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(54) Title: PLANTS HAVING ALTERED GROWTH AND/OR DEVELOPMENT AND A METHOD FOR MAKING THE SAME

(57) Abrégé/Abstract:
The present invention relates generally to the field of molecular biology and concerns a method for altering various aspects of plant growth and/or development by modulating expression in a plant of a nucleic acid encoding an UBiquitin-Specific Protease (UBP) of the UBP15 subfamily or a homologue thereof. The present invention also concerns plants having modulated expression of a nucleic acid encoding a UBP15 or a homologue thereof, which plants have altered growth and/or development relative to corresponding wild type plants or other control plants. The invention also provides constructs useful in the methods of the invention.

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(54) Title: PLANTS HAVING ALTERED GROWTH AND/OR DEVELOPMENT AND A METHOD FOR MAKING THE SAME

(57) Abstract: The present invention relates generally to the field of molecular biology and concerns a method for altering various aspects of plant growth and/or development by modulating expression in a plant of a nucleic acid encoding an UBiquitin-Specific Protease (UBP) of the UBP15 subfamily or a homologue thereof. The present invention also concerns plants having modulated expression of a nucleic acid encoding a UBP15 or a homologue thereof, which plants have altered growth and/or development relative to corresponding wild type plants or other control plants. The invention also provides constructs useful in the methods of the invention.

Plants having altered growth and/or development and a method for making the same

The present invention relates generally to the field of molecular biology and concerns a method for altering various aspects of plant growth and/or development by modulating expression in a plant of a nucleic acid encoding an **UB**iquitin-Specific **P**rotease (UBP) of the UBP15 subfamily or a homologue thereof. The present invention also concerns plants having modulated expression of a nucleic acid encoding such a UBP15 polypeptide or a homologue thereof, which plants have altered growth and/or development relative to corresponding wild type plants or other control plants. The invention also provides constructs useful in the methods of the invention.

The ever-increasing world population and the dwindling supply of arable land available for agriculture fuels research towards increasing the efficiency of agriculture. Conventional means for crop and horticultural improvements utilise selective breeding techniques to identify plants having desirable characteristics. However, such selective breeding techniques have several drawbacks, namely that these techniques are typically labour intensive and result in plants that often contain heterogeneous genetic components that may not always result in the desirable trait being passed on from parent plants. Advances in molecular biology have allowed mankind to modify the germplasm of animals and plants. Genetic engineering of plants entails the isolation and manipulation of genetic material (typically in the form of DNA or RNA) and the subsequent introduction of that genetic material into a plant. Such technology has the capacity to deliver crops or plants having various improved economic, agronomic or horticultural traits.

It has now been found that plant growth and/or development may be altered by modulating expression in a plant of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

Background

Ubiquitin-Specific Proteases (UBPs) are a conserved family of proteins in eukaryotes that play critical roles in protein de-ubiquitination. The covalent modification of proteins by ubiquitin play a central role in diverse cellular pathways such as cell cycle progression, signal transduction, transcriptional regulation, DNA repair, stress responses, endocytosis and apoptosis (Hochstrasser, 1996; Varshavsky, 1997; Hershko and Ciechanover, 1998; Weissman, 2001; Pickart, 2004). Protein ubiquitination is catalyzed by a cascade of three enzymes. Ubiquitn is first activated by ubiquitin-activating enzyme (E1), which forms a thiolester bond with the

ubiquitin C-terminus. Ubiquitin is then transferred to ubiquitin-conjugating enzyme (E2). Although some E2s can catalyze ligation of the ubiquitin C-terminus to the lysine residue of target proteins with the aid of ubiquitin ligases (E3s), other E2s transfer their conjugated ubiquitin to E3s before targetting to substrates. Target substrate proteins can be mono-ubiquitinated or multi-ubiquitinated by successive conjugation of the ubiquitin C-terminus to the lysine residue of the prior one through several possible linkages. The fate of ubiquitinated substrate proteins depends in part on the number of conjugated ubiquitin(s) and on the mode of linkage in the ubiquitin chain. The most common ubiquitination is multi-ubiquitin chain (ubiquitin number >=4) linked by Lys48, acting as a signal for protein degradation by 26S proteasome.

Cleavage of ubiquitin from proteins by de-ubiquitination enzymes (DUBs) can also affect the ubiquitinated substrate protein's activity and fate (Wilkinson, 1997; Amerik and Hochstrasser, 2004; Crosas et al., 2006; Hanna et al., 2006). Those DUBs are proteases that specifically cleave the peptide bond between ubiquitins or between the C-terminus of ubiquitin and covalently attached polypeptides. The currently known DUBs together carry out four types of essential biochemical functions: first, they generate mature ubiquitins from ubiquitin precursors (fused to ribosomal protein) and polyubiquitin gene products; secondly, they rescue proteins that are inappropriately ubiquitinated; thirdly, they cleave ubiquitin (chains) from attached substrate proteins; and fourth, release free ubiquitin monomers from multi-ubiquitin chains. The last three roles are accompanied by cleavage of the isopeptide bonds between the ubiquitin C-terminus Gly and Lys ε-amino residue of a target protein.

Cysteine proteases and metalloproteases are the two major groups of the DUB superfamily, with cysteine proteases being most numerous in eukaryotes (Nijman et al., 2005). All known metalloproteases have a JAMM domain for catalytic activity (Verma et al., 2002). The cysteine protease DUBs can be further divided into four families based on the organization of ubiquitin-protease catalytic center structure and organization (Wilkinson, 1997; Amerik and Hochstrasser, 2004; Nijman et al., 2005). Ubiquitin-Specific Proteases (UBP, or USPs as defined in mammals) possess catalytic triad residues in highly conserved cysteine box and histidine box (Hu et al., 2002). Ubiquitin C-terminal Hydrolases (UCHs), with similar catalytic triad residues in two conserved cysteine and histidine boxes (Johnston et al., 1997; Johnston et al., 1999), have a smaller overall protein size as well as a structural obstacle over the catalytic surface to restrict their ability to hydrolyze only small amides and esters at the C-terminus of ubiquitin (Amerik and Hochstrasser, 2004). Ovarian Tumor Proteases (OTUs) have a catalytic triad comparable to above two families in Cysteine and Histidine boxes but containing an OTU-related motif and being considered as a part of UBP family (Balakirev et al., 2003; Nanao et al., 2004). Lastly, Machado-Joseph Disease Protein Domain Proteases

(MJDs) possess a cysteine and histidine box-like domain but with rather low sequence similarity to the other three groups (Burnett et al., 2003; Scheel et al., 2003). The UBP family makes up the bulk of the cysteine proteases. All four types of the above mentioned DUB biochemical functions are found in UBPs family, while UCHs only perform their functions on small proteins and ubiquitin precursor.

In the model plant *Arabidopsis thaliana*, an *in silico* analysis of the completely sequenced genome revealed a total of 27 UBPs based on the presence of the conserved Cys and His boxes; those 27 UBPs were further divided into 14 subfamilies (Yan et al., 2000). Previous reports showed that UBP3 and UBP4 constitute one subfamily, possess UBP activity *in vitro* and are present in the nucleus (Chandler et al., 1997; Rao-Naik et al., 2000). Another member, UBP5, was shown also to have de-ubiquitination activity *in vitro* (Rao-Naik et al., 2000). A genetic analysis of UBP1 and UBP2, members of another subfamily, were reported to be required for resistance to the amino acid analog canavanine (Yan et al., 2000). Furthermore, a loss-of-function mutation in UBP14 was shown to be lethal in early embryo development (Doelling et al., 2001).

Summary

Surprisingly, it has now been found that modulating expression of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof gives plants altered growth and/or development relative to control plants.

According one embodiment, there is provided a method for altering plant growth and/or development relative to control plants, comprising modulating expression in a plant of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

Definitions

Polypeptide(s)/Protein(s)

The terms “polypeptide” and “protein” are used interchangeably herein and refer to amino acids in a polymeric form of any length, linked together by peptide bonds.

Polynucleotide(s)/Nucleic acid(s)/Nucleic acid sequence(s)/nucleotide sequence(s)

The terms "polynucleotide(s)", "nucleic acid sequence(s)", "nucleotide sequence(s)", “nucleic acid(s)”, “nucleic acid molecule” are used interchangeably herein and refer to nucleotides, either ribonucleotides or deoxyribonucleotides or a combination of both, in a polymeric unbranched form of any length.

Control plant(s)

The choice of suitable control plants is a routine part of an experimental setup and may include corresponding wild type plants or corresponding plants without the gene of interest. The control plant is typically of the same plant species or even of the same variety as the plant to be assessed. The control plant may also be a nullizygote of the plant to be assessed. Nullizygotes are individuals missing the transgene by segregation. A "control plant" as used herein refers not only to whole plants, but also to plant parts, including seeds and seed parts.

Homologue(s)

"Homologues" of a protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived.

A deletion refers to removal of one or more amino acids from a protein.

An insertion refers to one or more amino acid residues being introduced into a predetermined site in a protein. Insertions may comprise N-terminal and/or C-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than N- or C-terminal fusions, of the order of about 1 to 10 residues. Examples of N- or C-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)-6-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

A substitution refers to replacement of amino acids of the protein with other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1 to 10 amino acid residues. The amino acid substitutions are preferably conservative amino acid substitutions. Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company (Eds) and Table A below).

Table A: Examples of conserved amino acid substitutions

Residue	Conservative Substitutions	Residue	Conservative Substitutions
Ala	Ser	Leu	Ile; Val
Arg	Lys	Lys	Arg; Gln
Asn	Gln; His	Met	Leu; Ile
Asp	Glu	Phe	Met; Leu; Tyr
Gln	Asn	Ser	Thr; Gly
Cys	Ser	Thr	Ser; Val
Glu	Asp	Trp	Tyr
Gly	Pro	Tyr	Trp; Phe
His	Asn; Gln	Val	Ile; Leu
Ile	Leu, Val		

Amino acid substitutions, deletions and/or insertions may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulation. Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen in vitro mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

Derivatives

"Derivatives" include peptides, oligopeptides, polypeptides which may, compared to the amino acid sequence of the naturally-occurring form of the protein, such as the protein of interest, comprise substitutions of amino acids with non-naturally occurring amino acid residues, or additions of non-naturally occurring amino acid residues. "Derivatives" of a protein also encompass peptides, oligopeptides, polypeptides which comprise naturally occurring altered (glycosylated, acylated, prenylated, phosphorylated, myristoylated, sulphated etc.) or non-naturally altered amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents or additions compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence, such as a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein. Furthermore, "derivatives" also include fusions of the naturally-occurring form of the protein with tagging peptides such as FLAG,

HIS6 or thioredoxin (for a review of tagging peptides, see Terpe, Appl. Microbiol. Biotechnol. 60, 523-533, 2003).

Orthologue(s)/Paralogue(s)

Orthologues and paralogues encompass evolutionary concepts used to describe the ancestral relationships of genes. Paralogues are genes within the same species that have originated through duplication of an ancestral gene; orthologues are genes from different organisms that have originated through speciation, and are also derived from a common ancestral gene.

Domain

The term "domain" refers to a set of amino acids conserved at specific positions along an alignment of sequences of evolutionarily related proteins. While amino acids at other positions can vary between homologues, amino acids that are highly conserved at specific positions indicate amino acids that are likely essential in the structure, stability or function of a protein. Identified by their high degree of conservation in aligned sequences of a family of protein homologues, they can be used as identifiers to determine if any polypeptide in question belongs to a previously identified polypeptide family.

Motif/Consensus sequence/Signature

The term “motif” or “consensus sequence” or “signature” refers to a short conserved region in the sequence of evolutionarily related proteins. Motifs are frequently highly conserved parts of domains, but may also include only part of the domain, or be located outside of conserved domain (if all of the amino acids of the motif fall outside of a defined domain).

Hybridisation

The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to, for example, a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double

strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids.

The term “stringency” refers to the conditions under which a hybridisation takes place. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration, ionic strength and hybridisation buffer composition. Generally, low stringency conditions are selected to be about 30°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Medium stringency conditions are when the temperature is 20°C below T_m, and high stringency conditions are when the temperature is 10°C below T_m. High stringency hybridisation conditions are typically used for isolating hybridising sequences that have high sequence similarity to the target nucleic acid sequence. However, nucleic acids may deviate in sequence and still encode a substantially identical polypeptide, due to the degeneracy of the genetic code. Therefore medium stringency hybridisation conditions may sometimes be needed to identify such nucleic acid molecules.

The Tm is the temperature under defined ionic strength and pH, at which 50% of the target sequence hybridises to a perfectly matched probe. The T_m is dependent upon the solution conditions and the base composition and length of the probe. For example, longer sequences hybridise specifically at higher temperatures. The maximum rate of hybridisation is obtained from about 16°C up to 32°C below T_m. The presence of monovalent cations in the hybridisation solution reduce the electrostatic repulsion between the two nucleic acid strands thereby promoting hybrid formation; this effect is visible for sodium concentrations of up to 0.4M (for higher concentrations, this effect may be ignored). Formamide reduces the melting temperature of DNA-DNA and DNA-RNA duplexes with 0.6 to 0.7°C for each percent formamide, and addition of 50% formamide allows hybridisation to be performed at 30 to 45°C, though the rate of hybridisation will be lowered. Base pair mismatches reduce the hybridisation rate and the thermal stability of the duplexes. On average and for large probes, the Tm decreases about 1°C per % base mismatch. The Tm may be calculated using the following equations, depending on the types of hybrids:

1) DNA-DNA hybrids (Meinkoth and Wahl, Anal. Biochem., 138: 267-284, 1984):

$$T_m = 81.5°C + 16.6 \times \log_{10}[Na^+]^a + 0.41 \times \%[G/C^b] - 500 \times [L^c]^{-1} - 0.61 \times \% \text{ formamide}$$

2) DNA-RNA or RNA-RNA hybrids:

$$Tm = 79.8 + 18.5\ (\log_{10}[Na^+]^a) + 0.58\ (\%G/C^b) + 11.8\ (\%G/C^b)^2 - 820/L^c$$

3) oligo-DNA or oligo-RNA[d] hybrids:

For <20 nucleotides: $T_m = 2\ (l_n)$

For 20–35 nucleotides: $T_m = 22 + 1.46 (I_n)$

[a] or for other monovalent cation, but only accurate in the 0.01–0.4 M range.

[b] only accurate for %GC in the 30% to 75% range.

[c] L = length of duplex in base pairs.

[d] oligo, oligonucleotide; I_n, = effective length of primer = 2×(no. of G/C)+(no. of A/T).

Non-specific binding may be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein containing solutions, additions of heterologous RNA, DNA, and SDS to the hybridisation buffer, and treatment with Rnase. For non-homologous probes, a series of hybridizations may be performed by varying one of (i) progressively lowering the annealing temperature (for example from 68°C to 42°C) or (ii) progressively lowering the formamide concentration (for example from 50% to 0%). The skilled artisan is aware of various parameters which may be altered during hybridisation and which will either maintain or change the stringency conditions.

Besides the hybridisation conditions, specificity of hybridisation typically also depends on the function of post-hybridisation washes. To remove background resulting from non-specific hybridisation, samples are washed with dilute salt solutions. Critical factors of such washes include the ionic strength and temperature of the final wash solution: the lower the salt concentration and the higher the wash temperature, the higher the stringency of the wash. Wash conditions are typically performed at or below hybridisation stringency. A positive hybridisation gives a signal that is at least twice of that of the background. Generally, suitable stringent conditions for nucleic acid hybridisation assays or gene amplification detection procedures are as set forth above. More or less stringent conditions may also be selected. The skilled artisan is aware of various parameters which may be altered during washing and which will either maintain or change the stringency conditions.

For example, typical high stringency hybridisation conditions for DNA hybrids longer than 50 nucleotides encompass hybridisation at 65°C in 1x SSC or at 42°C in 1x SSC and 50% formamide, followed by washing at 65°C in 0.3x SSC. Examples of medium stringency hybridisation conditions for DNA hybrids longer than 50 nucleotides encompass hybridisation at 50°C in 4x SSC or at 40°C in 6x SSC and 50% formamide, followed by washing at 50°C in 2x SSC. The length of the hybrid is the anticipated length for the hybridising nucleic acid. When nucleic acids of known sequence are hybridised, the hybrid length may be determined by aligning the sequences and identifying the conserved regions described herein. 1×SSC is 0.15M NaCl and 15mM sodium citrate; the hybridisation solution and wash solutions may

additionally include 5x Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.5% sodium pyrophosphate.

For the purposes of defining the level of stringency, reference can be made to Sambrook et al. (2001) Molecular Cloning: a laboratory manual, 3rd Edition, Cold Spring Harbor Laboratory Press, CSH, New York or to Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989 and yearly updates).

Splice variant

The term "splice variant" as used herein encompasses variants of a nucleic acid sequence in which selected introns and/or exons have been excised, replaced, displaced or added, or in which introns have been shortened or lengthened. Such variants will be ones in which the biological activity of the protein is substantially retained; this may be achieved by selectively retaining functional segments of the protein. Such splice variants may be found in nature or may be manmade. Methods for predicting and isolating such splice variants are well known in the art (see for example Foissac and Schiex (2005) BMC Bioinformatics 6: 25).

Allelic variant

Alleles or allelic variants are alternative forms of a given gene, located at the same chromosomal position. Allelic variants encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp. SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms.

Gene shuffling/Directed evolution

Gene shuffling or directed evolution consists of iterations of DNA shuffling followed by appropriate screening and/or selection to generate variants of nucleic acids or portions thereof encoding proteins having a modified biological activity (Castle et al., (2004) Science 304(5674): 1151-4; US patents 5,811,238 and 6,395,547).

Regulatory element/Control sequence/Promoter

The terms "regulatory element", "control sequence" and "promoter" are all used interchangeably herein and are to be taken in a broad context to refer to regulatory nucleic acid sequences capable of effecting expression of the sequences to which they are ligated. The term "promoter" typically refers to a nucleic acid control sequence located upstream from the transcriptional start of a gene and which is involved in recognising and binding of RNA polymerase and other proteins, thereby directing transcription of an operably linked nucleic

acid. Encompassed by the aforementioned terms are transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a –35 box sequence and/or –10 box transcriptional regulatory sequences. The term “regulatory element” also encompasses a synthetic fusion molecule or derivative that confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ.

A “plant promoter” comprises regulatory elements, which mediate the expression of a coding sequence segment in plant cells. Accordingly, a plant promoter need not be of plant origin, but may originate from viruses or micro-organisms, for example from viruses which attack plant cells. The “plant promoter” can also originate from a plant cell, e.g. from the plant which is transformed with the nucleic acid sequence to be expressed in the inventive process and described herein. This also applies to other “plant” regulatory signals, such as “plant” terminators. The promoters upstream of the nucleotide sequences useful in the methods of the present invention can be modified by one or more nucleotide substitution(s), insertion(s) and/or deletion(s) without interfering with the functionality or activity of either the promoters, the open reading frame (ORF) or the 3'-regulatory region such as terminators or other 3' regulatory regions which are located away from the ORF. It is furthermore possible that the activity of the promoters is increased by modification of their sequence, or that they are replaced completely by more active promoters, even promoters from heterologous organisms. For expression in plants, the nucleic acid molecule must, as described above, be linked operably to or comprise a suitable promoter which expresses the gene at the right point in time and with the required spatial expression pattern.

For the identification of functionally equivalent promoters, the promoter strength and/or expression pattern of a candidate promoter may be analysed for example by operably linking the promoter to a reporter gene and assaying the expression level and pattern of the reporter gene in various tissues of the plant. Suitable well-known reporter genes include for example beta-glucuronidase or beta-galactosidase. The promoter activity is assayed by measuring the enzymatic activity of the beta-glucuronidase or beta-galactosidase. The promoter strength and/or expression pattern may then be compared to that of a reference promoter (such as the one used in the methods of the present invention). Alternatively, promoter strength may be assayed by quantifying mRNA levels or by comparing mRNA levels of the

nucleic acid used in the methods of the present invention, with mRNA levels of housekeeping genes such as 18S rRNA, using methods known in the art, such as Northern blotting with densitometric analysis of autoradiograms, quantitative real-time PCR or RT-PCR (Heid et al., 1996 Genome Methods 6: 986-994). Generally by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By "low level" is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts, to about 1/500,0000 transcripts per cell. Conversely, a "strong promoter" drives expression of a coding sequence at high level, or at about 1/10 transcripts to about 1/100 transcripts to about 1/1000 transcripts per cell.

Operably linked

The term "operably linked" as used herein refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest.

Constitutive promoter

A "constitutive promoter" refers to a promoter that is transcriptionally active during most, but not necessarily all, phases of growth and development and under most environmental conditions, in at least one cell, tissue or organ. Table B below gives examples of constitutive promoters.

Table B: Examples of constitutive promoters

Gene Source	Reference
Actin	McElroy et al, Plant Cell, 2: 163-171, 1990
HMGP	WO 2004/070039
CAMV 35S	Odell et al, Nature, 313: 810-812, 1985
CaMV 19S	Nilsson et al., Physiol. Plant. 100:456-462, 1997
GOS2	de Pater et al, Plant J Nov;2(6):837-44, 1992, WO 2004/065596
Ubiquitin	Christensen et al, Plant Mol. Biol. 18: 675-689, 1992
Rice cyclophilin	Buchholz et al, Plant Mol Biol. 25(5): 837-43, 1994
Maize H3 histone	Lepetit et al, Mol. Gen. Genet. 231:276-285, 1992
Alfalfa H3 histone	Wu et al. Plant Mol. Biol. 11:641-649, 1988
Actin 2	An et al, Plant J. 10(1); 107-121, 1996
34S FMV	Sanger et al., Plant. Mol. Biol., 14, 1990: 433-443
Rubisco small subunit	US 4,962,028
OCS	Leisner (1988) Proc Natl Acad Sci USA 85(5): 2553
SAD1	Jain et al., Crop Science, 39 (6), 1999: 1696
SAD2	Jain et al., Crop Science, 39 (6), 1999: 1696
Nos	Shaw et al. (1984) Nucleic Acids Res. 12(20):7831-7846

V-ATPase	WO 01/14572
Super promoter	WO 95/14098
G-box proteins	WO 94/12015

Ubiquitous promoter

A ubiquitous promoter is active in substantially all tissues or cells of an organism.

Developmentally-regulated promoter

A developmentally-regulated promoter is active during certain developmental stages or in parts of the plant that undergo developmental changes.

Inducible promoter

An inducible promoter has induced or increased transcription initiation in response to a chemical (for a review see Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108), environmental or physical stimulus, or may be “stress-inducible”, i.e. activated when a plant is exposed to various stress conditions, or a “pathogen-inducible" i.e. activated when a plant is exposed to exposure to various pathogens.

Organ-specific/Tissue-specific promoter

An organ-specific or tissue-specific promoter is one that is capable of preferentially initiating transcription in certain organs or tissues, such as the leaves, roots, seed tissue etc. For example, a “root-specific promoter” is a promoter that is transcriptionally active predominantly in plant roots, substantially to the exclusion of any other parts of a plant, whilst still allowing for any leaky expression in these other plant parts. Promoters able to initiate transcription in certain cells only are referred to herein as “cell-specific”.

A seed-specific promoter is transcriptionally active predominantly in seed tissue, but not necessarily exclusively in seed tissue (in cases of leaky expression). The seed-specific promoter may be active during seed development and/or during germination. The seed specific promoter may be endosperm and/or aleurone and/or embryo-specific.

Terminator

The term “terminator” encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3’ processing and polyadenylation of a primary transcript and termination of transcription. The terminator can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The terminator to be added may

be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

Modulation

The term “modulation” means in relation to expression or gene expression, a process in which the expression level is changed by said gene expression in comparison to the control plant, the expression level may be increased or decreased. The original, unmodulated expression may be of any kind of expression of a structural RNA (rRNA, tRNA) or mRNA with subsequent translation.

Expression

The term “expression” or “gene expression” means the transcription of a specific gene or specific genes or specific genetic construct. The term “expression” or “gene expression” in particular means the transcription of a gene or genes or genetic construct into structural RNA (rRNA, tRNA) or mRNA with or without subsequent translation of the latter into a protein. The process includes transcription of DNA and processing of the resulting mRNA product.

Increased expression/overexpression

The term “increased expression” or “overexpression” as used herein means any form of expression that is additional to the original wild-type expression level.

Methods for increasing expression of genes or gene products are well documented in the art and include, for example, overexpression driven by appropriate promoters, the use of transcription enhancers or translation enhancers. Isolated nucleic acids which serve as promoter or enhancer elements may be introduced in an appropriate position (typically upstream) of a non-heterologous form of a polynucleotide so as to upregulate expression of a nucleic acid encoding the polypeptide of interest. For example, endogenous promoters may be altered in vivo by mutation, deletion, and/or substitution (see, Kmiec, US 5,565,350; Zarling et al., WO9322443), or isolated promoters may be introduced into a plant cell in the proper orientation and distance from a gene of the present invention so as to control the expression of the gene.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added may be derived from, for example, the nopaline synthase or octopine

synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

An intron sequence may also be added to the 5' untranslated region (UTR) or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold (Buchman and Berg (1988) Mol. Cell biol. 8: 4395-4405; Callis et al. (1987) Genes Dev 1:1183-1200). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of the maize introns Adh1-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. For general information see: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, N.Y. (1994).

Endogenous gene

Reference herein to an “endogenous” gene not only refers to the gene in question as found in a plant in its natural form (i.e., without there being any human intervention), but also refers to that same gene (or a substantially homologous nucleic acid/gene) in an isolated form subsequently (re)introduced into a plant (a transgene). For example, a transgenic plant containing such a transgene may encounter a substantial reduction of the transgene expression and/or substantial reduction of expression of the endogenous gene. The isolated gene may be isolated from an organism or may be manmade, for example by chemical synthesis.

Decreased expression

Reference herein to “decreased epression” or “reduction or substantial elimination” of expression is taken to mean a decrease in endogenous gene expression and/or polypeptide levels and/or polypeptide activity relative to control plants. The reduction or substantial elimination is in increasing order of preference at least 10%, 20%, 30%, 40% or 50%, 60%, 70%, 80%, 85%, 90%, or 95%, 96%, 97%, 98%, 99% or more reduced compared to that of control plants.

For the reduction or substantial elimination of expression an endogenous gene in a plant, a sufficient length of substantially contiguous nucleotides of a nucleic acid sequence is required. In order to perform gene silencing, this may be as little as 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10 or fewer nucleotides, alternatively this may be as much as the entire gene (including the 5’ and/or 3’ UTR, either in part or in whole). The stretch of substantially

contiguous nucleotides may be derived from the nucleic acid encoding the protein of interest (target gene), or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest. Preferably, the stretch of substantially contiguous nucleotides is capable of forming hydrogen bonds with the target gene (either sense or antisense strand), more preferably, the stretch of substantially contiguous nucleotides has, in increasing order of preference, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the target gene (either sense or antisense strand). A nucleic acid sequence encoding a (functional) polypeptide is not a requirement for the various methods discussed herein for the reduction or substantial elimination of expression of an endogenous gene.

This reduction or substantial elimination of expression may be achieved using routine tools and techniques. A preferred method for the reduction or substantial elimination of endogenous gene expression is by introducing and expressing in a plant a genetic construct into which the nucleic acid (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of any one of the protein of interest) is cloned as an inverted repeat (in part or completely), separated by a spacer (non-coding DNA).

In such a preferred method, expression of the endogenous gene is reduced or substantially eliminated through RNA-mediated silencing using an inverted repeat of a nucleic acid or a part thereof (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest), preferably capable of forming a hairpin structure. The inverted repeat is cloned in an expression vector comprising control sequences. A non-coding DNA nucleic acid sequence (a spacer, for example a matrix attachment region fragment (MAR), an intron, a polylinker, etc.) is located between the two inverted nucleic acids forming the inverted repeat. After transcription of the inverted repeat, a chimeric RNA with a self-complementary structure is formed (partial or complete). This double-stranded RNA structure is referred to as the hairpin RNA (hpRNA). The hpRNA is processed by the plant into siRNAs that are incorporated into an RNA-induced silencing complex (RISC). The RISC further cleaves the mRNA transcripts, thereby substantially reducing the number of mRNA transcripts to be translated into polypeptides. For further general details see for example, Grierson et al. (1998) WO 98/53083; Waterhouse et al. (1999) WO 99/53050).

Performance of the methods of the invention does not rely on introducing and expressing in a plant a genetic construct into which the nucleic acid is cloned as an inverted repeat, but any

one or more of several well-known “gene silencing” methods may be used to achieve the same effects.

One such method for the reduction of endogenous gene expression is RNA-mediated silencing of gene expression (downregulation). Silencing in this case is triggered in a plant by a double stranded RNA sequence (dsRNA) that is substantially similar to the target endogenous gene. This dsRNA is further processed by the plant into about 20 to about 26 nucleotides called short interfering RNAs (siRNAs). The siRNAs are incorporated into an RNA-induced silencing complex (RISC) that cleaves the mRNA transcript of the endogenous target gene, thereby substantially reducing the number of mRNA transcripts to be translated into a polypeptide. Preferably, the double stranded RNA sequence corresponds to a target gene.

Another example of an RNA silencing method involves the introduction of nucleic acid sequences or parts thereof (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest) in a sense orientation into a plant. “Sense orientation” refers to a DNA sequence that is homologous to an mRNA transcript thereof. Introduced into a plant would therefore be at least one copy of the nucleic acid sequence. The additional nucleic acid sequence will reduce expression of the endogenous gene, giving rise to a phenomenon known as co-suppression. The reduction of gene expression will be more pronounced if several additional copies of a nucleic acid sequence are introduced into the plant, as there is a positive correlation between high transcript levels and the triggering of co-suppression.

Another example of an RNA silencing method involves the use of antisense nucleic acid sequences. An "antisense" nucleic acid sequence comprises a nucleotide sequence that is complementary to a "sense" nucleic acid sequence encoding a protein, i.e. complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA transcript sequence. The antisense nucleic acid sequence is preferably complementary to the endogenous gene to be silenced. The complementarity may be located in the “coding region” and/or in the “non-coding region” of a gene. The term “coding region" refers to a region of the nucleotide sequence comprising codons that are translated into amino acid residues. The term “non-coding region" refers to 5' and 3' sequences that flank the coding region that are transcribed but not translated into amino acids (also referred to as 5' and 3' untranslated regions).

Antisense nucleic acid sequences can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid sequence may be complementary to the entire nucleic acid sequence (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest), but may also be an oligonucleotide that is antisense to only a part of the nucleic acid sequence (including the mRNA 5' and 3' UTR). For example, the antisense oligonucleotide sequence may be complementary to the region surrounding the translation start site of an mRNA transcript encoding a polypeptide. The length of a suitable antisense oligonucleotide sequence is known in the art and may start from about 50, 45, 40, 35, 30, 25, 20, 15 or 10 nucleotides in length or less. An antisense nucleic acid sequence according to the invention may be constructed using chemical synthesis and enzymatic ligation reactions using methods known in the art. For example, an antisense nucleic acid sequence (e.g., an antisense oligonucleotide sequence) may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acid sequences, e.g., phosphorothioate derivatives and acridine substituted nucleotides may be used. Examples of modified nucleotides that may be used to generate the antisense nucleic acid sequences are well known in the art. Known nucleotide modifications include methylation, cyclization and 'caps' and substitution of one or more of the naturally occurring nucleotides with an analogue such as inosine. Other modifications of nucleotides are well known in the art.

The antisense nucleic acid sequence can be produced biologically using an expression vector into which a nucleic acid sequence has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Preferably, production of antisense nucleic acid sequences in plants occurs by means of a stably integrated nucleic acid construct comprising a promoter, an operably linked antisense oligonucleotide, and a terminator.

The nucleic acid molecules used for silencing in the methods of the invention (whether introduced into a plant or generated in situ) hybridize with or bind to mRNA transcripts and/or genomic DNA encoding a polypeptide to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid sequence which binds to DNA duplexes, through specific interactions in the major groove of the double helix. Antisense nucleic acid sequences may be introduced

into a plant by transformation or direct injection at a specific tissue site. Alternatively, antisense nucleic acid sequences can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense nucleic acid sequences can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid sequence to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid sequences can also be delivered to cells using the vectors described herein.

According to a further aspect, the antisense nucleic acid sequence is an a-anomeric nucleic acid sequence. An a-anomeric nucleic acid sequence forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gaultier et al. (1987) Nucl Ac Res 15: 6625-6641). The antisense nucleic acid sequence may also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) Nucl Ac Res 15, 6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215, 327-330).

The reduction or substantial elimination of endogenous gene expression may also be performed using ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid sequence, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) Nature 334, 585-591) can be used to catalytically cleave mRNA transcripts encoding a polypeptide, thereby substantially reducing the number of mRNA transcripts to be translated into a polypeptide. A ribozyme having specificity for a nucleic acid sequence can be designed (see for example: Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742). Alternatively, mRNA transcripts corresponding to a nucleic acid sequence can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (Bartel and Szostak (1993) Science 261, 1411-1418). The use of ribozymes for gene silencing in plants is known in the art (e.g., Atkins et al. (1994) WO 94/00012; Lenne et al. (1995) WO 95/03404; Lutziger et al. (2000) WO 00/00619; Prinsen et al. (1997) WO 97/13865 and Scott et al. (1997) WO 97/38116).

Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by strategies as described by, among others, Angell and Baulcombe ((1999) Plant J 20(3): 357-62), (Amplicon VIGS WO 98/36083), or Baulcombe (WO 99/15682).

Gene silencing may also occur if there is a mutation on an endogenous gene and/or a mutation on an isolated gene/nucleic acid subsequently introduced into a plant. The reduction or substantial elimination may be caused by a non-functional polypeptide. For example, the polypeptide may bind to various interacting proteins; one or more mutation(s) and/or truncation(s) may therefore provide for a polypeptide that is still able to bind interacting proteins (such as receptor proteins) but that cannot exhibit its normal function (such as signalling ligand).

A further approach to gene silencing is by targeting nucleic acid sequences complementary to the regulatory region of the gene (e.g., the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See Helene, C., Anticancer Drug Res. 6, 569-84, 1991; Helene et al., Ann. N.Y. Acad. Sci. 660, 27-36 1992; and Maher, L.J. Bioassays 14, 807-15, 1992.

Other methods, such as the use of antibodies directed to an endogenous polypeptide for inhibiting its function in planta, or interference in the signalling pathway in which a polypeptide is involved, will be well known to the skilled man. In particular, it can be envisaged that manmade molecules may be useful for inhibiting the biological function of a target polypeptide, or for interfering with the signalling pathway in which the target polypeptide is involved.

