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(54) **Title**: COMPOSITIONS AND METHODS FOR MANIPULATING THE DEVELOPMENT OF PLANTS

(57) **Abstract**: The invention provides a methods and materials for producing and selecting plants with at least one dwarfing-associated phenotype. The methods and materials relate to altering the expression, or activity, of an ARF3 poypeptide in the plant, and selecting plants with altered the expression, or activity, of an ARF3 poypeptide. The invention also provides plants produced or selected by the methods. The methods also involve crossing plants of the invention with other plants to produce further plants with at least one dwarfing-associated phenotype.

COMPOSITIONS AND METHODS FOR MANIPULATING THE DEVELOPMENT OF PLANTS

TECHNICAL FIELD

The invention relates to compositions and methods for the manipulation of plant development.

BACKGROUND

Dwarfing rootstocks have revolutionized the production of some tree and vine crops, by permitting high-density plantings that increase fruit yield in the early years of orchard establishment (Ferree and Carlson 1987; Webster and Wertheim 2003; Gregory and George 2011). The widespread use of dwarfing rootstocks has led to a steady increase in the efficiency of apple production over the past century (Hirst and Ferree 1995; Webster 1995).

'Malling9' ('M9') is the most frequently used apple dwarfing rootstock in both commercial and home orchards (Webster 1995). 'M9', originally called 'Jaune de Metz', was discovered as single seedling in the 1800s and was clonally propagated as a rootstock because of its effects on both precocity and vigour control of the grafted scion (Carrière 1897). At the beginning of the 20th century, all the apple rootstocks grown in Western Europe were collected at the East Malling Research Station (UK) and classified according to their effect on the grafted scion (Hatton 1917). Many of the apple rootstock varieties bred worldwide have parentage derived from this 'Malling' series, particularly 'M9' (Manhart 1995; Webster and Wertheim 2003). Progeny of 'M9' segregate for rootstock-induced dwarfing, indicating that this trait is determined by one or more genetic factors.

Dwarfing is a complex phenomenon, with some dwarfing-associated phenotypes being exhibited in the root stock plant, and other dwarfing-associated phenotypes being exhibited in scions grafted onto the root stock plants.

Phenotypes reported in M9 root stock plants include: altered xylem/phloem ratio, more phloem elements, smaller phloem elements, thicker bark, altered auxin transport, slower auxin transport, and reduced apical dominance. Grown as an un-grafted plant, M9 is also bushier than other types of non-grafted apples.

Based on the altered xylem/phloem phenotypes, researchers have suggested that dwarfing roots tocks function by altering the transport of water, nutrients or hormones. A number of studies have measured hormone concentration and/or movement in dwarfing rootstocks; auxin in particular seems to play a major role in rootstock induced dwarfing (Hooijdonk, Woolley et al. 2011). Soumelidou was the first to demonstrate that 'M9' apple stems transport auxin at a slower rate than non-dwarfing stems (Soumelidou K 1994). More recently, it has been shown that treating apple trees with NPA, a polar auxin transport inhibitor, phenocopies the effect of a dwarfing rootstock (van Hooijdonk 2010).

Despite M9 rootstocks being so widely used and the subjects of numerous studies, the underlying mechanism by which dwarfing rootstocks control both scion vigour and flowering remains unresolved.

In woody perennials where a dwarfing or vigour-reducing rootstock exists, the overall effect on the grafted scion is characterised by less vegetative growth, earlier termination of shoot growth, earlier competency to flower than non-grafted trees or trees on vigorous rootstocks (also called precocity), earlier phase change (a term which is related to earlier flowering, but also encompasses other traits, such as thorns, leaf shape, etc), a smaller canopy, reduced stem circumference (or TCA, Trunk Cross-sectional Area), weaker shoot system, reduced branch diameter.

The first detectable effects on apple scions grafted onto M9 rootstock are fewer and shorter sylleptic branches (axillary meristems that grow out in the same season they were initiated), more axillary flowers (these do not appear until the spring of year two, but are formed in summer of year 1), and a tendency for both the primary axis and secondary axes to terminate earlier (Seleznyova, Thorp et al. 2003; Seleznyova, Tustin et al. 2008; van Hooijdonk, Woolley et al. 2010; van Hooijdonk, Woolley et al. 2011).

An increased proportion of axillary floral buds along the primary axis can have a profound impact on the subsequent growth of the scion. In a floral bud, the sympodial "bourse" shoot that develops from an axillary meristem is much less vigorous than the monopodial shoot that continues growth from the apex of a vegetative bud. Bourse shoots do not begin extension until anthesis of the flowers and are developmentally delayed relative to monopodial shoots, which begin growth immediately after budbreak. The effects of increased flowering and reduced sylleptic shoot number and length in year one became amplified in successive growth seasons, and within three

years, scions grafted on dwarf or semi-dwarf rootstocks exhibited a distinctly reduced canopy size and branching density.

Quantitantive trait loci (QTL) associated with dwarfing have been identified in apple dwarf rootstock. For example, Pilcher *et al* (2008) generated a segregating rootstock population derived from a cross of 'M9' and the vigorous rootstock 'Robusta 5' ('R5'). The progeny were all grafted with 'Braeburn' scions and the scions were phenotyped over seven years. Using a bulked segregant analysis (comparing pooled rootstock DNAs from dwarfed and vigorous trees) of a the rootstock population, the authors identified a major dwarfing locus (*Dw1*) derived from 'M9' and located at the top of linkage group (LG) 5 (Pilcher, Celton et al. 2008) (Fig 1a). Some of the vigorous individuals in this population carried *Dw1*, suggesting there were one or more additional rootstock loci that influence dwarfing of the scion. Using an enlarged population from the same cross, a genetic map was constructed which enabled a multi-trait quantitative trait locus (QTL) analysis of rootstock-induced dwarfing (Celton, Tustin et al. 2009).

More recently Fazio et al characterised two dwarfing loci *Dw1* and *Dw2* and reported that the strongest degree of dwarfing was conferred by rootstock with both *Dw1* and *Dw2* whereas either *Dw1* or *Dw2* alone affected dwarfing (Celton et al 2009). The authors also reported the *Dw1* QTL to be located between the marking Hi22f12 and Hi04a08 defining an interval of 2.46 Mb.

The introduction of dwarfing into new apple cultivars is only currently achievable, through the laborious and slow procedures of breeding. Breeding of any fruit is also of course limited by the compatability of breeding species.

It would be beneficial to have tools or methods to introduce dwarfing, or dwarfing-associated phenotypes into new species where dwarfing technology is not yet available. Furthermore, even in species where dwarfing technology is available, it would also be advantageous to be able to more efficiently introduce dwarfing into certain cultivars, or root stock cultivars, that are well adapted to their local environment.

It is an object of the invention to provide materials and methods for producing dwarfing and/or at least one dwarfing-associated phenotype in plant, and/or at least to provide the public with a useful choice.

SUMMARY OF THE INVENTION

Method

In the first aspect the invention provides a method for producing a plant with at least one dwarfing-associated phenotype the method comprising altering the expression, or activity, of an ARF3 poypeptide in the plant.

In one embodiment the the method comprises increasing the expression of the ARF3 poypeptide in the plant.

In a further embodiment the method comprises transforming the plant to express the ARF3 poypeptide in the plant.

In a further embodiment the method comprises transforming the plant with polynucleotide encoding the ARF3 polypeptide.

In a further embodiment the polynucleotide is operably linked to a heterologous promoter.

In a further embodiment the method comprises modifying the sequence of an endogenous polynucleotide encoding the ARF3 polypeptide in the plant.

In one embodiment, modifying the endogenous polynucleotide alters the activity of the ARF3 poypeptide in the plant to induce the dwarfing-associated phenotype.

In one embodiment the dwarfing-associated phenotype is selected from:

a) altered auxin transport,
b) slower auxin transport,
c) reduced apical dominance,
d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,
j) reduced vigour,
k) less vegetative growth,

l) earlier termination of shoot growth,
m) earlier competence to flower,
n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length, and
y) reduced scion mass.

In one embodiment the dwarfing-associated phenotype is selected from a) to i). In a further embodiment the dwarfing-associated phenotype is selected from a) to h). In one embodiment a plant with at least one of these phenotypes is suitable for use as a rootstock plant. In a further embodiment the dwarfing-associated phenotype in this plant is at least one of reduced apical dominance, a bushier habit, an altered xylem/phloem ratio, an increased number of phloem elements, and reduced root mass.

In a further embodiment the dwarfing-associated phenotype is the competence to induce at least one of a) to y) in a scion grafted on to the plant. In a further embodiment the dwarfing-associated phenotype is the competence to induce at least one of a) to h) and j) to x) in a scion grafted on to the plant.

In a preferred embodiment the dwarfing-associated phenotype is the competence to induce at least one of j) to y) in a scion grafted on to the plant.

In a further embodiment the dwarfing-associated phenotype is the competence to induce at least one of: reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduced scion mass in a scion grafted on to the plant.

In a further embodiment the method includeds the step of grafting a scion on to a plant produced by the method.

In a further embodiment the dwarfing-associated phenotype is the competence to induce at least one of: reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, and reduced stem circumference, in a scion grafted on to the plant.

In a further embodiment the method includeds the step of grafting a scion on to a plant produced by the method.

In one embodiment the dwarfing-associated phenoytype is exhibited in a scion grafted onto the plant.

In one embodiment the dwarfing-associated phenoytype exhibited in the scion is at least one of j) to y). In one embodiment the dwarfing-associated phenoytype exhibited in the scion is at least one of j) to x).

In a further embodiment the dwarfing-associated phenoytype exhibited in the scion is at least one of: reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduced scion mass, in a scion grafted on to the plant.

In a further embodiment the dwarfing-associated phenoytype exhibited in the scion is at least one of: reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, and reduced stem circumference, in a scion grafted on to the plant.

In a further embodiment the invention provides a method of producing a plant with at least one dwarfing-associated phenotype selected from:

a) altered auxin transport,
b) slower auxin transport,
c) reduced apical dominance,
d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,

j) reduced vigour,
k) less vegetative growth,
l) earlier termination of shoot growth,
m) earlier competence to flower,
n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length, and
y) reduced scion mass,

the method comprising grafting a scion onto a plant produced by a method of the invention.

In this embodiment the at least one dwarfing-associated phenotype is preferably exhibited in the grafted scion. In this embodiment the grafted scion exhibits at least one of j) to y). In a further the grafted scion exhibits at least one of j) to x).

In a further embodiment the grafted scion preferably exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduce scion mass, in a scion grafted on to the plant.

In a further embodiment the grafted scion preferably exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, and reduced stem circumference, in a scion grafted on to the plant.

In a further embodiment the invention provides a method for producing a plant with at least one dwarfing-associated phenotype selected from:

a) altered auxin transport,
b) slower auxin transport,
c) reduced apical dominance,

d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,
j) reduced vigour,
k) less vegetative growth,
l) earlier termination of shoot growth,
m) earlier competence to flower,
n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length,
y) reduced scion mass,

the method comprising the steps:

A. providing a plant with altered the expression or activity of a ARF3 poypeptide,
B. grafting a scion onto the plant in A

wherein at least one of j) to y) is exhibited in the scion grafted on to the plant in A.

In a further embodiment at least one of j) to x) is exhibited in the scion grafted on to the plant in A.

In a further embodiment the grafted scion preferably exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduced scion mass, in a scion grafted on to the plant.

In a further embodiment the grafted scion preferably exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, and reduced stem circumference, in a scion grafted on to the plant.

In one embodiment the plant in A has increased expression of the ARF3 poypeptide.

In a further embodiment the plant in A has been transformed to express the ARF3 poypeptide.

In a further embodiment the plant in A is transgenic for a polynucleotide encoding the ARF3 polypeptide.

In a further embodiment the polynucleotide is operably linked to a heterologous promoter.

In a further embodiment the plant in A comprises a modification in an endogenous polynucleotide encoding the ARF3 polypeptide in the plant.

In a further embodiment the modification alters the activity of the ARF3 poypeptide in the plant to induce the dwarfing-associated phenotype.

ARF3 polypeptide/polynucleotides used in the methods of the invention

In one embodiment of the methods above the ARF3 polypeptide has a sequence with at least 70% identity to any one of SEQ ID NO:1 to 11, 28 and 29.

In a further embodiment the polypeptide has a sequence with at least 70% identity to SEQ ID NO:1 (MdARF3).

In a further embodiment the polypeptide has a sequence with at least 70% identity to SEQ ID NO:28 (MdARF3).

In most known ARF3 polypeptide sequences either a Serine or Proline residue is found at the position corresponding amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3) as shown in Figure 8.

In a further embodiment the polypeptide comprises a hydrophobic amino acid residue at the position corresponding amino acid residue 72 in SEQ ID NO:28 (MdARF3).

In a further embodiment the polypeptide comprises a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:28 (MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:2 (M9 MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:29 (M9 MdARF3).

In one embodiment the ARF3 polynucleotide is a polynucleotide that encodes and ARF3 polypeptide.

Modification of an endogenous polynucleotide

In one embodiment the modification results in expression of an ARF3 polypeptide with a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

In a preferred embodiment the hydrophobic amino acid is a Leucine residue.

In one embodiment the modification results in a codon encoding the Leucine residue.

In one embodiment the codon is found at a position corresponding to nucleotides 214 to 216 in the ARF3 polynucleotide of SEQ ID NO:12.

In one embodiment the codon is selected from: TTA, TTG, CTT, CTC, CTA and CTG.

In a preferred embodiment the codon is TTG.

Thus in a preferred embodiment, the modification results in a T nucleotide at a position corresponding to nucleotide 215 in the ARF3 polynucleotide of SEQ ID NO:12.

Polynucleotide encoding a M9 type ARF3 polypeptide

In a further aspect, the invention provides an isolated polynucleotide encoding an ARF3 polypeptide comprising a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

In a further embodiment the invention provides a polynucleotide encoding a variant of fragment of the ARF3 polypeptide.

In one embodiment, the hydrophobic amino acid residue is a Leucine residue.

Thus, in one embodiment, the invention provides an isolated polynucleotide encoding an ARF3 polypeptide comprising a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

In a further embodiment the ARF3 polypeptide comprising comprises at least 70% identity to SEQ ID NO:2 or 29 (MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:2 or 29 (M9 MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:29 (M9 MdARF3).

In one embodiment the polynucleotide has at least 70% identity to at least one of SEQ ID NO:14 and 15.

In a further embodiment the polynucleotide has at least 70% identity to SEQ ID NO:14.

In a further embodiment the polynucleotide has at least 70% identity to SEQ ID NO:15.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO:14 or 15.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO:14.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO:15.

Preferably the fragment of the ARF3 polypeptide comprises at least 50 contiguous amino acids, more preferably at least 100 contiguous amino acids, more preferably at least 150 contiguous amino acids, more preferably at least 200 contiguous amino acids, more preferably at least 250 contiguous amino acids, more preferably at least 300 contiguous amino acids, more preferably at least 350 contiguous amino acids, more preferably at least 400 contiguous amino acids, more preferably at least 450 contiguous amino acids of the polypeptide of the invention.

Preferably the fragment comprises the hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

Preferably the fragment comprises the hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:28 (MdARF3).

Preferably the hydrophobic amino acid residue is a Leucine residue.

Polynucleotide

In a further aspect the invention provides an isolated polynucleotide comprising the sequence of SEQ ID NO:14 or 15.

In one embodiment the polynucleotide comprising the sequence of SEQ ID NO:14.

In one embodiment the polynucleotide comprising the sequence of SEQ ID NO:15.

In a further embodiment the invention provides a variant or fragment of the polynucleotide.

Polypeptide

In a further aspect, the invention provides an isolated ARF3 polypeptide comprising a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

In a further embodiment the ARF3 polypeptide comprises a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

In a further embodiment the invention provides a variant of fragment of the ARF3 polypeptide.

In one embodiment, the hydrophobic amino acid residue is a Leucine residue.

Thus, in one embodiment, the invention provides an isolated ARF3 polypeptide comprising a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

In a further embodiment the ARF3 polypeptide comprising comprises at least 70% identity to SEQ ID NO:2 or 29 (M9 MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:2 (M9 MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:29 (M9 MdARF3).

Polypeptide fragment

Preferably the fragment comprises at least 50 contiguous amino acids, more preferably at least 100 contiguous amino acids, more preferably at least 150 contiguous amino acids, more preferably at least 200 contiguous amino acids, more preferably at least 250 contiguous amino acids, more preferably at least 300 contiguous amino acids, more preferably at least 350 contiguous amino acids, more preferably at least 400 contiguous amino acids, more preferably at least 450 contiguous amino acids of the polypeptide of the invention.

Preferably the fragment comprises the hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

Preferably the fragment comprises the hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO: 28 (MdARF3).

Preferably the hydrophobic amino acid residue is a Leucine residue.

Polynucleotide fragment / primers and probes

Preferably the polynucleotide fragment comprises at least 5 contiguous nucleotides, more preferably at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, more preferably at least 20 contiguous nucleotides, more preferably at least 21 contiguous nucleotides, more preferably at least 30 contiguous nucleotides, more preferably at least 50 contiguous nucleotides, more preferably at least 100 contiguous nucleotides, more preferably at least 150 contiguous nucleotides, more preferably at least 200 contiguous nucleotides, more preferably at least 250 contiguous nucleotides, more preferably at least 300 contiguous nucleotides, more preferably at least 350 contiguous nucleotides, more preferably at least 400 contiguous nucleotides, more preferably at least 450 contiguous nucleotides of the polynucleotide of the invention.

In a preferred embodiment, the fragment of the polynucleotide of the invention, encodes a polypeptide fragment of the invention.

In one embodiment the invention provides a primer consisting of a polynucleotide fragment of the invention.

In a further embodiment the invention provides a probe consisting of a polynucleotide fragment of the invention.

Construct

In a further embodiment the invention provides a construct comprising a polynucleotide of the invention.

In one embodiment the construct comprises the polynucleotide sequence operably linked to a heterologous promoter.

Cells

In a further embodiment the invention provides a cell comprising a polynucleotide of the invention.

Preferably the cell is transgenic for the polynucleotide. Preferably the transgenic cell, is transformed to comprise the polynucleotide of the invention. Alternatively, a predecessor of the cell has been transformed to comprise the polynucleotide, and the cell is an off-spring of the predecessor cell and has inheritied the polynucleotide that was transformed into the predecessor cell.

In a further embodiment the invention provides a cell comprising a genetic construct of the invention.

In a preferred embodiment the cell expresses the polynucleotide of the invention.

In a preferred embodiment the cell expresses the polypeptide of the invention.

In a preferred embodiment the cell is transformed or genetically modified to expresses the polynucleotide or polypeptide of the invention.

In one embodiment the cell is a plant cell.

Plant

In a further embodiment the invention provides a plant comprising a polynucleotide of the invention.

Preferably the plant is transgenic for the polynucleotide. Preferably the transgenic plant is transformed to comprise the polynucleotide of the invention. Alternatively, a predecessor of the plant has been transformed to comprise the polynucleotide, and the plant is an off-spring of the predecessor plant and has inheritied the polynucleotide that was transformed into the predecessor plant.

In a further embodiment the invention provides a plant comprising a genetic construct of the invention.

In a preferred embodiment the plant expresses the polynucleotide of the invention.

In a preferred embodiment the plant expresses the polypeptide of the invention.

In a preferred embodiment the plant is transformed or genetically modified to expresses the polynucleotide or polypeptide of the invention.

In one embodiment the plant comprises a plant cell of the invention.

In a further embodiment the plant has a dwarfing-associated phenotype as described above.

Plant parts

In a further embodiment the invention provides a part, propagule or progeny of a plant of the invention.

Preferably the part, propagule or progeny is transgenic for the polynucleotide. Preferably the transgenic part, propagule or progeny is transformed to comprise the polynucleotide of the invention. Alternatively, a predecessor of the plant (that provided the part, propagule or progeny) has been transformed to comprise the polynucleotide, and the part, propagule or progeny provided by an off-spring of the predecessor plant and has inheritied the polynucleotide that was transformed into the predecessor plant.

In a further embodiment the invention provides a part, propagule or progeny comprising a genetic construct of the invention.

In a preferred embodiment the part, propagule or progeny expresses the polynucleotide of the invention.

In a preferred embodiment the part, propagule or progeny expresses the polypeptide of the invention.

In a preferred embodiment the part, propagule or progeny is transformed or genetically modified to expresses the polynucleotide or polypeptide of the invention.

In one embodiment the part, propagule or progeny comprises a plant cell of the invention.

In one embodiment the plant cell, part, propagule or progeny can be rejgenrated into a plant with a dwarfing-associated phenotype as described above.

Marker assisted selection

In a further aspect the invention provides a method for identifying a plant with a genotype indicative of at least one dwarfing-associated phenotype, the method comprising testing a plant for at least one of:

a) altered expression of at least one ARF3 polypeptide,
b) altered expression of at least one ARF3 polynucleotide,
c) presence of a marker associated with altered expression of at least one ARF3 polypeptide,
d) presence of a marker associated with altered expression of at least one ARF3 polynucleotide,
e) presence of a marker associated with altered activity of at least one ARF3 polypeptide,

In one embodiment presence of any of a) to e) indicates that the plant has at least one dwarfing-associated phenotype.

In one embodiment dwarfing-associated phenotype is selected from those described above.

In one embodiment the altered expression is increased expression.

In one embodiment the marker associated with altered activity of at least one ARF3 polypeptide is presence of a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 (MdARF3).

In one embodiment, the hydrophobic amino acid residue is a Leucine residue.

Thus, in one embodiment, the invention the method inviolves identifying presence of a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 (MdARF3).

In a further embodiment the ARF3 polypeptide comprising comprises at least 70% identity to SEQ ID NO:2 (MdARF3).

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO:2 (M9 MdARF3).

Alternatively, the method involves detection of a polynucleotide encoding the Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 (MdARF3).

In a further embodiment the method provides the additional step of cultivating the identified plant.

In a further embodiment the method provides the additional step of breeding from the identified plant.

Methods for breeding plants with at least one dwarfing associated phenotype

In a further aspect the invention provides a method for producing a plant with at least one dwarfing-associated phenotype, the method comprising crossing one of:

a) a plant of the invention,
b) a plant produced by a method of the invention, and
c) a plant selected by a method of the invention,

with another plant, wherein the off-spring produced by the crossing is a plant with at least one dwarfing-associated phenotype.

In one embodiment dwarfing-associated phenotype is selected from those described above.

Method using plant of the invention

In a further embodiment the invention provides a method of producing a plant with at least one dwarfing-associated phenotype selected from:

a) altered auxin transport,

b) slower auxin transport,
c) reduced apical dominance,
d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,
j) reduced vigour,
k) less vegetative growth,
l) earlier termination of shoot growth,
m) earlier competence to flower,
n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length,
y) reduced scion mass,

the method comprising grafting a scion onto a plant of the invention, a plant produced by a method of the invnetion, or a plant selected by a method of the invention.

In one embodiment the dwarfing-associated phenotype is at least one of a) to h) and j) to x).

In this embodiment the at least one dwarfing associated phenotype is preferably exhibited in the grafted scion.

In this embodiment the grafted scion preferably exhibits at least one of j) to y). Alternatively, the grafted scion preferably exhibits at least one of j) to x).

In a further embodiment the grafted scion preferably exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduced scion mass in a scion grafted on to the plant.

In a further embodiment the grafted scion preferably exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, and reduced stem circumference, in a scion grafted on to the plant.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods and materials useful for producing or selecting plants with at least one dwarfing associated phenotype.

The dwarfing-associated phenotype may be exhibited in the plant produced or selected, or may be exhibited in scions grafted onto the plants used as root stock, as indicated in Table 1 below.

Table 1.

Dwarfing-associated phenotypes found in dwarfing rootstock plants	**Dwarfing-associated phenotypes found in scions grafted onto dwarfing rootstock plants**
bushier	reduced vigour
altered auxin transport	less vegetative growth
altered xylem/phloem ratio	earlier termination of shoot growth
more phloem elements	earlier competency to flower
smaller phloem elements	precocity
thicker bark	earlier phase change
slower auxin transport	smaller canopy
reduced apical dominance	reduced stem circumference
reduced root mass	reduced branch diameter
	fewer sylleptic branches
	shorter sylleptic branches

	more axillary flowers
	earlier terminating primary axis
	earlier terminating secondary axes
	reduced branching density
	reduced internode length
	reduced scion mass

The dwarfing-associated phenotype may be selected from:

a) altered auxin transport,
b) slower auxin transport,
c) reduced apical dominance,
d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,
j) reduced vigour,
k) less vegetative growth,
l) earlier termination of shoot growth,
m) earlier competence to flower,
n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length, and
y) reduced scion mass.

In one embodiment the plant exhibits at least 2, more preferably at least 3, more preferably at least 4, more preferably at least 5, more preferably at least 6, more

preferably at least 7, more preferably at least 8, more preferably at least 9, more preferably at least 10, more preferably at least 11, more preferably at least 12, more preferably at least 13, more preferably at least 14, more preferably at least 15, more preferably at least 16, more preferably at least 17, more preferably at least 18, more preferably at least 19, more preferably at least 20, more preferably at least 21, more preferably at least 22, more preferably all 23 of dwarfing associated phenotypes a) to w).

In a further embodiment the plant exhibits at least one of dwarfing associated phenotypes selected from a) to i). In one embodiment the plant exhibits at least 2, more preferably at least 3, more preferably at least 4, more preferably at least 5, more preferably at least 6, more preferably at least 7, more preferably at least 8, of dwarfing associated phenotypes a) to i). In one embodiment this a plant is suitable for use as a root stock.

The dwarfing-associated phenotype may also be the capacity to induce at least one of a) to y) in a scion grafted onto the plant. In a further embodiment the dwarfing-associated phenotype may also be the capacity to induce at least one of a) to y) in a scion grafted onto the plant.

The dwarfing-associated phenotypes are relative terms. In one embodiment the dwarfing associated phenotype is relative to that of a control plant.

The control plant may be any plant of the same type that is not transformed with the polynucleotide, or construct, of the invention of the invention, or used in a method of the invnetion. The control plant may also be transformed with an "empty" vector, wherein the empty vector does not include an insert sequence corresponding to a polynucleotide of the invention or used in a method of the invention.

For the selection methods the control plant may be a non-selected plant.

The phrases "altered auxin transport" and "slower auxin transport" means that auxin transport in the plant of the invention, or in a method of the invention, is altered or slower relative to that in a contol plant. Auxin transport may be measured by methods known to those skilled in the art and explified for example in (Ulmasov, Murfett et al. 1997; Ljung, Hull et al. 2005)

The phrase "apical dominance" is the phenomenon whereby the primary shoot axis suppresses outgrowth of axillary brances. Apical dominance may be assessed by methods known to those skilled in the art for example (Napoli, Beveridge et al. 1999; Shimizu-Sato and Mori 2001; Sussex and Kerk 2001; Bennett, Sieberer et al. 2006)

The phrases "an altered xylem/phloem ratio", "an increased number of phloem elements" and "smaller phloem elements" are known to those skilled in the art, and may be assessed microscopically, as described in the present Examples section (Ruzin 1999).

The phrase "thicker bark" is intended to take the standard meaning, known to those skilled in the art. Thickness of bark can be assessed by taking transverse sections, using hisological stains such as safranin/fast green to distinguish xylem from phleom and observing under a microscope (Ruzin 1999).

Bushiness of habit is a term well understood and easily assessed visually by those skilled in the art.

The phrase "reduced vigour" means a reduction in the number of metamers intintiated by extension growth units, resulting in fewer branches, shorter branches and shorter main axis (Costes and Guedon 2002; Seleznyova, Thorp et al. 2003).

The pharase "metamer" means the repeating unit of leaf, axillary meristem, node, and internode (Steeves and Sussex 1989).

The phrase "extension growth unit" means a vegetative shoot with internode expansion (Seleznyova, Thorp et al. 2003).

The phrase "less vegetative growth" means a higher proportion of floral buds relative to vegetative shoots.

The phrase "earlier termination of shoot growth" means a vegetative extension shoot that stops initiating new metamers earlier in the season, resulting in a shorter shoot (Böhlenius, Huang et al. 2006; Hsu, Adams et al. 2011).

The phrase "earlier competence to flower" means the ability of the plant to respond to flowering cues and begin floral development (Hsu, Liu et al. 2006).

The phrase "precocity" means a reduced period in which a plant is unable to begin floral development (Imamura, Nakatsuka et al. 2011).

The phrase "earlier phase change" means the same as "precocious", a plant that is able to respond to floral cues and begin floral development before others of the same age (Huijser and Schmid 2011; Willmann and Poethig 2011).

The phrase "smaller canopy " is a phrase well understood and easily assessed by those skilled in the art.

The phrase "stem circumference" can be easily assessed by those skilled in the art. Measurement of stem circumference can be replaced by measurement of " Trunk Cross-sectional Area " (TCA). TCA of a grafted scion is generally measured 20cm above the graft union for grafted trees. For non-tree plants the primary stem is measured in place of the trunk.

"Branch diameter" is a term well understood and easily assessed by those skilled in the art.

The term "sylleptic branches" means a vegetative bud that grows out without a dormancy period, i.e. in the same season it was initiated (Costes and Guedon 1997).

Number and length of sylleptic branches can be easily assessed by those skilled in the art.

The term "axillary flowers" means flowers that are flowers that form directly from an axillary meristem, as opposed to a "fruiting spur"(Fulford 1966).

The term "fruiting spur" means a very short shoot with very condensed internodes that terminates in a bud containing several leaves and an inflorescence"(Fulford 1966).

The phrase "an earlier teminating primary axis means a tree with a shorter primary axis, comprised of fewer nodes.

The phrase "earlier teminating secondary axes" means shorter branches comprised of fewer nodes.

The term "internode" is intended to take its standard meaning. Internode length can be easily assessed by those skilled in the art (Steeves and Sussex 1989).

Cells

In one embodiment the cell is a prokaryotic cell.

In a further embodiment the cell is a eukaryotic cell.

In one embodiment the cell is selected from a bacterial cell, a yeast cell, a fungal cell, an insect cell, algal cell, and a plant cell. In one embodiment the cell is a bacterial cell. In a further embodiment the cell is a yeast cell. In one embodiment the yeast cell is a *S. ceriviseae* cell. In further embodiment the cell is a fungal cell. In further embodiment the cell is an insect cell. In further embodiment the cell is an algal cell.

In a preferred embodiment the cell is a plant cell.

Plants

Plants or plant cells or the invention, or used in the methods of the invnetion, or used to source naturally occurring ARF3 sequences, may be from any species.

In one embodiment the plant cell or plant, is or is derived from a gymnosperm plant species.

In a further embodiment the plant cell or plant, is or is derived from an angiosperm plant species.

In a further embodiment the plant cell or plant, is or is derived from a from dicotyledonous plant species.

In a further embodiment the plant cell or plant, is or is derived from a monocotyledonous plant species.

Preferered plants in which to introduce dwafrfing associated pheotypes include those from any species that produces fruit.

Preferered plants from which to source naturally occurring ARF3 sequences include those from any species that produces fruit.

Preferred fruit producing plants include apple, avocado, pear, peach, cherry, plum, kiwifruit, grape, mango, and orange plants.

A preferred apple genus is *Malus*.

Preferred apple species include: *Malus angustifolia, Malus asiatica, Malus baccata, Malus coronaria, Malus doumeri, Malus florentina, Malus floribunda, Malus fusca, Malus halliana, Malus honanensis, Malus hupehensis, Malus ioensis, Malus kansuensis, Malus mandshurica, Malus micromalus, Malus niedzwetzkyana, Malus ombrophilia, Malus orientalis, Malus prattii, Malus prunifolia, Malus pumila, Malus sargentii, Malus sieboldii, Malus sieversii, Malus sylvestris, Malus toringoides, Malus transitoria, Malus trilobata, Malus tschonoskii, Malus x domestica, Malus x domestica x Malus sieversii, Malus x domestica x Pyrus communis, Malus xiaojinensis,* and *Malus yunnanensis*.

A particularly preferred apple species is M*alus x domestica*.

A preferred pear genus is *Pyrus*.

