GRF3*, such as the control of leaf size and senescence.

EXAMPLE #5

***rGRF3* outperforms *rGRF2* in increasing plant size and biomass accumulation**

As was previously showed, high levels of miR396 reduce considerably leaf (Rodriguez et al., 2010). On the other hand, plants expressing a miR396 resistant version of *GRF2* (*rGRF2*) accumulate high levels of *GRF2* that cause a slight decrease of leaf size (Rodriguez et al., 2010). It has been shown in Examples #1 and #2 that *rGRF3* plants also accumulate more biomass than wild-type plants. This example shows that *rGRF3* significantly outperforms *rGRF2* in increasing plant size and biomass accumulation.

To compare biomass accumulation in *rGRF2* and *rGRF3* lines, we measured fresh and dry weight of 40 day-old rosettes of 35S:*miR396*, wild-type, *rGRF2* and *rGRF3* plants (Figure 6). Plants with high levels of miR396 had a reduction of plant biomass of 25%. *rGRF2* plants have only a minor increase in biomass accumulation that was not statistically significant (Figure 6). *rGRF3* rosettes accumulated nearly 40% more biomass compared to wild-type plants, which is statistically significant (Figure 6).

Another remarkable difference between *rGRF2* and *rGRF3* plants was observed when comparing leaf morphology. Leaves of *rGRF2* plants have downward “rolling” shape, while leaves of *rGRF3* plants are bigger than wild-type leaves with no major change in leaf morphology (Figure 10). In this way, *rGRF3* produced plants with bigger leaves without affecting leaf morphology.

To analyze the correlation between biomass accumulation and *GRF* levels in *rGRF2* and *rGRF3* plants, one independent line of each *rGRF* transgenic line was selected. Then, *GRF2* and *GRF3* mRNA levels were measured by RT-PCR and the dry weight of 1 month-old rosettes of *rGRF2* and *rGRF3* plants. It was observed that a 25-fold increase in *GRF2* mRNA levels in *rGRF2* plants produced a biomass increase of only 30% (Figure 11). On the contrary, only a 2.5 fold increase in *GRF3* mRNA levels in *rGRF3* plants resulted in almost twice as much biomass accumulation compared with wild type Col-0 (Figure 11).

As a further comparison the effect of *rGRF2* or *rGRF3* expression with wild-type (Figure 55) was compared. Leaf area in *rGRF3* expressing plants was almost double that of wild-type and increased compared to *rGRF2* (Figure 55). When *rGRF2* was placed under the control of the *GRF3* promoter the increase in leaf area was not as significant as in *rGRF3*-expressing plants, showing that the differential activity of *rGRF3* and *rGRF2* is caused by their different primary sequences and not promoter strength and/or expression levels (Figure 55).

Materials and methods

The *Arabidopsis thaliana* Columbia (Col-0) accession was used as a wild-type. All transgenics are in the Col-0 background. Plants were grown in long photoperiods (16 hr light/8 hr dark) or in short photoperiods (8 hr light/16 hr dark) at 23 °C. See Table 1 for a list of binary plasmids generated and details on how transgenics plants were prepared. The miRNA target motif in *AtGRF3* or *AtGRF2* was altered introducing synonymous mutations in a cloned *AtGRF3* wild type genomic fragment using the QuikChange® Site Directed Mutagenesis Kit (Stratagene).

All constructs were cloned in the binary vector pCHF3 (Jarvis et al., 1998). T-DNA constructs were introduced into *Agrobacterium tumefaciens* strain ASE and *Arabidopsis* transgenics plants were obtained by floral-dip.

To determine biomass accumulation, complete rosettes were weighed to measure fresh weight. Then, tissue was dried at 60°C during 2 days and dry weight was measured.

For expression analysis by RT-PCR, RNA was prepared from apices of 20-day-old plants grown in short photoperiods, including developing leaves smaller than 3 mm. 0.5 to 1.0 µg of total RNA was treated with RQ1 RNase- free Dnase (Promega).

Then, first-strand cDNA synthesis was carried out using SuperScript™ III Reverse Transcriptase (Invitrogen). PCR reactions were performed in a Mastercycler® ep realplex thermal cycler (Eppendorf) using SYBRGreen I (Roche) to monitor dsDNA synthesis. qPCR for each gene was done on at least 3 biological replicates with technical duplicates for each biological replicate. The relative transcript level was determined for each sample, normalized using PROTEIN PHOSPHATASE 2A cDNA level (Czechowski et al., 2005).

Conclusions

- High levels of *rGRF2* are required to slightly increase plant biomass (e.g., 25 times more *GRF2* caused 30% biomass increase).
- Moderate increases of *GRF3* expression in *rGRF3* plants caused a high increase in biomass accumulation (e.g., 2.5 times more *GRF3* caused 85% biomass increase).
- High levels of *rGRF2* affect leaf development.
- Expression of *rGRF3* in plants leads to approximately 2 times increase in leaf area compared to wild-type
- Increased leaf area in *rGRF3* compared to *rGRF2* is dependent on the primary sequence of the genes and not a result of promoter strength

EXAMPLE #6

Arabidopsis *GRF3* and *GIF1* homologues are found in crop plants: *GRF* family in *Arabidopsis thaliana* and other plant species

The *GROWTH-REGULATING FACTOR (GRF)* family of transcription factors is a plant specific family of proteins defined by the presence of two highly conserved protein motifs, the QLQ and WRC (Kim et al., 2003). The QLQ domain is involved in protein-protein interactions with *GRF-INTERACTING FACTORS* proteins, and the WRC domain contains a functional nuclear localization signal and a DNA-binding motif consisting of three conserved cysteines and one histidine (Kim and Kende, 2004). The *GRF* family of transcription factors comprises nine members in *Arabidopsis* (Kim et al., 2003) (Figures 21 and 22), 12 in *Oryza sativa* (Choi et al., 2004) (Figures 23 and 24) and 14 in *Zea mays* (Zhang et al., 2008) (Figures 25 and 26). Besides, *GRFs* can be found in many other plant species (Zhang et al., 2011) (See selected examples from *Glycine max*, *Medicago truncatula*, *Prunus persica*, *Carica papaya* and *Populus trichocarpa* in Figures 27 to 34).

At least two other conserved regions can be found in *GRF* coding sequences. First, at the nucleotide level, only a subgroup of the *GRFs* from each species contains a miR396-target site. For example, only 7 of the nine *GRFs* found in *Arabidopsis* are miR396 targets (Figure 7 and 8) (Jones-Rhoades and Bartel, 2004).

Second, only a subgroup of the *GRFs* of each species contains the FFD conserved motif (Figure 8). For example, in *Arabidopsis* only *GRF3* and *GRF4* have the FFD motif. Furthermore, *GRFs* containing the miR396-targeting motif and the FFD motif, and with high homology to *Arabidopsis GRF3* can be found in rice, maize and many other plant species (Figures 7, 8, 22, 24, 26 31-34, 38).

GRFs expression patterns in Arabidopsis thaliana and Zea mays

GRF3 expression pattern was analyzed by RT-qPCR in developing leaves (Figure 15, left). The fifth rosette leaf was collected at three-day intervals, starting from the day that it first became visible (~1 mm) to the naked eye, which was 16 days after sowing (DAS). Next, the level of *GRF3* was determined by RT-qPCR. It was observed that this transcription factor was expressed during the early stages of leaf development (Figure 15, left). An expression atlas of *Arabidopsis* development (Schmid et al., 2005) indicates that mitosis specific genes are expressed in proliferating tissues (Figure 15, right). Consistent with a role of the *GRFs* as positive regulators of cell proliferation during organ growth, their expression profile is very similar to that of the mitosis specific genes (shown for *GRF3* in Figure 15, right).

To confirm the functional equivalency between *Arabidopsis* and *Zea mays GRFs* their expression patterns during maize leaf development were analysed using the Maize eFP browser (Li et al., 2010; Winter et al., 2007). As detailed in Figure 16, maize *GRFs*, in the same way as *Arabidopsis GRFs*, are coexpressed with mitosis specific genes.

GRF-INTERACTING FACTORS in Arabidopsis and crop plants

As described in example #2, *rGRF3* performance in plant productivity can be greatly enhanced by cooverexpression of *GRF-INTERACTING FACTOR 1*. This gene belongs to a small gene family composed by three members (*GIF1*, *GIF2* and *GIF3*) in *Arabidopsis*. Also, *GIF1* homologs are readily found in other plant species, such as

rice (Figure 9). The three *GIFs* in *Arabidopsis* are highly redundant, as mutants in *GIF1* can be complemented by the overexpression of *GIF2* or *GIF3* (Figure 43) (Lee et al., 2009). These results suggest that the enhancement of the *rGRF3* phenotype by overexpression of *GIF1* is also achieved by co-overexpression of *GIF2* and *GIF3*.

Materials and methods

RNA was prepared from apices of 20-day-old plants grown in short photoperiods, including developing leaves smaller than 3 mm. 0.5 to 1.0 µg of total RNA was treated with RQ1 RNase- free Dnase (Promega). Then, first-strand cDNA synthesis was carried out using SuperScript™ III Reverse Transcriptase (Invitrogen). PCR reactions were performed in a Mastercycler® ep realplex thermal cycler (Eppendorf) using SYBRGreen I (Roche) to monitor dsDNA synthesis. qPCR for each gene was done on at least 3 biological replicates with technical duplicates for each biological replicate. The relative transcript level was determined for each sample, normalized using PROTEIN PHOSPHATASE 2A cDNA level. Primer sequences are given in Table 2.

GRFs sequences from *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays* were obtained from Genbank using the accession numbers provided in the literature (Choi et al., 2004; Kim et al., 2003; Zhang et al., 2008). Pairwise sequence alignments and calculations of percentage of identity and similarity were performed with NEEDLE using the Needleman-Wunche alignment algorithm (Rice et al., 2000). Multiple sequence alignments of protein sequences were performed using MCOFFE (Moretti et al., 2007). The PHYLIP package version 3.67 (Felsenstein, 1989) was used to perform 100 bootstrap replicas of a neighbor joining (NJ) tree based on a JTT distance matrix. Trees were visualized using TreeView 1.6.6. (Page, 1996).

Conclusions

- GRFs in general and homologs (orthologues) of *GRF3* in particular exist in many plant species.
- GIFs also exist in many plant species.
- According to its function as a positive regulator of cell proliferation, *GRF3* is co-expressed with mitosis genes during leaf development in *Arabidopsis*. As expected for functional equivalent genes, *Zea mays* *GRFs* expression also co-expressed with mitosis genes during leaf development.

-The enhancement of the *rGRF3* phenotype by overexpression of *GIF1* might also be achieved by homologs (orthologues) from Arabidopsis and crop plants.

EXAMPLE #7

Introduction of *rGRF3* and *rGRF3*+*GIF* into *Brassica oleracea*

Materials and Methods

Plant material

A genetically uniform doubled haploid *Brassica oleracea* genotype, DH 1012 (Sparrow et al., 2004) was used in this study. This genotype is derived from a cross between a rapid cycling *B. oleracea alboglabra* (A12) and a *B. oleracea italica* Green Duke (GD33).

Bacterial strains

Transformations were carried out using the *Agrobacterium tumefaciens* strain AGL1 harbouring the appropriate plasmids pBRACT114 *rGRF3* and pBRACT114 *rGRF3*:*GIF1* and (see Figure 44) containing the neomycin phosphotransferase (*nptII*) selectable marker gene driven by the 35S promoter and the gene(s) of interest (namely *rGRF3* driven by its own promoter; or the combined construct which contained both *rGRF3* driven by its own promoter, and additionally *GIF* driven by the 35S promoter, respectively).

The cloning procedure used to make the transformation vector pBRACT114-*rGRF3* *GIF1* is described below. pBRACT114-*rGRF3* *GIF1* contains both the *rGRF3* gene driven by its native promoter and the coding region of *GIF1* over-expressed by the CaMV 35S promoter.

Digestion of ~1.7 µg of p*GRF3*:*GRF3r* DNA in a 20 µl total volume reaction with *PvuII* (Invitrogen) in the appropriate buffer was performed at 37 °C for 1 hour in a water bath. A 4950 bp fragment containing the *rGRF3* native promoter, coding region, 3'UTR and terminator was isolated by gel extraction.

The Brassica transformation vector pBRACT114 (www.bract.org) is based on pGreen (Hellens et al., 2000) and is Gateway™ (Invitrogen) compatible. Approximately 1µg of pBract114 was digested with restriction enzyme *StuI* (Roche) in the appropriate buffer for 1 hour at 37 °C. The linearised vector was dephosphorylated by incubation

at 37 °C for a further hour with shrimp alkaline phosphatase (SAP). The SAP was denatured by heating to 65°C for 15 minutes.

An overnight ligation reaction was performed at 14°C and contained the *rGRF3* fragment and the linear pBRACT114 at a 3:1 ratio respectively. Five units of T4 ligase (Invitrogen) were used in the 10 µl blunt end ligation. To 50 µl of ccdB competent *E. coli* cells (Invitrogen) 2 µl of the ligation reaction was added and transformation by heat shock. The cells were grown in 250 µl of SOC medium for 1 hour at 37 °C and shaken at 200 rpm. 20 µl and 100 µl of the culture was spread onto plates of solid LB medium (Sambrook and Russel, 2001) containing appropriate selection and incubated overnight at 37°C.

E. coli colonies were screened by direct colony PCR to ensure that they contained pBRACT114 with the insert in the desired orientation. Twelve PCR positive single colonies were transferred to 10 ml of liquid LB media containing the appropriate selection and incubated at 37°C shaken 220 rpm overnight. Plasmid DNA was isolated using a mini-prep kit (Qiagen). The integrity of the construct known as pBRACT114-*rGRF3* was confirmed by enzyme digestion and sequencing of the insertion sites.

Phase two of the cloning process to create pBRACT-*rGRF3 GIF1* used the Gateway™ (Invitrogen) system to recombine the coding region of *GIF1* downstream of the CaMV 35S promoter. The coding region of *GIF1* was amplified by PCR using high fidelity Platinum™ polymerase (Invitrogen) and Topo T/A cloned into the Gateway™ entry vector pCR8/GW/Topo® TA (Invitrogen). To 50 µl of chemically competent *E. coli* DH5-α cells (Invitrogen) 2 µl of the Topo reaction was added and transformation by heat shock. The cells were grown in 250 µl of SOC medium for 1 hour at 37 °C and shaken at 200 rpm. 20 µl and 100 µl of the culture was spread onto plates of solid LB medium (Sambrook and Russel, 2001) containing appropriate selection and incubated overnight at 37°C.

E. coli colonies were screened by direct colony PCR to ensure that they contained pCR8 with the *GIF1* amplicon in the desired orientation. Six PCR positive single colonies were transferred to 10 ml of liquid LB medium containing the appropriate selection and incubated at 37°C shaken 220 rpm overnight. Plasmid DNA was isolated using a plasmid mini-prep kit (Qiagen). The entry vector pCR8-*GIF1* was

checked by enzyme digestion. Sequencing of the entire *GIF1* coding region was performed to ensure its integrity.

A Gateway™ LR recombination reaction was performed to insert the *GIF1* coding region into pBRACT114-r*GRF3* between the gateway sites downstream of the CaMV 35S promoter. The 10 µl LR reaction contained ~ 100 ng of pBRACT114-r*GRF3* + 35 ng of pCR8-*GIF1* with 2 µl Gateway® LR Clonase™ II enzyme mix™ (Invitrogen) in TE buffer. The LR reaction was incubated at room temperature overnight. A proteinase K treatment was performed at 37 °C for 10 minutes. To 50 µl of chemically competent *E. coli* DH5-α cells (Invitrogen) 1 µl of the LR reaction was added and transformation by heat shock. The cells were grown in 250 µl of SOC medium for 1 hour at 37 °C and shaken at 200 rpm. 20 µl and 100 µl of the culture was spread onto plates of solid LB medium (Sambrook and Russel, 2001) containing appropriate selection and incubated overnight at 37°C.

Twelve single colonies were transferred to 10 ml of liquid LB media containing the appropriate selection and incubated at 37°C shaken 220 rpm overnight. Plasmid DNA was isolated using a mini-prep kit (Qiagen). The integrity of the construct known as pBRACT114-r*GRF3* *GIF1* was confirmed by enzyme digestion and sequencing of the *GIF1* insertion sites.

The plasmid pBRACT-r*GRF3* *GIF1* along with its helper plasmid pSoup (Hellens et al., 2000) was transformed into *Agrobacterium tumefaciens* strain AGL1 by electroporation. The plasmid p*GRF3*:r*GRF3* was also transformed by electroporation into *A. tumefaciens*. Briefly, 100 ng of plasmid DNA was added to 40 µl of electro-competent *A. tumefaciens* cells in a pre-chilled electroporation cuvette with 2mm electrode separation. The cells were electroporated in a GenePulser (Biorad) with the following settings 2.50kV, 25µFD and 400 Ohms. Immediately 300 µl of liquid LB medium was added to recover the cells, these were grown at room temperature, shaken at 180 rpm for 6 hours. The *A. tumefaciens* cultures were spread onto solid LB medium (Sambrook and Russel, 2001) containing appropriate selection and incubated at 28°C for 48 hours. Single colonies were selected and used to inoculate 10 ml of liquid LB media containing the appropriate antibiotics and incubated at 28°C, shaken at 200 rpm for 48 hours. Glycerol stocks and standard inoculums were prepared and stored at -80 °C. The plasmids were checked once again, by enzyme digestion, prior to embarking on the *Brassica* transformation experiments.

The *A. tumefaciens* was streaked onto solid LB medium (Sambrook and Russel, 2001) containing appropriate selection (and incubated at 28°C for 48 hours. A single colony was transferred to 10 ml of liquid LB media containing the appropriate selection and transferred to a 28°C shaker for 48 hours. A 50 µl aliquot of the resulting bacterial suspension was transferred to 10 ml of MGL liquid medium with selection and grown over night in a 28°C shaker. Overnight cultures were spun down at 3,000 rpm for 5 minutes at R.T. before being re suspended in liquid MS medium. Suspensions of $O.D_{650} = 0.3$ were used for inoculations (dilutions made using liquid MS medium).

Plant transformation

Seeds were surface sterilised in 100 % ethanol for 2 minutes, 15 % sodium hypochlorite plus 0.1% Tween-20TM for 15 minutes and rinsed three times for 10 minutes in sterile distilled water. Seeds were germinated on full strength MS (Murashige and Skoog, 1962) plant salt base, containing 3 % sucrose and 0.8 % phytagar (Difco) at pH 5.6. Prior to pouring, filter-sterilised vitamins were added to the medium; myo-Inositol (100mg/l), Thiamine-HCL (10mg/l), Pyridoxine (1mg/l) and Nicotinic acid (1mg/l). Seeds were sown at a density of 15 seed per 90 mm petri dish and transferred to a 10°C cold room overnight before being transferred to a 23°C culture room under 16 hour day length with $70\mu\text{mol m}^{-2} \text{sec}^{-1}$ illumination.

Based on the transformation protocol developed for *Brassica napus* (Moloney et al. 1989), and further developed by BRACT (www.bract.org), cotyledonary petioles excised from 4-day-old seedlings were dipped into an overnight suspension of *Agrobacterium*. Explants were maintained, 10 explants per plate, on co-cultivation medium (germination medium supplemented with 2mg/l 6-benzylaminopurine); with the petioles embedded and ensuring the cotyledonary lamella were clear of the medium. Cultures were maintained in growth rooms at 23°C with 16 hour day length, under scattered light of $40\mu\text{mol m}^{-2} \text{sec}^{-1}$ for 72 hours. After 72 hours explants were transferred to selection medium (co-cultivation medium supplemented with 160 mg/l timentin (or appropriate *Agrobacterium* eliminating antibiotic) and 15 mg/l kanamycin as the selection agent. Controls were established on kanamycin-free medium, as explants that had, and had not, been inoculated with *Agrobacterium*.

Shoot isolation and plant regeneration

Regenerating green shoots were excised and transferred to Gamborgs B5 medium

(Gamborg et al. 1968), containing 1 % sucrose, 0.8% Phytagar, 160mg/l timentin and 50 mg/l kanamycin. Where dense multiple shoots were isolated, further sub-culturing was made after shoot elongation to ensure a main stem was isolated thus reducing the likelihood of escapes and the frequency of multi-stemmed plants when transferred to the glasshouse. Shoots were maintained on Gamborgs B5 medium until roots developed. Plantlets were then transferred to sterile peat pots (Jiffy No.7) to allow further root development, before being transferred to the glasshouse.

Plant maintenance and seed production

Transgenic plants were maintained in a containment lit glasshouse (of 16-hour photoperiod, +18/12°C day/night) and self-pollinated, to generate the T₁ seed. Plants were covered with clear, perforated 'bread-bags' (Cryovac (UK) Ltd) as soon as they came into flower to prevent cross-pollination. The background genotype DH1012 is a self-compatible genotype and daily shaking of the 'bread-bag' was carried out to facilitate pollination. Pods were allowed to develop on the plant until fully swollen and were harvested when pods had dried and turned brown. Harvested pods were threshed when dry, and seed stored in the John Innes Centre seed store (+ 1.5°C, 7-10 relative humidity).

Molecular analysis

Leaf tissue from putative transgenic shoots (*in vitro*) was used for initial DNA extractions to PCR test for presence of the transgenes.

Copy number analysis by multiplexed real time PCR

The copy number of the transgene was measured using multiplexed real time PCR (TaqMan) assays, carried out by 'iDNA genetics' (www.idnagenetics.com). The *nptII* target gene was detected using a Fam labelled, Tamra quenched probe, and simultaneously an internal positive control gene was detected using a Vic labelled, Tamra quenched probe. The reactions were carried out using 5-20ng of genomic DNA from each sample, in a 20 µl reaction volume, with each sample assayed twice. The cycle threshold (Cts) for the Fam and Vic signals were found for each tube, and the average DeltaCt (CtFam - CtVIC) calculated for each sample. The samples were ranked by DeltaCt (where high delta Ct relates to samples with low numbers of copies, and low DeltaCt to high numbers of copies). Plant samples were classified with respect to reference samples (of known copy number).

Preliminary investigations show that enhanced growth and improved plant productivity is obtained in *Brassica* plants comprising the *AtrGRF3* or *AtrGRF3:GIF1*

Figure 49 shows data comparing *Brassica oleracea* plants transformed with *Arabidopsis rGRF3* and control plants (without the *At rGRF3*). Transforming *Brassica oleracea* plants with *At rGRF3* significantly improved growth and productivity of the plants. For example, at flowering the stem width 10 cm above soil level and the maximum stem width at flowering were both significantly greater in *Brassica oleracea* plants transformed with *At rGRF3* compared with control plants. These results were significant using either the t-test ($p < 0.01$) or regression analysis ($p = 0.008$).

Figure 56 shows data for *Brassica oleracea* plants transformed with *Arabidopsis rGRF3* (*rGRF3*) and a control of regeneration (TC). The widest stem width at flowering is increased in *rGRF3* when compared to the control (Figure 56). The figure also shows that the 10 cm stem weight is increased in *rGRF3* when compared to the control (Figure 56).

Root growth of transgenic *Brassica oleracea* plants expressing *Arabidopsis rGRF3* was measured. To do this, wild-type and transgenic plants were grown in vertical MS plates. Root length was measured in at least 10 plants for each genotype from 4 to 7 days after sowing (Figure 57, left). From the slope of these lines, the root growth rate was estimated (Figure 57, right).

Conclusions

- Transgenic *Brassica oleracea* plants expressing *Arabidopsis rGRF3* and *rGRF3:GIF1* show enhanced growth and improved plant productivity.
- Transgenic *Brassica oleracea* plants transformed with the miR396-resistant version of *GRF3* (named *rGRF3*) show a striking increase in root growth.

EXAMPLE #8

Expression in *Arabidopsis* of *GRF3* orthologues from soybean and rice also increases plant biomass

In Example #6, *GRFs* from other species than *Arabidopsis* were described. To test if these *GRFs* behave in a similar way to *Arabidopsis rGRF3*, selected sequences were introduced into *Arabidopsis*. The *GRFs* with the highest homology to *At-rGRF3* and

containing a FFD motif and a miR396 target site were selected from rice (Figure 37) and soybean (Figure 36). The GRF3 from soybean and rice were uncoupled from miR396 control by introducing mutations in the miRNA binding site as described previously for *Arabidopsis GRF3*.

A vector expressing these sequences from the *Arabidopsis* GRF3 promoter was prepared and then, *Arabidopsis* transgenic plants were obtained. In a similar way to plants expressing *At-rGRF3*, transgenic *Arabidopsis* plants expressing *Os-rGRF4* and *Gm-rGRF* had bigger leaves than wild-type plants (Figure 46). These transgenic plants expressing the soybean and rice *rGRF3* orthologues also had a delay in leaf senescence (not shown).

Materials and methods

The *Arabidopsis thaliana* Columbia (Col-0) accession was used as a wild type control. All transgenics are in the Col-0 background. Plants were grown in long photoperiods (16 hr light/8 hr dark) or in short photoperiods (8 hr light/16 hr dark) at 23 °C. See Table 1 for a list of binary plasmids generated and details on how transgenics plants were prepared. The miRNA target motif in *OsGRF4* and *Gm-GRF* was altered introducing mutations using the QuikChange® Site Directed Mutagenesis Kit (Stratagene) as described previously for *Arabidopsis GRF3*. The mutated miR396 motif in *Os-GRF4* and *Gm-GRF* is shown Figures 37 and Figure 36 respectively.

All constructs were cloned in the binary vector pCHF3 (Jarvis et al., 1998). T-DNA constructs were introduced into *Agrobacterium tumefaciens* strain ASE and *Arabidopsis* transgenics plants were obtained by floral-dip.

Leaf area was measured by first taking a photograph of detached fully expanded leaves, and then measuring the foliar area with the NIH software ImageJ (as described in Example #1 and other examples above).

Conclusions

rGRF3 orthologues from species other than *Arabidopsis* (e.g. at least rice and soybean) species can also increase plant size and biomass accumulation.

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We claim:

1. A method of producing a plant, comprising transforming a plant with a construct comprising an isolated nucleic acid encoding a growth regulatory factor (AtGRF-3) of SEQ ID NO: 81, operably linked with a native AtGRF3 promoter and a terminator.
2. The method of claim 1 wherein the method further comprises overexpressing a nucleic acid encoding a GRF-interacting factor (GIF), wherein the GIF comprises SEQ ID NO: 95.
3. A plant cell comprising a construct as defined in any one of claims 1-2.

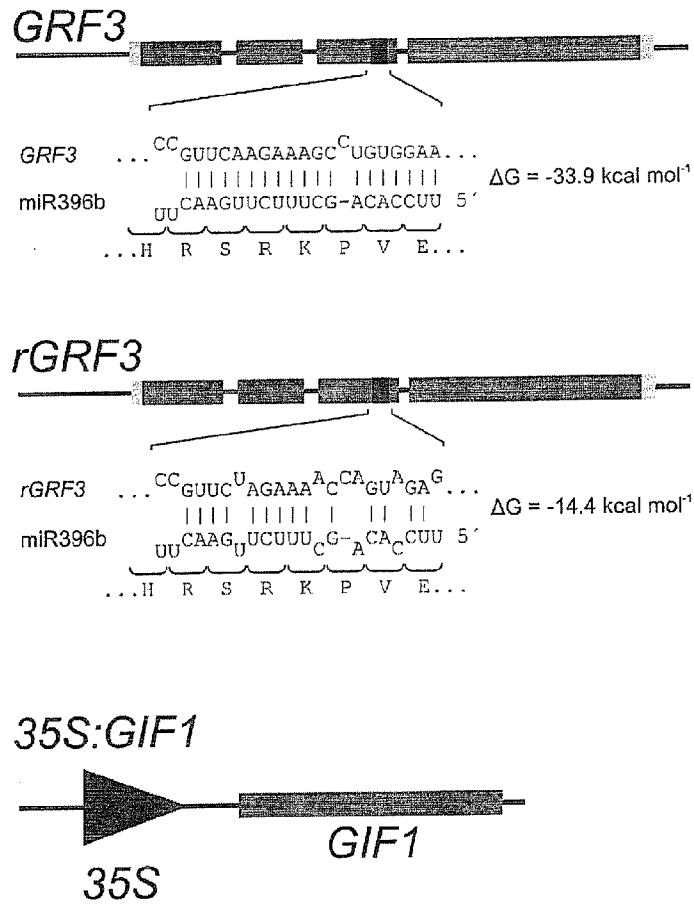


Figure 1

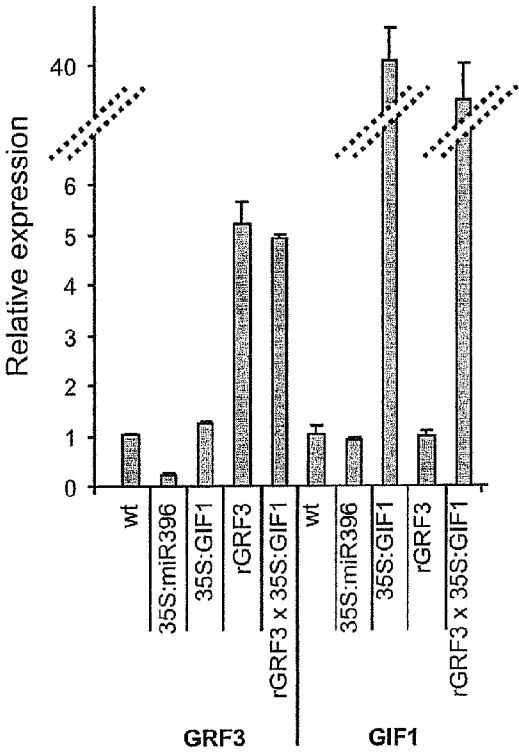


Figure 2

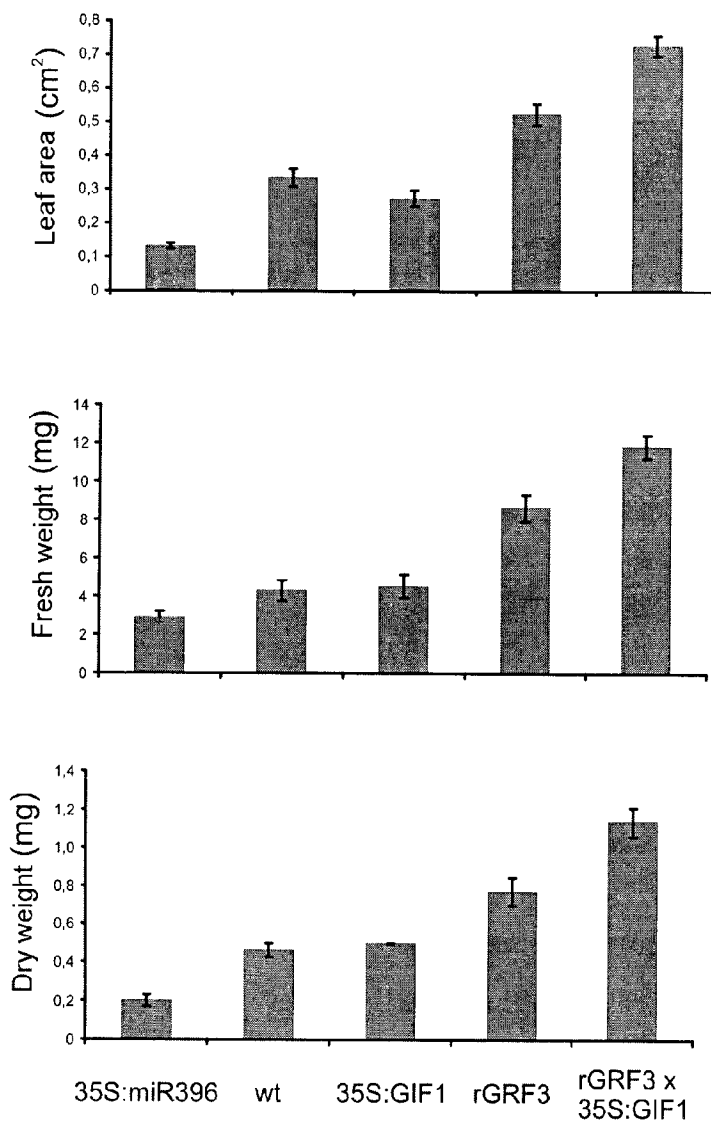
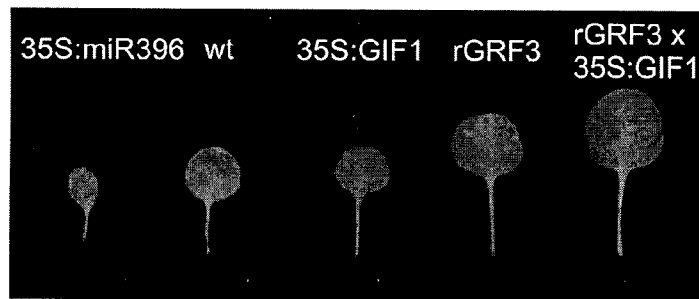