Alternatively, a screening program may be set up to identify in a plant population natural variants of a gene, which variants encode polypeptides with reduced activity. Such natural variants may also be used for example, to perform homologous recombination.

Artificial and/or natural microRNAs (miRNAs) may be used to knock out gene expression and/or mRNA translation. Endogenous miRNAs are single stranded small RNAs of typically 19-24 nucleotides long. They function primarily to regulate gene expression and/ or mRNA translation. Most plant microRNAs (miRNAs) have perfect or near-perfect complementarity with their target sequences. However, there are natural targets with up to five mismatches. They are processed from longer non-coding RNAs with characteristic fold-back structures by double-strand specific RNases of the Dicer family. Upon processing, they are incorporated in the RNA-induced silencing complex (RISC) by binding to its main component, an Argonaute protein. MiRNAs serve as the specificity components of RISC, since they base-pair to target nucleic acids, mostly mRNAs, in the cytoplasm. Subsequent regulatory events include target mRNA cleavage and destruction and/or translational inhibition. Effects of miRNA overexpression are thus often reflected in decreased mRNA levels of target genes.

Artificial microRNAs (amiRNAs), which are typically 21 nucleotides in length, can be genetically engineered specifically to negatively regulate gene expression of single or multiple genes of interest. Determinants of plant microRNA target selection are well known in the art. Empirical parameters for target recognition have been defined and can be used to aid in the design of specific amiRNAs, (Schwab et al., Dev. Cell 8, 517–527, 2005). Convenient tools for design and generation of amiRNAs and their precursors are also available to the public (Schwab et al., Plant Cell 18, 1121-1133, 2006).

For optimal performance, the gene silencing techniques used for reducing expression in a plant of an endogenous gene requires the use of nucleic acid sequences from monocotyledonous plants for transformation of monocotyledonous plants, and from dicotyledonous plants for transformation of dicotyledonous plants. Preferably, a nucleic acid sequence from any given plant species is introduced into that same species. For example, a nucleic acid sequence from rice is transformed into a rice plant. However, it is not an absolute requirement that the nucleic acid sequence to be introduced originates from the same plant species as the plant in which it will be introduced. It is sufficient that there is substantial homology between the endogenous target gene and the nucleic acid to be introduced.

Described above are examples of various methods for the reduction or substantial elimination of expression in a plant of an endogenous gene. A person skilled in the art would readily be able to adapt the aforementioned methods for silencing so as to achieve reduction of expression of an endogenous gene in a whole plant or in parts thereof through the use of an appropriate promoter, for example.

Selectable marker (gene)/Reporter gene

"Selectable marker", "selectable marker gene" or “reporter gene” includes any gene that confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells that are transfected or transformed with a nucleic acid construct of the invention. These marker genes enable the identification of a successful transfer of the nucleic acid molecules via a series of different principles. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance, that introduce a new metabolic trait or that allow visual selection. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as nptII that phosphorylates neomycin and kanamycin, or hpt, phosphorylating hygromycin, or genes conferring resistance to, for example, bleomycin, streptomycin, tetracyclin, chloramphenicol, ampicillin, gentamycin,

geneticin (G418), spectinomycin or blasticidin), to herbicides (for example bar which provides resistance to Basta®; aroA or gox providing resistance against glyphosate, or the genes conferring resistance to, for example, imidazolinone, phosphinothricin or sulfonylurea), or genes that provide a metabolic trait (such as manA that allows plants to use mannose as sole carbon source or xylose isomerase for the utilisation of xylose, or antinutritive markers such as the resistance to 2-deoxyglucose). Expression of visual marker genes results in the formation of colour (for example β-glucuronidase, GUS or β-galactosidase with its coloured substrates, for example X-Gal), luminescence (such as the luciferin/luceferase system) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof). This list represents only a small number of possible markers. The skilled worker is familiar with such markers. Different markers are preferred, depending on the organism and the selection method.

It is known that upon stable or transient integration of nucleic acids into plant cells, only a minority of the cells takes up the foreign DNA and, if desired, integrates it into its genome, depending on the expression vector used and the transfection technique used. To identify and select these integrants, a gene coding for a selectable marker (such as the ones described above) is usually introduced into the host cells together with the gene of interest. These markers can for example be used in mutants in which these genes are not functional by, for example, deletion by conventional methods. Furthermore, nucleic acid molecules encoding a selectable marker can be introduced into a host cell on the same vector that comprises the sequence encoding the polypeptides of the invention or used in the methods of the invention, or else in a separate vector. Cells which have been stably transfected with the introduced nucleic acid can be identified for example by selection (for example, cells which have integrated the selectable marker survive whereas the other cells die).

Since the marker genes, particularly genes for resistance to antibiotics and herbicides, are no longer required or are undesired in the transgenic host cell once the nucleic acids have been introduced successfully, the process according to the invention for introducing the nucleic acids advantageously employs techniques which enable the removal or excision of these marker genes. One such a method is what is known as co-transformation. The co-transformation method employs two vectors simultaneously for the transformation, one vector bearing the nucleic acid according to the invention and a second bearing the marker gene(s). A large proportion of transformants receives or, in the case of plants, comprises (up to 40% or more of the transformants), both vectors. In case of transformation with *Agrobacteria*, the transformants usually receive only a part of the vector, i.e. the sequence flanked by the T-DNA, which usually represents the expression cassette. The marker genes can subsequently be removed from the transformed plant by performing crosses. In another method, marker

genes integrated into a transposon are used for the transformation together with desired nucleic acid (known as the Ac/Ds technology). The transformants can be crossed with a transposase source or the transformants are transformed with a nucleic acid construct conferring expression of a transposase, transiently or stable. In some cases (approx. 10%), the transposon jumps out of the genome of the host cell once transformation has taken place successfully and is lost. In a further number of cases, the transposon jumps to a different location. In these cases the marker gene must be eliminated by performing crosses. In microbiology, techniques were developed which make possible, or facilitate, the detection of such events. A further advantageous method relies on what is known as recombination systems; whose advantage is that elimination by crossing can be dispensed with. The best-known system of this type is what is known as the Cre/lox system. Cre1 is a recombinase that removes the sequences located between the loxP sequences. If the marker gene is integrated between the loxP sequences, it is removed once transformation has taken place successfully, by expression of the recombinase. Further recombination systems are the HIN/HIX, FLP/FRT and REP/STB system (Tribble et al., J. Biol. Chem., 275, 2000: 22255-22267; Velmurugan et al., J. Cell Biol., 149, 2000: 553-566). A site-specific integration into the plant genome of the nucleic acid sequences according to the invention is possible. Naturally, these methods can also be applied to microorganisms such as yeast, fungi or bacteria.

Transgenic/Transgene/Recombinant

For the purposes of the invention, "transgenic", “transgene” or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

(a) the nucleic acid sequences encoding proteins useful in the methods of the invention, or

(b) genetic control sequence(s) which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or

(c) a) and b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original plant or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a

sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding nucleic acid sequence encoding a polypeptide useful in the methods of the present invention, as defined above – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

A transgenic plant for the purposes of the invention is thus understood as meaning, as above, that the nucleic acids used in the method of the invention are not at their natural locus in the genome of said plant, it being possible for the nucleic acids to be expressed homologously or heterologously. However, as mentioned, transgenic also means that, while the nucleic acids according to the invention or used in the inventive method are at their natural position in the genome of a plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acids takes place. Preferred transgenic plants are mentioned herein.

<u>Transformation</u>

The term “introduction” or “transformation” as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated there from. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell may then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

The transfer of foreign genes into the genome of a plant is called transformation. Transformation of plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation and regeneration of plants from plant tissues or plant cells may be utilized for transient or for stable transformation. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., (1982) Nature 296, 72-74; Negrutiu I et al. (1987) Plant Mol Biol 8: 363-373); electroporation of protoplasts (Shillito R.D. et al. (1985) Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A et al., (1986) Mol. Gen Genet 202: 179-185); DNA or RNA-coated particle bombardment (Klein TM et al., (1987) Nature 327: 70) infection with (non-integrative) viruses and the like. Transgenic plants, including transgenic crop plants, are preferably produced via *Agrobacterium*-mediated transformation. An advantageous transformation method is the transformation *in planta*. To this end, it is possible, for example, to allow the agrobacteria to act on plant seeds or to inoculate the plant meristem with agrobacteria. It has proved particularly expedient in accordance with the invention to allow a suspension of transformed agrobacteria to act on the intact plant or at least on the flower primordia. The plant is subsequently grown on until the seeds of the treated plant are obtained (Clough and Bent, Plant J. (1998) 16, 735–743). Methods for *Agrobacterium*-mediated transformation of rice include well known methods for rice transformation, such as those described in any of the following: European patent application EP 1198985 A1, Aldemita and Hodges (Planta 199: 612-617, 1996); Chan et al. (Plant Mol Biol 22 (3): 491-506, 1993), Hiei et al. (Plant J 6 (2): 271-282, 1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida et al. (Nat. Biotechnol 14(6): 745-50, 1996) or Frame et al. (Plant Physiol 129(1): 13-22, 2002), which disclosures are incorporated by reference herein as if fully set forth. Said methods are further described by way of example in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The nucleic acids or the construct to be expressed is preferably cloned into a vector, which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711). Agrobacteria transformed by such a vector can then be used in known manner for the transformation of plants, such as plants used as a model, like *Arabidopsis* (*Arabidopsis thaliana* is within the scope of the present invention not considered as a crop plant), or crop plants such as, by way of example, tobacco plants, for

example by immersing bruised leaves or chopped leaves in an agrobacterial solution and then culturing them in suitable media. The transformation of plants by means of *Agrobacterium tumefaciens* is described, for example, by Höfgen and Willmitzer in Nucl. Acid Res. (1988) 16, 9877 or is known inter alia from F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

In addition to the transformation of somatic cells, which then have to be regenerated into intact plants, it is also possible to transform the cells of plant meristems and in particular those cells which develop into gametes. In this case, the transformed gametes follow the natural plant development, giving rise to transgenic plants. Thus, for example, seeds of *Arabidopsis* are treated with Agrobacteria and seeds are obtained from the developing plants of which a certain proportion is transformed and thus transgenic [Feldman, KA and Marks MD (1987). Mol Gen Genet 208:274-289; Feldmann K (1992). In: C Koncz, N-H Chua and J Shell, eds, Methods in Arabidopsis Research. Word Scientific, Singapore, pp. 274-289]. Alternative methods are based on the repeated removal of the inflorescences and incubation of the excision site in the center of the rosette with transformed agrobacteria, whereby transformed seeds can likewise be obtained at a later point in time (Chang (1994). Plant J. 5: 551-558; Katavic (1994). Mol Gen Genet, 245: 363-370). However, an especially effective method is the vacuum infiltration method with its modifications such as the “floral dip” method. In the case of vacuum infiltration of *Arabidopsis*, intact plants under reduced pressure are treated with an agrobacterial suspension [Bechthold, N (1993). C R Acad Sci Paris Life Sci, 316: 1194-1199], while in the case of the ”floral dip” method the developing floral tissue is incubated briefly with a surfactant-treated agrobacterial suspension [Clough, SJ and Bent AF (1998) The Plant J. 16, 735-743]. A certain proportion of transgenic seeds are harvested in both cases, and these seeds can be distinguished from non-transgenic seeds by growing under the above-described selective conditions. In addition the stable transformation of plastids is of advantages because plastids are inherited maternally is most crops reducing or eliminating the risk of transgene flow through pollen. The transformation of the chloroplast genome is generally achieved by a process which has been schematically displayed in Klaus et al., 2004 [Nature Biotechnology 22 (2), 225-229]. Briefly the sequences to be transformed are cloned together with a selectable marker gene between flanking sequences homologous to the chloroplast genome. These homologous flanking sequences direct site specific integration into the plastome. Plastidal transformation has been described for many different plant species and an overview is given in Bock (2001) Transgenic plastids in basic research and plant biotechnology. J Mol Biol. 2001 Sep 21; 312 (3):425-38 or Maliga, P (2003) Progress towards commercialization of plastid transformation technology. Trends Biotechnol.

21, 20-28. Further biotechnological progress has recently been reported in form of marker free plastid transformants, which can be produced by a transient co-integrated maker gene (Klaus et al., 2004, Nature Biotechnology 22(2), 225-229).

T-DNA activation tagging

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

TILLING

The term “TILLING” is an abbreviation of “Targeted Induced Local Lesions In Genomes” and refers to a mutagenesis technology useful to generate and/or identify nucleic acids encoding proteins with modified expression and/or activity. TILLING also allows selection of plants carrying such mutant variants. These mutant variants may exhibit modified expression, either in strength or in location or in timing (if the mutations affect the promoter for example). These mutant variants may exhibit higher activity than that exhibited by the gene in its natural form. TILLING combines high-density mutagenesis with high-throughput screening methods. The steps typically followed in TILLING are: (a) EMS mutagenesis (Redei GP and Koncz C (1992) In Methods in Arabidopsis Research, Koncz C, Chua NH, Schell J, eds. Singapore, World Scientific Publishing Co, pp. 16–82; Feldmann et al., (1994) In Meyerowitz EM, Somerville CR, eds, Arabidopsis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 137-172; Lightner J and Caspar T (1998) In J Martinez-Zapater, J Salinas, eds, Methods on Molecular Biology, Vol. 82. Humana Press, Totowa, NJ, pp 91-104); (b) DNA preparation and pooling of individuals; (c) PCR amplification of a region of interest; (d) denaturation and annealing to allow formation of heteroduplexes; (e) DHPLC, where the presence of a heteroduplex in a pool is detected as an extra peak in the chromatogram; (f) identification of the mutant individual; and (g) sequencing of the mutant PCR product. Methods for TILLING are well known in the art (McCallum et al., (2000) Nat Biotechnol 18: 455-457; reviewed by Stemple (2004) Nat Rev Genet 5(2): 145-50).

Homologous recombination

Homologous recombination allows introduction in a genome of a selected nucleic acid at a defined selected position. Homologous recombination is a standard technology used routinely in biological sciences for lower organisms such as yeast or the moss *Physcomitrella*. Methods for performing homologous recombination in plants have been described not only for model plants (Offringa et al. (1990) EMBO J 9(10): 3077-84) but also for crop plants, for example rice (Terada et al. (2002) Nat Biotech 20(10): 1030-4; Iida and Terada (2004) Curr Opin Biotech 15(2): 132-8), and approaches exist that are generally applicable regardless of the target organism (Miller et al, Nature Biotechnol. 25, 778-785, 2007).

Increase/Improve/Enhance

The terms “increase”, “improve” or “enhance” are interchangeable and shall mean in the sense of the application at least a 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%, preferably at least 15% or 20%, more preferably 25%, 30%, 35% or 40% more of the factor in question in comparison to any control.

Plant

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

Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantae, in particular monocotyledonous and dicotyledonous plants including fodder or forage legumes, ornamental plants, food crops, trees or shrubs selected from the list comprising *Acer* spp., *Actinidia* spp., *Abelmoschus* spp., *Agave sisalana, Agropyron* spp., *Agrostis stolonifera, Allium* spp., *Amaranthus* spp., *Ammophila arenaria, Ananas comosus, Annona* spp., *Apium graveolens, Arachis spp, Artocarpus* spp., *Asparagus officinalis, Avena* spp. *(e.g. Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida), Averrhoa carambola, Bambusa sp., Benincasa hispida, Bertholletia excelsea, Beta vulgaris, Brassica* spp. *(e.g. Brassica napus, Brassica rapa* ssp. [canola, oilseed rape, turnip rape]), *Cadaba farinosa, Camellia sinensis, Canna indica, Cannabis sativa, Capsicum* spp., *Carex elata, Carica papaya, Carissa macrocarpa, Carya* spp., *Carthamus tinctorius, Castanea* spp., *Ceiba pentandra, Cichorium endivia,*

Cinnamomum spp., *Citrullus lanatus, Citrus* spp., *Cocos* spp., *Coffea* spp., *Colocasia esculenta, Cola* spp., *Corchorus sp., Coriandrum sativum, Corylus* spp., *Crataegus* spp., *Crocus sativus, Cucurbita* spp., *Cucumis* spp., *Cynara* spp., *Daucus carota, Desmodium* spp., *Dimocarpus longan, Dioscorea* spp., *Diospyros* spp., *Echinochloa* spp., *Elaeis (e.g. Elaeis guineensis, Elaeis oleifera), Eleusine coracana, Erianthus sp., Eriobotrya japonica, Eucalyptus sp., Eugenia uniflora, Fagopyrum* spp., *Fagus* spp., *Festuca arundinacea, Ficus carica, Fortunella* spp., *Fragaria* spp., *Ginkgo biloba, Glycine* spp. *(e.g. Glycine max, Soja hispida or Soja max), Gossypium hirsutum, Helianthus* spp. *(e.g. Helianthus annuus), Hemerocallis fulva, Hibiscus* spp., *Hordeum* spp. *(e.g. Hordeum vulgare), Ipomoea batatas, Juglans* spp., *Lactuca sativa, Lathyrus* spp., *Lens culinaris, Linum usitatissimum, Litchi chinensis, Lotus* spp., *Luffa acutangula, Lupinus* spp., *Luzula sylvatica, Lycopersicon* spp. *(e.g. Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme), Macrotyloma* spp., *Malus* spp., *Malpighia emarginata, Mammea americana, Mangifera indica, Manihot* spp., *Manilkara zapota, Medicago sativa, Melilotus* spp., *Mentha* spp., *Miscanthus sinensis, Momordica* spp., *Morus nigra, Musa* spp., *Nicotiana* spp., *Olea* spp., *Opuntia* spp., *Ornithopus* spp., *Oryza* spp. *(e.g. Oryza sativa, Oryza latifolia), Panicum miliaceum, Panicum virgatum, Passiflora edulis, Pastinaca sativa, Pennisetum sp., Persea* spp., *Petroselinum crispum, Phalaris arundinacea, Phaseolus* spp., *Phleum pratense, Phoenix* spp., *Phragmites australis, Physalis* spp., *Pinus* spp., *Pistacia vera, Pisum* spp., *Poa* spp., *Populus* spp., *Prosopis* spp., *Prunus* spp., *Psidium* spp., *Punica granatum, Pyrus communis, Quercus* spp., *Raphanus sativus, Rheum rhabarbarum, Ribes* spp., *Ricinus communis, Rubus* spp., *Saccharum* spp., *Salix sp., Sambucus* spp., *Secale cereale, Sesamum* spp., *Sinapis sp., Solanum* spp. *(e.g. Solanum tuberosum, Solanum integrifolium or Solanum lycopersicum), Sorghum bicolor, Spinacia* spp., *Syzygium* spp., *Tagetes* spp., *Tamarindus indica, Theobroma cacao, Trifolium* spp., *Triticosecale rimpaui, Triticum* spp. *(e.g. Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare), Tropaeolum minus, Tropaeolum majus, Vaccinium* spp., *Vicia* spp., *Vigna* spp., *Viola odorata, Vitis* spp., *Zea mays, Zizania palustris, Ziziphus* spp., amongst others.

Detailed description of the invention

Surprisingly, it has now been found that modulating expression in a plant of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof gives plants having altered growth and/or development relative to control plants. According to a first embodiment, the present invention provides a method for altering plant growth and/or development relative to control plants, comprising modulating expression in a plant of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

A preferred method for modulating (preferably, increasing) expression of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof is by introducing and expressing in a plant a nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

Any reference hereinafter to a "protein useful in the methods of the invention" is taken to mean a UBP polypeptide of the UBP15 subfamily or a homologue thereof as defined herein. Any reference hereinafter to a "nucleic acid useful in the methods of the invention" is taken to mean a nucleic acid capable of encoding such a UBP polypeptide. The nucleic acid to be introduced into a plant (and therefore useful in performing the methods of the invention) is any nucleic acid encoding the type of protein which will now be described, hereinafter also referred to as "UBP15 nucleic acid" or "UBP15 gene".

A "UBP15 polypeptide" as defined herein refers to any polypeptide comprising each of the following:

(i) a Cysteine box (Cys box); and

(ii) a Histidine box (His box); and

(iii) a ZnMYND zinc finger domain.

The Cys and His box are two well conserved motifs found in a conserved catalytic domain called the UBP domain (see Figure 1). The Cys box and the His box comprise the catalytic triad residues (Cys in Cys box, His and Asp/Asn in His box) (Amerik and Hochstrasser, 2004). The length of UBP domains vary from 300 to 900 amino acids in length, and despite sometimes having low overall sequence conservation, they typically display a conserved three dimensional structure. Within the UBP domains, the Cysteine in Cys box plays an essential role in catalytic activity and specific mutation in the Cysteine can abolish the de-ubiquitination activity of UBPs (Papa and Hochstrasser, 1993; Chandler et al., 1997; Rao-Naik et al., 2000; Yan et al., 2000; Baek et al., 2001; Doelling et al., 2001; Hanna et al., 2006).

The UBP15 subfamily includes UBP15, UBP16, UBP17, UBP18 and UBP19. Members of this subfamily and homologues thereof comprise a signature MYND type Zinc finger domain, which was reported to be a protein-protein interaction domain in mammals (Gross and McGinnis, 1996; Lutterbach et al., 1998a; Lutterbach et al., 1998b; Masselink and Bernards, 2000).

A genetic interaction analysis among members of the UBP15 subfamily revealed that UBP15 and UBP16, but not UBP17, have functional redundancy, even though UBP16 and UBP17 are equally related to UBP15. Mutation of another subfamily member, UBP19, leads to embryo lethality, while loss-of-function of its closest related member, UBP18, exhibits no visible defect.

UBP sequences useful in performing the methods of the invention may also be identified by including a query sequence in a UBP phylogenetic tree, such as the one depicted in Figure 1. UBP15 polypeptides or homologues thereof will cluster with the UPB15 subfamily rather than with any other subfamily.

Furthermore, UBP15 polypeptides and homologues thereof typically have UBP activity *In vitro*.

UBP15 polypeptides or homologues thereof useful in the methods of the invention typically have in increasing order of preference at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% overall sequence identity to any one of UBP15 (SEQ ID NO: 2), UBP16 (SEQ ID NO: 4), UBP17 (SEQ ID NO: 6), UBP18 (SEQ ID NO: 8) or UBP19 (SEQ ID NO: 10) from *Arabidopsis thaliana*. The overall sequence identity may be determined using a global alignment algorithm, such as the Needleman Wunsch algorithm in the program GAP (GCG Wisconsin Package, Accelrys), preferably with default parameters. Compared to overall sequence identity, the sequence identity will generally be higher when only conserved domains or motifs are considered.

The term “domain” and “motif” is defined in the “definitions” section herein. Specialist databases exist for the identification of domains, for example, SMART (Schultz et al. (1998) Proc. Natl. Acad. Sci. USA 95, 5857-5864; Letunic et al. (2002) Nucleic Acids Res 30, 242-244), InterPro (Mulder et al., (2003) Nucl. Acids. Res. 31, 315-318), Prosite (Bucher and Bairoch (1994), A generalized profile syntax for biomolecular sequences motifs and its function in automatic sequence interpretation. (In) ISMB-94; Proceedings 2nd International Conference on Intelligent Systems for Molecular Biology. Altman R., Brutlag D., Karp P., Lathrop R., Searls D., Eds., pp53-61, AAAI Press, Menlo Park; Hulo et al., Nucl. Acids. Res. 32:D134-D137, (2004)), or Pfam (Bateman et al., Nucleic Acids Research 30(1): 276-280 (2002)). A set of tools for *in silico* analysis of protein sequences is available on the ExPASy proteomics server (Swiss Institute of Bioinformatics (Gasteiger et al., ExPASy: the proteomics server for in-depth protein knowledge and analysis, Nucleic Acids Res. 31:3784-3788(2003)). Domains or motifs may also be identified using routine techniques, such as by sequence alignment.

Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch ((1970) J Mol Biol 48: 443-453) to find the global (i.e. spanning the complete sequences) alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. The BLAST algorithm (Altschul et al. (1990) J Mol Biol 215: 403-10) calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (NCBI). Homologues may readily be identified using, for example, the ClustalW multiple sequence alignment algorithm (version 1.83), with the default pairwise alignment parameters, and a scoring method in percentage. Global percentages of similarity and identity may also be determined using one of the methods available in the MatGAT software package (Campanella et al., BMC Bioinformatics. 2003 Jul 10;4:29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences.). Minor manual editing may be performed to optimise alignment between conserved motifs, as would be apparent to a person skilled in the art. Furthermore, instead of using full-length sequences for the identification of homologues, specific domains may also be used. The sequence identity values may be determined over the entire nucleic acid or amino acid sequence or over selected domains or conserved motif(s), using the programs mentioned above using the default parameters. For local alignments, the Smith-Waterman algorithm is particularly useful (Smith TF, Waterman MS (1981) J. Mol. Biol 147(1); 195-7).

Examples of nucleic acids encoding members of the UBP15 subfamily are represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 and 44. Such nucleic acids are useful in performing the methods of the invention. The nucleotide sequence represented by SEQ ID NO: 11 encodes the polypeptide sequence represented by SEQ ID NO: 12, a UBP from rice (Nipponbare strain) that is closely related to AtUBP15, and has 53% identity to AtUBP15 on a nucleotide level, 40% identity on a protein level and 54% similarity on a protein level. The nucleotide sequence represented by SEQ ID NO: 13 encodes the polypeptide sequence represented by SEQ ID NO: 14, a UBP from rice (Nipponbare strain) that is closely related to AtUBP16, and has 44% identity to AtUBP16 on a nucleotide level, 30% identity on a protein level and 44% similarity on a protein level to AtUBP16. The nucleotide sequence represented by SEQ ID NO: 15 encodes the polypeptide sequence represented by SEQ ID NO: 16, a UBP from rice (Nipponbare strain) that is closely related to AtUBP17, and has 40% identity to AtUBP17 on a nucleotide level, 33% identity on a protein level and 45% similarity on a protein level to AtUBP17. The nucleotide sequence represented by SEQ ID NO: 17 encodes the polypeptide sequence represented by SEQ ID NO: 18, a UBP from rice

(Nipponbare strain) that is closely related to AtUBP18, and has 34% identity to AtUBP17 on a nucleotide level, 30% identity on a protein level and 39% similarity on a protein level to AtUBP18. SEQ ID NO: 19 is a nucleotide sequence from maize (Zea mays) showing some similarity to SEQ ID NO: 11. SEQ ID NO: 19 is the longest cDNA sequence of maize obtained by assembling the ESTs of SEQ ID NOs 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 and 38. SEQ ID NO: 39 is another nucleotide sequence from maize showing some similarity to SEQ ID NO: 11. SEQ ID NO: 39 was assembled from the ESTs of SEQ ID NOs 40, 41, 42 and 43. SEQ ID NO: 44 is another nucleotide sequence from maize showing some similarity to SEQ ID NO: 11. SEQ ID NO: 44 was assembled from the ESTs of SEQ ID NOs 45, 46, 47, 48, 49, 50 and 51.

SEQ ID NOs 12, 14, 16 and 18 are examples of orthologues of the UBP polypeptides represented by SEQ ID NOs: 2, 4, 6 and 8, respectively. The terms “orthologues” and “paralogues” are as defined herein. Further orthologues and paralogues may readily be identified by performing a so-called reciprocal blast search. Typically, this involves a first BLAST involving BLASTing a query sequence (for example using any of sequences SEQ ID NOs 2, 4, 6, 8 or 10) against any sequence database, such as the publicly available NCBI database. BLASTN or TBLASTX (using standard default values) are generally used when starting from a nucleotide sequence, and BLASTP or TBLASTN (using standard default values) when starting from a protein sequence. The BLAST results may optionally be filtered. The full-length sequences of either the filtered results or non-filtered results are then BLASTed back (second BLAST) against sequences from the organism from which the query sequence is derived (where the query sequence is SEQ ID NO: 1 to SEQ ID NO: 10, the second BLAST would therefore be against *Arabidopsis* sequences). The results of the first and second BLASTs are then compared. A paralogue is identified if a high-ranking hit from the first blast is from the same species as from which the query sequence is derived, a BLAST back then ideally results in the query sequence being amongst the highest hits; an orthologue is identified if a high-ranking hit in the first BLAST is not from the same species as from which the query sequence is derived, and preferably results upon BLAST back in the query sequence being among the highest hits.

High-ranking hits are those having a low E-value. The lower the E-value, the more significant the score (or in other words the lower the chance that the hit was found by chance). Computation of the E-value is well known in the art. In addition to E-values, comparisons are also scored by percentage identity. Percentage identity refers to the number of identical nucleotides (or amino acids) between the two compared nucleic acid (or polypeptide) sequences over a particular length. In the case of large families, ClustalW may be used,

followed by a neighbour joining tree, to help visualize clustering of related genes and to identify orthologues and paralogues.

Nucleic acid variants may also be useful in practising the methods of the invention. Examples of such variants include nucleic acids encoding homologues and derivatives of any one of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8 or 10, the terms "homologue" and "derivative" being as defined herein. Also useful in the methods of the invention are nucleic acids encoding homologues and derivatives of orthologues or paralogues of any one of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8 or 10. Homologues and derivatives useful in the methods of the present invention have substantially the same biological and functional activity as the unmodified protein from which they are derived.

Further nucleic acid variants useful in practising the methods of the invention include portions of nucleic acids encoding UBP15 polypeptides or homologues thereof, nucleic acids hybridising to nucleic acids encoding UBP15 polypeptides or homologues thereof, splice variants of nucleic acids encoding UBP15 polypeptides or homologues thereof, allelic variants of nucleic acids encoding UBP15 polypeptides or homologues thereof and variants of nucleic acids encoding UBP15 polypeptides obtained by gene shuffling. The terms hybridising sequence, splice variant, allelic variant and gene shuffling are as described herein.

Nucleic acids encoding UBP15 polypeptides or homologues thereof need not be full-length nucleic acids, since performance of the methods of the invention does not rely on the use of full-length nucleic acid sequences. According to the present invention, there is provided a method for altering plant growth and development, comprising introducing and expressing in a plant a portion of any one of the nucleic acid sequences represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or a portion of a nucleic acid encoding an orthologue, paralogue or homologue of any of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18.

A portion of a nucleic acid may be prepared, for example, by making one or more deletions to the nucleic acid. The portions may be used in isolated form or they may be fused to other coding (or non-coding) sequences in order to, for example, produce a protein that combines several activities. When fused to other coding sequences, the resultant polypeptide produced upon translation may be bigger than that predicted for the protein portion.

Portions useful in the methods of the invention, encode a UBP15 polypeptide or a homologue thereof as defined herein, and have substantially the same biological activity as the amino acid sequences represented by any of SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18. Preferably, the portion is a portion of any one of the nucleic acids given in represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or is a portion of a nucleic acid encoding an orthologue or paralogue of any one of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18. Preferably the portion is at least 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2150, 1200, 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2600, 2650, 2700, 2750, 2800, 2850, 2900, 2950, 3000, 3050, 3100, 3150 or more consecutive nucleotides in length, the consecutive nucleotides being of any one of the nucleic acid sequences represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or of a nucleic acid encoding an orthologue or paralogue of any one of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18. Most preferably the portion is a portion of the nucleic acid of any one of SEQ ID NOs 1, 3, 5, 7 or 9. Preferably, the portion encodes a fragment of an amino acid sequence which, when used in the construction of a UBP phylogenetic tree, such as the one depicted in Figure 1, clusters with the UBP15 subfamily of proteins rather than with any other subfamily of UBPs.

Another nucleic acid variant useful in the methods of the invention is a nucleic acid capable of hybridising, under reduced stringency conditions, preferably under stringent conditions, with a nucleic acid encoding a UBP15 polypeptide or a homologue thereof as defined herein, or with a portion as defined herein.

According to the present invention, there is provided a method for altering plant growth and/or development, comprising introducing and expressing in a plant a nucleic acid capable of hybridizing to any one of the nucleic acids represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or comprising introducing and expressing in a plant a nucleic acid capable of hybridising to a nucleic acid encoding an orthologue, paralogue or homologue of any of the nucleic acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or, 18.

Hybridising sequences useful in the methods of the invention encode a UBP15 polypeptide as defined herein, having substantially the same biological activity as the amino acid sequences represented by any one of SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18. Preferably, the hybridising sequence is capable of hybridising to any one of the nucleic acids represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or to a portion of any of

these sequences, a portion being as defined above, or the hybridising sequence is capable of hybridising to a nucleic acid encoding an orthologue or paralogue of any one of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18. Most preferably, the hybridising sequence is capable of hybridising to a nucleic acid as represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15 or 17 or to a portion thereof.

Preferably, the hybridising sequence encodes a polypeptide with an amino acid sequence which, when full-length and used in the construction of a UBP phylogenetic tree, such as the one depicted in Figure 1, clusters with the subfamily of UBP15 polypeptides rather than with any other UBP subfamily.

Another nucleic acid variant useful in the methods of the invention is a splice variant encoding a UBP15 polypeptide or a homologue thereof as defined hereinabove, a splice variant being as defined herein.

According to the present invention, there is provided a method for altering plant growth and/or development, comprising introducing and expressing in a plant a splice variant of any one of the nucleic acid sequences represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or a splice variant of a nucleic acid encoding an orthologue, paralogue or homologue of any of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18.

Preferred splice variants are splice variants of a nucleic acid represented by any one of SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15 or 17, or a splice variant of a nucleic acid encoding an orthologue or paralogue of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16 or 18. Preferably, the amino acid sequence encoded by the splice variant, when used in the construction of a UBP phylogenetic tree, such as the one depicted in Figure 1, clusters with the subfamily of UBP15 polypeptides rather than with any other UBP subfamily.

Another nucleic acid variant useful in performing the methods of the invention is an allelic variant of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof as defined hereinabove, an allelic variant being as defined herein.

According to the present invention, there is provided a method for altering plant growth and/or development, comprising introducing and expressing in a plant an allelic variant of any one of the nucleic acids represented by SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or comprising introducing and expressing in a plant an allelic variant of a nucleic acid encoding

an orthologue, paralogue or homologue of any of the amino acid sequences represented by SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18.

The allelic variants useful in the methods of the present invention have substantially the same biological activity as any one of the UBP15 subfamily polypeptides represented by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16 or 18. Allelic variants exist in nature, and encompassed within the methods of the present invention is the use of these natural alleles. Preferably, the allelic variant is an allelic variant of any one of SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or an allelic variant of a nucleic acid encoding an orthologue or paralogue of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16 or 18. Preferably, the amino acid sequence encoded by the allelic variant, when used in the construction of a UBP phylogenetic tree, such as the one depicted in Figure 1, clusters with the UBP15 subfamily rather than with any other UBP subfamily.