Preferred pear species include: *Pyrus calleryana, Pyrus caucasica, Pyrus communis, Pyrus elaeagrifolia, Pyrus hybrid cultivar, Pyrus pyrifolia, Pyrus salicifolia, Pyrus ussuriensis* and *Pyrus x bretschneideri.*

A particularly preferred pear species are *Pyrus communis* and Asian pear *Pyrus x bretschneideri*.

A preferred avocado genus is *Persea.*

Preferred avacado species include *Persea americana* and *Persea gratissima.*

A preferred peach genus is *Prunus.*

Preferred peach species include: *Prunus africana, Prunus apetala, Prunus arborea, Prunus armeniaca, Prunus avium, Prunus bifrons, Prunus buergeriana, Prunus*

campanulata, Prunus canescens, Prunus cerasifera, Prunus cerasoides, Prunus cerasus, Prunus ceylanica, Prunus cocomilia, Prunus cornuta, Prunus crassifolia, Prunus davidiana, Prunus domestica, Prunus dulcis, Prunus fruticosa, Prunus geniculata, Prunus glandulosa, Prunus gracilis, Prunus grayana, Prunus incana, Prunus incisa, Prunus jacquemontii, Prunus japonica, Prunus korshinskyi, Prunus kotschyi, Prunus laurocerasus, Prunus laxinervis, Prunus lusitanica, Prunus maackii, Prunus mahaleb, Prunus mandshurica, Prunus maximowiczii, Prunus minutiflora, Prunus mume, Prunus murrayana, Prunus myrtifolia, Prunus nipponica, Prunus occidentalis, Prunus padus, Prunus persica, Prunus pleuradenia, Prunus pseudocerasus, Prunus prostrata, Prunus salicina, Prunus sargentii, Prunus scoparia, Prunus serrula, Prunus serrulata, Prunus sibirica, Prunus simonii, Prunus sogdiana, Prunus speciosa, Prunus spinosa, Prunus spinulosa, Prunus ssiori, Prunus subhirtella, Prunus tenella, Prunus tomentosa, Prunus triloba, Prunus turneriana, Prunus ursina, Prunus vachuschtii, Prunus verecunda, Prunus × yedoensis, Prunus zippeliana, Prunus alabamensis, Prunus alleghaniensis, Prunus americana, Prunus andersonii, Prunus angustifolia, Prunus brigantina, Prunus buxifolia, Prunus caroliniana, Prunus cuthbertii, Prunus emarginata, Prunus eremophila, Prunus fasciculata, Prunus fremontii, Prunus geniculata, Prunus gentryi, Prunus havardii, Prunus hortulana, Prunus huantensis, Prunus ilicifolia, Prunus integrifolia, Prunus maritima, Prunus mexicana, Prunus munsoniana, Prunus nigra, Prunus pensylvanica, Prunus pumila, Prunus rigida, Prunus rivularis, Prunus serotina, Prunus sphaerocarpa, Prunus subcordata, Prunus texana, Prunus umbellate and *Prunus virginiana.*

A particularly preferred peach species is *Prunus persica.*

A preferred kiwifruit genus is *Actinidia*.

Preferred kiwifruit species include: *Actinidia arguta, Actinidia arisanensis, Actinidia callosa, Actinidia carnosifolia*, *Actinidia chengkouensis*, *Actinidia chinensis*, *Actinidia chrysantha*, *Actinidia cinerascens*, *Actinidia cordifolia*, *Actinidia coriacea*, *Actinidia cylindrica*, *Actinidia deliciosa*, *Actinidia eriantha*, *Actinidia farinosa*, *Actinidia fasciculoides*, *Actinidia fortunatii*, *Actinidia foveolata*, *Actinidia fulvicoma*, *Actinidia glauco-callosa-callosa*, *Actinidia glaucophylla*, *Actinidia globosa*, *Actinidia gracilis*, *Actinidia grandiflora*, *Actinidia hemsleyana*, *Actinidia henryi*, *Actinidia holotricha*, *Actinidia hubeiensis*, *Actinidia indochinensis*, *Actinidia kolomikta*, *Actinidia laevissima*, *Actinidia lanceolata*, *Actinidia latifolia*, *Actinidia leptophylla*, *Actinidia liangguangensis*, *Actinidia lijiangensis*, *Actinidia linguiensis*, *Actinidia longicarpa*, *Actinidia macrosperma*,

Actinidia maloides, *Actinidia melanandra*, *Actinidia melliana*, *Actinidia obovata*, *Actinidia oregonensis*, *Actinidia persicina*, *Actinidia pilosula*, *Actinidia polygama*, *Actinidia purpurea*, *Actinidia rongshuiensis*, *Actinidia rubricaulis*, *Actinidia rubus*, *Actinidia rudis*, *Actinidia rufa*, *Actinidia rufotricha*, *Actinidia sabiaefolia*, *Actinidia sorbifolia*, *Actinidia stellato-pilosa-pilosa*, *Actinidia styracifolia*, *Actinidia suberifolia*, *Actinidia tetramera*, *Actinidia trichogyna*, *Actinidia ulmifolia*, *Actinidia umbelloides*, *Actinidia valvata*, *Actinidia venosa*, *Actinidia vitifolia* and *Actinidia zhejiangensis*.

Particularly preferred kiwifruit species are *Actinidia arguta, Actinidia chinensis* and *Actinidia deliciosa.*

A preferred orange genus is *Citrus*.

Preferred orange species include: *Citrus aurantiifolia, Citrus crenatifolia, Citrus maxima, Citrus medica, Citrus reticulata, Citrus trifoliata, Australian limes Citrus australasica, Citrus australis, Citrus glauca, Citrus garrawayae, Citrus gracilis, Citrus inodora, Citrus warburgiana, Citrus wintersii, Citrus japonica, Citrus indica* and *Citrus ×sinensis*.

Particularly preferred orange species are: *Citrus maxima, Citrus reticulate, Citrus* × *sinensis.*

A preferred grape genus is *Vitis*.

Preferred grape species include: *Vitis vinifera, Vitis labrusca, Vitis riparia, Vitis aestivalis, Vitis rotundifolia, Vitis rupestris, Vitis coignetiae, Vitis amurensis, Vitis vulpine.*

A particularly preferred grape species is *Vitis vinifera.*

A preferred avocado genus is *Persea.*

Preferred avacado species include *Persea americana* and *Persea gratissima.*

A preferred mango genus is *Mangifera*.

Preferred mango species include: *Mangifera foetida* and *Mangifera indica*.

A particularly preferred grape species is *Mangifera indica*.

A preferred plum genus is *Prunus*.

Preferred plum species include: *P. cerasifera, P. cocomilia, P. consociiflora, P. domestica, P. domestica ssp. insititia, P. simonii, P. spinosa, P. alleghaniensis, P. americana, P. angustifolia, P. hortulana, P. maritima, P. mexicana, P. nigra,* and *P. subcordata.*

A particularly preferred plum species is *Prunus domestica.*

Plant parts, propagues and progeny

The term "plant part" or grammatical equivalents thereof is intended to include any part of a plant, a tissue, an organ, a seed, a fruit, propagules and progeny of a plant.

The term 'propagule' means any part of a plant that may be used in reproduction or propagation, either sexual or asexual, including seeds and cuttings.

The plants of the invention may be grown and either self-ed or crossed with a different plant strain and the resulting progeny, comprising the polynucleotides or constructs of the invention, and/or expressing the ARF3 sequences of the invention, also form an part of the present invention.

Preferably the plants, plant parts, propagules and progeny comprise a polynucleotide or construct of the invention, and/or express a ARF3 sequence of the invention.

Marker assisted selection

Marker assisted selection (MAS) is an approach that is often used to identify plants that possess a particular trait using a genetic marker, or markers, associated with that trait. MAS may allow breeders to identify and select plants at a young age and is particularly valuable for fruit traits that are hard to measure at a young stage. The best markers for MAS are the causal mutations, but where these are not available, a marker that is in strong linkage disequilibrium with the causal mutation can also be used. Such information can be used to accelerate genetic gain, or reduce trait measurement costs, and thereby has utility in commercial breeding programs.

Methods for marker assisted selection are well known to those skilled in the art, for example: (Collard, B.C.Y. and D.J. Mackill, *Marker-assisted selection: an approach for*

precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B-Biological Sciences, 2008. 363(1491): p. 557-572.)

Markers

Markers for use in the methods of the invention may include nucleic acid markers, such as single nucleotide polymorphisms (SNPs), simple sequence repeats (SSRs or microsatellites), insertions, substitutions, indels and deletions.

Preferably the marker is in linkage disequilibrium (LD) with the trait.

Preferably the marker is in LD with the trait at a D' value of at least 0.1, more preferably at least 0.2, more preferably at least 0.3, more preferably at least 0.4, more preferably at least 0.5.

Preferably the marker is in LD with the trait at a R^2 value of at least 0.05, more preferably at least 0.075, more preferably at least 0.1, more preferably at least 0.2, more preferably at least 0.3, more preferably at least 0.4, more preferably at least 0.5.

The term "linkage disequilibrium" or LD as used herein, refers to a derived statistical measure of the strength of the association or co-occurrence of two independent genetic markers. Various statistical methods can be used to summarize linkage disequilibrium (LD) between two markers but in practice only two, termed D' and R^2, are widely used.

Markers linked, and or in LD, with the trait may be of any type including but not limited to, SNPs, substitutions, insertions, deletions, indels, simple sequence repeats (SSRs).

In the present invention, markers are associated with

a) altered expression of at least one ARF3 polypeptide,
b) altered expression of at least one ARF3 polynucleotide,
c) altered activity of at least one ARF3 polypeptide,

One marker associated with altered activity of at least one ARF3 polypeptide identified by the applicant is the presence of a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 (MdARF3).

In one embodiment, the hydrophobic amino acid residue is a Leucine residue.

Thus, in one embodiment, the invention the method involves identifying presence of a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 (MdARF3).

A further marker associated with altered activity of at least one ARF3 polypeptide identified by the applicant is the presence of a codon encoding the Leucine residue.

In one embodiment the codon is found at a position corresponding to nucleotides 214 to 216 in the ARF3 polynucleotide of SEQ ID NO:12.

In one embodiment the codon is selected from: TTA, TTG, CTT, CTC, CTA and CTG.

In a preferred embodiment the codon is TTG.

Thus in a preferred embodiment, the marker is a T nucleotide at a position corresponding to nucleotide 215 in the ARF3 polynucleotide of SEQ ID NO:12.

This marker defines the M9 allele of ARF3.

Other markers linked to the M9 allele of ARF3.

It would be most desirable to identify the presence of the M9 allele of ARF3 discussed above when selecting for at least one dwarfing associated phenotype. However, following the applicants present disclosure, those skilled in the art would know that it would also be possible to select for at least one dwarfing associated phenotype by identifying the presence of a marker linked to the M9 allele of ARF3. Selection methods utilising such linked markers also form part of the present invention. Methods for identify such linked markers are known to those skilled in the art.

Two other preferred markers for use in the marker assisted selection methods of the invention are Hi01c04 and Hi04a08.

The applicants have now shown that these are the closest markers defining the *Dw1* QTL interval.

Hi01c04

Hi01c04 is an SSR marker. Suitable primers for amplifying the Hi01c04 marker (and hybridising to the flanking sequences) are shown below.

Hi01c04 foward primer:

5'-GCTGCCGTTGACGTTAGAG-3'

Hi01c04 reverse primer:

5'- GTTTGTAGAAGTGGCGTTTGAGG -3'

The variable region between the flanking sequences is defined by the formula $(CTC)_n$

The whole sequence of the Hi01c04 is shown in SEQ ID NO:26

Hi04a08

Hi04a08 is also an SSR marker. Suitable primers for amplifying the Hi04a08 marker (and hybridising to the flanking sequences) are shown below.

Hi04a08 foward primer:

5'- TTGAAGGAGTTTCCGGTTTG -3'

Hi04a08 reverse primer:

5'- GTTTCACTCTGTGCTGGATTATGC -3'

The variable region between the flanking sequences is defined by the formula $(CTC)_n$

The whole sequence of the Hi04a08 is shown in SEQ ID NO:27

Methods for modifying endogenous polynucleotides

Some embodiments of the invention involve modifying and endogenous polynucleotide to induce a dwarfing associated phenotype in a plant, or scion grafted onto the plant.

Methods for modifying endogenous genomic DNA sequences in plants are known to those skilled in the art. Such methods may involve the use of sequence-specific nucleases that generate targeted double-stranded DNA breaks in genes of interest. Examples of such methods for use in plants include: zinc finger nucleases (Curtin et

al., 2011. Plant Physiol. 156:466–473. ; Sander, et al., 2011. Nat. Methods 8:67–69.), transcription activator-like effector nucleases or "TALENs" (Cermak *et al.*, 2011, Nucleic Acids Res. 39:e82 ; Mahfouz *et al.*, 2011 Proc. Natl. Acad. Sci. USA 108:2623–2628 ; Li *et al.*, 2012 Nat. Biotechnol. 30:390–392), and LAGLIDADG homing endonucleases, also termed "meganucleases" (Tzfira *et al.*, 2012. Plant Biotechnol. J. 10:373–389).

Targeted genome editing using engineered nucleases such as clustered, regularly interspaced, short palindromic repeat (CRISPR) technology, is an important new approach for generating RNA-guided nucleases, such as Cas9, with customizable specificities. Genome editing mediated by these nucleases has been used to rapidly, easily and efficiently modify endogenous genes in a wide variety of biomedically important cell types and in organisms that have traditionally been challenging to manipulate genetically. A modified version of the CRISPR-Cas9 system has been developed to recruit heterologous domains that can regulate endogenous gene expression or label specific genomic loci in living cells (Nature Biotechnology 32, 347–355 (2014). The system is applicable to plants, and can be used to regulate expression of target genes. (Bortesi and Fischer, Biotechnology Advances Volume 33, Issue 1, January–February 2015, Pages 41–52).

Those skilled in the art will thus appreciate that there are numerous ways in which the expression or activity of MdARF3 can be reduced or eliminated. Any such method is modified within the scope of the invention.

In certain embodiments of the invention, a genome editing technology (e.g. TALENs, a Zinc finger nuclease or CRISPR-Cas9 technology) can be used to modify one or more base pairs in a target ARF3 gene to create a codon encoding a hydrophobic amino acid, such as a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 (MdARF3). This approach effectively creates an M9 type ARF3 allele in the target plant.

Polynucleotides and fragments

The term "polynucleotide(s)," as used herein, means a single or double-stranded deoxyribonucleotide or ribonucleotide polymer of any length but preferably at least 15 nucleotides, and include as non-limiting examples, coding and non-coding sequences of a gene, sense and antisense sequences complements, exons, introns, genomic DNA, cDNA, pre-mRNA, mRNA, rRNA, siRNA, miRNA, tRNA, ribozymes, recombinant

polypeptides, isolated and purified naturally occurring DNA or RNA sequences, synthetic RNA and DNA sequences, nucleic acid probes, primers and fragments.

A "fragment" of a polynucleotide sequence provided herein is a subsequence of contiguous nucleotides.

The term "primer" refers to a short polynucleotide, usually having a free 3'OH group, that is hybridized to a template and used for priming polymerization of a polynucleotide complementary to the target. The primer may consist of a "fragment" of a polynucleotide as defined herein.

The term "probe" refers to a short polynucleotide that is used to detect a polynucleotide sequence that is complementary to the probe, in a hybridization-based assay. The probe may consist of a "fragment" of a polynucleotide as defined herein.

Polypeptides and fragments

The term "polypeptide", as used herein, encompasses amino acid chains of any length but preferably at least 5 amino acids, including full-length proteins, in which amino acid residues are linked by covalent peptide bonds. Polypeptides of the present invention, or used in the methods of the invention, may be purified natural products, or may be produced partially or wholly using recombinant or synthetic techniques.

A "fragment" of a polypeptide is a subsequence of the polypeptide that in some embodiments performs a function/activity of and/or influences three dimensional structure of the polypeptide.

The term "isolated" as applied to the polynucleotide or polypeptide sequences disclosed herein is used to refer to sequences that are removed from their natural cellular environment. The isolated sequence is preferably separated from the sequences that may be found flanking the sequence in its naturally occurring environment. An isolated molecule may be obtained by any method or combination of methods including biochemical, recombinant, and synthetic techniques.

The term "recombinant" refers to a polynucleotide sequence that is removed from sequences that surround it in its natural context and/or is recombined with sequences that are not present in its natural context.

A "recombinant" polypeptide sequence is produced by translation from a "recombinant" polynucleotide sequence.

The term "derived from" with respect to polynucleotides or polypeptides of the invention being derived from a particular genera or species, means that the polynucleotide or polypeptide has the same sequence as a polynucleotide or polypeptide found naturally in that genera or species. The polynucleotide or polypeptide, derived from a particular genera or species, may therefore be produced synthetically or recombinantly.

Variants

As used herein, the term "variant" refers to polynucleotide or polypeptide sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variants may be from the same or from other species and may encompass homologues, paralogues and orthologues. In certain embodiments, variants of the inventive polypeptides and polypeptides possess biological activities that are the same or similar to those of the inventive polypeptides or polypeptides. The term "variant" with reference to polypeptides and polypeptides encompasses all forms of polypeptides and polypeptides as defined herein.

Polynucleotide variants

Variant polynucleotide sequences preferably exhibit at least 50%, more preferably at least 51%, more preferably at least 52%, more preferably at least 53%, more preferably at least 54%, more preferably at least 55%, more preferably at least 56%, more preferably at least 57%, more preferably at least 58%, more preferably at least 59%, more preferably at least 60%, more preferably at least 61%, more preferably at least 62%, more preferably at least 63%, more preferably at least 64%, more preferably at least 65%, more preferably at least 66%, more preferably at least 67%, more preferably at least 68%, more preferably at least 69%, more preferably at least 70%, more preferably at least 71%, more preferably at least 72%, more preferably at least 73%, more preferably at least 74%, more preferably at least 75%, more preferably at least 76%, more preferably at least 77%, more preferably at least 78%, more preferably at least 79%, more preferably at least 80%, more preferably at least 81%, more preferably at least 82%, more preferably at least 83%, more preferably at least 84%, more preferably at least 85%, more preferably at least 86%, more preferably at least 87%, more preferably at least 88%, more preferably at least 89%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at

least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, and most preferably at least 99% identity to a sequence of the present invention. Identity is found over a comparison window of at least 20 nucleotide positions, preferably at least 50 nucleotide positions, more preferably at least 100 nucleotide positions, and most preferably over the entire length of a polynucleotide of the invention.

Polynucleotide sequence identity can be determined in the following manner. The subject polynucleotide sequence is compared to a candidate polynucleotide sequence using BLASTN (from the BLAST suite of programs, version 2.2.5 [Nov 2002]) in bl2seq (Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250), which is publicly available from the NCBI website on the World Wide Web at ftp://ftp.ncbi.nih.gov/blast/. The default parameters of bl2seq are utilized except that filtering of low complexity parts should be turned off.

The identity of polynucleotide sequences may be examined using the following unix command line parameters:

bl2seq –i nucleotideseq1 –j nucleotideseq2 –F F –p blastn

The parameter –F F turns off filtering of low complexity sections. The parameter –p selects the appropriate algorithm for the pair of sequences. The bl2seq program reports sequence identity as both the number and percentage of identical nucleotides in a line "Identities = ".

Polynucleotide sequence identity may also be calculated over the entire length of the overlap between a candidate and subject polynucleotide sequences using global sequence alignment programs (e.g. Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453). A full implementation of the Needleman-Wunsch global alignment algorithm is found in the needle program in the EMBOSS package (Rice,P. Longden,I. and Bleasby,A. EMBOSS: The European Molecular Biology Open Software Suite, Trends in Genetics June 2000, vol 16, No 6. pp.276-277) which can be obtained from the World Wide Web at http://www.hgmp.mrc.ac.uk/Software/EMBOSS/. The European Bioinformatics Institute server also provides the facility to perform EMBOSS-needle global alignments between two sequences on line at http:/www.ebi.ac.uk/emboss/align/.

Alternatively the GAP program may be used which computes an optimal global alignment of two sequences without penalizing terminal gaps. GAP is described in the

following paper: Huang, X. (1994) On Global Sequence Alignment. Computer Applications in the Biosciences 10, 227-235.

A preferred method for calculating polynucleotide % sequence identity is based on aligning sequences to be compared using Clustal X (Jeanmougin *et al.*, 1998, Trends Biochem. Sci. 23, 403-5.)

Polynucleotide variants of the present invention also encompass those which exhibit a similarity to one or more of the specifically identified sequences that is likely to preserve the functional equivalence of those sequences and which could not reasonably be expected to have occurred by random chance. Such sequence similarity with respect to polypeptides may be determined using the publicly available bl2seq program from the BLAST suite of programs (version 2.2.5 [Nov 2002]) from the NCBI website on the World Wide Web at ftp://ftp.ncbi.nih.gov/blast/.

The similarity of polynucleotide sequences may be examined using the following unix command line parameters:

bl2seq –i nucleotideseq1 –j nucleotideseq2 –F F –p tblastx

The parameter –F F turns off filtering of low complexity sections. The parameter –p selects the appropriate algorithm for the pair of sequences. This program finds regions of similarity between the sequences and for each such region reports an "E value" which is the expected number of times one could expect to see such a match by chance in a database of a fixed reference size containing random sequences. The size of this database is set by default in the bl2seq program. For small E values, much less than one, the E value is approximately the probability of such a random match.

Variant polynucleotide sequences preferably exhibit an E value of less than 1 x 10 -6 more preferably less than 1 x 10 -9, more preferably less than 1 x 10 -12, more preferably less than 1 x 10 -15, more preferably less than 1 x 10 -18, more preferably less than 1 x 10 -21, more preferably less than 1 x 10 -30, more preferably less than 1 x 10 -40, more preferably less than 1 x 10 -50, more preferably less than 1 x 10 -60, more preferably less than 1 x 10 -70, more preferably less than 1 x 10 -80, more preferably less than 1 x 10 -90 and most preferably less than 1 x 10-100 when compared with any one of the specifically identified sequences.

Alternatively, variant polynucleotides of the present invention, or used in the methods of the invention, hybridize to the specified polynucleotide sequences, or complements thereof under stringent conditions.

The term "hybridize under stringent conditions", and grammatical equivalents thereof, refers to the ability of a polynucleotide molecule to hybridize to a target polynucleotide molecule (such as a target polynucleotide molecule immobilized on a DNA or RNA blot, such as a Southern blot or Northern blot) under defined conditions of temperature and salt concentration. The ability to hybridize under stringent hybridization conditions can be determined by initially hybridizing under less stringent conditions then increasing the stringency to the desired stringency.

With respect to polynucleotide molecules greater than about 100 bases in length, typical stringent hybridization conditions are no more than 25 to 30° C (for example, 10° C) below the melting temperature (Tm) of the native duplex (see generally, Sambrook *et al.,* Eds, 1987, Molecular Cloning, A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press; Ausubel *et al.*, 1987, Current Protocols in Molecular Biology, Greene Publishing,). Tm for polynucleotide molecules greater than about 100 bases can be calculated by the formula Tm = 81. 5 + 0. 41% (G + C-log (Na+). (Sambrook *et al.,* Eds, 1987, Molecular Cloning, A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press; Bolton and McCarthy, 1962, PNAS 84:1390). Typical stringent conditions for polynucleotide of greater than 100 bases in length would be hybridization conditions such as prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65° C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

With respect to polynucleotide molecules having a length less than 100 bases, exemplary stringent hybridization conditions are 5 to 10° C below Tm. On average, the Tm of a polynucleotide molecule of length less than 100 bp is reduced by approximately (500/oligonucleotide length)° C.

With respect to the DNA mimics known as peptide nucleic acids (PNAs) (Nielsen *et al.*, Science. 1991 Dec 6;254(5037):1497-500) Tm values are higher than those for DNA-DNA or DNA-RNA hybrids, and can be calculated using the formula described in Giesen *et al.*, Nucleic Acids Res. 1998 Nov 1;26(21):5004-6. Exemplary stringent hybridization conditions for a DNA-PNA hybrid having a length less than 100 bases are 5 to 10° C below the Tm.

Variant polynucleotides of the present invention, or used in the methods of the invention, also encompasses polynucleotides that differ from the sequences of the invention but that, as a consequence of the degeneracy of the genetic code, encode a polypeptide having similar activity to a polypeptide encoded by a polynucleotide of the present invention. A sequence alteration that does not change the amino acid

sequence of the polypeptide is a "silent variation". Except for ATG (methionine) and TGG (tryptophan), other codons for the same amino acid may be changed by art recognized techniques, e.g., to optimize codon expression in a particular host organism.

Polynucleotide sequence alterations resulting in conservative substitutions of one or several amino acids in the encoded polypeptide sequence without significantly altering its biological activity are also included in the invention. A skilled artisan will be aware of methods for making phenotypically silent amino acid substitutions (see, e.g., Bowie *et al.*, 1990, Science 247, 1306).

Variant polynucleotides due to silent variations and conservative substitutions in the encoded polypeptide sequence may be determined using the publicly available bl2seq program from the BLAST suite of programs (version 2.2.5 [Nov 2002]) from the NCBI website on the World Wide Web at ftp://ftp.ncbi.nih.gov/blast/ via the tblastx algorithm as previously described.

Polypeptide variants

The term "variant" with reference to polypeptides encompasses naturally occurring, recombinantly and synthetically produced polypeptides. Variant polypeptide sequences preferably exhibit at least 50%, more preferably at least 51%, more preferably at least 52%, more preferably at least 53%, more preferably at least 54%, more preferably at least 55%, more preferably at least 56%, more preferably at least 57%, more preferably at least 58%, more preferably at least 59%, more preferably at least 60%, more preferably at least 61%, more preferably at least 62%, more preferably at least 63%, more preferably at least 64%, more preferably at least 65%, more preferably at least 66%, more preferably at least 67%, more preferably at least 68%, more preferably at least 69%, more preferably at least 70%, more preferably at least 71%, more preferably at least 72%, more preferably at least 73%, more preferably at least 74%, more preferably at least 75%, more preferably at least 76%, more preferably at least 77%, more preferably at least 78%, more preferably at least 79%, more preferably at least 80%, more preferably at least 81%, more preferably at least 82%, more preferably at least 83%, more preferably at least 84%, more preferably at least 85%, more preferably at least 86%, more preferably at least 87%, more preferably at least 88%, more preferably at least 89%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%,

and most preferably at least 99% identity to a sequences of the present invention. Identity is found over a comparison window of at least 20 amino acid positions, preferably at least 50 amino acid positions, more preferably at least 100 amino acid positions, and most preferably over the entire length of a polypeptide of the invention.

Polypeptide sequence identity can be determined in the following manner. The subject polypeptide sequence is compared to a candidate polypeptide sequence using BLASTP (from the BLAST suite of programs, version 2.2.5 [Nov 2002]) in bl2seq, which is publicly available from the NCBI website on the World Wide Web at ftp://ftp.ncbi.nih.gov/blast/. The default parameters of bl2seq are utilized except that filtering of low complexity regions should be turned off.

Polypeptide sequence identity may also be calculated over the entire length of the overlap between a candidate and subject polynucleotide sequences using global sequence alignment programs. EMBOSS-needle (available at http:/www.ebi.ac.uk/emboss/align/) and GAP (Huang, X. (1994) On Global Sequence Alignment. Computer Applications in the Biosciences 10, 227-235.) as discussed above are also suitable global sequence alignment programs for calculating polypeptide sequence identity.

A preferred method for calculating polypeptide % sequence identity is based on aligning sequences to be compared using Clustal X (Jeanmougin *et al.*, 1998, Trends Biochem. Sci. 23, 403-5.)

Polypeptide variants of the present invention, or used in the methods of the invention, also encompass those which exhibit a similarity to one or more of the specifically identified sequences that is likely to preserve the functional equivalence of those sequences and which could not reasonably be expected to have occurred by random chance. Such sequence similarity with respect to polypeptides may be determined using the publicly available bl2seq program from the BLAST suite of programs (version 2.2.5 [Nov 2002]) from the NCBI website on the World Wide Web at ftp://ftp.ncbi.nih.gov/blast/. The similarity of polypeptide sequences may be examined using the following unix command line parameters:

bl2seq –i peptideseq1 –j peptideseq2 -F F –p blastp

Variant polypeptide sequences preferably exhibit an E value of less than 1 x 10 -6 more preferably less than 1 x 10 -9, more preferably less than 1 x 10 -12, more preferably less than 1 x 10 -15, more preferably less than 1 x 10 -18, more preferably less than 1 x 10 -21, more preferably less than 1 x 10 -30, more preferably less than

1 x 10 -40, more preferably less than 1 x 10 -50, more preferably less than 1 x 10 -60, more preferably less than 1 x 10 -70, more preferably less than 1 x 10 -80, more preferably less than 1 x 10 -90 and most preferably 1x10-100 when compared with any one of the specifically identified sequences.

The parameter –F F turns off filtering of low complexity sections. The parameter –p selects the appropriate algorithm for the pair of sequences. This program finds regions of similarity between the sequences and for each such region reports an "E value" which is the expected number of times one could expect to see such a match by chance in a database of a fixed reference size containing random sequences. For small E values, much less than one, this is approximately the probability of such a random match.

Conservative substitutions of one or several amino acids of a described polypeptide sequence without significantly altering its biological activity are also included in the invention. A skilled artisan will be aware of methods for making phenotypically silent amino acid substitutions (see, e.g., Bowie *et al.*, 1990, Science 247, 1306).

Constructs, vectors and components thereof

The term "genetic construct" refers to a polynucleotide molecule, usually double-stranded DNA, which may have inserted into it another polynucleotide molecule (the insert polynucleotide molecule) such as, but not limited to, a cDNA molecule. A genetic construct may contain the necessary elements that permit transcribing the insert polynucleotide molecule, and, optionally, translating the transcript into a polypeptide. The insert polynucleotide molecule may be derived from the host cell, or may be derived from a different cell or organism and/or may be a recombinant polynucleotide. Once inside the host cell the genetic construct may become integrated in the host chromosomal DNA. The genetic construct may be linked to a vector.

The term "vector" refers to a polynucleotide molecule, usually double stranded DNA, which is used to transport the genetic construct into a host cell. The vector may be capable of replication in at least one additional host system, such as *E. coli*.

The term "expression construct" refers to a genetic construct that includes the necessary elements that permit transcribing the insert polynucleotide molecule, and, optionally, translating the transcript into a polypeptide. An expression construct typically comprises in a 5′ to 3′ direction:

a) a promoter functional in the host cell into which the construct will be transformed,
b) the polynucleotide to be expressed, and
c) a terminator functional in the host cell into which the construct will be transformed.

The term "coding region" or "open reading frame" (ORF) refers to the sense strand of a genomic DNA sequence or a cDNA sequence that is capable of producing a transcription product and/or a polypeptide under the control of appropriate regulatory sequences. The coding sequence may, in some cases, identified by the presence of a 5′ translation start codon and a 3′ translation stop codon. When inserted into a genetic construct, a "coding sequence" is capable of being expressed when it is operably linked to promoter and terminator sequences.

"Operably-linked" means that the sequenced to be expressed is placed under the control of regulatory elements that include promoters, tissue-specific regulatory elements, temporal regulatory elements, enhancers, repressors and terminators.

The term "noncoding region" refers to untranslated sequences that are upstream of the translational start site and downstream of the translational stop site. These sequences are also referred to respectively as the 5′ UTR and the 3′ UTR. These regions include elements required for transcription initiation and termination, mRNA stability, and for regulation of translation efficiency.

Terminators are sequences, which terminate transcription, and are found in the 3′ untranslated ends of genes downstream of the translated sequence. Terminators are important determinants of mRNA stability and in some cases have been found to have spatial regulatory functions.

The term "promoter" refers to nontranscribed cis-regulatory elements upstream of the coding region that regulate gene transcription. Promoters comprise cis-initiator elements which specify the transcription initiation site and conserved boxes such as the TATA box, and motifs that are bound by transcription factors. Introns within coding sequences can also regulate transcription and influence post-transcriptional processing (including splicing, capping and polyadenylation).

A promoter may be homologous with respect to the polynucleotide to be expressed. This means that the promoter and polynucleotide are found operably linked in nature.

Alternatively the promoter may be heterologous with respect to the polynucleotide to be expressed. This means that the promoter and the polynucleotide are not found operably linked in nature.