FIGURE 3

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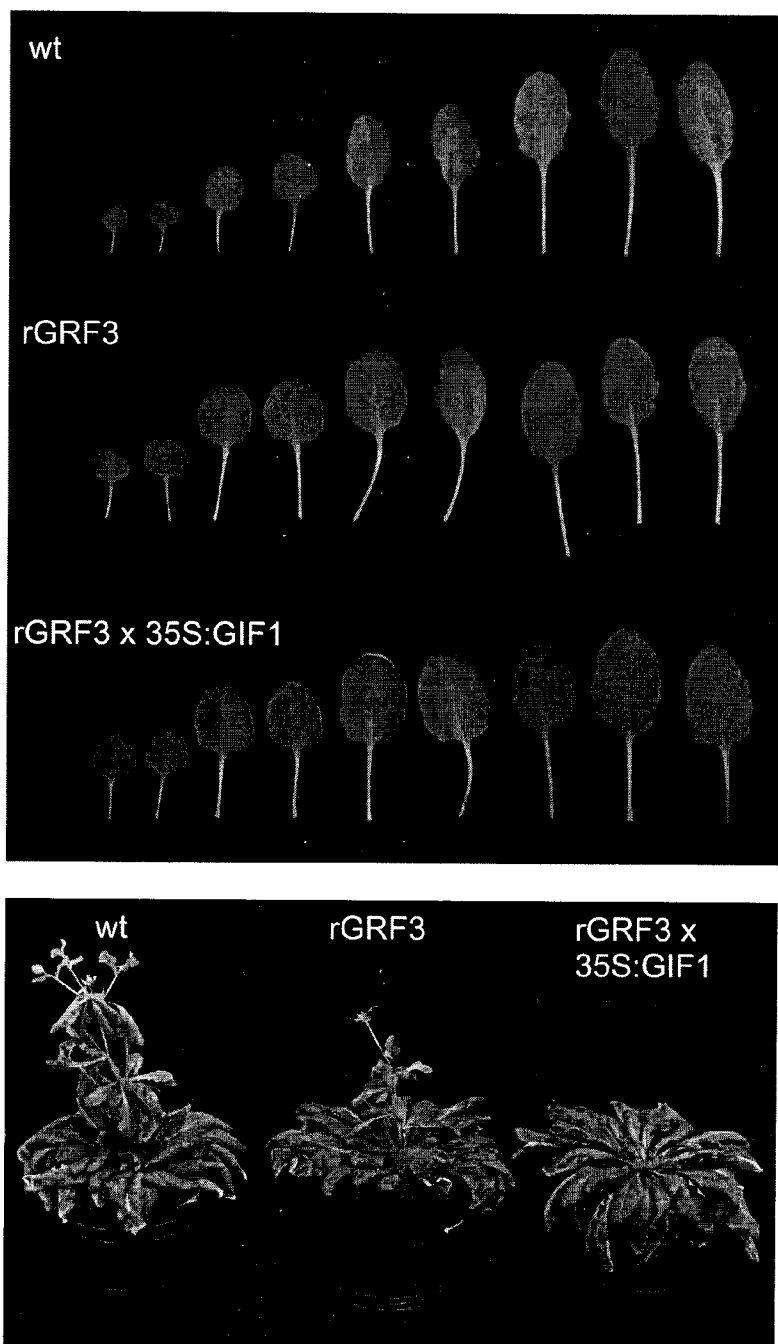
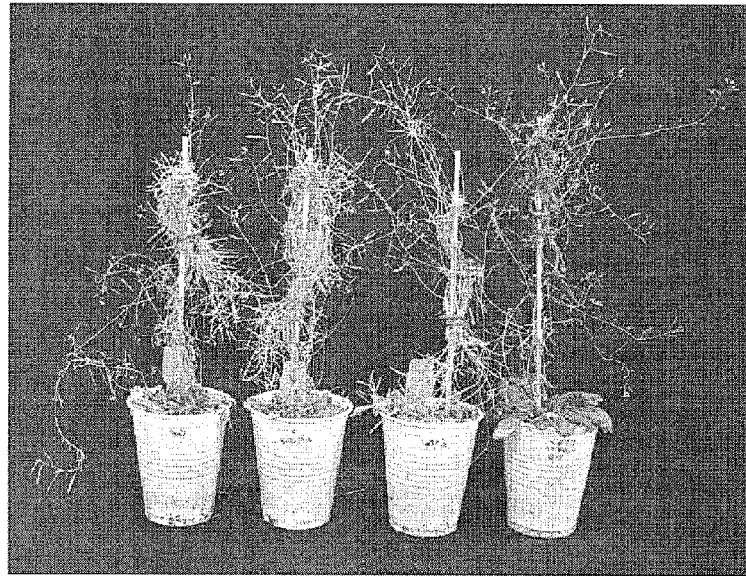


FIGURE 3 CONT.

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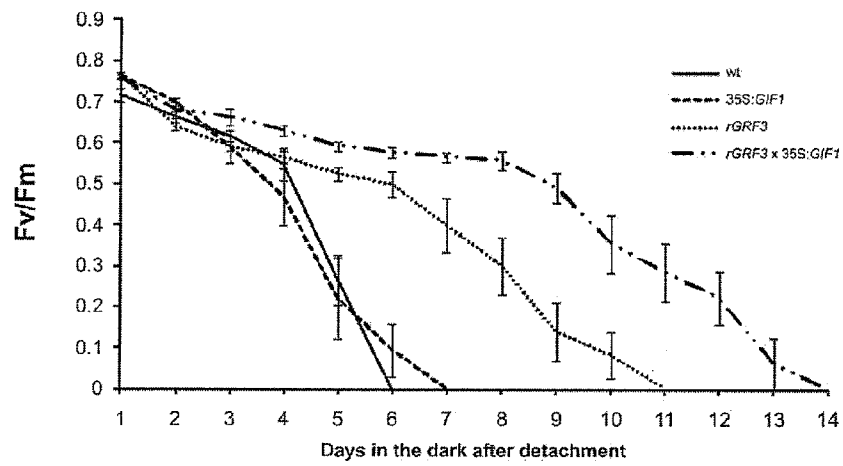


Figure 4

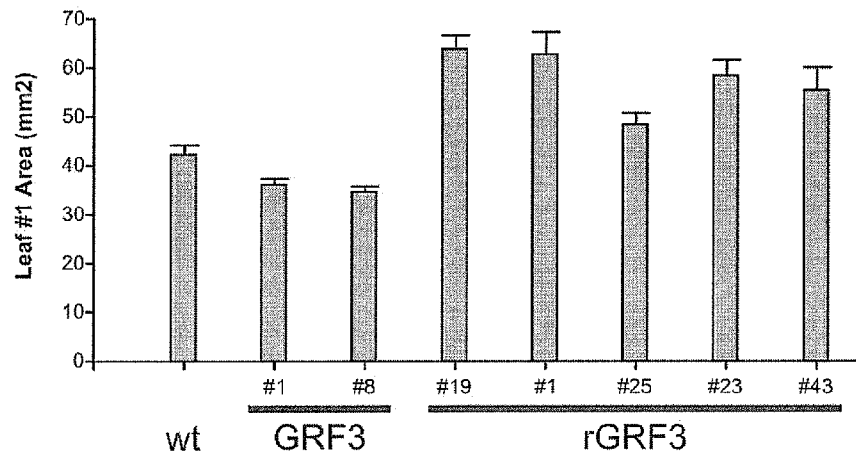


Figure 5

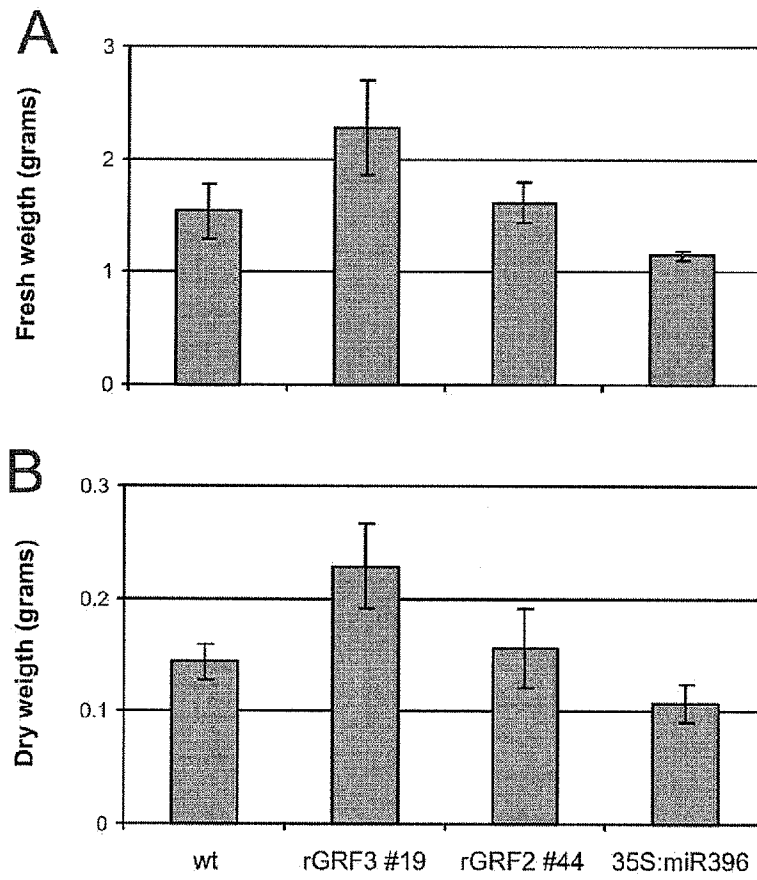


Figure 6

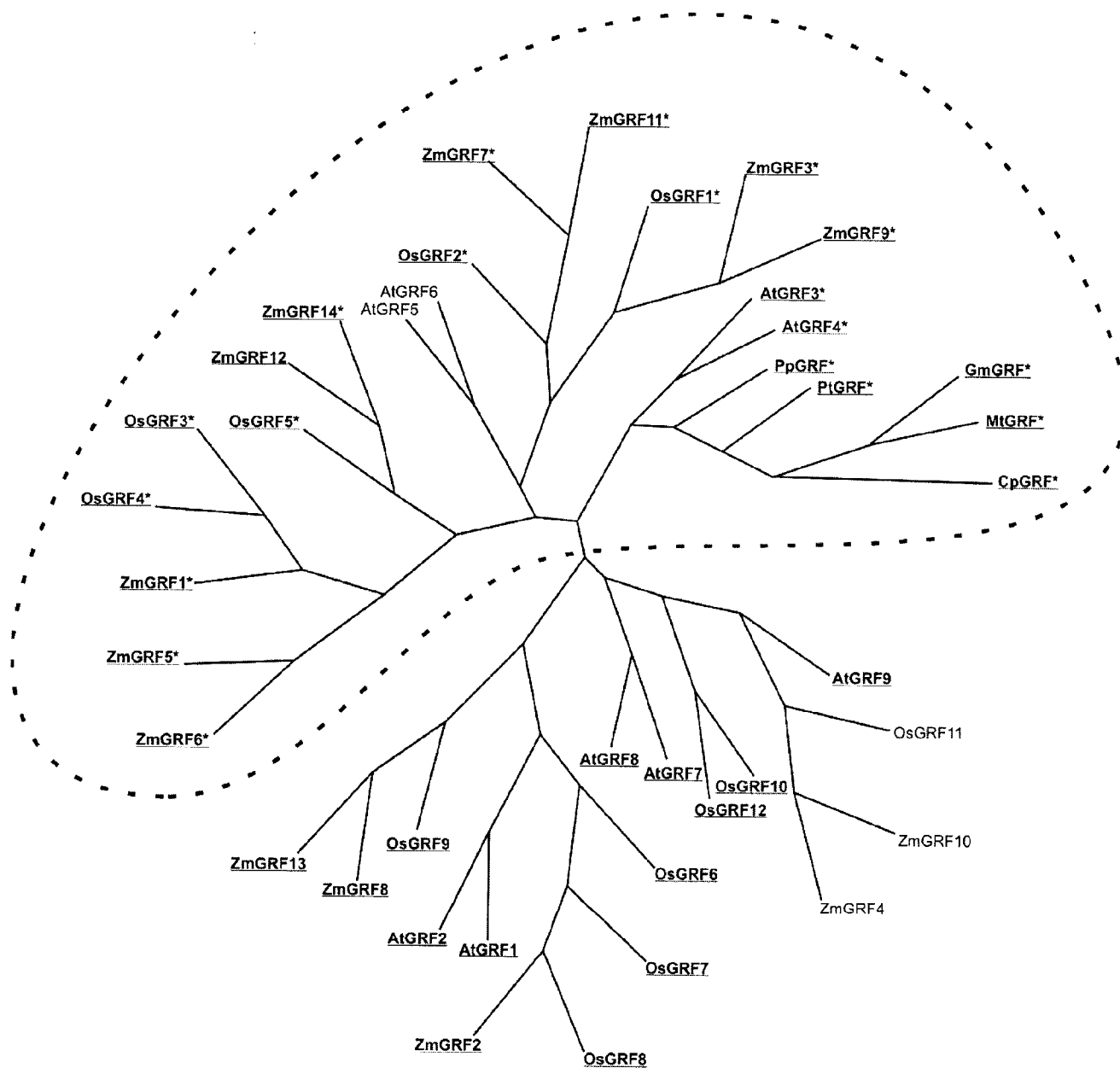


FIGURE 7

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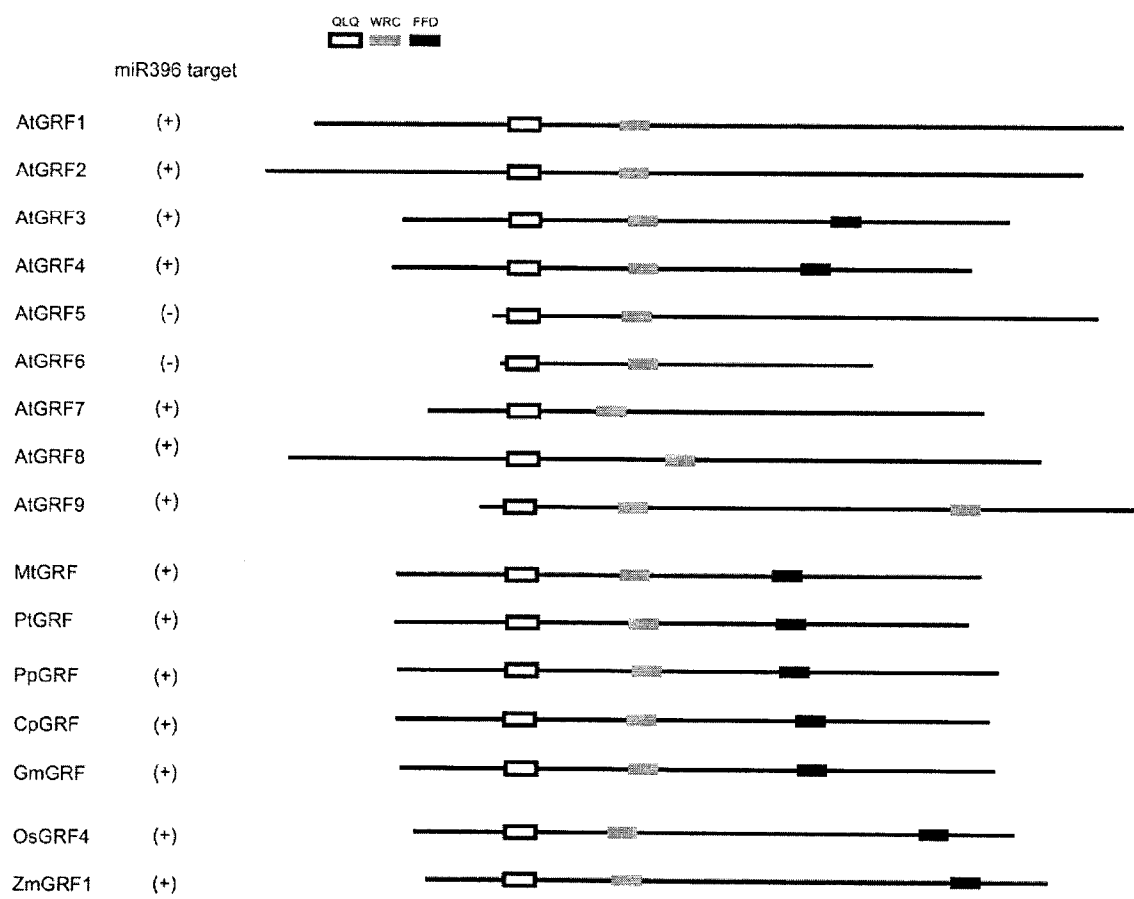


FIGURE 8

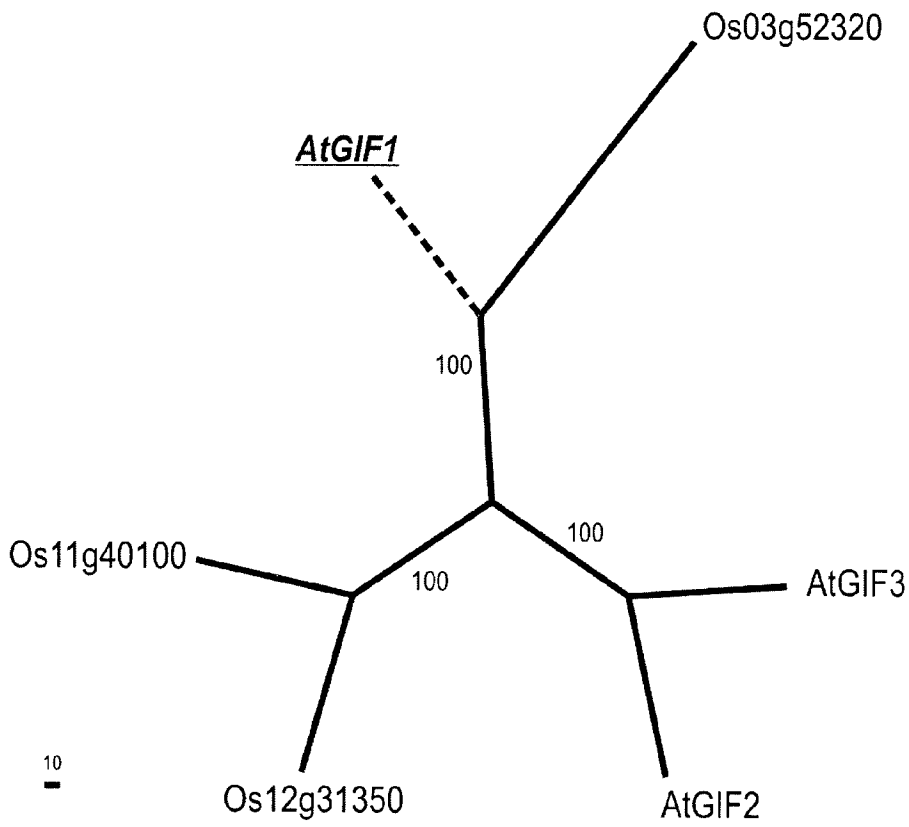


FIGURE 9

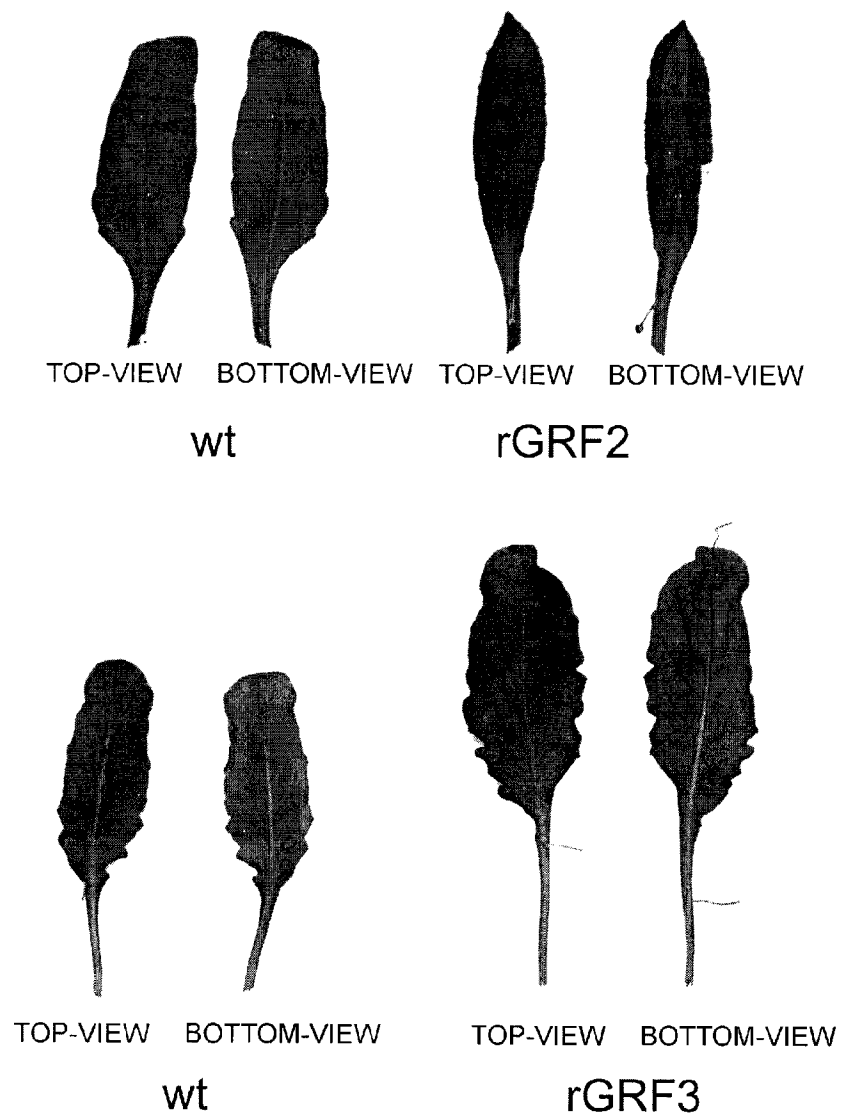


FIGURE 10

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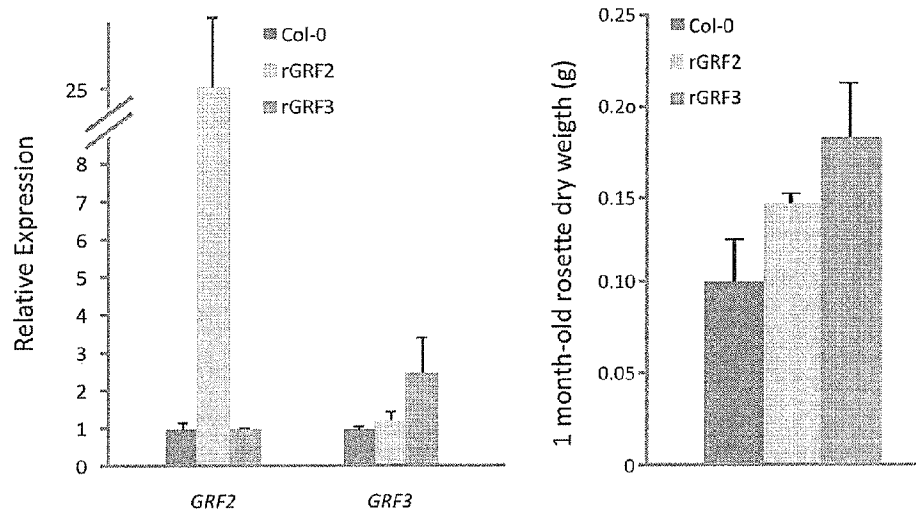


FIGURE 11

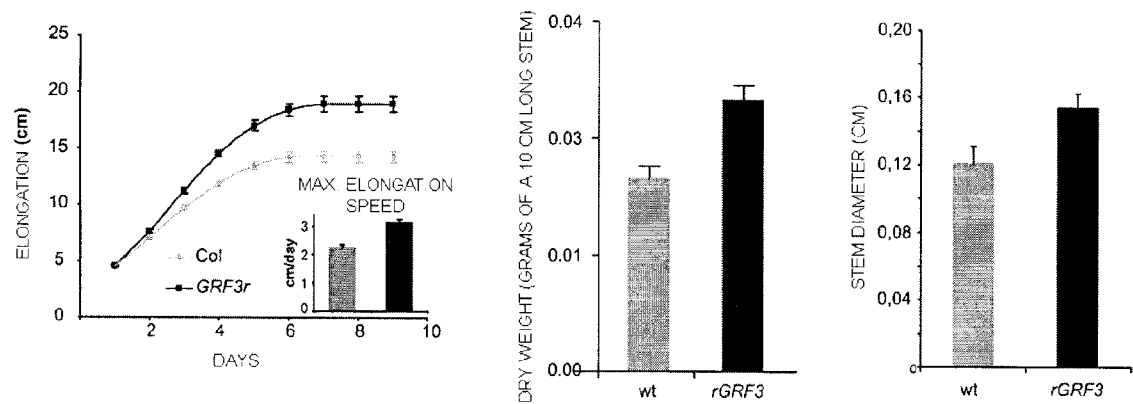


FIGURE 12

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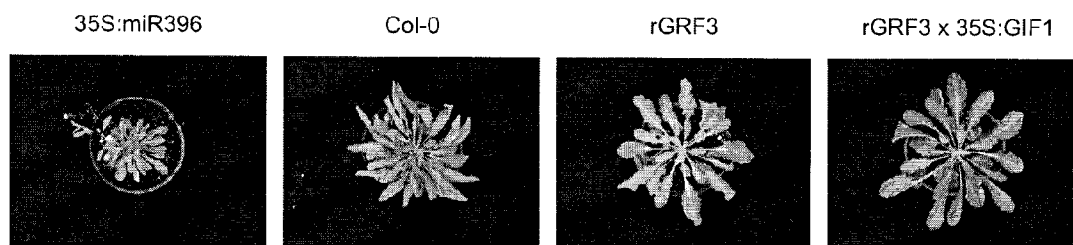


FIGURE 13

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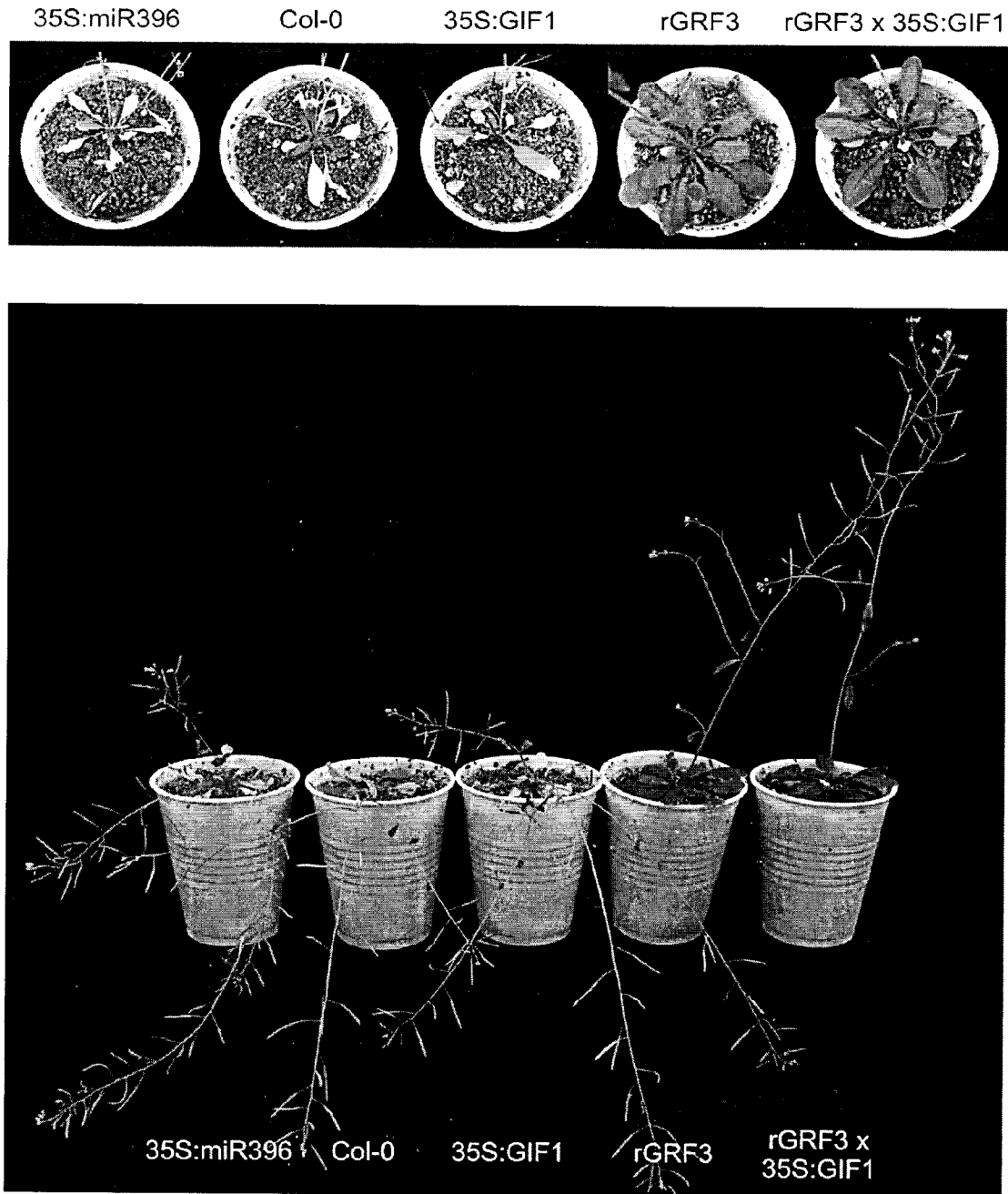