Gene shuffling or directed evolution may also be used to generate variants of nucleic acids encoding UBP15 polypeptides or homologues thereof as defined above; the term “gene shuffling” being as defined herein.

According to the present invention, there is provided a method for altering plant growth and/or development in plants, comprising introducing and expressing in a plant a variant of any one of the nucleic acid sequences represented by any one of SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39 or 44, or comprising introducing and expressing in a plant a variant of a nucleic acid encoding an orthologue, paralogue or homologue of any of the amino acid sequences represented by any one of SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18, which variant nucleic acid is obtained by gene shuffling.

Preferably, the amino acid sequence encoded by the variant nucleic acid obtained by gene shuffling, when used in the construction of a UBP phylogenetic tree such as the one depicted in Figure 1, clusters with the subfamily of UBP15 polypeptides rather than with any other UBP subfamily.

Furthermore, nucleic acid variants may also be obtained by site-directed mutagenesis. Several methods are available to achieve site-directed mutagenesis, the most common being PCR based methods (Current Protocols in Molecular Biology. Wiley Eds.).

Nucleic acids encoding UBP15 polypeptides may be derived from any natural or artificial source. The nucleic acid may be modified from its native form in composition and/or genomic environment through deliberate human manipulation. Preferably the UBP polypeptide-

encoding nucleic acid is from a plant, further preferably from a dicotyledonous plant, more preferably from the family Brassicaceae, most preferably the nucleic acid is from *Arabidopsis thaliana*.

Performance of the methods of the invention gives plants having altered plant growth and/or development.

The altered development may be in any part of a plant, in any cell, tissue or organ, particularly in the leaves. The altered development may be altered reproductive development. The altered development may cause altered plant phenotypes, such as altered leaf shape (particularly narrower and/or serrated and/or flat leaves); and altered phenotypes (such as altered flowering and/or changes in apical dominance and/or altered fertility). The altered development or phenotype may be caused by a change in cell proliferation.

The present invention encompasses plants or parts thereof (including seeds) obtainable by the methods according to the present invention. The plants or parts thereof comprise a nucleic acid transgene encoding a UBP15 polypeptide or a homologue thereof as defined above.

The invention also provides genetic constructs and vectors to facilitate introduction and/or expression in plants of nucleic acids encoding UBP15 polypeptides or homologues thereof. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells. The invention also provides use of a gene construct as defined herein in the methods of the invention.

More specifically, the present invention provides a construct comprising:

(a) a nucleic acid encoding a polypeptide that is a member of the UBP15 subfamily or a homologue thereof as defined above;

(b) one or more control sequences capable of driving expression of the nucleic acid sequence of (a); and optionally

(c) a transcription termination sequence.

Preferably, the nucleic acid encoding the UBP15 polypeptide or a homologue thereof is as defined above. The term “control sequence” and “termination sequence” are as defined herein.

Plants are transformed with a vector comprising any of the nucleic acids described above. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells containing the sequence of interest. The sequence of interest is operably linked to one or more control sequences (at least to a promoter).

Advantageously, any type of promoter, whether natural or synthetic, may be used to drive expression of the nucleic acid sequence. A constitutive promoter is particularly useful in the methods. Preferably the constitutive promoter is also a ubiquitous promoter. See the "Definitions" section herein for definitions of the various promoter types.

The constitutive promoter is preferably a CaMV35S promoter. Further preferably, the constitutive promoter is represented by a nucleic acid sequence substantially similar to SEQ ID NO: 52, most preferably the constitutive promoter is as represented by SEQ ID NO: 52. See Table B in the "Definitions" section herein for further examples of constitutive promoters.

Optionally, one or more terminator sequences may be used in the construct introduced into a plant. Additional regulatory elements may include transcriptional as well as translational enhancers. Those skilled in the art will be aware of terminator and enhancer sequences that may be suitable for use in performing the invention. An intron sequence may also be added to the 5' untranslated region (UTR) or in the coding sequence to increase the amount of the mature message that accumulates in the cytosol, as described in the definitions section. Other control sequences (besides promoter, enhancer, silencer, intron sequences, 3'UTR and/or 5'UTR regions) may be protein and/or RNA stabilizing elements. Such sequences would be known or may readily be obtained by a person skilled in the art.

The genetic constructs of the invention may further include an origin of replication sequence that is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

For the detection of the successful transfer of the nucleic acid sequences as used in the methods of the invention and/or selection of transgenic plants comprising these nucleic acids, it is advantageous to use marker genes (or reporter genes). Therefore, the genetic construct may optionally comprise a selectable marker gene. Selectable markers are described in more detail in the "definitions" section herein. The marker genes may be removed or excised

from the transgenic cell once they are no longer needed. Techniques for marker removal are known in the art, useful techniques are described above in the definitions section.

The invention also provides a method for the production of transgenic plants having altered growth and/or development relative to control plants, comprising introduction and expression in a plant of any nucleic acid encoding a UBP15 polypeptide or a homologue thereof as defined hereinabove.

More specifically, the present invention provides a method for the production of transgenic plants having altered growth and/or development, which method comprises:

(i) introducing and expressing in a plant or plant cell a UBP15 polypeptide-encoding nucleic acid; and

(ii) cultivating the plant cell under conditions promoting plant growth and development.

The nucleic acid of (i) may be any of the nucleic acids capable of encoding a UBP15 polypeptide or a homologue thereof as defined herein.

The nucleic acid may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of a plant). According to a preferred feature of the present invention, the nucleic acid is preferably introduced into a plant by transformation. The term “transformation” is described in more detail in the “definitions” section herein.

The genetically modified plant cells can be regenerated via all methods with which the skilled worker is familiar. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant. To select transformed plants, the plant material obtained in the transformation is, as a rule, subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected to a suitable selection by spraying. A further possibility consists in growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker such as the ones described above.

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

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed and homozygous second-generation (or T2) transformants selected, and the T2 plants may then further be propagated through classical breeding techniques. The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

The present invention clearly extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced by the parent in the methods according to the invention.

The invention also includes host cells containing an isolated nucleic acid encoding a UBP15 polypeptide or a homologue thereof as defined hereinabove. Preferred host cells according to the invention are plant cells. Host plants for the nucleic acids or the vector used in the method according to the invention, the expression cassette or construct or vector are, in principle, advantageously all plants, which are capable of synthesizing the polypeptides used in the inventive method.

The methods of the invention are advantageously applicable to any plant. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantae, in particular monocotyledonous and dicotyledonous plants including fodder or forage legumes, ornamental plants, food crops, trees or shrubs. According to a preferred embodiment of the present invention, the plant is a crop plant. Examples of crop

plants include soybean, sunflower, canola, alfalfa, rapeseed, cotton, tomato, potato and tobacco. Further preferably, the plant is a monocotyledonous plant. Examples of monocotyledonous plants include sugarcane. More preferably the plant is a cereal. Examples of cereals include rice, maize, wheat, barley, millet, rye, triticale, sorghum and oats.

The invention also extends to harvestable parts of a plant such as, but not limited to seeds, leaves, fruits, flowers, stems, roots, rhizomes, tubers and bulbs. The invention furthermore relates to products derived, preferably directly derived, from a harvestable part of such a plant, such as dry pellets or powders, oil, fat and fatty acids, starch or proteins.

According to a preferred feature of the invention, the modulated expression is increased expression. Methods for increasing expression of nucleic acids or genes, or gene products, are well documented in the art and examples are provided in the definitions section.

As mentioned above, a preferred method for modulating expression of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof is by introducing and expressing in a plant a nucleic acid encoding a UBP15 polypeptide or a homologue thereof; however the effects of performing the method, i.e. altered plant growth and/or development may also be achieved using other well known techniques, including but not limited to T-DNA activation tagging, TILLING, homologous recombination. A description of these techniques is provided in the definitions section.

The present invention also encompasses use of nucleic acids encoding UBP15 polypeptides or homologues thereof as described herein and use of these UBP15 polypeptides in altering growth and/or development in plants.

Nucleic acids encoding UBP15 polypeptide described herein, or the UBP15 polypeptides themselves, may find use in breeding programmes in which a DNA marker is identified which may be genetically linked to a UBP15 polypeptide-encoding gene. The nucleic acids/genes, or the UBP15 polypeptides themselves may be used to define a molecular marker. This DNA or protein marker may then be used in breeding programmes to select plants having altered growth and/or development as defined hereinabove in the methods of the invention.

Allelic variants of a UBP15 polypeptide-encoding nucleic acid/gene may also find use in marker-assisted breeding programmes. Such breeding programmes sometimes require introduction of allelic variation by mutagenic treatment of the plants, using for example EMS mutagenesis; alternatively, the programme may start with a collection of allelic variants of so

called "natural" origin caused unintentionally. Identification of allelic variants then takes place, for example, by PCR. This is followed by a step for selection of superior allelic variants of the sequence in question and which give altered growth and/or development. Selection is typically carried out by monitoring growth performance of plants containing different allelic variants of the sequence in question. Growth performance may be monitored in a greenhouse or in the field. Further optional steps include crossing plants in which the superior allelic variant was identified with another plant. This could be used, for example, to make a combination of interesting phenotypic features.

Nucleic acids encoding UBP15 polypeptides or homologues thereof may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. Such use of UBP15 polypeptide-encoding nucleic acids requires only a nucleic acid sequence of at least 15 nucleotides in length. The UBP15 polypeptide-encoding nucleic acids may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Sambrook J, Fritsch EF and Maniatis T (1989) Molecular Cloning, A Laboratory Manual) of restriction-digested plant genomic DNA may be probed with the UBP15-encoding nucleic acids. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) Genomics 1: 174-181) in order to construct a genetic map. In addition, the nucleic acids may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the UBP15 polypeptide-encoding nucleic acid in the genetic map previously obtained using this population (Botstein et al. (1980) Am. J. Hum. Genet. 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) Plant Mol. Biol. Reporter 4: 37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

The nucleic acid probes may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: Non-mammalian Genomic Analysis: A Practical Guide, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, the nucleic acid probes may be used in direct fluorescence in situ hybridisation (FISH) mapping (Trask (1991) Trends Genet. 7:149-154). Although current methods of FISH mapping favour use of large clones (several kb to several hundred kb; see Laan et al. (1995) Genome Res. 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods for genetic and physical mapping may be carried out using the nucleic acids. Examples include allele-specific amplification (Kazazian (1989) J. Lab. Clin. Med 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) Genomics 16:325-332), allele-specific ligation (Landegren et al. (1988) Science 241:1077-1080), nucleotide extension reactions (Sokolov (1990) Nucleic Acid Res. 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) Nat. Genet. 7:22-28) and Happy Mapping (Dear and Cook (1989) Nucleic Acid Res. 17:6795-6807). For these methods, the sequence of a nucleic acid is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

The methods according to the present invention result in plants having altered growth and/or development, as described hereinbefore. These traits may also be combined with other economically advantageous traits, such as yield-enhancing traits, tolerance to other abiotic and biotic stresses, traits modifying various architectural features and/or biochemical and/or physiological features.

Examples

The present invention will now be described with reference to the following examples, which are by way of illustration alone. The following examples are not intended to completely define or to otherwise limit the scope of the invention.

1. Plant Material and Growth Conditions

The wild-type *Arabidopsis thaliana* plants used in this study were of the Columbia-0 ecotype. The 38 T-DNA insertion lines (including *ubp15-1, ubp15-2* and *ubp19-1* those three lines with observed phenotypes) listed in Table 1 were obtained from the SALK collection. *UBP15* (or *UBP15C447A/S*)-overexpressing transgenic plants were obtained by transforming

P_{UBP15}:*UBP15* (or P_{UBP15}:*UBP15C447A/S*) into wild type background. *UBP15* complemented (or *UBP15C447A/S*) transgenic plants were obtained by transforming P_{UBP15}:*UBP15* (or P_{UBP15}:*UBP15C447A/S*) into *ubp15-1* background. P_{UBP19}:*GUS* transgenic plants were obtained by transforming the P_{UBP19}:*GUS* construct into the wild type background. *ubp15 ubp16*, *ubp15 ubp17* and *ubp16 ubp17* double mutants were obtained by crossing *ubp15-1* (or *ubp15-2*) with *ubp16-1* or *ubp17-1,* or by crossing *ubp16-1* with *ubp17-1*; homozygotes of F2 generation were confirmed by PCR analysis. Triple mutants *ubp15 ubp16 ubp17* were obtained by crossing *ubp15* with double mutants *ubp16 ubp17*; homozygotes of F2 generation were confirmed by PCR analysis.

Seeds were surface sterilized with 15% (v/v) NaOCl. After 48 h at 4°C for vernalization, seeds were placed onto 1×Murashige and Skoog (MS) plates (containing 1% sucrose, and 0.3% Phytagel) and placed in a chamber at 22°C under continuous light for 7 days before transferring to soil. Plants were then grown in standard long day (16 h light/8 h dark) growth rooms. Seeds grown in short day (8 h light/16 h dark) conditions were sown directly onto soil and were grown in a short day condition room.

2. Sequence Alignment and Phylogenetic Analysis

The search for the common domains shared among the 27 UBP proteins was performed by TAIR and NCBI CDART. The conserved domains within each protein were joined by eliminating variant regions and by alignment of the fragments using ClustalW (Thompson et al., 1994), followed by manual alignment.

A phylogenetic tree was constructed using MAGA version 3.1 (Kumar et al., 2004) on conserved positions of the alignment, using the neighbour-joining algorithm with 1000 bootstrap replicates.

3. Measurement of the Length of Seedlings, Root, Silique and Width and Length of Rosette Leaf

To obtain the root length of seedlings, wild type, *ubp15-1* and *ubp15-2* were grown vertically on plates. The root length of 20 randomly selected seedlings of either wild type or mutants was determined. 20 randomly selected mature siliques (older than 8 d after pollination) of wild type or mutants were measured. Mature rosette leaves from the first true leaf to the last one was dissected from wild type or *ubp15-1*, and the width and length of the leaves was measured.

4. Measurement of the Flowering Time and Rosette Leaf Number

The flowering time of wild type, *ubp15-1*, *UBP15*-overexpressing line was measured as the number of days from seed germination to the opening of the first flower. Rosette leaf numbers were counted at bolting.

5. RNA Isolation and RT-PCR

RNA was isolated using Trizol (Invitrogen). Before performing RT-PCR, 4 µgs RNA was treated with RNase-Free DNase (Premega) to avoid contamination of DNA, followed by chloroform: phenol (1:1) extraction to avoid the affect of the ions. A portion of RNA was then used for PCR by At3g04120/*GAPDH* primers to confirm elimination of the DNA. The remaining sample was subjected to the RT reaction using a Superscript™ II RNase H- Reverse Transcriptase kit (Invitrogen) and random primers. The reaction mixture was diluted 10 times and 1 µL was subjected to the PCR using rTaq DNA polymerase (TaKaRa). At3g04120/*GAPDH* was used as an internal control. Linearity of the PCR reaction was monitored by comparing relative amounts of PCR products after 22, 30, and 35 cycles. Forward and reverse primer sequences, used for detection of gene transcripts, were as follows At3g04120/*GAPDH*, 5'-CACTTGAAGGGTGGTGCCAAG-3' and 5'-CCTGTTGTCGCCAACGAAGTC-3'; *UBP15*, 5'-TCGAGAGGCAACAGTTATGCTG-3' and 5'-CTCAGGCCTCCAGTAACTGTAAGTTCTATCCTG-3'.

6. RNA Gel Blot Analysis

RNA blot was performed as previously described (Yang et al., 2005). The total RNA from different regions of leaves was extracted and 20µgs of total RNA for each lane was separated on an agarose gel, transferred to a Hybond-N+ membrane (Amersham Biosciences), and hybridized with a *UBP15*-specific probe. 18S rRNA served as the loading control. Primers were as follows: *UBP15*, 5'-TCGAGAGGCAACAGTTATGCTG-3' and 5'-CTCAGGCCTCCAGTAACTGTAAGTTCTATCCTG-3'.

7. Transient Expression in Onion Epidermal Cells

The procedure for transient expression in living onion (*Allium cepa*) epidermal cells using particle bombardment was performed as previously described (Ang et al., 1998). After bombardment, onion cell layers were incubated for 24 h at 22°C in the light. The cell layers were then examined by confocal microscopy.

8. Constructs of Transgenic Lines

To obtain *UBP15*-overexpressing and *UBP15* complemented plants, a *Hind*III/*Xba*I-digested *UBP15* promoter (1.8kb upstream of ATG) fragment and an *Xba*I/*Stu*I *UBP15* CDS fragment

(2.8kb) was inserted into pCAMBIA1300 binary vector. The resulting construct was then transformed into wild type and *ubp15-1* by means of *Agrobacterium*-mediated transformation. Constructs mutated in Cys site P_{UBP15}*:UBP15C447A* and P_{UBP15}*:UBP15C447S* were also made in the same way and transformed into wild type and *ubp15-1*. Transgenic plants were selected with hygromycin (20 µg/mL; Roche) on MS plates.

To make *35S:GFP-UBP15* (or *35S:UBP15-GFP*) constructs, *35S* as well as *GFP*-fused full-length *UBP15* CDS (or full-length *UBP15* CDS fused *GFP*) was subcloned into the *Xba*I/*Sph*I site of pCAMBIA1300.

To detect tissue expression pattern of *UBP19* using GUS staining, a *BamH*I/*Kpn*I fragment of *UBP19* promoter (1.2kb) was inserted into pCAMBIA1381Z and transformed into a wild type background using *Agrobacterium*-mediated transformation. Transgenic lines were selected using hygromycin (20 µg/mL; Roche).

9. In vitro DUB Activity Assay

The ability of UBP15 to cleave ubiquitin linked via α-amino linkages was performed in *E. coli.* using his-tagged substrates polyubiquitin UBQ10 and ubiquitin-extension protein UBQ1. Each of the two α-amino substrates (UBQ1 in pET28a and UBQ10 in pACYCDuet-1) were co-expressed with either GST or GST-UBP15 or GST-UBP15C447A/S in pGEX4T-3 in *E. coli.* strain NovaBlue (DE3) under standard conditions (22°C induce) (Novagen). Lysates were analyzed on a Western blot with anti-ubiquitin antibody (Sigma) or anti-his antibody (Sigma). ECL chemiluminescences system (Amersham) was used for detection.

10. Tissue Fixation and Embedding for Histological Sections

To observe the transverse structure of leaves, samples were fixed for more than 24 h in FAA (50% ethanol, 5% acetic acid, and 5% formaldehyde) at room temperature, dehydrated in a graded series of mixture of H_2O: 95% alcohol: tert-butyl alcohol (4:5:1, 3:5:2, 1.5:5:3.5, 0:5:5, 0:2.5:7.5, 100% tert-butyl alcohol twice) with a 2 h incubation in each solution and then infiltrated by adding paraffin gradually (tert-butyl alcohol: paraffin as 3:1, 2:2, 1:3, 0:4, 0:4). Embedded tissues were cut into 8 µm sections with a microtome (Leica RM2255). Sections were placed onto poly-L lysine coated slides, deparaffinized and rehydrated using an ethanol series (ethanol: H_2O as 1:0, 1:0, 9.5:0.5, 9:1, 8:2, 7:3, and 1:1) with a 5 min incubation in each solution. Sections were then stained with 1% safranin O and 0.02% fast green. The cover glasses were adhibited on slides by resin. The numbers of adaxial epidermal and palisade cells were counted under a microscope.

11. Microarray Analysis and Quantitative RT-PCR

The procedures used in the microarray experiments and data analysis were described previously (Ma et al., 2002). Early emerged 9th rosette leaves of wild type and *ubp15-1* were used as samples. Total RNA was isolated using RNAwiz reagent (Ambion) and purified by the RNeasy kit (Qiagen). For each sample, 50 µg of total RNA was labeled with aminoallyl-dUTP (aa-dUTP, Sigma-Aldrich) by reverse transcription. The aminoallyl-dUTP-labeled cDNAs were purified using a Microcon YM-30 filter (Millipore) and resuspended in 0.1 M $NaHCO_3$. The purified cDNAs were further fluorescently labeled by conjugating monofunctional Cy3 or Cy5 dye (Amersham) to the aminoallyl functional groups. Pair-wise combinations of two selected samples were used to simultaneously probe one slide, and four independent biological replicates were performed (two replicates with dyes exchange). Hybridized slides were scanned with a GenePix 4000B scanner (Axon), and independent TIFF images for Cy3 and Cy5 channels were used for subsequent analysis by GenePix Pro5.0 software package.

To confirm the microarray result, real-time PCR was performed using the ABI SYBR Green PCR master mix in a volume of 20 µL on an ABI 7900 system. The PCR mixture consisted of 0.3 µL of cDNA, 0.6 µM primers, and 1x master mix. In every real-time PCR run, At1g42970/*GAPDH* was used as an internal control to normalize for variation in the amount of cDNA template. The results of a t test confirmed it to be a constitutively expressed gene. The primer sequences used were: At1g42970/*GAPDH*, 5'-TCTTTCCCTGCTCAATGCTCCTC-3' and 5'-TTTCGCCACTGTCTCTC CTCTAAC-3'. 7 genes down regulated and 2 genes up regulated were picked for quantitative RT-PCR test. Primers used to quantify those genes were At1g71030/*MYB*, 5'-CATTTGCCTGACCTAAACATTG-3' and 5'- AAGCGTTTCTTGACCTGTTGA-3'; At5g57660/*TRANSCRIPT FACTOR*, 5'-GGCTCATCCACCACCGTT-3' and 5'-GGGAGAGGCTCTGTTTTCGTC-3'; At5g67030/*ABA1*, 5'-GGGCTTGGTCCTCTGTCTT-3' and 5'–GTGAGTCTGCAACTAGGTGGC3'; At5g35970/*HELICASE-LIKE*, 5'-CCACAGGGCTCGGAGGTAT-3' and 5'-TCGTAAGTAAGGGCATCGGC-3'; At3g49160/*PYRUVATE KINASE FAMILY PROTEIN*, 5'-TCCAGCAGGTCTCACATAAACAA-3' and 5'-CTGCTGCTAAGAGATGTGACCG-3'; At1g73480/*HYDROLASE*, 5'-AATGGCGGTGGAAACAATG-3' and 5'-ACGACGCGAAACGGAAGGAG-3'; At5g24470/*APRR5*, 5-TACCCTACGCCAACCCCTAT-3' and 5'-ATGTGATTGCCTATTGCACTATGT-3'; At3g20810/*TRANSCRIPTION FACTOR,* 5'-CCTCAATGCTGTTGCTGGTAA-3' and 5'-TGGGCAAGATAAGTAGGCTCC-3'; At4g21990/*APR3*, 5'-CTGTCAACACGCTTCACGC-3' and 5'-TCTTTCCGGTTCTCTAACTTCATC-3'. To determine the specificity of those primers, the amplified products were subjected to melt curve analysis using the machine's standard

method. Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 1 min. The reported values were averages of six independent trials (two biological replicates and three technical replicates). Relative expression levels were calculated as follows. Transcript levels of those genes were normalized relative to a standard (*GAPDH)* using the formula $\Delta C_T = C_T$ (*gene X*) mean of technical repeat $- C_T$ (*GAPDH*) mean of technical repeat either in wild type or *ubp15-1*. Then, the $\Delta Ct_{(wild\ type)}$ and $\Delta Ct_{(ubp15\text{-}1)}$ of each gene was obtained. Wild type was used as the standard for the comparison of expression levels. Relative expression levels were then calculated using the equation $2^{-[\Delta CT\ (ubp15\text{-}1) - \Delta CT\ (wild\ type)]}$. Next, the C_T average of 2 biological repeats was calculated.

12. Detection of GUS Activity

P_{UBP19}*:GUS* transgenic lines were grown on MS plates. Seedlings were washed gently with 100 mM sodium phosphate buffer, pH 7.0, and then stained for 4 h at 37°C in 2 mM X-glucuronide dissolved in 0.1 mM potassium ferricyanide, 0.1 mM potassium ferrocyanide, 10% Triton X-100, 100 µg/ml chloramphenicol, and 500 mM sodium phosphate buffer, pH 7.0 (Weigel and Glazebrook, 2002; Byrne et al., 2003). The tissue was then washed in 100 mM sodium phosphate buffer followed by 95% and 70% ethanol at room temperature. Microscopy was performed by a dissection microscope (Leica MZFLIII) with a 10x objective, and images were captured by Canon digital camera (Power Shot S70).

13. Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are listed in Table 1.

14. The UBP Genes exhibited non-identical expression profiles

To obtain expression patterns of the *UBP* genes, a previously described microarray data set was examined covering 18 different *Arabidopsis* organs, including cauline leaves, rosette leaves, pistil one day before pollination, pistil one day after pollination, silique 3 day after pollination, silique 8 day after pollination, stem, sepal, stamen, petal, seed, cultured cell, root dark, root white light, hypocotyl dark, hypocotyl white light, cotyledon dark and cotyledon white light (Ma et al., 2005; Supplemental Table 1). In that described study, 24 of 27 *UBP* genes were covered by the microarray used and expression for all 24 genes was detected in one or more tissues. Among those 24 genes, *UBP9* and *UBP10* were found to be essentially identical and their oligo probes were cross-hybridized to each other, thus not enabling definitive definition of the expression of both genes. The expression of the three genes not covered by this microarray, *UBP13*, *UBP14* and *UBP20,* were reported in a prior study (Yan et al., 2000). As a

sample case, the tissue-specific expression profiles for the 5 genes in *UBP15* subfamily were present in Supplemental Figure 2. The 5 genes appear to have distinct expression profiles.

15. Genome Wide Isolation and Analysis of T-DNA Insertional Mutants of the UBP Genes

As a first step in the functional analysis of the UBP gene family, the North America *Arabidopsis* Resource Center database was searched for all available T-DNA insertion lines for the UBP gene family members. A total of 38 T-DNA insertion lines corresponding to 25 UBP genes were obtained and verified, as summarized in Table 1 with the locations of the T-DNA insertion sites for each line. Following PCR-based genotyping, forward and reverse primers (Supplemental Table 2) were designed. Examination of possible phenotypes of those individual mutant homozygous lines revealed that only 5 loss-of-function mutant lines in 3 genes of 2 subfamilies exhibited visible phenotypes (Table 1). Two independent T-DNA alleles for *UBP14* exhibited recessive embryo lethality, similar to a previous report (Doelling et al., 2001). Another three mutant lines belonging to the *UBP15* subfamily, with two alleles for *UBP15*, exhibited a leaf morphology phenotype, and one allele for *UBP19* exhibited embryo lethality. The 5 genes in this subfamily had not previously been characterised and so were selected for in-depth analysis.

A search for homologous genes of AtUBP15 subfamily in *Rice* (*Oriza Sativa*) to find out whether they have similar functions was carried out. Four genes were found with high similarity, and were aligned with the 5 AtUBP15 subfamily genes. The resultant phylogenetic tree is shown in Supplemental Figure 3. One of the rice genes was even more similar to AtUBP15 than other Arabidopsis genes.

16. The Two ubp15 Mutant Alleles Exhibited Similar Leaf Development Defects

The two T-DNA insertion lines for *UBP15*, Salk_018601 and Salk_015611, designated *ubp15-1* and *ubp15-2*, were identified from the Salk collection and have T-DNA insertions in the 12th and 8th exon of the *UBP15* gene respectively (Figure 2A). Semi-quantitative RP-PCR showed the mRNA expression level could not be detected in either of the two mutants (Figure 2B), suggesting both are null alleles. Both lines segregated a single, recessive Mendelian trait (described below) similar to each other co-segregating with the T-DNA, indicating the trait was caused by a single insertion in *UBP15*. Compared to wild type adult plants, both mutants give smaller plants and narrow, serrated leaves, with the leaf phenotype becoming more severe in the later rosette leaves (Figure 2C). The mutants also exhibited shorter roots in seedling stage (Figure 2D), smaller flowers (Figure 2E), shorter siliques (Figure 2F) as well

as short and slim stems. Those morphopmetric changes (silique length, root length, primary stem length and primary stem diameter) are summarized in Table 2.

The morphology of rosette leaves using *ubp15-1* were further analysed. The rosette leaves of wild type *Arabidopsis* grown in long day condition (16 h light/8 h dark) gradually change their overall patterns, starting with true leaves round and flat, slowing become more ovule shaped and a little downward-curled, eventually become long and narrow shape, with severe downward-curled margin. While the rosette leaves of *ubp15-1* mutants are more flat even at a later stage and consistently produce fewer rosette leaves (10.8 versus 12.6 of wild type) before bolting (Figure 2C). The ratio of length and width of *ubp15-1* and wild type was plotted and defined as a leaf index value (Tsukaya, 2006), and revealed a much stronger reduction in width than in length of the rosette leaf blade in the mutant (Figure 2G). On the contrary, cauline leaves of *ubp15-1* did not show any obvious difference compared to the wild type.

Consistent with the reduced number of vegetative leaves, *ubp15-1* also exhibited an early flowering phenotype, with average flowering time of 39.1 (±2.02) days versus the 41.5 (±3.79) days for wild type (Table 2). Even in short day condition (8 h light/16 h dark), *ubp15-1* was also slight early flowering (Table 2). Also noted was that *ubp15-1* rosette leaves had less weight (Table 2) and were thinner, implying alteration of the cell structure.

17. UBP15 expressed highly in rosette leaves and reproductive tissues, and localized to both cytosol and nucleus

The presence of defects in *ubp15-1* rosette leaves and flowers suggested involvement of UBP15 in regulation of vegetative and reproductive development. To support this, and to verify the prior microarray analysis, a semi quantitative RT-PCR was employed to examine tissue specific expression of *UBP15.* As shown in Figure 3A, the mRNA level of *UBP15* was highly accumulated in the rosette leaves and inflorescences, while low in roots, siliques and cotyledons, intermediate level in stems and cauline leaves. This expression pattern is largely consistent with microarray data (Supplemental Table 1 and Supplemental Figure 2).

The spatial expression pattern of *UBP15* within a rosette leaf was further examined. As in Figure 3B, rosette leaves at early, middle, and late stages were selected and dissected into center and margin regions for separate RNA preparations (Figure 3B upper part, shown by the black lines) and RNA blot analysis (Figure 3B, bottom). *UBP15* mRNA level increased from early leaf to late leaf, together with a more evident higher expression in margin of leaf in late stage. These leaf expression patterns during development were consistent with a role of

UBP15 in defining the leaf pattern and shapes of leaves margin, such as serrating and curling.

To assess the subcellular distribution of UBP15, constructs containing either *35S:GFP-UBP15* or *35S:UBP15-GFP* were transformed into onion epidermal cell. Transient expression showed the fusion proteins ubiquitously present in both cytosol and nucleus, and the result for *35S:GFP-UBP15* is shown in Figure 3C.

18. UBP15 Possess De-Ubiquitinatination Activity in vitro and This Biochemical Activity Is Essential for Function in vivo

To verify UBP15 is a bona fide de-ubiquitinating enzyme, *UBP15* as well as two mutated forms (changed the conserved catalytic center Cysteine^{447} residue to Alanine or Serine) were co-expressed as GST fusion proteins in *E. coli.* The his tagged UBQ1 or UBQ10 poly-ubiquitin proteins were used as substrates for *in vitro* DUB activity assay with recombinant GST-UBP15 wild type or mutant form proteins. Immunoblot analysis with anti-ubiquitin antibody showed UBP15 was capable of cleaving the two substrates and that Cysteine^{447} is essential for this DUB activity (Figures 4A and 4B respectively).

To test the essential role of DUB activity of UBP15 in plant development, we introduced wild type *UBP15* and its two Cysteine^{447} mutant forms under the control of the *UBP15* native promoter into wild type *Arabidopsis* and *ubp15-1* background by means of Agrobacterium-mediated transformation. For the transgenic lines with wild type background, we obtained in total 36 independent T_0 generation plants for the wild type *UBP15* transgene. Half of the lines (18 out of 36) exhibited no phenotypic changes, whereas the other half displayed interesting differences from wild type, opposite to the *ubp15* mutant phenotypes (Figure 4C, middle left). For those lines with wild type transgenes in *ubp15-1* background, 5 independent lines showed wild type phenotype (Figure 4C, middle right), thus functionally rescued the mutant defect. RNA gel blot analysis with two representative lines from each groups (Figure 4D) revealed that high level expression of the transgene in wild type background was responsible for the abnormal phenotype opposite to *ubp15-1* mutant, while expression level similar to endogenous gene confer phenotype rescue in *ubp15-1* mutant and no phenotype effect in wild type background. Where the wild type transgene failed to rescue the *ubp15-1* phenotype, those transgenic lines only expressed truncated *ubp15* mRNA and failed to express wild type mRNA.

Introduction of *UBP15C447A* or *UBP15C447S* mutant transgenes under the control of the *UBP15* native promoter into wild type, and *ubp15-1*, failed to cause overexpression

phenotype in wild type background and complementation of mutant phenotype in *ubp15-1* background, as the RNA gel blot analysis showed normal or even higher level expression of mutant genes (Figures 4C and 4E). In contrast, some lines with *UBP15C447A* in wild type exhibited phenotype similar to *ubp15-1* mutant (Figure 4C, bottom left, and 4E), which was opposite to the UBP15-overexpression in wild type background, as did transgenic lines of wild type overexpressing *UBP15C447S* (data not shown). Those lines tended to express the mutant transgene at higher levels, likely caused a dominant negative interference with endogenous protein function. These results imply that DUB activity is essential for UBP15 function *in vivo*.

19. Over-expression of UBP15 Displaying Phenotypes Opposite to ubp15-1 Mutants

We further examined the phenotypes of *UBP15*-overexpressing lines (wild type transgenes in wild type background) above. One obvious phenotype was the larger overall status of the plants as well as each rosette leaf. The rosette leaves of those over-expressing lines were rounder than of wild types starting at very early stage and were severely downwardly-curled in the middle to later stages (Figure 4C, middle left). Later developed rosette leaves, such as the ninth rosette leaf, were not only downwardly-curled, but also sometimes displayed a knot in the leaf tip region (arrow in Figure 5B, compare 5A and 5B), possibly caused by the uneven proliferation of the cells in different positions within a leaf. In addition, the *UBP15*-overexpressing line was also late flowering, with the greater number of rosette leaves (16.2) before bolting than that of wild type, and the delay in flowering time under long day conditions (Table 2). The fresh weight of the rosette leaves of *UBP15*-overexpressing lines per area was also increased compared to wild type (Table 2).