In certain embodiments the ARF3 polynucleotides/polypeptides of the invention may be andvantageously expessed under the contol of selected promoter sequences as described below.

Vegetative tissue specific promoters

An example of a vegetative specific promoter is found in US 6,229,067; and US 7,629,454; and US 7,153,953; and US 6,228,643.

Pollen specific promoters

An example of a pollen specific promoter is found in US 7,141,424; and US 5,545,546; and US 5,412,085; and US 5,086,169; and US 7,667,097.

Seed specific promoters

An example of a seed specific promoter is found in US 6,342,657; and US 7,081,565; and US 7,405,345; and US 7,642,346; and US 7,371,928. A preferred seed specific promoter is the napin promoter of *Brassica napus* (Josefsson *et al*., 1987, J Biol Chem. 262(25):12196-201; Ellerström *et al*., 1996, Plant Molecular Biology, Volume 32, Issue 6, pp 1019-1027).

Fruit specific promoters

An example of a fruit specific promoter is found in US 5,536,653; and US 6,127,179; and US 5,608,150; and US 4,943,674.

Non-photosynthetic tissue preferred promoters

Non-photosynthetic tissue preferred promoters include those preferentially expressed in non-photosynthetic tissues/organs of the plant.

Non-photosynthetic tissue preferred promoters may also include light repressed promoters.

Light repressed promoters

An example of a light repressed promoter is found in US 5,639,952 and in US 5,656,496.

Root specific promoters

An example of a root specific promoter is found in US 5,837,848; and US 2004/0067506 and US 2001/0047525.

Tuber specific promoters

An example of a tuber specific promoter is found in US 6,184,443.

Bulb specific promoters

An example of a bulb specific promoter is found in Smeets *et al.*, (1997) Plant Physiol. 113:765-771.

Rhizome preferred promoters

An example of a rhizome preferred promoter is found Seong Jang *et al.*, (2006) Plant Physiol. 142:1148-1159.

Endosperm specific promoters

An example of an endosperm specific promoter is found in US 7,745,697.

Corm promoters

An example of a promoter capable of driving expression in a corm is found in Schenk *et al.*, (2001) Plant Molecular Biology, 47:399-412.

Photosythetic tissue preferred promoters

Photosythetic tissue preferred promoters include those that are preferrentially expressed in photosynthetic tissues of the plants. Photosynthetic tissues of the plant include leaves, stems, shoots and above ground parts of the plant. Photosythetic tissue preferred promoters include light regulated promoters.

Light regulated promoters

Numerous light regulated promoters are known to those skilled in the art and include for example chlorophyll a/b (Cab) binding protein promoters and Rubisco Small Subunit (SSU) promoters. An example of a light regulated promoter is found in US 5,750,385. Light regulated in this context means light inducible or light induced.

Transgene

A "transgene" is a polynucleotide that is introduced into an organism by transformation. The transgene may be derived from the same species or from a different species to the organism into which the transgene is introduced. In one embodiment the transgene is a naturally occurring sequence. In a further embodiment the transgene is a non-naturally occurring sequence. The transgene may be synthesized or produced by recombinant methods.

Host cells

Host cells may be derived from, for example, bacterial, fungal, yeast, insect, mammalian, algal or plant organisms. Host cells may also be synthetic cells. Preferred host cells are eukaryotic cells. A particularly preferred host cell is a plant cell, particularly a plant cell in a tissue of a plant.

A "transgenic plant" refers to a plant which contains new genetic material as a result of genetic manipulation or transformation. The new genetic material may be derived from a plant of the same species as the resulting transgenic plant or from a different species. Subsequent offspring or generations of the plant that still contain the new genetic material are also transgenic plants according to the invention.

Methods for isolating or producing polynucleotides

The polynucleotide molecules of the invention can be isolated by using a variety of techniques known to those of ordinary skill in the art. By way of example, such polypeptides can be isolated through use of the polymerase chain reaction (PCR) described in Mullis *et al.*, Eds. 1994 The Polymerase Chain Reaction, Birkhauser, incorporated herein by reference. The polypeptides of the invention can be amplified using primers, as defined herein, derived from the polynucleotide sequences of the invention.

Further methods for isolating polynucleotides of the invention include use of all, or portions of, the polypeptides having the sequence set forth herein as hybridization probes. The technique of hybridizing labelled polynucleotide probes to polynucleotides

immobilized on solid supports such as nitrocellulose filters or nylon membranes, can be used to screen the genomic or cDNA libraries. Exemplary hybridization and wash conditions are: hybridization for 20 hours at 65°C in 5. 0 X SSC, 0. 5% sodium dodecyl sulfate, 1 X Denhardt's solution; washing (three washes of twenty minutes each at 55°C) in 1. 0 X SSC, 1% (w/v) sodium dodecyl sulfate, and optionally one wash (for twenty minutes) in 0. 5 X SSC, 1% (w/v) sodium dodecyl sulfate, at 60°C. An optional further wash (for twenty minutes) can be conducted under conditions of 0.1 X SSC, 1% (w/v) sodium dodecyl sulfate, at 60°C.

The polynucleotide fragments of the invention may be produced by techniques well-known in the art such as restriction endonuclease digestion, oligonucleotide synthesis and PCR amplification.

A partial polynucleotide sequence may be used, in methods well-known in the art to identify the corresponding full length polynucleotide sequence. Such methods include PCR-based methods, 5'RACE (Frohman MA, 1993, Methods Enzymol. 218: 340-56) and hybridization- based method, computer/database –based methods. Further, by way of example, inverse PCR permits acquisition of unknown sequences, flanking the polynucleotide sequences disclosed herein, starting with primers based on a known region (Triglia *et al.*, 1998, Nucleic Acids Res 16, 8186, incorporated herein by reference). The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template. Divergent primers are designed from the known region. In order to physically assemble full-length clones, standard molecular biology approaches can be utilized (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987).

It may be beneficial, when producing a transgenic plant from a particular species, to transform such a plant with a sequence or sequences derived from that species. The benefit may be to alleviate public concerns regarding cross-species transformation in generating transgenic organisms. For these reasons among others, it is desirable to be able to identify and isolate orthologues of a particular gene in several different plant species.

Variants (including orthologues) may be identified by the methods described.

Methods for identifying variants

Physical methods

Variant polypeptides may be identified using PCR-based methods (Mullis *et al.*, Eds. 1994 The Polymerase Chain Reaction, Birkhauser). Typically, the polynucleotide sequence of a primer, useful to amplify variants of polynucleotide molecules of the invention by PCR, may be based on a sequence encoding a conserved region of the corresponding amino acid sequence.

Alternatively library screening methods, well known to those skilled in the art, may be employed (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987). When identifying variants of the probe sequence, hybridization and/or wash stringency will typically be reduced relatively to when exact sequence matches are sought.

Polypeptide variants may also be identified by physical methods, for example by screening expression libraries using antibodies raised against polypeptides of the invention (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987) or by identifying polypeptides from natural sources with the aid of such antibodies.

Computer based methods

The variant sequences of the invention, including both polynucleotide and polypeptide variants, may also be identified by computer-based methods well-known to those skilled in the art, using public domain sequence alignment algorithms and sequence similarity search tools to search sequence databases (public domain databases include Genbank, EMBL, Swiss-Prot, PIR and others). See, e.g., Nucleic Acids Res. 29: 1-10 and 11-16, 2001 for examples of online resources. Similarity searches retrieve and align target sequences for comparison with a sequence to be analyzed (i.e., a query sequence). Sequence comparison algorithms use scoring matrices to assign an overall score to each of the alignments.

An exemplary family of programs useful for identifying variants in sequence databases is the BLAST suite of programs (version 2.2.5 [Nov 2002]) including BLASTN, BLASTP, BLASTX, tBLASTN and tBLASTX, which are publicly available from (ftp://ftp.ncbi.nih.gov/blast/) or from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894 USA. The NCBI server also provides the facility to use the programs to screen a number of publicly available sequence databases. BLASTN compares a nucleotide query sequence against a nucleotide sequence database. BLASTP compares an amino acid query sequence against a protein sequence database.

BLASTX compares a nucleotide query sequence translated in all reading frames against a protein sequence database. tBLASTN compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames. tBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. The BLAST programs may be used with default parameters or the parameters may be altered as required to refine the screen.

The use of the BLAST family of algorithms, including BLASTN, BLASTP, and BLASTX, is described in the publication of Altschul *et al.*, Nucleic Acids Res. 25: 3389-3402, 1997.

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, BLASTX, tBLASTN, tBLASTX, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN, BLASTP, BLASTX, tBLASTN and tBLASTX algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see by chance when searching a database of the same size containing random contiguous sequences. The Expect value is used as a significance threshold for determining whether the hit to a database indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the database screened, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in that database is 1% or less using the BLASTN, BLASTP, BLASTX, tBLASTN or tBLASTX algorithm.

Multiple sequence alignments of a group of related sequences can be carried out with CLUSTALW (Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680, http://www-igbmc.u-strasbg.fr/BioInfo/ClustalW/Top.html) or T-COFFEE (Cedric Notredame, Desmond G. Higgins, Jaap Heringa, T-Coffee: A novel method for fast and accurate multiple sequence alignment, J. Mol. Biol. (2000) 302: 205-217)) or PILEUP, which uses progressive, pairwise alignments. (Feng and Doolittle, 1987, J. Mol. Evol. 25, 351).

Pattern recognition software applications are available for finding motifs or signature sequences. For example, MEME (Multiple Em for Motif Elicitation) finds motifs and signature sequences in a set of sequences, and MAST (Motif Alignment and Search Tool) uses these motifs to identify similar or the same motifs in query sequences. The MAST results are provided as a series of alignments with appropriate statistical data and a visual overview of the motifs found. MEME and MAST were developed at the University of California, San Diego.

PROSITE (Bairoch and Bucher, 1994, Nucleic Acids Res. 22, 3583; Hofmann *et al.*, 1999, Nucleic Acids Res. 27, 215) is a method of identifying the functions of uncharacterized proteins translated from genomic or cDNA sequences. The PROSITE database (www.expasy.org/prosite) contains biologically significant patterns and profiles and is designed so that it can be used with appropriate computational tools to assign a new sequence to a known family of proteins or to determine which known domain(s) are present in the sequence (Falquet *et al.*, 2002, Nucleic Acids Res. 30, 235). Prosearch is a tool that can search SWISS-PROT and EMBL databases with a given sequence pattern or signature.

Methods for isolating polypeptides

The polypeptides of the invention, or used in the methods of the invention, including variant polypeptides, may be prepared using peptide synthesis methods well known in the art such as direct peptide synthesis using solid phase techniques (e.g. Stewart *et al.*, 1969, in Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco California, or automated synthesis, for example using an Applied Biosystems 431A Peptide Synthesizer (Foster City, California). Mutated forms of the polypeptides may also be produced during such syntheses.

The polypeptides and variant polypeptides of the invention, or used in the methods of the invention, may also be purified from natural sources using a variety of techniques that are well known in the art (e.g. Deutscher, 1990, Ed, Methods in Enzymology, Vol. 182, Guide to Protein Purification,).

Alternatively the polypeptides and variant polypeptides of the invention, or used in the methods of the invention, may be expressed recombinantly in suitable host cells and separated from the cells as discussed below.

Methods for producing constructs and vectors

The genetic constructs of the present invention comprise one or more polynucleotide sequences of the invention and/or polynucleotides encoding polypeptides of the invention, and may be useful for transforming, for example, bacterial, fungal, insect, mammalian or plant organisms. The genetic constructs of the invention are intended to include expression constructs as herein defined.

Methods for producing and using genetic constructs and vectors are well known in the art and are described generally in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987 ; Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing, 1987).

Methods for producing host cells comprising polynucleotides, constructs or vectors

The invention provides a host cell which comprises a genetic construct or vector of the invention.

Host cells comprising genetic constructs, such as expression constructs, of the invention are useful in methods well known in the art (e.g. Sambrook *et al.*, Molecular Cloning : A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987 ; Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing, 1987) for recombinant production of polypeptides of the invention. Such methods may involve the culture of host cells in an appropriate medium in conditions suitable for or conducive to expression of a polypeptide of the invention. The expressed recombinant polypeptide, which may optionally be secreted into the culture, may then be separated from the medium, host cells or culture medium by methods well known in the art (e.g. Deutscher, Ed, 1990, Methods in Enzymology, Vol 182, Guide to Protein Purification).

Methods for producing plant cells and plants comprising constructs and vectors

The invention further provides plant cells which comprise a genetic construct of the invention, and plant cells modified to alter expression of a polynucleotide or polypeptide of the invention, or used in the methods of the invention. Plants comprising such cells also form an aspect of the invention.

Methods for transforming plant cells, plants and portions thereof with polypeptides are described in Draper *et al.*, 1988, Plant Genetic Transformation and Gene Expression. A Laboratory Manual. Blackwell Sci. Pub. Oxford, p. 365; Potrykus and Spangenburg, 1995, Gene Transfer to Plants. Springer-Verlag, Berlin.; and Gelvin *et al.*, 1993, Plant

Molecular Biol. Manual. Kluwer Acad. Pub. Dordrecht. A review of transgenic plants, including transformation techniques, is provided in Galun and Breiman, 1997, Transgenic Plants. Imperial College Press, London.

Methods for genetic manipulation of plants

A number of plant transformation strategies are available (e.g. Birch, 1997, Ann Rev Plant Phys Plant Mol Biol, 48, 297, Hellens *et al.*, (2000) Plant Mol Biol 42: 819-32, Hellens *et al.*, Plant Meth 1: 13). For example, strategies may be designed to increase expression of a polynucleotide/polypeptide in a plant cell, organ and/or at a particular developmental stage where/when it is normally expressed or to ectopically express a polynucleotide/polypeptide in a cell, tissue, organ and/or at a particular developmental stage which/when it is not normally expressed. The expressed polynucleotide/polypeptide may be derived from the plant species to be transformed or may be derived from a different plant species.

Transformation strategies may be designed to reduce expression of a polynucleotide/polypeptide in a plant cell, tissue, organ or at a particular developmental stage which/when it is normally expressed. Such strategies are known as gene silencing strategies.

Genetic constructs for expression of genes in transgenic plants typically include promoters for driving the expression of one or more cloned polynucleotide, terminators and selectable marker sequences to detect presence of the genetic construct in the transformed plant.

The promoters suitable for use in the constructs of this invention are functional in a cell, tissue or organ of a monocot or dicot plant and include cell-, tissue- and organ-specific promoters, cell cycle specific promoters, temporal promoters, inducible promoters, constitutive promoters that are active in most plant tissues, and recombinant promoters. Choice of promoter will depend upon the temporal and spatial expression of the cloned polynucleotide, so desired. The promoters may be those normally associated with a transgene of interest. In one embodiment the promoter is not normally associated with a transgene of interest. Such a promoter may be described as a heterologous promoter, with respect to the transgene.

The promoters may be derived from genes of other plants, viruses, and plant pathogenic bacteria and fungi. Those skilled in the art will, without undue experimentation, be able to select promoters that are suitable for use in modifying and modulating plant traits using genetic constructs comprising the polynucleotide

sequences of the invention. Examples of constitutive plant promoters include the CaMV 35S promoter, the nopaline synthase promoter and the octopine synthase promoter, and the Ubi 1 promoter from maize. Plant promoters which are active in specific tissues respond to internal developmental signals or external abiotic or biotic stresses are described in the scientific literature. Exemplary promoters are described, e.g., in WO 02/00894 and WO2011/053169, which is herein incorporated by reference.

Exemplary terminators that are commonly used in plant transformation genetic construct include, e.g., the cauliflower mosaic virus (CaMV) 35S terminator, the *Agrobacterium tumefaciens* nopaline synthase or octopine synthase terminators, the *Zea mays* zein gene terminator, the *Oryza sativa* ADP-glucose pyrophosphorylase terminator and the *Solanum tuberosum* PI-II terminator.

Selectable markers commonly used in plant transformation include the neomycin phophotransferase II gene (NPT II) which confers kanamycin resistance, the aadA gene, which confers spectinomycin and streptomycin resistance, the phosphinothricin acetyl transferase (*bar* gene) for Ignite (AgrEvo) and Basta (Hoechst) resistance, and the hygromycin phosphotransferase gene (hpt) for hygromycin resistance.

Use of genetic constructs comprising reporter genes (coding sequences which express an activity that is foreign to the host, usually an enzymatic activity and/or a visible signal (e.g., luciferase, GUS, GFP) which may be used for promoter expression analysis in plants and plant tissues are also contemplated. The reporter gene literature is reviewed in Herrera-Estrella *et al.*, 1993, Nature 303, 209, and Schrott, 1995, In: Gene Transfer to Plants (Potrykus, T., Spangenberg. Eds) Springer Verlag. Berline, pp. 325-336.

The following are representative publications disclosing genetic transformation protocols that can be used to genetically transform the following plant species: Rice (Alam *et al.*, 1999, Plant Cell Rep. 18, 572); apple (Yao *et al.*, 1995, Plant Cell Reports 14, 407-412); maize (US Patent Serial Nos. 5, 177, 010 and 5, 981, 840); wheat (Ortiz *et al.*, 1996, Plant Cell Rep. 15, 1996, 877); tomato (US Patent Serial No. 5, 159, 135); potato (Kumar *et al.*, 1996 Plant J. 9, : 821); cassava (Li *et al.*, 1996 Nat. Biotechnology 14, 736); lettuce (Michelmore *et al.*, 1987, Plant Cell Rep. 6, 439); tobacco (Horsch *et al.*, 1985, Science 227, 1229); cotton (US Patent Serial Nos. 5, 846, 797 and 5, 004, 863); grasses (US Patent Nos. 5, 187, 073 and 6. 020, 539); peppermint (Niu *et al.*, 1998, Plant Cell Rep. 17, 165); citrus plants (Pena *et al.*, 1995, Plant Sci.104, 183); caraway (Krens *et al.*, 1997, Plant Cell Rep, 17, 39); banana (US

Patent Serial No. 5, 792, 935); soybean (US Patent Nos. 5, 416, 011 ; 5, 569, 834 ; 5, 824, 877 ; 5, 563, 04455 and 5, 968, 830); pineapple (US Patent Serial No. 5, 952, 543); poplar (US Patent No. 4, 795, 855); monocots in general (US Patent Nos. 5, 591, 616 and 6, 037, 522); brassica (US Patent Nos. 5, 188, 958 ; 5, 463, 174 and 5, 750, 871); cereals (US Patent No. 6, 074, 877); pear (Matsuda *et al.*, 2005, Plant Cell Rep. 24(1):45-51); Prunus (Ramesh *et al.*, 2006 Plant Cell Rep. 25(8):821-8; Song and Sink 2005 Plant Cell Rep. 2006 ;25(2):117-23; Gonzalez Padilla *et al.*, 2003 Plant Cell Rep.22(1):38-45); strawberry (Oosumi *et al.*, 2006 Planta. 223(6):1219-30; Folta *et al.*, 2006 Planta Apr 14; PMID: 16614818), rose (Li *et al.*, 2003), Rubus (Graham *et al.*, 1995 Methods Mol Biol. 1995;44:129-33), tomato (Dan *et al.*, 2006, Plant Cell Reports V25:432-441), apple (Yao *et al.*, 1995, *Plant Cell Rep.* **14**, 407–412), Canola (Brassica napus L.).(Cardoza and Stewart, 2006 Methods Mol Biol. 343:257-66), safflower (Orlikowska *et al.*, 1995, Plant Cell Tissue and Organ Culture 40:85-91), ryegrass (Altpeter *et al.*, 2004 Developments in Plant Breeding 11(7):255-250), rice (Christou *et al.*, 1991 Nature Biotech. 9:957-962), maize (Wang *et al.*, 2009 In: Handbook of Maize pp. 609-639) and *Actinidia eriantha* (Wang *et al.*, 2006, Plant Cell Rep. 25,5: 425-31). Transformation of other species is also contemplated by the invention. Suitable methods and protocols are available in the scientific literature.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner. In some embodiments, the term "comprising" (and related terms such as "comprise and "comprises") can be replaced by "consisting of" (and related terms "consist" and "consists").

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows identification of the rootstock dwarfing loci, *Dw1*. a) Using a bulked segregant analysis, a major dwarfing locus (*Dw1*) from 'M9' was identified at the top

of linkage group (LG) 5. The markers flanking *Dw1* were NZraAM18-700 (developed by Plant & Food Research, not publically avaliable) and CH03a09 (publically available). b) A multi-trait QTL analysis identified *Dw1* as having a very strong influence on rootstock induced dwarfing. The markers flanking *Dw1* are Hi01c04a and CH03a09.

Figure 2 shows genetic markers flanking o *Dw1* according to the applicant, and that described by Fazio *et al*. a) Markers flanking our *Dw1* are shown in red and extend from 4.72 Mb to 7.62Mb. b) Markers flanking the Fazio *et al Dw1* are shown in green. The distal marker CH05b06z is not mapped. c) The proximal marker CH05b06z maps elsewhere, and the distal most maps incorrectly. d) The distal marker C3843 does not map to LG5. Based on the markers that do map, this would place the Fazio *et al Dw1* more distal than ours.

Figure 3 shows recombinant Dwarf & Semi-Dwarf individuals narrow the genomic interval containing *Dw1* to <1.1Mb. Parents and progeny are listed along the left most column, phenotypes in the next column over, each the remaining columns are genotypes for genetic markers sequentially ordered along LG5. Pink indicates the 'M9' allele and green the 'R5' allele. Individuals highlighted in yellow are recombinant over the interval. Only dwarfed (D) and semi-dwarfed (SD) individuals are informative, as some intermediate (I) and vigorous (V) individuals carry *Dw1.*

Figure 4 shows the number of trees in each flowering class and composition of classes by *Dw1* and *Dw2* genotype. Flowering was assessed by estimating the total number of flower clusters on each tree in the spring of year two, and placing them into quartiles relative to the most highly floral trees, ie, 1%-25%, 26-50%, 51-75%, 76-100%. Trees with no flowers were also recorded. Data is from 109 trees from the first population, replicate 1.

Figure 5 shows the average year seven TCA of trees in each genotypic class. The number of individuals in each class is given in parentheses, error bars indicate standard error. Average TCAs were compared to the group with neither *Dw1* nor *Dw2* by ANOVA, asterisks indicate the means are significantly different with a p value of $\leq$ 0.001. Data is from 303 trees from the second population.

Figure 6 shows the composition of each phenotypic class by *Dw1* and *Dw2* genotype. Trees from both populations (449 trees in total) were visually assessed after seven years of growth and placed into one of five phenotypic classes, D=dwarf, SD=semi-dwarf, I=intermediate, V=vigorous, and VV=very vigorous.

Figure 7 shows quantitative RT-PCR of ARF3. For each time point, RNA was isolated

and analysed from vascular-enriched tissue from 4-6 separate biological replicates of each genotype. Error bars indicate standard error for biological replicates.

Figure 8 shows an amino acid line up of ARF3 proteins from plants. ARF3 proteins have a highly conserved B3 DNA binding domain, an auxin response element and a tasi-ARF recognition site. 'M9' is heterozygous for a non-synonymous SNP that changes a conserved Serine/Proline to a Leucine (indicated by red box)

Figure 9 shows a table demonstrating % similarity between ARF 3 proteins. Proteins were aligned using MUSCLE and the phylogenetic tree used to generate this table was constructed with PHYML, using JTT substitution model and 1,000 bootstrap interations

Figure 10 shows over-expression of 'M9' ARF3 in petunia. a) Non-transformed and b-f) 35S:'M9' ARF3 flowers. Three independent lines showed incomplete petal fusion at the tube (b-c), irregular petal margins (d), and vascular patterning defects (e). (f) shows a close up of the abaxial (outside) of the flower, revealing incomplete petal fusion and vascular patterning defects.

Figure 11 shows over-expression of 'M9' ARF3 in petunia. a) untransfomed and b) 35S:M9 ARF3 flower showing petaloid stamen that appear in two lines .

Figure 12 shows over-expression of 'M9' ARF3 in tobacco. (a) un-transformed and (b-c) 35S: M9 ARF3. Vascular patterning defects were observed in several lines (arrows in b and c). One line showed an asymmetric leaf phenotype (arrowheads in c).

Figure 13 shows the vascular patterning defects in the 'M9' ARF3 overexpression tobacco plants.

Figure 14 shows 'M9' overexpression plants exhibiting reduced height, thick stems, shorter internodes and more axillary outgrowth compared to wild-type tobacco.

Figure 15 shows floral phenotypes of 35S:ARF3 in tobacco. Extra petaloid organs are common (arrows in a, c, e) as well as patterning defects, irregular vascular patterning (arrows in a, b) and unfused tube (arrow in d).

Figure 16 shows irregular vascular development in 35S:ARF3 in tobacco. Sections of (a) untransformed and (b-d) 35S: M9 ARF3 tobacco petioles. Tobacco has a co-lateral arrangement of xylem surrounded by phloem on both abaxial (AB) and adaxial (AD) sides. The M9 ARF3 over-expression lines show irregular vascular patterning, with more inner phloem cells (red arrows in b-d).

Figure 17 shows a summary of *Dw1* and *Dw2* genotyping of rootstock accessions. SSR makers were used to genotype rootstock accessions for the presence of *Dw1* and *Dw2*. A green square indicates the presence of a single allele of *Dw1*, yellow represents *Dw2*. The very dwarfing rootstock 'M27' is homozygous for *Dw1*, suggesting that *Dw1* is a semi-dominant mutation.

Figure 18 shows that a pear rootstock QTL maps to the same position as *Dw1*. a) A rootstock QTL affecting scion flowering, shoot growth and TCA (Trunk Cross-sectional Area) was detected on LG5, in the same position as *Dw1*. One major difference between the two QTLs, the pear QTL controlling early flowering is on the same position, but on the other chromosome, ie derived from the other parent. An HRM marker detecting the ARF3 SNP in apple was screened over the pear population. In b—d, individuals scored as "AA" were statistically different than siblings scored as "AB" for b) flowering, c) primary axis growth and d) TCA. *= p value <0.001, very significant.

Figure 19 illustrates a grafting experiment to demonstrate effect on scion. A – illustrates that one apical meristem is allowed to grow out. B – shows the grafted non-transformed wild-type stem. C – shows thwe graft junction. D - shows the "rootstock" which can be 35S:Dw1 (M9 mutant allele), 35S:dw1 (M793 non-dwarf allele) or non-transformed (WT).

Figure 20 shows the phenotypic characteristics of scions grafted onto 4 different "rootstocks" as indicated. Panel A (left side) shows shoot length of the grafted scion. Panel B (right side) shows days to flowering of the grafted scion. Values were compared to WT/WT by ANOVA, ** = p-value < 0.01, * = p-value <0.05.

Figure 21 shows the phenotypic characteristics of scions grafted onto 4 different "rootstocks" as indicated. Panel A (left side) shows number of nodes on the grafted scion. Panel B (right side) shows Trunk Cross-sectional Area (TCA) of the grafted scion. Values were compared to WT/WT by ANOVA, ** = p-value < 0.01, * = p-value <0.05.

Figure 22 shows the total scion dry weight of scions grafted onto 4 different "rootstocks" (same root stocks as in Figures 21 and 22). Values were compared to WT/WT by ANOVA, ** = p-value < 0.01, * = p-value <0.05.

Figure 23 shows the total leaf area of scions grafted onto 4 different "rootstocks"

(same root stocks as in Figures 21 and 22). Values were compared to WT/WT by ANOVA, * = p-value <0.05.

Figure 24 shows tree dry weight accumulation during the first year of growth. 'Royal Gala' scions were grafted to 'M793' (vigorous), 'M9' (dwarfing) or 'M27' (very dwarfing). At each time point, six composite trees of each rootstock genotype were severed at the graft junction, a) scion and b) rootstock were dried and weighed. Values were compared by ANOVA and the only significant differences detected between vigorous and dwarfing rootstocks was at the final time point (* = p-value <0.001). Error bars are SE.

Figure 25 shows average primary and total lateral root length of two week old seedlings. Seedlings were germinated on media, grown for two weeks, then harvested for photography. Digital images were measured using Image J. Error bars are standard error.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:	Sequence type	Common name	Species	Reference
1	Polypeptide	Apple	*Malus domestica*	MdARF3
2	Polypeptide	Apple	*Malus domestica*	MdARF3 'M9'
3	Polypeptide	Arabidopsis	*Arabidopsis thaliana*	ARF3/ETTIN
4	Polypeptide	Bean	*Phaseolus vulgaris*	PvARF3
5	Polypeptide	Tomato	*Solanum lycopersicum*	SlARF3
6	Polypeptide	Mandarin orange	*Citrus clemantina*	CcARF3
7	Polypeptide	Strawberry	*Frageria vesca*	FvARF3
8	Polypeptide	Plum	*Prunus persica*	PpARF3
9	Polypeptide	Pear	*Pyrus communis*	PcARF3
10	Polypeptide	Poplar	*Populus tremula*	PtARF3
11	Polypeptide	Grape	*Vitis vinefera*	VvARF3
12	Polynucleotide	Apple	*Malus domestica*	MdARF3 (cDNA)
13	Polynucleotide	Apple	*Malus domestica*	MdARF3 (gDNA)
14	Polynucleotide	Apple	*Malus domestica*	MdARF3 'M9'(cDNA)
15	Polynucleotide	Apple	*Malus domestica*	MdARF3 'M9'(gDNA)
16	Polynucleotide	Arabidopsis	*Arabidopsis*	ARF3/ETTIN (cDNA)

			thaliana	
17	Polynucleotide	Arabidopsis	*Arabidopsis thaliana*	ARF3/ETTIN (gDNA)
18	Polynucleotide	Bean	*Phaseolus vulgaris*	PvARF3 (cDNA)
19	Polynucleotide	Tomato	*Lycopersicum esculentum*	LeARF3 (cDNA)
20	Polynucleotide	Mandarin orange	*Citrus clemantina*	CcARF3 (cDNA)
21	Polynucleotide	Strawberry	*Frageria vesca*	FvARF3 (cDNA)
22	Polynucleotide	Plum	*Prunus persica*	PpARF3 (cDNA)
23	Polynucleotide	Pear	*Pyrus communis*	PcARF3 (cDNA)
24	Polynucleotide	Poplar	*Populus tremula*	PtARF3 (cDNA)
25	Polynucleotide	Grape	*Vitis vinefera*	VvARF3 (cDNA)
26	Polynucleotide	Apple	*Malus domestica*	Hi01C04 marker
27	Polynucleotide	Apple	*Malus domestica*	Hi04A08 marker

EXAMPLES

The invention will now be illustrated with reference to the following non-limiting examples.

It is not the intention to limit the scope of the invention to the present example only. As would be appreciated by a skilled person in the art, many variations are possible without departing from the scope of the invention.

Example 1: Refining the genomic region containing the *Dw1* loci

Background

In a previous QTL study, the closest genetic markers that defined *Dw1* were Hi01c04 and Ch03a09 (Figure 1), which are located at 4.72 and 7.62 Mb respectively on the reference golden delicious genome (Celton et al 2009). More recently Fazio and co-workers (Fazio et al 2014) found a more distal position for *Dw1*, between Hi22f12 (2.69Mb) and Hi04a08 (5.15Mb) (Figure 2).

In the present work, the applicants developed genetic markers based on genomic sequence from the interval between 4.5 Mb and 7.2 Mb on linkage group 5 (LG5). By screening these markers over the parents and progeny of their rootstock population, the applicants were able to identify recombinants within this interval (i.e. had a chromosomal cross over between 'M9' and 'R5'). Intermediate and vigorous recombinants were not informative, because some of the individuals carried *Dw1*. However, all dwarfed and semi-dwarfed individuals carried *Dw1*, so these recombinants were informative in defining the interval that contains *Dw1*. Based on four dwarfed and two semi-dwarfed recombinant individuals, the applicants were able to narrow the genomic interval containing *Dw1* to a smaller region, between 4.75 Mb and 5.80 Mb (Figure 3).

This region defines an interval of 1.05 Mb (5.80-4.75 Mb).

Although this is a smaller interval, this region could still contain over 100 genes. It is also possible that the genetic determinant of dwarfing at the *Dw1* locus would be a micro RNA (miRNA) or other non-protein encoding gene. Furthermore, prior to the present application, there were no obvious candidate gene/s, or even classes of candidate genes that might be responsible for the dwarfing effect of the the *Dw1* locus.