FIGURE 14

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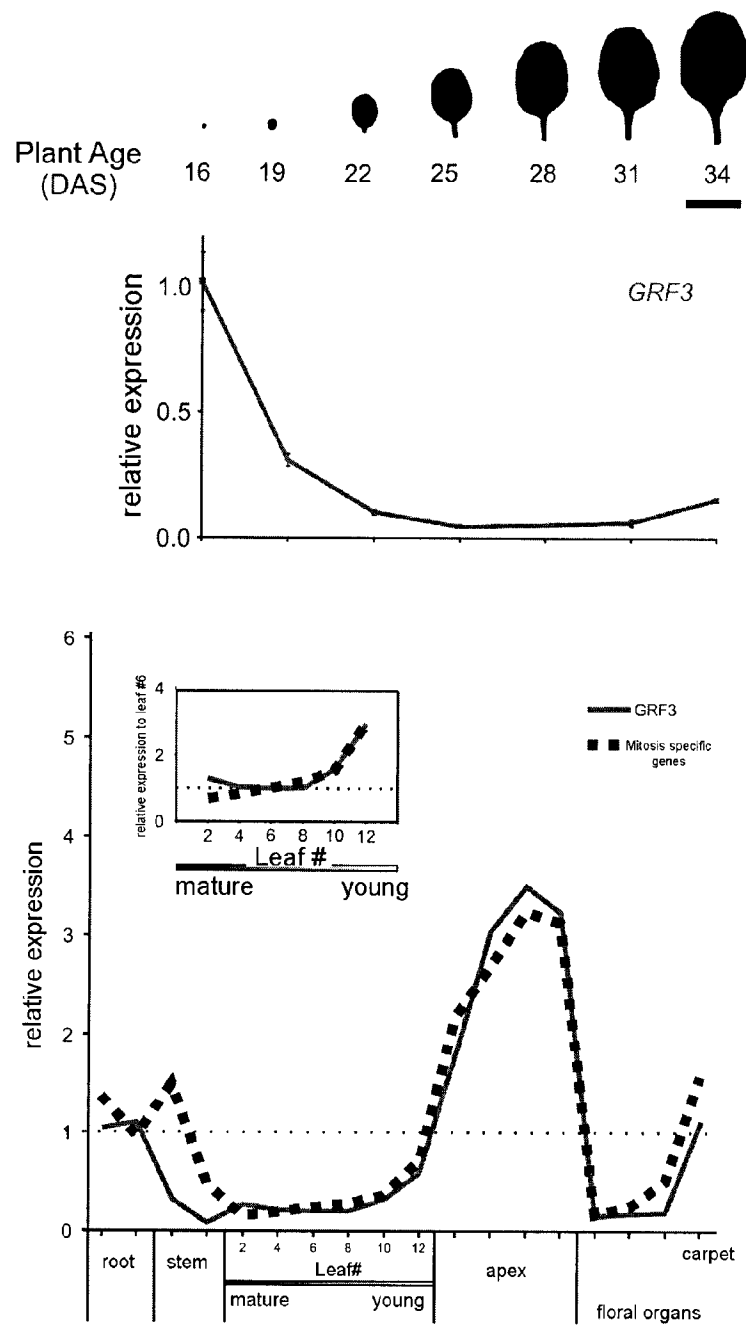


FIGURE 15

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MAIZE

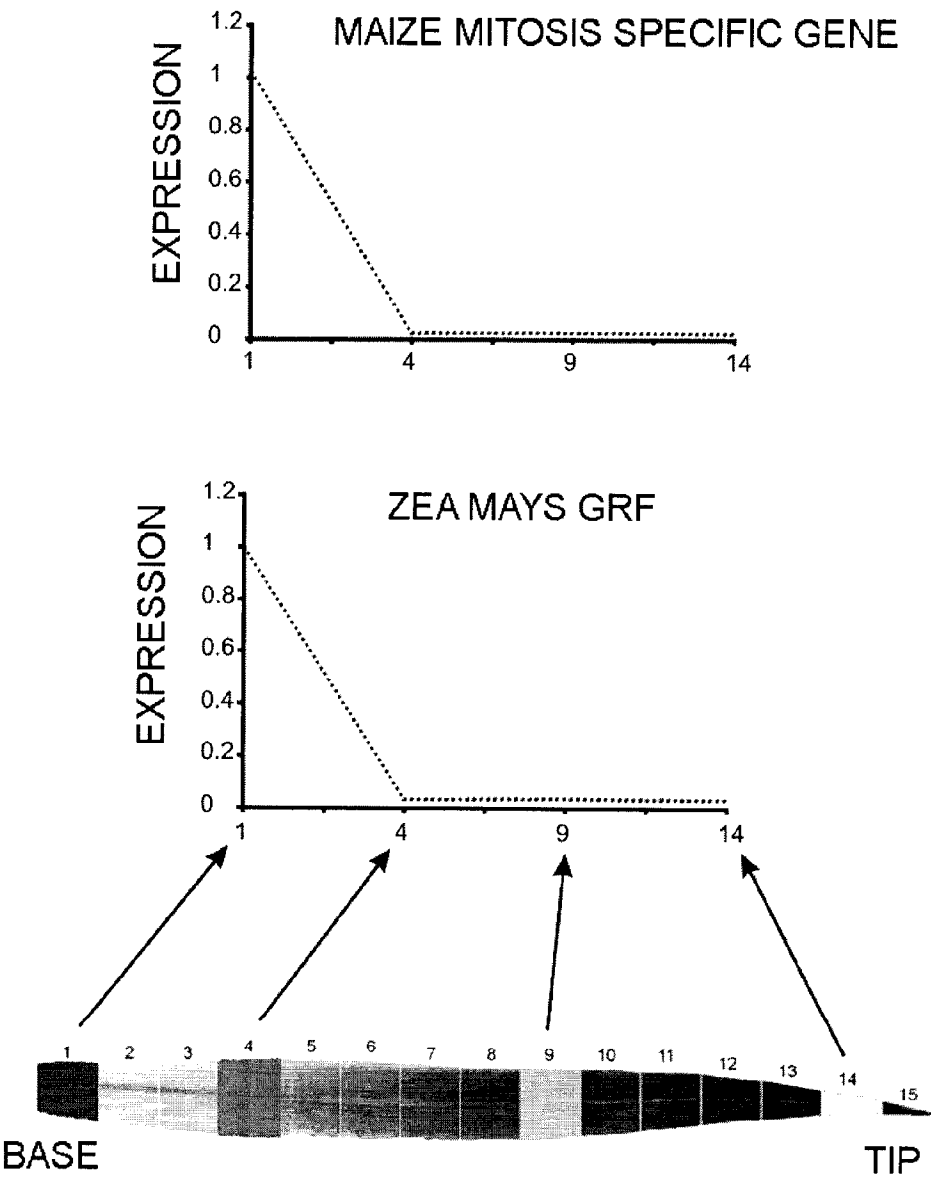
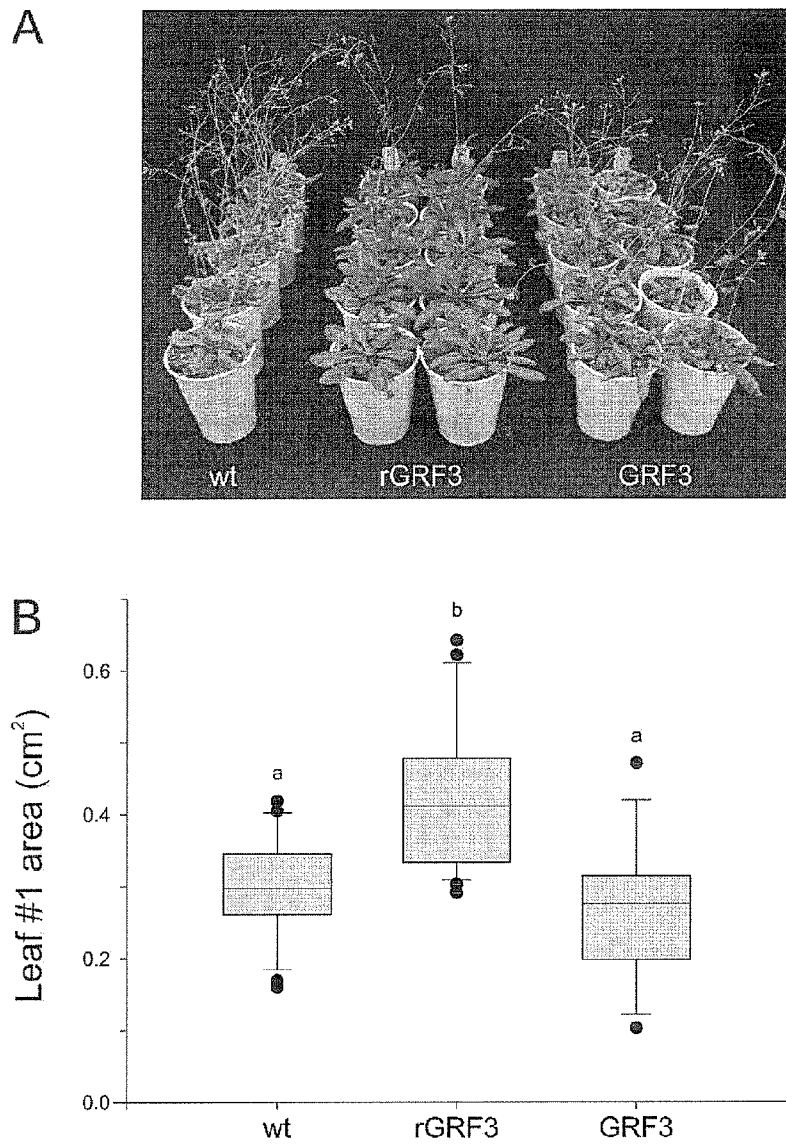


FIGURE 16

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FIGURE 17



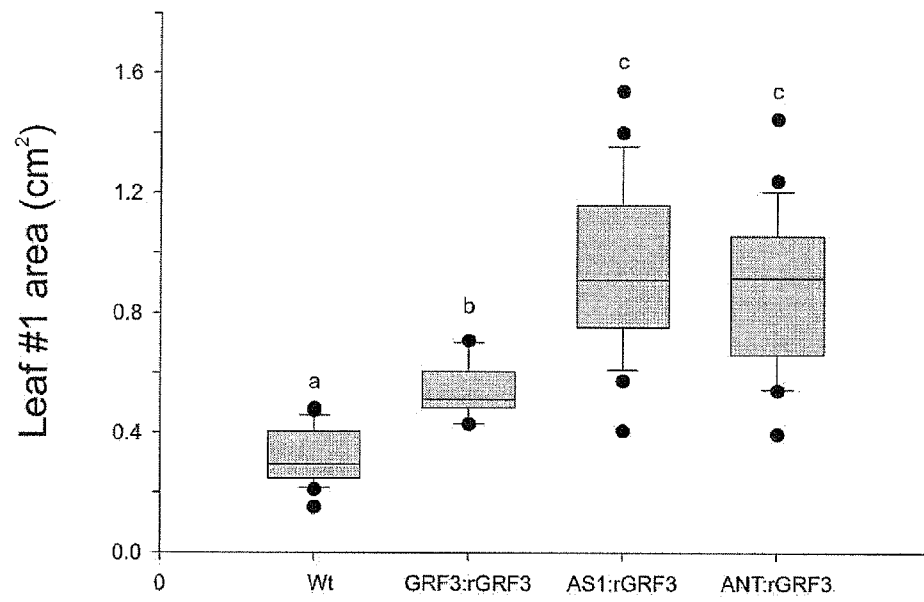


FIGURE 18

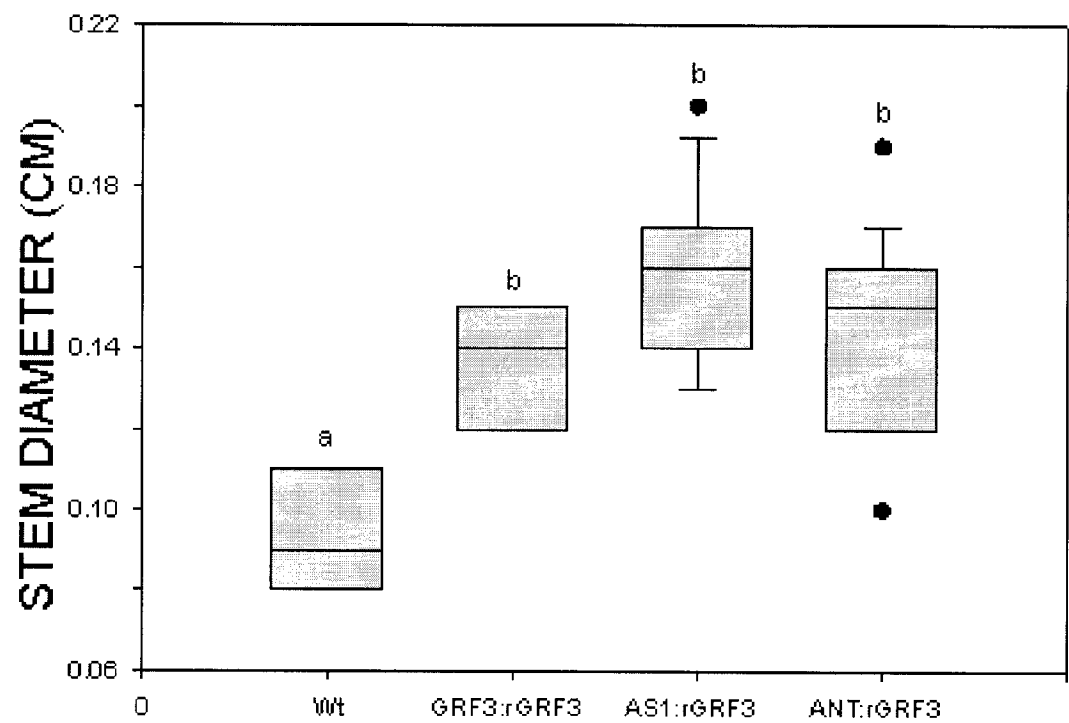


FIGURE 19

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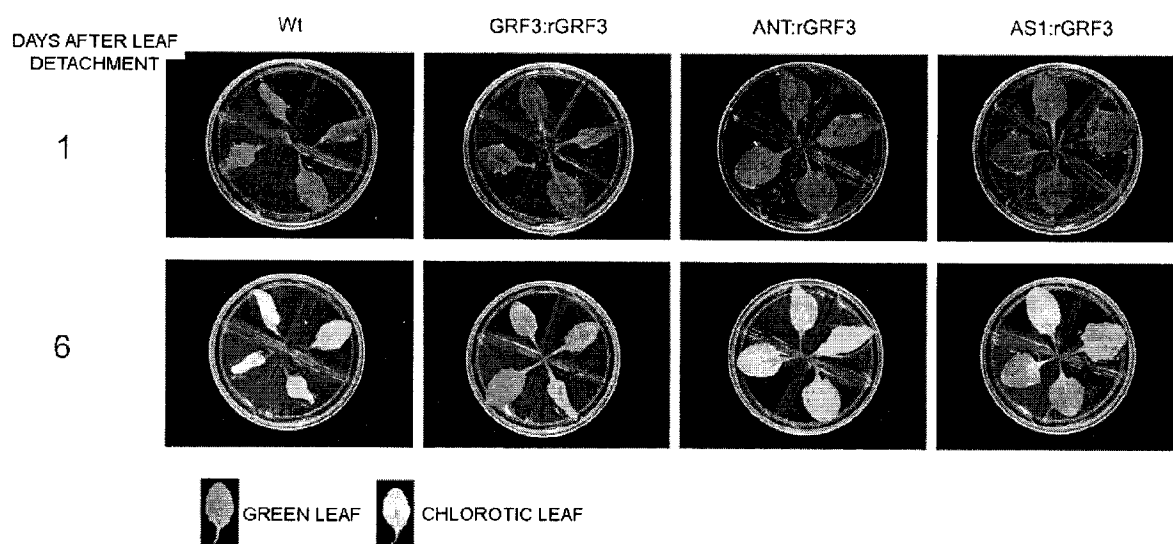


FIGURE 20

23186597.1

FIGURE 21

>AtGRF1 (SEQ ID No. 40)

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>AtGRF2 (SEQ ID No. 87)

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>AtGRF3 (SEQ ID No. 2)

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CTTCTTCTAAACTCTCCTCTTTGTCTCCTGATTCTTCCTCCAGGTTCCCCAAGAT

GGGGAGCTTCTTTAGCTGGGCACAGTGGCAAGAACTTGAAGTACAAGCTCTGAT
CTACAGGTACATGTTGGCTGGTGTCTGCTGTTCCCTCAGGAGCTCCTTTTACCAAT
CAAGAAAAGCCTTCTCCATCTATCTCCTTCCTACTTTCTTCACCATCCTCTTCAAC
ACCTACCTCATTACCAACCTGCTTGGTATTTGGGAAGGGCAGCGATGGATCCTG
AGCCAGGCAGATGCAGGAGAACGGATGGTAAGAAGTGGAGATGTTCAAGAGAC
GTCTTCGCTGGCCACAAGTATTGCGAGCGCCACATGCACCGTGGCCCGCAACCG
TTCAAGAAAGCCTGTGGAAACTCCAACCACCGTCAATGCAACTGCCACGTCCA
TGGCTTCATCAGTAGCAGCCGCAGCCACCACTACAACAGCAACAACAACATCTA
CGTTTGCTTTTGGTGGTGGTGGTGGTAGTGAGGAAGTGGTTGGTCAAGGAGGA
TCTTTCTTCTTCTCTGGCTCTTCTAACTCTTCATCTGAACTTCTCCACCTTAGTCA
AAGTTGTTCCGAGATGAAGCAAGAAAGCAACAACATGAACAACAAGAGGCCATA
CGAGTCCCACATCGGATTCAGTAACAACAGATCAGATGGAGGACACATCCTGAG
GCCCTTCTTTGACGATTGGCCTCGTTCTTCGCTCCAAGAAGCTGACAATAGTTC
AAGCCCCATGAGCTCAGCCACTTGTCTCTCCATCTCCATGCCCGGGAAGCTCTTC
CTCAGACGTCTCTCTGAAGCTGTCCACAGGCAACGAAGAGGGAGCCCGGAGCA
ACAACAATGGGAGAGATCAGCAAAACATGAGCTGGTGGAGCGGTGGAGGTTCC
AACCACCATCATCACAACATGGGCGGACCATTGGCCGAAGCCCTGAGATCTTCT
TCCTCATCTTCCCCAACCAAGTGTCTCCATCAGCTTGGTGTCTCGACACAAGCC
TTTCATTGA

>AtGRF4 (SEQ ID No. 19)

ATGGACTTGCAACTGAAACAATGGAGAAGTCAGCAGCAGAATGAGTCAGAAGAA
CAAGGCTCTGCTGCAACTAAGATATCAAACCTTTTTCTTTGATCAGATTCAGTCCC
AAACTGCTACTTCTGCTGCTGCGGCTCCTCTTCCTCTCTTTGTCCCTGAACCCA
CTTCTTCCTCTTCTTTCTCTTGCTTCTCTCCTGACTCTTCTAATTCTTCTTCTTCT
CCAGGTTCTCAAGATGGGAACTTCTTCAGCTGGGCACAGTGGCAAGAACTTG
AGCTACAAGCACTGATCTATAGATACATGTTGGCTGGTGTCTTCTGTTCCCTCAAGA
GCTTCTCTTACCTATTAAGAAAAGTCTCCTCCATCAATCTCCTATGCATTTCTTC
ACCATCCTCTTCAACATAGTTTTCTCATCACCAACCTTCTTGGTATTGGGGAAG
AGGAGCAATGGATCCTGAGCCAGGGAGGTGTAAGAGAACTGACGGCAAGAAAT
GGAGATGTTCAAGGGATGTTGTAGCGGGCCACAAGTATTGTGACCGCCACATT
CACCGTGGAAGAAACCGTTCAAGAAAGCCTGTGGAAACCGCCACAACCACCAT
CACAACGACAGCCACAACAACCGCATCTTCTTTTGTCTTAGGTGAGGAGCTTGG
TCATGGACCAAACAACAACCACTTCTTCTCCTCTGGTTCATCTCAACCTCTCCAC
CTTAGTCATCAACAAAGTTGTTCTTCAGAGATGAAACAAGAAAGCAACAACAACA
AGAGGCCATATGAAGCTAACAGTGGATTCAGCAATGGAAGATCAGACGATGGTC
ACATCTTGAGGCATTTCTTTGACGATTGGCCACGATCATCAGACTCTACCTCCA

GTCCAATGAGCTCATCCACTTGTCATCTTTCAATCTCCATGCCCCGGTAACAACAC
GTCCTCAGATGTTTCTCTAAACTTTCCACAGGCAATGAAGAAGAAGAAGAGAA
CATGAGAAATAACAACAATGAGAGGGAGCAAATGAATTGGTGGAGCAATGGAG
GGAATCACCACAACAATATGGGAGGACCATTAGCTGAGGCTTTGAGGTCAGCTT
CTTCGACGTCAAGTGTTCTTCATCAGATGGGAATCTCTACTCAAGTTTTTCATTA
A

>AtGRF5 (SEQ ID No. 41)

ATGATGAGTCTAAGTGGAAGTAGCGGGAGAACAATAGGAAGGCCTCCATTTACA
CCAACACAATGGGAAGAACTGGAACATCAAGCCCTAATCTACAAGTACATGGTC
TCTGGTGTTCCGTGTCCACCTGAGCTCATCTTCTCCATTAGAAGAAGCTTGGAC
ACTTCCTTGGTCTCTAGACTCCTTCCTCACCAATCCCTTGGATGGGGGTGTTAC
CAGATGGGATTTGGGAGAAAACCAGATCCAGAGCCAGGAAGATGCAGAAGAAC
AGATGGTAAGAAATGGAGATGCTCAAGAGAAGCTTACCCAGATTCAAGTACTG
TGAAAAACACATGCACAGAGGAAGAAACCGTGCCAGAAAATCTCTTGATCAGAA
TCAGACAACAACAACCTCCTTTAACATCACCATCTCTCTCATTACCAACAACAAC
AACCCAAGTCCCACCTTGTCTTCTTCTTCTCCTCTAATTCCTCTTCTACTACTTA
TTCTGCTTCTTCTTCTTCAATGGATGCCTACAGTAACAGTAATAGGTTTGGGCTT
GGTGGAAGTAGTAGTAACACTAGAGGTTATTTCAACAGCCATTCTCTTGATTATC
CTTATCCTTCTACTTCACCCAAACAACAACAACAACTCTTCATCATGCTTCCGC
TTTGTCACCTTCATCAAAATACTAATTCTACTTCTCAGTTCAATGTCTTAGCCTCTG
CTACTGACCACAAAGACTTCAGGTACTTTCAAGGGATTGGGGAGAGAGTTGGAG
GAGTTGGGGAGAGAACGTTCTTTCCAGAAGCATCTAGAAGCTTTCAAGATTCTC
CATACCATCATCACCAACAACCGTTAGCAACAGTGATGAATGATCCGTACCACC
ACTGTAGTACTGATCATAATAAGATTGATCATCATCACACATACTCATCCTCATCA
TCATCTCAACATCTTCATCATGATCATGATCATAGACAGCAACAGTGTTTTGTTTT
GGGCGCCGACATGTTCAACAAACCTACAAGAAGTGTCTTGCAAACCTCATCAAG
ACAAGATCAAAATCAAGAAGAAGATGAGAAAGATTCATCAGAGTCGTCCAAGAA
GTCTCTACATCACTTCTTTGGTGAGGACTGGGCACAGAACAAGAACAGTTCAGA
TTCTTGCTTGACCTTTCTTCCCACTCAAGACTCGACACTGGTAGCTAA

>AtGRF6 (SEQ ID No. 42)

ATGGCTACAAGGATTCCATTCACAGAATCACAATGGGAAGAACTTGAAAACCAA
GCTCTTGTGTTCAAGTACTTAGCTGCAAATATGCCTGTTCCACCTCATCTTCTCT
TCCTCATCAAAAGACCCTTTCTCTTCTCTTCTTCTTCTTCTTCATCTTCTTCTCAA
GCTTCTTCTCTCCCACTCTTTCTCCACACTTTGGGTGGAATGTGTATGAGATGG
GAATGGGAAGAAAGATAGATGCAGAGCCAGGAAGATGTAGAAGAACTGATGGC

AAGAAATGGAGATGCTCTAAAGAAGCTTACCCTGACTCTAAGTACTGTGAGAGA
CATATGCATAGAGGCAAGAACCGTTCTTCCTCAAGAAAGCCTCCTCCTACTCAA
TTCCTCCAAATCTCTTTCTCGACTCTTCTTCCAGAAGAAGAAGTGGATACA
TGGATGATTTCTTCTCCATAGAACCTTCCGGGTCAATCAAAGCTGCTCTGGCT
CAGCAATGGAAGATAATGATGATGGCTCATGTAGAGGCATCAACAACGAGGAGA
AGCAGCCGGATCGACATTGCTTCATCCTTGGTACTGACTTGAGGACACGTGAGA
GGCCATTGATGTTAGAGGAGAAGCTGAAACAAAGAGATCATGATAATGAAGAAG
AGCAAGGAAGCAAGAGGTTTTATAGTTTTCTTGATGAATGGCCTTCTT

>AtGRF7 (SEQ ID No. 43)

ATGGACTTTCTCAAAGTTTCAGACAAGACAACAATTCCATATAGAAGTGATTCTT
TGTTTAGTTTGAATCAGCAACAATACAAAGAGTCTTCTTTTGGATTGAGAGACAT
GGAGATTCATCCGCATCCTACTCCATATGCAGGAAATGGACTTTTGGGTTGTTAT
TACTATTACCCTTTCACAAACGCACAATTGAAGGAGCTTGAGAGACAAGCAATG
ATCTACAAGTACATGATCGCATCTATTCTGTTCTTTCGATCTACTTGTTTCTTC
ACCATCCTCTGCCTCTCCTTGTAACAATAAAACATCGCCGGAGATTTAGAGCC
GGGAAGATGCCGGAGAACAGACGGAAAGAAATGGAGATGCGCGAAAGAAGTC
GTCTCTAATCACAAATACTGTGAGAAACACTTACACAGAGGTCGTCTCTCGTTCA
AGAAAGCATGTGGAACTCCTTATTCTCGCCCTAACAACAATGGTGGTTCTGTG
AAAAACAGAGATCTCAAAAAGCTTCCTCAAAAGTTATCTAGTAGTTCCATCAAAG
ACAAAACACTTGAGCCAATGGAGGTTTCATCATCAATCTCAAACATAGAGACTC
CAGAGGAAGTGAGAAATTTACTGTATTGGCAACAACAGAGCAAGAGAACAAGTA
TCTGAATTTCATAGATGTATGGTCCGATGGAGTAAGATCATCTGAAAAACAGAGT
ACAACCTCAACACCTGTTTCTTCTTCCAATGGCAATCTCTCTCTTTACTCGCTTGA
TCTCTCAATGGGAGGAAACAACCTTAATGGGCCAAGACGAAATGGGCCTGATACA
AATGGGCTTAGGTGTAATCGGGTCGGGTAGTGAGGATCATCACGGGTATGGTC
CTTATGGTGTGACTTCTTCACTAGAGGAGATGTCAAGCTGGCTTGCTCCGATGT
CTACCACACCTGGTGGACCATTAGCGGAGATACTGAGGCCGAGTACGAATTTG
GCGATCTCTGGTGATATCGAATCGTATAGCTTGATGGAGACTCCCACTCCAAGC
TCGTCCCCGTCTAGAGTGATGAAGAAGATGACTAGTTCAGTGTCCGACGAAAGC
AGCCAGGTTTAG

>AtGRF8 (SEQ ID No. 44)

ATGAGGATGCTTCTTGGGATTCTTACGTAGACAAGTCGGTTCTTTCCAACCTCTG
TTCTTGAGAGAGGGCAAGCAGGATAAAAGCAAACCTATTGTTAGTCGACAAATGCC
ATTATGAGCTTGATGTTGAAGAACGCAAGGAAGATTTTGTGGTGGGTTTGGATT
TGGTGTGTAGAAAATTCGCATAAAGACGTTATGGTGCTACCTCATCATCACTAT

TATCCATCATATTCATCACCTTCCTCTTCTTTGTGTTACTGTTCTGCTGGTGT
TAGCGATCCCATGTTCTCTGTTTCTAGCAATCAGGCTTACACTTCTTCTCACAGT
GGTATGTTACACCCGCCGGTTCTGGTTCTGCTGCTGTGACTGTAGCAGATCCT
TTTTTCTCCTTGAGCTCTTCAGGGGAAATGAGAAGAAGTATGAACGAAGATGCT
GGTGCAGCTTTCAGCGAAGCTCAATGGCATGAGCTTGAGAGGCAGAGGAATAT
ATACAAGTACATGATGGCTTCTGTTCCCTGTTCCCTCCAGAGCTTCTCACACCCTTT
CCCAAGAACCACCAATCAAACACTAACCCGGATGTGGATACATATAGGAGTGGA
ATGTTTAGTATTTATGCTGATTACAAGAATCTGCCGTTGTCTATGTGGATGACAG
TAACTGTGGCAGTGGCGACAGGAGGCTCATTGCAGCTGGGGATTGCTTCAAGC
GCAAGCAATAACACGGCTGATCTGGAGCCATGGAGGTGCAAGAGAACAGATGG
GAAGAAATGGAGGTGCTCTAGAAACGTGATTCTGATCAGAAATACTGTGAGAG
ACACACACACAAGAGCCGTCCTCGTTCAAGAAAGCATGTGGAAATCATCTCACC
AATCATCTCACCACAATGACATTCGTACGGCTAAGAATGATACTAGCCAGCTTGT
GAGAACTTATCCTCAGTTTTACGGACAACCTATAAGCCAGATCCCTGTGCTTTCT
ACTCTTCCGTCTGCCTCCTCTCCATATGATCACACAGAGGACTGAGGTGGTTT
ACGAAAGAAGATGATGCCATTGGAACCTTAAACCCGGAGACTCAAGAAGCTGTC
CAGCTGAAAGTTGGATCAAGCAGAGAGCTCAAACGGGGATTGATTATGATCTG
AATTCAGGCAGAAAGAGCCAATAGTAGACCAGAGCTTTGGAGCATTGCAGGGT
CTATTAAGTCTAAACCAGACACCACAACATAACCAAGAAACAAGACAGTTTGTG
TAGAAGGAAAGCAAGATGAAGCGATGGGAAGCTCTCTGACACTCTCAATGGCT
GGAGGAGGCATGGAGGAAACAGAGGGAACAAACCAGCATCAGTGGGTTAGCC
ATGAAGGTCCATCATGGCTCTATTCAACAACACCAGGTGGACCATTGGCTGAAG
CACTGTGTCTCGGTGTCTCCAACAACCCAAGTTCTAGTACTACTACTAGTAGCT
GCAGCAGAAGCTCAAGCTAA

>AtGRF9 (SEQ ID No. 45)

ATGCAGAGCCCTAAAATGGAGCAGGAGGAGGTTGAGGAGGAGAGGATGAGGA
ATAAGTGGCCGTGGATGAAGGCGGCGCAGTTAATGGAGTTTCGGATGCAAGCT
TTGGTGTATAGATACATAGAGGCTGGTCTCCGTGTGCCTCATCATCTCGTGGTG
CCTATTTGGAACAGTCTTGCTCTCTCTTCTCCTCCAATTACAACATCACTCTTC
TTCTCTGTTGAGTAACAAGGGAGTAACCCATATCGACACGTTGGAACTGAACC
AACTAGGTGCAGGAGAACAGATGGGAAGAAATGGCGCTGTAGCAACACGGTCC
TTCTATTGAGAAGTACTGTGAACGGCACATGCATAGAGGTCGTAAACGTTCAA
GAAAGCTTGTGGAAATCTTCTTCTGAGGTTGCTTCATCATCAACCAATACGACA
ACACTTATGGTTTGGATAGGTATAACGAGAGTCAGAGTCATCTTCATGGGACAA
TCTCGGGTTCTAGTAATGCGCAGGTAGTTACCATTGCTTCACTGCCTAGTGCCA
GATCCTGTGAAAATGTCATTCCGTCCGTCTTTAGTGATCTCTGAATTCACAAACAA

AAGTGTGAGTCACGGCAGAAAGAACATGGAGATGAGTTATGATGACTTTATTAA
TGAAAAAGAGGCGAGTATGTGTGTTGGAGTTGTTCTTCAAGGTGATGAGAG
CAAACCTTCGGTTCAAAAGTTCTTCCCTGAGGTATCTGATAAATGCTTAGAAGCT
GCAAAATTCTCAAGCAACAGGAAGAATGATATAATTGCAAGAAGCAGAGAATGG
AAGAATATGAATGTTAATGGTGGTTTGTTCATGGTATCCACTTTTCTCCAGACA
CTGTTCTTCAAGAACGTGGTTGTTTTGTTTACAAGGAGTTGAAACAGACAATGA
ACCAGGAAGGTGCCGAAGAACAGATGGGAAGAAGTGGAGATGCAGCAAAGATG
TTTTGTCTGGTCAGAACTACTGCGATAAGCACATGCATAGAGGTATGAAGAAGA
AGCATCCAGTTGATACTACTAACTCACATGAGAATGCCGGGTTTAGCCCGTTAA
CCGTGGAAACAGCTGTTAGATCGGTTGTGCCTTGCAAAGATGGAGATGACCAG
AAGCATTCTGTTTCAGTCATGGGAATTACACTGCCCCGAGTTTCTGATGAGAAG
AGCACTAGCAGTTGCAGTACCGACACTACCATTACTGACACAGCTTTAAGGGGT
GAAGACGACGATGAGGAGTACTTGTCTTTGTTTTACCAGGTGTTTAG