There was also an evident flower phenotype in *UBP15*-overexpressing lines. Compared to the wild type, *UBP15*-overexpressing lines exhibited larger flowers (compare Figures 5I and 5J) with a high rate of abnormality in petal or stamen number (by 91.3%, 40 out of 46 in one plant) in the early flowers (Figures 5C and 5D, 5E, 5K). However this flower pattern abnormality was less frequent in late flowers. The overexpression lines also has siliques larger than wild type (Figure 5L), opposite to the ubp15-1 mutants. Flower and silique abnormalities do not affect fertility, unless in extreme cases where damaged sexual organs prevented fertilization (Figure 5H and 5M). An increased apical domanance in over-expressing lines was seen compared to the reduce apical dominance in *ubp15* mutants (Table 2 and Supplemental Figure 4). In some extreme cases of *UBP15* over-expression plants, positions for expected secondary bolts in the only main bolt degenerated into a flower then silique (Figure 5G) contrasting to wild type (Figure 5F).

20. The ubp15 Loss-of-function Mutants and UBP15-overexpressing Plants Display Opposite Abnormality In Leaf Cell Proliferation

To further characterize the role of UBP15 in *Arabidopsis* leaf development, the morphogenetic patterns of all leaves, from cotyledons, true leaves, to cauline leaves from loss-of-function mutants, over-expressing plants, and wild type were compared. As shown in Figure 6A, wild type plants had about 11 rosette leaves, while both two *ubp15* mutants had about 9 rosette leaves. In contrast, *UBP15*-overexpressing line produced about 14 rosette leaves. Rosette leaves in the two mutants were narrow, serrated and flat, but were more round in early leaves and downwardly-curled in later leaves in *UBP15*-overexpressing plants. The cauline leaves did not exhibit obvious differences amongst those lines examined, except two mutants had slightly narrower leaves.

To examine the cellular basis for their small plant size and narrow leaf lamina of the *ubp15* mutants, transverse sections of leaves from mutants as well as *UBP15*-overexpressing line were compared with wild type leaves. The cartoon in the right of Figure 6B illustrates the positions of representative sections routinely obtained and analyzed, which was in the middle region of the leaf. The left cartoon in Figure 5B was the section model of the middle region of wild type. Six leaves (leaves with odd numbers in Figure 6A) were selected for measurement, from the first cotyledon to the first, the third, the fifth, the ninth true leaf and the first grown cauline leaf. Those true leaves later than the ninth were not included for uniformity, because two mutants produced only 9~10 true leaves. Adaxial epidermal cell number and palisade cell numbers in each line were counted in serial sections (3~4) under microscope.

For adaxial epidermal cells, the cell number of each position in each line is shown in Figure 6C. At the beginning of leaf development, the adaxial epidermal cell numbers across the lamina among those lines was similar, which was ~30 cells in the cotyledon of each line, but the difference increased with the development of the leaves, and severely differed in the ninth rosette leaf, cell numbers across the lamina of which were ~290 in wild type, ~180 in two mutants (decreased by ~40%) and ~400 in *UBP15*-overexpressing line (increased by ~40%). It was consistent with the phenotype observed, for distinction became noticeable in late development phases. Adaxial epidermal cell number of cauline leaf was higher than that of any rosette leaves. This may possibly be caused by more density in the adaxial epidermal cell number across the cauline leaf. Similar results were observed in the measuring of palisade cell number (Supplemental Figure 5). Mutation of *UBP15* gave a decrease in adaxial epidermal and palisade cell number in a lateral direction, while overexpression of *UBP15* led to the opposite.

21. Arabidopsis UBP15 is involved in leaf cell layer organization

To extend the findings of cell number alteration, the cellular structure of the leaves of those lines was examined. Based on the result that late developed rosette leaves exhibited severe phenotype, the fully expanded ninth rosette leaf was chosen for comparison of the transverse sections of cellular structure in the middle region of the lines (Figure 6B). As Figure 7A left first wild type shown, leaf had an organized internal anatomy containing a layer of vertically packed palisade cells beneath the adaxial surface, and ~4 layers spongy parenchyma cells, interspersed with air spaces, were loosely arranged below the palisade layer. *ubp15-1* and *ubp15-2* also exhibited similar structure but the number of spongy cell layers in mutants was less than that of wild type, whereas that of *UBP15*-overexpressing line was more (Figure 7A, from left, the second to the fourth). The spongy cell layers in wild type was generally ~4, but in two mutants the spongy cell layers were ~3, while in *UBP15*-overexpressing line the spongy cell layers reached ~5. The defects in cell layer structure in *ubp15-1* was rescued by expression of its corresponding wild-type UBP15, confirming that these *ubp15-1* phenotype was caused directly by disruption of the *UBP15* gene (data not shown). These results were consistent with the fresh weight results for less weight corresponding to the less cell layers (Table 2), greater weight resulting from more cell layers (see above). A slight increase in the cell size of mutants was detected, implying partial compensation of the loss of cell number.

The mid-vein and peripheral structure of those lines was also compared. The vascular bundle of wild type was composed of several layers of xylem and phloem surrounded by layers of parenchyma cell (Figure 7B, left first). In contrast, two mutants showed decreased thickness. The vascular bundles of the two mutants were slimmer than of the wild type due to decreased cell number in xylem and phloem. In addition, the numbers of parenchyma cells surrounding the vascular bundles of two mutants was also reduced. In *UBP15*-overexpressing line, opposite results were obtained, showing advanced vascular bundle, strong both in xylem and phloem (Figure 7B, from left, the second to the fourth).

22. Transcriptome Analysis Shows UBP15 Influences Expression Level of 804 Genes (adjusted P<0.15)

To examine the cell cycle effects in *ubp15-1* and to explore the possible role of UBP15 in regulating the transcription of other genes, we performed microarray assay to conduct a genome wide expression analysis on the ninth rosette leaf immediately emerged. Microarray analysis also confirmed no expression of *UBP15* mRNA in *ubp15-1* (Supplemental Table 3). Statistical analysis showed a total of 804 out of 20,000 genes were differentially expressed in *ubp15-1* compared with wild type at adjusted P<0.15 (4% of the expressed genes), among

which 406 (50.5%) were up regulated and 398 (49.5%) were down regulated with fold change expression ranging from 1.72 to 8.35 and 0.09 to 0.56, respectively (Supplemental Table 3). To confirm the trends observed in the transcriptome analysis, we randomly picked 9 genes with 7 down regulated and 2 up regulated and performed real-time PCR. Expression levels of those genes was consistent with that of microarray result at 100% (Figure 8), suggesting microarray assay was reliable. At1g42970/*GAPDH* served as an internal control.

To characterize the biological processes involved, representatives of those genes influenced by the *ubp15-1* mutant were analyzed for gene ontology (GO) (Maere et al., 2005) (Table 3). Two genes related to cell cycle were differentially expressed in *ubp15-1* (Table 3). Because the flowering time was also influenced in *ubp15-1*, genes controlling flowering were the focus. Floral homeotic gene CAL, positively regulating flower development, was up regulated 1.98 fold in *ubp15-1* background, while another one, MAF5, negatively regulated flower development was down regulated 0.31 fold (Table 3).

Other categories influenced by *ubp15-1* comprised genes involved in biosynthetic metabolism, chlorophyll biosynthesis, photosynthesis, signal transduction and also included a large number of transcription factors (Table 3). Thus, besides effects on some phenotype-related genes such as cell cycle and flowering control, the *ubp15-1* mutant was in general defective in numerous plant metabolism pathways at the transcriptional level, suggesting secondary and not only primary effects on the observed growth defects.

23. UBP15 and UBP16 are partially Redundant but not with UBP17

UBP16 and UBP17 are two closely related proteins (Figure 1), suggesting they may have functional redundancy. Knockout lines of the two genes (*ubp16-1*, *ubp17-1* and *ubp17-2*) (Figure 9A) did not show any observable phenotype. *ubp16-1* was crossed with *ubp17-1* or *ubp17-2* to examine whether there was any defect in double mutants. Homozygote F2 generation of crossed lines did not show noticeable phenotype, indicating there maybe other proteins for redundancy. Subsequently crossed were *ubp15* mutants with the double mutant *ubp16 ubp17*. In the F2 generation, three gene types of double mutants *ubp15 ubp16*, *ubp15 ubp17* and *ubp16 ubp17* were obtained, as well as a triple mutant *ubp15 ubp16 ubp17* (Figure 9B and 9C, *ubp16 ubp17* not shown). Double mutant *ubp15 ubp16* showed a similar but more severe phenotype to the *ubp15* mutant, indicating there may be functional redundancy of the two genes. *ubp15 ubp16* exhibited dwarf plants and aborted siliques, rosette leaves, which were narrower than that of *ubp15* mutants (Figure 9C). The alteration in cellular level was also conspicuous, only 2 spongy cell layers (Figure 7A, right first) and strikingly degenerated vascular bundle (Figure 7B, right first). In the case of the ninth leaf,

the adaxial epidermal cell number cross the leaf lamina decreased by 60% over that of wild type (Supplemental Figure 6), as did the palisade cell number (data not shown). In contrast, *ubp15 ubp17* did not further the defect of *ubp15* mutants, implying those two gene may work in two different pathways to regulate cellular function. The triple mutant *ubp15 ubp16 ubp17* was similar to the double mutant *ubp15 ubp16*, further confirming UBP17 involves in another regulating pathway from UBP15 and UBP16, although it had higher sequence homology to UBP16.

The data suggest that the UBP15 mutation disrupted rosette leaf development, and its effect was more prominent when combined with UBP16. The effect of UBP16 was only detectable in the presence of UBP15. There was no detectable effect of the UBP17 mutation on this process. These results indicate that UBP15 is a major regulator of the shape of rosette leaves and development of the whole plants. UBP16 is also involved in these processes, although its contribution was less prominent than that of UBP15.

24. UBP19 May be Involved in Embryo Development While the Function of UBP18 is Unknown

Figure 9A shows UBP18 and UBP19 gene structure with two T-DNA mutants each. *ubp19-1*, with T-DNA inserted in the 5'UTR region 195bp upstream of ATG, exhibited recessive embryo lethality, with ~1/4 (13/47) aborted embryos in one heterozygote silique (Figure 9B, arrow indicated yellow embryos). Further statistical analysis of an average of 10 siliques in each heterozygote line showed the ratio of the normal: abnormal was ~3:1 (372/136), confirming it was a recessive embryo lethal line. The embryo development in a specific heterozygote silique, from mature stage tracing back to globular stage was examined. It was found that *ubp19-1* stopped developing at globular stage (Figure 10C).

It is therefore proposed that UBP19 is involved in early stage embryo development. A *GUS* construct driven by a 1.2kb *UBP19* promoter was made to transform wild types and to examine tissue expression pattern. It was found that *UBP19* was ubiquitously expressed, including in the whole young seedling, except the root meristemetic region (Figure 11A) (Enlarged in Figure 11A was the detailed demonstration of root tip region.), in the vascular tissues and stomas of mature rosette leaves (Figure 11B) as well as in inflorescence. Detailed examination found *GUS* expressed in the sepal and petal vascular tissues, also in anthers and styles (Figure 11C). GUS was detected in the tip and basal regions of siliques but hardly within the embryos (Figure 11D).

Another *ubp19* mutant, 205bp upstream of ATG, did not show any obvious phenotype, indicating the region between 195bp to 205bp upstream of ATG was essential for the function of UBP19.

Neither of the two mutants inserted in *UBP18* exon and intron exhibited any observable phenotype, implying potential functional redundancy between UBP18 and other proteins in this family.

25. UBP15 is Involved in Rosette Leaf Development

Phenotype of *ubp15* mutants and *UBP15*-overexpressing lines provided a basis for further analysis of this gene in plant leaf development. Both *ubp15* mutants showed rosette leaves that were narrow, serrated and flat, whereas *UBP15*-overexpressing lines produced an opposite phenotype with rosette leaves that were round (early developed) and downwardly-curled (late developed) (Figures 2A and Figure 5B). The cell number of transverse sections (both palisade and adaxial epidermal cells across the leaf lamina) of *ubp15* mutants decreased whereas that of *UBP15*-overexpressing line dramatically increased compared to wild types. This indicates that UBP15 alters leaves shape by cell proliferation, possibly by regulating cell cycle proteins. Recently, *hub1* was found to exhibit narrower rosette leaves, caused by cell number decrease and a microarray assay discovered that the amount of genes related to cell cycle and cytokinesis were changed (Fleury et al., 2007). In this study, although microarray data showed there were only two genes related to cell cycle altered (ICK1 and Cycle-like F box domain containing protein), their expression level was consistent with the cell number decrease. The rosette leaves in mutants became flat while they were downwardly-curled in *UBP15*-overexpressing lines. The expression level of *UBP15* in rosette leaves increased in the leaf margin with the development of plants (Figure 3B), suggesting that UBP15 specifically (at least partly) determines the margin of rosette leaves. This was similar to *iamt1-D*, which displayed dramatic hyponastic leaf phenotypes caused by increased expression levels of the *IAMT1* gene, and which was found to be specifically expressed in the margin of rosette leaves by GUS expression assay (Qin et al., 2005).

The leaf index increased in *ubp15-1* mutants because of narrower rosette leaves (although leaf length was shorter but less altered and lead to a large leaf index value). Polarized growth of leaf blades in the leaf-width direction is governed by polar elongation of leaf cells or polar cell proliferation (Tsukaya, 2006). Genes altered cell proliferation in leaf-width direction reported until now are *GIF1* (also named *AN3*) (Kim and Kende, 2004), *GRF5* (Horiguchi et al., 2005), and *HUB1* (Kim and Kende, 2004; Horiguchi et al., 2005; Fleury et al., 2007). They all are important in positive control of cell number in leaf width direction. *ubp15* mutants

altered adaxial epidermal and palisade cell numbers in leaf width direction, but microarray data did not show there was any change of the expression level of *GIF1* (*AN3*), *GRF5* or *HUB1* in *ubp15-1* background. This may because of distinct pathways involved by UBP15 and those genes.

26. The Cysteine Amino Acid of UBP15 is Critical for its Function in vitro and in vivo

In vitro co-expression assay showed UBP15 is a bona fide ubiquitin specific protease and Cys^{447} is critical for this activity. It was found that UBP15 can only cleave α-linked peptide (polyubiquitin and ubiquitin-extension gene) (Figure 5A), not ε-linked isopeptide (multiubiquitin chain linked by iso-peptide) (data not shown), suggesting it may only function on α-linked peptide or can only recognize the site of substrate-proximal end of the chain. UBP3, 4 and 5 proteins were reported to contain nuclear localization signal and were deduced to only function on isopeptide-linked substrates (Chandler et al., 1997; Rao-Naik et al., 2000). The subcellular localization of UBP15, ubiquitous expression throughout the cell, suggests that this enzyme may recognize more than one substrate to execute its diverse functions; or it has other function addition of de-ubiquitination.

In this study, we first proved in vivo function of Cys^{447} of UBP15 in leaf development. While expressing UBP15 wild type construct in *ubp15-1* can rescue the mutant phenotype, neither overexpressing UBP15C447A or UBP15C447S showed complementation of mutant deficiency. This implied $Cysteine^{447}$ was the functional residue of UBP15. On the other hand, transgenic lines of wild type with constructs containing wild type UBP15 exhibited downwardly-curled mature rosette leaves while those with constructs containing mutation in Cys447Ala or Cys447Ser showed *ubp15-1* mutant phenotype. This result confirmed the phenotype of rosette leaves was caused by overexpression of *UBP15* and that the Cys^{447} is critical for this function. It also implies that it is the de-ubiquitination activity but not other domains (such as Zinc finger MYND) that cause the abnormability. Recently found gene *HUB1* (Fleury et al., 2007), an E3 ligase mono-ubiquitinating H2B, with similar phenotype as *ubp15* mutants, possibly shares the same pathway as UBP15.

There is also another result that transgenic lines with construct containing *UBP15* driven by 35S promoter in wild type background did not show any phenotype, nor did the mutant can be rescued (data not shown). This suggested the native promoter may provide a favorable spatial structure for better executing the function of exogenous proteins.

27. UBP15 Regulates Transcription of Many Genes

A microarray assay was performed to analyze the possible role of *UBP15* in regulating other genes at a transcriptional level. Two genes related to cell cycle were found to be altered and may directly cause the cell number changes in *ubp15-1*, although not like HUB1, which altered cell cycle proteins across the genome (24 genes).

We also found two genes controlling flowering were altered and this alteration caused the phenotype we observed. MAF5 and Floral homeotic gene CAL, regulate flowering in opposition, both showed altered *ubp15-1* and caused the early flower phenotype.

Although numerous other genes were up- or down-regulated in *ubp15-1*, they seem to be the secondary effectors instead of proximal downstream targets.

28. Functional Redundancy among the UBP15 Subfamily Members

UBP15 subfamily members encode proteins containing zinc finger MYND domains. They can be further divided into 3 groups based on the sequence similarity. UBP16 and UBP17 have 50% amino acid identity in their two conserved domains, while UBP18 and UBP19 have 68% identity in the full length. But UBP15 is less similar to other four (UBP15 to UBP16 is 37% identity in the UBP domain and to UBP18 is 45% identity in the full length), and this explains the *ubp15* mutant phenotype. To see if UBP16 and UBP17 have functional redundancy, the two mutants of the two genes were crossed to give double mutants. Homozygotes of double mutants did not show any phenotype suggesting they may work in different pathways. Subsequently, the double mutant was crossed with *ubp15* mutants to examine whether there is any alteration. In the F2 generation, only *ubp15 ubp16* but no others intensified the phenotype of *ubp15* mutants. This indicated that UBP15 and UBP16 may have functional redundancy. Triple mutant *ubp15 ubp16 ubp17* exhibited similar phenotypes as *ubp15 ubp16* further suggesting UBP17 did not involve in the function shared by the other two. On the other hand, *ubp16* mutant did not display any visible phenotype, but it intensified the phenotype of *ubp15* mutants, suggesting that in *Arabidopsis*, UBP15 is a master regulator in this pathway. Functional redundancy was also observed in another subfamily UBP1 and UBP2, whose double mutants but not each mutant were sensitive to Canavanine (Yan et al., 2000).

The sequence homology of UBP18 and UBP19 also imply functional redundancy. However, only one mutant (*ubp19-1*) out of four showed recessive embryo lethality. Furthermore, insertion in the 195bp upstream of *UBP19* 5'UTR caused a phenotype, whereas inserting

205bp upstream of *UBP19* 5'UTR did not show any phenotype. This implies the region from 195bp tracing to 205bp upstream of *UBP19* 5'UTR is critical for the function of UBP19.

On the other hand, although UBP16 and UBP17 are more related to each other than to UBP15 in protein sequences, the exhibited phenotype of *ubp15 ubp16* similar to *ubp15* mutant suggested UBP16 was more related to UBP15 instead of UBP17 in function. This also applies to the group of UBP18 and UBP19, since the phenotype can only be detected in the *ubp19* mutant but not in *ubp18* mutants. This shows that proteins closely related at a sequence level are not always functionally redundant.

Description of figures

The present invention will now be described with reference to the following figures in which:

Figure 1. Phylogenetic Analysis of 27 UBPs family of *Arabidopsis*.

The Phylogenetic tree of 27 UBPs. The branch lengths of the tree are proportional to divergence. The 0.1 scale represents 10% change. Bootstrap values are shown in percentages at nodes. 27 proteins can be subdivided into 14 subfamilies differing in distinct colors based on the domain similarity. Multiple alignments used for this analysis are shown in Supplemental Figure 1.

Proteins with conserved domains are plotted on a schematically scale. Each colored box represents a domain. Black lines represent the length of UBP proteins, while the length of the domains can be estimated by the binding black lines. aa, amino acids; UBP, ubiquitin specific protease; ZnF, zinc finger; MYND, myeloid, Nervy, and DEAF-1; DUSP, domain in ubiquitin-specific proteases; UBQ, ubiquitin homologues; MATH, meprin and TRAF homology; UBA, ubiquitin-associated.

Figure 2. Characterization of the Ubiquitin-Specific Protease gene *UBP15*.

(A) Gene structure of *UBP15*. There are 14 exons dispersed within the 4.37kb genomic region. Black boxes represent the exons while lines between those exons are introns. White boxes in the two ends stand for 5' and 3' UTRs. Two T-DNA knockout lines inserted in the 8th and 12th exon respectively, each resulting in destroyed UBP domain.

(B) Expression of *UBP15* in the wild type and the homozygous *ubp15-1* and *ubp15-2* by RT-PCR with the primer of *UBP15FP* and *UBP15RP*. At3g04120/*GAPDH* served as an internal control.

(C) One-month-old plants of wild type, homozygous *ubp15-1* and homozygous *ubp15-2*. Bar=1cm.

(D) 12-d-old seedlings of wild type, homozygous *ubp15-1* and homozygous *ubp15-2*. Bar=1cm.

(E) Flowers of wild type, homozygous *ubp15-1* and homozygous *ubp15-2*. Bar=1mm.

(F) Siliques of wild type, homozygous *ubp15-1* and homozygous *ubp15-2*. Bar=1cm. Plants above all grow in 16 light/8 dark condition.

(G) Length and width ratio of the rosette leaves of wild type and *ubp15-1* in short day condition (8 h light/16 h dark). Error bars represent the standard deviation of the 7 repeats.

Figure 3. Tissue and developmental expression patterns of UBP15, and subcellular localization of UBP15 in onion epidermal cells.

(A) RT-PCR analysis of tissue expression pattern of *UBP15*. At3g04120/*GAPDH* as an internal control.

(B) Northern blot analysis of *UBP15* expression pattern in leaf center and margin regions in three developmental stages, P1, P2 and P3. Leaves were dissected in two parts: center and margin as the lines shown in the figure. Equal amounts of total RNA from the different samples were used, and gel blot was hybridized and labeled with *UBP15* gene-specific probes. The rRNA band pattern was used to show equal loading. C, center region; M, margin region. Bar=1cm.

(C) Subcellular localization of UBP15 in onion epidermal cells. Bar=100μm.

Figure 4. UBP15 encodes a functional deubiquitinating enzyme capable of cleaving the polypeptides, and its Cys^{447} is essential for the function in vitro and in vivo.

(A) UBP15 can cleave substrate UBQ1 (ubiquitin-extension protein). Co-expressed plasmids in *E. coli.* Novablue (DE3) strain (Novagen) was UBQ1 with GST vector (lane 1), GST-UBP15 (lane 2), GST-UBP15C447A (lane 3) and GST-UBP15C447S (lane 4). The cleaved products were detected by immunoblot analysis with anti-ubiquitin antibody.

(B) UBP15 can cleave substrate UBQ10 (hexameric polyubiquitin). Co-expressed plasmids in *E. coli.* Novablue (DE3) strain (Novagen) was UBQ10 with GST vector (lane 1), GST-UBP15 (lane 2), GST-UBP15C447A (lane 3) and GST-UBP15C447S (lane 4). The cleaved products were detected by immunoblot analysis with anti-ubiquitin antibody. Cleaved products are shown as white arrows.

(C) Expression of *UBP15* in *ubp15-1* recapitulates its wild type function, while overexpression of *UBP15*, but not *UBP15C447A* or *UBP15C447S*, causes phenotype with round (early developed) and curled down (mature) rosette leaves opposite to the *ubp15-1* mutants. Samples one-month-old from left to right and up to bottom are: wild

type, *ubp15-1*, *UBP15*-overexpressing line, *UBP15*-complemented line, *UBP15C447A*-overexpressing line and *UBP15C447A*-overexpressing in *ubp15-1* line. Bar=1cm.

(D) RNA gel blot analysis of the *UBP15* gene expression in transgenic lines. RNA levels from wild type (1), *ubp15-1* (2), wild type with *UBP15* transgene (3~6) and *ubp15-1* with *UBP15* transgene (7~9) plants were analyzed. Total RNA was isolated from rosette leaves grown for 4 weeks. Equal amounts of total RNA from the different plant samples were used, and gel blot was hybridized and labeled with *UBP15* gene-specific probes. The rRNA band pattern was used to show equal loading. The upper band (upper arrow) was the full length of the *UBP15* mRNA, while the lower band (lower arrow) represented the truncated mRNA. Lane3, 4 were the samples with *UBP15*-overexpressing phenotype while lane 5, 6 were similar to wild type without obviously *UBP15*-overexpressing phenotype. Lane 7, 8 but not 9 were rescued samples for they did not exhibit mutants phenotype but as normal as wild type. Line 9 sample showed *ubp15-1* mutant phenotype.

(E) RNA gel blot analysis of the *UBP15C447A* gene expression in transgenic lines.

Figure 5. Comparison of wild type and *UBP15*-overexpressing line.

(A) Ninth rosette leaf of wild type. Bar=1mm.

(B) Ninth rosette leaf of *UBP15*-overexpressing line. Bar=1mm.

(C) Top view of wild type flower. Bar=1mm.

(D) (E) (K) Ectopic flowers of *UBP15*-overexpressing line. Bar=1mm.

(I) Side view of the flower of wild type. Bar=1mm.

(J) Side view of the flower of *UBP15*-overexpressing line. Bar=1mm.

(F) Secondary stems of wild type. Bar=1cm.

(G) Degenerated secondary stems of *UBP15*-overexpressing line. Bar=1cm.

(H) Siliques of wild type. Bar=1cm.

(M) Abnormal siliques of *UBP15*-overexpressing line. Bar=1cm.

(L) Comparison the normal siliques of wild type (left) and *UBP15*-overexpressing line (right). Bar=1cm.

Figure 6. Comparison of the transverse sections of rosette leaves across the lamina in four lines.

(A) From top to the bottom: wild type, *ubp15-1*, *ubp15-2* and *UBP15*-overexpressing line. In each panel, two cotyledons and rosette leaves from the first to the last as well as two first cauline leaves are placed from left to the right. Arrows indicate positions of cauline leaves. Bar=1cm.

(B) Models demonstrate the position of transverse sections.

(C) Comparison of adaxial epidermal cell number in transverse sections across the rosette leaves lamina. Error bars represent standard deviation of three biological repeats.

Figure 7. Histological comparison of the transverse sections of the ninth rosette leaf middle region.

(A) Comparison of the cell layer in transverse sections of wild type, *ubp15-1*, *ubp15-2*, *UBP15*-overexpressing line and *ubp15 ubp16*. ad, adaxial; pa, palisade; sm, spongy mesophyll; ab, abaxial; x, xylem; p, phloem. Bar=0.1mm.

(B) Comparison of the mid vein structure in transversal sections of wild type, *ubp15-1*, *ubp15-2*, *UBP15*-overexpressing line and *ubp15 ubp16*. Bar=0.1mm.

Figure 8. Real-time PCR confirm the microarray results.

7 down regulated genes and 2 up regulated genes are randomly picked for real-time PCR analysis which confirms microarray result.

Figure 9. Characterization of the *UBP16* and *UBP17*.

(A) Gene structure of *UBP16* (left) and *UBP17* (right).

(B) 12-d-old seedlings of wild type, *ubp15-1*, *ubp15 ubp17*, *ubp15 ubp16* and *ubp15 ubp16 ubp17*. Bar=1cm.

(C) 2-month-old plants of wild type, *ubp15-1*, *ubp15 ubp17*, *ubp15 ubp16* and *ubp15 ubp16 ubp17*. Bar=1cm.

Figure 10. Characterization of the *UBP18* and *UBP19*.

(A) Gene structure of *UBP18* (up panel) and *UBP19* (bottom panel).

(B) *ubp19* exhibits recessive embryo lethality. The upper silique is *ubp19* Heterzygote and the lower is wild type. Arrows refer to those abnormal homozygote embryos. Bar=1mm.

(C) Microscopic examination of embryos of wild type (left) and *ubp19* homozygote (right). *bup19* homozygote stagnates developing at global stage. Bar=20μm.

Figure 11. Tissue expression pattern of *UBP19*. The expression pattern of *UBP19* was determined using P_{UBP19}:*GUS* transgenic lines.

(A) 12-d-old seedling of the P_{UBP19}:*GUS* line. Bar=1mm. Figure in the right bottom is 6 fold enlarged of the root tip region.

(B) Mature rosette leaf of the P_{UBP19}:*GUS* line. Bar=1mm.

(C) Inflorescence of the P_{UBP19}:*GUS* line. Figure in the left bottom is 2 fold of a flower. Bar=1mm.

(D) Silique of the P_{UBP19}:*GUS* line. Bar=1mm.

Table 1. Analysis of T-DNA insertion lines for *UBP* genes.

Arabidopsis Genome ID	Protein	Mutant allele	T-DNA line	Insertion site	Phenotype
At2g32780	UBP1	*ubp1-2*	SALK_086190	Promoter	ND
At1g04860	UBP2	*ubp2-2*	SALK_064103	1st exon	ND
		ubp2-3	SALK_059858	1st exon	ND
At4g39910	UBP3	*ubp3-1*	SALK_112950	3rd exon	ND
At2g22310	UBP4	*ubp4-1*	SALK_043210	4th exon	ND
At2g40930	UBP5	*ubp5-1*	SALK_08839	5'UTR	ND
At1g51710	UBP6	*ubp6-1*	SALK_108832	14th intron	ND
At3g21280	UBP7	*ubp7-1*	SALK_014223	ND	ND
At5g22030	UBP8	*ubp8-1*	SALK_034744	5th intron	ND
		ubp8-2	SALK_149329	6th intron	ND
		ubp8-3	SALK_088692	7th exon	ND
At4g10570	UBP9	*ubp9-1*	SALK_141485	5'UTR	ND
At4g10590	UBP10	*ubp10-1*	SALK_093503	9th exon	ND
At1g32850	UBP11	*ubp11-1*	SALK_043515	8th intron	ND
At5g06600	UBP12		NONE		
At3g11910	UBP13	*ubp13-1*	SALK_128312	5th exon	ND
		ubp13-2	SALK_024054	6th exon	ND
		ubp13-3	SALK_130784	17th exon	ND
		ubp13-4	SALK_132368	21th intron	ND
At3g20630	UBP14	*ubp14-3*	SALK_050151	9th exon	Recessive embryo lethal
		ubp14-4	SALK_012863	19th exon	Recessive embryo lethal
At1g17110	UBP15	*ubp15-1*	SALK_018601	12th exon	Recessive rosette leaves
					narrow and serrated
		ubp15-2	SALK_015611	8th exon	Recessive rosette leaves
					narrow and serrated
At4g24560	UBP16	*ubp16-1*	SALK_023552	5th exon	ND
At5g65450	UBP17	*ubp17-1*	SALK_087726	9th exon	ND
		ubp17-2	SALK_113300	12th exon	ND
		ubp17-3	SALK_009641	8th intron	ND
At4g31670	UBP18	*ubp18-1*	SALK_101685	2nd intron	ND
		ubp18-2	SALK_126252	4th exon	ND
At2g24640	UBP19	*ubp19-1*	SALK_084566	5' UTR	Recessive embryo lethal
		ubp19-2	SALK_117787	5' UTR	ND
At4g17890	UBP20		NONE		

At5g46740	UBP21	*ubp21-1*	SALK_079015	1st exon	ND
At5g10790	UBP22		NONE		
At5g57990	UBP23	*ubp23-1*	SALK_121772	10th exon	ND
At4g30890	UBP24	*ubp24-1*	SALK_001531	3rd exon	ND
At3g14400	UBP25	*ubp25-1*	SALK_088458	5' UTR	ND
		ubp25-2	SALK_111336	2nd exon	ND
At3g49600	UBP26	*ubp26-1*	SALK_024392	4th intron	ND
At4g39370	UBP27	*ubp27-1*	SALK_067020	5' UTR	ND
		ubp27-2	SALK_027968	1st exon	ND

38 T-DNA knockout lines corresponding to the 24 out of 27 *UBP* genes are screened for their phenotype. Only 5 lines belong to 3 genes exhibit observable phenotype.

Table 2. Morphometric analysis of wild type, *ubp15-1* mutant, and *UBP15* over-expressing plants.

Body parameters	**Wild type**	***ubp15-1***	**UBP15 over-expressing plants**
Number of rosette leaves (LD)[a]	12.58 ± 0.95 (n=26)	10.82 ± 1.36 (n=18)	16.21 ± 1.63 (n=26)
Flowering time (LD) (day)[a]	41.54 ± 3.79 (n=26)	39.13 ± 2.02 (n=46)	46.07 ± 2.37 (n=26)
Number of rosette leaves (SD)[b]	15.92 ± 2.11 (n=7)	13.08 ± 1.77 (n=7)	20.22 ± 2.66 (n=7)
Flowering time (SD) (day)[b]	69.04 ± 1.41 (n=8)	65.69 ± 4.66 (n=21)	75.45 ± 4.87 (n=8)
Fresh weight of the rosette leaves (mg/cm^2)[b]	13.24 ± 0.48 (n=24)	10.74 ± 0.29 (n=40)	15.6 ± 0.46 (n=24)
Silique length (cm)[c]	1.36 ± 0.08 (n=50)	1.09 ± 0.08 (n=50)	1.42 ± 0.07(n=40)
Root length (cm)[d]	5.87 ± 0.75 (n=22)	4.64 ± 0.74 (n=22)	6.84 ± 0.69 (n=26)
Primary stem length (cm)[c]	26.09 ± 2.68 (n=11)	23.77 ± 2.98 (n=15)	29.25 ± 4.44 (n=6)
Primary stem diameter (mm)[c]	0.80 ± 0.08 (n=14)	0.65 ± 0.09 (n=18)	0.98 ± 0.07 (n=8)

a. Measurements were taken from bolting plants. Plants grow in 16 h light/8 h dark.

b. Measurements were taken from bolting plants. Plants grow in 8 h light/16 h dark.

c. Measurements were taken from plants 60 d after sowing. Plants grow in 16 h light/8 h dark.

d. Measurements were taken from plant 14 d after sowing. Plants grow in 16 h light/8 h dark.