_Dw1_ has a more significant effect than _Dw2_ on rootstock-induced dwarfing

To elucidate the relative contributions of *Dw1* and *Dw2* to dwarfing of the scion, the applicants examined three of the most robust phenotypes associated with dwarfing, i.e. early flowering (spring of year two), final TCA (year seven), and overall visual assessment (year seven) of scions grafted to rootstocks carrying various combinations of *Dw1* and *Dw2*.

Early flowering was assessed in the spring of year two by estimating the number of floral clusters on 109 trees from the first population. The majority of the trees with the highest degree of flowering had been grafted onto rootstocks carrying both *Dw1* and

Dw2 (50%), or *Dw1* alone (41.7%) (Figure 4). Conversely, the trees with no flowers or the fewest flowers were predominantly grafted onto rootstocks carrying *Dw2* alone (33.9%), or neither dwarfing locus (44.6%).

After seven years of growth, the TCA of 303 trees from the second population were measured. Trees grafted onto rootstocks carrying both *Dw1* and *Dw2* exhibited the lowest average TCA, only 23% of that of scions on rootstocks with neither loci. Rootstocks with *Dw1* alone reduced scion TCA to 73% of those with neither rootstock loci. Surprisingly, trees grafted onto rootstocks with *Dw2* alone had the highest TCA of all (Figure 5).

As rootstock-induced dwarfing becomes more pronounced over successive growth cycles, an expert visual assessment of the whole tree phenotype after seven years provided an overall measure of scion vigour. When 449 grafted trees from both populations were compared, a clear trend relating rootstock genotype to phenotypic class was observed. All the dwarfed and semi-dwarfed trees were grafted onto rootstocks with *Dw1* and *Dw2* or *Dw1* alone, whereas the vigorous and very vigorous trees had rootstocks carrying *Dw2* alone, *Dw1* alone, or neither locus (Figure 6). Nearly 40% of the vigorous trees were on rootstocks carrying *Dw2*, indicating that this locus alone is not sufficient to dwarf the scion.

However in contrast to the recent work of Fazio et al (Fazio, Wan et al. 2014) the present study does indicate that the *Dw1* loci can influence dwarfing alone (i.e. even in the absence of *Dw2*).

Other dwarfing and semi-dwarfing rootstocks carry *Dw1* and *Dw2*

Genetic markers linked to *Dw1* and *Dw2* were screened over 41 rootstock accessions that confer a range of effects on scion growth. The majority of dwarfing and semi-dwarfing rootstock accessions screened carried marker alleles linked to both *Dw1* and *Dw2* (Foster et al, 2015 and Figure 17). This suggests that most apple dwarfing rootstocks have been derived from the same genetic source.

Example 2: A pear rootstock QTL influencing scion size and flowering

Pear does not have a true dwarfing rootstock, such as 'M9', although some rootstocks are known to influence scion size and flowering. A pear segregating rootstock population was generated by crossing 'Old Home' to 'Louis Bon Jersey'. The progeny were grafted

with 'Comice', and scions were phenotyped for 4 years. A QTL influencing scion size and flowering was identified at the top of LG5, in the exact location as *Dw1* (Figure 18, PFR, unpublished). No QTL corresponding to *Dw2* was identified. Pear and apple are very closely related and show strong synteny of gene order along their orthologous chromosomes. This finding raises the exciting possibility that *Dw1* predates the divergence of apple and pear and that the same gene may be influencing both the apple and pear QTL.

Example 3: Identification of ARF3 as a candidate gene for *Dw1*

The applicants found that there are approximately 168 annotated genes within the 1.1 Mb interval (unpublished). Based on expressed sequence ESTs from the Plant and Food proprietary *Malus* database (Newcomb, Crowhurst et al. 2006) and RNA seq experiments (unpublished), the applicants estimated the number of expressed genes is about 100.

The applicants identified an Auxin Response Factor 3 (ARF3) transcription factor gene present in the refined *Dw1* interval, which they showed to be upregulated in M9 rootstock, as a candidate gene for the *Dw1* QTL effect.

Many hypotheses to explain the mechanism of dwarfing rootstocks implicate auxin, but the genetic basis of any auxin effect is completely unknown. ARF3 is a member of a large family of Auxin Response Factors, transcription factors that activate or repress downstream genes in response to auxin. ARF3/ETTIN was first discovered as a gene required for normal patterning of floral organs in Arabidopsis (Sessions and Zambryski 1995; Sessions, Nemhauser et al. 1997). It was later discovered that ARF3 and the transcription factor KANADI mediate both auxin flow and organ polarity, which includes vascular patterning (Pekker, Alvarez et al. 2005; Izhakia and Bowman 2007; Kelley, Arreola et al. 2012). ARF3 also has a key role in promoting phase change (transition to flowering), increased ARF3 expression leads to earlier flowering, loss of ARF3 function delays flowering. (Fahlgren, Montgomery et al. 2006; Hunter, Willmann et al. 2006).

ARF3 is up-regulated in 'M9' and 'M27' relative to vigorous rootstocks

The applicants used quantitative real time PCR (qRT-PCR) to compare ARF3 expression in vascular-enriched tissue from 'M9' and another dwarfing rootstock 'M27' with a vigorous rootstock, 'M793' (Figure 7). ARF3 expression was about four times higher in

'M9' than 'M793' at all time points. In 'M27', ARF3 expression was 2-4 times higher levels than 'M793'.

'M9' has a mutation in the ARF3 gene

To identify any 'M9'-specific DNA changes that might alter gene expression or function/activity the applicants performed genomic sequencing of 'M9'. This revealed that the 'M9' MdARF3 (MDP000173151) carried a single nucleotide polymorphism (SNP) that changed a conserved Serine to a Leucine. Figure 8 shows an amino acid line-up with the 'M9', the reference MdARF3 proteins and ARF3 proteins from a variety of plants. This SNP alter the function of the ARF3 protein.

The 'M9' ARF3 SNP as a genetic marker in apple and pear

To test if the SNP identified in the 'M9'ARF3 segregates with dwarfing individuals, the applicants used primers that amplify the SNP in a High Resolution Melting (HRM) analysis over the entire 'M9' x 'R5' rootstock population. The results showed clear segregation of a distinct melting curve with all individuals that were previously identified as having *Dw1*. The same marker was also tested on the pear rootstock population and showed clear segregation with one curve associated with high flowering individuals, another with low or no flowering trees.

Example 4: Transgenic expression of ARF3 in petunia and tobacco

To test if the higher expression and/or the non-synonymous SNP in the 'M9' ARF3 cause phenotypes associated with dwarfing rootstocks, the applicants made transgenic lines of both tobacco and petunia that over-express either the 'M9' or the reference allele of ARF3. These are hence referred to as M9 ARF3 and wt ARF3 respectively. Petunia and tobacco were chosen as models because they are both amenable to grafting.

The applicants generated 10 independent lines expressing 35S: M9 ARF3, but the applicants were unable to recover 35S: wt ARF3 petunias. The applicants verified that the plants were expressing the construct by q-RT-PCR. Three independent lines of the 35S:M9 ARF3 had a floral phenotype, ranging from irregular petal margins, incomplete tube fusion, vascular defects, and petaloid stamens (Figures 10, 11). Microscopic analysis of the irregular petal margins revealed small patches of inverted petal polarity, which is consistent with the known function of ARF3 in adaxial-abaxial patterning.

The applicants generated 10 M9 ARF3 and 10 wt ARF3 over-expression lines in tobacco. The applicants verified that all T_0 plants were expressing the construct. Preliminary analysis indicates that several of the plants exhibit irregular vascular patterning in the leaves (Figure 13). Two plants have asymmetric leaves, with half of the blade missing entirely or double midveins (Figure 12 b, c). The most extreme line of 35S: M9 ARF 3 (#6) is much shorter than wild-type with thick stems, and decreased apical dominance, creating a bushy phenotype (Figure 14). The lines with the highest ARF3 expression flowered earlier than the others. Early flowering is also seen in dwarfed scions in apple. Many of the M9 and wt ARF3 plants have floral phenotypes. These include incomplete fusion of the tube, patterning defects, and extra petaloid organs (Figure 15).

To examine the vascular patterning defects in more detail, petioles from untransformed and ARF3 over-expression plants were fixed, sectioned and stained with safranin fast green. Figure 16 shows representative micrographs illustrating that 35S:M9 ARF3 plants have irregular vascular patterning, with more inner phloem cells, consistent with the similar phenotype seen in M9 apple rootstock.

Phenotypic analysis of the ARF3 over-expression tobacco plants, can also be carried out on plants produced from T_1 seed.

Plants transformed to express ARF3 and M9 ARF3 can be phenotyped, as can scions grafted onto the transgenic, and control plants.

Such phenotyping can involve a detailed architectural analysis to document metamer initiation rate, the outgrowth and size of axillary brances, the size and node number of the primary shoot, and time to flowering.

Growth chambers can also be used to test if the transgenic plants have an altered sensitivity to long days or short days.

Further histological analysis can also be undertaken to compare vascular development between transgenic lines and untrasformed controls.

Example 5: Transgenic expression of ARF3 in apple

The constructs described in Example 4 above were transformed into apple, to further assess the phenotypic effect of higher expression and/or the non-synonymous SNP.

Plantlettes generated, can be tested to verify that ARF3 is over-expressed using qRT-PCR. Transgenic lines can be assessed for dwarfing-associated phenotypes by comparing the overall plant architecture (main axis hight, outgrowth of axillary branches, etc) with un-transformed controls. To examine any changes to the vasculature, tissue can be fixed, sectioned, stained and photographed on a microscope to compare with untransformed controls.

Once plantlettes have generated roots and are large enough, they can be grafted with un-transformed controls. Scions can be assessed for dwarfing-associated phenotypes by comparing the number of growth units on the primary and secondary axis, comparing the number and size of sylleptic and prolleptic shoots, and eventually the number of flowers.

Example 6: Transgenic expression of ARF3 in pear.

The constructs described in Example 4 above were transformed into pear, to further assess the phenotypic effect of higher expression and/or the non-synonymous SNP.

Plantlettes generated, can be tested to verify that ARF3 is over-expressed using qRT-PCR. Transgenic lines can be assessed for dwarfing-associated phenotypes by comparing the overall plant architecture (main axis hight, outgrowth of axillary branches, etc) with un-transformed controls. To examine any changes to the vasculature, tissue can be fixed, sectioned, stained and photographed on a microscope to compare with untransformed controls.

Once plantlettes have generated roots and are large enough, they can be grafted with un-transformed controls. Scions can be assessed for dwarfing-associated phenotypes by comparing the number of growth units on the primary and secondary axis, comparing the number and size of sylleptic and prolleptic shoots, and eventually the number of flowers.

Example 7: Determine if the 'M9' SNP alters protein function

Transient expression experiments in *Nicotiana benthamiana* (Martin, Kopperud et al. 2009), can be used to further assess the function of the non-synonomous SNP in the 'M9' ARF3. First an an auxin responsive reporter line, DR5:LUC (Ulmasov, Murfett et al. 1997) can be generated. This reporter will result in an enzyme that generates fluorescent compound in response to auxin.

The reporter construct can be co-expressed with either the 'M9' or wt ARF3 and the fluorescent compound measured after 1-3 days. These experiments can also be repeated with application of exogenous auxin to compare auxin sensitivity.

Example 8: Determine if pear has altered ARF3 sequence and/or expression.

ARF3 expression in pear can be assessed by qRT-PCR to determine if "dwarfish" individuals from the pear rootstock population have higher expression of ARF3 than vigorous individuals. To determine if the same non-synonomous SNP exists "dwarfish" individuals, the pear ARF3 gene can be amplified and sequenced.

Example 9: Examination of the phenotype of apple seedlings genotyped for *Dw1* and *Dw2*

Seedlings derived from controlled crosses can be genotyed for *Dw1* and *Dw2* to identify individuals that have zero, one or two copies of *Dw1*, and either zero or one copy of *Dw2*. ARF3 expression in apple seedlings and young trees can be assessed. Seedlings/trees can be measured for differences in metamer number of primary and secondary axes, the outgrowth of axillary shoots, and the time to flowering. Stem vascular development can also be assessed histologically.

Example 10: Tree dry weight accumulation during the first year of growth.

'Royal Gala' scions were grafted to 'M793' (vigorous), 'M9' (dwarfing) or 'M27' (very dwarfing). At each time point (60, 120, 180 and 300 days after bud break [DABB]), four to six composite trees of each rootstock genotype were severed at the graft junction. Scion and rootstock material was oven dried at 60°C to a constant mass and

weighed. Dry weights of scion include scion budwood, primary axis, sylleptic shoots and leaves, whilst dry weights of rootstock include roots and rootstock stem. Values were compared by ANOVA and the only significant differences detected between vigorous and dwarfing rootstocks was at the final time point (* = p-value <0.001). The results are shown in Figure 24. Error bars are SE.

Example 11: Grafting Experiments

Methods of grafting

Tobacco plants were grown in pots until plants had 10-15 leaves. In this experiment, all scions were wild-type tobacco, the "rootstocks" were wild-type, M9 ARF3 (2 independent lines, 2 and 6) and 35S: 793 (wt) ARF3 (line 4). We note M27 has the same ARF3 allele as M9, thus M27 contains the M9 allele of ARF3. In Figures 20 to 23, the M9 ARF3 rootstock lines are labelled M27 2-1 and M27 6-16 and the WT ARF3 rootstock line is labelled M793 4-3.

At the time of grafting, a horizontal cut was made through the "rootstock" stem at the very top of node 4-5. A "V"-shaped notch was cut vertically into the stem, 5-10mm deep. The wild-type scion was cut from the base of the plant such that the base was approximately the diameter of the "rootstock". Leaves and shoot tip were removed and a piece of stem containing 2 nodes (each with an axillary meristem) was cut into a wedge shape at the bottom end. The scion was inserted into the "rootstock" notch and the junction was secured with a small piece of parafilm. Plants were placed in a mist tent to recover. After one week, all leaves from the "rootstock" were removed. Once it became apparent that one or more axillary meristems of the scion was growing out, the other was removed.

The scion shoots were grown until the first flower was fully extended, this date was considered the flowering date. The time between grafting date and the flowering date is the days to flowering. Once plants had flowered, architectural data was collected from the scion. The shoot length and node number was measured from the axil to the uppermost leaf base, this does not include the original scion stem segment, only the shoot that grew from the axillary meristem. The scion shoot diameter was measured at the base of the shoot using an electronic calliper. Trunk circumference area (TCA) was calculated with the formula: $(\text{diameter}/2)^2 \pi$ and is given in mm^2. The area of each leaf was measured with an electronic leaf scanner, total leaf area is the sum of

all leaves on a plant and is given in cm^2. The scions were dried and weighed to determine dry weight (gm). Each line was compared to WT/WT by one way- ANOVA to determine significant differences.

Results

As ungrafted plants, 35S: M9 ARF3 line 6, hereafter referred to as line 6, show the most extreme phenotype. 35S: M9 ARF3 line 2 (line 2) has the mildest phenotype and 35S:793 ARF3 line 4 is undistinguishable from wild-type.

Relative to the WT/WT homografts, the WT scions on line 6 rootstocks were significantly shorter (Figure 20). Scions on line 2 and line 4 had slightly shorter lengths, but these were not significant.

Scions on all three transgenic rootstocks flowered slightly earlier than the WT/WT (Figure 21).

Line 6 had significantly fewer nodes than WT/WT (Figure 20).

Scions on both line 2 and line 6 had a smaller TCA than WT/WT. Line 6 was significantly different than WT/WT (Figure 21).

Scions on line 2 and line 6 had a smaller dry weight than WT/WT. Line 6 was significantly different than WT/WT (Figure 22).

Although lines 2, 6 and 4 had less total leaf area, only line 6 was significantly different from WT/WT (Figure 23).

To our knowledge, there has been no report of dwarfing rootstocks causing smaller leaf size in scions.

Seedling root measurements

35S: M9 ARF3, 35S: wt ARF3 and wild-type tobacco seeds were sterilized in 2% bleach for 30 minutes, rinsed in distilled H2O, 3x, for 10 minutes each, then plated on MS media containing Kanamycin (for the transgenic seeds) or just MS (wild-type). Two weeks after plating, seedlings were removed from the media, excess media was

removed and seedlings were photographed on a grid using a stereo microscope equipped with a digital camera. Primary and lateral root length were measured from digital images using Image J, total lateral root length is the sum of all lateral root lengths. (see Figure 25).

In terms of shoot length, node number, TCA, scion dry weight, and scion mass, the effect of line 6 on the scion appears to replicate the effect of the 'M9' dwarfing rootstock.

Summary of data shown in transgenic plants, and grafted scions.

The phenotypes shown in transgenic plants over-expressing M9 ARF1 or WT ARF1, and in WT plants grafted onto transgenic plants over-expressing M9 ARF1 or WT ARF1, in comparison to the known phenotypes in known root stock and dwarfed grafted scions are summarised in the tables below.

Table 2 – Phenotypes shown in transgenic plants over-expressing M9 ARF1 or WT ARF1

Known dwarfing-associated phenotypes found in dwarfing rootstock plants (previous data)	**Shown in plants over-expressing M9 ARF1 (this study)**	**Shown in plants over-expressing WT ARF1 (this study)**
bushier	Yes	No
altered xylem/phloem ratio	Yes	No
more phloem elements	Yes	No
reduced apical dominance	Yes	No
reduced root mass	Yes	Yes

Table 3 - Phenotypes shown in WT plants grafted onto transgenic plants over-expressing M9 ARF1 or WT ARF1

Known dwarfing-associated phenotypes found in scions grafted onto dwarfing rootstock plants (previous data)	**Shown in WT "scions" grafted on to "rootstock" plants over-expressing M9 ARF1 (this study)**	**Shown in WT "scions" grafted on to "rootstock" plants over-expressing M9 ARF1 (this study)**
reduced vigour	Yes	Yes
less vegetative growth	Yes	Yes
earlier termination of shoot growth	Yes	Yes
smaller canopy	Yes	No
reduced stem circumference	Yes	No

reduced scion mass	Yes	No

Materials and Methods

Plant material

A rootstock population derived from crosses between *Malus* x *domestica* 'Malling9' ('M9') and *Malus robusta* 5 ('R5') was used for QTL analysis. For the first population, 135 seedlings were planted in 1998 and grown as stoolbeds to produce multiple rooted stocks of each genotype. The rootstocks were cleft grafted with 'Braeburn' scions, grown in the nursery for two years, then transplanted into the Plant & Food Research orchard (Havelock North, New Zealand) as described by Pilcher *et al*.(Pilcher, Celton et al. 2008) Replicates of the original 135 rootstocks were propagated in 2000 and planted in the orchard as one-year-old grafted trees. Of the replicated trees, 112 individuals from replicate two, and 57 individuals from replicate three were phenotyped for QTL analysis. The second population consisted of 350 seedlings, which were grafted as described above and planted in the orchard as one-year-old trees in 2004. From the second population, 81 individuals were evaluated for the QTL analysis and 314 survived until final phenotypic assessment in year seven. Trees were grown with in-row spacing of 1.5 m between trees and a double wire trellis as support, in a complete randomized block design. Scions grafted onto 'M9' and 'R5' were planted throughout as controls. Trees were not pruned, to allow full expression of the rootstock effects on scion growth. Once trees began fruiting, chemical thinning sprays were applied to avoid over-cropping and limb breakage.

Forty-one (41) apple rootstock accessions (*Malus* spp.) representing rootstock varieties used in major apple-growing regions in the world were used for pedigree analysis of *Dw1* and *Dw2*.

Phenotypic analysis

Rootstock effects on the development of 'Braeburn' scions were assessed using multiple methods, over seven years, within the two populations. Table 1 presents the specific traits that were assessed for the QTL analysis in each population/replicate and the sample size phenotyped. Height, internode number, and average internode length of the scion were recorded at the end of the first year of growth after grafting (year one). Flowering was scored by estimating the total number of flower clusters on each tree in

the spring of year two, and placing them into quartiles relative to the most highly floral trees, i.e., 1-25% had the fewest flowers, 75-100% had the most flowers. Trees without any flowers in year two were recorded as "0". Trunk Cross-sectional Area (TCA) was measured 20 cm above the graft junction at the end of each year from year two to year seven. From year two to year seven, the overall vigour of each tree was assessed annually by comparing trunk size, crown height and spread, branch density and vigour. For the QTL analysis, an overall dwarfing phenotype (DW%) was assigned in year seven, with 100% = very vigorous, 80% = vigorous, 60% = intermediate, 40% = semi-dwarfed, and 20% = dwarfed.

The 41 rootstocks accessions used for the pedigree analysis were classified according to their dwarfing effect in accordance with the literature and in-house Plant & Food Research professional expertise.

DNA isolation and genotyping of 'M9' x 'R5' rootstock population and rootstock accessions

Total genomic DNA was extracted from leaves and quantified according to Gardiner *et al.*(Gardiner, Bassett et al. 1996) Leaf material was collected from 135 seedlings from the first 'M9' x 'R5' population and 350 from the second population. Leaves of the rootstock accessions were collected from the Plant & Food Research germplasm collection in Havelock North, NZ, or from the USDA-ARS collection in Geneva, NY, USA.

For *Dw1* and *Dw2* genotyping of the entire population of 'M9' x 'R5' rootstocks, polymerase chain reaction (PCR) products containing single nucleotide polymorphisms (SNP) were amplified on a LightCycler480 instrument (Roche Diagnostics) and screened using the High Resolution Melting (HRM) technique as described by Chagné *et al.*(Chagné, Gasic et al. 2008) Supplementary Table 1 lists the position of markers on the 'Golden Delicious' genome(Velasco, Zharkikh et al. 2010) and primer sequence.

Markers detecting SSRs located on LG5 and LG11 were employed to genotype the 41 rootstock accessions. Hi01c04, Hi04a08, CH03a09 and CH02d08 were developed by Silfverberg-Dilworth *et al.*(Silfverberg-Dilworth, Matasci et al. 2006) and Liebhard *et al.*(Liebhard, Gianfranceschi et al. 2002) Two new SSR markers (MDP0000365711 and MDP00024370) located at the top of LG11 were developed using the Plant & Food Research *Malus* genome database(Newcomb, Crowhurst et al. 2006), with the programmes Sputnik and Primer3. The M13 sequence TGTAAAACGACGGCCAGT was

added to the 5′ end of the forward primer to enable the use of Schuelke's (Schuelke 2000) approach to fluorescent labelling. PCR reactions were performed and analysed on an ABI 3500 Genetic Analyzer (Applied Biosystems) as described by Hayden *et al.* (Hayden, Nguyen et al. 2008)

QTL analysis

The parental genetic maps for 'M9' and 'R5' were constructed using a total of 316 loci amplified from 296 primer pairs as described in Celton *et al.*(Celton, Tustin et al. 2009) The maps span a total of 1,175.7 and 1,086.7 cM for 'M9' and 'R5' respectively.(Celton, Tustin et al. 2009) The linkage phase of the markers was determined using JoinMap® 3.0 (Kyazma, NL). QTL analysis was performed for all growth traits using MapQTL® 5 Software (Kyazma, NL). Traits evaluated over multiple years and replicates were analysed separately. Interval mapping (IM), followed by multiple QTL model (MQM) analysis using the best markers obtained by IM as co-factors, was used for normally quantitative traits. Only additive models were considered for the QTL analysis. The threshold for QTL genome-wide significance was calculated after 1,000 permutations. Kruskal-Wallis analysis was used for ordinal traits such as the estimated number of flower clusters and expert assessment of dwarfing.

RNA purification

For RNA-seq, tissue was collected from the rootstock stem of two M.793 and two M.9 individuals in November (60 DABB, ~90 days after grafting). M.27 was not included in the RNA-seq experiment because suitable material was not available. For qRT-PCR expression analysis, 30 'Royal Gala' trees grafted onto M.9, M.27 and M.793 rootstocks were grown as previously described. Tissue was collected for RNA purification in November, January, March and July (60, 120, 180 and 300 DABB respectively). For each time point, four to six trees of each genotype were selected for uniform scion growth to minimize any effects due to differential tree size. RNA was pooled from four shoots from each of the rootstock accessions shown in Fig. 5. For all other experiments, RNA from each individual was extracted and analysed separately. For all collections, the outer bark was removed, vascular tissue was scraped off with a scalpel, and snap frozen in liquid nitrogen. Tissue was harvested between four and five hours after sunrise for all time points. Total RNA was isolated and cDNA generated as described in (Janssen et al. 2008). The quality and concentration of the RNA samples

was assessed with an RNA Nano kit (Agilent) and only samples with a RIN value of 8 or higher were further analyzed by sequencing or qRT-PCR.

RNA sequencing and data processing

RNA was sent to Axeq/Macrogen for library preparation and sequencing using an Illumina Hiseq 2000 instrument. Individual samples were run as a multiplexed sample on one lane to produce 100 nucleotide paired end sequence reads. The first 13 bases of all RNAseq reads were trimmed using an in-house perl script. Adapters were removed using fastq-mcf from the ea-utils package (Aronesty 2011) using a minimum read retention length of 50 and a minimum quality score threshold of 20. Quality score analysis was performed using fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) both before and after trimming. Trimmed reads were mapped to the reference using bowtie2 (Langmead and Salzberg 2012) using the following settings: -a --end_to_end --sensitive. SAM file to BAM file conversion was undertaken using samtools (Li et al. 2009). Raw read counts and reads per kilobase per million (RPKM) values were extracted from BAM files using the multicov option of bedtools (Quinlan and Hall 2010) and either an in-house R script or cufflinks (Trapnell et al. 2010). Apple homologues of Arabidopsis flowering genes were determined by BLASP value and tested by reciprocal BLASTP. Differentially expressed genes were selected using the Limma package (Smyth 2005) in BioConductor, genes were selected using an adjusted P value of < 0.05 and fold change cutoff > 6 (Smyth 2005).

Transformation of ARF3 into plants

Primers were designed to amplify the MdARF3 gene, from from 100bp upstream of the start codon to 50bp 5' of the stop codon. Single products were amplified from cDNA derived from 'Royal Gala' or 'M9' meristem enriched tissue. These products were cloned into an expression vector (pHEX), which uses the cauliflower mosaic virus (CaMV) 35S promoter to drive expression and contains the neomycin phoshotransferase II gene (NPTII) to confer kanamycin resistance. Agrobacterium tumefaciens strain GV3-101 transformed with either the 'Royal Gala' ("wt") or the 'M9' ARF3 was used to transform leaf discs from *N. tabacum* ('Samsun'), petunia ('Mitchell') or apple transformation cell lines. Callus formation and regeneration of plantlettes are as described in (Kotoda and Wada 2005).

Histology

Stem and petiole sections were fixed overnight in FAA (3.7% Formaldehyde, 50% EtOH, 5% Acetic Acid), processed and embedded in paraffin as described in Ruzin (Ruzin 1999). Tissue was sectioned to 10□m on a rotary microtome, and slides were stained using a safranin/fast green procedure to distinguish xylem from phloem.

Grafting

Scions can be grafted onto rootstocks using cleft grafting or chip-budding depending on the material (Stoltz and Strang 1982; Webster and Wertheim 2003; Crasweller 2005).

References

Bennett, T., T. Sieberer, et al. (2006). "The Arabidopsis MAX Pathway Controls Shoot Branching by Regulating Auxin Transport." Current Biology **16**(6): 553-563.

Böhlenius, H., T. Huang, et al. (2006). "CO/FT Regulatory Module Controls Timing of Flowering and Seasonal Growth Cessation in Trees." Science **312**(5776): 1040-1043.

Carrière, E. A. (1897). "Un nouveau sujet pour greffer les pommes." Revue Horticole: 436-437.

Celton, J. M., D. S. Tustin, et al. (2009). "Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from Malus ESTs and Pyrus genomic sequences." Tree Genetics & Genomes **5**(1): 93-107.

Chagné, D., K. Gasic, et al. (2008). "Development of a set of SNP markers present in expressed genes of the apple." Genomics **92**(5): 353-358.

Costes, E. and Y. Guedon (1997). "Modelling the sylleptic branching of one-year-old trunks of apple cultivars." Journal of American Society for Horticultural Science **122**: 53-62.

Costes, E. and Y. Guedon (2002). "Modelling branching patterns on 1-year-old trunks of six apple cultivars." Annals of Botany **89**(5): 513-524.

Fahlgren, N., T. A. Montgomery, et al. (2006). "Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA Affects Developmental Timing and Patterning in Arabidopsis." Current Biology **16**(9): 939-944.

Fazio, G., Y. Wan, et al. (2014). "Dw2, a New Dwarfing Locus in Apple Rootstocks and Its Relationship to Induction of Early Bearing in Apple Scions." Journal of the American Society for Horticultural Science **139**(2): 87-98.

Ferree, D. C. and R. F. Carlson (1987). Apple rootstocks. Rootstocks for Fruit Crops. R. C. Rom and R. F. Carlson. New York, USA, John Wiley and Sons**:** 107-143.

Fulford, R. M. (1966). "The Morphogenesis of Apple Buds: II. The Development of the Bud." Annals of Botany **30**(1): 25-38.

Gardiner, S., H. Bassett, et al. (1996). "A detailed linkage map around an apple scab resistance gene demonstrates that two disease resistance classes both carry the V f gene." Theoretical and Applied Genetics **93**(4): 485-493.

Gregory, P. J. and T. S. George (2011). "Feeding nine billion: the challenge to sustainable crop production." Journal of Experimental Botany **62**(15): 5233-5239.

Hatton, R. G. (1917). "'Paradise' apple socks." Journal of the Royal Horticultural Society **42**: 361-399.

Hayden, M., T. Nguyen, et al. (2008). "Multiplex-Ready PCR: A new method for multiplexed SSR and SNP genotyping." BMC Genomics **9**(1): 80.

Hirst, P. M. and D. C. Ferree (1995). "Rootstock Effects on the Flowering of `Delicious' Apple. I. Bud Development." Journal of the American Society for Horticultural Science **120**(6): 1010-1017.

Hooijdonk, B. v., D. Woolley, et al. (2011). "Rootstocks Modify Scion Architecture, Endogenous Hormones, and Root Growth of Newly Grafted 'Royal Gala' Apple Trees." Journal of the American Society for Horticultural Science **136**(2): 93-102.

Hsu, C.-Y., Y. Liu, et al. (2006). "Poplar FT2 Shortens the Juvenile Phase and Promotes Seasonal Flowering." The Plant Cell **18**(8): 1846-1861.

Hsu, C. Y., J. P. Adams, et al. (2011). "FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar." Proceedings of the National Academy of Sciences of the United States of America **108**(26): 10756-10761.

Huijser, P. and M. Schmid (2011). "The control of developmental phase transitions in plants." Development **138**(19): 4117-4129.

Hunter, C., M. R. Willmann, et al. (2006). "Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis." Development **133**(15): 2973-2981.

Imamura, T., T. Nakatsuka, et al. (2011). "The Gentian Orthologs of the FT/TFL1 Gene Family Control Floral Initiation in Gentiana." Plant and Cell Physiology **52**(6): 1031-1041.

Izhakia, A. and J. L. Bowman (2007). "KANADI and Class III HD-Zip Gene Families Regulate Embryo Patterning and Modulate Auxin Flow during Embryogenesis in Arabidopsis." The Plant Cell **19**: 495-508.

Kelley, D. R., A. Arreola, et al. (2012). "ETTIN (ARF3) physically interacts with KANADI proteins to form a functional complex essential for integument development and polarity determination in Arabidopsis." Development **139**(6): 1105-1109.

Kotoda, N. and M. Wada (2005). "MdTFL1, a TFL1-like gene of apple, retards the transition from the vegetative to reproductive phase in transgenic Arabidopsis." Plant Science **168**(1): 95-104.

Liebhard, R., L. Gianfranceschi, et al. (2002). "Development and characterisation of 140 new microsatellites in apple (Malus x domestica Borkh.)." Molecular Breeding **10**(4): 217-241.

Ljung, K., A. K. Hull, et al. (2005). "Sites and Regulation of Auxin Biosynthesis in Arabidopsis Roots." The Plant Cell Online **17**(4): 1090-1104.

Manhart, W. (1995). Apples for the Twenty-First Century. Portland, OR, North American Tree.