FIGURE 22

>AtGRF1 (SEQ ID No. 46)

MDLGVRVSGHETVSSPGQTELGSGFSNKQERSGFDGEDCWRSSKLSRTSTDGFS
 SSPASAKTLSFHQGIPLLRSTTINDPRKGQEHMLSFSSASGKSDVSPYLQYCRNSG
 YGLGGMNNTSNMHGNLLTGVKGPFSLTQWAELEQQALIYKYITANVPVPSSLLLSL
 KKSFFPYGSLPPNSFGWGSFHLGFSGGNMDPEPGRCRRTDGKKWRCSRDAVPD
 QKYCERHINRGRHRSRKPVEGQNGHNTNAAAAASAAAAASTAAVSKAAAGTSAVA
 MRGSDNNNSLAAAVGTQHHTNNQSTD LANRVQNSRGASVFPATMNLQSKETHP
 KQSNNPFEFGLISSDSLLNPSHKQASYATSSKGFSGSYLDFGNQAKHAGNHNNVDS
 WPEELKSDWTQLSMSIPMAPSSPVQDKLALSPLRLSREFDPAIHMGLGVNTEFLDP
 GKKTNNWIPISWGNNSMGGPLGEVLNSTTNSPKFGSSPTGVLQKSTFGSLSNSS
 SASSTIIGDNNNKNGDGKDPLGPTTLMNTSATAPSL

>AtGRF2 (SEQ ID No. 47)

MDIGVHVLGSVTSNENESLGLKELIGTKQDRSGFIGEDCLQRSLKLARTTTTAE
 NLSSSVAAAYCKTMSFHQGIPLMRSASPLSSDSRRQEQMLSFSDKPDALDFSKYV
 GLDNSSNNKNSLSPFLHQIPPPSYFRSSGGYGGGMMMNMSMQGNFTGVKGPFT
 LTQWAELEQQALIYKYITANVPVPSSLLISIKKSFYPYGSLPPSSFGWGTFHLGFAGG
 NMDPEPGRCRRTDGKKWRCSRDAVPDQKYCERHINRGRHRSRKPVEVQSGQNG
 TAAAASKAVTTPQQPVVAGNTNRSNARASSNRSLAIGSQYINPSTESLPNNRGVSIY
 PSTVNLQPKESPVIHQHRNNNNPFEFGHISSDSLLNPNTAKTYGSSFLDFSSNQE
 HSGNHNHNSWPEELTSDWTQLSMSIPIASSSPSSTHNNNNNAQEKTTLSPLRLSREL
 DLSIQTDETTIEPTVKKVNTWIPISWGNLSLGGPLGEVLNSTTNSPTFGSSPTGVLQK
 STFCSLSNSSSVSSPIAENNRHNGDYFHYTT

>AtGRF3 (SEQ ID No. 20)

MDLQLKQWRSQQQQQHQTSEEQPSAAKIPKHVFDQIHSHTATSTALPLFTPEPTS
 SKLSSLSPDSSSRFPKMGSFFSWAQWQELELQALIYRYMLAGAAVPQELLPIKKS
 LHLSPSYFLHHPLQHLPHYQPAWYLGRAAMDPEPGRCRRTDGKKWRCSRDVFAG
 HKYCERHMHGRNRNRKPVETPTTVNATATSMASVAAAATTTTATTTSTFAFGG
 GGGSEEVVGQGGGSSFFSGSSNSSSELLHLSQSCSEMKQESNNMNNKRPYESHIG
 FSNRSDGGHILRPFFDWPRSSLQEADNSSSPMSSATCLSISMPGNSSSDVSLKL
 STGNEEGARSNNNGRDQQNMSWWSGGGSNNHHHNMGGPLAEALRSSSSSSPT
 SVLHQLGVSTQAFH

>AtGRF4 (SEQ ID No. 21)

MDLQLKQWRSQQQNESEEQGSAAATKISNFFFDQIQSQTATSAAAAPLPLFVPEPTS
SSSFSCFSPDSSNSSSSSRFLKMGNFFSWAQWQELELQALIYRYMLAGASVPQEL
LLPIKKSLLHQSPMHFLHPLQHSFPHHQPSWYWGRGAMDPEPGRCKRTDGKKW
RCSRDVVAGHKYCDRHIHRGRNRSRKPVETATTTITTTATTTASSFVLGEELGHGP
NNNHFFSSGSSQPLHLHQSSCSSEMKESSNNNKRPEANSFGFSNGRSDDGHL
RHEFDWPRSSDSTSSPMSSSTCHLSISMPGNNTSSDVSLKLSTGNEEEEEENMRN
NNNEREQMNWWSNGGNHHNNMGGPLAEALRSASSTSSVLHQMISTQVFH

>AtGRF5 (SEQ ID No. 48)

MMSLSGSSGRTIGRPPFTPTQWEELEHQALIYKYMVSGVPVPELIFSIRRLDTSL
VSRLPHQSLGWGCYQMGFGRKPDPEPGRCCRRTDGKKWRCSREAYPDSKYCEK
HMHRGRNRARKSLDQNTTTTTLTSPSLSFTNNNNPSPTLSSSSSSNSSSTTYSAS
SSSMDAYSNSNRFLGGSSSNTRGYFNHSLDYPYPSTSPKQQQQTLHHASALSL
HQNTNSTSQFNVLASATDHKDFRYFQGIGERVGGVGERTFFPEASRSFQDSPYHH
HQQPLATVMNDPYHHHCSTDHNNKIDHHHTYSSSSSSQHLHHDHHRQQQCFVLGA
DMFNKPTRSVLANSSRQDQNEEDEKDSSESSKSLHHFFGEDWAQNKNSSDSW
LDLSSHSRLDTGS

>AtGRF6 (SEQ ID No. 49)

MATRIPTESQWEELENQALVFKYLAANMPVPPHLLFLIKRPFLFSSSSSSSSSSFF
SPTLSPHFGWNVYEMGMGRKIDAEPGRCCRRTDGKKWRCSKEAYPDSKYCERHM
HRGKNRSSSRKPPTQFTPNLFLDSSRRRRSGYMDDFFSIEPSGSIKSCSGSAME
DNDDGSCRGINNEEKQPDHCFILGTDLRTREPLMLEEKLKQRDHDNEEEQGSK
RFYRFLDEWPSSKSSVSTSLFI

>AtGRF7 (SEQ ID No. 50)

MDFLKVSDKTTIPYRSDSLFSLNQQQYKESSFGFRDMEIHPHPTPYAGNLLGCYY
YYPFTNAQLKELERQAMIYKMIASIPVPFDLLVSSPSSASPCNNKNIAGDLEPGR
RRTDGKKWRCACEVVSNNHKYCEKHLHRGRPRSRKHVEPPYSRPNNNGGSVKNR
DLKKLPQKLSSSSIKDKTLEPMEVSSSISNYRDSRGSEKFTVLATTEQENKYLNFIDV
WSDGVRSSSEKQSTTSTPVSSSNGNLSLYSLDLMSGGNNLMGQDEMGLIQMGLGVI
GSGSEDHHGYGPYGVTSLEEMSSWLAPMSTTPGGPLAEILRPSTNLAISGDIESY
SLMETPTPSSSPSRVMKKMTSSVSDESSQV

>AtGRF8 (SEQ ID No. 51)

MRMLLGIPYVDKSVLSNSVLERGKQDKSKLLLVDKCHYELDVEERKEDFVGGFGFG
VVENSHKDVMLPHHHYYPSYSSPSSSSSLCYCSAGVSDPMFSVSSNQAYTSSHS
MFTPAGSGSAAVTVADPFFSLSSSGEMRRSMNEDAGAAFSEAQWHELERQRNIYK
YMMASVPVPPELLTPFPKNHQSNTNPDVDTYRSGMFSIYADYKNLPLSMWMTVTV
AVATGGSLQLGIASSASNNTADLEPWRCRTDGKKWRC SRNVIPDQKYCERHTHK
SRPRSRKHVESSHQSSHHNDIRTAKNDSQLVRTYPQFYGQPISQIPVLSTLPSASS
PYDHRGLRWFTKEDDAIGTLNPETQEAVQLKVGSSRELKRGFDYDLNFRQKEPIV
DQSFGALQGLLSLNQTPQHNQETRQFVVEGKQDEAMGSSLTLSMAGGGMEETEG
TNQHQWWSHEGPSWLYSTTPGGPLAEALCLGVSNNPSSSTTTSSCSRSSS

>AtGRF9 (SEQ ID No. 52)

MQSPKMEQEEVEEERMNRNKWPWMKAAQLMEFRMQALVYRYIEAGLRVPHHLVVP
IWNSLALSSSSNYYHSSSLLSNKGVTHIDTLETEPTRCRRTDGKKWRC SNTVLLFE
KYCERHMRGRKR SRKLVESSEVASSSTKYDNTYGLDRYNESQSHLHGTISGSS
NAQVVTIASLPSARSCENVIRPSLVISEFTNKS VSHGRKNMEMSYDDFINEKEASMC
VGVVPLQGDESKPSVQKFFPEVSDKCLEAAKFSSNRKNDIARSREWKNMNVNGG
LFHGIHFSPDTV LQERGC FRLQG VETDNEPGRCRRTDGKKWRC SKDVL SGQKYC
DKHMHHRGMKKKHPVDTTNSHENAGFSPLTVETAVRSVVPCKDGDDQKHSVVMG
ITLPRVSDEKSTSSCSTDTTITD TALRGEDDDEEYLSLFSPGV

FIGURE 23

>OsGRF1 (SEQ ID No. 3)

ATGATGATGATGAGCGGTCGCCCCGAGCGGCGGCGCCGGCGGAGGTCCGTACC
CGTTCACGGCGTCGCAGTGGCAGGAGCTGGAGCACCAGGCGCTCATCTACAA
GTACATGGCGTCCGGGACTCCCATCCCCTCCGACCTCATCCTCCCCCTCCGCC
GCAGCTTCCTCCTCGACTCCGCCCTCGCCACCTCCCCTTCCCTCGCCTTCCCTC
CCCAACCTTCACTGGGGTGGGGTTGCTTTGGCATGGGGTTTGGGCGGAAGGC
GGAGGACCCGGAGCCAGGGCGATGCCGGCGTACGGACGGCAAGAAGTGGCG
GTGCTCCAAGGAGGCGTACCCGGACTCCAAGTACTGCGAGAAGCACATGCACC
GTGGCAAGAACCGTTCAAGAAAGCCTGTGGAAATGTCCTTGGCCACGCCGCC
GCCGCCGTCTCCTCCGCCACCTCCGCCGCGTCGAACACCTCCGCCGGCGTC
GCCCCACCACCACCACCACCTCCTCCCGGCGCCCTCCTACAGCCGCCCGG
CGCCGCACGACGCGGCGCCGTACCAGGCGCTCTACGGCGGGCCCTACGCCG
CGGCCACCGCGCGCACCCCCGCCGCCGCGGCGTACCACGCGCAGGTGAGCC
CGTTCCACCTCCAGCTCGACACCACCCACCCGCACCCGCCGCCGTCTACTAC
TCCATGGACCACAAGGAGTACGCGTACGGGCACGCCACCAAGGAGGTGCACG
GCGAGCACGCCTTCTTCTCCGATGGCACCAGAGGGAGCACCACCACGCCGC
CGCCGGGCACGGCCAGTGGCAGTTCAAGCAGCTCGGCATGGAGCCCAAGCAG
AGCACCACGCCTCTCTTCCCGGGCGCCGGCTACGGCCACACCGCGGCGTCCG
CGTACGCCATTGATCTTTCAAAAGAGGACGACGATGAGAAAGAGAGGCGGCAA
CAGCAGCAGCAGCAGCAGCAGCACTGCTTCCTCCTGGGCGCCGACCTCC
GTCTGGAGAAGCCGGCGGGCCACGACCACGCGGCGGCGGCGCAGAAACCTCT
CCGCCACTTCTTCGACGAGTGGCCGCATGAGAAGAACAGCAAGGGCTCCTGGA
TGGGGCTCGAAGGCGAGACGCAGCTGTCCATGTCCATCCCCATGGCCGCCAAC
GACCTCCCGATCACCACCACCTCCCGCTACCACAATGATGATTAA

>OsGRF2 (SEQ ID No. 4)

ATGGCGATGCCCTTTGCCTCCCTGTGCGCGGCGAGCCGACCACCGGCCCTCCTT
CATCTTCCCCTTCTGCCGCTCCTCCCCTCTCTCCGCGGTCCGGGAGGAGGCGC
AGCAGCACATGATGGGCGCGAGGTGGGCGGCGGCGGTGGCCAGGCCGCCGC
CCTTCACGGCGGCGCAGTACGAGGAGCTGGAGCAGCAGGCGCTCATATACAA
GTACCTCGTCGCCGGCGTGCCCGTCCCGGCGGATCTCCTCCTCCCCATCCGCC
GTGGCCTCGACTCACTCGCCTCGCGCTTCTACCACCACCCTGTCCTTGGATAC
GGTTCCTACTTCGGCAAGAAGCTGGACCCGGAGCCCGGACGGTGCCGGCGTA
CGGACGGCAAGAAGTGGCGGTGCTCCAAGGAGGCCGCGCCGGACTCCAAGTA
CTGTGAGCGACACATGCACCGCGGCCGCAACCGTTCAAGAAAGCCTGTGGAA

GCGCAGCTCGTCGCCCCCACTCGCAGCCCCCGCCACGGCGCCGGCCGCC
GCCGTACCTCCACCGCCTTCCAGAACCACTCGCTGTACCCGGCGATTGCTAA
TGGCGGCGGCGCCAACGGAGGCGGTGGTGGTGGTGGCGGTGGCGGCAGCGC
GCCTGGCTCGTTCGCCTTGGGGTCTAATACTCAGCTGCACATGGACAATGCTG
CGTCTTACTCGACTGTTGCTGCTGGTGCCGGAACAAAGATTTTCAGGTATTCTG
CTTATGGAGTGAGACCATTGGCAGATGAGCACAGCCCACTCATCACTGGAGCTA
TGGATACCTCTATTGACAATTCGTGGTGCTTGCTGCCTTCTCAGACCTCCACATT
TTCAGTTTCGAGCTACCCTATGCTTGAAATCTGAGTGAGCTGGACCAGAACAC
CATCTGCTCGCTGCCGAAGGTGGAGAGGGAGCCATTGTCATTCTTCGGGAGCG
ACTATGTGACCGTCGACTCCGGGAAGCAGGAGAACCAGACGCTGCGCCCCCTTT
TTCGACGAGTGGCCAAAGGCAAGGGACTCCTGGCCTGATCTAGCTGATGACAA
CAGCCTTGCCACCTTCTCTGCCACTCAGCTCTCGATCTCCATTCCAATGGCAAC
CTCTGACTTCTCGACCACCAGCTCACGATCACACAACGGTATATACTCCCGATG
A

>OsGRF3 (SEQ ID No. 5)

ATGGCGATGCCCTTTGCCTCCCTGTCGCCGGCAGCCGACCACCGGCCCTCCTT
CATCTTCCCCTTCTGCCGCTCCTCCCCTCTCTCCGCGGTGGGGAGGAGGCGC
AGCAGCACATGATGGGCGCGAGGTGGGCGGCGGCGGTGGCCAGGCCGCCGC
CCTTCACGGCGGCGCAGTACGAGGAGCTGGAGCAGCAGGCGCTCATATACAA
GTACCTCGTCGCCGGCGTGCCCGTCCCGGCGGATCTCCTCCTCCCCATCCGCC
GTGGCCTCGACTCACTCGCCTCGCGCTTCTACCACCACCCTGTCCTTGATAC
GGTTCCTACTTCGGCAAGAAGCTGGACCCGGAGCCCGGACGGTGCCGGCGTA
CGGACGGCAAGAAGTGGCGGTGCTCCAAGGAGGCCGCGCCGGACTCCAAGTA
CTGTGAGCGACACATGCACCGCGGCCGCAACCGTTCAAGAAAGCCTGTGGAA
GCGCAGCTCGTCGCCCCCACTCGCAGCCCCCGCCACGGCGCCGGCCGCC
GCCGTACCTCCACCGCCTTCCAGAACCACTCGCTGTACCCGGCGATTGCTAA
TGGCGGCGGCGCCAACGGAGGCGGTGGTGGTGGTGGCGGTGGCGGCAGCGC
GCCTGGCTCGTTCGCCTTGGGGTCTAATACTCAGCTGCACATGGACAATGCTG
CGTCTTACTCGACTGTTGCTGCTGGTGCCGGAACAAAGATTTTCAGGTATTCTG
CTTATGGAGTGAGACCATTGGCAGATGAGCACAGCCCACTCATCACTGGAGCTA
TGGATACCTCTATTGACAATTCGTGGTGCTTGCTGCCTTCTCAGACCTCCACATT
TTCAGTTTCGAGCTACCCTATGCTTGAAATCTGAGTGAGCTGGACCAGAACAC
CATCTGCTCGCTGCCGAAGGTGGAGAGGGAGCCATTGTCATTCTTCGGGAGCG
ACTATGTGACCGTCGACTCCGGGAAGCAGGAGAACCAGACGCTGCGCCCCCTTT
TTCGACGAGTGGCCAAAGGCAAGGGACTCCTGGCCTGATCTAGCTGATGACAA
CAGCCTTGCCACCTTCTCTGCCACTCAGCTCTCGATCTCCATTCCAATGGCAAC

CTCTGACTTCTCGACCACCAGCTCACGATCACACAACGGTATATACTCCCGATG
A

>OsGRF4 (SEQ ID No. 6)

ATGGCGATGCCGTATGCCTCCCTGTCTCCGGCGGTGGCCGACCACCGCTCGTC
CCCGGCAGCCGCGACCGCCTCCCTCCTCCCTTCTGCCGCTCCACCCCGCTCT
CCGCGGGCGGTGGTGGCGTCGCGATGGGGGAGGACGCGCCGATGACCGCGA
GGTGGCCGCCGGCGGCGGCGGCGAGGCTGCCGCCGTTACCGCGGCGCAGT
ACGAGGAGCTGGAGCAGCAGGCGCTCATATACAAGTACCTGGTGGCAGGCGT
GCCCCGTCCCGCCGGATCTCGTGCTCCCCATCCGCCGCGGACTCGACTCCCTC
GCCGCCCGCTTCTACAACCATCCCGCCCTTGATATGGTCCGTA CTTCGGCAA
GAAGCTGGACCCAGAGCCAGGGCGGTGCCGGCGTACGGACGGCAAGAAATGG
CGGTGCTCGAAGGAGGCCGCGCCGGATTCCAAGTACTGCGAGCGCCACATGC
ACCGCGGCGCGCAACCGTTCAAGAAAGCCTGTGGAAACGCAGCTGGTCGCCCA
GTCCCAACCGCCCTCATCTGTTGTCGGTTCTGCGGCGGCGCCCCTTGCTGCTG
CCTCCAATGGCAGCAGCTTCCAAAACCACTCTCTTTACCCTGCTATTGCCGGCA
GCAATGGCGGGGGCGGGGGGAGGAACATGCCCAGCTCATTTGGCTCGGCGTT
GGGTTCTCAGCTGCACATGGATAATGCTGCCCTTATGCAGCTGTTGGTGGTG
GAACAGGCAAAGATCTCAGGTATACTGCTTATGGCACAAGATCTTTGGCGGATG
AGCAGAGTCAACTCATTACTGAAGCTATCAACACATCTATTGAAAATCCATGGCG
GCTGCTGCCATCTCAGAACTCGCCATTTCCCTTTCAAGCTATTCTCAGCTGGG
GGCACTAAGTGACCTTGGTCAGAACACCCCCAGCTCACTTTCAAAGGTTCAAG
GCAGCCACTTTCTGTTCTTTGGGAACGACTATGCGGCTGTGATTCTGTGAAGCA
AGAGAACCAGACGCTGCGTCCCTTCTTTGATGAGTGGCCAAAGGGAAGGGATT
CATGGTCAGACCTCGCTGATGAGAATGCTAATCTTTGTCATTCTCAGGCACCC
AACTGTGATCTCCATACCAATGGCATCCTCTGACTTCTCGGCGGCCAGTTCTC
GATCAACTAATGGTGACTGA

>OsGRF5 (SEQ ID No. 53)

ATGCTGAGCTCGTCGCCCTCGGCGGCGGCGCCGGGGATAGGAGGGTACCAGC
CGCAGCGCGGGGCGGCGGTCTTCACGGCGGCGCAGTGGGCGGAGCTGGAGC
AGCAGGCGCTCATTTACAAGTACCTCGTCGCCGGTGTCCCCGTCCCGGGCGAT
CTCCTCCTCCCAATCCGCCCCCACTCCTCCGCCGCCGCCACCTACTCCTTCGC
CAACCCCGCCGCCGCGCCCTTCTACCACCACCACCACCACCCCTCTCTGAGCT
ATTATGCCTACTATGGCAAGAAGCTTGACCCTGAGCCGTGGCGTTGCCGCCGC
ACCGACGGCAAGAAGTGGCGGTGCTCCAAGGAGGCGCACCCCGACTCCAAGT
ACTGCGAGCGCCACATGCACCGTGGCCGCAACCGTTCAAGAAAGCCTGTGGA

ATCCAAGACCGCTGCCCCCTGCGCCCCAGTCGCAGCCCCAGCTGTCCAATGTCA
CGACCGCGACTCACGACACCGATGCGCCTCTCCCGTCACTCACTGTGGGTGCT
AAAACCCACGGTCTGTCCCTTGGTGGTGCTGGCTCGTCGCAGTTCCATGTCGA
CGCACCATCGTACGGCAGCAAGTACTCTCTTGGAGCTAAAGCTGATGTGGGTG
AACTGAGCTTCTTCTCAGGAGCATCAGGAAACACCAGGGGCTTCACCATTGATT
CTCCAACAGATAGCTCATGGCATTCACTGCCTTCCAGTGTACCCCCATACCCGA
TGTCAAAGCCAAGGGACTCTGGCCTCCTACCAGGTGCCTACTCCTACTCCCACC
TTGAACCTTCACAGGAATTGGCCAGGTCACCATCGCCTCGCTGTCCCAAGAG
CAGGAGCGCCGCTCTTTTGGTGGTGGAGCGGGGGGGATGCTAGGAAATGTGA
AGCACGAGAACCAGCCGCTGAGGCCTTTCTTCGATGAGTGGCCTGGGAGGCGA
GACTCGTGGTCGGAGATGGATGAGGAGAGGTCCAACCAGACCTCCTTCTCGAC
AACCCAGCTCTCGATCTCCATCCCGATGCCCAGATGTGATTGA

>OsGRF6 (SEQ ID No. 54)

ATGCAGGGTGCAATGGCCAGGGTGAGGGGTCCCTTCACGCCGTCTCAGTGGAT
CGAGCTGGAGCACCAGGCGCTGATATACAAGTACTTGGCTGCGAATAGCCCTG
TACCACACAGCCTCCTCATCCCCATCAGGAGGAGCCTCACATCGCCCTACTCAC
CTGCCTACTTTGGCTCAAGCACATTGGGATGGGGATCTTTCCAGCTGGGCTACT
CCGGCAGCGCGGATCCGGAGCCCGGCCGGTGCCGCCGGACGGACGGCAAGA
AATGGCGGTGCTCGAGGGATGCGGTGCGCGACCAGAAGTACTGTGAGCGACA
CATGAACCGGGGACGCCACCGTTCAAGAAAGCATGTGGAAGGCCAGCCTGGC
CATGCCGCGAAAGCGATGCCCGCGGCGGTGGCAGCAGCCGCTGCCTCTGCTA
CCCAGCCTAGTGCTCCGGCCGCCACAGTGGCGGAGCTGTTGCTGGCCTCGC
TATCAACCATCAGCACCAGCAAATGAAGAACTACGCTGCCAACACTGCCAATCC
TTGCTCTCTGCAATATAGCAGGGATCTGGCAAACAAGCATAATGAGAGTGAACA
AGTGCAAGACTCAGACAGTCTCTCGATGCTGACTTCCATTAGCACGAGAAATAC
GGGCAGCCTGTTTCCGTTCTCAAACAACATAATCCTTTTGAAGTGTCCAACTCA
AGGCCAGATTTTGGCCTAGTATCACCTGATTCACTGATGAGTTCTCCTCATAGCT
CCTTGGAGAACGTCAATTTGCTCACTTCGCAGAGTCTGAATGAACAACAGAGTT
CAGTTTCCCTTCAACACTTTGTGGACTGGCCAAGGACACCTGCACAAGGAGCTC
TCGCATGGCCTGATGCTGAAGACATGCAAGCTCAGAGAAGCCAGCTCTCAATAT
CTGCTCCAATGGCGTCTTCTGACCTGTCATCAGCCTCAACATCTCCCATCCATG
AGAAGCTGATGTTGTCACTTAACTGAGCCGTGAATATAGTCCTATTGGTCT
CGTTTTTGCAGCAAATAGAGATGAGGTTAACCAGGGAGAAGCAAACCTGGATGC
CTATGTTCCGTGATTCTTTGATGGGCGGACCATTGGGAGAGGTTTTAAACCAAGA
ATAACAACATGGAAGCAAGGAATTGCCTATCGGAGTCTCTGAATCTTTTAAATGA
TGGCTGGGATTCAAGCTCAGGGTTTGATTCACTCCCGAGTTGGTGTCTGCAGAA

GACCACCTTTGGATCAGTATCCAGTAGCACCGGAAGCAGTCCTAGACTGGAGA
ATCATAGTGTATTATGATGGCAACAGTAACCTGCGGGATGATCTCGGTTCAAGTTG
TTGTAAATCATCCGAGCATCCGCCTGGTGTGA

>OsGRF7 (SEQ ID No. 55)

ATGGCAATGGCGACCCCTACGACCAACGGCAGCTTCCTTCTTGGATCAGGGTT
GGATTGTGGAAGCTCAGATGTGGCAAGAATGCAGGGGGTTTTAGCAAGGGTTA
GGGGGCCATTACACCAACACAATGGATGGAGCTGGAGCACCAGGCTCTGATC
TACAAGCACATTGTGGCGAATGCGCCGGTACCGGCCGGCTTGCTCCTCCCAT
CAGGAGAAGCCTCCATCCACCAGTGTTCCACACTTCTCCTCTGGTGGCATTCT
TGGCTCCAGCTCCTTGGGATGGGGGTCATTTCAAGCTGGGCTATTCTGGGAGTG
CTGACTCCGAGCCCCGGGAGATGCCGTGGAACCGATGGCAAGAAATGGCGGTG
CTCGAGAGACGCAGTTGTGACCAAAAGTACTGCGAGCGGCACATAAACCGGG
GTCGCCACCGTTCAAGAAAGCATGTGGAAGGCCAATCTAGCCATGCCGCAAAA
GCAACGGTTCCCGCCATAGCACAACCACCCATTGGTGCATCTAATGGCAAATTG
TCAGGCAGCCATGGTGTGTCAAATGAGCTCACGAAAACCTTGGCTACTAACAGG
ATGATGTTGGATAAAGCAAATCTTATTGAACGCTCCAGGACTACACTAATCAGC
AACACAACATCCTACAGAACAACACAAAAGGTGATAATTGGTCTGAAGAGATGT
CCTCACAAGCAGACTATGCAGTAATCCCTGCTGGCTCTCTCATGAACACACCGC
AATCGGCGAATTTAAATCCAATTCCCCAGCAACAACGCTGTAAGCAGTCACTCTT
TGGCAAAGGGATACAGCATGATGACATTCAGCTGTCGATATCCATTCCCGTGGA
TAACTCCGACTTACCCACTAACTACAACAAGGCTCAAATGGACCATGTAGTAGG
CGGTTTCATCGAATGGCGGAAACAACACGCGAGCAAGTTGGATACCGGGCTCCT
GGGAAGCGTCCATAGGTGGACCTCTGGGTGAGTTCTTACCAACACCAGCAGC
GCATCAGACGACAAAGGCAAAAGCCGCCACCCGCCATCTTTGAACCTCTTAGCT
GATGGACATACTACAAGTCCACAGCTGCAATCGCCCACCGGAGTCCTGCAGAT
GACTAGCTTCAGTTCAGTGCCCAGCAGCACTGTTAGTAGTCCTGCAGGCAGCCT
CTGCAATGGCTTGCTCACTTCAGGCCTGGTGAATGCCCAGACTGTCCAAACACT
GTGA

>OsGRF8 (SEQ ID No. 56)

ATGCTGAGCTCTTGTGGTGGCCATGGCCATGGAAATCCAAGAAGCTTGCAAGAA
GAACACCATGGCAGATGTGGTGAGCAGCAAGGTGGAGGAGGAGGAGGGGC
AAGAGCAAGAGCAAGATGGGTTCTTGGTGAGAGAGGCAAGGGCATCCCCACCA
TCTCCATCTTCTTCATCATTTCTTGGATCCACAAGCTCTTCTTGTCTGGAGGAG
GAGGAGGAGGGCAGATGTTGAGCTTCTCCTCCCCCAATGGAACAGCAGGGTTG

GGCTTGAGCTCAGGAGGAAGCATGCAGGGGGTCTTGGCAAGGGTCAGGGGGC
CGTTCACCCCAACACAGTGGATGGAGCTGGAGCACCAGGCACTGATCTACAAG
CACATTGCTGCAAATGTTTCTGTCCCTTCCAGCTTGCTCCTCCCCATCAGGAGA
AGCCTCCATCCATGGGGATGGGGATCATTCCCTCCTGGCTGTGCTGATGTAGA
ACCCAGAAGATGCCGCCGCACAGACGGCAAGAAGTGGCGGTGCTCCAGAGAT
GCTGTTGGGGATCAGAAGTATTGTGAGCGACACATAAACCGTGGTCGCCATCG
TTCAAGAAAGCATGTGGAAGGCCGAAAGGCGACACTCACCATTGCAGAACCAT
CCACGGTTATTGCTGCTGGTGTATCATCTCGCGGCCACACTGTGGCTCGGCAG
AAGCAGGTGAAAGGCTCAGCTGCTACTGTCTCTGATCCTTTCTCGAGACAATCC
AACAGGAAATTTCTGGAGAAACAGAACGTTGTGACCAATTGTCTCCCATGGAT
TCATTTGATTTCTCATCCACACAATCTTCTCCAACTATGACAATGTAGCATTGTC
ACCACTGAAGTTGCACCATGATCATGATGAATCTTACATCGGGCATGGAGCAGG
CAGTTCATCAGAAAAAGGCAGTATGATGTACGAAAGTCGGTTAACAGTCTCTAA
GGAAACACTTGATGATGGACCTTTAGGTGAAGTTTTCAAAGAAAGAATTGCCAA
TCAGCTTCTACAGAAATCTTAACTGAAAAATGGACTGAGAACCCCAACTTACATT
GCCCATCTGGAATCCTACAAATGGCTACTAAGTTCAATTCAATTTCCAGCGGCAA
CACAGTAAATAGTGGTGGCACCGCAGTGGAGAATCTTATCACTGATAATGGATA
TCTTACTGCAAGAATGATGAATCCTCATATTGTCCCAACACTTCTCTAA

>OsGRF9 (SEQ ID No. 57)