Table 3. Microarray Data for Selected Genes with Various Functions

AGI Code	**Fold Change**	**Gene Description and Putative Function**	**GO Category**
		Proteins related to cell cycle	
At2g23430	1.72	ICK1	Negative regulation of cell division and promoter of endoreduplication.
At1g77880	0.3	Cyclin-like F box domain containing protein	
		Proteins regulating flowering	
At1g26310	1.98	Floral homeotic gene CAL	Positive regulation of flower development

At5g65080	0.31	MAF5	Negative regulation of flower development, vernalization response
		Transcription factors	
At1g74080	4.26	MYB122	Encodes a putative transcription factor
At3g07650	2.39	CONSTANS gene family	Negative regulation of long-day photoperiodism, flowering
At4g17980	2.32	No apical meristem (NAM) family protein	Transcription factor activity
At3g20810	2.05	jmjC domain-containing transcription factor	Transcription factor activity
At3g23250	2.03	MYB15	Transcription factor activity ,response to kinds of hormone
At1g21910	1.92	ERF/AP2 transcription factor	Transcription factor activity, TINY-like protein
At2g31220	1.86	Basic helix-loop-helix (bHLH) family protein	Transcription factor activity
At2g19810	0.56	Zinc finger (CCCH-type) family protein	Transcription factor activity
At5g57660	0.51	B-box type zinc finger family protein	Transcription factor activity
At1g71030	0.42	MYB family transcription factor	Transcription factor activity, response to kinds of hormone, mainly in leaves.
At4g16780	0.42	Homeobox-leucine zipper protein 4	Transcription factor activity, response to cytokinin stimulus
At3g04070	0.45	No apical meristem (NAM) family protein	Transcription factor activity
		Biosynthetic metabolism	
At5g38710	4.81	Proline oxidase	Glutamate biosynthesis, proline catabolic process, located in MT
At4g21990	3.31	APS REDUCTASE 3	Sulfate assimilation, located in chloroplast
At3g12430	3.24	3'-5' exonuclease	Nucleic binding, 3'-5' exonuclease activity
At5g06290	2.53	2-cys peroxiredoxin	Located in chloroplast, antioxidant activity
At3g24420	2.48	Hydrolase, alpha/beta fold family protein	Located in endomembrane system
At5g61440	2.04	Thioredoxin family protein	Thiol-disulfide exchange intermediate activity, located in chloroplast
At1g51760	1.96	IAA-amino acid hydrolase 3	IAA-Ala conjugate hydrolase activity
At1g76130	1.92	Alpha-amylase	Carbohydrate metabolic process,located in extracellular region
At4g19170	0.54	Putative nine-cis-epoxycarotenoid dioxygenase	Located in plastoglobule
At5g35970	0.48	DNA helicase-like	DNA-binding protein, located in chloroplast
At1g73480	0.47	Alpha/beta fold family hydrolase	Aromatic compound metabolism, located in chloroplast
At1g77760	0.47	Nitrate reductase 1 (NR1)	
At3g49160	0.43	Pyruvate kinase -like protein	Pyruvate kinase activity, involved in glycolysis

At1g77760	0.43	Nitrate reductase	Response to light stimulus, nitric oxide biosynthetic process
At5g24470	0.42	APRR5	Circadian rhythm, photomorphogenesis
At5g11330	0.41	Monooxygenase family protein	Electron transport, metabolic process, Located in endomembrane system
At1g32900	0.39	Starch synthase	Transferase activity, transferring glycosyl groups, located in chloroplast
At5g48490	0.28	Lipid transfer protein (LTP) family protein	Lipid binding and transport, located in endomembrane system
		Polysaccharide metabolism	
At5g25980	4.53	Glycosyl hydrolase family 1	Hydrolase activity, hydrolyzing O-glycosyl compounds
At1g55850	3.57	Cellulose synthase family protein	Polysaccharide biosynthetic process, cell wall biosynthetic process
At3g44990	2.52	Xyloglucan: xyloglucosyl transferase	Hydrolase activity, acting on glycosyl bonds, located in chloroplast
At2g32290	2.3	Putative 1,4-alpha-D-glucan maltohydrolase	Polysaccharide catabolic process
At3g21750	2.17	UDP-glucosyl transferase family protein	Transferring glycosyl groups
		Signal transduction	
At5g39670	3.03	Calcium-binding EF hand family protein	
At1g14320	2.52	Wilm's tumor suppressor protein-related	Involved in translation
At5g35735	2.04	Auxin-responsive family protein	Dopamine beta-monooxygenase activity, located in membrane
At1g61370	2.1	S-locus lectin protein kinase family protein	Protein amino acid phosphorylation, located in endomembrane system
At1g51760	1.96	IAA amino acid hydrolase (IAR3)	Proteolysis, located in endomembrane system
At5g67030	0.55	Zeaxanthin epoxidase	Abscisic acid biosynthetic process
At5g45830	0.52	Tumor-related protein like	
At4g12980	0.51	Auxin-responsive family protein,putative	Dopamine beta-monooxygenase activity, located in membrane
At4g28950	0.47	ROP GTPase gene family protein	Protein transport, small GTPase mediated signal transduction
At5g45820	0.26	CBL-interacting protein kinase 20 (CIPK20)	Kinase activity
		Photosynthesis	
At3g17040	0.5	Tetratricopeptide repeat (TPR)-containing protein	Chloroplast precursor
At3g59400	0.37	GUN4	Chlorophyll biosynthetic process, located in chloroplast
At1g44446	0.34	Chlorophyll a oxygenase / chlorophyll b synthase	Chlorophyll biosynthetic process

Supplemental Figure 1. Alignment of 27 UBP proteins.

Highly conserved amino acids are shaded in black while less conserved ones are shaded in gray. Numbers above the alignment indicate the amino acid position in the consensus.

Supplemental Figure 2. Gene expression pattern of *UBP15* subfamily.

Organs from 1 to 18 are cauline leaves, rosette leaves, pistil one day before pollination, pistil one day after pollination, silique 3 day after pollination, silique 8 day after pollination, stem, sepal, stamen, petal, seed, cultured cell, root dark, root white light, hypocotyl dark, hypocotyl white light, cotyledon dark and cotyledon white light.

Supplemental Figure 3. Phylogenetic Analysis of AtUBP15 subfamily and its homologues in *Rice*.

The Phylogenetic tree of 9 UBPs in *Arabidopsis* or *Rice*. Bootstrap values are shown in percentages at nodes.

Supplemental Figure 4. Two month old lines of wild type, two *ubp15* mutants and *UBP15*-overexpressing line.

Two-month-old wild type, *ubp15-1*, *ubp15-2*, *UBP15*-overexpressing line and rescued line. Mutants are weak, with early flowering time and more secondary stems while *UBP15*-overexpressing line showed opposite phenotype with late flower and strong apical dominance. Bar=1cm.

Supplemental Figure 5. Comparison of palisade cell number in transverse sections across the lamina of rosette leaf. Error bars represent standard deviation of three biological repeats.

Supplemental Figure 6. Comparison of the ninth rosette leaf of wild type and mutants as well as transgenic lines.

(A) Mature ninth rosette leaf of wild type, *ubp15-1*, *ubp15-2*, *UBP15*-overexpressing line and *ubp15 ubp16*. Bar=1cm.

(B) Comparison of adaxial epidermal cell numbers of transverse sections in three regions of the ninth rosette leaf.

Supplemental Tables

Supplemental Table 1. Microarray data of 27 *UBP* genes expression in various organs.

Gene code	gene name	Cauline leaf	Rosette leaf	Pistil one day after pollenation	Pistil one day before pollenation	Silique 3 day after pollenation	Silique 8 day after pollenation
At4g10590	UBP10	253.2371134	538	318.9969539	178.5757576	209.7058824	69
At4g10570	UBP9	253.2371134	538	318.9969539	178.5757576	209.7058824	69
At1g32850	UBP11	14.17808219	19	10.58646617	12.07272727	38.05147059	14.80555556
At2g40930	UBP5	114.5257732	497.5804196	350.327381	404.625	337.3897059	70
At5g22030	UBP8	123.0618557	309.4912281	218.0594178	315.5220588	311	89.97222222
At4g30890	UBP24	248	584.2237762	655.5600733	568	736.5073529	168.5555556
At4g39910	UBP3	172.9726027	350.6993007	302.4974359	372.8897059	208.8602941	130.9722222
At2g22310	UBP4	38.41237113	66.24561404	63.11683054	48	28	22
At5g06600	UBP12	1141	1519.146853	1139.097744	599.6121212	1186.024096	247.1388889
At3g49600	UBP26	190	480.2807018	330.575188	343.5955882	313.1325301	219
At1g51710	UBP6	621	1231.754386	891.3714671	781.1764706	754.2647059	574
At3g21280	UBP7	71	292.9370629	145.1863505	160.5073529	136.1397059	107
At2g32780	UBP1	7	8.251748252	51.91666667	29.67878788	15.22058824	35
At1g04860	UBP2	211.2680412	381.2307692	367.7518797	222.8424242	306.9485294	149
At4g39370	UBP27	46.94845361	74.52631579	127.5	61.02941176	57	77.9
At5g10790	UBP22	379.1443299	600.3508772	273.7743975	238.0147059	289.1911765	91
At5g46740	UBP21	120.9278351	158.4335664	383.9718913	241.4545455	222.3897059	124.1388889
At5g57990	UBP23	491	1614.736842	1162.335897	1227.911765	1056	726.6111111
At3g14400	UBP25	407.5979381	719.3859649	611.4935415	376.2666667	496.0240964	315.4722222
At4g24560	UBP16	152.2268041	443.9440559	509.277381	546.8235294	367.1686747	142.3611111
At5g65450	UBP17	59.04123711	193.0909091	134.8794872	150.9090909	152.2058824	85
At1g17110	UBP15	711.3402062	1031.982456	1031.665293	758.0666667	845	514.7777778
At4g31670	UBP18	142	404.3356643	203.3615577	209.3308824	181.8014706	188
At2g24640	UBP19	56	222	205.9252747	238.0147059	203	84.27777778

Gene code	gene name	Stem	Sepal	Stamen	Petal	Seed	Cultured cell
At4g10590	UBP10	266	504.8139535	661.641791	290	95.75543478	963.8555417
At4g10570	UBP9	266	504.8139535	661.641791	290	95.75543478	963.8555417
At1g32850	UBP11	52	91.84883721	64.74129353	134	-3.02173913	34.26132223
At2g40930	UBP5	315.9270073	246.3023256	270	798	67.02173913	1217.020979
At5g22030	UBP8	444	29.51162791	451.2704918	886.7755102	116.8865489	417.7322314
At4g30890	UBP24	646.8076923	562.5348837	707	1767.030612	191.9293478	1559.865613
At4g39910	UBP3	524.2627737	458.3953488	750.5721393	1617	68.875	1084.045697
At2g22310	UBP4	143.0192308	90.53488372	101	160	-10.30366848	65.2861244
At5g06600	UBP12	1903.386861	204.5813953	1246	2006	401.0923913	2426.601399
At3g49600	UBP26	551.4615385	195.755814	215.6721311	465.122449	213.3043478	560.5384615
At1g51710	UBP6	1669.846154	406.5465116	901	1469	394.9476902	6227.830303
At3g21280	UBP7	263.1094891	138.7674419	125	294.505102	71.46195652	274.6139657
At2g32780	UBP1	21	67.65116279	39.12935323	118	30.04415761	28.70312881
At1g04860	UBP2	324	590.1046512	639.5870647	256.4693878	261.9293478	819.7538462

At4g39370	UBP27	43	114.4302326	122.3681592	116	248.3913043	64.60289855
At5g10790	UBP22	289	409.6046512	407	834	139.798913	371.109589
At5g46740	UBP21	243.5192308	135.1860465	118.0995025	146.7091837	72.24456522	93.65178979
At5g57990	UBP23	2079.445255	431.5581395	346	929.1581633	534.28125	3446.150487
At3g14400	UBP25	697.0576923	277.3837209	758	214.0867347	207.9673913	1548.92372
At4g24560	UBP16	351.75	318.744186	454	542	93.54076087	1062.276923
At5g65450	UBP17	98.78832117	178.4767442	108.8507463	455	276.5108696	88.66155158
At1g17110	UBP15	851.6730769	379.5930233	534.2935323	207.5663265	210.125	641.6596737
At4g31670	UBP18	398.0875912	309.0348837	322	880	130.9728261	574.4164103
At2g24640	UBP19	331	172.5348837	269.6368159	547.7142857	112.7391304	556.7737557

Gene code	gene name	Root dark	Root white light	Hypocotyl dark	Hypocotyl white light	Cotyledon dark	Cotyledon white light
At4g10590	UBP10	424.8228346	438.28125	173.40625	131.5095238	393.2027027	370.7176157
At4g10570	UBP9	424.8228346	438.28125	173.40625	131.5095238	393.2027027	370.7176157
At1g32850	UBP11	51.77382175	85.8125	17.91666667	36.67142857	44.11560694	39.55357143
At2g40930	UBP5	325.4406923	344.65625	97.63541667	123.1904762	353.9322034	313.0154762
At5g22030	UBP8	279.9637111	408.21875	129.2604167	143.8857143	419.0860927	422.2265816
At4g30890	UBP24	414.0984252	531.8125	188.71875	313.3857143	467.1186441	429.1309524
At4g39910	UBP3	462.4347826	666.46875	311.2708333	294.1071429	540.1788079	598.1203056
At2g22310	UBP4	128.5507246	111.09375	20.54166667	46.95714286	62.12582781	101.2047619
At5g06600	UBP12	1517.831793	2085.65625	416.6458333	406.9738095	1479	1652.075033
At3g49600	UBP26	425.0354331	616.53125	202.71875	183.6095238	397.9595376	378.8214286
At1g51710	UBP6	1981.102362	3926.3125	569.9583333	668.4071429	889	1159.790698
At3g21280	UBP7	105.6540784	209.125	42.21875	91.56428571	131.4277457	177.4428571
At2g32780	UBP1	62.84468789	34.8125	20.13541667	28.72619048	26.65317919	26.14784053
At1g04860	UBP2	502.2960018	594.59375	183.71875	200.4095238	438.0397351	410.7517483
At4g39370	UBP27	92.94123017	106.28125	28.96875	47.19047619	109	73.32009044
At5g10790	UBP22	410.9566929	420.59375	106.6354167	127.0714286	470.7118644	562.8515873
At5g46740	UBP21	146.8093119	215.71875	63.33333333	84.58809524	145.2138728	122.7753913
At5g57990	UBP23	832.6963369	1380.65625	540.7708333	948.9547619	817.1125828	1300.761526
At3g14400	UBP25	730.0184868	1210.8125	323.5520833	322.2595238	837.2774566	704.6364653
At4g24560	UBP16	345.6990291	359.90625	148.8854167	179.4928571	431.9653179	379.5714286
At5g65450	UBP17	171.0468619	137.28125	48.69791667	90.40714286	195.8543046	184.5927003
At1g17110	UBP15	787.4924113	1596.84375	409.7291667	498.3833333	1680.243243	1613.125
At4g31670	UBP18	388.7701246	783.5625	268.8229167	230.0404762	196.7288136	236.5464286
At2g24640	UBP19	216.5870136	405.65625	110.78125	162.3714286	315.8513514	298.2021164

Supplemental Table 2. Primers designed to identify the genotype of T-DNA insertion lines.

T-DNA insertion lines	Forward (FP) and reverse (RP) primers
SALK_086190	FP: 5’-TGCGTGAAGGAATTCAGATCCA-3’
	RP: 5’-TTCAGCGTTATATCTAAAAGAATTG-3’
SALK_064103	FP: 5’-GCTTCGCTTACGTTATACCACGC-3’
	RP: 5’-CTGAAGCCTCGGGAGTTGGTT-3’
SALK_059858	FP: 5’-CGGGTCTTCTCCGCTACACCT-3’

	RP: 5’-CCTTTGGTGGTTGCAGATTCG-3’
SALK_112950	FP: 5’-TGTGCACAACACCATTTGCCT-3’
	RP: 5’-TCTCTCCCTTGTGCAGGCTCTT-3’
SALK_043210	FP: 5’-TGTGATTGGGTTTGGTTTGGG-3’
	RP: 5’-TCTCTTGACCTGCTTGGCTGA-3’
SALK_088398	FP: 5’-GTGCTGCTACTGCTGCTTCCC-3’
	RP: 5’-CAACAGCAGCTAAATCAAAAAGG -3’
SALK_108832	FP: 5’-AAAATGTGGTCCAAGTGGATGG-3’
	RP: 5’-TGAGAAGGAAACTCACATGACTGGA-3’
SALK_014223	FP: 5’-CCAATTACAGTGCGTTCCAAGC-3’
	RP: 5’-TGGCCAACTTTGTTAGATGTTTCA-3’
SALK_034744	FP: 5’-GACCAAGGGGATTCCAAATGC-3’
	RP: 5’-TTTCTGGTTGCAGGGCCAATA-3’
SALK_149329	FP: 5’-AGCGGGAAATCCACATATGCC-3’
	RP: 5’-CCTTTCCAATGGTTTTCAGGC-3’
SALK_088692	FP: 5’-CGTAAGCAGCCGAGGTCTTGA-3’
	RP: 5’-TCCAAGCGGTTGAATGTGCTT-3’
SALK_141485	FP: 5’-TGCAATGATGCTAATTGGATCAAGA -3’
	RP: 5’-TTTTATTATGCTTCTGTTCCTTTTT -3’
SALK_093503	FP: 5’-AGCATCAGGAAGGTGGCCATT -3’
	RP: 5’-TCGGTTACCATTTCCTTCCATTG -3’
SALK_043515	FP: 5’-CACTAGGAAACCAGTGCCTTCG -3’
	RP: 5’- ACACTTTGGGCCCCTGTCACT -3’
SALK_128312	FP: 5’-CCCTCCACAACAGTTCCCTTG-3’
	RP: 5’-TTGGAATGGAGTCAAGTTACCGC-3’
SALK_024054	FP: 5’-CGCACTATGAACCCCAACACC -3’
	RP: 5’-GAAAGGTTGGATGCTTGTTTTG -3’
SALK_130784	FP: 5’-GCTTTTGTTGGAACAGATGTCAA-3’
	RP: 5’-TCCTCATGTAGGAAGAGGTAGCCA-3’
SALK_132368	FP: 5’-CCAAGCTTCTCAGCCACCCTT-3’
	RP: 5’-TGTTGGCAGGCTAATGGTGAAA-3’
SALK_050151	FP: 5’-GCCGAAAAGGAGTATCGTTCCA-3’
	RP: 5’-CAAGGTAGATGCCATTGCCCA-3’
SALK_012863	FP: 5’-GGAGGCAAATTAAAAAGACAGCGA-3’
	RP: 5’-ATGCACCAATCTCCCACCAGA-3’
SALK_018601	FP: 5’-ATGGTGAACCGGAGCTTTTCC-3’
	RP: 5’-CCAGGTTAAATGCCTGAGGTGTG-3’
SALK_015611	FP: 5’-TCACACCTCAGGCATTTAACC-3’
	RP: 5’-TTGTGGAAACAGGTATTGTCTC-3’
SALK_023552	FP: 5’-TACGCAAATGAAAGACCATGA-3’
	RP: 5’- TGGGTTTGAGAAGCTGGTCGT -3’
SALK_087726	FP: 5’-GGTGAATCATATGGGTTTTGCTTT-3’
	RP: 5’- TTTGAACCAATCTCCATCAAGGG-3’
SALK_113300	FP: 5’-TGCTTCTTTATGCAAGGTGAATGA-3’
	RP: 5’-CATACTCCCTCCGTTTTCACAA-3’

SALK_009641	FP: 5'-AAAGGCAAGGGGAGGAGAATC-3'
	RP: 5'-GAAGCTCGGGAAAATGGATGG-3'
SALK_101685	FP: 5'-TGAGCATCCTCCTGTCTTCCA -3'
	RP: 5'-TTTTTCACATTGTTACCCAAAAA -3'
SALK_126252	FP: 5'- TGAGCATCCTCCTGTCTTCCA -3'
	RP: 5'- TTTTTCACATTGTTACCCAAAAA -3'
SALK_084566	FP: 5'-TCGGCGATGGTCTCTATCGAA -3'
	RP: 5'-GGTTGATAACAATTTACCAAAGTCG -3'
SALK_117787	FP: 5'-TGCGTGAAGGAATTCAGATCCA -3'
	RP: 5'-TTCAGCGTTATATCTAAAAGAATTG-3'
SALK_079015	FP: 5'-TTTAAGTTTTCTAGACACTATTTTT-3'
	RP: 5'-GGGAGAAAGCCGAGAGTCTGTG-3'
SALK_121772	FP: 5'-TGTAACCTCGATCCCTCAGCATC-3'
	RP: 5'-TTGCCAAATGGGATGAGGAAA -3'
SALK_001531	FP: 5'-CCTTCCCAGTAACCGAGGCTCT-3'
	RP: 5'-CCTTTTGTGCAGCTCCTCCAG -3'
SALK_088458	FP: 5'-CGGAGAAAACCAACCAAGCAA -3'
	RP: 5'-ACAGCTATTGCCGGTGTAGCG -3'
SALK_111336	FP: 5'-TGAACGTTGCAAATTCATTCGAT -3'
	RP: 5'-CCGATGCGCCTAACAAGATTTC -3'
SALK_024392	FP: 5'-TTGTGGAAACACCCCACAAAA -3'
	RP: 5'-TTGGCTTCGTCTATGGGCTGA -3'
SALK_067020	FP: 5'-TTCTCAAAACATTCGCAGTGGC -3'
	RP: 5'-AATAGACCGTGCTGTTGGGCA -3'
SALK_027968	FP: 5'-TTTCAAATCAAATAAGCTAAAAAG -3'
	RP: 5'-TGGCTTGTCAAATTGAAATTTTTG -3'

Supplemental Table 3. Transcriptome analysis of *ubp15-1*.

Gene Code	ID	M	W	M/W
At1g17110	AF302665	110	1959	0.06
At1g28375	AC010155	21	221	0.09
At4g01525	AC069551	25	236	0.11
At1g14550	AC010657	32	204	0.16
At3g59970	AF181966	39	239	0.16
At5g05820.a	F15569	45	270	0.17
At3g29000	AB025615	37	209	0.18
At4g12900	AL079349	47	247	0.19
At1g38450	AC006918	50	233	0.22
At1g41720	AC006918	50	233	0.22
At2g06330	AC006918	50	233	0.22
At1g42360	AC006918	50	233	0.22
At1g42370	AC006918	50	233	0.22
At1g37160	AC006918	50	233	0.22

At5g39890	AB010077	57	256	0.22
At1g63530	AC008047	56	253	0.22
At1g71150	AC016972	55	244	0.22
At1g32060	AC074309	517	2159	0.24
At5g66990	AB026640	73	302	0.24
At1g35690	AC007887	81	322	0.25
At5g41315	AB006707	60	230	0.26
At3g02120	AC011664	76	290	0.26
At4g02240	AL096882	64	244	0.26
At5g65330	AL096882	64	244	0.26
At5g45820	AB016870	495	1874	0.26
At2g06410	AC006918	69	244	0.28
At5g48490	AB020745	2248	7953	0.28
At4g28440	AV557403	62	217	0.29
At1g62710	AF367254	85	281	0.3
At1g77880	AC009243	83	276	0.3
At1g48980	AC016041	61	203	0.3
At3g60500	AL138646	62	206	0.3
At3g01650	AC009325	71	241	0.3
At5g01600	AF326869	5374	17383	0.31
At5g16580	AB008270	72	231	0.31
At1g14500	AC012188	71	228	0.31
At5g65080	AF214485	114	363	0.31
At4g07938	AC006423	63	200	0.31
At5g02720	AL162973	66	212	0.31
At5g58830	AB016885	120	386	0.31
At1g43830	AC006423	63	200	0.31
At2g38690	AC005499	90	287	0.31
At2g02690	AC002521	64	201	0.32
At1g51960	AC006216	160	502	0.32
At1g48660	AC073555	116	364	0.32
At5g19840	AB024038	71	218	0.33
At1g65920	AC009513	123	376	0.33
At4g24080	AC002343	94	282	0.33
At3g55240	AL132954	508	1545	0.33
At1g44446	AB021316	3029	8868	0.34
At4g39090	AL035679	2180	6320	0.34
At4g38590	AL035540	74	220	0.34
At4g12440	AL049730	80	231	0.34
At4g20450	AL080253	104	308	0.34
At2g29670	AF375460	6906	19504	0.35
At4g31360	AL021633	190	547	0.35

At1g53930	AC006577	112	318	0.35
At3g63160	BE039458	1963	5579	0.35
At2g34940	AC004238	113	320	0.35
At3g26210	AB024038	515	1421	0.36
At5g44510	AB017065	73	202	0.36
At1g32810	AC006424	153	421	0.36
At4g13490.a	AA404812	4980	13728	0.36
At3g58650	AL137082	87	244	0.36
At5g62080	AB016880	81	217	0.37
At4g09930	AL049481	110	297	0.37
At1g47730	AC007519	110	296	0.37
At2g22240	U30250	235	626	0.37
At3g59400	AL356014	6379	17232	0.37
At3g01080	AC008261	102	276	0.37
At2g46590	AC006418	145	386	0.38
At4g20970	AL080282	95	248	0.39
At3g27500	AB025626	78	200	0.39
At1g17740	AB010407	105	267	0.39
At4g26460	AL022223	100	256	0.39
At5g54190	U29699	880	2255	0.39
At3g56790	AL390921	111	287	0.39
At3g46760	AL096859	80	207	0.39
At1g32900	AC006424	158	406	0.39
At5g49140	AB023028	107	268	0.4
At4g36195	AL022141	97	242	0.4
At4g35960	AL022373	96	242	0.4
At3g30130	AY046045	221	555	0.4
At1g74980	AY045856	476	1195	0.4
At1g77410	AC078898	85	214	0.4
At1g49800	AC079674	112	280	0.4
At1g28470	AV567286	152	381	0.4
At1g56350	AY046045	221	555	0.4
At2g18150	AC007212	152	383	0.4
At1g05700	AC007153	108	273	0.4
At4g29230	AL161574	126	313	0.4
At3g52040	AL049711	94	236	0.4
At1g63570	AC008047	151	378	0.4
At5g11330	AL360314	1143	2805	0.41
At3g13760	AP001307	151	368	0.41
At1g27220	AC004557	85	207	0.41
At1g04710	AC002376	144	356	0.41
At5g51670	AB025607	198	477	0.41

At1g63840	H77052	242	585	0.41
At2g31670	AV540982	214	519	0.41
At4g20380	U87834	101	247	0.41
At3g18780	U41998	687	1650	0.42
At3g03590	AC009327	190	450	0.42
At3g27160	AY039901	6880	16566	0.42
At4g16780	X68146	1165	2800	0.42
At1g56080	AC009894	115	272	0.42
At3g25080	AB026647	163	389	0.42
At3g10440	AC011560	231	555	0.42
At2g11010	AB028615	133	315	0.42
At2g46570	AC006418	84	200	0.42
At1g71030	Z68157	3598	8496	0.42
At1g41630	AC006250	238	572	0.42
At1g41600	AC006250	238	572	0.42
At1g41660	AC006250	238	572	0.42
At4g23700	AL035394	142	336	0.42
At4g10220	AF096373	89	211	0.42
At5g37230	AB017069	104	245	0.42
At5g37250	AB017069	104	245	0.42
At5g37270	AB017069	104	245	0.42
At5g03350	AL162751	2212	5268	0.42
At5g24470	AF027408	407	972	0.42
At1g79910	AC011717	104	247	0.42
At1g62690	AC007190	137	317	0.43
At5g06980	AY042849	657	1544	0.43
At1g75100	AC013258	117	271	0.43
At1g30080	AC022455	108	251	0.43
At2g35310	AC004667	171	397	0.43
At1g44542	AC084807	133	312	0.43
At1g50720	AC079027	120	276	0.43
At2g47150	AC004411	103	238	0.43
At2g46140	AC005397	112	259	0.43
At3g51150	AL132980	93	218	0.43
At3g42230	AL138645	168	390	0.43
At3g49160	AL132956	485	1128	0.43
At4g36470	AL161589	146	340	0.43
At1g36960	AC051631	86	202	0.43
At1g66100	AF380652	6908	15905	0.43
At2g47860	AC005309	117	275	0.43
At3g60570	AL138646	116	260	0.44
At1g08630	AC003981	93	213	0.44

At3g04360	AC016829	89	201	0.44
At5g26700	AF058914	111	255	0.44
At1g04570	AC002376	117	263	0.44
At2g19070	AC002392	92	206	0.44
At2g04440	AC006951	113	256	0.44
At2g41300	AC005662	107	245	0.44
At4g23970	AL078468	114	254	0.45
At1g43880	AC006423	147	331	0.45
At1g27490	AC004557	116	260	0.45
At1g65300	AC004512	106	236	0.45
At3g04070	AC011698	152	335	0.45
At1g38196	AC006423	147	331	0.45
At3g58350	AL137081	180	401	0.45
At1g80640	AC018849	96	214	0.45
At3g45390	AL132953	115	254	0.45
At1g14390	AC012188	188	407	0.46
At1g37130	AF367272	4767	10361	0.46
At5g51760	AB010074	132	289	0.46
At2g11760	AC074109	217	468	0.46
At2g12010	AC074109	217	468	0.46
At2g34980	AC004238	100	215	0.46
At5g39330	AB009054	171	370	0.46
At4g12210	AL080318	103	224	0.46
At4g12220	AL080318	103	224	0.46
At3g45650	AL138657	239	519	0.46
At5g62130	AB016880	343	745	0.46
At3g59060	AL163527	2960	6497	0.46
At1g38340	AC074109	217	468	0.46
At5g27700	AV534288	124	271	0.46
At1g38149	AC074109	217	468	0.46
At4g07733	AC074109	217	468	0.46
At3g16320	AC001645	119	260	0.46
At1g09150	AC003114	104	227	0.46
At1g20030	AC022472	330	699	0.47
At1g19010	AF360209	217	458	0.47
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At3g48800	AL132963	186	397	0.47
At4g00320	AL161471	202	429	0.47
At3g19050	AP000735	149	315	0.47
At5g46330	AB010698	101	216	0.47
At1g73480	AY045929	1324	2806	0.47
At1g77760	AC012193	1190	2545	0.47

At5g08480	AB006697	120	255	0.47
At3g51830	U72504	886	1904	0.47
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At4g18250	AL021713	116	249	0.47
At3g19700	AP000417	186	400	0.47
At3g48940	AL132967	98	207	0.47
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At1g06210	AC025290	265	546	0.48
At4g17460	U09332	2470	5158	0.48
At1g44860	AC007264	125	259	0.48
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At1g43740	AC009526	126	262	0.48
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At1g42400	AC007264	125	259	0.48
At2g31900	AC006533	127	261	0.48
At3g02380	AF370149	221	459	0.48
At4g27310	AL030978	621	1281	0.49
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At1g12490	AC025416	138	282	0.49
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At2g15730	AC006248	139	283	0.49
At5g16190	AL391148	141	287	0.49
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At5g53040	AB018116	108	221	0.49
At5g64170	AB008266	736	1512	0.49
At1g13650	AC027656	1432	2937	0.49
At5g10660	AL392144	125	255	0.49
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At4g29650	AL079344	247	497	0.5
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At1g63420	AF372939	221	441	0.5
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At3g25960	AB023041	434	835	0.52
At2g16980	AC002354	204	389	0.52
At4g10670	AF080119	208	402	0.52
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At5g45830	AB016870	306	590	0.52
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At1g64860	AB004821	2996	5515	0.54
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At4g04130	AL161499	222	411	0.54
At5g62780	AB009053	150	278	0.54
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At2g34320	AC004481	156	284	0.55
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At2g19810	AC005169	516	927	0.56
At5g44690	AB016874	140	251	0.56
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At1g10040	AC004122	352	173	2.04
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At2g42730	AC006931	248	121	2.05
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At5g20620	L05363	11449	5488	2.09
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At3g09790	L05363	11449	5488	2.09
At1g65350	L05363	11449	5488	2.09
At1g55060	L05363	11449	5488	2.09
At3g62250	L05363	11449	5488	2.09
At4g02890	L05363	11449	5488	2.09
At4g16690	AL161544	1258	601	2.1
At1g08640	AF370182	372	178	2.1
At2g02815	AF175994	283	134	2.1
At3g62150	AL138651	293	140	2.1
At1g56430	AC058785	641	306	2.1
At1g61370	AC004255	444	211	2.1
At3g24100	AF370331	463	220	2.1
At4g02140	AL161493	252	119	2.11
At3g46190	AL355775	210	100	2.11
At3g56260	AL163763	349	166	2.11
At2g21170	AF247559	20479	9650	2.12
At1g80280	AV547254	221	104	2.12
At3g21760	AF372973	1223	575	2.13
At1g14600	AC010657	413	193	2.14

At3g15650	AB017071	416	194	2.14
At2g25510	AC006300	14882	6928	2.15
At4g11440	AL050399	402	187	2.15
At5g03000	AL163002	249	116	2.15
At1g50560	AC012561	241	111	2.16
At3g04120	AC016829	19007	8803	2.16
At5g26000	AY045681	4323	1999	2.16
At1g15620	AC013453	277	128	2.16
At1g65400	AF325110	2725	1260	2.16
At1g55290	AC027034	243	112	2.16
At1g50540	AC012561	241	111	2.16
At3g46890	AL096859	16966	7863	2.16
At1g61400	AC004255	248	115	2.16
At1g61430	AC004255	248	115	2.16
At1g61440	AC004255	248	115	2.16
NoAnno	AF003102	550	254	2.17
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At4g01530	AL161492	212	98	2.17
At3g21750	AB025634	825	381	2.17
At2g18120	AC007212	258	119	2.17
At4g22020	AJ002892	3194	1468	2.18
At1g21310	AB031821	278	128	2.18
At1g49490	AJ002892	3194	1468	2.18
At4g27440	AY042883	279	128	2.18
At3g46250	AL355775	207	95	2.18
At2g40200	AF085279	212	97	2.19
At4g01310	AL161491	15461	7075	2.19
At5g65980	AB011474	204	93	2.19
At3g63290	AV543251	247	113	2.19
At5g27280	AF007271	303	139	2.19
At4g35100	AY049238	16586	7554	2.2
At2g22760	AC005617	203	92	2.2
At2g45960	AY049238	16586	7554	2.2
At2g16850	AY049238	16586	7554	2.2
At2g39310	AC004697	205	93	2.2
At3g61430	AY049238	16586	7554	2.2
At4g19700	AL024486	975	443	2.2
At2g14800	AC004705	483	219	2.21
At2g03020	AC004138	362	164	2.21
At3g60810	AL162295	261	118	2.21
At3g52145	AI995315	206	93	2.22
At2g16600	U40399	24863	11149	2.23

At2g30570	U93215	8840	3958	2.23
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At5g13510	AL391710	21808	9770	2.23
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At5g06540	AP002543	307	136	2.26
At3g15530	AC024081	1342	592	2.27
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At2g27385	AV531683	327	144	2.28
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At1g30260	AC073506	4248	1858	2.29
At2g32290	AC005700	434	189	2.3
At3g46430	AL133298	19794	8588	2.3
At4g16370	AL161543	3197	1382	2.31
At1g44810	AY042861	277	120	2.32
At4g17980	AL021889	357	154	2.32
At5g52050	AB015478	478	205	2.33
At3g50820	AJ145957	16012	6873	2.33
At5g42900	AB008264	7894	3370	2.34
At4g30750	AY037251	209	89	2.36
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At2g01300	AC006200	1094	461	2.37
At5g08280	X73535	25523	10707	2.38
At5g62300	AF370460	27616	11598	2.38
At5g50610	AB025619	222	93	2.38
At5g50710	AB025619	222	93	2.38
At3g07650	AC009176	4686	1965	2.39
At5g11780	AL163814	274	114	2.39
At4g32430	AL034567	394	164	2.4
At5g02500	X74604	20275	8429	2.41
At3g54890	AF326866	9157	3789	2.42
At3g59340	AF370505	692	284	2.43
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At2g21420	AC006841	260	106	2.45
At4g36010	AF360165	958	388	2.47
At4g13940	AF325037	26706	10790	2.48
At1g32080	AC084165	20969	8468	2.48