Martin, K., K. Kopperud, et al. (2009). "Transient expression in Nicotiana benthamiana fluorescent marker lines provides enhanced definition of protein localization, movement and interactions in planta." Plant J **59**(1): 150-162.

Napoli, C. A., C. A. Beveridge, et al. (1999). "Reevaluating concepts of apical dominance and the control of axillary bud outgrowth." Curr Top Dev Biol **44**: 127-169.

Newcomb, R. D., R. N. Crowhurst, et al. (2006). "Analyses of Expressed Sequence Tags from Apple." Plant Physiology **141**(1): 147-166.

Pekker, I., J. P. Alvarez, et al. (2005). "Auxin Response Factors Mediate Arabidopsis Organ Asymmetry via Modulation of KANADI Activity." The Plant Cell Online **17**(11): 2899-2910.

Pilcher, R. L. R., J.-M. Celton, et al. (2008). "Genetic Markers Linked to the Dwarfing Trait of Apple Rootstock 'Malling 9'." Journal of the American Society for Horticultural Science **133**(1): 100-106.

Ruzin, S. E. (1999). Plant Microtechnique and Microscopy. New York, NY, USA, Oxford University Press.

Schuelke, M. (2000). "An economic method for the fluorescent labeling of PCR fragments." Nat Biotech **18**(2): 233-234.

Seleznyova, A. N., T. G. Thorp, et al. (2003). "Application of architectural analysis and AMAPmod methodology to study dwarfing phenomenon: the branch structure of 'Royal Gala" apple grafted on dwarfing and non-dwarfing rootstock/interstock combinations." Annals of Botany **91**: 665-672.

Seleznyova, A. N., D. S. Tustin, et al. (2008). "Apple dwarfing rootstocks and interstocks affect the type of growth units produced during the annual growth cycle: Precocious transition to flowering affects the composition and vigour of annual shoots." Annals of Botany **101**(5): 679-687.

Sessions, A., J. L. Nemhauser, et al. (1997). "ETTIN patterns the Arabidopsis floral meristem and reproductive organs." Development **124**(22): 4481-4491.

Sessions, R. A. and P. C. Zambryski (1995). "Arabidopsis gynoecium structure in the wild and in ettin mutants." Development **121**(5): 1519-1532.

Shimizu-Sato, S. and H. Mori (2001). "Control of Outgrowth and Dormancy in Axillary Buds." Plant Physiology **127**(4): 1405-1413.

Silfverberg-Dilworth, E., C. L. Matasci, et al. (2006). "Microsatellite markers spanning the apple (Malus x domestica Borkh.) genome." Tree Genetics & Genomes **2**(4): 202-224.

Soumelidou K, M. D., Battey NH, Barnett JR, John P (1994). "Auxin transport capacity in relation to the dwarfing effect of apple rootstocks." Journal of Horticultural Science **69**: 719-725.

Steeves, T. A. and I. M. Sussex (1989). Patterns in Plant Development. New York, N.Y., Cambridge University Press.

Sussex, I. M. and N. M. Kerk (2001). "The evolution of plant architecture." Current Opinion in Plant Biology **4**(1): 33-37.

Ulmasov, T., J. Murfett, et al. (1997). "Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements." The Plant Cell Online **9**(11): 1963-1971.

van Hooijdonk, B., D. Woolley, et al. (2011). "Rootstocks Modify Scion Architecture, Endogenous Hormones, and Root Growth of Newly Grafted 'Royal Gala' Apple Trees." Journal of the American Society for Horticultural Science **136**(2): 93-102.

van Hooijdonk, B. M., D. J. Woolley, et al. (2010). "Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot signalling by auxin, gibberellin, and cytokinin." Journal of Horticultural Science & Biotechnology **85**(1): 59-65.

van Hooijdonk, B. M., Woolley, D.J., Warrington, I.J. and Tustin, D.S. (2010). "Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot signalling by auxin, gibberellin, and cytokinin." The Journal of Horticultural Science & Biotechnology **85**(1): 59-65.

Velasco, R., A. Zharkikh, et al. (2010). "The genome of the domesticated apple (Malus x domestica Borkh.)." Nat Genet **42**: 833 - 839.

Webster, A. D. (1995). "Rootstock and interstock effects on deciduous fruit tree vigour, precocity and yield productivity." New Zealand Journal of Crop and Horticultural Science **23**: 373 - 382.

Webster, A. D. and S. J. Wertheim (2003). Apple Rootstocks. Apples: Botany, Production and Uses. D. C. Ferree and I. J. Warrington. Wallingford, UK, CABI Publishing, CAB International.

Willmann, M. R. and R. S. Poethig (2011). "The effect of the floral repressor FLC on the timing and progression of vegetative phase change in Arabidopsis." Development **138**(4): 677-685.

CLAIMS

1. A method for producing a plant with at least one dwarfing-associated phenotype the method comprising altering the expression, or activity, of an ARF3 poypeptide in the plant.

2. The method of claim 1 comprising increasing the expression of the ARF3 poypeptide in the plant.

3. The method of claim 1 or 2 comprising transforming the plant to express the ARF3 poypeptide in the plant.

4. The method of claim 3 comprising transforming the plant with polynucleotide encoding the ARF3 polypeptide.

5. The method of claim 4 wherein polynucleotide is operably linked to a heterologous promoter.

6. The method of claim 1 comprising modifying the sequence of an endogenous polynucleotide encoding the ARF3 polypeptide in the plant.

7. The method of claim 6 wherein modifying the endogenous polynucleotide alters the activity of the ARF3 poypeptide in the plant to induce the dwarfing-associated phenotype.

8. The method of any one of claims 1 to 7 wherein the dwarfing-associated phenotype is selected from:

a) altered auxin transport,
b) slower auxin transport,
c) reduced apical dominance,
d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,
j) reduced vigour,
k) less vegetative growth,

l) earlier termination of shoot growth,
m) earlier competence to flower,
n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes, and
x) shorter intenode length
y) reduced scion mass.

9. The method of claim 8 wherein the dwarfing-associated phenotype is selected from a) to i).

10. The method of claim 8 wherein the dwarfing-associated phenotype is at least one of reduced apical dominance, a bushier habit, an altered xylem/phloem ratio, an increased number of phloem elements and reduced root mass.

11. The method of any one of claims 1 to 7 in which the dwarfing-associated phenotype is the competence to induce at least one of:
a) altered auxin transport,
b) slower auxin transport,
c) reduced apical dominance,
d) an altered xylem/phloem ratio,
e) an increased number of phloem elements,
f) smaller phloem elements,
g) thicker bark,
h) a bushier habit,
i) reduced root mass,
j) reduced vigour,
k) less vegetative growth,
l) earlier termination of shoot growth,
m) earlier competence to flower,

n) precocity,
o) earlier phase change,
p) smaller canopy,
q) reduced stem circumference,
r) reduced branch diameter,
s) fewer sylleptic branches,
t) shorter sylleptic branches,
u) more axillary flowers,
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length, and
y) reduced scion mass

in a scion grafted on to the plant.

12. The method of claim 11 wherein the dwarfing-associated phenotype is the competence to induce at least one of j) to y) in a scion grafted on to the plant.

13. The method of claim 11 wherein the dwarfing-associated phenotype is the competence to induce at least one of: reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduced scion mass, in a scion grafted on to the plant.

14. The method of any one of claims 1 to 8 wherein the method includes the step of grafting a scion on to a plant produced by the method.

15. The method of claim 14 in which the dwarfing-associated phenoytype is exhibited in a scion grafted onto the plant.

16. The method of claims 15 in which the dwarfing-associated phenoytype exhibited in the scion is at least one of j) to y).

17. A method for producing a plant with at least one dwarfing-associated phenotype selected from:

j) reduced vigour,
k) less vegetative growth
l) earlier termination of shoot growth
m) earlier competence to flower
n) precocity

o) earlier phase change
p) smaller canopy,
q) reduced stem circumference
r) reduced branch diameter
s) fewer sylleptic branches
t) shorter sylleptic branches
u) more axillary flowers
v) an earlier teminating primary axis,
w) earlier teminating secondary axes,
x) shorter intenode length
y) reduced scion mass

the method comprising the steps:

A. providing a plant with altered the expression or activity of a ARF3 poypeptide,
B. grafting a scion onto the plant in A

wherein at least one of j) to y) is exhibited in the scion grafted on to the plant in A.

18. The method of claim 18 the phenotype exhibited in the scion is at least one of: reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference, and reduced scion mass, in a scion grafted on to the plant.

19. The method of any one of claims 1 to 18 in which the ARF3 polypeptide has a sequence with at least 70% identity to any one of SEQ ID NO:1 to 11, 28 and 29.

20. The method of claim 19 in which the ARF3 polypeptide has a sequence with at least 70% identity to SEQ ID NO:1 or 28 (MdARF3).

21. The method of claim 19 or 20 in which the ARF3 polypeptide comprises a hydrophobic amino acid residue at the position corresponding amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

22. The method of claim 21 in which the ARF3 polypeptide comprises a Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

23. The method of any one of claims 19 to 22 in which the ARF3 polypeptide comprises the sequence of SEQ ID NO:2 or 29 (M9 MdARF3)

24. The method of claim 1 in which the modification results in expression of an ARF3 polypeptide with a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

25. The method of claim 24 in which the hydrophobic amino acid is a Leucine residue.

26. An isolated polynucleotide encoding an ARF3 polypeptide, or a fragment or variant thereof, comprising a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

27. The isolated polynucleotide of claim 26 wherein the hydrophobic amino acid residue is a Leucine residue.

28. The isolated polynucleotide of claim 26 or 27 wherein the ARF3 polypeptide comprises at least 70% identity to SEQ ID NO:2 or 29 (MdARF3).

29. The isolated polynucleotide of any one of claim 26 to 28 wherein the polypeptide comprises the sequence of SEQ ID NO:2 or 29 (M9 MdARF3).

30. The isolated polynucleotide of claim 26 that has at least 70% identity to at least one of SEQ ID NO:14 and 15.

31. An isolated polynucleotide comprising the sequence of SEQ ID NO:14 or 15, or a fragment of variant thereof.

32. An isolated ARF3 polypeptide, or a fragment or variant thereof, comprising a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

33. A construct comprising a polynucleotide of any one of claims 26 to 31.

34. The construct of claim 33 in which the polynucleotide sequence is operably linked to a heterologous promoter.

35. A cell comprising at least one of:
a) a polynucleotide a polynucleotide of any one of claims 26 to 31, or
b) a construct of claim 33 or 34.

36. A plant comprising at least one of:
a) a polynucleotide a polynucleotide of any one of claims 26 to 31, or
b) a construct of claim 33 or 34.

37. A part, propagule or progeny of a plant of claim 36, comprising the polynucleotide or construct.

38. A method for identifying a plant with a genotype indicative of at least one dwarfing-associated phenotype, the method comprising testing a plant for at least one of:

a) altered expression of at least one ARF3 polypeptide,
b) altered expression of at least one ARF3 polynucleotide,
c) presence of a marker associated with altered expression of at least one ARF3 polypeptide,
d) presence of a marker associated with altered expression of at least one ARF3 polynucleotide,
e) presence of a marker associated with altered activity of at least one ARF3 polypeptide,

wherein presence of any of a) to e) indicates that the plant has at least one dwarfing-associated phenotype.

39. The method of claim 38 in which the marker associated with altered activity of at least one ARF3 polypeptide is presence of a hydrophobic amino acid residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

40. The method of claim 39 in which the the hydrophobic amino acid residue is a Leucine residue.

41. The method of claim 40 in which the method involves detection of a polynucleotide encoding the Leucine residue at a position corresponding the amino acid residue 72 in SEQ ID NO:1 or 28 (MdARF3).

42. The method of any one of claims 38 to 41 includes an additional step of at least one of:
a) cultivating the identified plant, and
b) breeding from the identified plant.

43. A method for producing a plant with at least one dwarfing-associated phenotype, the method comprising crossing one of:

a) a plant of claim 36,

b) a plant produced by a method of any one of claims 1 to 25, and

c) a plant identified by a method of any one of claims 38 to 41,

with another plant, wherein the off-spring produced by the crossing is a plant with at least one dwarfing-associated phenotype.

44. In a further embodiment the invention provides a method of producing a plant with at least one dwarfing-associated phenotype selected from:

a) altered auxin transport,

b) slower auxin transport,

c) reduced apical dominance,

d) an altered xylem/phloem ratio,

e) an increased number of phloem elements,

f) smaller phloem elements,

g) thicker bark,

h) a bushier habit,

i) reduced root mass,

j) reduced vigour,

k) less vegetative growth,

l) earlier termination of shoot growth,

m) earlier competence to flower,

n) precocity,

o) earlier phase change,

p) smaller canopy,

q) reduced stem circumference,

r) reduced branch diameter,

s) fewer sylleptic branches,

t) shorter sylleptic branches,

u) more axillary flowers,

v) an earlier teminating primary axis,

w) earlier teminating secondary axes,

x) shorter intenode length,

y) reduced scion mass,

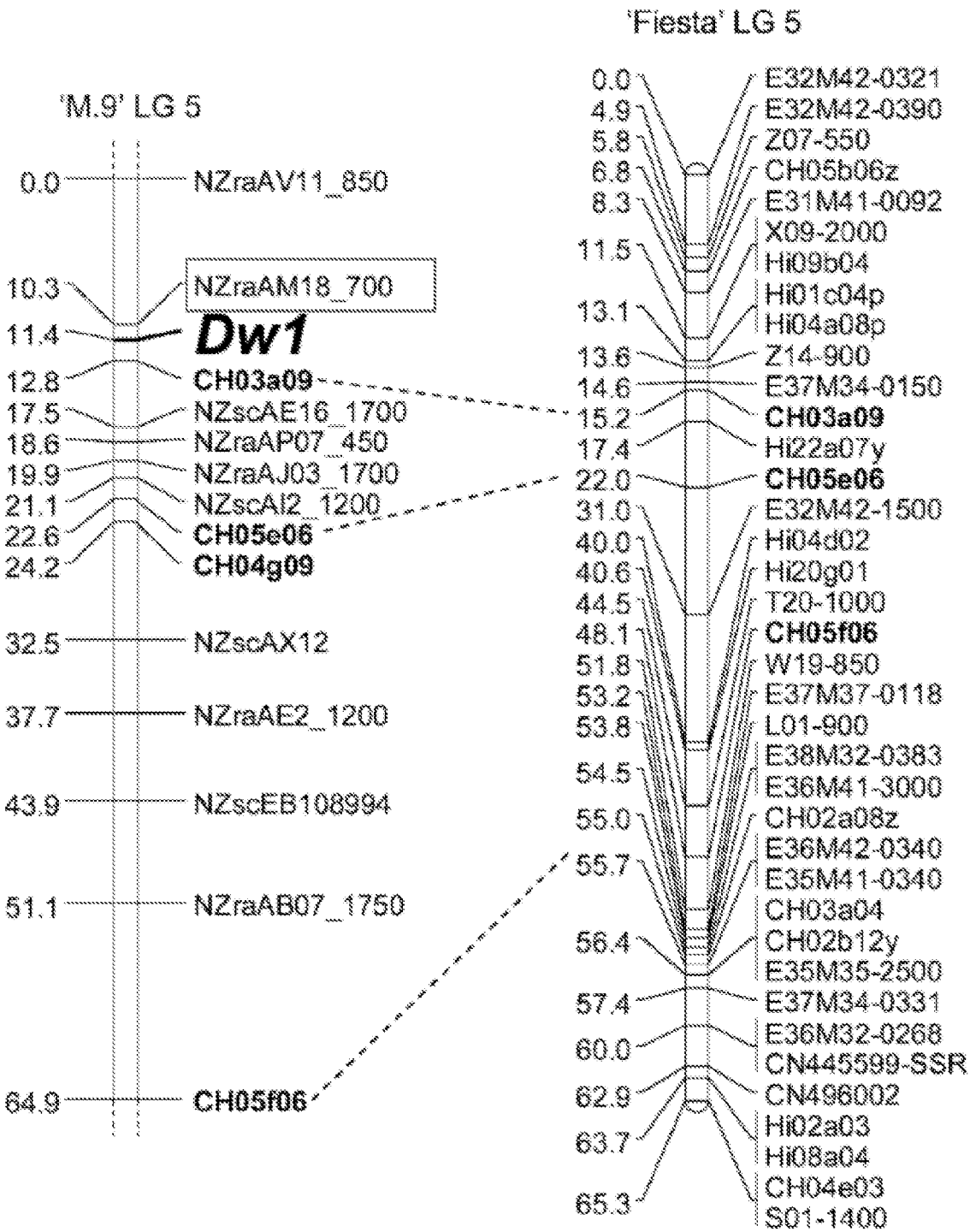
the method comprising grafting a scion onto at least one of:

A) a plant of claim 36,
B) a plant produced by a method of any one of claims 1 to 25, and
C) a plant identified by a method of any one of claims 38 to 41.

45. The method of claim 44 in which the at least one dwarfing associated phenotype is exhibited in the grafted scion.

46. The method of claim 45 in which the grafted scion exhibits at least one of j) to y).

47. The method of claim 46 in which the grafted scion exhibits at least one of reduced vigour, less vegetative growth, earlier termination of shoot growth, a smaller canopy, reduced stem circumference and reduced scion mass.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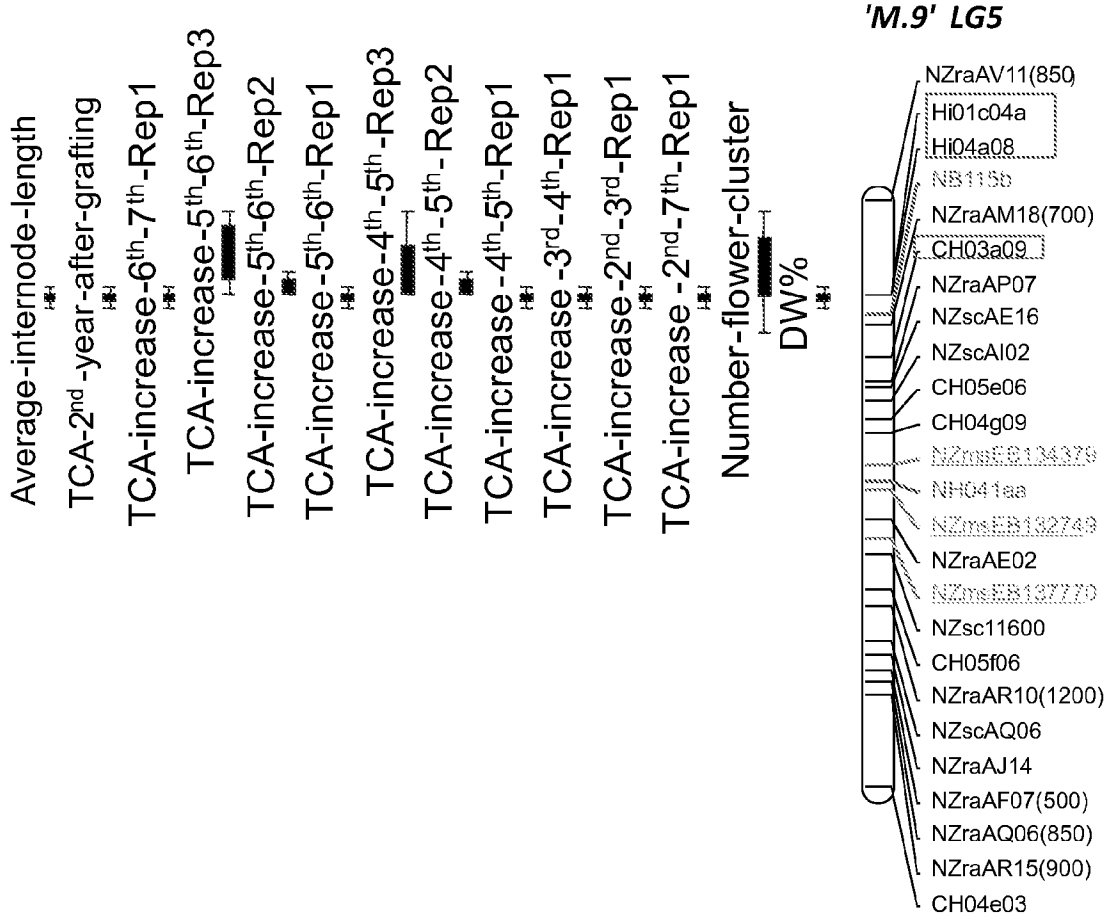
0.0 NZraAV11_850
10.3 NZraAM18_700
11.4 Dw1
12.8 CH03a09
17.5 NZscAE16_1700
18.6 NZraAP07_450
19.9 NZraAJ03_1700
21.1 NZscAI2_1200
22.6 CH05e06
24.2 CH04g09
32.5 NZscAX12
37.7 NZraAE2_1200
43.9 NZscEB108994
51.1 NZraAB07_1750
64.9 CH05f06
'Fiesta' LG 5
0.0 E32M42-0321
4.9 E32M42-0390
5.8 Z07-550
6.8 CH05b06z
8.3 E31M41-0092
11.5 X09-2000
Hi09b04
13.1 Hi01c04p
Hi04a08p
13.6 Z14-900
14.6 E37M34-0150
15.2 CH03a09
17.4 Hi22a07y
22.0 CH05e06
31.0 E32M42-1500
40.0 Hi04d02
40.6 Hi20g01
44.5 T20-1000
48.1 CH05f06
51.8 W19-850
53.2 E37M37-0118
53.8 L01-900
E38M32-0383
54.5 E36M41-3000
55.0 CH02a08z
55.7 E36M42-0340
E35M41-0340
CH03a04
56.4 CH02b12y
E35M35-2500
57.4 E37M34-0331
60.0 E36M32-0268
CN445599-SSR
62.9 CN496002
63.7 Hi02a03
Hi08a04
65.3 CH04e03
S01-1400

FIGURE 1a


Average-internode-length
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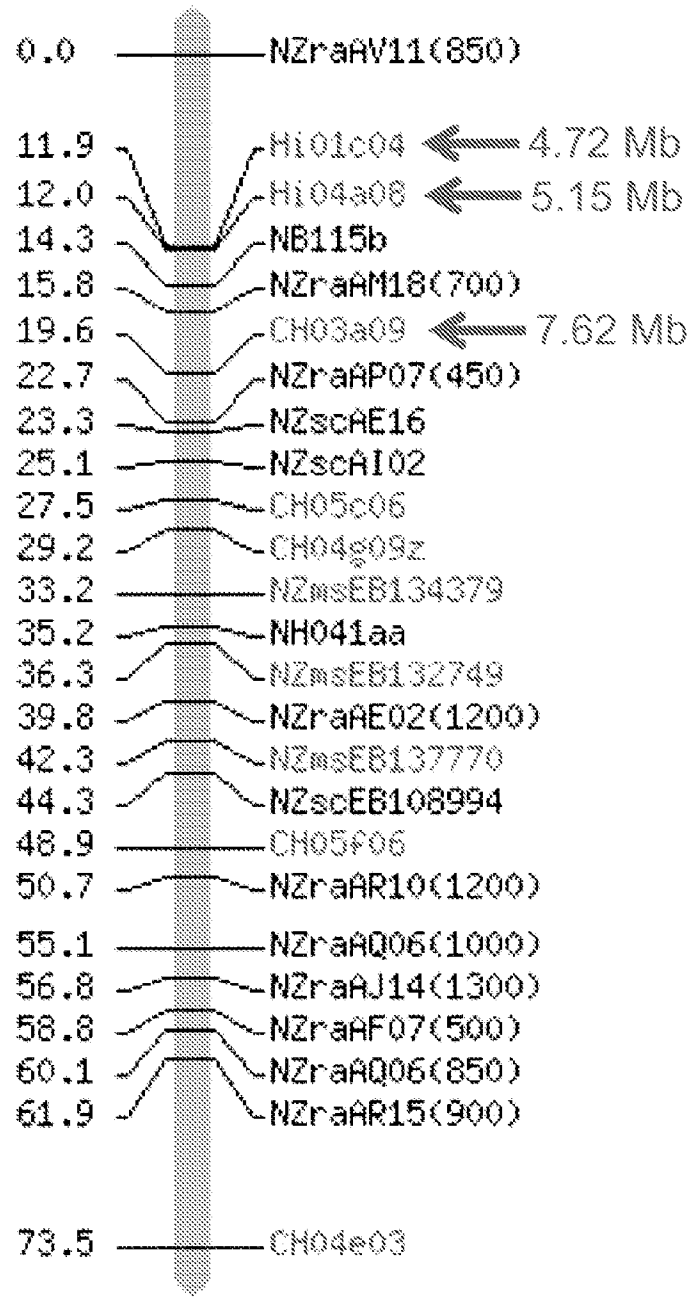
TCA-increase-5th-6th-Rep3
TCA-increase-5th-6th-Rep2
TCA-increase-5th-6th-Rep1
TCA-increase-4th-5th-Rep3
TCA-increase-4th-5th-Rep2
TCA-increase-4th-5th-Rep1
TCA-increase-3rd-4th-Rep1
TCA-increase-2nd-3rd-Rep1
TCA-increase-2nd-7th-Rep1
Number-flower-cluster
DW%
'M.9' LG5
NZraAV11(850)
Hi01c04a
Hi04a08
NB115b
NZraAM18(700)
CH03a09
NZraAP07
NZscAE16
NZscAI02
CH05e06
CH04g09
NZmsEB134379
NH041aa
NZmsEB132749
NZraAE02
NZmsEB137770
NZsc11600
CH05f06
NZraAR10(1200)
NZscAQ06
NZraAJ14
NZraAF07(500)
NZraAQ06(850)
NZraAR15(900)
CH04e03

FIGURE 1b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11.9
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NB115b
15.8
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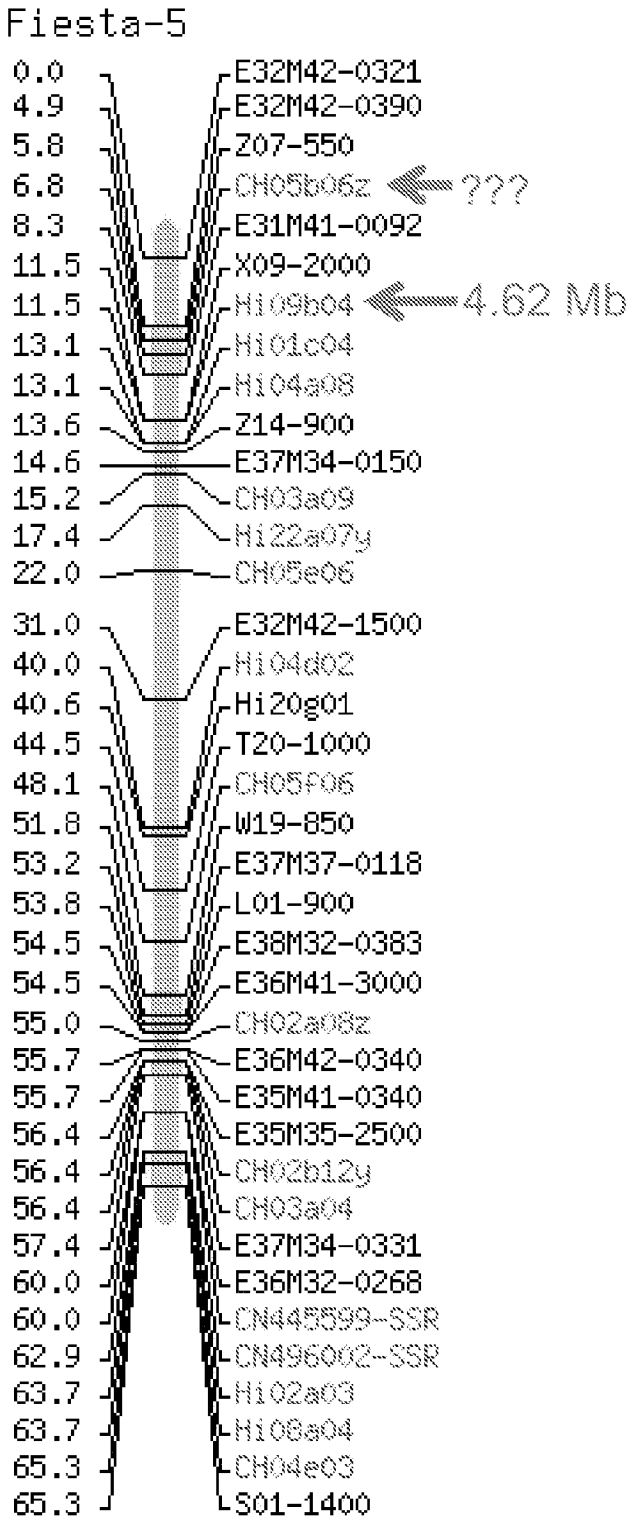
19.6
CH03a09
7.62 Mb
22.7
NZraAP07(450)
23.3
NZscAE16
25.1
NZscAI02
27.5
CH05c06
29.2
CH04g09z
33.2
NZmsEB134379
35.2
NH041aa
36.3
NZmsEB132749
39.8
NZraAE02(1200)
42.3
NZmsEB137770
44.3
NZscEB108994
48.9
CH05f06
50.7
NZraAR10(1200)
55.1
NZraAQ06(1000)
56.8
NZraAJ14(1300)
58.8
NZraAF07(500)
60.1
NZraAQ06(850)
61.9
NZraAR15(900)
73.5
CH04e03

FIGURE 2a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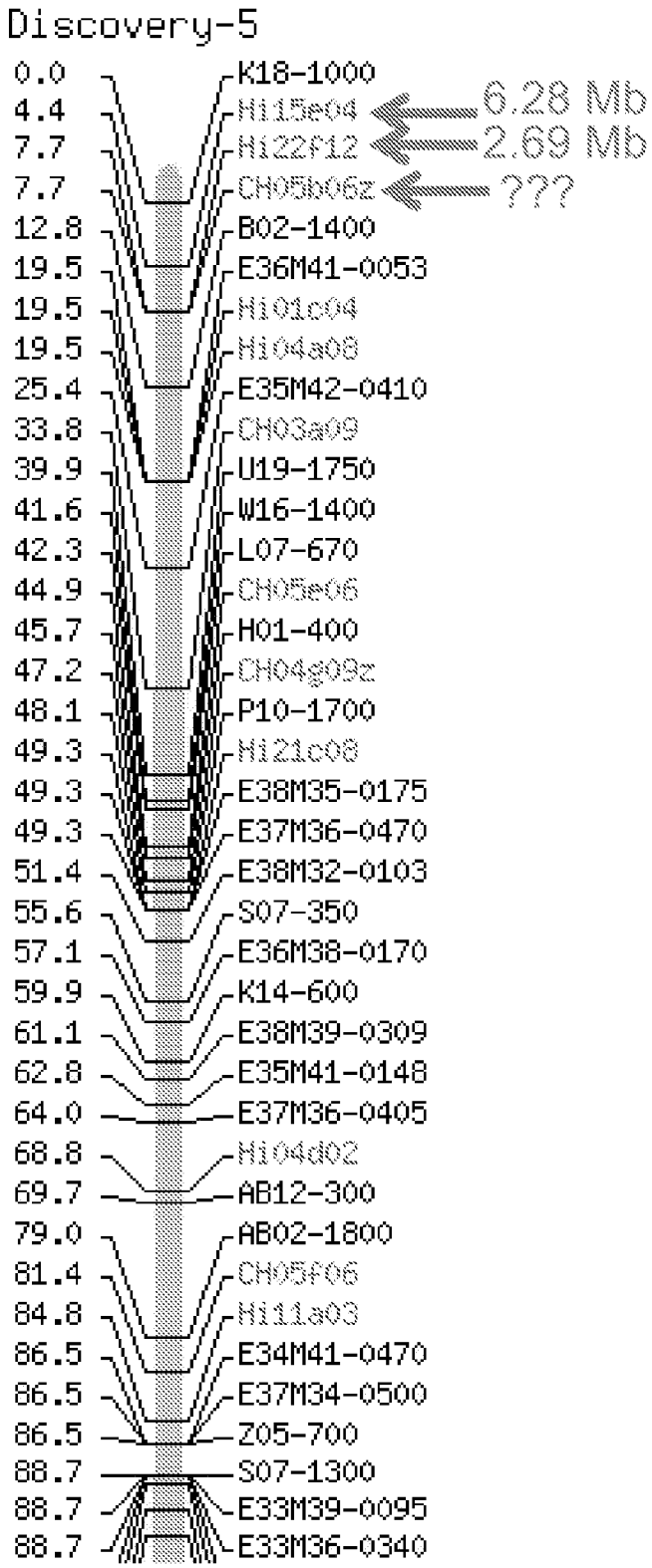
4.9 E32M42-0390
5.8 Z07-550
6.8 CH05b06z ???
8.3 E31M41-0092
11.5 X09-2000
11.5 Hi09b04 4.62 Mb
13.1 Hi01c04
13.1 Hi04a08
13.6 Z14-900
14.6 E37M34-0150
15.2 CH03a09
17.4 Hi22a07y
22.0 CH05e06
31.0 E32M42-1500
40.0 Hi04d02
40.6 Hi20g01
44.5 T20-1000
48.1 CH05f06
51.8 W19-850
53.2 E37M37-0118
53.8 L01-900
54.5 E38M32-0383
54.5 E36M41-3000
55.0 CH02a08z
55.7 E36M42-0340
55.7 E35M41-0340
56.4 E35M35-2500
56.4 CH02b12y
56.4 CH03a04
57.4 E37M34-0331
60.0 E36M32-0268
60.0 CN445599-SSR
62.9 CN496002-SSR
63.7 Hi02a03
63.7 Hi08a04
65.3 CH04e03
65.3 S01-1400

FIGURE 2b


Discovery-5
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7.7 Hi22f12
7.7 CH05b06z
12.8 B02-1400
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19.5 Hi01c04
19.5 Hi04a08
25.4 E35M42-0410
33.8 CH03a09
39.9 U19-1750
41.6 W16-1400
42.3 L07-670
44.9 CH05e06
45.7 H01-400
47.2 CH04g09z
48.1 P10-1700
49.3 Hi21c08
49.3 E38M35-0175
49.3 E37M36-0470
51.4 E38M32-0103
55.6 S07-350
57.1 E36M38-0170
59.9 K14-600
61.1 E38M39-0309
62.8 E35M41-0148
64.0 E37M36-0405
68.8 Hi04d02
69.7 AB12-300
79.0 AB02-1800
81.4 CH05f06
84.8 Hi11a03
86.5 E34M41-0470
86.5 E37M34-0500
86.5 Z05-700
88.7 S07-1300
88.7 E33M39-0095
88.7 E33M36-0340
6.28 Mb
2.69 Mb
???