ATGTTTGCTGACTTCTCTGCTGCTGCCATGGAGCTTGGAGAGGTGTTGGGCTTG
CAAGGACTCACAGTGCCATCCACCAAGGAGGGTGATCTGAGCCTCATCAAGAG
AGCTGCTGCTGGTAGCTTCACCCAGGCTGCTGCTGCATCATACCCTTCCCCCTT
TCTTGATGAACAGAAGATGCTCAGATTGCCAAGGCTGCTCACACATTGCCATC
AGGTTTGGATTTTGGGAGGGAAAATGAGCAGAGGTTCTTGTTGTCTAGGACCAA
GAGGCCTTTCACTCCCTCACAGTGGATGGAGCTGGAGCACCAGGCTCTCATTTA
CAAGTATCTCAATGCAAAGGCCCTATACCTTCCAGCCTGCTCATTTCAATCAGC
AAAAGCTTCAGATCATCAGCTAACAGAATGAGCTGGAGGCCTCTCTATCAAGGC
TTCCCAAATGCAGACTCTGACCCAGAACCTGGAAGATGCCGTGCAACAGATGG
CAAGAAATGGCGGTGTTCAAAGGAGGCCATGGCCGACCACAAGTATTGTGAGA
GGCACATCAACAGAAACCGCCACCGTTCAAGAAAGCCTGTGGAAAACCAAAGT
AGAAAGACTGTGAAAGAGACACCGTGTGCTGGCTCATTGCCATCTTCTGTGGG
CAGGGCAGCTTCAAGAAGGCCAAAAGTTAATGAAATGAAGCCACGCAGTATCAGC
TATTGGACAGATAGTTTGAACAGGACAATGGCGAACAAAGAGAAAGGAAACAAA
GCTGCTGAAGAAAACAATGGCCCACTGCTAAATTTAACGAATCAACAGCCAACA
TTGTCCCTGTTCTCTCAGTTGAAGCAACAGAACAAACCGGAGAAGTTCAATACA
GCAGGAGACAGTGAATCGATTTCTTCAAATACCATGTTGAAGCCTTGGGAGAGC

AGCAACCAGCAGAACAACAAAAGCATTCTTTACCAAGATGCATGATCGTGGA
TGCCTTCAGTCAGTCCTTCAGAAATTTAGCTTGCCTAAGGACGAGAAAATGGAG
TTTCAGAAAAGCAAAGATTCCAATGTCATGACAGTTCCATCAACTTTCTATTCT
CGCCAGAGGACCCACGCGTCAGCTGCCATGCACCTAATATGGCACAAATGCAA
GAGGATAGCATCTCAAGTTCTTGGGAGATGCCTCAAGGTGGACCTCTAGGTGA
GATCTTGACAAACTCCAAAAATCCTGACGATTCAATCATGAAACCAGAAGCAAG
GCCATATGGTTGGTTACTGAACCTCGAGGATCATGCAATGTGA

>OsGRF10 (SEQ ID No.58)

ATGGATGAGGAGAAGGAAGCCGACTCGCCGCAGCCACCGTCCAAGCTGCCTC
GCCTCTCCGGCGCTGACCCGAATGCCGGAGTGGTGACCATGGCAGCACCCCC
GCCGCCGGTGGGTCTTGGGCTGGGGCTTGGACTCGGCGGCGACAGCCGCGG
CGAGCGTGACGTGGAAGCGTCGGCGGCGGGCGGCGCACAAGGCGACGGCGCT
GACGTTTCATGCAGCAGCAGGAGCTGGAGCACCAGGTGCTCATCTACCGCTACT
TCGCCGCGGGCGCGCCCGTGCCGGTGACCTCGTGCTCCCCATCTGGAAGAG
CGTCGCGTCCTCCTCCTTCGGCCCCGACCGCTTCCCTTCCCTGGCAGTGATGG
GGTTGGGGAACCTGTGCTTCGACTACCGGAGCAGCATGGAGCCGGACCCAGG
GCGGTGCAGGCGCACGGACGGCAAGAAGTGGCGGTGCTCGCGCGACGTGGT
GCCGGGGCACAAGTACTGCGAGCGGCACGTCCACCGCGGACGCGGGCCGTTCA
AGAAAGCCTGTGGAAGCCTCCGCGGGCGCCACCCGGCGAACAACGGCGGC
GGCGGTGGCATCGTCTTCTCCCCACCAGCGTCCTCCTCGCCCACGGCACCGC
GCGCGCCACCTGA

>OsGRF11 (SEQ ID No. 59)_

ATGGCGGCGGAGGGGGAGGCCAAGAAGGACAGCGCCAGCAACCCTCCCGGG
GGAGGAGGCGGCGGAGGTGGAGGGGAGGAGGAGGAGGATAGCAGCCTGGCT
GTCGGGGAGGCGGCGGTCTGGGGTGGGCGAGGCTGGTGGAGGAGGAGGAGGA
GGGGAGAAGGCGGATCGAGAGGAGGAGGAGGGGAAGGAGGATGTGGAGGAG
GGCGGCGTGTGTAAGGATCTGGTGCTCGTCGAGGACGCCGTCCCCGTGAGG
ATCCGGAGGAAGCCGCGAGCAACTGCAGCACTTCAGGAAGAAATGAAAGCGCTC
GTTGAATCCGTCCCAGTTGGTGCTGGGGCGGCATTACCGCGATGCAACTACA
GGAGCTTGAGCAGCAATCTCGTGCTACCAAGTATATGGCTGCCCGTGTGCCTGT
GCCTACTCATCTCGTCTTCCCAATATGGAAGAGTGTTACTGGTGCATCTTCTGAA
GGCGCCCAGAAGTACCCGACATTGATGGGGTTGGCAACACTCTGCTTGGACTT
TGGAAGAACCAGAACCAAGACCTGGGAGGTGCCGGCGAACTGATGGAAAGA
AGTGGCGGTGCTGGAGAAATGCAATTGCAAATGAGAAATATTGCGAACGCCATA
TGACCCGTGGCCGCAAGCGTCCTGTACAGCTTGTTGTCGAGGATGACGAGCCT

GATTCTACCTCAGGGTCGAAACCAGCATCTGGCAAGGCCACCGAAGGTGGCAA
GAAGACTGATGACAAGAGCTCAAGTAGCAAGAAGCTTGCAGTGGCAGCACCAG
CTGCTGTGGAGTCTACATGA

>OsGRF12 (SEQ ID No. 60)

ATGTTGGCCGAGGGAAGGCAAGTCTACTTGCCGCCGCCGCCGCCGTCCAAGCT
TCCTCGTCTCTCCGGCACCGATCCAACCGACGGCGTGGTGACGATGGCAGCGC
CGTCGCCGCTGGTTCTTGGGCTGGGTCTCGGTCTGGGCGGCAGCGGCAGCGA
CAGCAGTGGGAGCGACGCGGAAGCGTCTGCGGCCACCGTGCGGGAGGCGCG
GCCGCCGTCGGCGCTGACGTTCATGCAGCGGCAGGAGCTGGAGCAGCAGGTG
CTCATCTACCGCTACTTCGCCGCCGGCGCGCCTGTGCCGGTTCACCTCGTGCT
GCCCATATGGAAGAGCATCGCCGCCGCCTCCTCGTTCGGCCCGCAAAGCTTTC
CCTCCCTGACGGGCCTGGGGAGCCTGTGCTTCGACTACAGGAGCAGCATGGA
GCCGGAGCCGGGGCGGTGCCGGCGCACGGACGGCAAGAAGTGCGGGTGCTC
GCGCGACGTGGTGCCGGGGCACAAGTATTGCGAGCGGCACGTCCACCGTGGC
CGCGGCCGTTCAAGAAAGCCTATGGAAGCCTCTGCAGCAGTCGCTCCCACATA
TCTCCCGGTCCGGCCGGCACTCCACACCGTCGCCACCCTCGCCACCAGCGCG
CCATCGCTGTCGCACCTCGGTTTCTCCTCCGCCAGCAAAGTGCTCCTCGCCCA
CACCACCACCGGCACCACGCGCGCTACTTGA

FIGURE 24

>OsGRF1 (SEQ ID No. 22)

MMMMSGRPSGGAGGGRYPFTASQWQELEHQALIYKYMASGTPIPSDLILPLRRSF
 LLDSALATSPSLAFPPQPSLGWGCFCGMGFRKAEDPEPGRCRRTDGKKWRCSKE
 AYPDSKYCEKHMHRGKNRSRKPVEMSLATPPPPSSSATSASNTSAGVAPTTTTT
 SSPAPSYSRPAPHDAAPYQALYGGPYAAATARTPAAAAYHAQVSPFHLQLDTHP
 HPPPSYYSMDHKEYAYGHATKEVHGEHAFFSDGTEREHHHAAAGHGQWQFKQLG
 MEPKQSTTPLFPGAGYGHTAASPYAIDLSKEDDDEKERRQQQQQQQQHCFLG
 ADLRLEKPAGHDHAAAAQKPLRHFFDEWPHEKNSKGSWMGLEGETQLSMSIPMA
 ANDLPITTTSTRYHNDD

>OsGRF2 (SEQ ID No. 23)

MMAGGGSGRCLFTATQWQELEHQALIYKYMAAGAPVPPDLLHLRHRAAAAAAAD
 VDTVPSLAFPPHHLGWGCYGAAAQYGRRVEDPEPGRCRRTDGKKWRCSREAY
 GESKYCEKHMHRGKNRSRKPVEMPPPAAAVYRPSALSISPPPHDADAPSYGAGA
 GAPLQLHLD SFHASTSPPPSYHRYAHTSSAPLFPSSAAGYGGWSLSKEHCLTLG
 GAAADLSLDKPADHHHDATSATTEKPLRRFFDEWPRSDDGRTPWDGTQLSISIPTA
 AAASPD LAIAGAASRYHSNGDHLRTSE

>OsGRF3 (SEQ ID No. 24)

MAMPFASLSPAADHRPSFIFPFCRSSPLSAVGEEAQQHMMGARWAAAVARPPPFT
 AAQYEELEQQALIYKYL VAGVPVPADLLLPIRRGLDSLASFYHHPVLGYGSYFGKK
 LDPEPGRCRRTDGKKWRCSKEAAPDSKYCERHMHRGRNRSRKPVEAQLVAPHS
 QPPATAPAAAVTSTAFQNHSLYPAIANGGGGANGGGGGGGGGGSAPGSFALGSNT
 QLHMDNAASYSTVAAGAGNKDFRYSAYGVRPLADEHSPLITGAMDTSIDNSWCLLP
 SQTSTFSVSSYPMLGNLSELDQNTICSLPKVEREPLSFFGSDYVTVDSGKQENQTL
 RPFDEWPKARDSWPD LADDNSLATFSATQLSISIPMATSDFTTSSRSHNGIYSR

>OsGRF4 (SEQ ID No. 25)

MAMPYASLSPAVADHRSSPAAATASLLPFCRSTPLSAGGGGVAMGEDAPMTARW
 PPAARLPPFTAAQYEELEQQALIYKYL VAGVPVPPDLVLPPIRRGLDSLAAFYNH
 PALGYGPYFGKKLDPEPGRCRRTDGKKWRCSKEAAPDSKYCERHMHRGRNRSR
 KPVETQLVAQSQPPSSVVGSAAPLAAASNGSSFQNHSLYPAIAGSNGGGGGGRNM
 PSSFGSALGSQLHMDNAAPYAAVGGGTGKDLRYTAYGTRSLADEQSQLITEAINTSI
 ENPWRLLP SQNSPFPLSSYSQLGALSDLGQNT PSSLSKVQRQPLSFFGNDYAAVD

SVKQENQTLRPFFDEWPKGRDSWSDLADENANLSSFSGTQLSISIPMASSDFSAAS
SRSTNGD

>OsGRF5 (SEQ ID No. 61)

MLSSSPSAAAPGIGGYQPQRGAAVFTAAQWAELEQQALIYKYLAVGVVPVPGDLLPI
RPHSSAAATYSFANPAAAPFYHHHHHPSLSYAYYGKKLDPEPWRCRRTDGKKW
RCSKEAHPDSKYCERHMRGRNRSRKPVESKTAAPAPQSQPQLSNVTTATHDTD
APLPSLTVGAKTHGLSLGGAGSSQFHVDAPSYGSKYSLGAKADVGEISSFGASG
NTRGFTIDSPTDSSWHSLPSSVPPYPMSKPRDSGLLPGAYSYSHLEPSQELGQVTI
ASLSQEQERRSFSGGAGGMLGNVKHENQPLRPFFDEWPGRRDSWSEMDEERSN
QTSFSTTQLSISIPMPRCGSPIGPRLP

>OsGRF6 (SEQ ID No. 62)

MQGAMARVRGPFTPSQWIELEHQALIYKYLAANSPVPHSLLPIRRSLTSPYSPAYF
GSSTLGWGSFQLGYSGSADPEPGRCRRTDGKKWRCSRDAVADQKYCERHMNRG
RHRSRKHVEGQPGHAAKAMPAAVAAAAASATQPSAPAAHSGGAVAGLAINHQHQ
QMKNYAANTANPCSLQYSRDLANKHNESEQVQDSDSLSMILTSISTRNTGSLFPFSK
QHNPFEVSNRPDFGLVSPDSLMSSPHSSLENVNLLTSQSLNEQQSSVSLQHFDV
WPRTPAQGALAWPDAEDMQAQRSQLSISAPMASSDLSSASTSPIHEKMLMLPLKLS
REYSPIGLGAANRDEVNQGEANWMPMFRDSLMGGPLGEVLTNNNNMEARNCLS
ESLNNLLNDGWDSSSGFDSSPVGVQLKTTFGSVSSSTGSSPRENHSVYDGNSNLR
DDLGSVVVNHPISIRLV

>OsGRF7 (SEQ ID No. 63)

MAMATPTTNGSFLLGSGLDGSSDVARMQGVLARVRGPFTPTQWMELEHQALIYK
HIVANAPVPAGLLLPIRRSLHPPVFPFHSSGGILGSSSLGWGSFQLGYSGSADSEPG
RCRRTDGKKWRCSRDAVVDQKYCERHINRGRHRSRKHVEGQSSHAAKATVPAIA
QPPIGASNGKLSGSHGVSNELTKTALNRMMLDKANLIERSQDYTNQQHNILQNN
KGDNWSEEMSSQADYAVIPAGSLMNTPQSANLNPIQQQRCKQSLFGKGIQHDDI
QLSISIPVDNSDLPTNYNKAQMDHVVGSSNGGNNTASWIPGSWEASIGGPLGEF
FTNTSSASDDKGKSRHPPSLNLLADGHTTSPQLQSPTGVLQMTSFSSVPSSTVSSP
AGSLCNGLLTSGLVNAQTVQTL

>OsGRF8 (SEQ ID No. 64)

MLSSCGGHGHGNPRSLQEEHHGRCGEQQGGGGGGGQEQEQDGFLVREARASP
PSPSSSSFLGSTSSSSCGGGGGGGQMLSFSSPNGTAGLGLSSGGSMQGVLARVRG
PFTPTQWMELEHQALIYKHIAANVSVPSLLLPIRRSLHPWGWGSFPFGCADVEPR

RCRRTDGKKWRCSRDAVGDKY CERHINRGRHRSRKHVEGRKATLTIAEPSTVIA
AGVSSRGHTVARQKQVKGSAATVSDPFSRQSNRK FLEKQNVVDQLSPMDSFDFS
STQSSPNYDNVALSPLKLHHDHDESYIGHGAGSSSEKGSMMYESRLTVSKETLDD
GPLGEVFKRKNCQSASTEILTEKWTENPNLHCP SGILQMATKFNSISSGNTVNSGG
TAVENLITDNGYLTARMMNPHIVPTLL

>OsGRF9 (SEQ ID No. 65)

MFADFSAAAMELGEVLGLQGLTVPSTKEGDL SLIKRAAAGSFTQAAAASYPSPFLLD
EQKMLRFAKAAHTLP SGLDFGRENEQRFLLSRTKRPFTPSQWMELEHQALIYKYL N
AKAPIPSSLLISISK SFRSSANRMSWRPLYQGFPNADSDPEPGRCRRTDGKKWRCS
KEAMADHKY CERHINRNRHRSRKPVENQSRKTVKETPCAGSLPSSVGQGSFKKAK
VNEMKPRSISYWTD SLNRTMANKEKGNKAAEENNGPLLNL TNQQPTLSLFSQLKQ
QNKPEKFNTAGDSE SSSNTMLKPWESSNQNNKSIPFTKMHDRGCLQSVLQNFS
LPKDEKMEFQKSKDSNVMTVPSTFYSSPEDPRV SCHAPNMAQMQEDSISSSWEM
PQGGPLGEILTNSKNPDDSIMKPEARPYGWLLNLEDHAM

>OsGRF10 (SEQ ID No. 66)

MDEEKEADSPQPPSKLPRLSGADPNAGVVTMAAPPPVGLGLGLGLGGDSRGER
DVEASAAAAHKATALTFMQQQELEHQVLIYRYFAAGAPVPVHLVLPWKSVASSSF
GPHRFPSLAVMGLGNLCFDYRSSMEPD PGRCRRTDGKKWRCSRDRVPGHKYCE
RHHVHRGRGRSRKPVEASAAATPANNGGGGGGIVFSPTSVLLAHGTARAT

>OsGRF11 (SEQ ID No. 67)

MAAEGEAKKDSASNPPGGGGGGGGGEEEEEDSSLAVGEAAVGVGEAGGGGGGG
EKADREEEEGKEDVEEGGVCKDLVLVEDAVPVEDPEEAAATAALQEEMKALVESV
PVGAGAAFTAMQLQELEQQSRVYQYMAARVPVPTHLVFPIWKSVTGASSEGAQKY
PTLMGLATLCLDFGKNPEPEPGRCRRTDGKKWRCWRNAIANEKY CERHMHGRGRK
RPVQLVVEDDEPDSTSGSKPASGKATEGGKKTDDKSSSSK KLAVAAPAAVEST

>OsGRF12 (SEQ ID No. 68)

MLAEGRQVYLPPPPPSKL PRLSGTDPTDGVVTMAAPSPLVLGLGLGLGGSGSDSS
GSDAEASAATVREARPPSALT FMRQEELEQQVLIYRYFAAGAPVPVHLVLPWK SIA
AASSFGPQSFP SLTGLGSLCFDYRSSMEPEPGRCRRTDGKKWRCSRDRVPGHKY
CERHVVHRGRGRSRKPMEASAAVPTYLPVRPALHTVATLATSAPSLSHLGFSSASK
VLLAHTTTGTTRAT

FIGURE 25

>ZmGRF1 (SEQ ID No. 7)

gacagggttgagatggcgatgccgtatgcctctcttcccccggcaggcgccgcccaccacgctcctccacagccacg
 gcgtccctcgctccctctcgccgtccaccccgctctccggggcgccggctgggggaggaggacgcccaggcgga
 gcgcgagggtggccggccgcgaggccgggtggtgcggttcacgcccgcgagtaggagctggagcagcaggc
 gctcatatacaagtacctggtggccggcggtcccggtccgcccgatctcggttccaatccgccggcgccctcgactcc
 ctgcctaccgcttctacggccaacccacactcgggtacggaccgtacctggggaggaaactggatccggagcccg
 gccggtgccggcgaaacggacggcaagaagtggcggtgctccaaggaagccgcccggactccaagtactgca
 gcgccacatgcaccgcggccgcaacccgttcaagaaagcctgtggaacgcagctgcgccccagtcccaaccg
 cccgccgcccggcggtctccgccgtccgcccctggcagccgcgcgcccgcgcccaccaacggcagcggcttc
 cagaaccactctctacccggccatcgccggcagcactggtggtggaggaggagttggcggggtccggcaatatctc
 ctccccgttctcctcgatggggggatcgctcagctgcacatggacagtgtgacagctactcctacgcagctcttg
 tggtggaactgcaaaggatctcaggtacaacgcttacggaataagatctctggcggacgagcacaaccagctgac
 gcagaagccatcgactcgatagagagccagaggcgctcccccagctcgctgttcccgctctcgagctaccac
 atctcgggcgctggggcgacctggggcgccagaacagcacggtgagctcgctgcgaagatggagaagcagca
 gccgcccctcgctctcctagggaaacgacaccggggccggcatggccatgggctccgctccgcgaagcaggaggg
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FIGURE 26

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SRKHVEGRKATPTTADPTMAVSGGSLLHSHAVAWQQQKSSAANVTDPFSLGSN
RNLLDKQNLGDQFSVSTSMDSFDFSSSHSSPNQAKVAFSPVAMQHEHDQLYLVHG
AGSSAENVNKSQDGQLLVSRRETIDDGPLGEVFKGKSCQSASADILTDHWTSTRDLR
PPTGVLQMSSSNTVPAENHTSNSSYLMARMANSQTVPTLH

>ZmGRF3 (SEQ ID No. 27)

MMMMSSGRAGGGATAGRYPFTASQWQEEHQALIKYKCLASGKPIPSYLMPLRRI
LDSALATSPSLAYPPQPSLGWGCFGMGFTRKADEDPEPGRRCRRTDGKKWRCSKE
AYPDSKYCEKHMHRGKNRSRKPVEMSLATPAPAPAPAAATTATATSSPAPSYHRP
AHDATPSPYHALYGGGGGGGGSPYSASARPGATGGGGAYHHAQHVSFHLHLET
THPHPPPPYNYSADQRDYAYGHAAAKEVGEHAFFSDGAGERVDRQAAAGQWQF
RQLGVETKPGPTPLFPVAGYGHGAASPYGVELGKDDDEQEERRRQHCFVLGADL
RLERPSSGHGHGHGHDHDDAAAAQKPLRPFDEWPHQKGDKAGSWMGLDGETQ
LSMSIPMAATDLPVTSRFRNDE

>ZmGRF4 (SEQ ID No. 76)

MAAEGEAKNPSSGGGEGGNPQHQQAVQAAPAEPPMAQGEAVQEAGAQTGQEPE
GEKANRDGEGSAGEKDDGACRDLVLVEDPEVLAVEDPEEAAATAALQEEMKALVA
SVPDGAGAAFTAMQLQEEQQSRVYQYMAARVPVPTHLVFPVWKSVTGASSEGA
QKYPTLLGLATLCLDFGKNPEPEPGRRCRRTDGKKWRCWRNTIPNEKYCERRMHR

GRKRVPQWEEAEPDSASGSKSAPGKATEGAKKVGDKSPGSKKLAVAAAAAAAQ
ST

>ZmGRF5 (SEQ ID No. 28)

MGMAMPFASPSPAADHRPSSLLPFCRAAPLSAAGEDAAQQHAMSGRWAARPALF
TAAQYEELEHQALIYKYL VAGVPVPPDLLLPLRRGFVFHQPPALGYGPYFGKKVDPE
PGRCCRRTDGKKWRCSKEAAPDSKYCERHMRGRNRSRKPVEAQLAPPPHAQPP
QQQQAPAPAAGFQNHSLYPSILNGNGGGGLGAGAGGGTFLGPTSQ LHMDSAAA
YATAAGGGSKYLRYSAYGVKSLSDEHSTLLSGGMDPSMMDNSWRLLPSQNNTFQ
ATSYPVFGTSLGDESTIASLPKTQREPLSFFGSDFVTAAKQENQTLRP FFDEWPKS
RDSWP ELGEDGSLGFSATQLSIPMATSDFSNTSSRSPGGIPSR

>ZmGRF6 (SEQ ID No. 29)

MAMPFASLSPAADHRPSSLLPYCRAAPLSAVGEDAAAQAAAAQQQQHAMSGRWAA
RPPALFTAAQYEELEHQALIYKYL VAGVPVPPDLLLPLRRGFVYHQPALGYGPYFGK
KVDPEPGRCCRRTDGKKWRCSKEAAPDSKYCERHMRGRNRSRKPVEAQLVPPP
HAQPQQQAPAPTAGFQSHPMYPSILAGNGGGGGGVGGGAGGGTFLGPTSQ LR
MDSAAAYATAADGGSKDLRYSAYGVKSLSDEHSQ LPPGGGGGMDASMDNSWRLL
PSQTAATFQATSYPLFGALSGLDESTIASLPKTQREPLSFFGSDFVTPKQENQTLRP
FFDEWPKSRDSWP ELNEDNSLGSSATQLSTSIPMAPSDFNTSSRSPNGIPSR

>ZmGRF7 (SEQ ID No. 30)

MMLSGHGGGRRLFTASQWQELEHQALIFKYMASGAPVPHDLVLPLRLATGVDTAP
SLAFPPQPSPSLAYWGCY GAGAPFVGRKAAEDTEPGRCCRRTDGKKWRCSREAHG
DSKYCEKHIHRGKSRSRKPVEVTSSPAAGAAAAYRPSAISTISPPRAADAPPPSLAY
PQQHLLHGASSSAAARAPAGALQLHLDASLHAAAASPPPPSYHRYAHYTPPASSL
FPGGGYGYDYDYGQSKELRRRHFHALGADLSLDKPLPEPDTGSDEKQPLRR FFDE
WPRESGDMAADDATQLSISIPAASPSDLAATSASAAAARFHNGE

>ZmGRF8 (SEQ ID No. 77)

MSAEFCAAAGVAMELGVDALGLQQGIAITAPSPRSDLG LLLKRAGLTQAAAAAP
YPSPFLDGEKMLRFSKAAHTSHSGLDFGGPGEQAFLLSRTKMPFTPSQWMELGHQ
ALIYKYLNAKAPIPSSLLISISKSRSSNRVSWRPLYQGYTNADSDPEPGRCCRRTDG
KKWRCSKEAMADHKYCERHINRNRHRSRKPVENQPKTTKEVPAAAGSLPCAGP
QGSLKKAKVND SKPGTVSYWADSLNRTMLSREKANKPTEDSSLLLTSTNSQPTWS
LLSQLKQQNKPKDLGPTLENESNPDTILKAWGGNQPSHKSSISTERHDAESLQSVL

QNLSLAQNEKMESEKDKYSDSVLVSSSTFYSAGGPRATCLTPNMTQVKQDCISSSW
EMPQGGPLGEILTNSKNSKDLSKCKPRSYGWLLNLDHAP

>ZmGRF9 (SEQ ID No. 31)

MMMMSGRAATAGRYPFTASQWQELEHQALIYKCLASGKPIPSYLMPPLRRLDSAL
ATSPSLAAFQPQPSLWGGCGFMGFSRKPADEDPEPGRCRRTDGKKWRCSKEA
YPDSKYCEKHMHRGKNRSRKPVEMSLATPAPPASSAATTSTSPAPSYHRPAPAAH
DAVPYHAPYGAAYHHTQTQVMSPFHLHLETTHPHPPPPPPYYADQRDYAYGKEV
GERAFFSDGAGERDRQQQAAGQWQFKQLGTMEATKPCPTPTPLLPAAGYGVGQA
KEDEEEETRRQQQHCFLVLGADLRLAERPSGAHDDAAQKPLRHFFDEWPHEKGS
KAGWWIGGLDGETTQLSMSIPMAAADLPVTSRYRT

>ZmGRF10 (SEQ ID No. 78)

MTAEGEAKNPSAGGGGDNPPQHQAAPAPAPAQGEVAQEAAVQGTGQEQRDKA
DREVQGGAGEKDDGACRDLVLVEDPEVLAVEDPEEEAATAALQEEMKALVASIPD
GAGAAFTAMQLQELEQQSRVYQYMAARVPVPTHLVFPWKSVTGASSEGAQKYP
TLMGLATLCLDFGKNPEPEPGRCRRTDGKKWRCWRNTIPNEKYCERHMHRGRKR
PVQVFLEDDEPDSASGSKPAAPGKATEGAKKADDSKSPSSKKLAVAAPAAVQST

>ZmGRF11 (SEQ ID No. 32)

MMLSGHGGGRRLFTASQWQELEHQALIFKYMASGAPVPHDLVLPLRLATGVDTAP
SLAFPPQPSPSLAYWGCYGAGAPFGRKAEDPEPGRCRRTDGKKWRCSREAHGD
SKYCEKHIHRGKSRSRKPVEVTSPAAYRPSAFSISPPRAADAPPPPPGLGHPQQQH
LRHGALSPAGRAHAAGALQLHLDSSLHAASPPPSYHRYAHSHAHYTPPPPSLYDY
GQSKELREAAELRRRHFFHALGADLSLDKPLADAGAAEKPLRRFFDEWPRERGDTR
PSWAGAEDATQLSISIPAASPSSDHAASAAARCHNDGSDRCIS

>ZmGRF12 (SEQ ID No. 79)

MLSSASSAGAAMGMGGGYQHQLPLPQRGAAAVFTAAQWAELEQQALIYKYL
AGVPVPPDLLRPAPHAAAFSFASPAASPFYHHHHHPSLSYYAYYGKKLDPEPWR
CRRTDGKKWRCSKEAHPDSKYCERHMHRGRNRSRKPVESKTASSPPQLSTVTT
TTTREAAAATPLESLAGAGGKAHGLSLGGGAGSSHLSVDASNTHFRYGSKYPLGA
KSDAGELSFFSGAPGNSRGFTIDSPADNSWHSLSNPVPPFTLSKGRDSGLLPGAPP
VVVQQQRGRRRWWVAGEREAGEPAAEALLRRVAWDAGLVVGDGRREVQ

>ZmGRF13 (SEQ ID No. 80)

MSAEFCAAAAGAVAMELGVDVMGLQQGIAAATGPSSGDSDLGLLKRAGLAQAAT
SYSPSPFLDQQKMLRFSKAAAHTSPSGLDFGGGPSEQAFLLSRTKRPFTPSQWME
LEHQALIYKYLNKAPIPSSLLVSISKSFRSSNRVSWRPLYQGYANADSDPEPGRCR
RTDGKKWRCSKEAMPDHKYCERHINRNRHRSRKPVENQPRKTSKEVPTAAAGSL
PCAGPQGSLLKAKVNDKSKPGTGSYWTDSLNRMTLSREKANKPTEDESLLLSSTKN
SQPTLSLLTQLKQQNKPKDLGPTPENEPNSDTMLKAWGGSHHKNISSTQRHDAES
LQSVLQNFSLAQNDRLESEKNRYSDSVLVSSAFYSADGPQTTCLTPNMTQVQQDCI
SSSWEMPQGGPLGEILTNSKISEDSSKCGSRSYGWLLNLDHAP

>ZmGRF14 (SEQ ID No. 33)

MLSSASSAAGAAMGMGGGGYAHQPPPQRAVFTAAQWAELEQQALIYKYL MAGVP
VPPDLLLPVRPGPAAAFSAGPAAASPFYHQHPSLSYYAYYGKKLDPEPWRCRR
TDGKKWRCSKEAHPDSKYCERHMHGRNRNRSRKPVESKTASSSSPAHPSPQLST
VTTTAPLEPLAAAGGKVHGLSLGGGAAGSSHLGVDASNAHYRYGSNRYPLGAKPD
GGELSSFFSGASSGNNSRGGFTIDSPSDNNSWHSALASSVPPFTLSTKSGDSGLLP
GAYASYSQSHSHMEPPRELQGVTIASLAQEQERQQPFSGGMLGNVKQENQNQPL
RPFDEWPGRADSWPPEMDGAPRAGRTSFSSSTTQLSISIPMPRCELHLRNQNS

FIGURE 27

>GmGRF (*Glycine max*, Glyma03g35010) (SEQ ID No. 16)