At4g01260	AL161491	227	91	2.48
At4g31830	AL049607	292	118	2.48
At3g24420	AP000382	8999	3623	2.48
At2g28900	AY045593	21483	8645	2.49
At1g63820	AC010852	214	86	2.5
At5g16400	AF144386	19378	7696	2.52
At1g14320	AY045866	22985	9128	2.52
At1g27710	AC012375	213	85	2.52
At1g75690	AC006434	24943	9882	2.52
At3g44990	X92975	865	343	2.52
At5g06290	AF326871	22183	8782	2.53
At3g15630	AB017071	22541	8902	2.53
At5g01100	AL137189	595	235	2.53
At1g73810	AC012679	279	110	2.54
At5g14610	AL163792	30717	12004	2.56
At5g42530	AB016888	7812	3054	2.56
At2g28800	U89272	22626	8809	2.57
At2g36970	AC006922	308	120	2.57
At1g77110	AC002291	255	99	2.58
At1g28410	AC010155	257	99	2.6
At1g32990	AF325023	20190	7763	2.6
At5g63580	AB005234	209	80	2.62
At2g21800	AC007019	204	78	2.63
At2g33450	AC002332	25052	9518	2.63
At2g23910	AC005170	699	266	2.63
At2g19150	AC002392	206	78	2.64
At5g23660	AF095641	2931	1106	2.65
At3g21470	AB019232	401	151	2.66
At5g13630	Z68495	21783	8155	2.67
At2g42220	AY045616	20113	7535	2.67
At2g03190	AC005313	206	77	2.68
At1g44880	AC020576	245	91	2.68
At2g10350	AC020576	245	91	2.68
At4g03970	AC020576	245	91	2.68
At3g42530	AC020576	245	91	2.68
At3g01310	AC010676	212	79	2.68
At3g45680	AL157735	258	95	2.72
At3g45690	AL157735	258	95	2.72
At2g10940	AC006429	8529	3119	2.73
At5g46110	AY037211	7130	2614	2.73
At2g41430	AC004625	8374	3041	2.75
At1g48300	AC007932	2604	935	2.78

At1g28760	AC007508	310	111	2.8
At1g44040	AC022314	360	129	2.8
At1g08790	AC003981	277	99	2.81
At5g63090	AB008265	223	80	2.81
At3g49910	AF370158	22291	7877	2.83
At3g12840	AB024033	220	77	2.84
At2g29280	AC004561	496	175	2.84
At1g12610	AC025417	293	103	2.85
At2g28460	AC006587	256	89	2.86
At2g38240	AC003028	282	98	2.87
At2g05070	AY045787	20173	6958	2.9
At2g05100	AY045787	20173	6958	2.9
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At1g49500	AF370563	22168	7513	2.95
At5g58310	AB019228	1438	487	2.95
At3g45520	AL161500	215	72	2.97
At4g04380	AL161500	215	72	2.97
At3g25290	AB026647	307	103	2.99
At3g01500	AC009325	616	205	3
At4g24420	AL078637	202	67	3
At3g43600	AB005805	19762	6576	3.01
At5g11370	AL360314	236	78	3.01
At2g28000	AC006929	27972	9297	3.01
At5g39670	AB012243	291	96	3.03
At5g24780	AF386930	375	123	3.05
At1g23310	AF360195	31336	10247	3.06
At2g34420	X64460	7379	2403	3.07
At3g21640	AJ224640	20590	6686	3.08
At5g15960	X55053	22316	7183	3.11
At5g15970	X55053	22316	7183	3.11
At3g01600	AC009325	233	75	3.11
At4g32540	AL050398	303	97	3.12
At2g11820	AB047398	207	66	3.12
At4g17350	AL161546	215	69	3.13
At1g04270	AY048221	22066	7025	3.14
At2g17180	AC007127	345	109	3.15
At4g21280	AL021960	7348	2313	3.18
At3g53290	AL132958	228	71	3.2
At5g44980	AB010693	210	65	3.22
At2g30950	AF135189	29571	9161	3.23
At3g12430	AC069474	270	83	3.24

At4g32490	AL034567	261	81	3.25
At1g69990	AC002062	249	76	3.26
At5g05250	AB010692	1176	358	3.28
At3g56360	AL163972	1291	391	3.3
At1g72150	AY045913	24133	7291	3.31
At4g21990	U53865	1684	509	3.31
At2g30840	AC004669	308	93	3.32
At1g41810	AC022456	240	71	3.37
At5g29090	AC022456	240	71	3.37
At5g32610	AC022456	240	71	3.37
At3g59170	AL356014	217	64	3.41
At5g37470	AP000607	223	65	3.45
At2g25040.a	AV558611	287	83	3.46
At3g32370.a	AV558611	287	83	3.46
At5g37300	AB017069	753	217	3.48
At4g22214	AL021712	256	73	3.51
At1g48700	AC073555	275	78	3.52
At1g55850	AC002304	267	75	3.57
At1g04920	AC004809	362	99	3.64
At4g03280	AJ243702	1486	400	3.71
At1g35660.a	AC007887	203	55	3.73
At4g27110	AL035680	201	51	3.9
At2g04820	AC006955	341	85	4.01
At1g75910	AC007396	205	51	4.04
At3g21980	AB028622	215	52	4.15
At3g27830	AP000371	25310	6009	4.21
At1g74080	AC016662	352	83	4.26
At2g17850	AC003952	277	65	4.29
At2g45570	AC003680	251	58	4.33
At3g56040	AV548493	280	62	4.49
At5g25980	AF360348	28538	6306	4.53
At3g43580	AL391734	222	48	4.61
At5g38710	AB011478	376	78	4.81
At3g12760	AB024033	271	50	5.41
At1g32920.a	BE522104	235	28	8.35

W, wild type; M, mutant.

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Claims:

1. A method for altering plant growth and/or development, comprising modulating expression in a plant of a nucleic acid encoding a **UB**iquitin-Specific **P**rotease (UBP) of the UBP15 subfamily or a homologue thereof comprising the following motifs:
   (i) a Cys box;
   (ii) a His box; and
   (iii) a ZnMYND zinc finger domain.

2. Method according to claim 1, wherein said altered growth and/or development is selected from one or more of: altered cell proliferation, altered leaf development, altered reproductive development.

3. Method according to any one of claims 1 to 3, wherein said modulated expression is effected by introducing and expressing in a plant a nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

4. Method according to any preceding claim, wherein said nucleic acid encoding a UBP15 polypeptide is represented by any one of SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 39, or 44, or is a portion of such a nucleic acid, or a nucleic acid capable of hybridising with such a nucleic acid.

5. Method according to any preceding claim, wherein said nucleic acid sequence encodes an orthologue or paralogue of any of SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16 or 18.

6. Method according to any one of claims 3 to 5, wherein said nucleic acid is operably linked to a constitutive promoter, preferably to a CaMV35S promoter.

7. Method according to any preceding claim, wherein said nucleic acid encoding a UBP15 polypeptide or a homologue thereof is of plant origin, preferably from a dicotyledonous plant, further preferably from the family Brassicaceae, more preferably from the genus Arabidopsis, most preferably from Arabidopsis thaliana.

8. Plant or part thereof, including seeds, obtainable by a method according to any preceding claim, wherein said plant or part thereof comprises a recombinant nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

9. Construct comprising:
   (i) nucleic acid encoding a UBP15 polypeptide or a homologue thereof as defined in claims 1 or 2;
   (ii) one or more control sequences capable of driving expression of the nucleic acid sequence of (a); and optionally
   (iii) a transcription termination sequence.

10. Construct according to claim 9, wherein one of said control sequences is a constitutive promoter, preferably a CaMV35S promoter.

11. Use of a construct according to claim 9 or 10 in a method for making plants having altered growth and/ort development relative to control plants.

12. Plant, plant part or plant cell transformed with a construct according to claim 9 or 10.

13. Method for the production of a transgenic plant having altered growth and/or development, comprising:
    (i) introducing and expressing in a plant a nucleic acid encoding a UPB15 polypeptide or a homologue thereof as defined in claim 1; and
    (ii) cultivating the plant cell under conditions promoting plant growth and development.

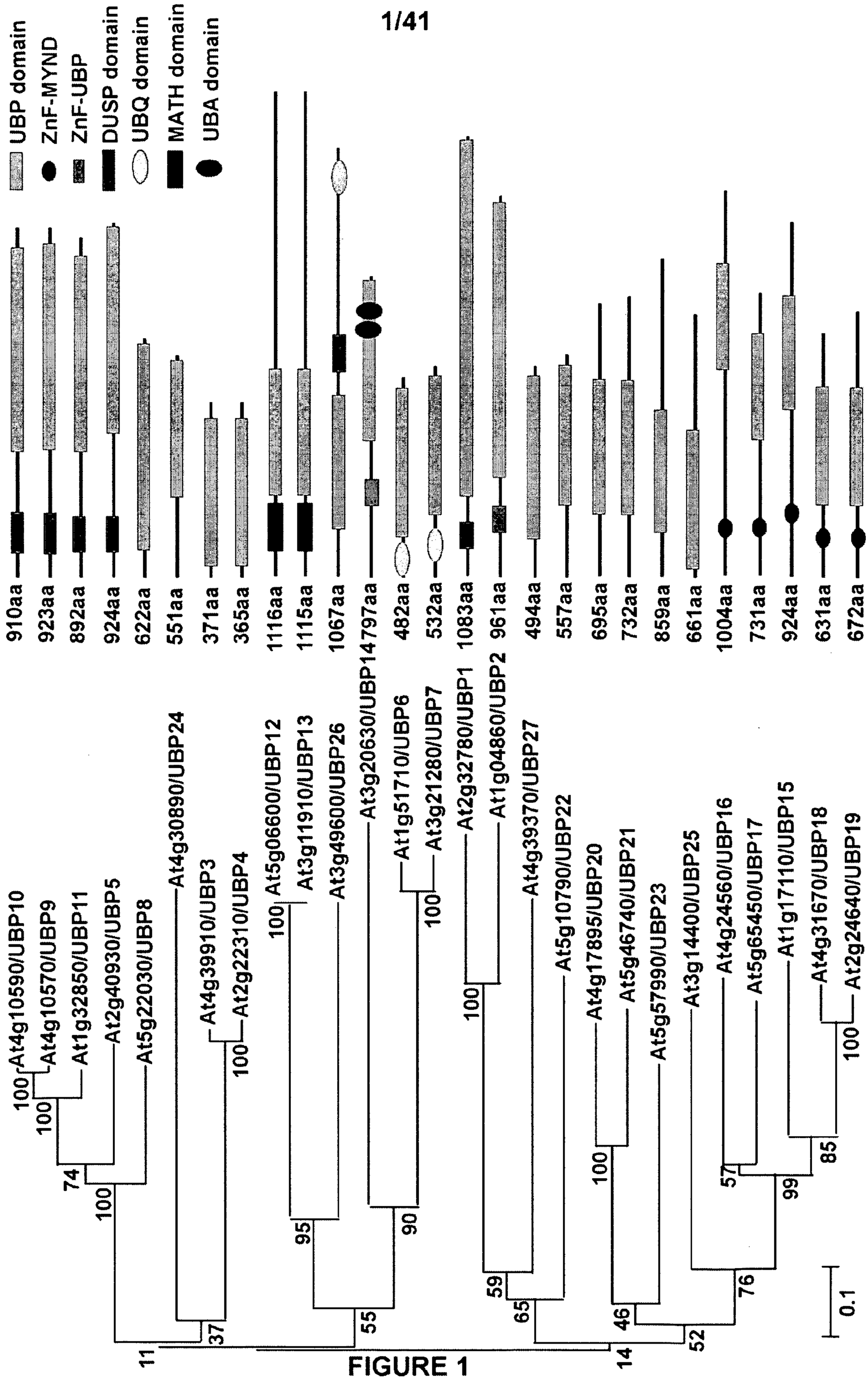
14. Transgenic plant having altered growth and/or development relative to control plants, resulting from modulated expression of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof.

15. Transgenic plant according to claim 8, 12 or 14, or a transgenic plant cell derived thereof, wherein said plant is a crop plant or a monocot or a cereal, such as rice, maize, wheat, barley, millet, rye, triticale, sorghum and oats.

16. Harvestable parts of a plant according to claim 15, wherein said harvestable parts are preferably shoot biomass and/or seeds.

17. Products derived from a plant according to claim 15 and/or from harvestable parts of a plant according to claim 16.

18. Use of a nucleic acid encoding a UBP15 polypeptide or a homologue thereof in altering plant growth and/or development relative to control plants.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ZnF-UBP
DUSP domain
UBQ domain
MATH domain
UBA domain
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923aa
892aa
924aa
622aa
551aa
371aa
365aa
1116aa
1115aa
1067aa
14797aa
482aa
532aa
1083aa
2 961aa
494aa
557aa
695aa
732aa
859aa
661aa
1004aa
731aa
924aa
631aa
672aa
At4g10590/UBP10
At4g10570/UBP9
At1g32850/UBP11
At2g40930/UBP5
At5g22030/UBP8
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At2g22310/UBP4
At5g06600/UBP12
At3g11910/UBP13
At3g49600/UBP26
At3g20630/UBP
At1g51710/UBP6
At3g21280/UBP7
At2g32780/UBP1
At1g04860/UBP
At4g39370/UBP27
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At5g57990/UBP23
At3g14400/UBP25
At4g24560/UBP16
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100
100
74
100
11
37
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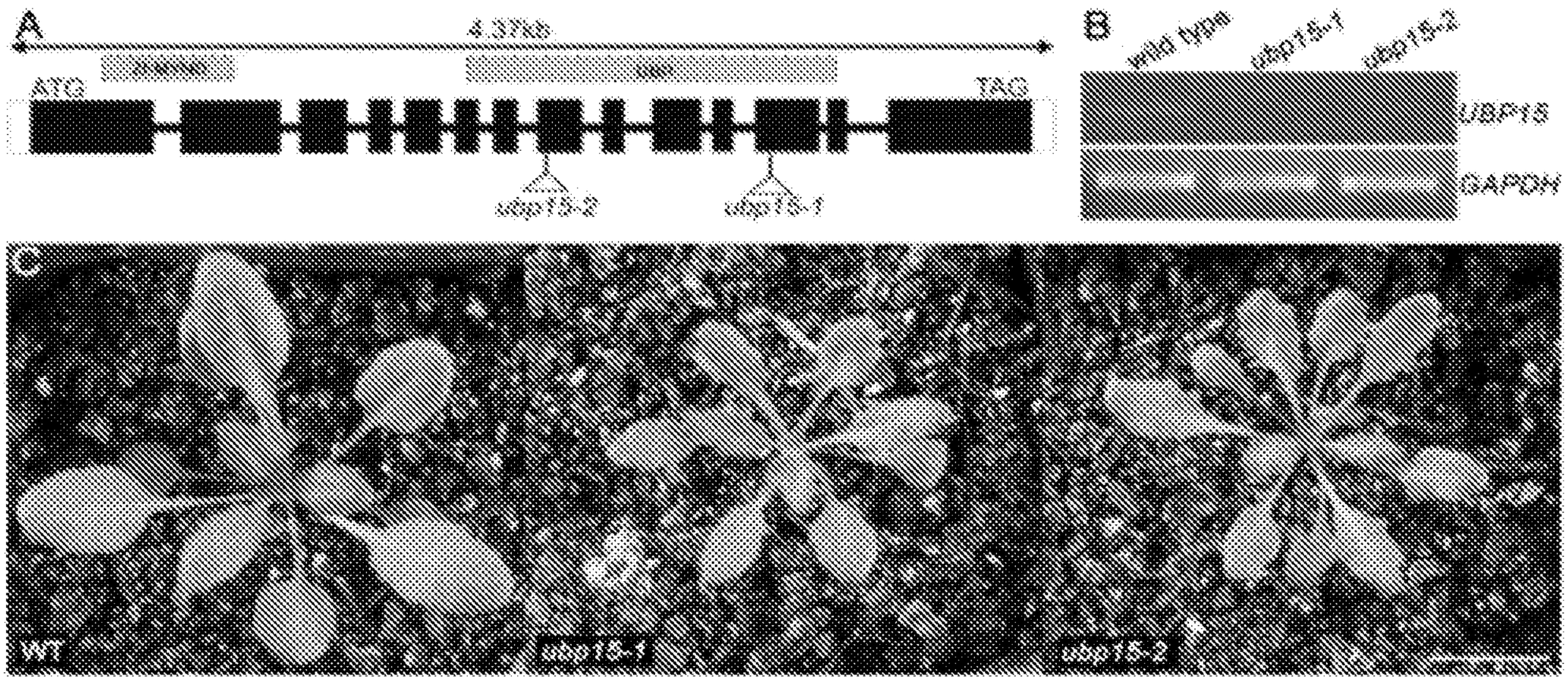
95
55
90
100
59
65
100
46
52
57
99
85
76
0.1

FIGURE 1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ubp15-1
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ubp15-2
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D
WT
ubp15-1
ubp15-2
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ubp15-1
ubp15-2
F
WT
ubp15-1
ubp15-2
G
wild type (n=8)
ubp15-1 (n=8)
Length/width ratio
3.0
2.5
2.0
1.5
1.0
1st
2nd
3rd
4th
5th
6th
7th
8th
9th
10th
11th
12th
13th
14th
15th
Rosette leaf position

FIGURE 2

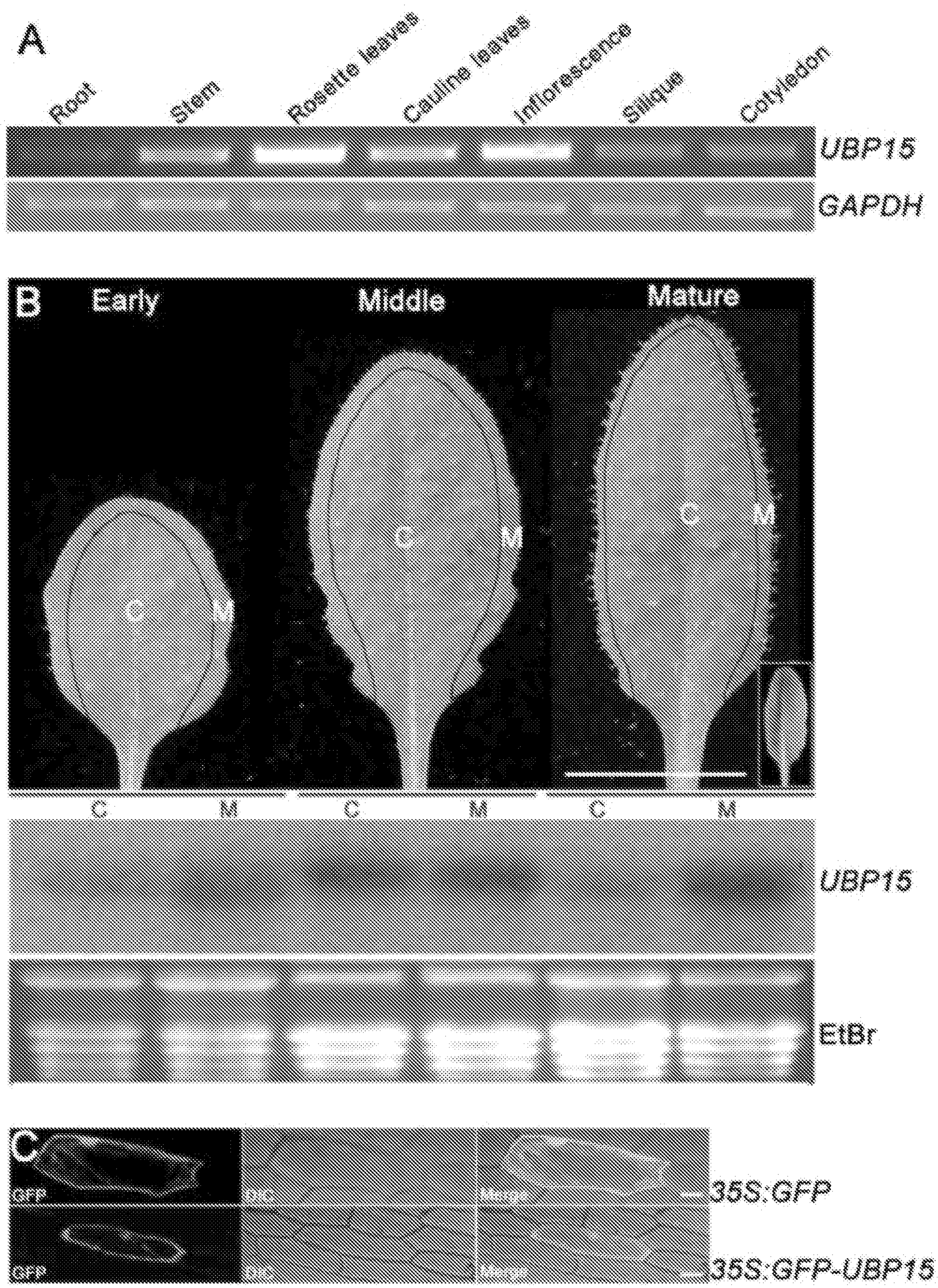

A
Root
Stem
Rosette leaves
Cauline leaves
Inflorescence
Silique
Cotyledon
UBP15
GAPDH
B
Early
Middle
Mature
C
M
C
M
C
M
C
M
C
M
C
M
UBP15
EtBr
C
GFP
BF
Merge
35S:GFP
35S:GFP-UBP15