FIGURE 2c

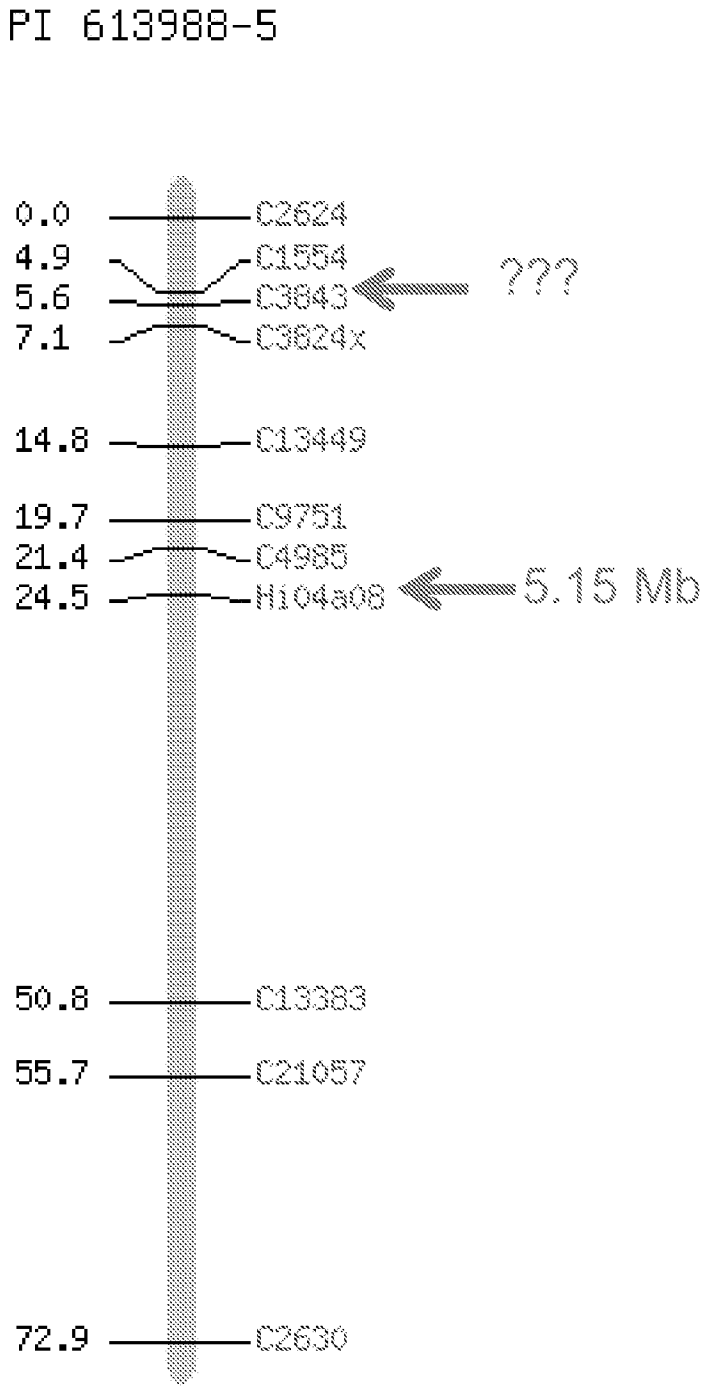
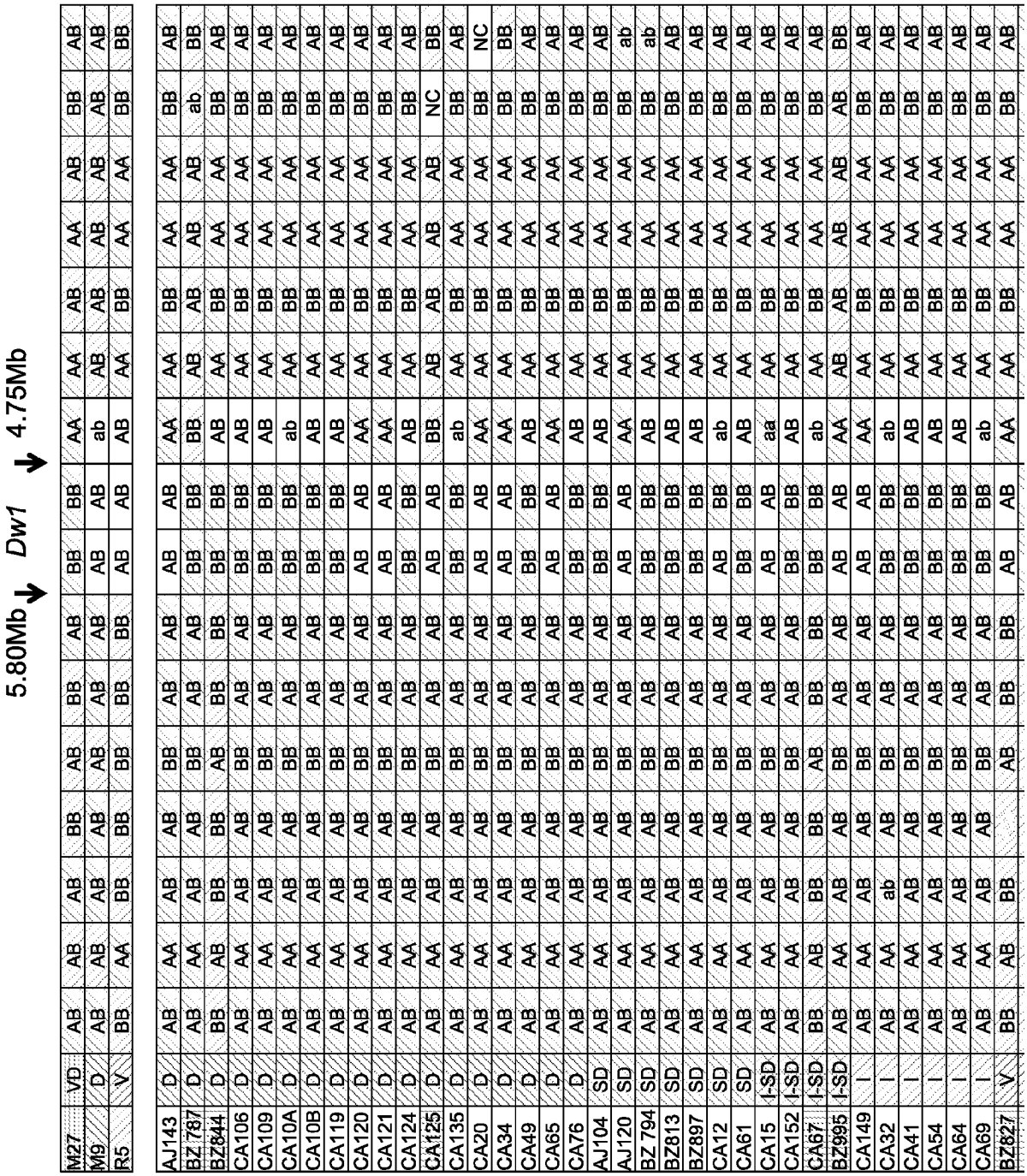

PI 613988-5
0.0
4.9
5.6
7.1
14.8
19.7
21.4
24.5
50.8
55.7
72.9
C2624
C1554
C3843
C3824x
C13449
C9751
C4985
Hi04a08
C13383
C21057
C2630
???
5.15 Mb

FIGURE 2d


5.80Mb
Dw1
4.75Mb
M27 VD AB AB AB BB AB AB BB BB AA AA AB AB BB AB
M9 D AB AB AB AB AB AB AB AB ab AB AB AB AB AB
R5 V BB AA BB BB BB BB AB AB AB AB BB AA AA AB
AJ143 D AB AA AB AB BB AB AB AB AB AA AA AA BB AA AA BB AB
BZ 787 D AB AA AB AB BB AB AB BB BB BB AB AB AB ab BB
BZ844 D BB AB BB BB AB BB BB BB BB AB AA BB AA AA BB AB
CA106 D AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA109 D AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA10A D AB AA AB AB BB AB AB BB BB ab AA BB AA AA BB AB
CA10B D AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
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CA121 D AB AA AB AB BB AB AB AB AB AA AA BB AA AA BB AB
CA124 D AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA125 D AB AA AB AB BB AB AB AB AB BB AB AB AB AB NC BB
CA135 D AB AA AB AB BB AB AB BB BB ab AA BB AA AA BB AB
CA20 D AB AA AB AB BB AB AB AB AB AA AA BB AA AA BB NC
CA34 D AB AA AB AB BB AB AB AB AB AA AA BB AA AA BB BB
CA49 D AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA65 D AB AA AB AB BB AB AB AB AB AA AA BB AA AA BB AB
CA76 D AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
AJ104 SD AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
AJ120 SD AB AA AB AB BB AB AB AB AB AA AA BB AA AA BB ab
BZ 794 SD AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB ab
BZ813 SD AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
BZ897 SD AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA12 SD AB AA AB AB BB AB AB AB BB ab AA BB AA AA BB AB
CA61 SD AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA15 I-SD AB AA AB AB BB AB AB AB AB aa AA BB AA AA BB AB
CA152 I-SD AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA67 I-SD BB AB BB AB BB BB BB BB BB ab AA BB AA AA BB AB
BZ995 I-SD AB AA AB AB BB AB AB AB AB AA AB AB AB AB AB BB
CA149 I AB AA AB AB BB AB AB AB AB AA AA BB AA AA BB AB
CA32 I AB AA ab AB BB AB AB BB BB ab AA BB AA AA BB AB
CA41 I AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA54 I AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
CA64 I AB AA AB AB BB AB AB BB BB AB AA BB AA AA BB AB
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BZ827 V BB AB BB AB BB BB AB AB AB AA AA BB AA AA BB AB

FIGURE 3

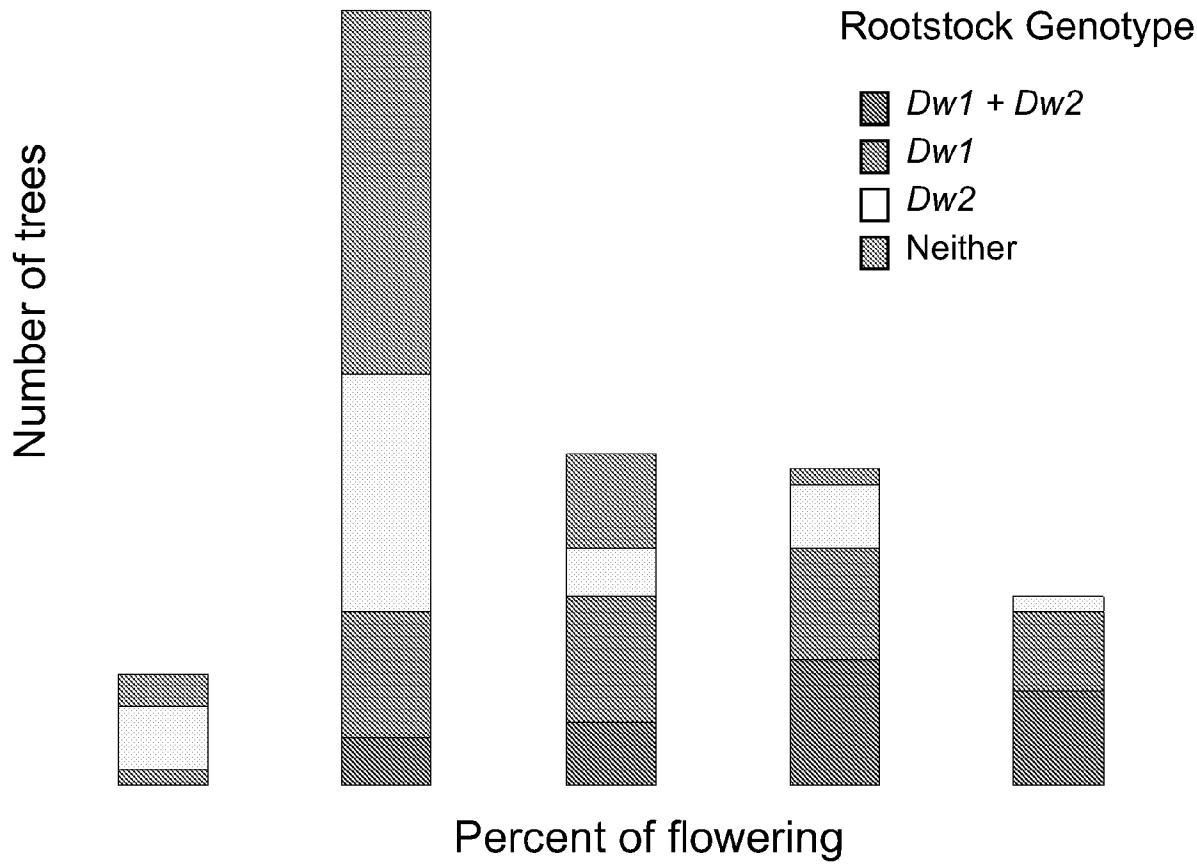
Rootstock Genotype
Dw1 + Dw2
Dw1
Dw2
Neither
Number of trees
Percent of flowering

FIGURE 4

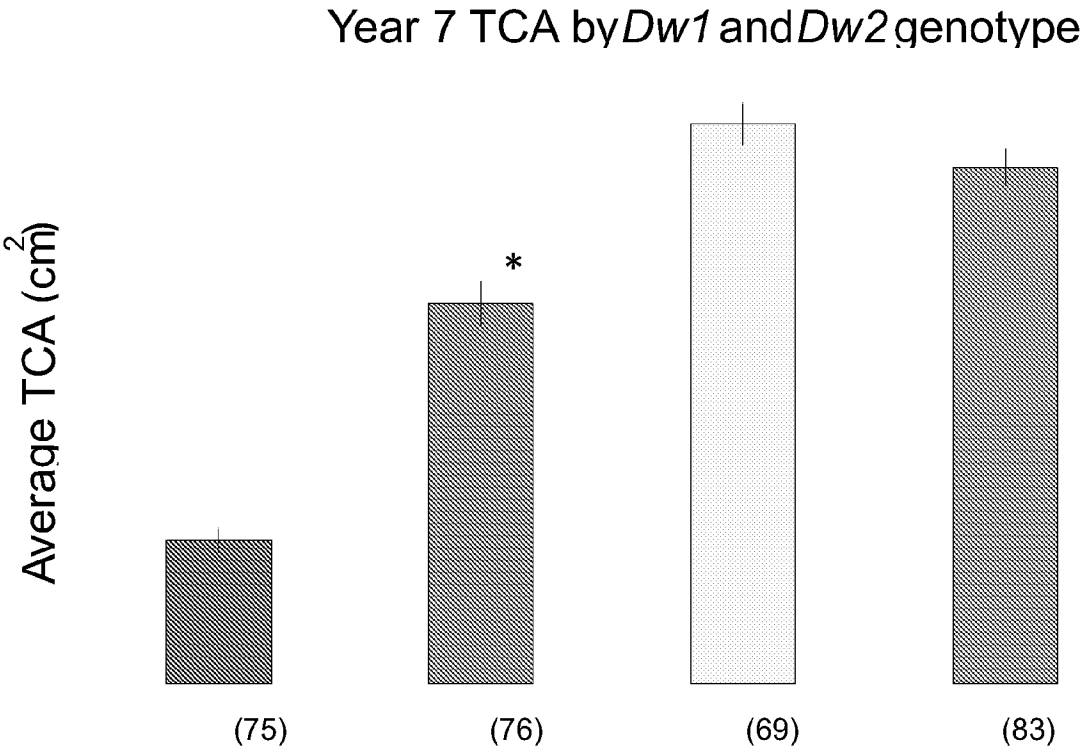

FIGURE 5

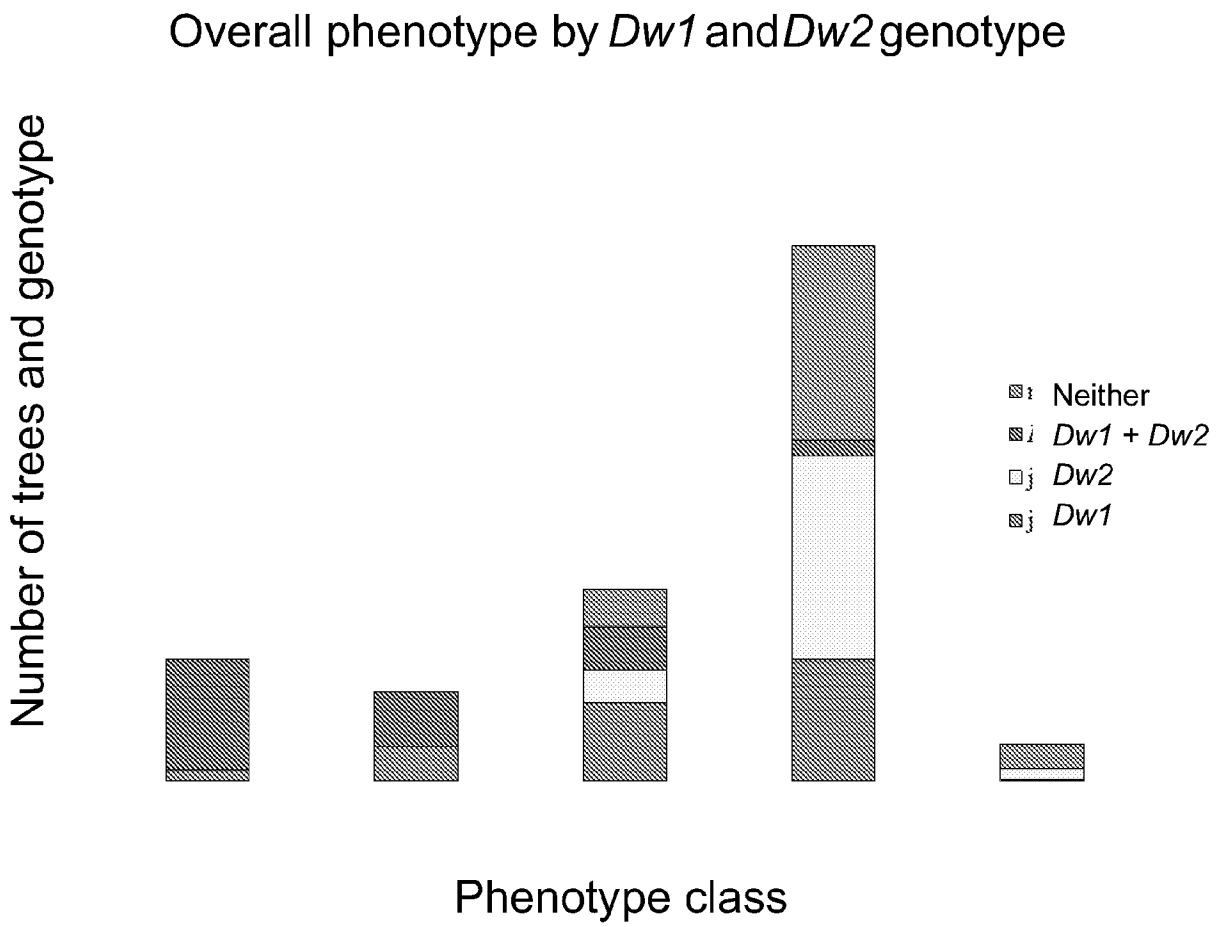
Overall phenotype by *Dw1* and *Dw2* genotype
Number of trees and genotype
Neither
Dw1 + *Dw2*
Dw2
Dw1
Phenotype class

FIGURE 6

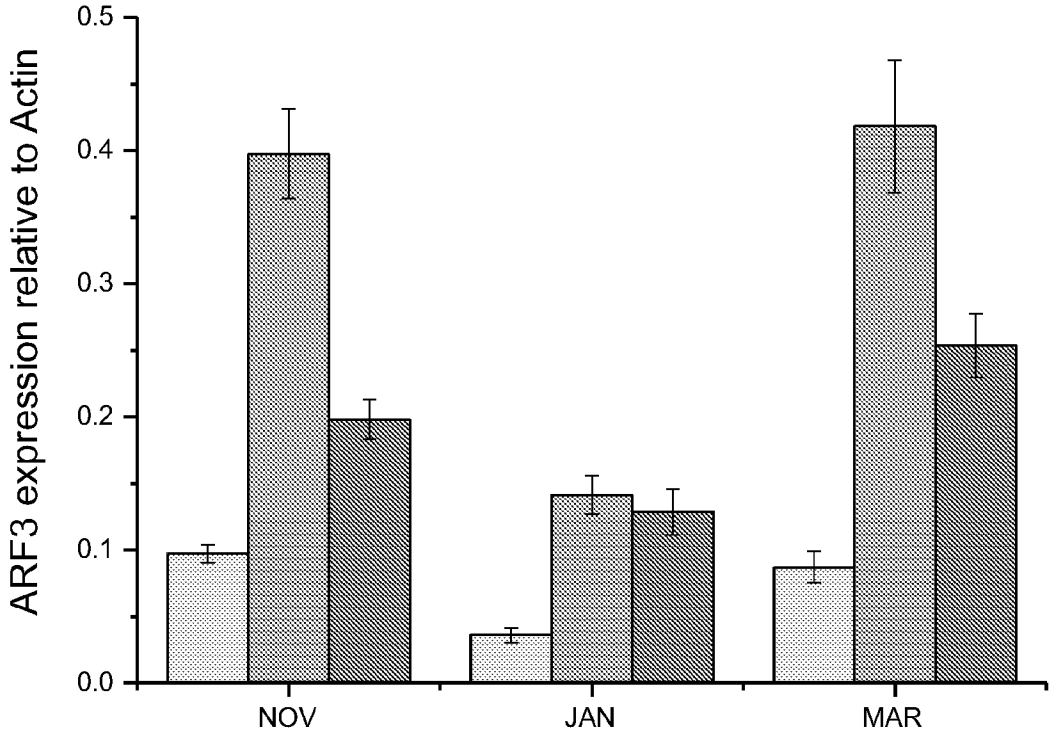
ARF3 expression relative to Actin
0.5
0.4
0.3
0.2
0.1
0.0
NOV
JAN
MAR

FIGURE 7

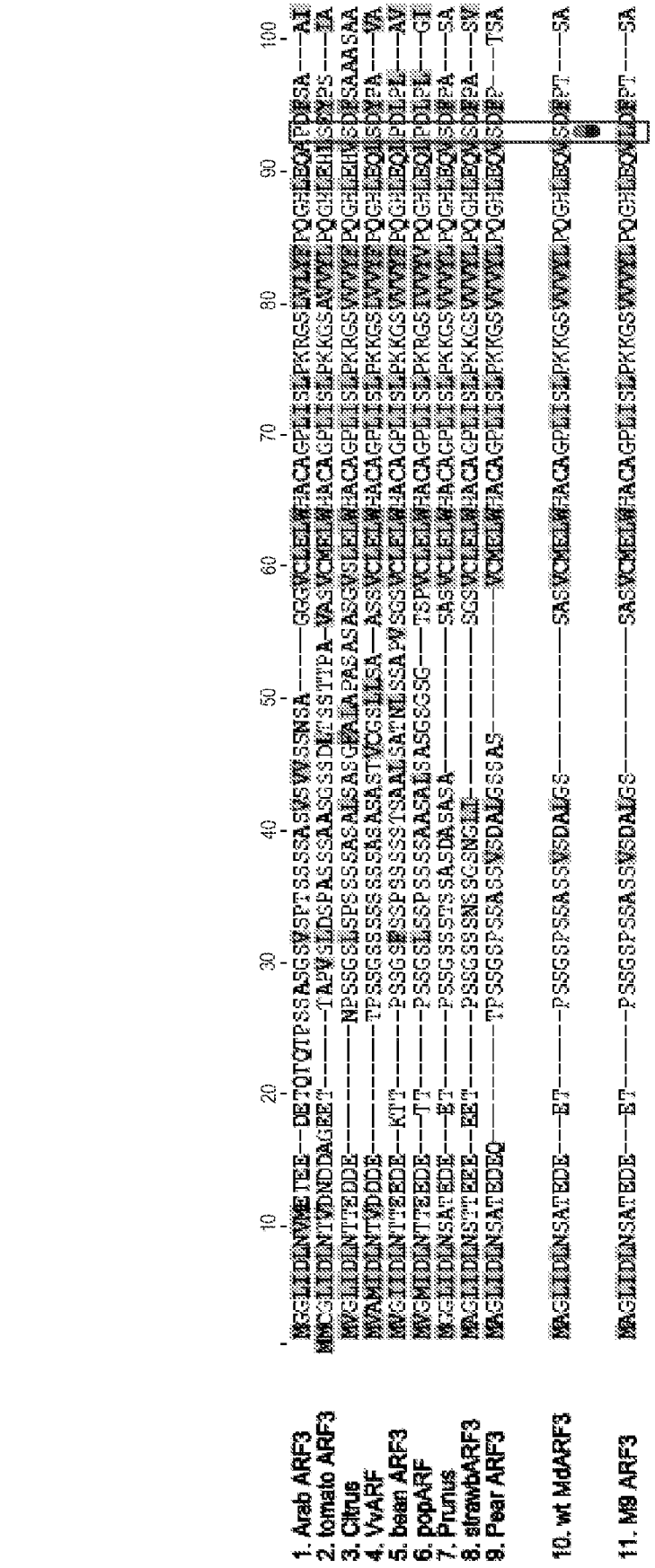
1. Arab ARF3
2. tomato ARF3
3. Citrus
4. VvARF
5. bean ARF3
6. popARF
7. Prunus
8. strawbARF3
9. Pear ARF3
10. wt MdARF3
11. M9 ARF3

FIGURE 8

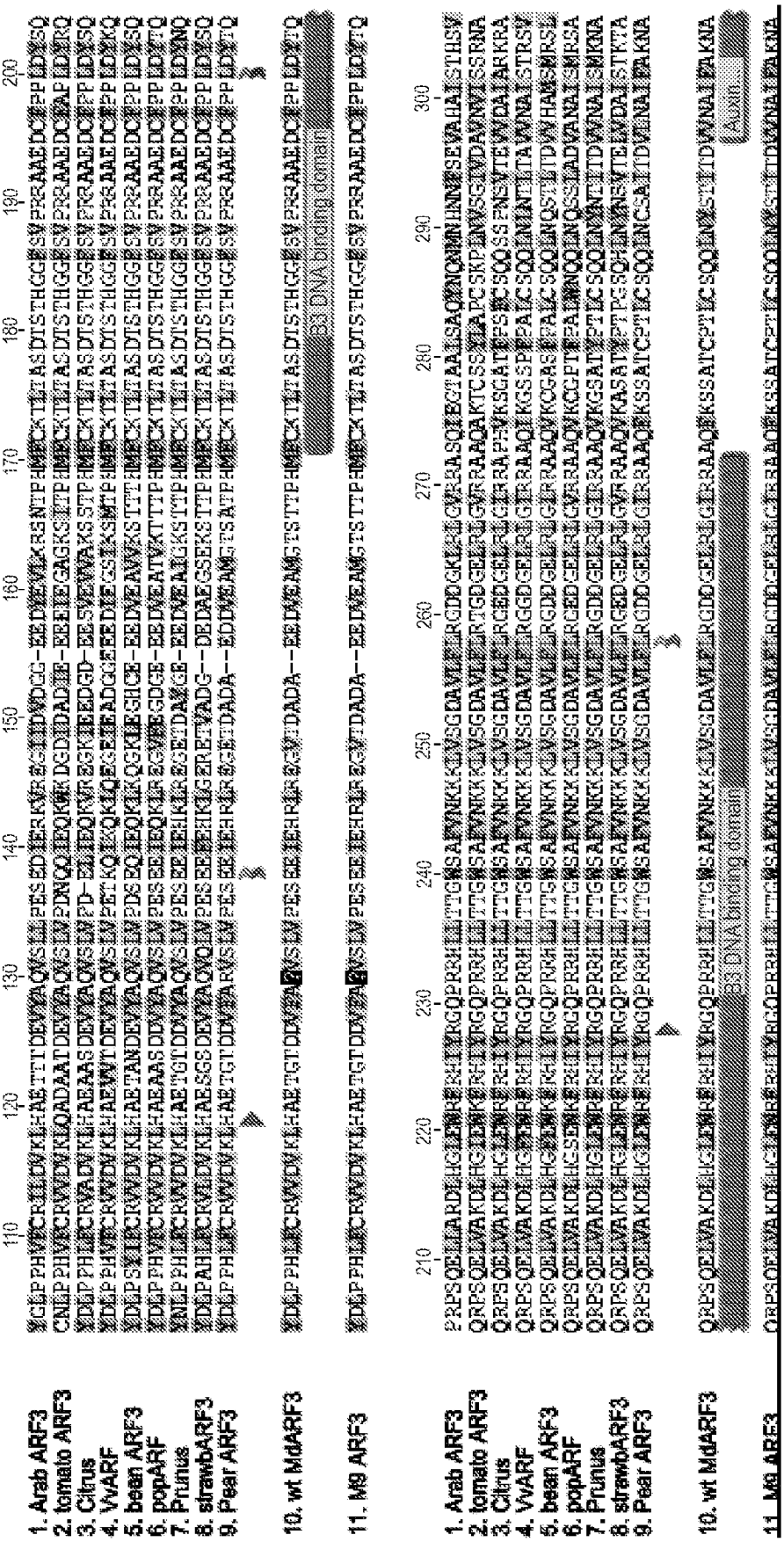

FIGURE 8
(continued)