ATGGACTTCCATCTGAAGCAATGGAGAAACCAGCACGAGTCAGAGGAACAACAT
TCTACAAAGATGCCAAAACCTTCTCCCTGAATCCCATCAACAACAACAGCCATCAG
CCTCTGCACTCCCTTTGTTTGTACCTGAACCCAACAGCAGCAAAGTCAGCACCC
TATTATTTCCCAGGATGGGGAGCTACTTCAGCTTGTCTCAGTGGCAGGAGCTTG
AGTTGCAGGCTTTGATATTCAGGTACATGTTGGCTGGTGTCTGCTGTTCTCCTG
AACTCCTTCAACCAATCAAGAAAAGCCTTCTTCATTCTCCACACTATTACCTCCA
TCACCCTCTCCAACATTACCAACCTTCTGCTTGGTATTGGGGTAGAGGAGCGAT
GGATCCGGAGCCAGGGCGGTGCCGGAGAACCGACGGCAAGAAGTGGCGCTGT
TCGAGGGACGTGGTGGCTGGGCAAAAGTACTGTGAGCGCCACATGCACCGTG
GAAGAAACCGTTCAAGAAAGCCTGTGGAACTACCCACACCAACTAGTGCTATTA
ACAATTGTGGTGTAACTGGAGTTGGATCCCTAGGACCAGGTGCTTCATCATCTT
CCATTTGTTCAACCACCCTTAGCTTCTGCTTCATTCAAATCTCCTTTTGATCTTCAT
CTTGATGAACGTTCTCTGCGGACCAAGAATGAAGACGAAGATCATGTGGGTGG
GGATGGCAGATCAGGTGGAGGTGGTGGCCATATGCTGAGGCATTTCTTCGATG
ATTGGCCACGATCACTCCAAGACTCTGACAACGTTGAAAACAATGCTGCTGCTG
GCCGTAGCCTCTCTATTTCAATGCCCGGTGCTTCCTCGGATGTGTCAATTGAAAT
TGTCACCGGGCTATGGAGAGGACTCGGGCCCAGGAAATGAGAATGTAAGCCTC
GAGCCAGAGCAGCTGCAGTTGAATTGGGCCGGAGGATGGGCCTCGTCTAATCA
AGTGGCTTCGATGGGAGGTCCACTTGCTGAGGCACTCAGATCATCTACTTCAAC
CTCATCTCCCACTAGTGTTTTGCATCGTCACTTGCCTCGTGGATCTGAGACCAG
CTTTATTAGCACCTGA

FIGURE 28

>MtGRF (*Medicago truncatula*) (SEQ ID No. 17)

Atggactttcctacgaaacaatggagaaaccaacagcatgagtcagagaaacaacattccacaaagatgccaaa
acttctcaccctgctcaatctcaatcccaatcccattcccatcaacaatcacctgcacttctttgtttctacctcaacca
acaccaaagtcaccaactgtgcagattcagcattacctccaacaacagattcccagaataggaatgggaagccattt
cagcttatctcaatggcaagaacttgagttacaagctttgatatttaggtacatgttggttggtgctctgttctctgaactt
cttcaacctatcaagaaaagtcttctcattcatctcctattttctcatcattatcaacctacagcattgttgcaaagtggtga
ttggggaagaggagcaatggatccagagccaggtcggtgccggagaaacagacggtaaaaaagtggcggtgtgcga
gggatgtggtggctggacaaaagtactgtgaaagacacatgcatcggtgtagaaaccgttcaagaaagcctgttg
aacttcccacaccaactagtaatggcggtggatctttctctgtttgtcttctatttttcacagcctcttgcacttctcattca
aatctccttttgatcttcactttactgaacgctccactgggacaaaattgaagagaagagccttatgtgaaagcgatgatc

atgtgggtggggatggaagaccaggtgggcaaagtctaaggcatttcttgacgattggccacgatcactgcaagact
ctgacaatgctgaaaacaatgggtgggtcatcctccacatgtctctcaatttcaatgccaggaaataacaacacttcttctt
cttcttcagatgtgtcattgaaattgtccactggctatggagaagaaccatgtccaagaaatgagaatgtgggcctagta
caaactgagcagcaacaacaacaacttcaattgaattggatcggaggatggaattcaggtaatcaagtgtcttcaatg
ggaggaccacttgctgaggcacttagatcatctacttcaacttctcacctactagtgtttacatcaattgccacgttgttct
ggttctcaaaccagctacattagcacctaa

FIGURE 29

>PtGRF (*Populus trichocarpa*) (SEQ ID No. 18)

ATGGACTTCCATCTGAAGCAATGGAGAAACCAGCATGAGGAGTCAGGGCAACA
ACCCCTCTGCAAAGATGCCAAAACCTCCTCATGGATCCCCATCAACCACAACAACA
TCCACACTCATCTGGGTCTGCTGCCTTCCCTTTGTTTCTACCCGAGCCCAGCTG
CAAAAATAGTAACCTGTCAGCATTTCTGATTCAAACACAGCTGCAAACACCAGA
CTTCCTAAGATCATGGGGAATTACTTTAGCCTGGAACAGTGGCAAGAGCTAGAG
CTGCAGGCTTTGATCTACAGATTCATGTTAGCCGGTGCAGCTATTCCTCCGGAG
CTCCTCCAACCAATCAAGAAAACCCCTTCTTCATTCTCACCCCCCTCCATATTTCC
TCCATCATCCTCTTCAATTACATTGCTCTTATTATCAGCCATCTTGGTATTGGGG
AAGAGCAGCCATGGATCCGGAGCCAGGTCCGTGCCGGAGAACAGATGGGAAG
AAATGGCGGTGCTCCAGAGACGTGGTGGCAGGGCACAAGTATTGCGAGCGCC
ACTTGCACCGTGGCCGCAAC**CGTTCAAGAAAGCCTGTGGAA**AATCCCACACCT
ACAATATCCACTAACATCACTTGCATTGGTATTGGAGAATTGGACCAAACCTACCT
TTTCATTGTTTTGTTTTTGTCTTAATCTTCTTGCTCACCCCTTATTGCAGCTCCAAA
ACTGAAAGCAAGGGCTTAATTGGACCACCACCTCCAAATGAGGTTGGTAACAGG
TCTGATGGCCACATTCTGTGGCATTTTTTTTGATGACTGGCCACGATCCGTTGAT
GAATCCGACAATATGAATGCTGGAAGCTCAATGAACTCTTTAACCTGCCTCTCC
GTTTCAATGCCTGGAAACTCACCCAGCATCAGATGTGTCATTGAAATTGTCCACTG
GGAATAATATTGCAGAGGAGGAGCCGGAGCCAGTCCCAGCCCCGATCCCTAGA
GGCAATACAAGCAATTGGGCTGCTGCAGGATGGGGCACAAAATTACAAACCA
GGTGGTGACTTCAATGGGGGGACCTCTTGCTGAGGCGCTGAGGTCCTCCACTA
CCAAACTCATCTCCACGAATGTTCTGCACCAGTTATGTCGC
CCCACTGTTTCTGA

FIGURE 30

>PpGRF (*Prunus persica*) (SEQ ID No. 15)

ATGGACTTTCACCTCAAGCAATGGAGAAACCAGCAGCATGAGTCAGAGGAACAA
CATTCTGCAAAGATACCAAACTTCACCTTGAGCCCCATCCCACTCAGAGCCA
TCTGGGTATGCTCTCCCTCTGTTTGTTCTTGAGCCCAACAGCAAATGATCAGC
ACCCTGTCAGCGTTTTCTGAATCTACACCAGCATCTGCCTCCACCAGATTTCCC
AAAATGGGGAGCTATTTTCAGCTTCTCCCAGTTGCAGGAGCTTGAGCTGCAGGCT
TTGATATTCAGGTACATGTTAGCTGGTGCTGCTGTTCCCTCCTGAACTTCTTCAGC
CAATCAGGAAAAGCCTTCTCCACTCTCCTCCATATTTTCTCCACCACCCTCTTCA
ACAGTACCCTCATTTTCAGCCTGCTTTGTTGCAATCAGGGTATTGGGGAAGAGC

AGCCATGGATCCAGAGCCAACAAGGTGTAGAAGGACAGATGGCAAGAAATGGA
 GGTGTTCTAGAGATGTGGTGGCTGGTCAGAAGTACTGTGAGCGCCATGTGCAC
 CGTGGCAGAAACCGTTCAAGAAAGCCTGTGGAAGCCACCACTGCTGCTGCTG
 GTGGTGGTGGTGGAGGGACAAGTGATATTGCTACCAACACCACCACCAAGACA
 TCATCTAGTGGGGCCCATTTTACTCTTTCTGGGTCATCATCATCCCCTTCAATTG
 ATCTGCTTCATCTCAACCAGAGTTCCTCAGAGCCCAAAGCTGAGAATAGGAGCC
 TCTTTGAACCCACAGTGAGGTCTCCGGGAGTGCTAAATCCGACAGCCATGTCT
 TGCGGCCTTTTTTTTGTGACTGGCCGGGGAAGCTCCAAGAACTGGACAATGCA
 CGAACCAATGCTGGCTCAATGAACTCTGCCACCAGCCTCTCCATTTGATACGG
 GGAAATTCCTCCTCGGATGTGTCACTGAAATTGTCTACCGGCAATGGAGTTGAG
 ACAGGGCGCCTGGACGGCCATGCTGAGCGCGAGCAGCCACAATTGAATTGGC
 CTGCCGGATGGGGAACAAACCAATGGCTTCCATGGGAGGGCCGCTTGCGGA
 GGCCCTTAGGTCCTCCTCCAATTCCTCACCAACCAGTGTTCTACATCA
 GTTGCCCCGCAGCTCCGCCTCAGAACTAGCTTTATCAGCACTTGA

FIGURE 31

>MtGRF (*Medicago truncatula*) (SEQ ID No. 36)

MDFPTKQWRNQHESEKQHSTKMPKLLHPAQSSQSHSHQQSPALPLFLPQPNT
 KVTNLSDSALPSNNRFPRIKMGSHFSLSQWQELELQALIFRYMLVGASVPELLQPI
 KKSLLHSSPYFLHHYQPTALLQSGYWGRGAMDPEPGRCRRTDGKKWRCCARDVVA
 GQKYCERHMRGRNRSRKPVELPTPTSNGGGSFSALSSISSQPLVTSSFKSPFDL
 HFTERSTGKIEEKSLCESDDHVGGDGRPGGQMLRHFFDDWPRSLQSDNAENN
 GGSSSTCLISIMPGNNNTSSSSSDVSLKLSTGYGEEPCPRNENVGLVQTEQQQQQ
 LQLNWIGGWNSGNQVSSMGGPLAEALRSSTSTSSPTSVLHQLPRCSGSQTSYIST

FIGURE 32

>GmGRF (*Glycine max*, Glyma03g35010) (SEQ ID No. 35)

MDFHLKQWRNQHESEEQHSTKMPKLLPESHQQQPSASALPLFVPEPNSSKVSTL
 LFPRMGSYFSLSQWQELELQALIFRYMLAGAAVPELLQPIKKSLLHSPHYLLHHPL
 QHYQPSAWYWGRGAMDPEPGRCRRTDGKKWRCSRDVVAGQKYCERHMRGR
 NRSRKPVELPTPTSAINNCGVTGVGSLGPGASSSSICSPPLASAFKSPFDLHLDER
 SSGTKNEDEDHVGGDGRSGGGGGHMLRHFFDDWPRSLQSDNVENNAAAGRSL
 SISIMPGASSDVSLKLSTGYGEDSGPGNENVSLPEQLQLNWAGGWASSNQVASM
 GGPLAEALRSSTSTSSPTSVLHRLPRGSETSFIST

FIGURE 33

>PtGRF (*Populus trichocarpa*) (SEQ ID No. 37)

MDFHLKQWRNQHEESGQQPSAKMPKLLMDPHQPQQHPHSSGSAAFLFLPEPSC
 KNSNLSAFPDSNTAANTRLPKIMGNYFSLEQWQELELQALIYRFMLAGAAIPPELLQ
 PIKKTLLHSHPPPYFLHHPLQLHCSYYQPSWYWGRAAMDPEPGRCRRTDGKKWR
CSRDVVAGHKYCERHLHRGRNRSRKPVENPTPTISTNITCIGIGELDQTTFSLFCFC
 FNLLAHPYCSSLTESKGLIGPPPNEVGNRSDGHILWHFFDDWPRSVDESDNMNA
 GSSMNSLTCLSVSMPGNPASDVSLKLSTGNNIAEEEEPEPVPAPIPRGNTSNWAAA
 GWGTKITNQVVTSMGGPLAEALRSSTTKLISHECSAPVMSPHCF

FIGURE 34

>PpGRF (*Prunus persica*) (SEQ ID No. 34)

MDFHLKQWRNQQHESEEQHS AKIPKLHLEPHPHSEPSGYALPLFVPEPNSKMISTL
 SAFSESTPASASTRFPKMGSYFSFSQLQELELQALIFRYMLAGAAVPPELLQPIRKS
 LLHSPPYFLHHPLQQYPHFQ PALLQSGYWGRAAMDPEPTRCRRTDGKKWRCSR
 DVVAGQKYCERHVHRGRNRSRKPVEATTAAAGGGGGGTSDIATNTTTKTSSSGAHF
 TLSGSSSSPSIDLLHLNQSSSEPKAENRSLFEPHSEVSGSAKSDSHVLRPFFDDWP
 GKLELDNARTNAGSMNSATSLSISIRGNSSSDVSLKLSTGNGVETGRLDGHAERE
 QPQLNWPAGWGTNQMASMGGPLAEALRSSNSNSSPTSVLHQLPRSSASETSFIS
 T

FIGURE 35

>At-rGRF3 (SEQ ID No. 81)

ATGGATTTGCAACTGAAACAATGGAGAAGCCAGCAGCAGCAACAACATCAGACA
GAGTCAGAAGAACAACCTTCTGCAGCTAAGATACCAAAACATGTCTTTGACCAG
ATTCATTCTCACACTGCAACTTCTACTGCTCTTCCTCTCTTTACCCCTGAGCCTA
CTTCTTCTAAACTCTCCTCTTTGTCTCCTGATTCTTCCTCCAGGTTCCCCAAGAT
GGGGAGCTTCTTTAGCTGGGCACAGTGGCAAGAACTTGAAGTACAAGCTCTGAT
CTACAGGTACATGTTGGCTGGTGCTGCTGTTCCCTCAGGAGCTCCTTTTACCAAT
CAAGAAAAGCCTTCTCCATCTATCTCCTTCCTACTTTCTTCACCATCCTCTTCAAC
ACCTACCTCATTACCAACCTGCTTGGTATTTGGGAAGGGCAGCGATGGATCCTG
AGCCAGGCAGATGCAGGAGAACGGATGGTAAGAAAGTGGAGATGTTCAAGAGAC
GTCTTCGCTGGCCACAAGTATTGCGAGCGCCACATGCACCGTGGCCGCAACCG
TTCTAGAAAaCCaGTaGAgACTCCAACCACCGTCAATGCAACTGCCACGTCCATG
GCTTCATCAGTAGCAGCCGCAGCCACCACTACAACAGCAACAACAACATCTACG
TTTGCTTTTGGTGGTGGTGGTGGTAGTGAGGAAGTGGTTGGTCAAGGAGGATC
TTTCTTCTTCTCTGGCTCTTCTAACTCTTCATCTGAACTTCTCCACCTTAGTCAA
GTTGTTCCGAGATGAAGCAAGAAAAGCAACAACATGAACAACAAGAGGCCATACG
AGTCCCACATCGGATTCAAGTAACAACAGATCAGATGGAGGACACATCCTGAGGC
CCTTCTTTGACGATTGGCCTCGTTCTTCGCTCCAAGAAGCTGACAATAGTTCAA
GCCCCATGAGCTCAGCCACTTGTCTCTCCATCTCCATGCCCCGGGAACCTTTCCT
CAGACGTCTCTCTGAAGCTGTCCACAGGCAACGAAGAGGGAGCCCGGAGCAAC
AACAATGGGAGAGATCAGCAAAACATGAGCTGGTGGAGCGGTGGAGGTTCCAA
CCACCATCATCAACATGGGCGGACCATTGGCCGAAGCCCTGAGATCTTCTTC
CTCATCTTCCCCAACCAAGTGTCTCCATCAGCTTGGTGTCTCGACACAAGCCTTT
CATTGA

FIGURE 36

>rGmGRF (Glyma03g35010) (SEQ ID No. 82)

ATGGACTTCCATCTGAAGCAATGGAGAAACCAGCACGAGTCAGAGGAACAACAT
TCTACAAAGATGCCAAAACCTTCTCCCTGAATCCCATCAACAACAACAGCCATCAG
CCTCTGCACTCCCTTTGTTTGTACCTGAACCCAACAGCAGCAAAGTCAGCACCC
TATTATTTCCCAGGATGGGGAGCTACTTCAGCTTGTCTCAGTGGCAGGAGCTTG
AGTTGCAGGCTTTGATATTCAGGTACATGTTGGCTGGTGTCTGCTGTTCCCTCCTG
AACTCCTTCAACCAATCAAGAAAAGCCTTCTTCATTCTCCACACTATTACCTCCA
TCACCCCTCTCCAACATTACCAACCTTCTGCTTGGTATTGGGGTAGAGGAGCGAT
GGATCCGGAGCCAGGGCGGTGCCGGAGAACCGACGGCAAGAAGTGGCGCTGT
TCGAGGGACGTGGTGGCTGGGCAAAAGTACTGTGAGCGCCACATGCACCGTG
GAAGAAACCGTTCTAGAAAaCCaGTaGAgCTACCCACACCAACTAGTGCTATTAAC
AATTGTGGTGTAACTGGAGTTGGATCCCTAGGACCAGGTGCTTCATCATCTTCC
ATTTGTTACCAACCCTTAGCTTCTGCTTCATTCAAATCTCCTTTTGATCTTCATCT
TGATGAACGTTCTCTGGGACCAAGAATGAAGACGAAGATCATGTGGGTGGGG
ATGGCAGATCAGGTGGAGGTGGTGGCCATATGCTGAGGCATTTCTTCGATGATT
GGCCACGATCACTCCAAGACTCTGACAACGTTGAAAACAATGCTGCTGCTGGCC
GTAGCCTCTCTATTTCAATGCCCGGTGCTTCCTCGGATGTGTCATTGAAATTGTC
CACGGGCTATGGAGAGGACTCGGGCCCAGGAAATGAGAATGTAAGCCTCGAGC
CAGAGCAGCTGCAGTTGAATTGGGCCGGAGGATGGGCCTCGTCTAATCAAGTG
GCTTCGATGGGAGGTCCACTTGCTGAGGCACTCAGATCATCTACTTCAACCTCA
TCTCCCACTAGTGTTTTGCATCGTCACTTGCCTCGTGGATCTGAGACCAGCTTTA
TTAGCACCTGA

FIGURE 37

>rOsGRF4.1 (SEQ ID No. 83)

ATGCCTCCCTGTCTCCGGCGGTGGCCGACCACCGCTCGTCCCCGGCAGCCGC
GACCGCCTCCCTCCTCCCTTCTGCCGCTCCACCCCGCTCTCCGCGTAAGCAA
CGCGAACCCGCGGCTACAACCCATTTTCTTGGCTCCAGTGGTGCATGTGACAA
CACGGTGAGACGTTGTGTGTGGGTGGGTGGGTGCAGGGGCGGTGGTGGCGTC
GCGATGGGGGAGGACGCGCCGATGACCGCGAGGTGGCCGCCGGCGGGCGGCG
GCGAGGCTGCCGCCGTTACCGCGGCGCAGTACGAGGAGCTGGAGCAGCAGG
CGCTCATATACAAGTACCTGGTGGCAGGCGTGCCCGTCCCGCCGGATCTCGTG
CTCCCCATCCGCCGCGGACTCGACTCCCTCGCCGCCCGCTTCTACAACCATCC
CGCCCTTGATATGGTCCGTACTTCGGCAAGAAGCTGGACCCAGAGCCAGGGC

GGTGCCGGCGGTACGGACGGCAAGAAATGGCGGTGCTCGAAGGAGGCCGCGCC
GGATTCCAAGTACTGCGAGCGCCACATGCACCGCGGCCGCAACCGTTCTAGAA
AaCCaGTaGAgACGCAGCTGGTCGCCCAGTCCCAACCGCCCTCATCTGTTGTCG
GTTCTGCGGCGGCGCCCTTGCTGCTGCCTCCAATGGCAGCAGCTTCCAAAAC
CACTCTCTTTACCCTGCTATTGCCGGCAGCAATGGCGGGGGCGGGGGGAGGA
ACATGCCCAGCTCATTTGGCTCGGCGTTGGGTTCTCAGCTGCACATGGATAATG
CTGCCCCCTTATGCAGCTGTTGGTGGTGGAACAGGCAAAGATCTCAGGTATACTG
CTTATGGCACAAGATCTTTGGCGGATGAGCAGAGTCAACTCATTACTGAAGCTA
TCAACACATCTATTGAAAATCCATGGCGGCTGCTGCCATCTCAGAACTCGCCAT
TTCCCCCTTTCAAGCTATTCTCAGCTGGGGGCACTAAGTGACCTTGGTCAGAACA
CCCCCAGCTCACTTTCAAAGGTTTCAGAGGCAGCCACTTTCGTTCTTTGGGAACG
ACTATGCGGCTGTCGATTCTGTGAAGCAAGAGAACCAGACGCTGCGTCCCTTCT
TTGATGAGTGGCCAAAGGGAAGGGATTTCATGGTCAGACCTCGCTGATGAGAAT
GCTAATCTTTCGTCATTCTCAGGCACCCAAGTGTGATCTCCATACCAATGGCAT
CCTCTGACTTCTCGGCGGCCAGTTCTCGATCAACTAATGGTGACTGA

FIGURE 38

GRF#	Identity (%)	Similarity (%)	Gaps (%)
AtGRF1	26	37	35
AtGRF2	23	33	41
AtGRF3	100	100	0
AtGRF4	69	75	14
AtGRF5	23	30	50
AtGRF6	21	27	55
AtGRF7	36	41	41
AtGRF8	23	33	34
AtGRF9	19	27	48
OsGRF1	21	32	49
OsGRF2	25	34	43
OsGRF3	26	35	45
OsGRF4	26	38	39
OsGRF5	25	32	47
OsGRF6	24	33	45
OsGRF7	24	33	40
OsGRF8	24	32	39
OsGRF9	26	37	32
OsGRF10	23	30	54
OsGRF11	15	21	54
OsGRF12	20	23	62
ZmGRF1	27	37	40
ZmGRF2	26	35	33
ZmGRF3	21	30	51
ZmGRF4	14	20	65
ZmGRF5	24	33	41
ZmGRF6	23	33	44
ZmGRF7	24	31	48
ZmGRF8	24	33	31
ZmGRF9	20	30	52
ZmGRF10	15	23	60
ZmGRF11	25	35	40
ZmGRF12	28	35	36
ZmGRF13	23	34	30
ZmGRF14	23	30	51
PpGRF	54	63	19
GmGRF	51	60	17
MtGRF	45	55	26
PtGRF	51	60	17

FIGURE 39

>JD36 (SEQ ID No. 84)

TTGATCCCGAGGGGAACCCCTGTGGTTGGCATGCACATACAAATGGACGAACGG
ATAAACCTTTTACGCCCCTTTTAAATATCCGTTATTCTAATAAACGCTCTTTTCTC
TTAGgtttaccgcgaatatatcctgtcaAACACTGATAGTTTAACTGAAGGCGGGAAACGA
CAATCTGATCCAAGCTCAAGCTGCTCTAGCCAATACGCAAACCGCCTCTCCCCG
CGCGTTGGCCGATTCAATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAA
GCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACC
CCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG
ATAACAATTTACACAGGAAACAGCTATGACCATGATTACGAATTCGGTCCCCA
GATTAGCCTTTTCAATTTCAAGAAAGATGCTAACCCACAGATGGTTAGAGAGGCT
TACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATC
AAATACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGC
ATCAAGAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTAT
GGACGATTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCT
CTAAAAAGGTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAG
AGGACCTAACAGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCT
TACGACTCAATGACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACAC
TTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGA
GACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGC
TATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATG
CCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTT
CCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGG
GATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTT
CATTTTCATTTGGAGAGAACACGGGGGACGAGCTCGGTACCCGGGGATCCCCCG
GACCATGCAACAGCACCTGATGCAGATGCAGCCCATGATGGCTGGTTACTAC
CCCAGCAATGTTACCTCTGATCATATCCAACAGTACTTGGACGAAAACAAATC
GTTGATTCTGAAGATTGTTGAGTCTCAAACTCTGGAAAGCTTAGCGAATGCG
CCGAGAATCAAGCAAGGCTTCAACGCAACCTAATGTACCTAGCTGCAATAGC
AGATTCTCAGCCTCAGCCACCAAGTGTGCATAGCCAGTATGGATCTGCTGGTG
GTGGGATGATTACGGGAGAAGGAGGGTCACACTATTTGCAGCAGCAACAAGC
GA CTCAACAGCAACAGATGACTCAGCAGTCTCTAATGGCGGCTCGATCTTCAA
TGTTGTATGCTCAGCAACAGCAGCAGCAGCAGCCTTACGCGACGCTTCAGCA
TCAGCAATTGCACCATAGCCAGCTTGGAATGAGCTCGAGCAGCGGAGGAGGA
GGAAGCAGTGGTCTCCATATCCTTCAGGGAGAGGCTGGTGGGTTTCATGATTT

TGGCCGTGGGAAGCCGGAATGGGAAGTGGTGGTGGCGGTGAAGGCAGAGG
AGGAAGTTCAGGGGATGGTGGAGAAACCCTTTACTTGAAATCATCAGATGATG
GGAATTGAAAGGTCGACTTACCCATACGACGTTCCAGACTACGCTTCTTTGGGTG
GTTCTAGCCCAAGCTCAGAGCTCCACCGCGGTGGCGGCCGCATCTTTTACCCA
TACGATGTTCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGGA
TGA CTGACCTGCAGAGCTTTCGTTCGTATCATCGGTTTCGACAACGTTCGTCA
AGTTCAATGCATCAGTTTCATTGCGCACACACCAGAATCCTACTGAGTTCGAG
TATTATGGCATTGGGAAACATGTTTTCTTGTACCATTTGTTGTGCTTGTAATT
ACTGTGTTTTTATTCGGTTTTCGCTATCGAACTGTGAAATGGAAATGGATGGA
GAAGAGTTAATGAATGATATGGTCCTTTTGTTCAATCTCAAATTAATATTATTG
TTTTTCTCTTATTGTTGTGTGTTGAATTTGAAAATATAAGAGATATGCAAACA
TTTTGTTTTGAGTAAAAATGTGTCAAATCGTGGCCTCTAATGACCGAAGTTAAT
ATGAGGAGTAAACACTTGTAGTTGTACCATTATGCTTATTCAGTGGCAACA
AATATATTTTCAGACCTAGAAAAGCTGCAAATGTTACTGAATACAAGTATGTCC
TCTTGTGTTTTAGACATTTATGAACTTTCCTTTATGTAATTTTCCAGAATCCTTGT
CAGATTCTAATCATTGCTTTATAATTATAGTTATACTCATGGATTTGTAGTTGAG
TATGAAAATATTTTTTAATGCATTTTATGACTTGCCAATTGATTGACAACATGCA
TCAATCGAAGCTTGCATGCCTGCAGGTCTGCTGAGCCTCGACATGTTGTGCGC
AAAATTCGCCCTGGACCCGCCCAACGATTTGTCGTCACTGTCAAGGTTTGACCT
GCACTTCATTTGGGGCCACATACACCAAAAAATGCTGCATAATTCTCGGGGC
AGCAAGTCGGTTACCCGGCCGCCGTGCTGGACCGGGTTGAATGGTGCCCGTAA
CTTTCGGTAGAGCGGACGGCCAATACTCAACTTCAAGGAATCTCACCCATGCGC
GCCGGCGGGGAACCGGAGTTCCTTCAGTGAACGTTATTAGTTCGCCGCTCGG
TGTGTCGTAGATACTAGCCCCTGGGGCCTTTTGAAATTTGAATAAGATTTATGTA
ATCAGTCTTTTAGGTTTGACCGGTTCTGCCGCTTTTTTTAAAATTGGATTTGTAAT
AATAAACGCAATTGTTTGTTATTGTGGCGCTCTATCATAGATGTCGCTATAAAC
CTATTCAGCACAATATATTGTTTTCATTTTAATATTGTACATATAAGTAGTAGGGT
ACAATCAGTAAATTGAACGGAGAATATTATTCATAAAAAATACGATAGTAACGGGT
GATATATTCATTAGAATGAACCGAAACCGGCGGTAAGGATCTGAGCTACACATG
CTCAGGTTTTTTACAACGTGCACAACAGAATTGAAAGCAAATATCATGCGATCAT
AGGCGTCTCGCATATCTCATTAAAGCAGGACTCTAGGATCGATCCCCCGGGTCA
TCACATCTCGGTGACGGGCAGGACCGGACGGGGCGGTACCGGCAGGCTGAAG
TCCAGCTGCCAGAAACCCACGTATGCCAGTTCCCGTGCTTGAAGCCGGCCGC
CCGCAGCATGCCGCGGGGGGCATATCCGAGCGCCTCGTGATGCGCACGCTC
GGGTCGTTGGGCAGCCCGATGACAGCGACCACGCTCTTGAAGCCCTGTGCCTC
CAGGGACTTCAGCAGGTGGGTGTAGAGCGTGGAGCCCAGTCCCGTCCGCTGG
TGCGGGGGGGAGACGTACACGGTTGACTCGGCCGTCCAGTCGTAGGCGTTGC

GTGCCTTCCAGGGGCCCCGCGTAGGCGATGCCGGCGACCTCGCCGTCCACCTC
GGCGACGAGCCAGGGATAGCGCTCCCGCAGACGGACGAGGTCGTCCGTCCAC
TCCTGCGGTTCTGCGGCTCGGTACGGAAGTTGACCGTGCTTGTCTCGATGTA
GTGGTTGACGATGGTGCAGACCGCCGGCATGTCCGCCTCGGTGGCACGGCGG
ATGTCGGCCGGGCGTCGTTCTGGGCTCATGGTAATTGTAAATAGTAATTGTAAT
GTTGTTGTTGTTTGTGTTGTTGTTGGTAATTGTTGTAAAAATAGAGCTCTTATACTCG
AGGAATTCgctagagTCGATTTGGTGTATCGAGATTGGTTATGAAATTCAGATGCTA
GTGTAATGTATTGGTAATTTGGGAAGATATAATAGGAAGCAAGGCTATTTATCCA
TTTCTGAAAAGGCGAAATGGCGTCACCGCGAGCGTCACGCGCATTCCGTTCTT
GCTGTAAAGCGTTGTTTGGTACACTTTTGACTAGCGAGGCTTGGCGTGTACAGCG
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AGAATACCCGCGAAATTCAGGCCCGGTTGCCATGTCCTACACGCCGAAATAAAC
GACCAAATTAGTAGAAAAATAAAAACTgaCTCgGATACTTACGTACGTCTTGCG
CACTGATTTGAAAAATCTCAATATAAACAAAGACGGCCACAAGAAAAAACCAAAA
CACCGATATTCATTAATCTTATCTAGTTTCTCAAAAAAATTCATATCTTCCACACG
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TCAAAAGTTTTTATTTATCCGACATGGCGCGACCGACCTCAACGAGAAGGAAA
TTGTCGTGAACGGTGAGAAGCTCTGGGGCGTGCAAGGTTCCGGAACGAACATC
GGTCTCAATGCAAAAGGGGAACGCCAGGCTCTgttgccccTCGAAATTCGGCGTT
AATTCAGTACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAAGCGTCAAT
TTgtttacaccacaatatatcctgcccaCCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCG
GCACAAAATCACCACTCGATACAGGCAGCCCATCAGTCCGGGACGGCGTCAGC
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AAGAACGGCAACTAAGCTGCCGGGTTTGAACACGGATGATCTCGCGGAGGGT
AGCATGTTGATTGTAACGATGACAGAGCGTTGCTGCCTGTGATCAATTCCGGGCA
CGAACCCAGTGGACATAAGCCTCGTTTCGGTTCGTAAGCTGTAATGCAAGTAGCG
TAACTGCCGTACGCAACTGGTCCAGAACCTTGACCGAACGCAGCGGTGGTAA
CGGCGCAGTGGCGGTTTTTCATGGCTTCTTGTTATGACATGTTTTTTGGGGTAC
AGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTGATGT
TTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAACA
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GTAGAAGTCACCATTTGTTGTGCACGACGACATCATTCCGTGGCGTTATCCAGCT
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TTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGCAAGA
GAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGT
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CTGCTTTTATTATTTTAAAGCGTGCATAATAAGCCCTACACAAATTGGGAGATATA
TCATGCATGACCAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGAC
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCT
GCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATC
AAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATAC
CAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGT
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CGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGC
CACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTC
GGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTA
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TCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGA
TTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAG
CCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTG
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ACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCATCACCGAAACG
CGCGAGGCAGGGTGCCTTGATGTGGGCGCCGGCGGTGAGTGGCGACGGCG
CGGCTTGTCCGCGCCCTGGTAGATTGCCTGGCCGTAGGCCAGCCATTTTTGAG
CGGCCAGCGGCCGCGATAGGCCGACGCGAAGCGGCGGGGCGTAGGGAGCGC
AGCGACCGAAGGGTAGGCGCTTTTTGCAGCTCTTCGGCTGTGCGCTGGCCAGA
CAGTTATGCACAGGCCAGGCGGGTTTTAAGAGTTTTAATAAGTTTTAAAGAGTTT