FIGURE 3

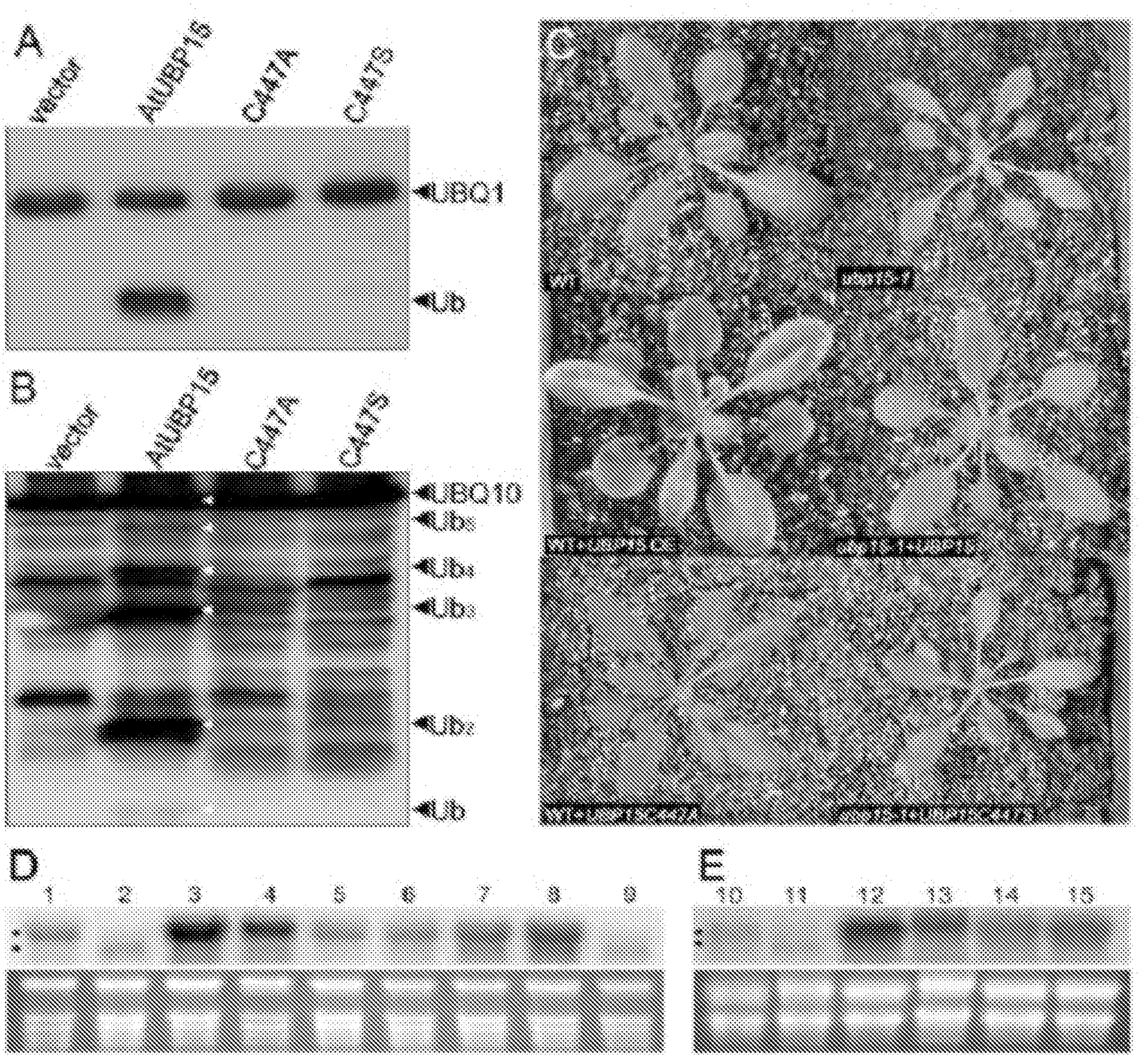


FIGURE 4

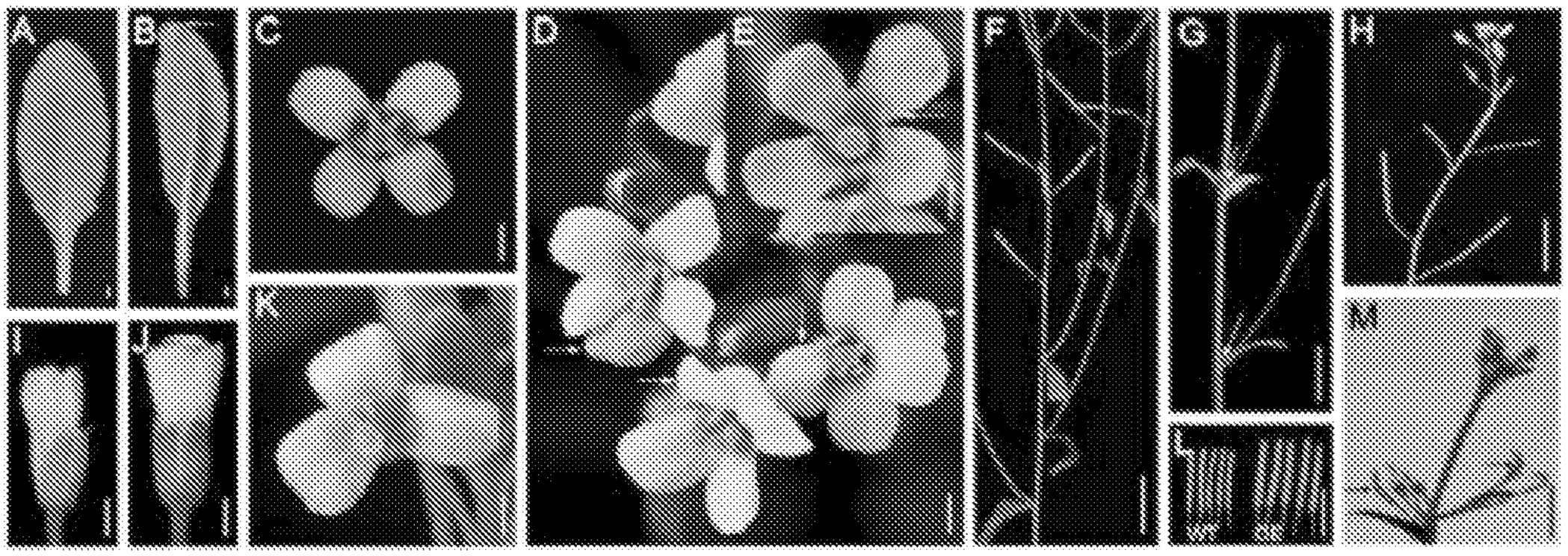


FIGURE 5

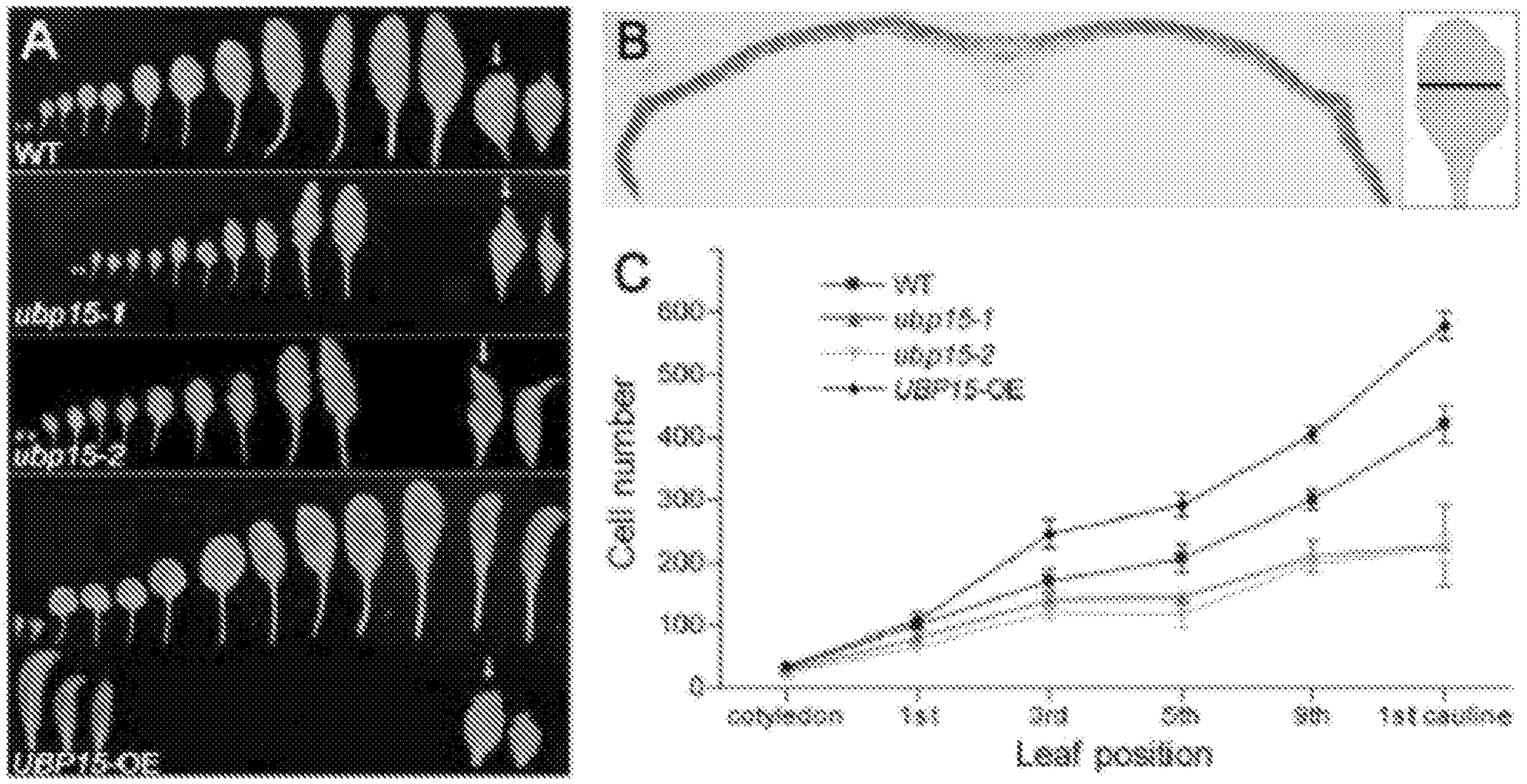


FIGURE 6

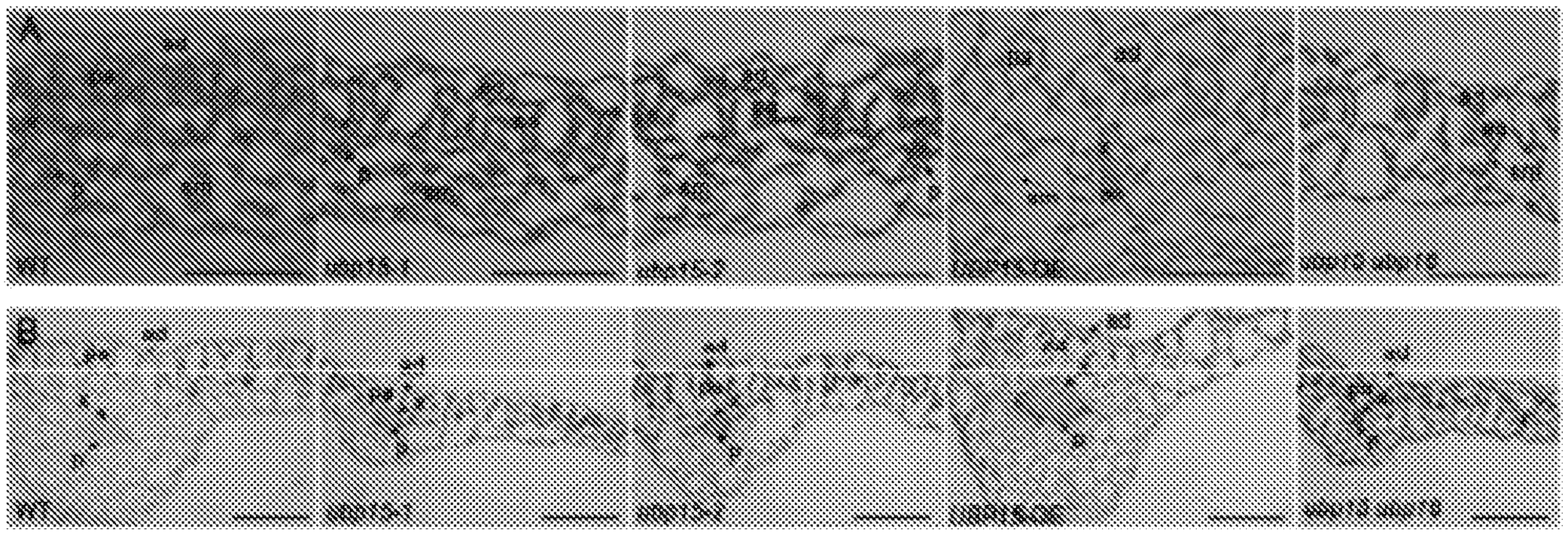


FIGURE 7

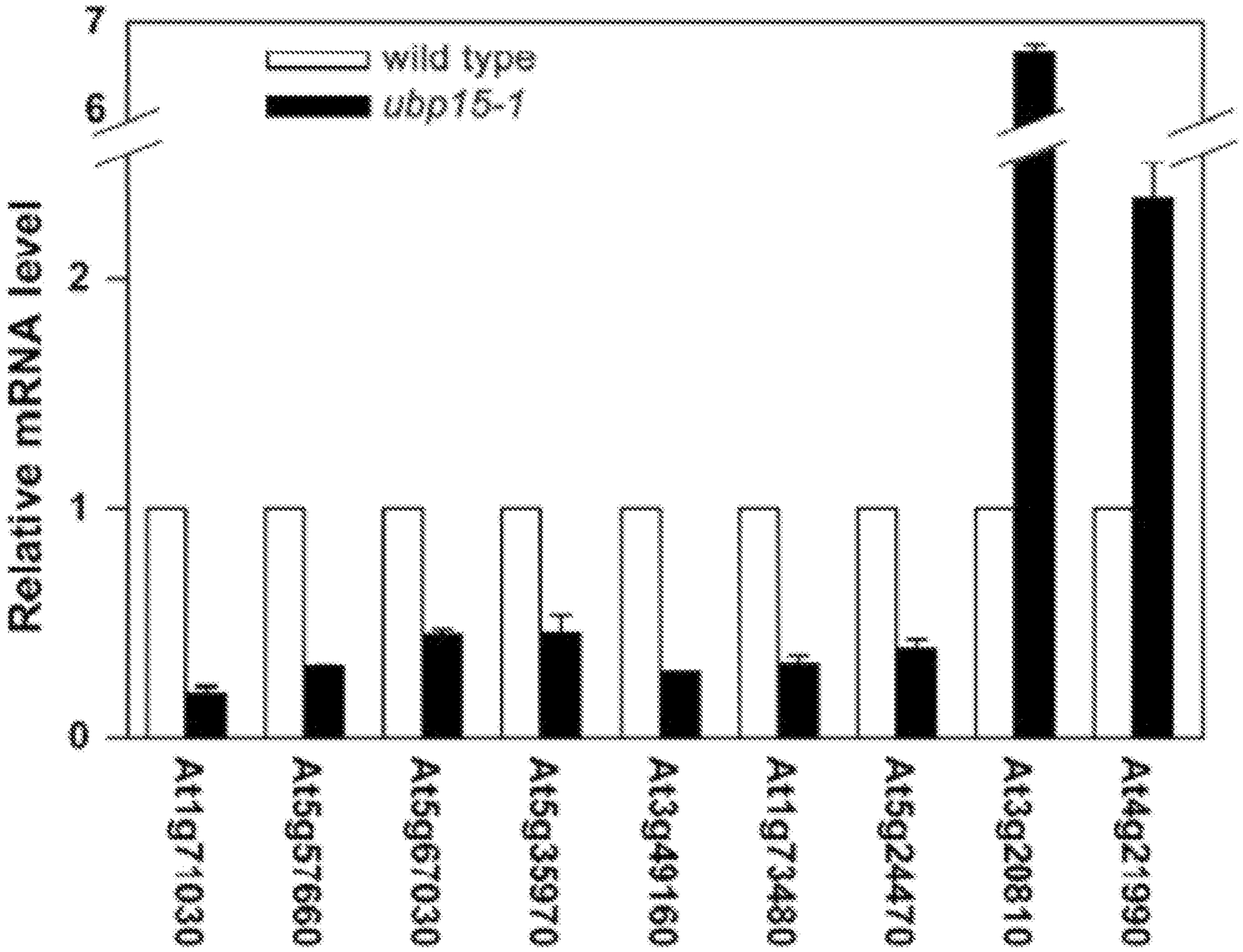


FIGURE 8

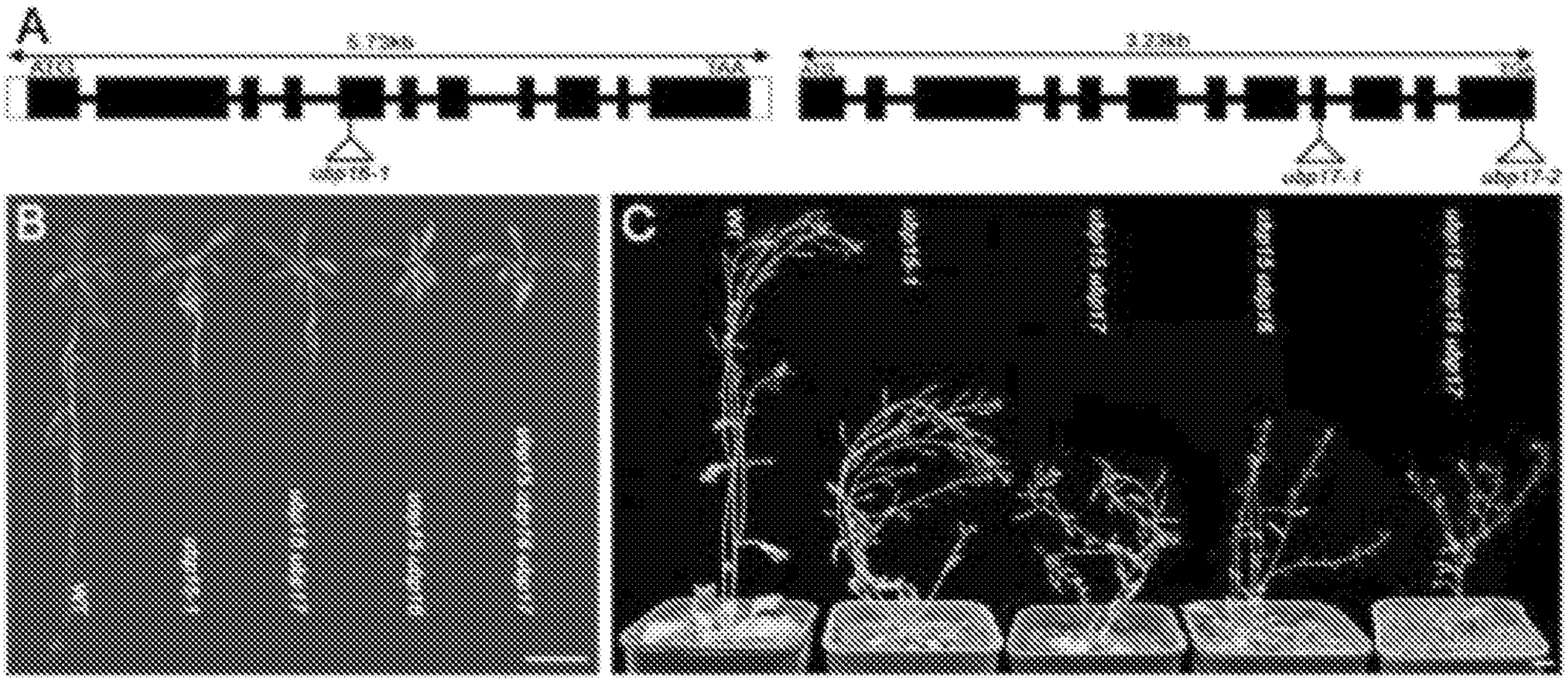
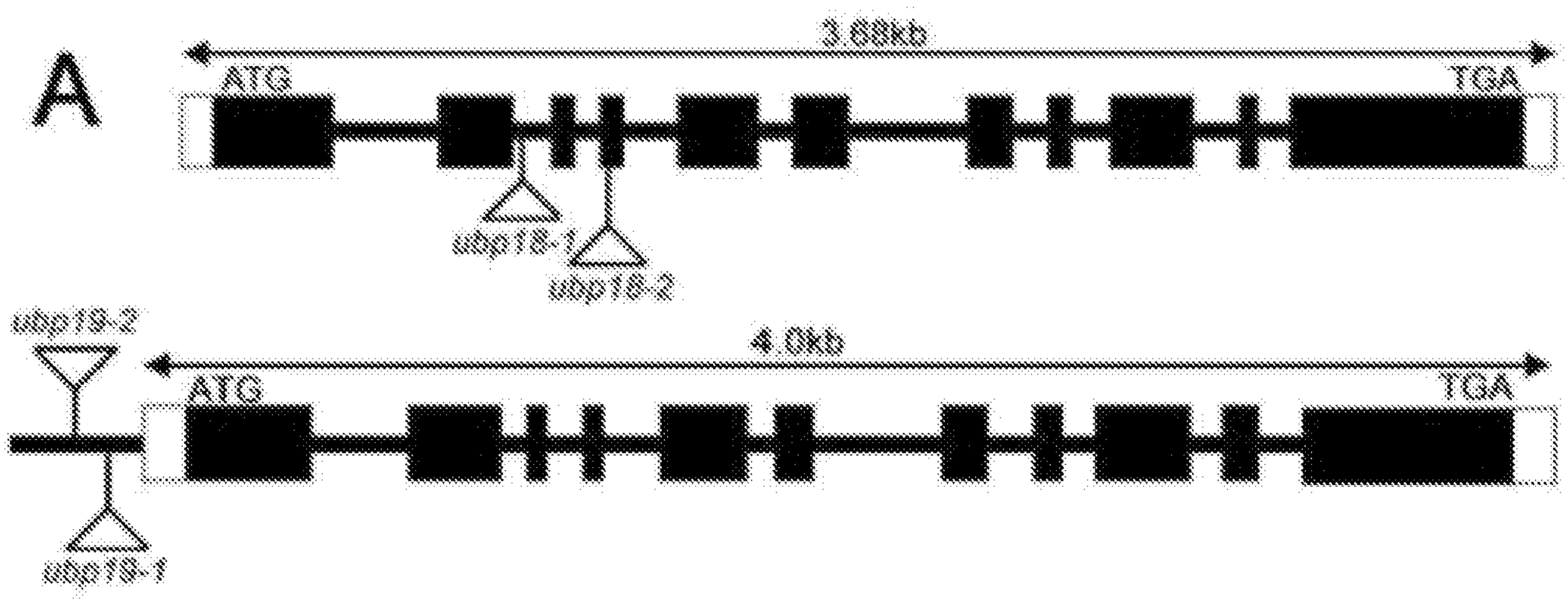


FIGURE 9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TGA
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ubp19-2
ubp19-2
4.0kb
ATG
TGA
ubp19-1
B
C
WT
ubp19-1

FIGURE 10

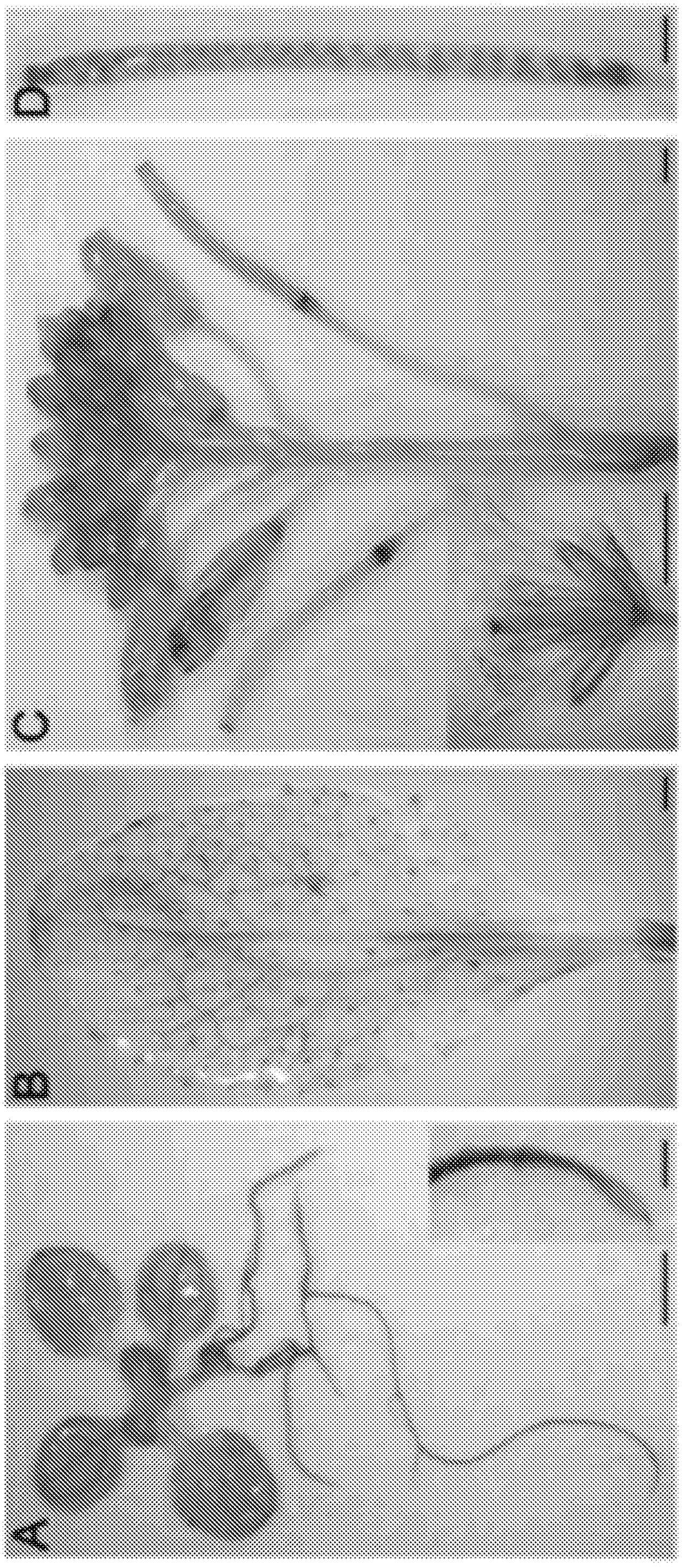


FIGURE 11

SUPPLEMENTAL FIGURE 1

```
                1                                                  50
UBP9      (1)  NLKQGNLYFVISKRWYTSWQEYVEN----------SANECSTGESSEAP
UBP10     (1)  NLKEGNLYFVISKRWYTSWEKYVEQ----------STKEYISGESSEAS
UBP11     (1)  -LKEGNLYFVISNRWYTRWQRFVGL----------LTEEFRSGEPSEVT
UBP5      (1)  NSKEGDTFYLITQRWWQEWIEYVNQDQPCNTNDGSSLSEHCDSPGSSTLK
UBP8      (1)  --------------------------------------------------
UBP24     (1)  --------------------------------------------------
UBP3      (1)  --------------------------------------------------
UBP4      (1)  --------------------------------------------------
UBP12     (1)  --------------------------------------------------
UBP13     (1)  --------------------------------------------------
UBP26     (1)  --------------------------------------------------
UBP14     (1)  --------------------------------------------------
UBP6      (1)  --------------------------------------------------
UBP7      (1)  --------------------------------------------------
UBP1      (1)  --------------------------------------------------
UBP2      (1)  --------------------------------------------------
UBP27     (1)  --------------------------------------------------
UBP22     (1)  --------------------------------------------------
UBP20     (1)  --------------------------------------------------
UBP21     (1)  --------------------------------------------------
UBP23     (1)  --------------------------------------------------
UBP25     (1)  --------------------------------------------------
UBP15     (1)  --------------------------------------------------
UBP16     (1)  --------------------------------------------------
UBP17     (1)  --------------------------------------------------
UBP18     (1)  --------------------------------------------------
UBP19     (1)  --------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                51                                                  100
UBP9      (40) RPGPIDNHDIIES---DSDINDPQLRRLLVEGEDYVLVPKQVWKRLVEWY
UBP10     (40) RPGPIDNHDIIES---ESDVNDPQLRRLLMERVDYVLVPQEVWKRLVEWY
UBP11     (39) RPGPIDNHDIIDS---ESDASDPQLRMMLEEGVDYTLVQQEVWRKLVKWY
UBP5      (51) KPSRIDNSDLIYDSSLEDPSNTSEIIETLQEGRDYVLLPQEVWNQLRSWY
UBP8       (1) ----------------------------------------------------
UBP24      (1) ----------------------------------------------------
UBP3       (1) ----------------------------------------------------
UBP4       (1) ----------------------------------------------------
UBP12      (1) ----------------------------------LKFTWTIPNFSRQ
UBP13      (1) ----------------------------------LKFTWTIPMFTRL
UBP26      (1) ----------------------------------------------------
UBP14      (1) ----------------------------------------------------
UBP6       (1) ----------------------------------------------------
UBP7       (1) ----------------------------------------------------
UBP1       (1) ----------------------------------------------------
UBP2       (1) ----------------------------------------------------
UBP27      (1) ----------------------------------------------------
UBP22      (1) ----------------------------------------------------
UBP20      (1) ----------------------------------------------------
UBP21      (1) ----------------------------------------------------
UBP23      (1) ----------------------------------------------------
UBP25      (1) ----------------------------------------------------
UBP15      (1) ----------------------------------------------------
UBP16      (1) ----------------------------------------------------
UBP17      (1) ----------------------------------------------------
UBP18      (1) ----------------------------------------------------
UBP19      (1) ----------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    101                                                 150
UBP9          (87)  SGGPPIERKLICQGFYTRSYSVEVYPLCLMLTDGRDESRTVIRLGKQASI
UBP10         (87)  SGGPPIERKLICQGFYTRSYSVEVYPLCLMLTDGRDESRTVIRLGKQASI
UBP11         (86)  KGGPPVPRKLISQGFYTKSFSVEVYLLCLTLTDSRDESTTIIRLSKQASI
UBP5         (101)  GGGPTLARRVISSGLSQTELAVEVYPLRLQLLLMPKSDHSAIRISKKETI
UBP8          (1)   ------------------------------------------------
UBP24         (1)   ------------------------------------------------
UBP3          (1)   ------------------------------------------------
UBP4          (1)   ------------------------------------------------
UBP12         (14)  NTRKHYSDVFVVGGYKWRILIFPKGNNVDHLSMYLDVSDAASLPYGWSRY
UBP13         (14)  NTRKHYSDVFVVGGYKWRILIFPKGNNVDHLSMYLDVADAANLPYGWSRY
UBP26         (1)   ------------------------------------------------
UBP14         (1)   ----------------------------------CSKCDKTENLWLN
UBP6          (1)   ------------------------------------TVSVKWQKK
UBP7          (1)   -----------------------------------LTVSVKWQKK
UBP1          (1)   ----------------------------------RNAIWLCLECGYY
UBP2          (1)   ----------------------------------AIWLCLECGCY
UBP27         (1)   ------------------------------------------------
UBP22         (1)   ------------------------------------------------
UBP20         (1)   ------------------------------------------------
UBP21         (1)   ------------------------------------------------
UBP23         (1)   ------------------------------------------------
UBP25          (1)  ------------------------------------------------
UBP15         (1)   ------------------------------------------------
UBP16         (1)   ------------------------------------------------
UBP17         (1)   ------------------------------------------------
UBP18         (1)   ------------------------------------------------
UBP19         (1)   ------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    151                                                 200
UBP9       (137)  RELYEKVCAMTGVPQEKAHIWDYFDKRKNGLLDPLSYKSLEESSLHMDQD
UBP10      (137)  RELYEKVCALTGVPQEKAHIWDYFDKRKNGLLDSLSYKSLEESSLHMDQD
UBP11      (136)  GQLYEMVCAGKGVAKEKARIWDYFEKKKSVLLDPSSEQSVEEAGLQFNQD
UBP5       (151)  RELHRRACEIFDLDSEHVRIWDYYGHQKYSLMNDLD-KTLDDANLQMDQD
UBP8         (1)  ------------------------------------------------
UBP24        (1)  ------------------------------------------------
UBP3         (1)  ------------------------------------------------
UBP4         (1)  ------------------------------------------------
UBP12       (64)  AQFSLAVVNQIHTRYTVRKETQHQFNARESDWGFTSFMPLSELYDPSRGY
UBP13       (64)  SQFSLAVVNQVNNRYSIRKETQHQFNARESDWGFTSFMPLSELYEPTRGY
UBP26        (1)  ------------------------------------------------
UBP14       (14)  LTDGMILCGRKNWDGTGGNNHAVEHYKETAYPLAVKLGTITADLEAADVY
UBP6        (10)  VLDGIEIDVSLPPYVFKAQLYDLTGVPPERQKIMVKGGLLKDD---GDWA
UBP7        (11)  VFESIEIDTSQPPFVFKAQLYDLSGVPPERQKIMVKGGLLKDD---ADWS
UBP1        (14)  VCGDVGLPTEAQSHVMGHNRLTRHRLVIQCKNPQLRWCFSCQSLLPFDNE
UBP2        (12)  VCGGVGLPNGPQSHVLRHSRVTRHRLVIQWENPQLRWCFPCQLLLPVEKE
UBP27        (1)  ------------------------------------------------
UBP22        (1)  ------------------------------------------------
UBP20        (1)  ------------------------------------------------
UBP21        (1)  ------------------------------------------------
UBP23        (1)  ------------------------------------------------
UBP25        (1)  ------------------------------------------------
UBP15        (1)  ----------------CARCFGPAKTRCSRCKSVRYCSGKCQIIHWRVA
UBP16        (1)  ----------------CPVCYCLATTRCSRCKAVRYCSGKCQIIHWRQG
UBP17        (1)  ----------------CAVCLYPTTTRCSQCKSVRYCSSKCQILHWRRG
UBP18        (1)  ----------------CSVCGNFSTKKCSRCKSVRYCSAECQRSDWSSG
UBP19        (1)  ----------------CSVCGKATTKKCSRCKSVRYCSAACQTSDWKSG
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    201                                                 250
UBP9       (187)  ILVEVD---------GLSNLGNTCFMNSALQCLAHTPPIVEY--FLQ-DY
UBP10      (187)  ILLEVD---------GLSNLGNTCFMNSALQCLAHTPPIVEY--FLQ-DY
UBP11      (186)  ILLEVDGSA---SSQGLQNLGNTCFMNSTLQCLAHTPPIVEY--FLQ-DY
UBP5       (200)  ILVEVLDINGTLSSAGLLNLGNTCFMNSAIQCLVHTPEFASY--FQE-DY
UBP8         (1)  ---------------GLQNLGNTCFMNSSLQCLAHTPKLVDF--FLG-EY
UBP24        (1)  ----PR---------GLINAGNLCFLNATLQALLSCSPFVQL--LQK-IQ
UBP3         (1)  ---------------GFENFGNTCYCNSVLQALYFCVPFREQ--LLEYYT
UBP4         (1)  ---------------GFENFGNTCYCNSVLQALYFCAPFREQ--LLEHYA
UBP12      (114)  LVNDTVLVEAEVGFVGLKNQGATCYMNSLLQTLYHIPYFRKAVYHMPTTE
UBP13      (114)  LVNDTVLIEAEVGFVGLKNQGATCYMNSLLQTLYHIPYFRKAVYHMPTTE
UBP26        (1)  ---------------GLTNLGATCYANSILQCLYMNTAFREG--VFSVEV
UBP14       (64)  SYPEDDSVLDPLLAEGLVNLGNSCYLAATMQIVFSTHSFISR--YFSHQS
UBP6        (57)  AIGVKDGQKLMMMGTGLVNLGNTCYMNSTVQCLKSVPELKSA--LSNYS-
UBP7        (58)  TLGLKNGQKLMMMGTGLVNLGNTCYMNSTMQCLISVPELKSE--LSNY--
UBP1        (64)  ENG------------GLVNLGNTCFFNSVMQNLLSLDQLREH--FLKEDL
UBP2        (62)  DNGEKKD--------GLVNLGNTCFFNSIMQNLLSLDRLRDH--FLKENG
UBP27        (1)  ---------------GLQNLGNNCFLNVILQALASCKDFRSFLQWVLEDA
UBP22        (1)  ------R--------GLNNLGSTCFMNAVLQALVHAPPLRNF--WLSGQH
UBP20        (1)  -----GA--------GLWNLGNSCFLNSVFQCFTHTVPLIES--LLSFRY
UBP21        (1)  -----GA--------GLYNSGNTCFIASVLQCFTHTVPLIDS--LRSFMY
UBP23        (1)  -----GA--------GLQNLGNTCFLNSVLQCLTYTEPLAAT--LQTAAH
UBP25        (1)  -----PL--------GLRNLGNTCYLNSVLQCLTFTPPLANF--CLTHKH
UBP15       (34)  HK---PR--------GLVNCGNSCYANAVLQSLTCTKPLVAY--LLRRSH
UBP16       (34)  HKDECPC--------GLINVGNSCFANVVFQCLMFTPPLTTY--FLQQFH
UBP17       (34)  HKEECPF--------GLVNLGNSCYANAVLQCLAFTRPLISY--LIRGLH
UBP18       (34)  HQRNCPC--------GLMNCGNSCFANVILQCLSWTRPLVAY--LLEKGH
UBP19       (34)  HKLKCPC--------GLTNCGNSCFANVVLQCLSWTRPLVAY--LLERGH
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                  251                                                 300
UBP9      (225)  SDDINR-----DNPLGMCGELAIAFGDL------------LKKL--WSS-
UBP10     (225)  SDDINR-----DNPLGMCGELAIAFGDL------------LKKL--WSS-
UBP11     (230)  RSDINA-----KNPLGMRGELAIAFGEL------------LRKL--WSS-
UBP5      (247)  HQEINW-----QNPLGMVGELALAFGDL------------LRKL--WAP-
UBP8       (33)  SKEINL-----DNPLGMKGEIALAFGDL------------LRSL--WAP-
UBP24      (35)  LQDI-----------------------------------------------
UBP3       (34)  SNKSVAD----AEEN-----LMTCLADL------------FSQIS--SQK
UBP4       (34)  NNK--AD----AEEN-----LLTCLADL------------FSQIS--SQK
UBP12     (164)  NDAPT------AS-------IPLALQSL------------FYK----LQY
UBP13     (164)  NDAPT------AS-------IPLALQSL------------FYK----LQY
UBP26      (34)  HVLK--------QNP-----VLDQIARL------------FAQLH--ASQ
UBP14     (112)  LKMAFE-----MAPADPTLDLNMQLTKLGHGLLSGKYSMPATQKDATTGD
UBP6      (104)  -----------LAARSNDVDQTSHMLTV------------ATRELFGELD
UBP7      (104)  -----------QSARTKDVDQTSHMLTV------------ATRELFSELD
UBP1      (100)  S--VSG-------------PLVSSLKEL------------FAESNSEASV
UBP2      (102)  SG-VGG-------------PLASSLRKL------------FTETKPEAG-
UBP27      (36)  RGSLAGE--QEE-QL----PLTFALSAL------------LQELGTVGS-
UBP22      (35)  NRDLCPR--RTMGLL----CLPCDLDVI------------FSAM--FSG-
UBP20      (36)  EVPCHCG----NEFF----CVIRAIRYH------------IEAA--LRP-
UBP21      (36)  GNPCNCG----NEKF----CVMQALRDH------------IELA--LRS-
UBP23      (36)  QKYCH------VAGF----CALCAIQKH------------VRTA---RQA
UBP25      (36)  SSHCDTYVDGERKRD----CPFCIVEKR------------IARS---LSV
UBP15      (71)  SRSCS------GKDW----CLMCELEQH------------VMM----LRE
UBP16      (74)  SRACT------KKEQ----CFTCGFEKL------------VVK----AKE
UBP17      (74)  SKTCR------KKSW----CFVCEFEHL------------ILK----ARG
UBP18      (74)  KRECM------RNDW----CFLCEFQTH------------VER----ASQ
UBP19      (74)  KRECR------RNDW----CFLCEFENH------------LDR----ANY
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                  301                                                 350
UBP9      (255) GR-NAVAPRAFKTKLARFAPQFSGYN-----QHDSQELLAFLLDGLHEDL
UBP10     (255) GR-NSVAPRAFKTKLARFAPQFSGYN-----QHDSQELLAFLLDGLHEDL
UBP11     (260) GQ-NTVAPRAFKTKLARFAPQFSGYN-----QHDSQEMLAFLLDGLHEDL
UBP5      (277) GR-TPIAPRPFKAKLARFAPQFSGYN-----QHDSQELLAFLLDGLHEDL
UBP8       (63) GA-STVAPRTFKAKLARFAPQFSGFN-----QHDSQELLAFLLDGLHEDL
UBP24      (39) -------PKADSPTLAAFSEFISELD-----VPSS---------------
UBP3       (61) KKTGVIAPKRFVQRLKKQNELFRSYM-----HQDAHEFLNYLLNEVVD--
UBP4       (59) KKTGVIAPKRFVQRLKKQNELFRSYM-----HQDAHEFLNYLLNELVE--
UBP12     (185) ND-TSVATKELTKSFGWDT--YDSFM-----QHDVQELNRVLCEKLED--
UBP13     (185) ND-TSVATKELTKSFGWDT--YDSFM-----QHDVQELNRVLCEKLED--
UBP26      (57) K--SFVDSDAFVKTLELDN-----GV-----QQDTHEFLTLLLSLLER--
UBP14     (157) PRQEGIPPRMFKNVIAASHAEFSSMR-----QQDALDFFLHLVGKVER--
UBP6      (131) RSVNAVSPSQFWMVLRKKYPQFSQLQNGMHMQQDAEECWTQLLYTLSQ--
UBP7      (131) KSVKAVAPMPFWMVLQKKYPQFAQLHNGNHMQQDAEECWTQMLYTLSQ--
UBP1      (123) FR-NEINPRDLFFSVCSQAPQFRGYQ-----QHDSHELLRCLLDGLSIEE
UBP2      (125) LK-SVINPRAFFGSFCSKAPQFRGYD-----QHDSHELLRCLLDSLSTEE
UBP27      (66) RR-SVSNPRKVMVTLTDYAKNFNLTS-----QQDAAEALLHLISSLQEEI
UBP22      (64) DR-TPYSPAHLLYSWWQHSTNLATYE-----QQDSHEFFISLLDRIHE--
UBP20      (63) ER-CPIAPYFFFDNLNYFSPDFQRYQ-----QEDAHEFLQAFLEKLEI--
UBP21      (63) SG-YGINIDRFRDNLTYFSSDFMINH-----QEDAHEFLQSFLDKLER--
UBP23      (61) NG-RILAPKDLVSNLRCISRNFRNCR-----QEDAHEYMINLLECMHK--
UBP25      (67) DL-TTDAPNKISSCLKIFAEHFKLGR-----QEDAHEFLRYVIDACHN--
UBP15      (95) SG-GPLSASRILSHMRSINCQIGDGS-----QEDAHEFLRLLVASMQS--
UBP16      (98) EK-SPLSPNGLLSQLQNIGIFLGNGK-----EEDAHEFLRFVVDTMQS--
UBP17      (98) GE-SPLSPIKILSKLQKIGKHLGPGK-----EEDAHEFLRCAVDTMQS--
UBP18      (98) SR-FPFSPMNIISRLTNIGGTLGYGR-----QEDAHEFMRYAIDMMQS--
UBP19      (98) SR-FPFSPMNIISRLPNIGGNLGYGR-----QEDAHELMRFAIDMMQS--
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    351                                                 400
UBP9       (299) NKVKRKPYIELKDSDSR---------PDDEVAEELWNYHKARNDS-----
UBP10      (299) NKVKRKPYIELKDSDSR---------PDDEVAEELWNYHKARNDS-----
UBP11      (304) NKVKRKPYIEAKDSDGR---------PDDEVAEEKWKYHKARNDS-----
UBP5       (321) NRVKHKPYINSRDADGR---------PDEEVADEFWKNHIARNDS-----
UBP8       (107) NRVKNKPYVEAKDGDGR---------PDAEVADEYWRNHVARNDS-----
UBP24       (62) -------S-------------------------------SIRNNV-----
UBP3       (104) -------ILEKEAKATKTE---H-ETSSSSSPEKIANGLKVPQANGVVHK
UBP4       (102) -------ILEKETQATKAD---N-ETSS--SPEKIANVLKAPLANG-VHK
UBP12      (225) --------KMKG-------------------------TVVEG-------
UBP13      (225) --------KMKG-------------------------TVVEG-------
UBP26       (93) -------CLLHSGVKAK--------------------------------
UBP14      (200) --------ASNTTPDLD-------------------PSRSFKFGIEEK--
UBP6       (179) --------SLKAPTSSE-------------------GADAVKALFGVN--
UBP7       (179) --------SLKLPSPSE-------------------DPDAVKALFGLN--
UBP1       (167) SSLRKKLGVSDSNDSSTYQKPTLIDSVFGGEISSTVSCLECGHFSK----
UBP2       (169) SALRKKRGVSDNDEKST----TLIESVFGGETSSIVSCMECGHSSK----
UBP27      (110) VVCYRP---SQSSNLSD--------ILF-----S--RNLRMLAPSE----
UBP22      (106) -----------NEGKSK--------CLY-----Q--DNEECQCIT-----
UBP20      (105) ---------CGSDRTSF-----------------RGDITSQD-------
UBP21      (105) ---------CCLDPKNQ-----------------LGSVSSQDLN-----
UBP23      (103) --------CSLPSGVP-------------------SESSDAYRRS-----
UBP25      (109) --------TSLRLKKLR----------------Y-NGNEPFNGNS-----
UBP15      (137) --------ICLERLGGE----------------T-KVDPRLQETT-----
UBP16      (140) --------VCIKASEYD----------------M-TKSSKLEDTT-----
UBP17      (140) --------VFLKEAP-------------------AAGPFAEETT-----
UBP18      (140) --------VCLDEFGGE----------------K-IVPPRSQETT-----
UBP19      (140) --------VCLDEFGGE----------------K-VVPPRAQETT-----
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    401                                                 450
UBP9      (335) ----VIVD--------------------------------------------------
UBP10     (335) ----VIVD--------------------------------------------------
UBP11     (340) ----VIVD--------------------------------------------------
UBP5      (357) ----IIVD--------------------------------------------------
UBP8      (143) ----IIVD--------------------------------------------------
UBP24      (69) ----TVVE--------------------------------------------------
UBP3      (143) EPIVTWVHN-------------------------------------------------
UBP4      (138) EPIVTWVHK-------------------------------------------------
UBP12     (234) -----TIQQ-------------------------------------------------
UBP13     (234) -----TIQK-------------------------------------------------
UBP26     (103) ----TIVQD-------------------------------------------------
UBP14     (221) -----ILCP-------------------------------------------------
UBP6      (200) -----LQS--------------------------------------------------
UBP7      (200) -----LLN--------------------------------------------------
UBP1      (213) -VYEPFMDLSLPVPSMKLPPKKQQILSQAKEVLKNGAVSKDSEVVSAKPA
UBP2      (211) -VYEPFLDLSLPVPFKKSPPKKPQPVSRAKKAKLP-----PKRVP--KNV
UBP27     (138) -GLHGLMEL-------------K----RWHKHLRG---------------
UBP22     (125) ----------------------------HK--------------------
UBP20     (121) ----------------------------------------------------
UBP21     (123) -----IVDN--------------------------------------------
UBP23     (121) -----LVHK--------------------------------------------
UBP25     (129) -----VVKE--------------------------------------------
UBP15     (157) -----LVQH--------------------------------------------
UBP16     (160) -----LIGL--------------------------------------------
UBP17     (157) -----LVGL--------------------------------------------
UBP18     (160) -----LIQY--------------------------------------------
UBP19     (160) -----LIQY--------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    451                                                 500
UBP9      (339) --------VCQG-----------------QYKSTLVCPVCGK----ISI-
UBP10     (339) --------VCQG-----------------QYKSTLVCPACGK----ISI-
UBP11     (344) --------VFQG-----------------QYKSTLVCPDCGK----ISI-
UBP5      (361) --------VCQG-----------------QYKSTLVCPICNK----VSV-
UBP8      (147) --------VCQG-----------------QYKSTLVCPICKK----VSV-
UBP24      (73) --------AGR------------------------------------
UBP3      (152) --------IFQG-----------------ILTNETRCLRCET----VTA-
UBP4      (147) --------IFQG-----------------ILTNETRCLRCET----VTA-
UBP12     (238) --------LFEG-----------------HHMNYIECINVDF----KST-
UBP13     (238) --------LFEG-----------------HHMNYIECINVDY----KST-
UBP26     (108) --------LFSG-----------------SVSHVTTCSKCGRDS-EASS-
UBP14     (225) ---------S-GK---------------VGYNKREDCILSLNIPLHEATN
UBP6      (203) ------------------------------RLHCQESG----EESS-
UBP7      (203) ------------------------------RLHCQESS----EESS-
UBP1      (262) SDHNSTVPLFPSDHKIQSRPETSDNETDLVLLSDVSDTAPSTE---AKG-
UBP2      (253) SKVSKVSKVLPG--MVLSELNSSGKS---MAVTADSDTSCSSL---APL-
UBP27     (155) --------PFDG-----------------ILGSTLMCRTCSSQ---ISL-
UBP22     (127) --------AFSG-----------------LLRSDVTCTTCGS----TST-
UBP20     (121) --------VFSG-----------------RLISGLRCCNCDY----VSE-
UBP21     (127) --------VFGG-----------------GLMSTLCCCNCNS----VSN-
UBP23     (125) --------IFGG-----------------SLRSQVKCEQCSH----CSN-
UBP25     (133) --------IFGG-----------------ALQSQVKCLSCGA----ESN-
UBP15     (161) --------MFGG-----------------RLRSKVKCLRCDH----ESE-
UBP16     (164) --------TFGG-----------------YLRSKIKCMKCQV----KSE-
UBP17     (161) --------TFGG-----------------YLHSKIKCMACLH----KSE-
UBP18     (164) --------IFGG-----------------LLQSQVQCTVCNH----VSD-
UBP19     (164) --------IFGG-----------------LLQSQVQCTACSN----VSD-
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    501                                                 550
UBP9      (359) --TFDPFMYLSVPLPSTLTRSMTITVFYCDGSRLPMPYTVIVPKQGSIRD
UBP10     (359) --TFDPFMYLSVPLPSTLTRSMTVTVFYCDGSHLPMPYTVIVPKNGSIRD
UBP11     (364) --TFDPFMYLSLPLPSSRTRSMTVTVFYGDGSHLPMPYTVTVPKDGSCRD
UBP5      (381) --TFDPFMYLSLPLQFNTTRAITVTVFSCDKTALPSTITVNVSKQGRCRD
UBP8      (167) --MFDPFMYLSLPLPCTSMRTMDLTVMSADGSSLPIPLTVNVPKFGKFED
UBP24      (76) --PFRPAMFEGV------LRNFTPDVLN----------------------
UBP3      (172) --RDETFLDLSLDIE-------------------------------------
UBP4      (167) --RDETFLDLSLDIE-------------------------------------
UBP12     (258) --RKESFYDLQLDVKG-----------------------------------
UBP13     (258) --RKESFYDLQLDVKG-----------------------------------
UBP26     (131) --KMEDFYALELNVK-----------------------------------
UBP14     (250) KDELEAFHKQKAGKGLEEND-----------------------------
UBP6      (215) --ETESVYSLKCHISHEVNH-----------------------------
UBP7      (215) --ETESVFSLKCHISHEVNH-----------------------------
UBP1      (308) --VNQILVGSTETL----MHDNDRTGKTVPDKEDVRATQSNEETSASGIS
UBP2      (294) --DNGPVLETPSVL----TLDNNQASES--------ASQSDTGFDGS-WL
UBP27     (176) --EFQFFHTLPLSP----LLHH----------------------GG---
UBP22     (147) --TYDPFIDISLT-------------------------------------
UBP20     (141) --TYEKSVGLSLEI----ED-----------------------------
UBP21     (147) --TFEPSLGWSLEI----ED-----------------------------
UBP23     (145) --KFDPFLDLSLDI----S------------------------------
UBP25     (153) --KADEIMDISLEI----L------------------------------
UBP15     (181) --RYENIMDLTLEI----YG-----------------------------
UBP16     (184) --LREKMMDLTVEI----DG-----------------------------
UBP17     (181) --RPELMMDLTVEI----DG-----------------------------
UBP18     (184) --QYENMMDLIVEM----HG-----------------------------
UBP19     (184) --QYENMMDLTVEI----HG-----------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    551                                                 600
UBP9      (407) LITALGTAC----------CLAEDE------------------------
UBP10     (407) LITALGTAC----------LLAEDE------------------------
UBP11     (412) LSNALGTAC----------CLDNDE------------------------
UBP5      (429) LIQALTNAC----------SLKQSE------------------------
UBP8      (215) LHKALVTAC----------SLPEEE------------------------
UBP24      (96) ------------------------------------------------
UBP3      (185) ------------------------------------------------
UBP4      (180) ------------------------------------------------
UBP12     (272) ------------------------------------------------
UBP13     (272) ------------------------------------------------
UBP26     (144) ------------------------------------------------
UBP14     (270) ------------------------------------------------
UBP6      (233) ------------------------------------------------
UBP7      (233) ------------------------------------------------
UBP1      (352) AVIDEAQVCGCPDLEQSSSSANQGADEELALMVADSQVLYMPYKDHLFYD
UBP2      (329) DFIGPETSGDETNLDMQEDGIDNVITAEVNQIVPSPNIV----------
UBP27     (194) ------------------------------------------------
UBP22     (158) ------------------------------------------------
UBP20     (155) ------------------------------------------------
UBP21     (161) ------------------------------------------------
UBP23     (158) ------------------------------------------------
UBP25     (166) ------------------------------------------------
UBP15     (195) ------------------------------------------------
UBP16     (198) ------------------------------------------------
UBP17     (195) ------------------------------------------------
UBP18     (198) ------------------------------------------------
UBP19     (198) ------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                601                                                650
UBP9      (422) ----------S------------------------------LLL-AEVYD
UBP10     (422) ----------S------------------------------LLL-AEVYD
UBP11     (427) ----------S------------------------------LLL-AEVYD
UBP5      (444) ----------E------------------------------LKL-AEIRN
UBP8      (230) ----------T------------------------------LLV-TEVYN
UBP24      (96) ------------------------------------------NMSG
UBP3      (185) --------------------------------------------------
UBP4      (180) --------------------------------------------------
UBP12     (272) --------------------------------------------------
UBP13     (272) --------------------------------------------------
UBP26     (144) --------------------------------------------------
UBP14     (270) --------------------------------------------------
UBP6      (233) --------------------------------------------------
UBP7      (233) --------------------------------------------------
UBP1      (402) DYMVAEASSSFVSGDHEPKKDYFDFSSFLDEPEIREGPVFRPLSKSEVYE
UBP2      (368) ------ANSSVSSGDQ----------------TLEGNTERLMQD---YE
UBP27     (194) --------------------------------------------------
UBP22     (158) --------------------------------------------------
UBP20     (155) --------------------------------------------------
UBP21     (161) --------------------------------------------------
UBP23     (158) --------------------------------------------------
UBP25     (166) --------------------------------------------------
UBP15     (195) --------------------------------------------------
UBP16     (198) --------------------------------------------------
UBP17     (195) --------------------------------------------------
UBP18     (198) --------------------------------------------------
UBP19     (198) --------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    651                                                 700
UBP9      (431) HKIFRYFEIPLDSLSAIKDDEHIVAYRLNQIPKGSRKAKLEILHGGQERA
UBP10     (431) HKIFKYFENPLDSLSSIKDDEHIVAYRLNQMPKGSGKAKLEILHGGQKRP
UBP11     (436) HKVFKYYENPRELLNGIKDNEHIVAYRFKQMHKGPGKVKLEILHGEQEK-
UBP5      (453) NFIHRLFEDPLIPLSSIKDDDHLAAYKLSKSSENTTLLRLVLRRRDQKAG
UBP8      (239) NRIIRFLEEPTDSLTLIRDGDKLVVYRLKKDANNS--PLIVYMHQKLEEQ
UBP24     (100) RPR---QEDAQEFLSFIMDQMHDELLKLKE--------------------
UBP3      (185) ----------------------------------------------------
UBP4      (180) ----------------------------------------------------
UBP12     (272) ----------------------------------------------------
UBP13     (272) ----------------------------------------------------
UBP26     (144) ----------------------------------------------------
UBP14     (270) ----------------------------------------------------
UBP6      (233) ----------------------------------------------------
UBP7      (233) ----------------------------------------------------
UBP1      (452) AGFKADCS-DDKTVSAGKGEASSSFISSDHEQNIDYVDFSSFFDEPEISE
UBP2      (392) EIAKAEANLDEKDVQAMQSDEC--------------------------P
UBP27     (194) -------------YNIMSG--C-----------------------------
UBP22     (158) -------------LDSMNG--F-----------------------------
UBP20     (155) ----------------------------------------------------
UBP21     (161) ----------------------------------------------------
UBP23     (158) ----------------------------------------------------
UBP25     (166) ----------------------------------------------------
UBP15     (195) ----------------------------------------------------
UBP16     (198) ----------------------------------------------------
UBP17     (195) ----------------------------------------------------
UBP18     (198) ----------------------------------------------------
UBP19     (198) ----------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    701                                                 750
UBP9      (481) VLDSVRGSDVKLFGTPFVTYVNT-EPLSGTDIDAVISGFLSPLHK-----
UBP10     (481) ILESVRGRDVKLFGTPFVTYVNT-EPLSGADIDAVLSRFLSPLHK-----
UBP11     (485) --SSDRGP--KCFGTPLVTYINK-EPLSGTDIATSISGLLSPLRR-----
UBP5      (503) --ERESTVQLKPCGTPLLSSASCGDALTKGKIHCLVQNMLSPFRREE---
UBP8      (287) FISGKSSPTWKAFGIPLVSRLC--DVENGSDVENLYLKLLSSFKMP----
UBP24     (127) --QS-----------PKVTASK--------------------------
UBP3      (185) ----------------------------------------------
UBP4      (180) ----------------------------------------------
UBP12     (272) ----------------------------------------------
UBP13     (272) ----------------------------------------------
UBP26     (144) ----------------------------------------------
UBP14     (270) ----------------------------------------------
UBP6      (233) ----------------------------------------------
UBP7      (233) ----------------------------------------------
UBP1      (501) RPFFRPLSKSEVSEAGFKADCSDDKTVSAGKGEASSSFVSSDHEQNIDYV
UBP2      (415) -------ATSGISAEFSQASCIG---CDPGIGESSS---SVNP-------
UBP27     (201) ----------------TLEHCL--------------------------
UBP22     (165) ----------------SPADCR--------------------------
UBP20     (155) ----------------------------------------------
UBP21     (161) ----------------------------------------------
UBP23     (158) ----------------------------------------------
UBP25     (166) ----------------------------------------------
UBP15     (195) ----------------------------------------------
UBP16     (198) ----------------------------------------------
UBP17     (195) ----------------------------------------------
UBP18     (198) ----------------------------------------------
UBP19     (198) ----------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    751                                                 800
UBP9      (525) -----VHAPSKIHNGSDNGHLADAT-VDQASGILSSPDTEIDNASD---R
UBP10     (525) -----VHAPSKIHNGSENGHLPDAT-VDEASEILSSPDTEIDDASD---R
UBP11     (525) -----VHMSCVVNSGNENGHVP-----DESSRSILSRDTETEDN-D---R
UBP5      (548) ----SVGKKGNSDSSIPERRSARFNNTEEEDKVGGLKKAKKSNSSDLGAS
UBP8      (331) ----TEFFTENLENPTEEEATDKTD-TDGTTSVEDTNSTDVKETTES-LP
UBP24     (136) --------SSVISSANDDG--------DEWETVGPKNKSAVT--------
UBP3      (185) ---------------------------------------------------
UBP4      (180) ---------------------------------------------------
UBP12     (272) ---------------------------------------------------
UBP13     (272) ---------------------------------------------------
UBP26     (144) ---------------------------------------------------
UBP14     (270) ---------------------------------------------------
UBP6      (233) ---------------------------------------------------
UBP7      (233) ---------------------------------------------------
UBP1      (551) DFSSFFDEPEISERPFFRPLSKSEVSEAGFMAVSGNDKTVRAGKGET---
UBP2      (445) -----WDEEELP-----LVVADSQILYMPYKEISCNDKSVEG-ECEA---
UBP27     (207) ------------------------------------K--KF--------
UBP22     (171) ------------------------------------K--NR--------
UBP20     (155) ---------------------------------------------------
UBP21     (161) ---------------------------------------------------
UBP23     (158) ------------------------------------K-------------
UBP25     (166) ------------------------------------Q-------------
UBP15     (195) ------------------------------------W-------------
UBP16     (198) ------------------------------------D-------------
UBP17     (195) ------------------------------------D-------------
UBP18     (198) ------------------------------------D-------------
UBP19     (198) ------------------------------------D-------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                  801                                                 850
UBP9      (566) ELSFRIFLTDERGLNIKP-LQSESSISPGTVTRVLVEWNEGEHERYDSSY
UBP10     (566) ELSFRIFLTDERGLNFKP-LQSESSISLGIATRVLVEWNEGEHERYDSSY
UBP11     (561) ELSLSLLR-DYYSFNLQP-LESDSVVNPGSVTKVLVKWNEKEHEKYDSSY
UBP5      (594) KLSLQLIDEDNKTINLPDNEAEAMKLPSSATVTIYLDWTPELSGMYDITC
UBP8      (375) DPVLRLYLTDDRGNSIEAEMLKEKPVNKSKRLNVLARWPVKELDVYDTCL
UBP24     (162) -----------RTQSFVP--SELSEIFGGQLKSVVKAKGTKASATVQPYL
UBP3      (185) ----------------------------------------------------
UBP4      (180) ----------------------------------------------------
UBP12     (272) ----------------------------------------------------
UBP13     (272) ----------------------------------------------------
UBP26     (144) ----------------------------------------------------
UBP14     (270) ----------------------------------------------------
UBP6      (233) ----------------------------------------------------
UBP7      (233) ----------------------------------------------------
UBP1      (598) -------FSSFMSGDNE-RNIDYVEFTNRIFDDRGTSERPVFGPPSKAKV
UBP2      (481) -------SSSFVTGDHEPQNSDFVDFGG-LFDEPETTEGPVFGPPSKAEA
UBP27     (210) -----------LNTEKV---ENYFCYRC----------------W----
UBP22     (174) -----------YSGGPS---VN----------------------------
UBP20     (155) ----------------------------------------------------
UBP21     (161) ----------------------------------------------------
UBP23     (159) ----------------------------------------------------
UBP25     (167) ----------------------------------------------------
UBP15     (196) ----------------------------------------------------
UBP16     (199) ----------------------------------------------------
UBP17     (196) ----------------------------------------------------
UBP18     (199) ----------------------------------------------------
UBP19     (199) ----------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    851                                                 900
UBP9       (615) LSDLPEVHKTS--FSAKKTRQESISLFSCLEAFLAEEPLG-PDDMWFCPS
UBP10      (615) LSDLPEVHKTS--FSAKKTRQESISLFSCLEAFLAEEPLG-PDDMWFCPS
UBP11      (609) LNDLPKVHKN---VLAKKTMQEGISLFSCLEAFLAEEPLG-PDDMWYCPG
UBP5       (644) LESLPEVLKYG--PTTKKARSEPLSLYACLEAFLREEPLV-PDEMWFCPQ
UBP8       (425) LSSLPEVSKS-----GTKRPQESVSLFKCLEAFLTEEPLG-PDDMWYCPG
UBP24      (199) LLHL-DIHPD-----GVQGIEDALHLFSAQEDLEGYRASV-TGKTGVVS-
UBP3       (185) --Q---NSS----------------ITSCLKNFSSTETLH-AEDKFFCDK
UBP4       (180) --Q---NSS----------------ITSCLKNFSSTETLH-AEDKFFCDK
UBP12      (272) ------CKD----------------VYASFDKYVEVERLE-GDNKYHAEG
UBP13      (272) ------CKD----------------VYASFDKYVEVERLE-GDNKYHAEG
UBP26      (144) --G---LKS----------------LDASLNDYLSLEQLN-GDNQYFCGS
UBP14      (270) ----MRSSD------EI--VRPRVPLEACLANFASSEPIE----DYYSSA
UBP6       (233) ----LHEG-----------------LKHGLKGELEKT----------SPA
UBP7       (233) ----LHEG-----------------LKHGLKGELEKT----------SPS
UBP1       (640) SEAGFVAVSSDSDPAVLDESDSPVSVDRCLAQFTKHEILS-EDNAWHCEN
UBP2       (523) SGVGFMAFSSESDPEEIDDSDLPVSVERCLGHFTKHEILS-DDNAWNCEN
UBP27      (225) HGAALKYLS-----------------VIGAAETEIEKLR-SCGGEDQCD
UBP22      (182) --AIMPTLS-----------------GCLDFFTRSEKL----GPDQKLN
UBP20      (155) ------VDT----------------LGSALESFTRVEKLD---EQLTCDN
UBP21      (161) ------VNT----------------LWKALESFTCVEKLE---DQLTCDN
UBP23      (159) ------ADS----------------LQRALSRFTAVELLDNGAKVYQCER
UBP25      (167) ------SSS----------------VKESLQKFFQSEILD-GNNKYRCES
UBP15      (196) ------VES----------------LQDALTQFTRPEDLD-GENMYRCSR
UBP16      (199) ------IST----------------LDDALRRFTRTEILD-GENKYRCGS
UBP17      (196) ------IGS----------------LEEALAQFTAYEVLD-GENRYFCGR
UBP18      (199) ------AGS----------------LEECLDQFTAEEWLH-GDNMYKCDR
UBP19      (199) ------AVS----------------LEECLDQFTAKEWLQ-GDNLYKCDR
```