	Arab ARF3	tomato ARF3	Citrus	VvARF	bean ARF3	popARF	Prunus	strawbARF3	Pear ARF3	wt MdARF3	M9 ARF3
Arab ARF3		42.2%	46.9%	44.6%	46.9%	48.0%	47.2%	45.4%	45.5%	46.7%	46.7%
tomato ARF3	42.2%		50.9%	55.6%	55.1%	59.7%	53.3%	49.1%	50.8%	53.1%	53.0%
Citrus	46.9%	50.9%		64.0%	66.6%	61.9%	64.1%	59.5%	60.0%	60.7%	60.6%
VvARF	44.6%	55.6%	64.0%		64.7%	60.0%	63.4%	58.3%	59.1%	58.8%	58.7%
bean ARF3	46.9%	55.1%	66.6%	64.7%		68.6%	63.3%	58.8%	59.0%	62.1%	62.1%
popARF	48.0%	59.7%	61.9%	60.0%	68.6%		59.8%	57.3%	58.0%	58.9%	58.9%
Prunus	47.2%	53.3%	64.1%	63.4%	63.3%	59.8%		72.7%	77.6%	81.1%	80.9%
strawbARF3	45.4%	49.1%	59.5%	58.3%	58.8%	57.3%	72.7%		68.3%	69.5%	69.3%
Pear ARF3	45.5%	50.8%	60.0%	59.1%	59.0%	58.0%	77.6%	68.3%		89.2%	89.1%
wt MdARF3	46.7%	53.1%	60.7%	58.8%	62.1%	58.9%	81.1%	69.5%	89.2%		99.1%
M9 ARF3	46.7%	53.0%	60.6%	58.7%	62.1%	58.9%	80.9%	69.3%	89.1%	99.1%	

FIGURE 9

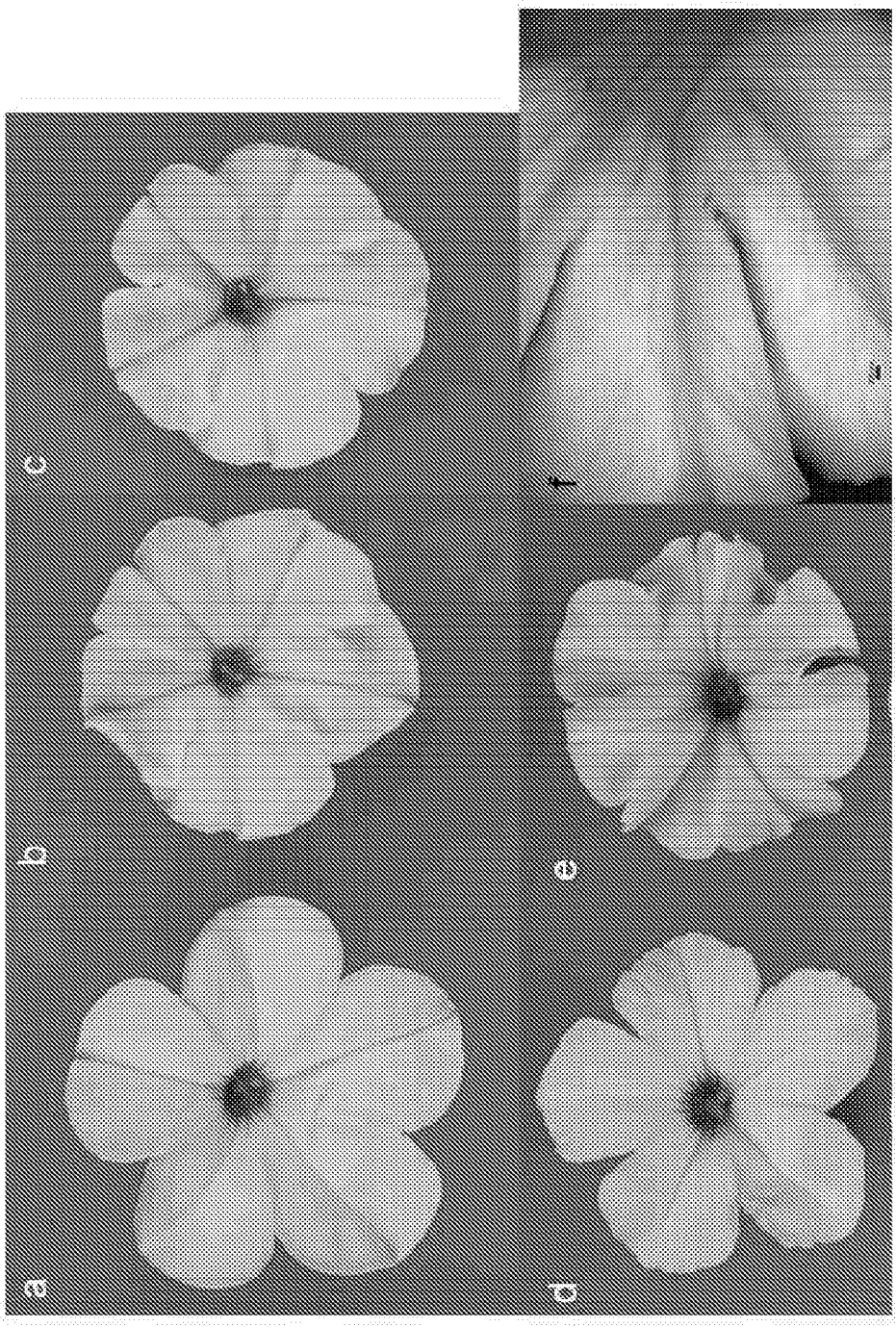

FIGURE 10

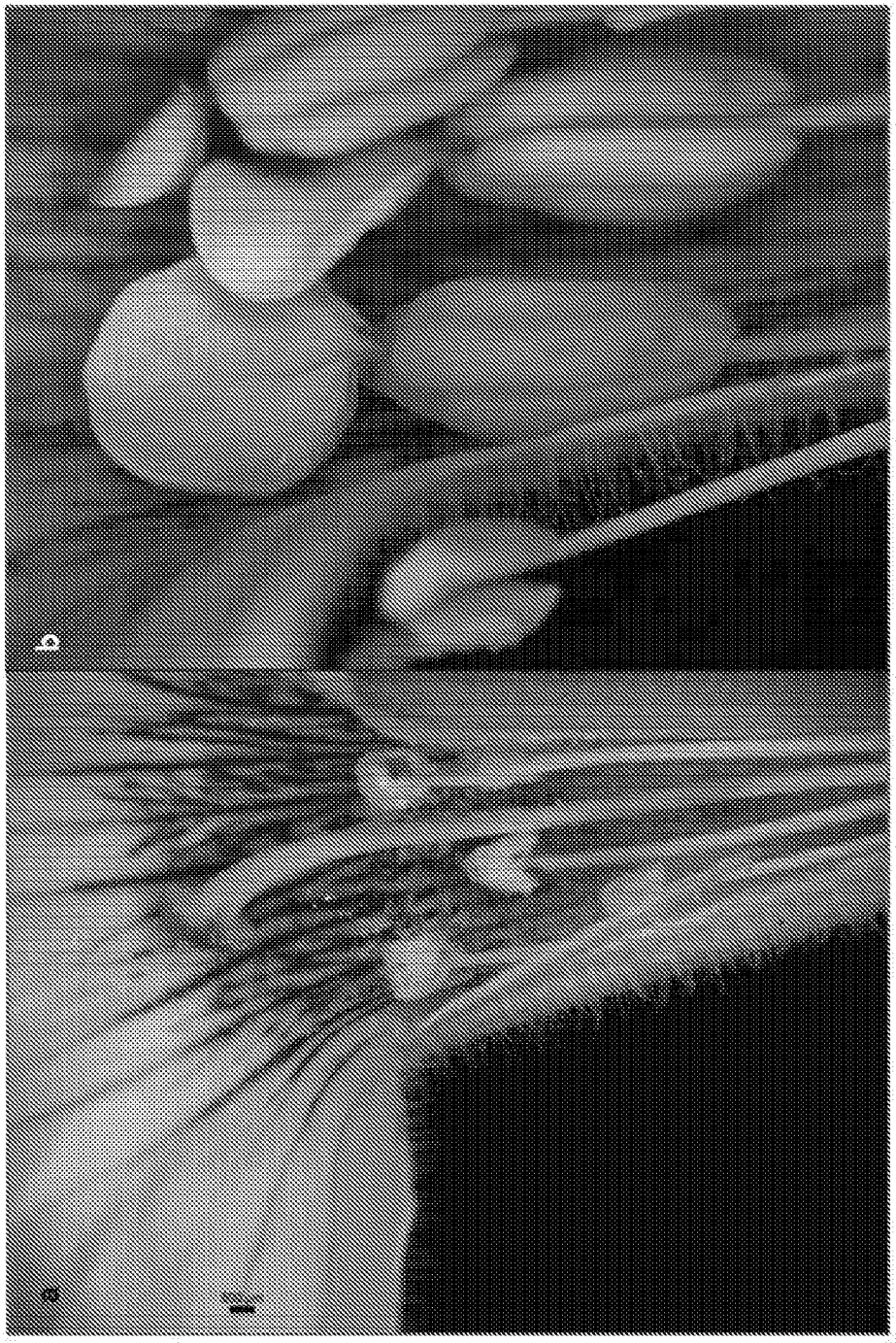

FIGURE 11

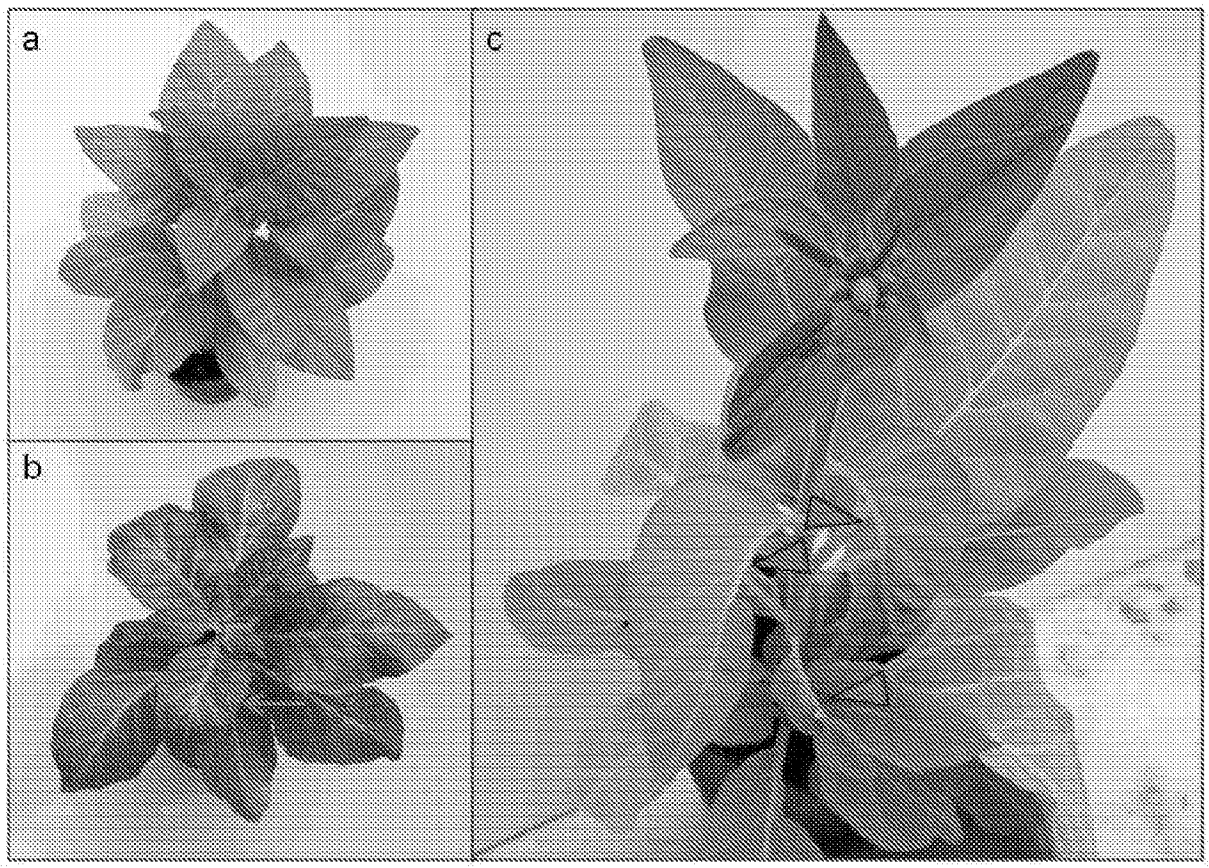

FIGURE 12

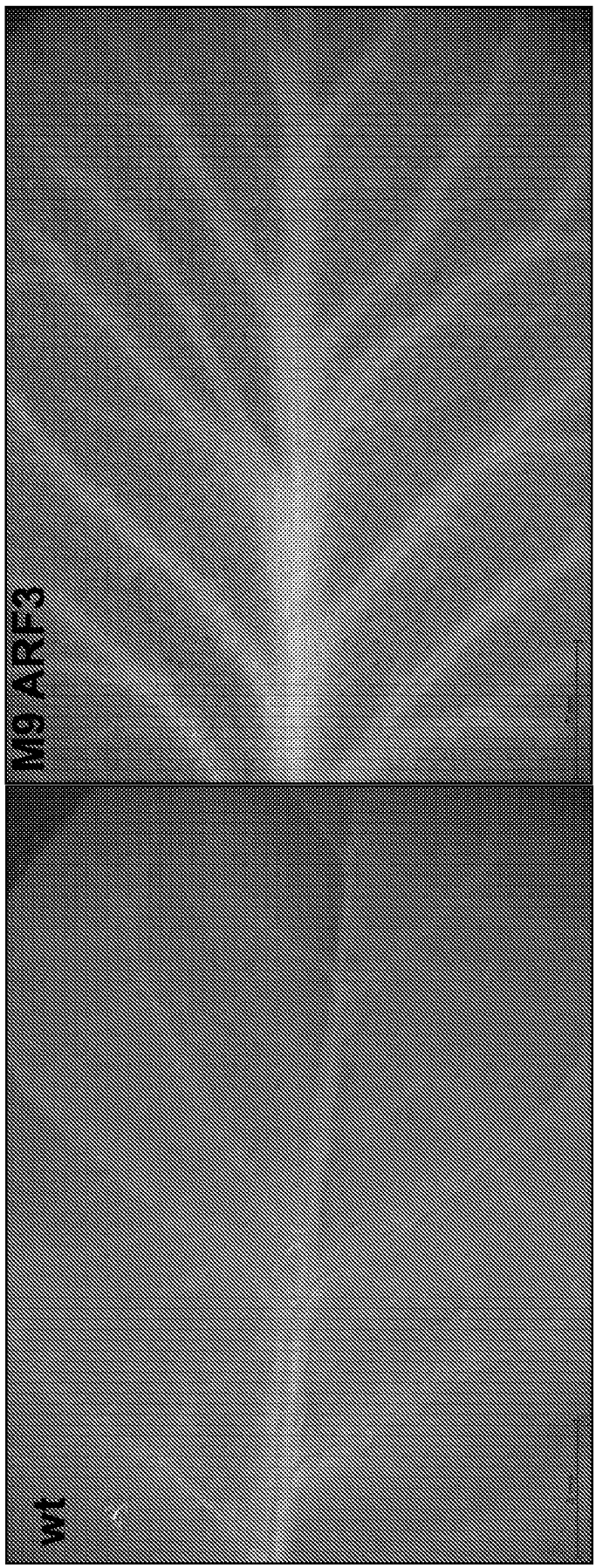

FIGURE 13

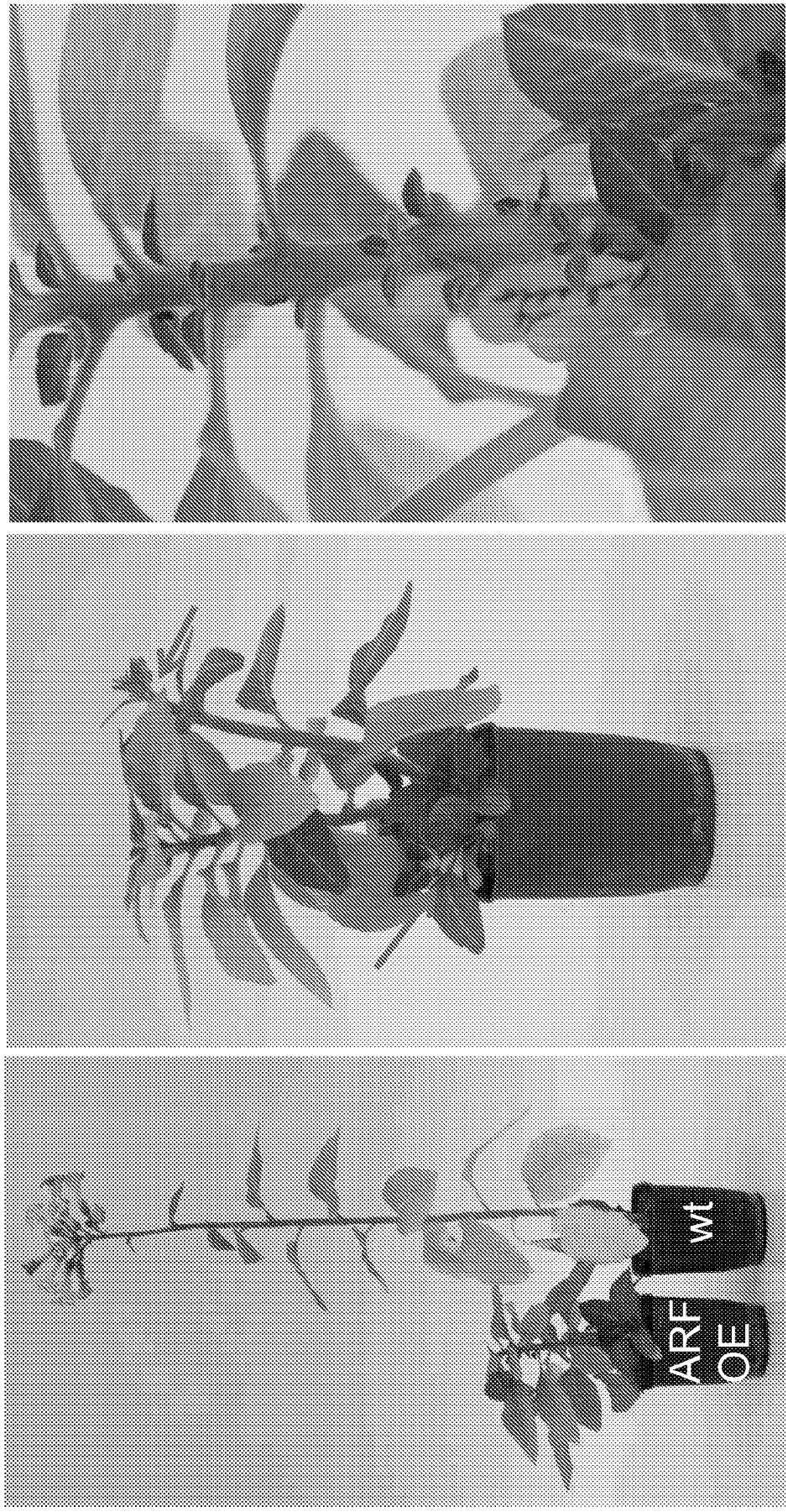

FIGURE 14

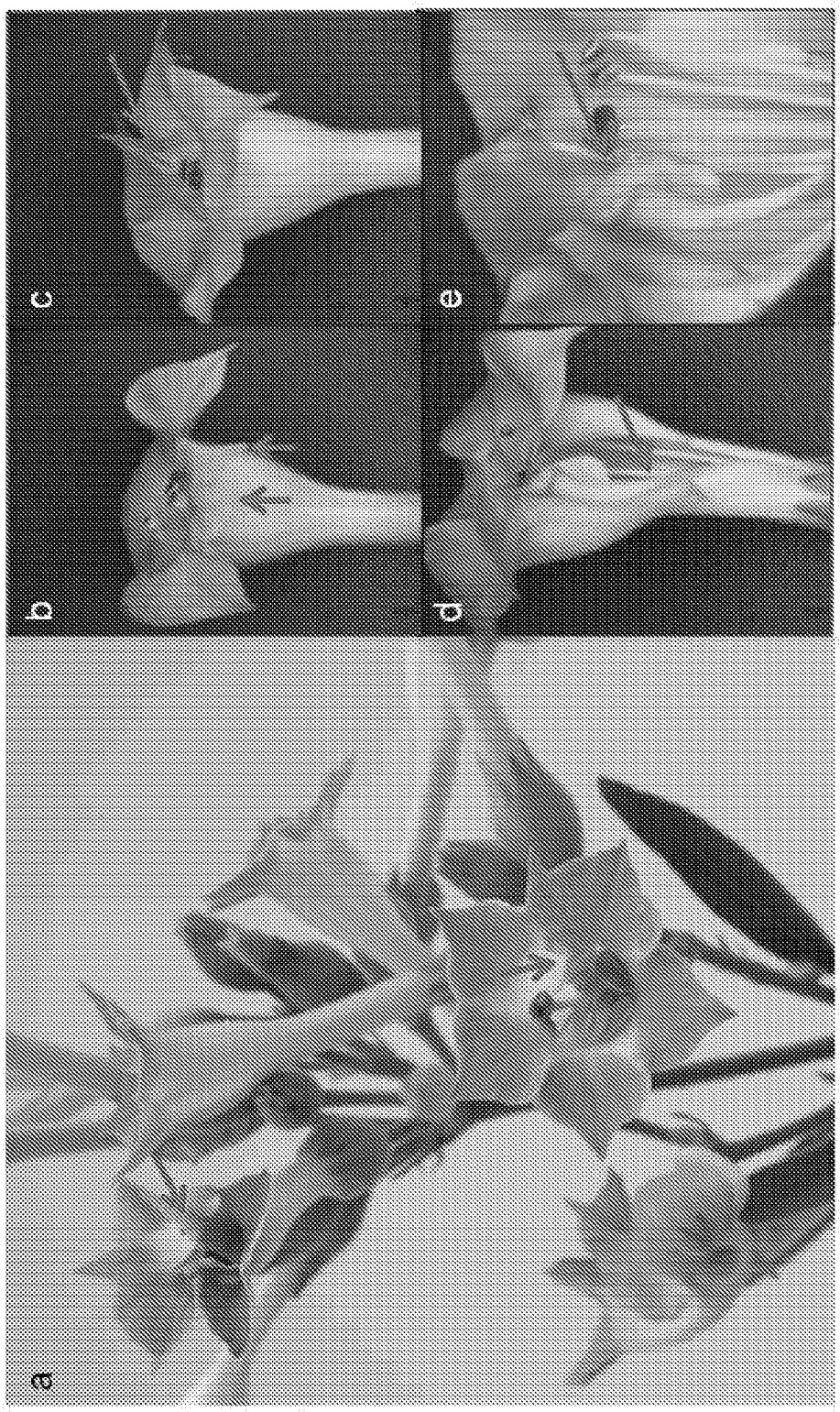

FIGURE 15

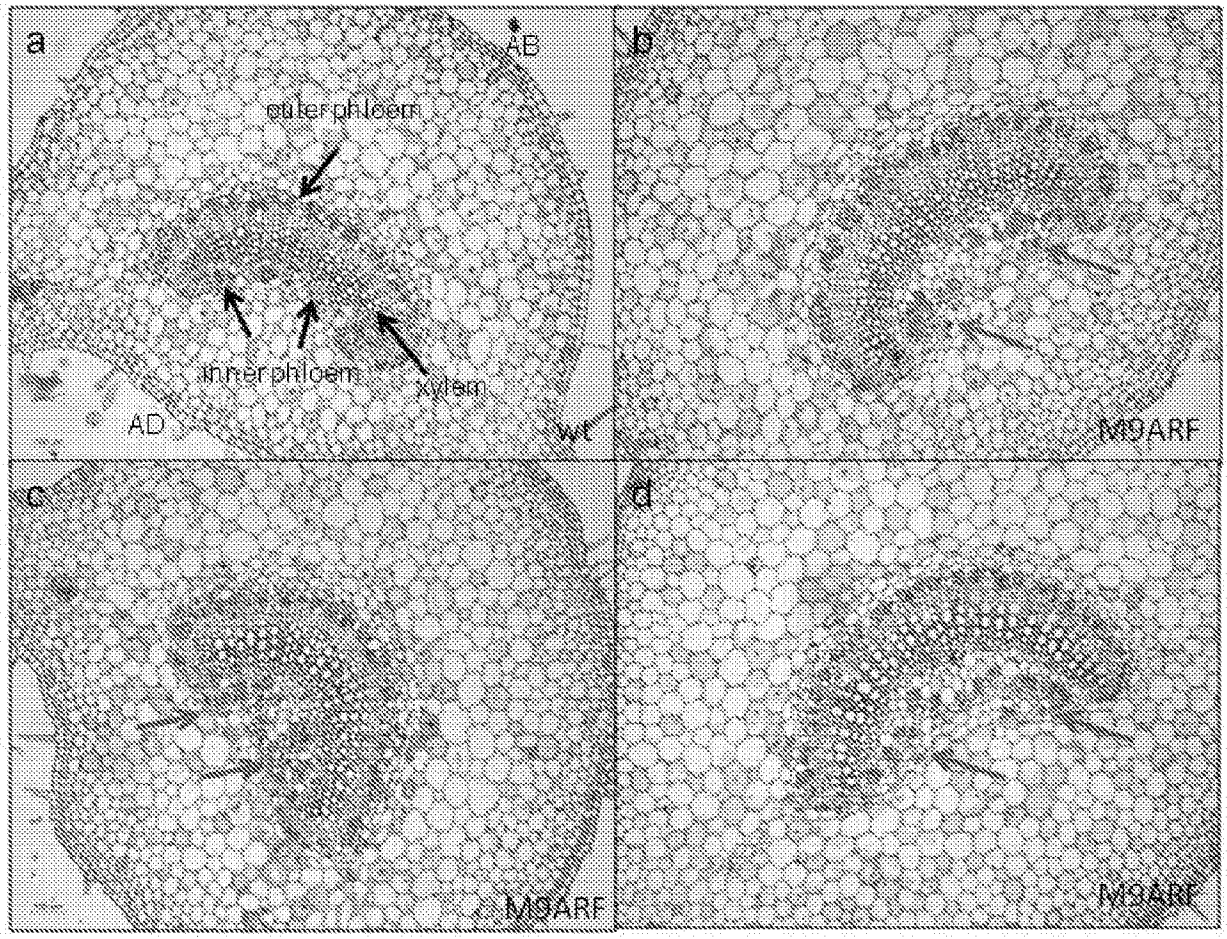
a
AB
outer phloem
inner phloem
xylem
AD
wt
b
M9ARF
c
M9ARF
d
M9ARF

FIGURE 16

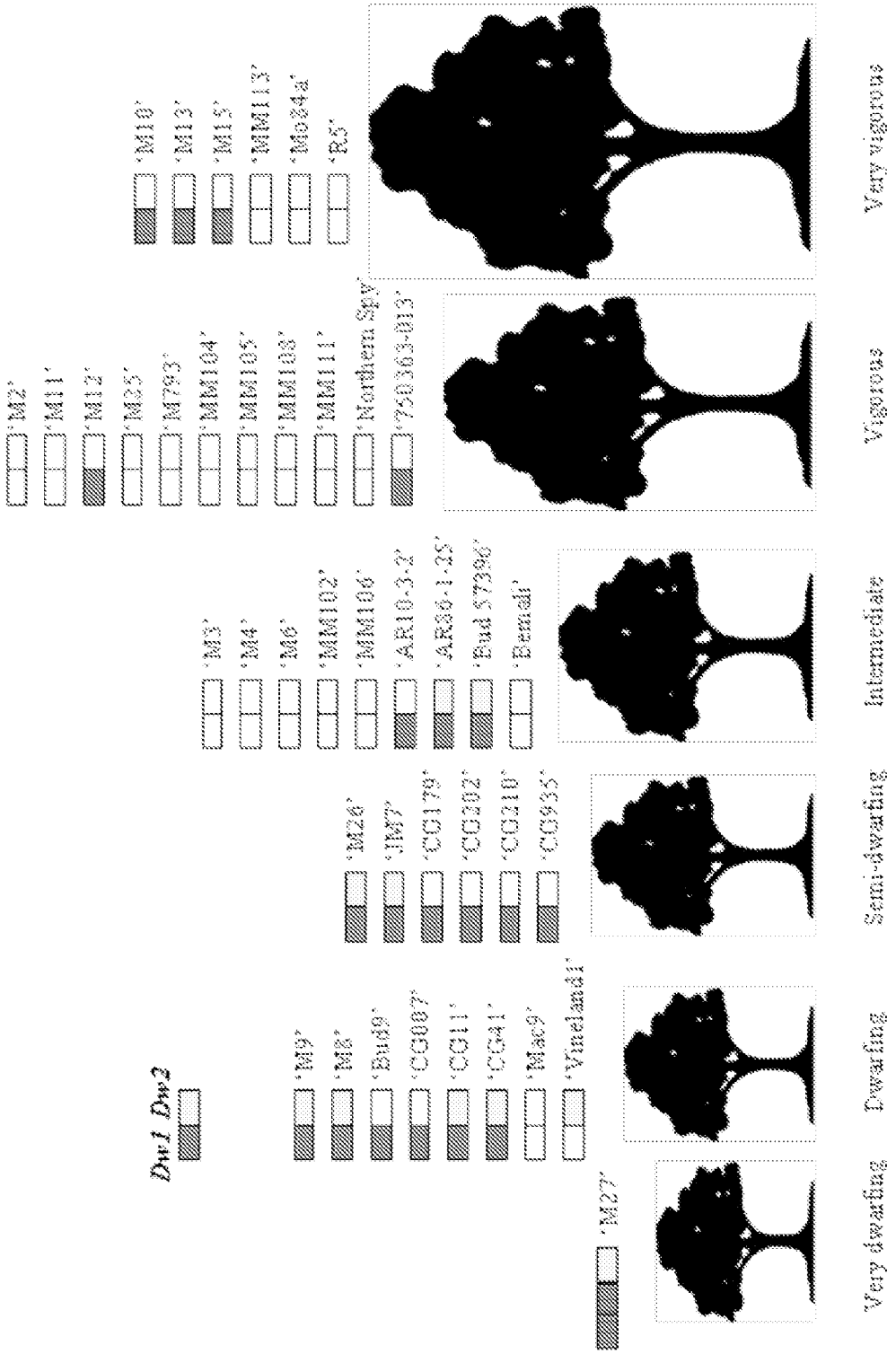
Dw1 Dw2
'M27'
'M9'
'M8'
'Bud9'
'CG007'
'CG11'
'CG41'
'Mac9'
'Vineland1'
'M26'
'JM7'
'CG179'
'CG202'
'CG210'
'CG935'
'M3'
'M4'
'M6'
'MM102'
'MM106'
'AR10-3-2'
'AR30-1-25'
'Bud 5779'
'Bemadi'
'M2'
'M11'
'M12'
'M25'
'M793'
'MM104'
'MM105'
'MM108'
'MM111'
'Northern Spy'
'750363-013'
'M10'
'M13'
'M15'
'MM113'
'Mo84a'
'R5'
Very dwarfing
Dwarfing
Semi-dwarfing
Intermediate
Vigorous
Very vigorous