TAGGCGGAAAAATCGCCTTTTTCTCTTTATATCAGTCACTTACATGTGTGACC
GGTTCCCAATGTACGGCTTTGGGTTCCCAATGTACGGGTTCCGGTTCCCAATGT
ACGGCTTTGGGTTCCCAATGTACGTGCTATCCACAGGAAAGAGACCTTTTCGAC
CTTTTTCCCCTGCTAGGGCAATTTGCCCTAGCATCTGCTCCGTACATTAGGAAC
CGGCGGATGCTTCGCCCTCGATCAGGTTGCGGTAGCGCATGACTAGGATCGGG
CCAGCCTGCCCCGCCTCCTCCTTCAAATCGTACTCCGGCAGGTCATTTGACCC
GATCAGCTTGCGCACGGTGAAACAGAACTTCTTGAACCTCTCCGGCGCTGCCACT
GCGTTCGTAGATCGTCTTGAACAACCATCTGGCTTCTGCCTTGCCTGCGGCGC
GGCGTGCCAGGCGGTAGAGAAAACGGCCGATGCCGGGATCGATCAAAAAGTAA
TCGGGGTGAACCGTCAGCACGTCCGGGTTCTTGCCTTCTGTGATCTCGCGGTA
CATCCAATCAGCTAGCTCGATCTCGATGTACTCCGGCCGCCCGGTTTCGCTCTT
TACGATCTTGTAGCGGCTAATCAAGGCTTCACCCTCGGATACCGTCACCAGGCG
GCCGTTCTTGGCCTTCTTCGTACGCTGCATGGCAACGTGCGTGGTGTTTAACCG
AATGCAGGTTTCTACCAGGTCGTCTTTCTGCTTTCCGCCATCGGCTCGCCGGCA
GAACTTGAGTACGTCCGCAACGTGTGGACGGAACACGCGGCCGGGCTTGTCTC
CCTTCCCTTCCCGGTATCGGTTTCATGGATTTCGGTTAGATGGGAAACCGCCATCA
GTACCAGGTCGTAATCCCACACACTGGCCATGCCGGCCGGCCCTGCGGAAACC
TCTACGTGCCCCGTCTGGAAGCTCGTAGCGGATCACCTCGCCAGCTCGTCGGTC
ACGCTTCGACAGACGGAAAACGGCCACGTCCATGATGCTGCGACTATCGCGGG
TGCCACGTCATAGAGCATCGGAACGAAAAATCTGGTTGCTCGTCGCCCTTGG
GCGGCTTCCTAATCGACGGCGCACCGGCTGCCGGCGGTTGCCGGGATTCTTTG
CGGATTTCGATCAGCGGCCGCTTGCCACGATTACCGGGGCGTGCTTCTGCCTC
GATGCGTTGCCGCTGGGCGGCCTGCGCGGCCTTCAACTTCTCCACCAGGTCAT
CACCCAGCGCCGCGCCGATTTGTACCGGGCCGGATGGTTTGCGACCGTCACG
CCGATTCTCGGGCTTGGGGGTTCCAGTGCCATTGCAGGGCCGGCAGACAACC
CAGCCGCTTACGCCTGGCCAACCGCCCGTTCTCCACACATGGGGCATTCCAC
GGCGTCGGTGCTGTTGTTCTTGATTTTCCATGCCGCCTCCTTTAGCCGCTAA
AATTCATCTACTCATTTATTCATTTGCTCATTTACTCTGGTAGCTGCGCGATGTAT
TCAGATAGCAGCTCGGTAATGGTCTTGCCTTGGCGTACCGCGTACATCTTCAGC
TTGGTGTGATCCTCCGCCGGCAACTGAAAGTTGACCCGCTTCATGGCTGGCGT
GTCTGCCAGGCTGGCCAACGTTGCAGCCTTGCTGCTGCGTGCGCTCGGACGG
CCGGCACTTAGCGTGTTTGTGCTTTTGCTCATTTTCTCTTTACCTCATTAACCTCAA
ATGAGTTTTGATTTAATTTAGCGGCCAGCGCCTGGACCTCGCGGGCAGCGTC
GCCCTCGGGTTCTGATTCAAGAACGGTTGTGCCGGCGGCGGCAGTGCCTGGG
TAGCTCACGCGCTGCGTGATACGGGACTCAAGAATGGGCAGCTCGTACCCGGC
CAGCGCCTCGGCAACCTCACCGCCGATGCGCGTGCCCTTTGATCGCCCGCGACA
CGACAAAGGCCGCTTGTAGCCTTCATCCGTGACCTCAATGCGCTGCTTAACCA

GCTCCACCAGGTCGGCGGTGGCCCATATGTCGTAAGGGCTTGGCTGCACCGG
AATCAGCACGAAGTCGGCTGCCTTGATCGCGGACACAGCCAAGTCCGCCGCCT
GGGGCGCTCCGTTCGATCACTACGAAGTCGCGCCGGCCGATGGCCTTCACGTC
GCGGTCAATCGTCGGGCGGTTCGATGCCGACAACGGTTAGCGGTTGATCTTCCC
GCACGGCCGCCCAATCGCGGGCACTGCCCTGGGGATCGGAATCGACTAACAG
AACATCGGCCCCGGCGAGTTGCAGGGCGCGGGCTAGATGGGTTGCGATGGTC
GTCTTGCTGACCCGCCTTTCTGGTTAAGTACAGCGATAACCTTCATGCGTTCC
CCTTGCGTATTTGTTTATTTACTCATCGCATATACGCAGCGACCGCATGACG
CAAGCTGTTTTACTCAAATACACATCACCTTTTTAGACGGCGGCGCTCGGTTTCT
TCAGCGGCCAAGCTGGCCGGCCAGGCCGCCAGCTTGGCATCAGACAAACCGG
CCAGGATTTTCATGCAGCCGCACGGTTGAGACGTGCGCGGGCGGCTCGAACAC
GTACCCGGCCGCGATCATCTCCGCCTCGATCTCTTCGGTAATGAAAAACGGTTC
GTCCTGGCCGTCTTGGTGCGGTTTCATGCTTGTTCTTGGCGTTCATTCTCG
GCGGCCGCCAGGGCGTCGGCCTCGGTCAATGCGTCCTCACGGAAGGCACCGC
GCCGCCTGGCCTCGGTGGGCGTCACTTCCTCGCTGCGCTCAAGTGCGCGGTA
CAGGGTCGAGCGATGCACGCCAAGCAGTGCAGCCGCCTCTTTCACGGTGCGG
CCTTCCTGGTCGATCAGCTCGCGGGCGTGCGCGATCTGTGCCGGGGTGAGGG
TAGGGCGGGGGCCAACTTCACGCCTCGGGCCTTGGCGGCCTCGCGCCCGCT
CCGGGTGCGGTTCGATGATTAGGGAACGCTCGAACTCGGCAATGCCGGCGAAC
ACGGTCAACACCATGCGGCCGGCCGGCGTGGTGGTGTGCGCCACGGCTCTG
CCAGGCTACGCAGGCCCGCGCCGGCCTCCTGGATGCGCTCGGCAATGTCCAG
TAGGTGCGGGGTGCTGCGGGCCAGGCGGTCTAGCCTGGTCACTGTCAACAACG
TCGCCAGGGCGTAGGTGGTCAAGCATCCTGGCCAGCTCCGGGCGGTGCGGCC
TGGTGCCGGTGATCTTCTCGGAAAACAGCTTGGTGCAGCCGGCCGCGTGCACT
TCGGCCCGTTGGTTGGTCAAGTCCTGGTTCGTGCGTGCTGACGCGGGCATAGCC
CAGCAGGCCAGCGGCGGCGCTCTTGTTTCATGGCGTAATGTCTCCGGTTCTAGT
CGCAAGTATTCTACTTTATGCGACTAAAACACGCGACAAGAAAACGCCAGGAAA
AGGGCAGGGCGGCAGCCTGTGCGTAAGTCTAGGACTTGTGCGACATGTCGTTT
TCAGAAGACGGCTGCACTGAACGTCAGAAGCCGACTGCACTATAGCAGCGGAG
GGGTTGGATCAAAGTACT

FIGURE 40

>RER32 (SEQ ID No. 85)

TTGATCCCGAGGGGAACCCCTGTGGTTGGCATGCACATACAAATGGACGAACGG
ATAAACCTTTTACGCCCCTTTTAAATATCCGTTATTCTAATAAACGCTCTTTTCTC
TTAGGTTTACCCGCCAATATATCCTGTCAAACACTGATAGTTTAAACTGAAGGCG
GGAAACGACAATCTGATCCAAGCTCAAGCTGCTCTAGCCAATACGCAAACCGCC
TCTCCCCGCGCGTTGGCCGATTCAATATGCAGCTGGCACGACAGGTTTCCCG
ACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATT
AGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTG
TGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGAAttgaaa
acgcaggcccatftacATCCTCCAAAACAAAAGAAGCAATAGAATCCGGA
ACTGAATTA
AAAACATACAAACCAAGAGGTAAAGAAAACGCATAGTTAGCTAACCCGTCCGCA
AGACGATTGACCTCCCTATACACGTGAGAAATACGGACTAACCAGTCCCTTGAT
ATGAAGCCATAACACAAACGTAGTAGGAAAGATAGAGGATGAGAATCCGGTATC
CCTGTCTGTAAAAACCCAACACGATCTTCGAATCCACTTCCAGCTCAAGGCGT
GTTATTCCTTGCTCCCAGACTATGTGTAACCCATAGTAGAGTCCCCACA
ACTCTG
CTAGTGGCGCTGAACAGATTTTCGATATTCAAAGCAAAAACCCCAACCCAGTTCT
CGTTCCCATCACGCACCGCACCTCCCGCTGCTGCTAACCCGGGATTCTCTCTC
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gtcttctgtaacccaaccaacaagactttcataagctattaaatcagaaccttggaagacaaaaaaggggaaaaaac
cattatccttaagtaaccaacacttctctctctttcttca**ATGGATTGCAACTGAAACAATGGAGA**
AGCCAGCAGCAGCAACAACATCAGACAGAGTCAGAAGAACAACCTTCTGCAG
CTAAGATACCAAAACATGTCTTTGACCAGATTCATTCTCACACTGCAACTTCTA
CTGCTCTTCCTCTCTTTACCCCTGAGCCTACTTCTTCTAAACTCTCCTCTTTGTC
TCCTGATTCTTCCTCCAGGTTCCCCAgtgagtcctttctcctcttatcttcttcttgataaagaa
ttagacttttcattcatatagtttgtttaattgattttgattcctttttgtagAGATGGGGAGCTTCTTTAG

CTGGGCACAGTGGCAAGAACTTGAAC TACAAGCTCTGATCTACAGGTACATGT
TGGCTGGTGCTGCTGTTCCCTCAGGAGCTCCTTTTACCAATCAAGAAAAGCCTT
CTCCATCTATCTCCTTCCTACTTTCTTCACCATCCTCTTCAACACCTACCTCATT
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GGATGGTAAGAAGTGGAGATGTTCAAGAGACGTCTTCGCTGGCCACAAGTAT
TGCGAGCGCCACATGCACCGTGGCCGCAACCGTTCTAGAAAaCCaGTaGAgAC
TCCAACCACCGTCAATGCAACTGCCACGTCCATGGCTTCATCAGTAGCAGCCG
CAGCCACCACTACAACAGCAACAACAACATCTACGTTTGCTTTTGGTGGTGGT
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TACGAGTCCCACATCGGATTCAGTAACAACAGATCAGATGGAGGACACATCC
TGAGGCCCTTCTTTGACGATTGGCCTCGTTCTTCGCTCCAAGAAGCTGACAAT
AGTTCAAGCCCCATGAGCTCAGCCACTTGTCTCTCCATCTCCATGCCCGGGAA
CTCTTCCTCAGACGTCTCTCTGAAGCTGTCCACAGGCAACGAAGAGGGAGCC
CGGAGCAACAACAATGGGAGAGATCAGCAAAACATGAGCTGGTGGAGCGGT
GGAGGTTCCAACCACCATCATCACAACATGGGCGGACCATTGGCCGAAGCCC
TGAGATCTTCTTCCTCATCTTCCCCAACCAGTGTTCTCCATCAGCTTGGTGTCT
CGACACAAGCCTTTTCATTGAaccagtgtaaaaccaacacacaacaatgcggttttactgtgttttggttttatc
caaatttctgtataaagaggggagggtttgtgtcctcttcccttttctttaagatttccctgtatctgtagccttctctgca
gattttatctcctcaaagatttgggttttgaaattcatgtctaataaggatctacgatgaagcttaggcaaaaatgTCGAC
CTGCAGAGCTTTCGTTTCGTATCATCGGTTTCGACAACGTTTCGTCAAGTTCAATG
CATCAGTTTCATTGCGCACACACCAGAATCCTACTGAGTTCGAGTATTATGGC
ATTGGGAAACATGTTTTTCTTGTACCATTGTGTTGTGCTTGTAATTTACTGTGTTT

TTTATTCGGTTTTCGCTATCGAACTGTGAAATGGAAATGGATGGAGAAGAGTT
AATGAATGATATGGTCCTTTTGTTCAATTCTCAAATTAATATTATTTGTTTTTCTC
TTATTTGTTGTGTGTTGAATTTGAAAATATAAGAGATATGCAAACATTTTGTTTT
GAGTAAAAATGTGTCAAATCGTGGCCTCTAATGACCGAAGTTAATATGAGGAG
TAAACACTTGTAGTTGTACCATTATGCTTATTCAGTGGCAACAAATATATTTT
CAGACCTAGAAAAGCTGCAAATGTTACTGAATACAAGTATGTCCTCTTGTTT
TAGACATTTATGAACTTTCCTTTATGTAATTTTCCAGAATCCTTGTCAGATTCTA
ATCATTGCTTTATAATTATAGTTATACTCATGGATTTGTAGTTGAGTATGAAAAT
ATTTTTTAATGCATTTTATGACTTGCCAATTGATTGACAACATGCATCAATCGAA
GCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTT
ACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGC
GAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCCGAGCCTGAATGGCGA
ATGCTAGAGCAGCTTGCCAACATGGTGGAGCACGACACTCTCGTCTACTCCAAG
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCTATTGAGACTTTTCAACAAA
GGGTAATATCGGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTCA
TCAAAAGGACAGTAGAAAAGGAAGGTGGCACCTACAAATGCCATCATTGCGATA
AAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGA
CCCCACCCACGAGGAGCATCGTGGAAGAAAGACGTTCCAACCACGTCTTC
AAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACTCTCGTCTACTC
CAAGAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCTATTGAGACTTTTCAA
CAAAGGGTAATATCGGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCAC
TTCATCAAAGGACAGTAGAAAAGGAAGGTGGCACCTACAAATGCCATCATTGC
GATAAAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGAT
GGACCCCCACCCACGAGGAGCATCGTGGAAGAAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACA
ATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCAATTTG
GAGAGGACACGCTGAAATCACCAGTCTCTCTCTACAAATCTATCTCTCTCGATTG
GCAGATCTGTGATCGACCATGGGGATTGAACAAGATGGATTGCACGCAGGTT
CTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACA
ATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGGCGCCCGG
TTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTCCAGGACGAGG
CAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCT
CGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCG
GGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATG
GCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGA
CCACCAAGCGAAACATCGCATCGAGCGAGCACGTAAGTCCGATGGAAGCCGGTC
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CTGTTCCGCCAGGCTCAAGGCGCGCATGCCCCACGGCGAGGATCTCGTCGTGA
CACATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTG
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CCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCCGGATCGATCCTCTAG
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AATTAATTCGGCGTTAATTCAGTACATTA AAAACGTCCGCAATGTGTTATTAAGTT
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CAGTCCGGGACGGCGTCAGCGGGAGAGCCGTTGTAAGGCGGCAGACTTTGCT
CATGTTACCGATGCTATTCGGAAGAACGGCAACTAAGCTGCCGGGTTTGAAACA
CGGATGATCTCGCGGAGGGTAGCATGTTGATTGTAACGATGACAGAGCGTTGC
TGCCTGTGATCAATTCGGGCACGAACCCAGTGGACATAAGCCTCGTTCGGTTC
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CGTTACGCCGTGGGTGATGTTTGATGTTATGGAGCAGCAACGATGTTACGCAG
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AGAGCGAGATTCTCCGCGCTGTAGAAGTCACCATTGTTGTGCACGACGACATCA
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GCCCCGTCATACTTGAAGCTAGACAGGCTTATCTTGGACAAGAAGAAGATCGCTT
GGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAAAGGCGAGA
TCACCAAGGTAGTCGGCAAATAATGTCTAGCTAGAAATTCGTTCAAGCCGACGC

CGCTTCGCCGGCGTTAACTCAAGCGATTAGATGCACTAAGCACATAATTGCTCA
CAGCCAAACTATCAGGTCAAGTCTGCTTTTATTATTTTAAAGCGTGCATAATAAG
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TTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGA
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GCGGTGTTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAC
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GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC
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ACTCCGGCCGCCCGGTTTCGCTCTTTACGATCTTGTAGCGGCTAATCAAGGCTT
CACCCTCGGATACCGTCACCAGGCGGCCGTTCTTGGCCTTCTTCGTACGCTGC
ATGGCAACGTGCGTGGTGTTTAACCGAATGCAGGTTTCTACCAGGTCGTCTTTC
TGCTTTCGCCATCGGCTCGCCGGCAGAACTTGAGTACGTCCGCAACGTGTGG
ACGGAACACGCGGCCGGGCTTGTCTCCCTTCCCTTCCCGGTATCGGTTTCATGG
ATTCCGTTAGATGGGAAACCGCCATCAGTACCAGGTCGTAATCCCACACACTGG
CCATGCCGGCCGGCCCTGCGGAAACCTCTACGTGCCCCGTCTGGAAGCTCGTAG
CGGATCACCTCGCCAGCTCGTCGGTCACGCTTCGACAGACGGAAAACGGCCAC
GTCCATGATGCTGCGACTATCGCGGGTGCCACGTCATAGAGCATCGGAACGA
AAAAATCTGGTTGCTCGTCGCCCTTGGGCGGCTTCCTAATCGACGGCGCACCG
GCTGCCGGCGGTTGCCGGGATTCTTTGCGGATTCGATCAGCGGCCGCTTGCCA
CGATTACCCGGGGCGTGCTTCTGCCTCGATGCGTTGCCGCTGGGCGGCCTGC
GCGGCCTTCAACTTCTCCACCAGGTCATCACCCAGCGCCGCGCCGATTTGTAC
CGGGCCGGATGGTTTGCGACCGTCACGCCGATTCTCGGGCTTGGGGGTTCC
AGTGCCATTGCAGGGCCGGCAGACAACCCAGCCGCTTACGCCTGGCCAACCG
CCCGTTCCCTCCACACATGGGGCATTCCACGGCGTCGGTGCCTGGTTGTTCTTG
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CTCATTTACTCTGGTAGCTGCGCGATGTATTCAGATAGCAGCTCGGTAATGGTC
TTGCCTTGCGTACCGCGTACATCTTCAGCTTGGTGTGATCCTCCGCCGGCAAC
TGAAAGTTGACCCGCTTCATGGCTGGCGTGTCTGCCAGGCTGGCCAACGTTGC
AGCCTTGCTGCTGCGTGCCTCGGACGGCCGGCACTTAGCGTGTTTGTGCTTT
TGCTCATTTTCTCTTTACCTCATTAACCTCAAATGAGTTTTGATTTAATTTACGCGG
CCAGCGCCTGGACCTCGCGGGCAGCGTCGCCCTCGGGTTCTGATTCAAGAAC
GGTTGTGCCGGCGGCGGCAAGTGCCCTGGGTAGCTCACGCGCTGCGTGATACGG
GACTCAAGAATGGGCAGCTCGTACCCGGCCAGCGCCTCGGCAACCTCACCGC
CGATGCGCGTGCCCTTTGATCGCCCCGCGACACGACAAAGGCCGCTTGTAGCCTT
CCATCCGTGACCTCAATGCGCTGCTTAACCAGCTCCACCAGGTCGGCGGTGGC
CCATATGTCGTAAGGGCTTGGCTGCACCGGAATCAGCACGAAGTCGGCTGCCT
TGATCGCGGACACAGCCAAGTCCGCCGCCTGGGGCGCTCCGTCGATCACTAC
GAAGTCGCGCCGGCCGATGGCCTTCACGTCGCGGTCAATCGTCGGGCGGTGC
ATGCCGACAACGGTTAGCGGTTGATCTTCCCGCACGGCCGCCCAATCGCGGGC
ACTGCCCTGGGGATCGGAATCGACTAACAGAACATCGGCCCCGGCGAGTTGCA
GGGCGCGGGCTAGATGGGTTGCGATGGTCGTCTTGCTGACCCGCCTTTCTGG
TTAAGTACAGCGATAACCTTCATGCGTTCCCTTGCGTATTTGTTTATTTACTCAT
CGCATCATATACGCAGCGACCGCATGACGCAAGCTGTTTTACTCAAATACACAT

CACCTTTT TAGACGGCGGCGCTCGGTTTCTTCAGCGGCCAAGCTGGCCGGCCA
GGCCGCCAGCTTGGCATCAGACAAACCGGCCAGGATTT CATGCAGCCGCACGG
TTGAGACGTGCGCGGGCGGCTCGAACACGTACCCGGCCGCGATCATCTCCGC
CTCGATCTCTTCGGTAATGAAAAACGGTTCGTCCTGGCCGTCCTGGTGCGGTTT
CATGCTTGTTCTCTTGCGGTTCACTCTCGGCGGCCGCCAGGGCGTCGGCCTC
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CAGTGCGAGCCGCTCTTTCACGGTGCGGCCTTCCTGGTCGATCAGCTCGCGGG
CGTGCGCGATCTGTGCCGGGGTGAGGGTAGGGCGGGGGCCAACTTCACGCC
TCGGGCCTTGCGCGCCTCGCGCCCGCTCCGGGTGCGGTCGATGATTAGGGAA
CGCTCGAACTCGGCAATGCCGGCGAACACGGTCAACACCATGCGGCCGGCCG
GCGTGGTGGTGTCGGCCACGGCTCTGCCAGGCTACGCAGGCCCGCGCCGGC
CTCCTGGATGCGCTCGGCAATGTCCAGTAGGTGCGGGGTGCTGCGGGCCAGG
CGGTCTAGCCTGGTCACTGTCAACACGTCGCCAGGGCGTAGGTGGTCAAGCAT
CCTGGCCAGCTCCGGGCGGTGCGGCCTGGTGCCGGTGATCTTCTCGGAAAC
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GTCGTGGTGCTGACGCGGGCATAGCCAGCAGGCCAGCGGCGGCGCTCTTG
TTCATGGCGTAATGTCTCCGTTCTAGTCGCAAGTATTCTACTTTATGCGACTAA
AACACGCGACAAGAAAACGCCAGGAAAAGGGCAGGGCGGCAGCCTGTGCGGT
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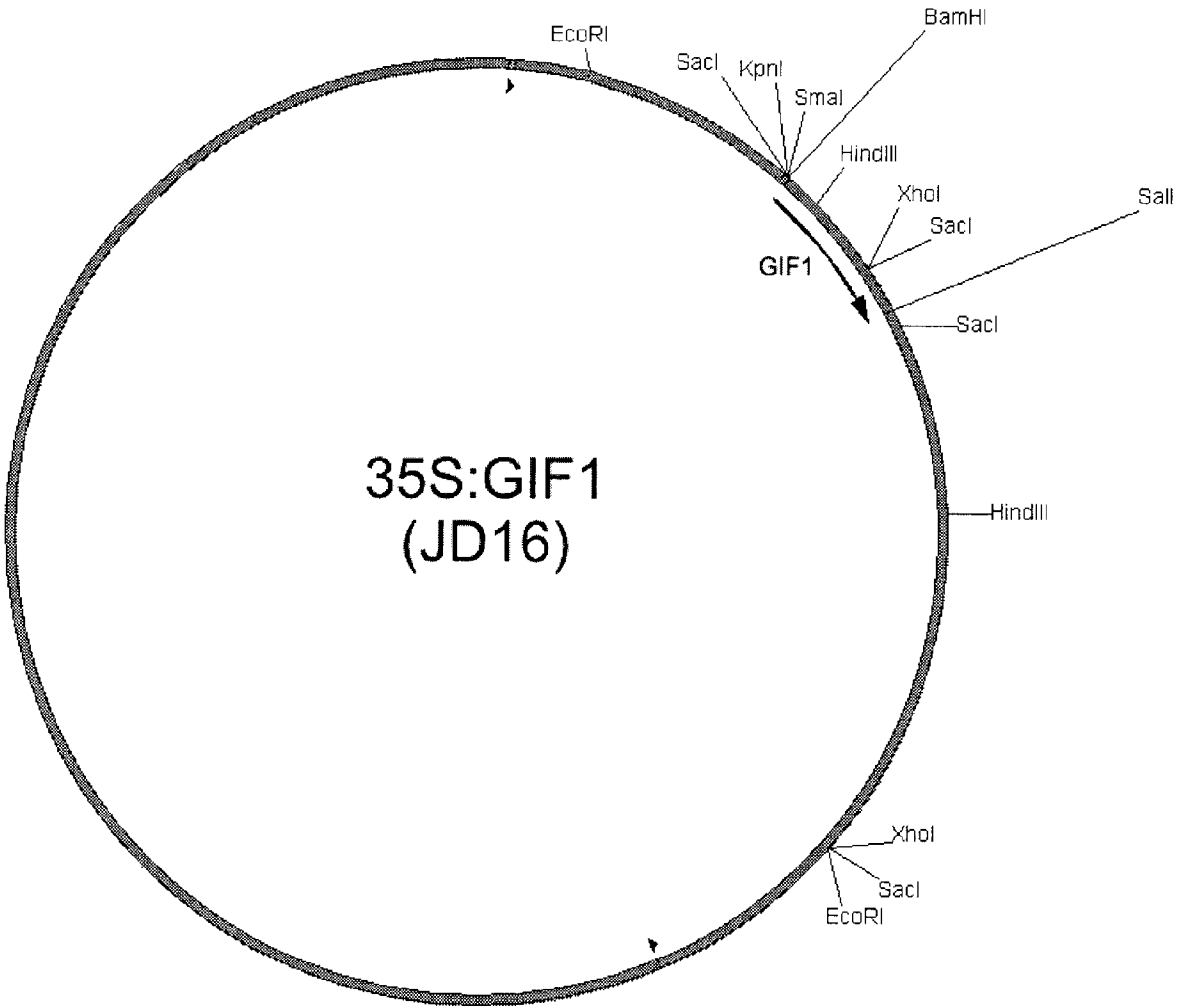