FIGURE 17

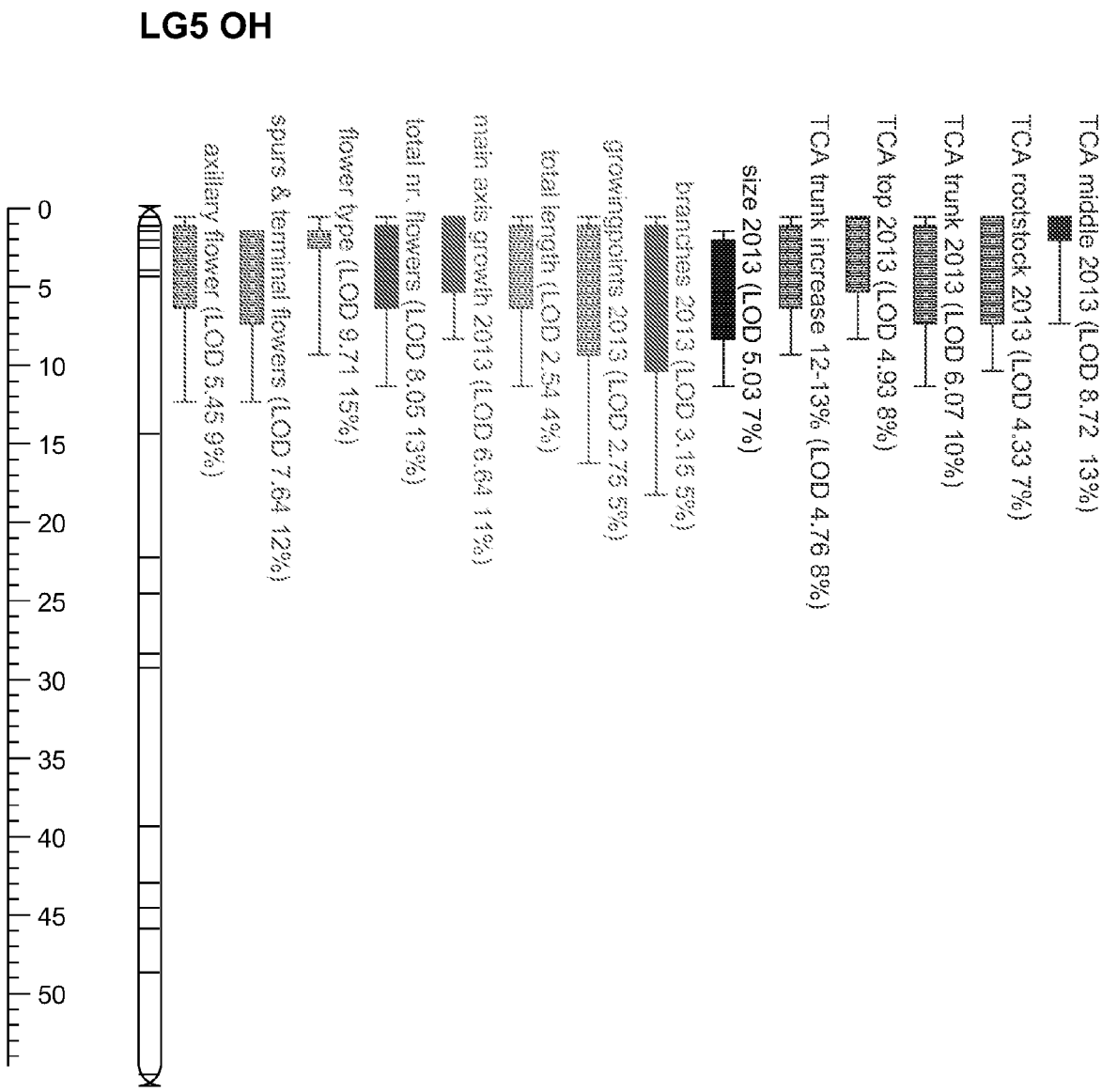
LG5 OH
TCA middle 2013 (LOD 8.72 13%)
TCA rootstock 2013 (LOD 4.33 7%)
TCA trunk 2013 (LOD 6.07 10%)
TCA top 2013 (LOD 4.93 8%)
TCA trunk increase 12-13% (LOD 4.76 8%)
size 2013 (LOD 5.03 7%)
branches 2013 (LOD 3.15 5%)
growingpoints 2013 (LOD 2.75 5%)
total length (LOD 2.54 4%)
main axis growth 2013 (LOD 6.64 11%)
total nr. flowers (LOD 8.05 13%)
flower type (LOD 9.71 15%)
spurs & terminal flowers (LOD 7.64 12%)
axillary flower (LOD 5.45 9%)
0
5
10
15
20
25
30
35
40
45
50

FIGURE 18a

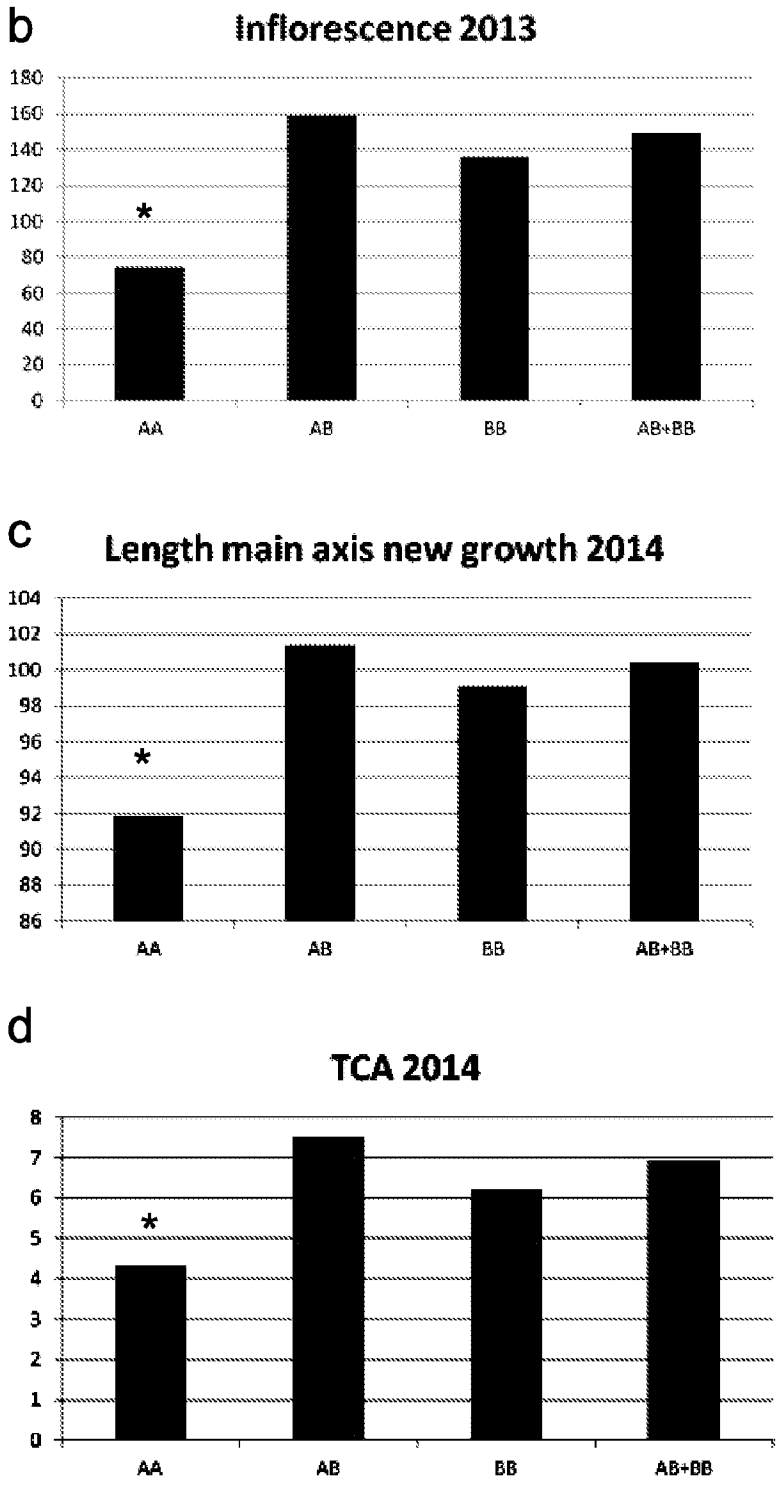
b
Inflorescence 2013
180
160
140
120
100
80
60
40
20
0
*
AA
AB
BB
AB+BB
c
Length main axis new growth 2014
104
102
100
98
96
94
92
90
88
86
*
AA
AB
BB
AB+BB
d
TCA 2014
8
7
6
5
4
3
2
1
0
*
AA
AB
BB
AB+BB

FIGURE 18b

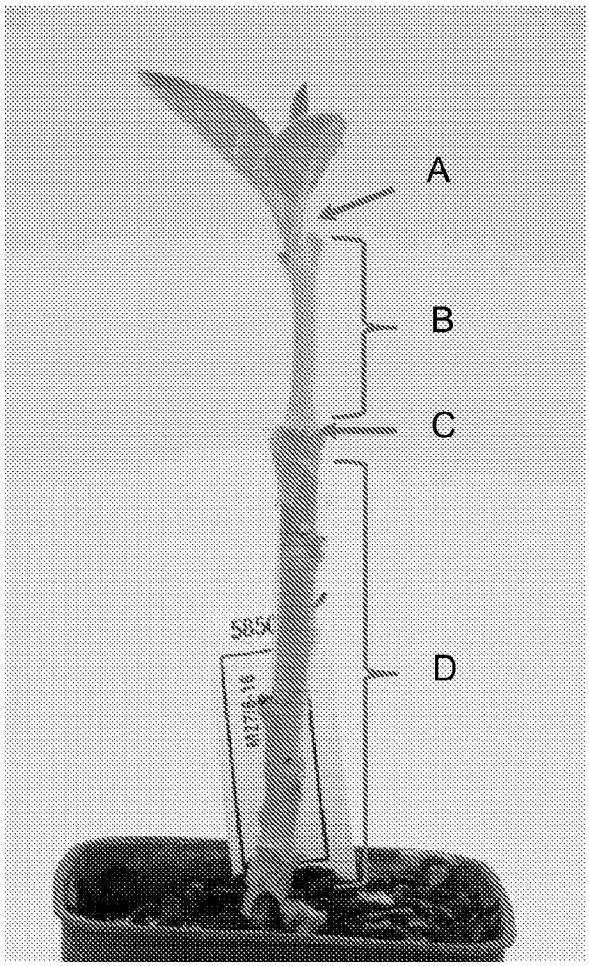

FIGURE 19

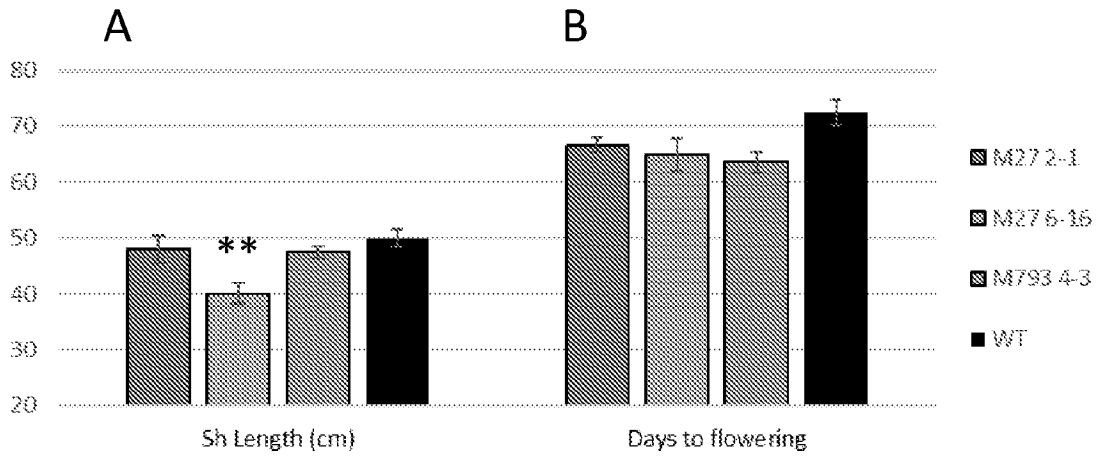

FIGURE 20

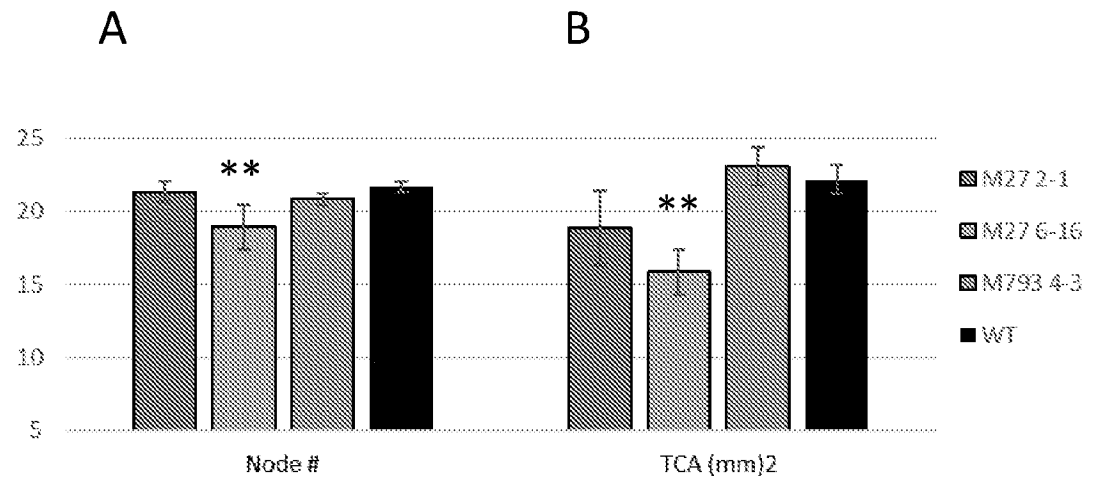

FIGURE 21

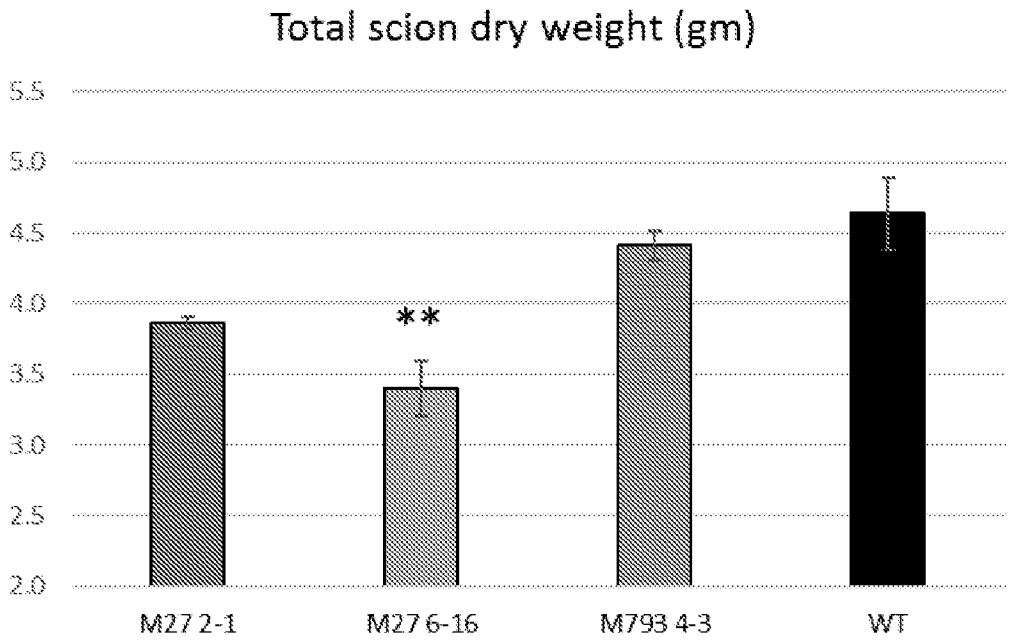

FIGURE 22

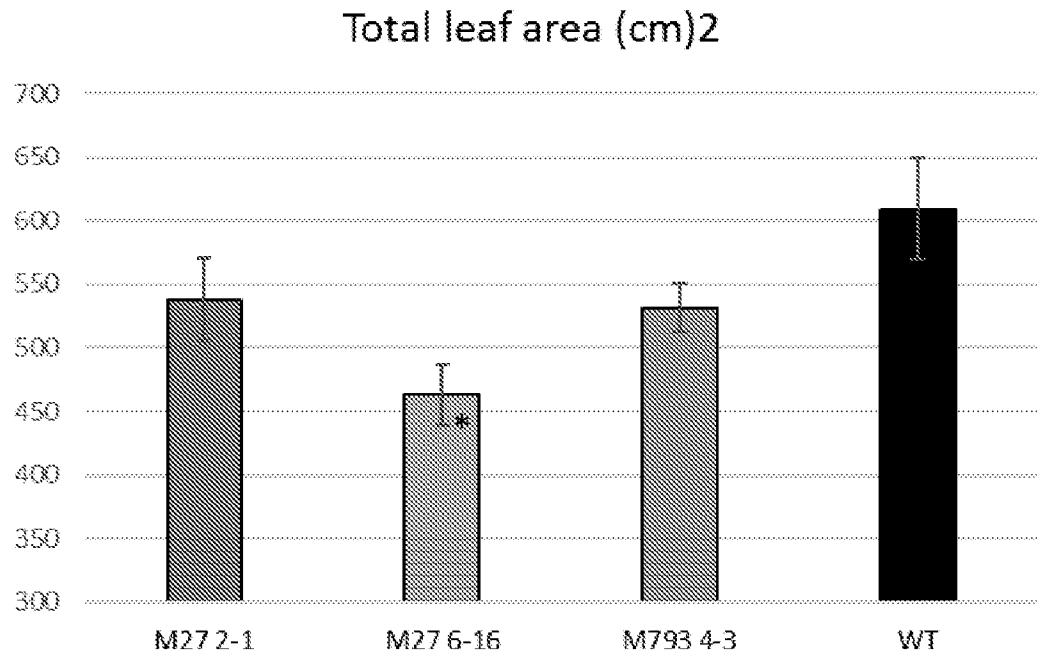

FIGURE 23

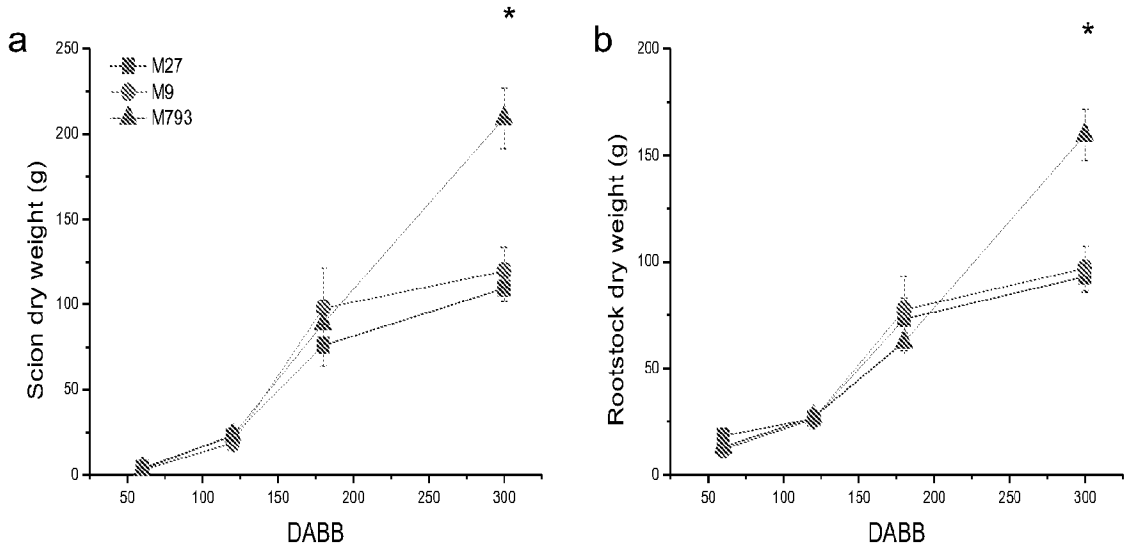

FIGURE 24

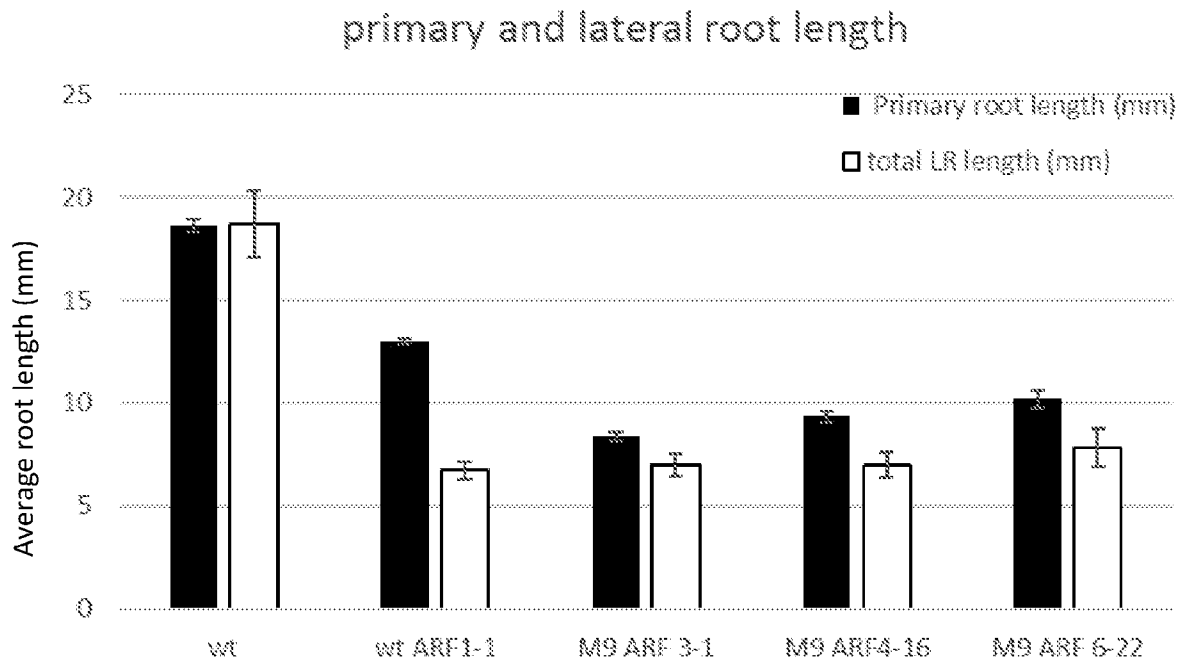

FIGURE 25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2016/057631

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/82 (2006.01) A01H 1/06 (2006.01) C12N 15/67 (2006.01) C12Q 1/68 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

WPIAP, EPODOC, MEDLINE, BIOSIS, EMBASE, CAPLUS, CABA & keywords: ARF3, auxin response factor, ETT, auxin, dwarf, plant height, and like terms.

GenomeQuest: Search of SEQ ID NOs:1-11, 14-15, 28-29 and "LEQVLDFPTSA" motif

Internal databases provided by IP Australia and external databases (ESPACENET, PATENTSCOPE): Inventor and applicant names

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 February 2017	15 February 2017
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Dr Jessie Williams AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832865

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/IB2016/057631**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FAHLGREN, N. et al., "Regulation of *AUXIN RESPONSE FACTOR3* by *TAS3* ta-siRNA affects developmental timing and patterning in Arabidopsis", CURRENT BIOLOGY. 2006, vol. 16, pages 939-944 Pages 939 & 941, Supplement; Abstract; Figures 2 & 3	1-18, 38, 42-47
X	US 2015/0143581 A1 (MONSANTO TECHNOLOGY LLC) 21 May 2015 SEQ ID NOs: 29887, 14965	26-27, 30, 33-37
X	US 2010/0240061 A1 (BUTRUILLE, D. et al.) 23 September 2010 SEQ ID NO: 4979	26-27, 30, 33-37
A	WO 2013/034722 A1 (INSTITUT NATIONAL POLYTECHNIQUE DE TOULOUSE) 14 March 2013	
A	LUO, X.-C. et al., "Genomewide identification and expression analysis of the ARF gene family in apple", JOURNAL OF GENETICS. 2014, vol. 93, no. 3, pages 785-797	
A	LIU, X. et al., "AUXIN RESPONSE FACTOR3 integrates the functions of AGAMOUS and APETALA2 in floral meristem determinacy", PLANT JOURNAL. 2014, vol. 80, no. 4, pages 629-641	

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2016/057631**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2015/0143581 A1	21 May 2015	US 2015143581 A1	21 May 2015
		AR 037699 A1	01 Dec 2004
		AR 053269 A1	25 Apr 2007
		AU 2006247506 A1	23 Nov 2006
		AU 2006247506 B2	06 Dec 2012
		BR PI0610816 A2	27 Jul 2010
		CA 2625583 A1	23 Nov 2006
		CN 101248182 A	20 Aug 2008
		CN 101248182 B	27 Nov 2013
		EP 1882038 A2	30 Jan 2008
		EP 1882038 B1	14 Sep 2016
		JP 2008539784 A	20 Nov 2008
		JP 5016594 B2	05 Sep 2012
		MX 2007014298 A	08 Feb 2008
		MX 291669 B	03 Nov 2011
		NZ 563198 A	30 Apr 2010
		US 2007020621 A1	25 Jan 2007
		US 7365185 B2	29 Apr 2008
		US 2006195932 A1	31 Aug 2006
		US 7408052 B2	05 Aug 2008
		US 2004123339 A1	24 Jun 2004
		US 7491806 B2	17 Feb 2009
		US 2006101541 A1	11 May 2006
		US 7547774 B2	16 Jun 2009
		US 2008127376 A1	29 May 2008
		US 7557201 B2	07 Jul 2009
		US 2008263730 A1	23 Oct 2008
		US 7560542 B2	14 Jul 2009
		US 2008276335 A1	06 Nov 2008
		US 7572902 B2	11 Aug 2009
		US 2003135870 A1	17 Jul 2003
		US 7598361 B2	06 Oct 2009
		US 7625738 B1	01 Dec 2009
		US 2008005809 A1	03 Jan 2008

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2016/057631**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 7635764 B2	22 Dec 2009
		US 2007220622 A1	20 Sep 2007
		US 7655469 B2	02 Feb 2010
		US 7655770 B1	02 Feb 2010
		US 2007143878 A1	21 Jun 2007
		US 7655777 B2	02 Feb 2010
		US 2007118921 A1	24 May 2007
		US 7790958 B2	07 Sep 2010
		US 2004172684 A1	02 Sep 2004
		US 7834146 B2	16 Nov 2010
		US 2007039076 A1	15 Feb 2007
		US 7868149 B2	11 Jan 2011
		US 2005097631 A1	05 May 2005
		US 7888557 B2	15 Feb 2011
		US 2009293158 A1	26 Nov 2009
		US 7919608 B2	05 Apr 2011
		US 2008227639 A1	18 Sep 2008
		US 8058516 B2	15 Nov 2011
		US 2009144848 A1	04 Jun 2009
		US 8106174 B2	31 Jan 2012
		US 2009217414 A1	27 Aug 2009
		US 8299321 B2	30 Oct 2012
		US 2011099655 A1	28 Apr 2011
		US 8324482 B2	04 Dec 2012
		US 2012291154 A1	15 Nov 2012
		US 9000265 B2	07 Apr 2015
		US 2012227131 A1	06 Sep 2012
		US 9290773 B2	22 Mar 2016
		US 2012317677 A1	13 Dec 2012
		US 9322031 B2	26 Apr 2016
		US 2014130204 A1	08 May 2014
		US 9487797 B2	08 Nov 2016
		US 2003233670 A1	18 Dec 2003
		US 2004031072 A1	12 Feb 2004
		US 2004034888 A1	19 Feb 2004
		US 2010293669 A2	18 Nov 2010

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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2016/057631**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2004116682 A1	17 Jun 2004
		US 2004121321 A1	24 Jun 2004
		US 2004123340 A1	24 Jun 2004
		US 2004123343 A1	24 Jun 2004
		US 2010269213 A2	21 Oct 2010
		US 2011131679 A2	02 Jun 2011
		US 2004181830 A1	16 Sep 2004
		US 2004214272 A1	28 Oct 2004
		US 2004216190 A1	28 Oct 2004
		US 2005022266 A1	27 Jan 2005
		US 2005108791 A1	19 May 2005
		US 2006030698 A1	09 Feb 2006
		US 2006195941 A1	31 Aug 2006
		US 2006236419 A1	19 Oct 2006
		US 2010293663 A2	18 Nov 2010
		US 2006253933 A1	09 Nov 2006
		US 2006265782 A1	23 Nov 2006
		US 2007006338 A1	04 Jan 2007
		US 2007011783 A1	11 Jan 2007
		US 2007016974 A1	18 Jan 2007
		US 2007061916 A1	15 Mar 2007
		US 2007067865 A1	22 Mar 2007
		US 2007192889 A1	16 Aug 2007
		US 2010269214 A2	21 Oct 2010
		US 2011067143 A2	17 Mar 2011
		US 2007234438 A1	04 Oct 2007
		US 2007277258 A1	29 Nov 2007
		US 2007283459 A1	06 Dec 2007
		US 2008034453 A1	07 Feb 2008
		US 2008066199 A1	13 Mar 2008
		US 2008066201 A1	13 Mar 2008
		US 2008072348 A1	20 Mar 2008
		US 2008072354 A1	20 Mar 2008
		US 2008104730 A1	01 May 2008
		US 2008114160 A1	15 May 2008
		US 2008115246 A1	15 May 2008

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2016/057631**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2008134369 A1	05 Jun 2008
		US 2008168582 A1	10 Jul 2008
		US 2008229439 A1	18 Sep 2008
		US 2008256662 A1	16 Oct 2008
		US 2008256663 A1	16 Oct 2008
		US 2008276334 A1	06 Nov 2008
		US 2008301838 A1	04 Dec 2008
		US 2009019601 A1	15 Jan 2009
		US 2009044297 A1	12 Feb 2009
		US 2009049573 A1	19 Feb 2009
		US 2009077685 A1	19 Mar 2009
		US 2009081687 A1	26 Mar 2009
		US 2009089901 A1	02 Apr 2009
		US 2009093620 A1	09 Apr 2009
		US 2009100536 A1	16 Apr 2009
		US 2009113574 A1	30 Apr 2009
		US 2009158452 A1	18 Jun 2009
		US 2009172833 A1	02 Jul 2009
		US 2009183270 A1	16 Jul 2009
		US 2009193538 A1	30 Jul 2009
		US 2009241220 A1	24 Sep 2009
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		US 2009276922 A1	05 Nov 2009
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		US 2010024079 A1	28 Jan 2010
		US 2010037356 A1	11 Feb 2010
		US 2010071097 A1	18 Mar 2010
		US 2010077513 A1	25 Mar 2010
		US 2010095400 A1	15 Apr 2010
		US 2010100987 A1	22 Apr 2010
		US 2010122381 A1	13 May 2010
		US 2010122384 A1	13 May 2010
		US 2010162622 A1	01 Jul 2010
		US 2010186127 A1	22 Jul 2010
		US 2011020526 A1	27 Jan 2011
		US 2011179531 A1	21 Jul 2011

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2016/057631**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2011185456 A1	28 Jul 2011
		US 2011214206 A1	01 Sep 2011
		US 2011231959 A1	22 Sep 2011
		US 2011258734 A1	20 Oct 2011
		US 2011277178 A1	10 Nov 2011
		US 2012096599 A1	19 Apr 2012
		US 2012216318 A1	23 Aug 2012
		US 2013074202 A1	21 Mar 2013
		US 2013097737 A1	18 Apr 2013
		US 2013167263 A1	27 Jun 2013
		US 2013191942 A1	25 Jul 2013
		US 2013326723 A1	05 Dec 2013
		US 2013326724 A1	05 Dec 2013
		US 2013333071 A1	12 Dec 2013
		US 2013340112 A1	19 Dec 2013
		US 2014090114 A1	27 Mar 2014
		US 2014130203 A1	08 May 2014
		US 2014325713 A1	30 Oct 2014
		US 2015082481 A1	19 Mar 2015
		US 2015152146 A1	04 Jun 2015
		US 2015191739 A1	09 Jul 2015
		US 2015197763 A1	16 Jul 2015
		US 2015337329 A1	26 Nov 2015
		US 2016264980 A1	15 Sep 2016
		US 2016264984 A1	15 Sep 2016
		US 2016272990 A1	22 Sep 2016
		UY 29533 A1	29 Dec 2006
		WO 2006124678 A2	23 Nov 2006
		WO 2009021041 A2	12 Feb 2009
		ZA 200709564 B	26 Nov 2008

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/IB2016/057631**

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Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2010/0240061 A1	23 September 2010	US 2010240061 A1	23 Sep 2010
		US 2006135758 A1	22 Jun 2006
WO 2013/034722 A1	14 March 2013	WO 2013034722 A1	14 Mar 2013
		EP 2568047 A1	13 Mar 2013

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.