FIGURE 41

23186597.1

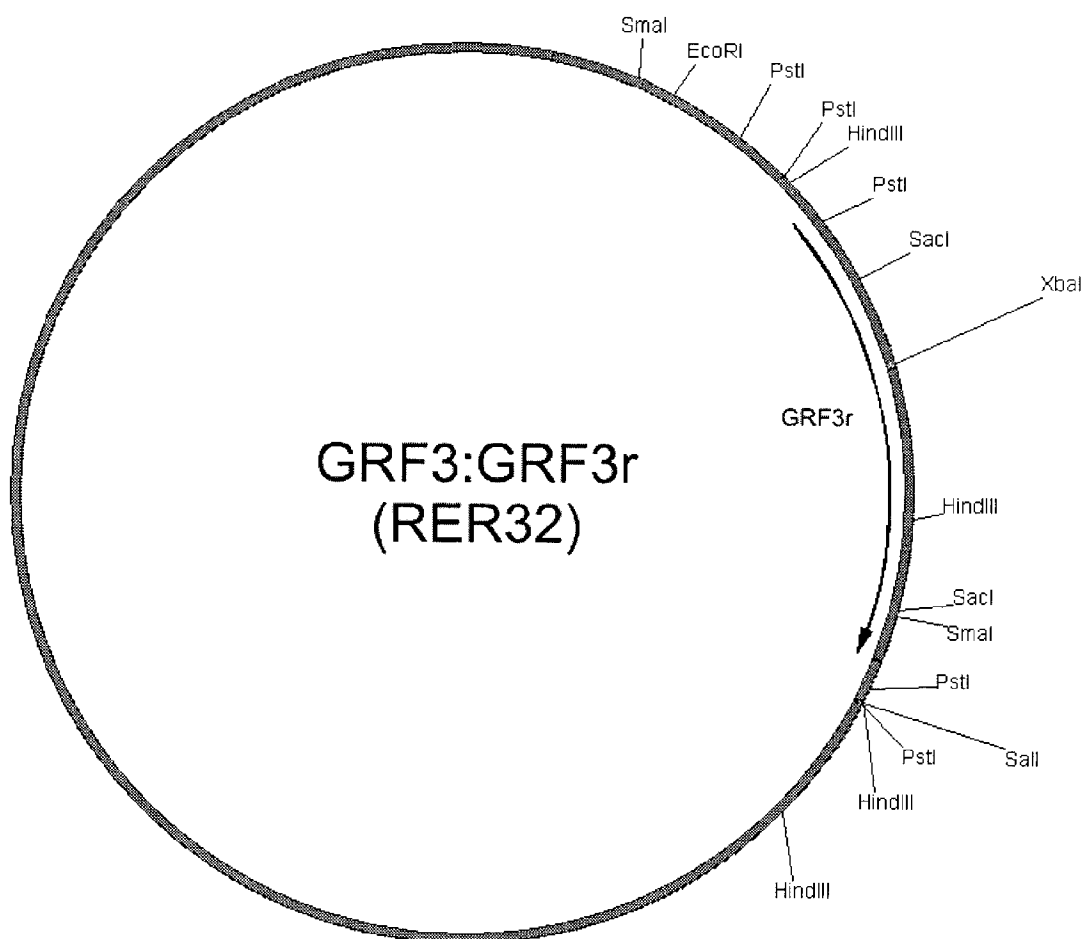


FIGURE 42

23186597.1

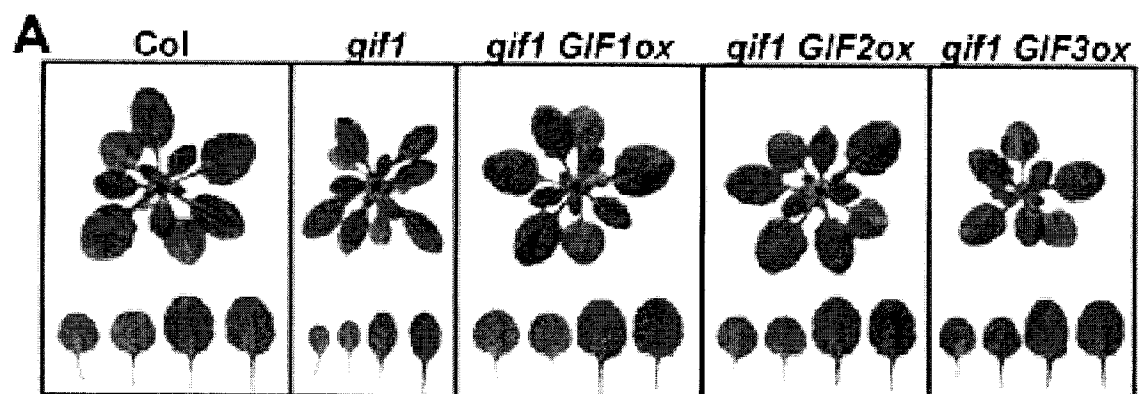


FIGURE 43

23186597.1

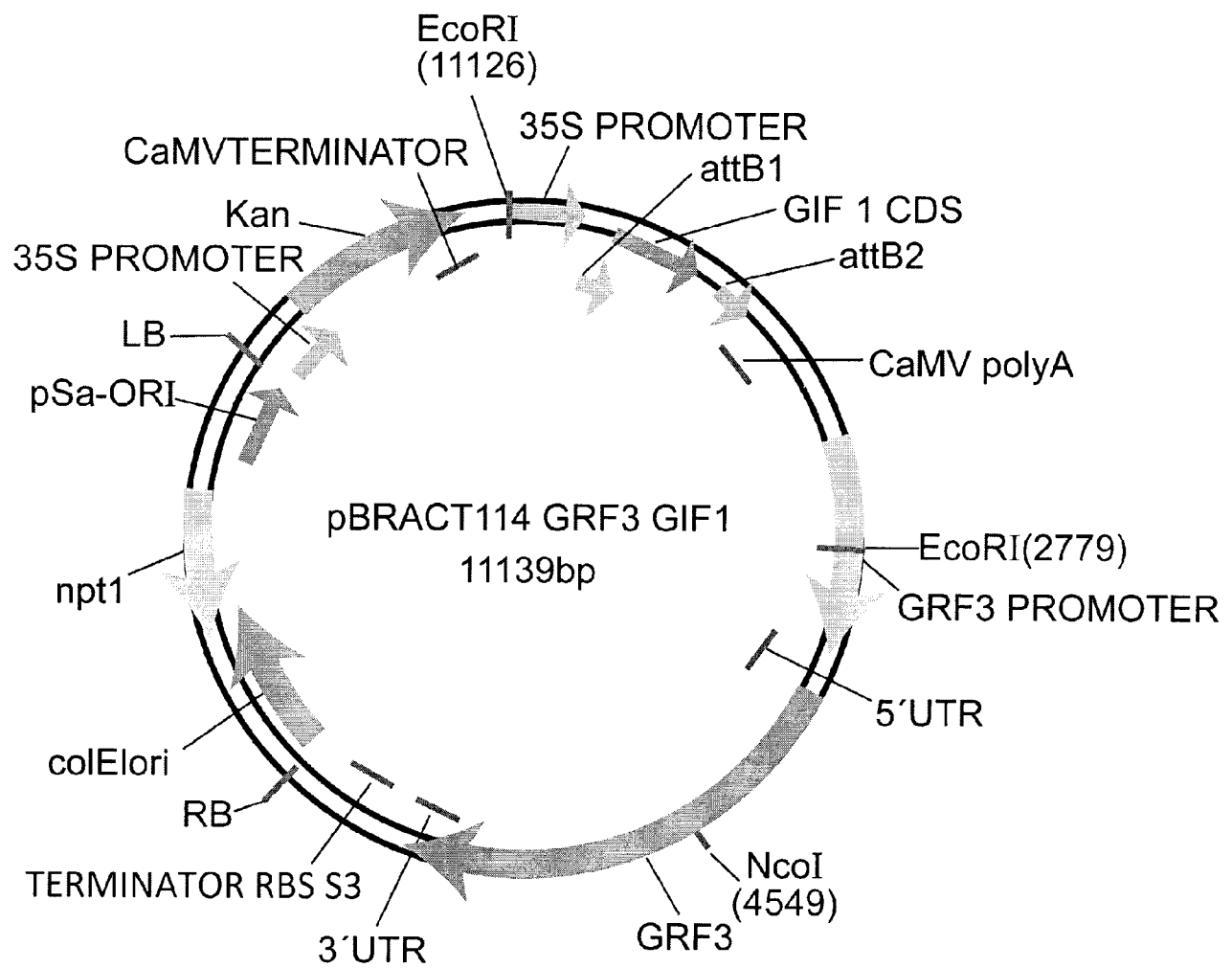


FIGURE 44

23186597.1

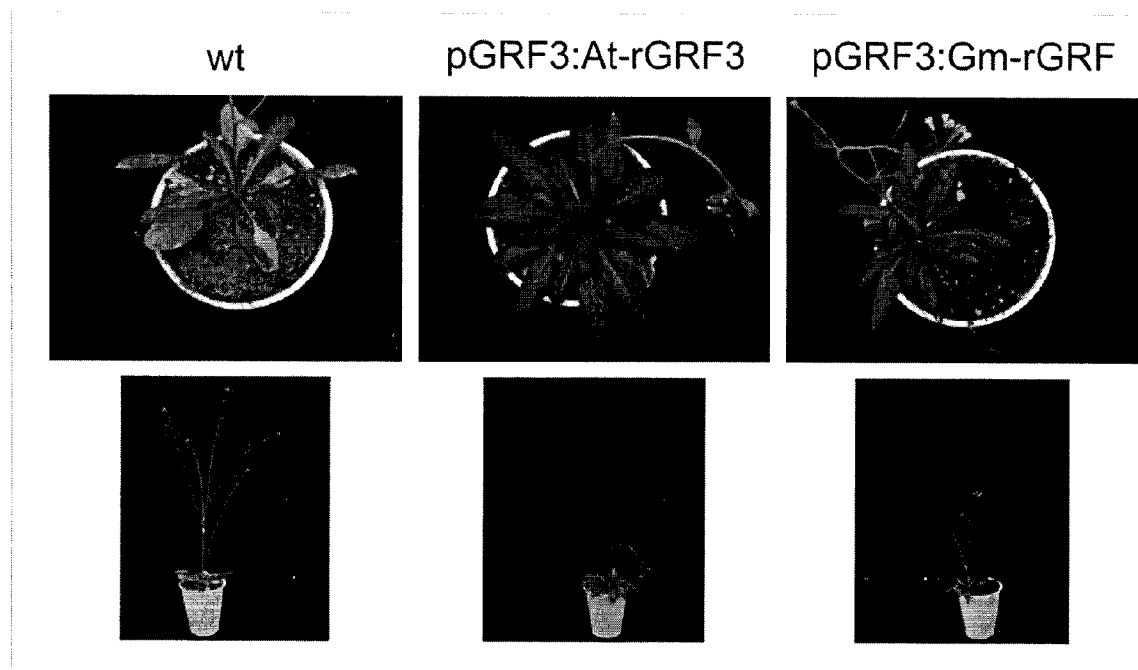


FIGURE 45

23186597.1

FIGURE 46

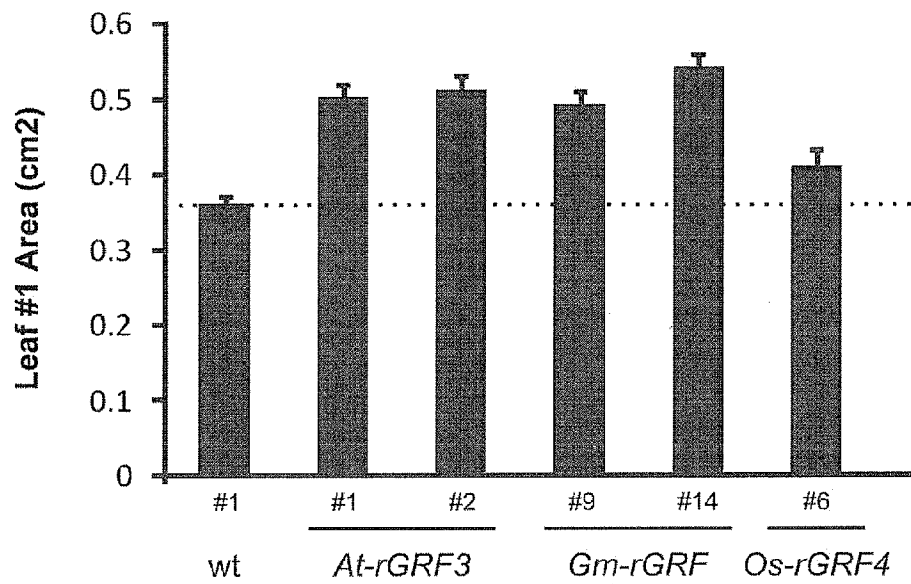


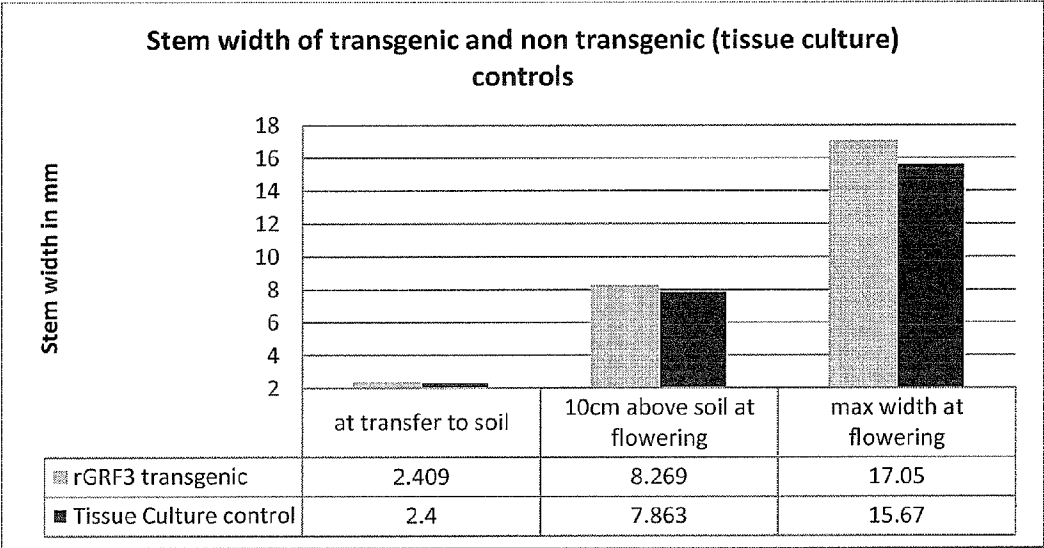
FIGURE 47

>CpGRF (*Carica papaya*) (SEQ ID No. 88)
ATGGACTTGCATCTGAAACAATGGAGGAACCAGCATGAGTCAGAGCAACAACCTTCT
GCGAAGATAACCAAACTTCTCCTTGATCCACATCAACAAAACCCATCTGCCTCAGCT
TCTGCTTCTCTTGCACTCCCTTTGTTTGTACCTGAACAGCCCTCTACCAAACTCACC
AACCTGTCAGCGTTGCCAGATTCATCCTCTAGATTTCCCAAGATGGGAAGCTACTTT
AGTTTGGCTCAGTGGCAGGAGCTGGAGTTGCAGGCTTTGATCTACAGATACATGTTA
GCTGGTGCTGCCGTTCCCTCCCGAGCTCCTCCAGCCAATCAAGAAAAGTCTCCTTCAC
TCTTCTCCATATTTCCCTCCATCATCCTCTTCAACATTACGCTCATTTATCAGCCTGCT
TGGTATTGGAGCAGAGCTGCCCTGGATCCGGAGCCGGGTCGGTGCCGGAGAACAGAT
GGAAAGAAATGGAGGTGCTCAAGAGATGTGGTGGCTGGCCAGAAATATTGCGAGCGC
CACATGCACCGTGCCGCAAC**CGTTCAAGAAAGCCTGTGGAAAT**CCCCACGCCGAAC
ACCACCGCCGCCGTCACTCCACTCTCCGTAGCCGCCCTCAACGGTTTCTTCTCTGGGT
GCTGGTGGTGGTGGTCTCGGTGGCAGCGACACTTTCAAATCCACCGGTCCAATCTCC
ATGACATTGCCGGCAATGGTGGCTAATGGGCCGAGCTTCGGCCTCGCCGGACCGGCT
AGCTCCGCTGATCTCCTGCACCTGAATCATAGTTCTCAGAGTTCAGGATTGAGAAC
AAGGGCCTCTTTGAAGCCCAAACGAAGTTGACAACAGACCTGACGGCCACATTCTA
AGGCATTTTTTTGATGATTGGCCCCGATCACTTCAAGAACCTGATAATGCTGGGAGG
AATGCTAGCCCTATGAGCTCCTCCACCTGTCTCACAATTTATCCTCCGATGTGTCTG
TTGAAACTGTCAACTGGTAATGCAGATGAACTCACCACCAGGGACGGCGAAAGGGAT
CAACTGCAGTTGAATTGGGCTGCCGGATGGGCGACAAACCAAATGGGAGGACCTCTA
GCTGAGGCATTGCGTTCCCTCCACTTCAAATTCCTTCACCCACCAGTGTCTTACATCAG
TTGCCGCGGAACCTCTGCCACAGAATCTAGTTACGTTAGCACCTGTGTTTAG

FIGURE 48

>CpGRF (*Carica papaya*) (SEQ ID No. 89)
MDLHLKQWRNQHESEQQPSAKIPKLLLDPHQQNPASASASLALPLFVPEQPSTKLT
NLSALPDSSSRFPKMGSYFSLAQWQELELQALIYRYMLAGAAVPELLQPIKKSLLH
SSPYFLHHPLQHYAHYQPAWYWSRAALDPEPGRRCRRTDGKKWRC**SRD**VVAGQKYCER
HMRGRNRSRKPVETPTNTAAVTPLSVAASTVSSLGAGGGGLGGSDTFKSTGPIS
MTLPAMVANGPSFGLAGPASSADLLHLNHSSEFRIENKGLFEAQNEVDNRDPGHIL
RHE**FD**DWPRSLQEPDNAGRNASPMSSSTCLTISSSDVSLKLSTGNADELTTTRDGERD
QLQLNWAAGWATNQMGGPLAEALRSSTSNSSPTSVLHQLPRNSATESSYVSTCV

FIGURE 49



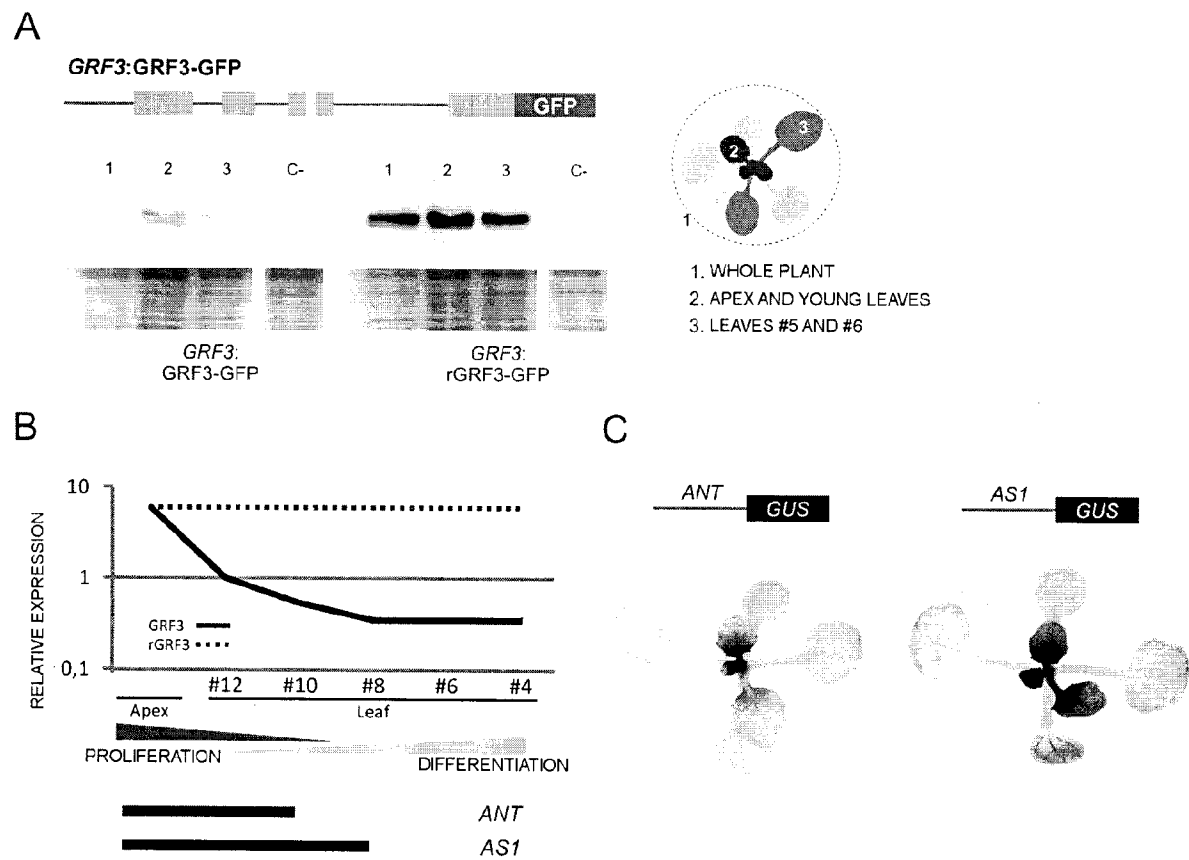


FIGURE 50

23186597.1

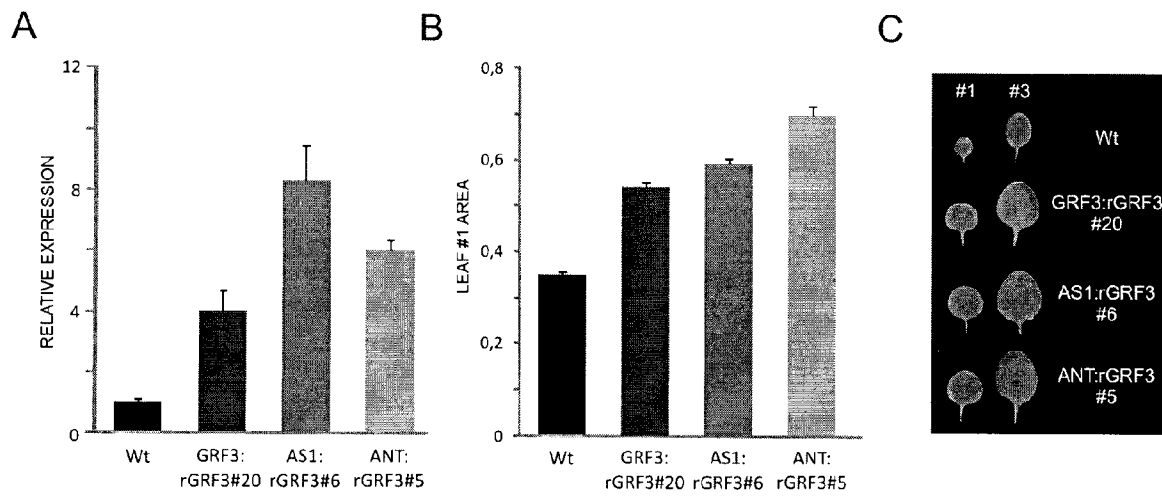


FIGURE 51

23186597.1

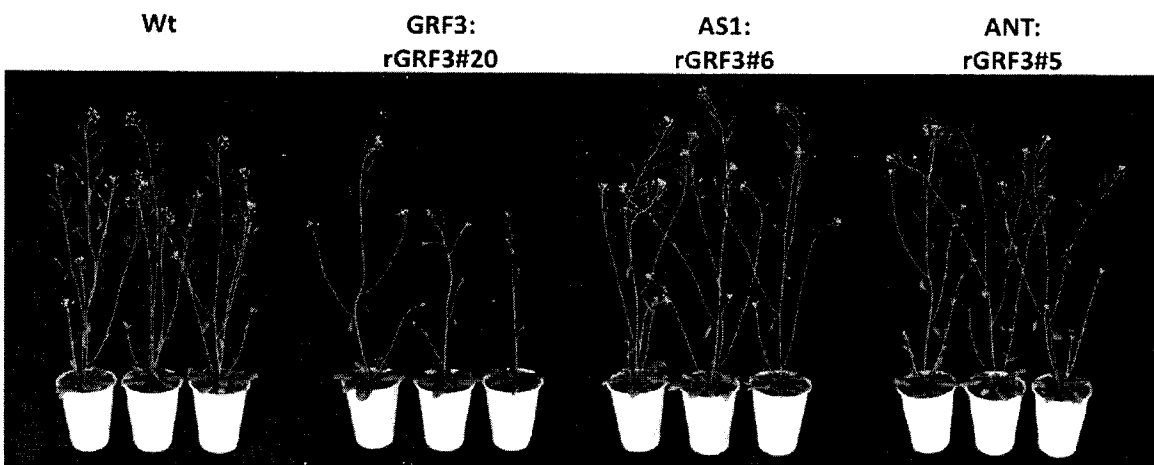
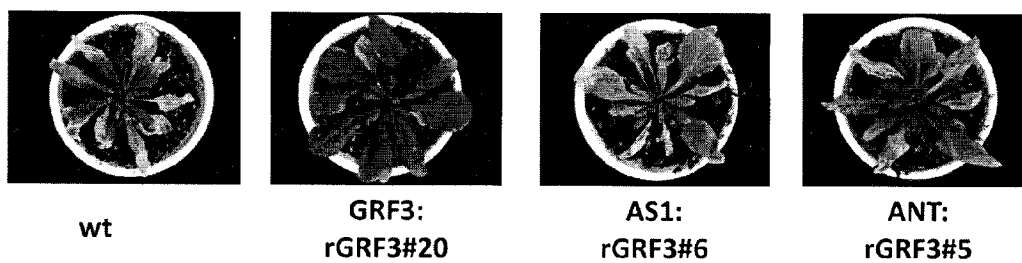


FIGURE 52

23186597.1

A



B

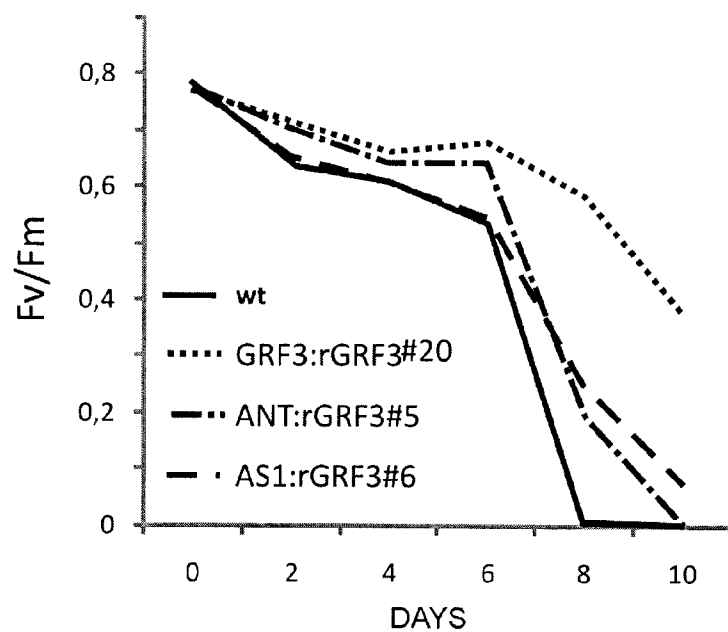
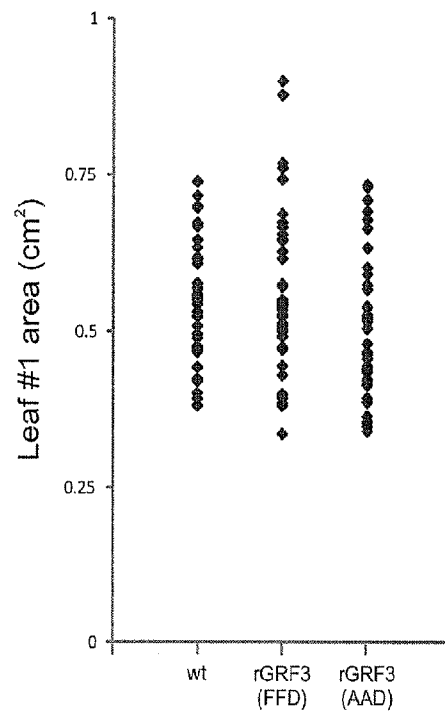


FIGURE 53

23186597.1

FIGURE 54



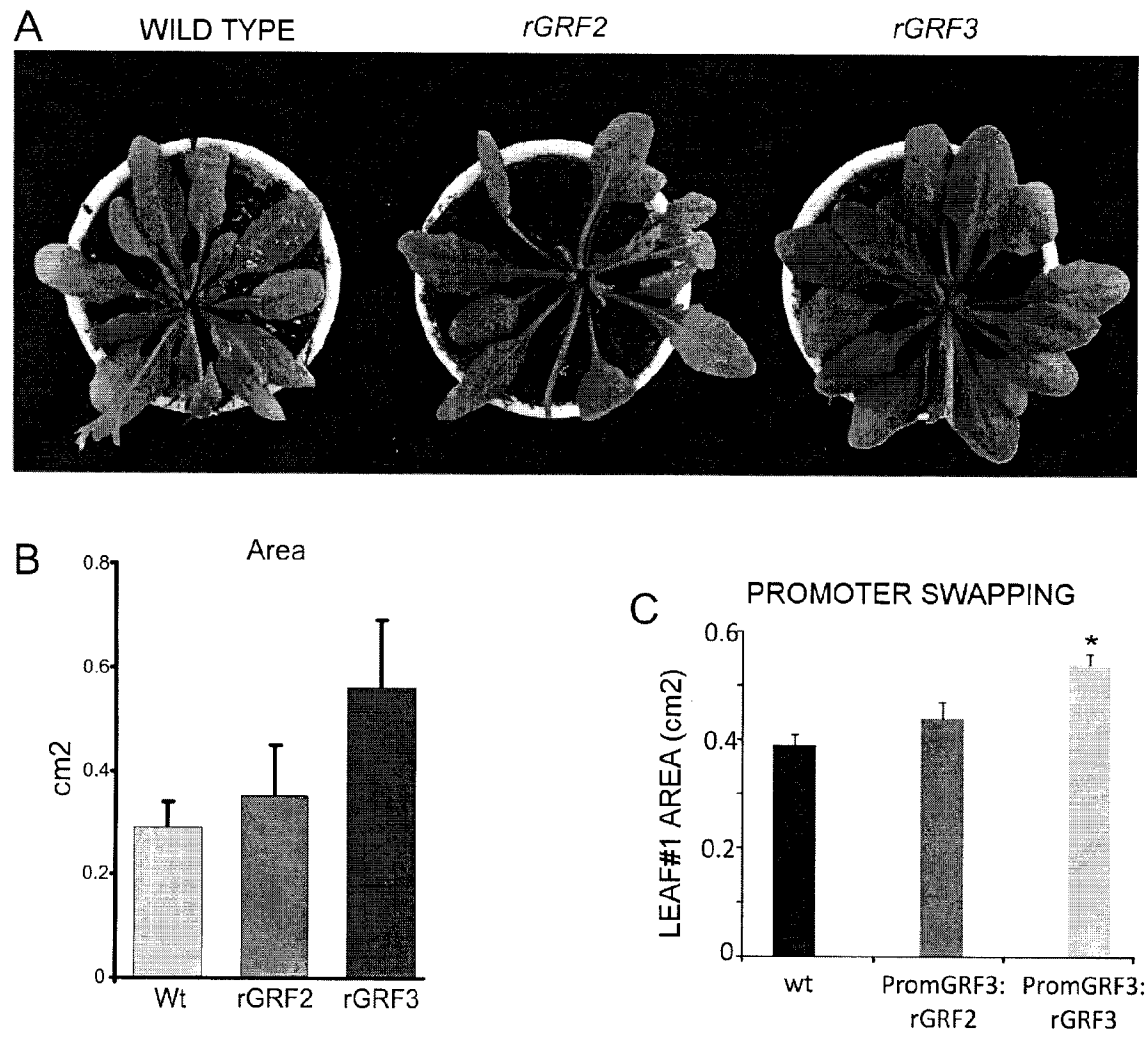


FIGURE 55

23186597.1

FIGURE 56

Construct	Copy No.	Glass house randomised position order	widest stem width at Flowering mm	10cm stem weight (g)
rGRF3	1	3	18.67	3.5
rGRF3	1	4	17.60	2.0
rGRF3	2	7	15.88	2.4
rGRF3	1	13	14.46	1.9
rGRF3	2	14	16.31	2.2
rGRF3	2	15	17.18	2.9
rGRF3	1	16	17.81	2.4
rGRF3	2	20	17.47	2.3
rGRF3	2	21	16.71	2.7
rGRF3	1	31	16.87	2.4
rGRF3	2	33	14.92	2.5
rGRF3	1	38	17.40	3.0
rGRF3	2	40	20.92	2.4
rGRF3	1	41	16.08	3.5
rGRF3	2	42	14.20	2.6
rGRF3	1	55	19.75	3.7
rGRF3	2	56	17.56	3.7
TC	0	1	16.29	2.4
TC	0	6	15.34	3.0
TC	0	8	16.36	2.3
TC	0	9	16.63	2.7
TC	0	10	15.34	2.5
TC	0	19	12.80	2.5
TC	0	23	13.02	1.8
TC	0	26	15.29	2.3
TC	0	28	16.60	1.7
TC	0	29	16.38	2.1
TC	0	32	15.88	2.2
TC	0	34	17.67	2.2
TC	0	36	15.41	3.2
TC	0	37	16.36	1.9
TC	0	43	16.02	2.4
TC	0	47	15.86	2.0
TC	0	48	14.02	3.7
TC	0	49	16.72	3.2
TC	0	51	15.02	3.2
TC	0	39	16.38	3.4

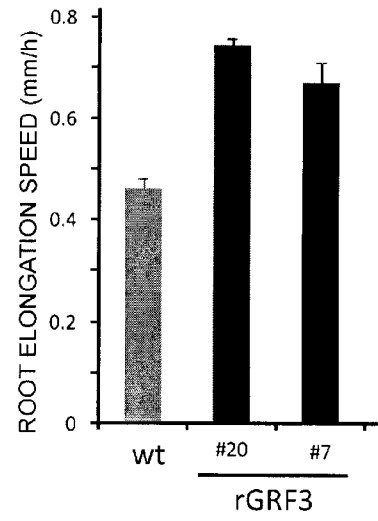
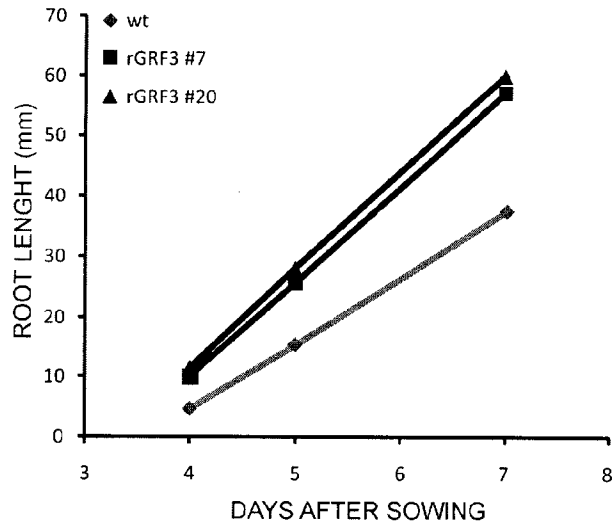


FIGURE 57

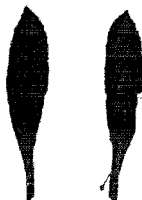
23186597.1



TOP-VIEW

BOTTOM-VIEW

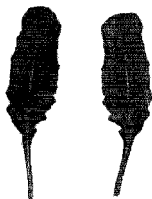
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TOP-VIEW

BOTTOM-VIEW

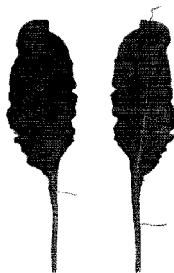
rGRF2



TOP-VIEW

BOTTOM-VIEW

wt



TOP-VIEW

BOTTOM-VIEW

rGRF3