SUPPLEMENTAL FIGURE 1 (continued)

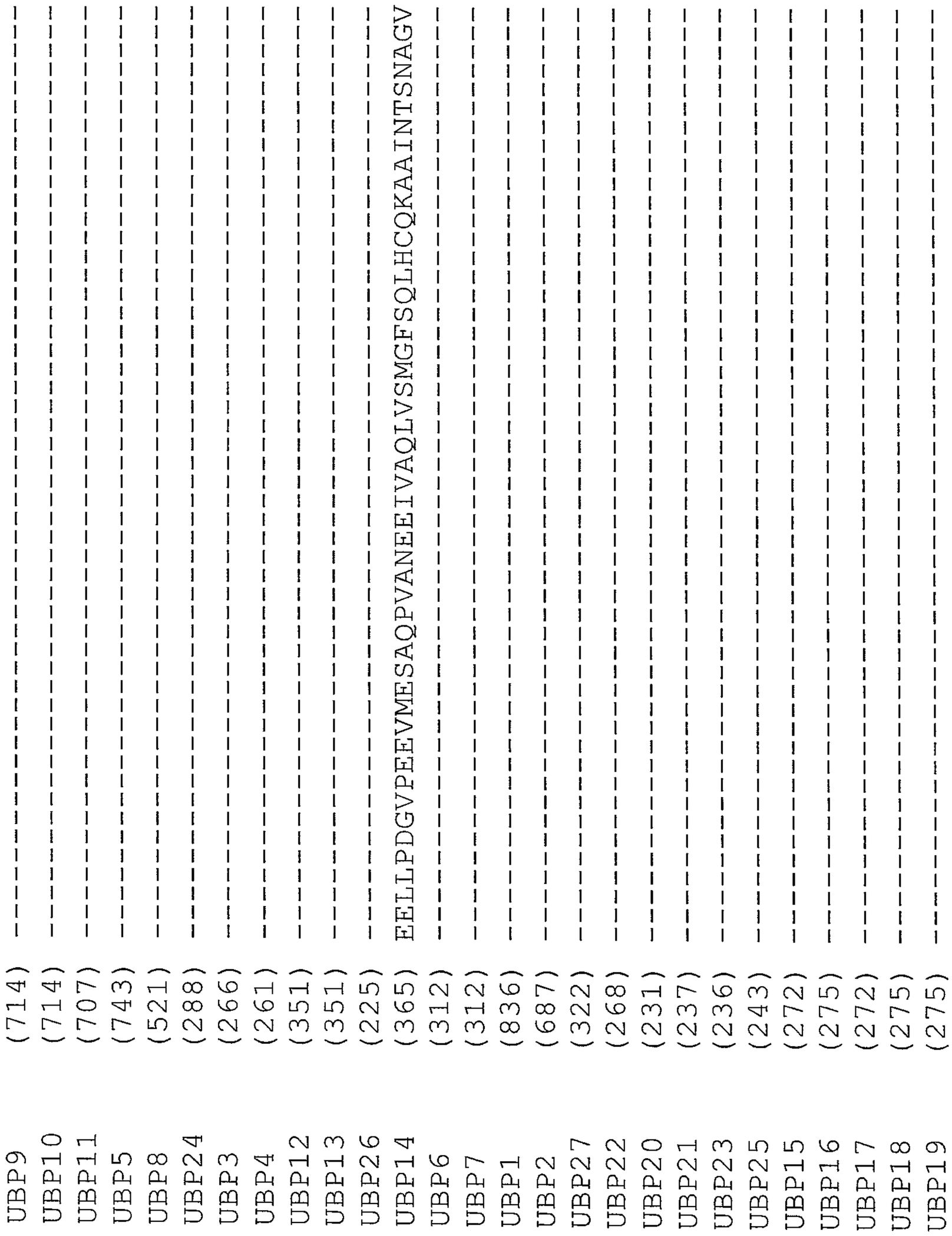
```
                    901                                                 950
UBP9      (662) CKE--------------------------------------------------
UBP10     (662) CKE--------------------------------------------------
UBP11     (655) CKE--------------------------------------------------
UBP5      (691) CNE--------------------------------------------------
UBP8      (469) CKE--------------------------------------------------
UBP24     (241) -----------------------------------------------------
UBP3      (213) CCS--------------------------------------------------
UBP4      (208) CCS--------------------------------------------------
UBP12     (299) -HG--------------------------------------------------
UBP13     (299) -HD--------------------------------------------------
UBP26     (172) CNA--------------------------------------------------
UBP14     (304) LKG--------------------------------------------------
UBP6      (252) LGR--------------------------------------------------
UBP7      (252) LGR--------------------------------------------------
UBP1      (689) CSKNLKLQRLREKRRTKEGLSNRWVNENGASSAFDECRDSSLNQSCIDLE
UBP2      (572) CSKNLKLQRLREKRKSNE----------------DESR-SS------NTS
UBP27     (256) CKTSLHLQRMP---WSNS-----------------------------------
UBP22     (208) CQS------------CGE-----------------------------------
UBP20     (180) CNE--------------------------------------------------
UBP21     (186) CKE--------------------------------------------------
UBP23     (187) CKQ--------------------------------------------------
UBP25     (194) CEK--------------------------------------------------
UBP15     (223) CAG--------------------------------------------------
UBP16     (226) CKS--------------------------------------------------
UBP17     (223) CKS--------------------------------------------------
UBP18     (226) CSD--------------------------------------------------
UBP19     (226) CDD--------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                951                                                  1000
UBP9      (665) ----------------------------HRQANKKLDLWKLPDI--LV
UBP10     (665) ----------------------------HRQANKKLDLWKLPDI--LV
UBP11     (658) ----------------------------HRQANKKLDLWKLPDI--LV
UBP5      (694) ----------------------------RRQASKKLDLWRLPEV--LV
UBP8      (472) ----------------------------HRQAIKKLDLWRLPEI--LV
UBP24     (241) --------------------------------ASKSIKIQKLSKI--MI
UBP3      (216) ----------------------------LQEAQKRMKIKKPPHI--LV
UBP4      (211) ----------------------------LQEAQKRMKIKKPPHI--LV
UBP12     (301) ----------------------------LQDAKKGVLFIDFPPV--LQ
UBP13     (301) ----------------------------LQDAKKGVLFIDFPPV--LQ
UBP26     (175) ----------------------------RVDATRCIKLRTLPPV--IT
UBP14     (307) ----------------------------MTTAIKTTGLTSFPDY--LV
UBP6      (255) ----------------------------TALYVKESLIDSLPRY--LT
UBP7      (255) ----------------------------TAVYVKESLIDSLPRY--LT
UBP1      (739) NGYKAAPPITKLPNCKEEESAIDDGFVGEENTKQAPITSVTETPLLGGET
UBP2      (599) NGWVKE----------NEDEGFGETEILAVKQDPNDTSCVKDHSSDGRKA
UBP27     (271) ----------------------------YSHILKQLIIARFPKL--LC
UBP22     (214) ----------------------------KRESSKQMSIRRLPLL--LC
UBP20     (183) ----------------------------KVSKEKQLLLDKLPLV--AT
UBP21     (189) ----------------------------KVTKEKQLRFDKLPPV--AT
UBP23     (190) ----------------------------KVKAKKQLTVSKAPYV--LT
UBP25     (197) ----------------------------LVTARKQMSILQAPNI--LV
UBP15     (226) ----------------------------YVRARKELSIHEAPNI--LT
UBP16     (229) ----------------------------YERAKKKLKITEPPNV--LT
UBP17     (226) ----------------------------YQKAKKKLMILEGPNI--LT
UBP18     (229) ----------------------------YVKACKRLTIRRAPNI--LT
UBP19     (229) ----------------------------YVKACKRLSIRCAPNI--LT
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                1001                                                1050
UBP9      (683) FHLKRFTYSRYLK---NKIDTFVNFPVH-------DLDLSK---------
UBP10     (683) FHLKRFTYSRYLK---NKIDTFVNFPVH-------DLDLSK---------
UBP11     (676) FHLKRFTYSRYFK---NKIDTLVNFHIH-------DLDLSK---------
UBP5      (712) IHLKRFSYSRSMK---HKLETFVNFPIH-------DLDLTK---------
UBP8      (490) IHLKRFSYSRFMK---NKLEAYVDFPLD-------NLDLSS---------
UBP24     (256) LHLMRFSYGSQGS---TKLRKGVKFPLEL------NLNRSH---------
UBP3      (234) IHLKRFKYIEQLG-RYKKLSYRVVFP--------LELKLSN---------
UBP4      (229) IHLKRFKYMEQLG-RYKKLSYRVVFP--------LELKLSN---------
UBP12     (319) LQLKRFEYDFMRDT-MVKINDRYEFP--------LELDLDR---------
UBP13     (319) LQLKRFEYDFMRDT-MVKINDRYEFP--------LQLDLDR---------
UBP26     (193) FQLKRCIFLPKTT-AKKKITSSFSFP--------QVLDMGS---------
UBP14     (325) LHMRKFVME-EGW-VPKKLDVYIDVP--------DVIDISHMRSKGLQPG
UBP6      (273) VQFVRFFWKRESN-QKAKILRKVDYP--------LVLDIFDLCSEDLR--
UBP7      (273) VQFVRFFWKRESN-QKAKILRKVDYP--------LELDIYDLCSEDLR--
UBP1      (789) ISSQPASDNECENWEDLAVDSEEVI-VKRDARKKVLINKAPPVLTIHL--
UBP2      (639) ARIHSADESESKGTQDEDEDSEKVITVKRDATKKVLINKAPPVLTIHL--
UBP27     (289) IQVQRASFNMFEEF---KLSGHIAF-------P-LVLNLS----LFTP--
UBP22     (232) LHVKRFEHSLTRKTS-RKIDSYLQY-------P-FRLNMSP---YLSS--
UBP20     (201) FHLKRFKNNGLY---MEKIYKHVKIP--------LEIDLQP---------
UBP21     (207) FHLKRFTNDGVT---MEKIFDHIEFP--------LELDLSP---------
UBP23     (208) VHLKRFEAHRSE-----KIDRKVDFT--------SAIDMKP---------
UBP25     (215) IQLKRFGGIFGG-----KIDKAISFG--------EILVLSN---------
UBP15     (244) IVLKRFQEGRYG-----KINKCISFP--------EMLDMIP---------
UBP16     (247) IALKRFQAGKFG-----KLNKLIRFP--------ETLDLAP---------
UBP17     (244) VVLKRFQSDNFG-----KLSKPIHFP--------ELLDISP---------
UBP18     (247) IALKRYQGGRYG-----KLNKRISFP--------ETLDLNP---------
UBP19     (247) IALKRFQGGRFG-----KLNKRISFP--------ETFDLGP---------
```



SUPPLEMENTAL FIGURE 1 (continued)

SUPPLEMENTAL FIGURE 1 (continued)

```
                    1101                                                1150
UBP9      (714) --------------------------------------------------
UBP10     (714) --------------------------------------------------
UBP11     (707) --------------------------------------------------
UBP5      (743) --------------------------------------------------
UBP8      (521) --------------------------------------------------
UBP24     (288) --------------------------------------------------
UBP3      (266) --------------------------------------------------
UBP4      (261) --------------------------------------------------
UBP12     (351) --------------------------------------------------
UBP13     (351) --------------------------------------------------
UBP26     (225) --------------------------------------------------
UBP14     (415) EEAMNWLLSHMDDPDIDAPISHQTSDIDQSSVDTLLSFGFAEDVARKALK
UBP6      (312) ------------KKLEAPR-QKLREEEGK--------------KLGLQ
UBP7      (312) ------------KKLEAPR-QKLRDIEGQ--------------KLGLQ
UBP1      (836) ----------------------------------------K---RFS
UBP2      (687) ----------------------------------------K---RFS
UBP27     (322) ----------------------------------------S---SIG
UBP22     (268) ----------------------------------------S---IIG
UBP20     (231) --------------------------------------------------
UBP21     (237) --------------------------------------------------
UBP23     (236) --------------------------------------------------
UBP25     (243) --------------------------------------------------
UBP15     (272) --------------------------------------------------
UBP16     (275) --------------------------------------------------
UBP17     (272) --------------------------------------------------
UBP18     (275) --------------------------------------------------
UBP19     (275) --------------------------------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                1151                                                1200
UBP9      (714) ----------------------YVKNKNG-QS-Y----------LYEL
UBP10     (714) ----------------------YVKNKND-QS-Y----------LYEL
UBP11     (707) ----------------------YVKNEDG-QS-Y----------LYEL
UBP5      (743) ----------------------YVANKNL-SQPQ----------LYEL
UBP8      (521) ----------------------YISYKNG-QTTY----------RYML
UBP24     (288) ----------------------LVSLSN--ES-L----------RYEL
UBP3      (266) ----------------------TVEPYA--D--V----------EYSL
UBP4      (261) ----------------------TVDEYV--D--I----------EYSL
UBP12     (351) ---ED-----------------GKYLSPDADRSVRN----------LYTL
UBP13     (351) ---ED-----------------GRYLSPDADKSVRN----------LYTL
UBP26     (225) ----------------------RLAESS--QNKL----------TYDL
UBP14     (465) ASGGDIEKATDWVFNNPNASVSDMDVSSSNSAQTPAQSG-LPDGGGKYKL
UBP6      (333) TSAKSGSKDSDVKMTDAEASANGSGESSTVNPQEGTSSEKETHMTGIYDL
UBP7      (333) ASAKSSSKGDDVKMTDAEGSSNQSGESSTGDQQEGASP----HMTGIYDL
UBP1      (840) QDARGRVSKLSGHVDFQEFIDLSKYMDTRCSEEDEP----------VYRL
UBP2      (691) QDLRGRLSKLNGHVAFKEVIDLRQYMDSRCSGEDPP----------VYRL
UBP27     (326) VNIEERIEMSS----------EYQKPEAS-KNHG----------MYRL
UBP22     (272) KRFGNRIFAFDG-----E----GEYDSSSSS-SPSA----------EFEI
UBP20     (231) ----------------------YMRNIQENEVST----------KYHL
UBP21     (237) ----------------------FMSSNHDPEVST----------RYHL
UBP23     (236) ----------------------FVSGPHE-GNL----------KYTL
UBP25     (243) ----------------------FMSKASK-DPQP----------EYKL
UBP15     (272) ----------------------FMTRTGD-VPP----------LYML
UBP16     (275) ----------------------YVSGGSE-KSH----------DYKL
UBP17     (272) ----------------------YMSDPNH-GDHP----------VYSL
UBP18     (275) ----------------------YMSEGGD-GSD----------VYKL
UBP19     (275) ----------------------YMSGGGE-GSD----------VYKL
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                1201                                                1250
UBP9      (728) YAVSNHYGG-LGG---GHYTAYAKLIDDN-----------KWYHFDD--
UBP10     (728) YAVSNHYGG-LGG---GHYTAYAKLIDDN-----------EWYHFDD--
UBP11     (721) YAISNHYGG-LGG---GHYTAYAKLMDET-----------KWYNFDD--
UBP5      (758) YALTNHYGG-MGS---GHYTAHIKLLDDS-----------RWYNFDD--
UBP8      (536) YAISNHYGS-MGG---GHYTAYVHHGGD-----------RWYDFDD--
UBP24     (301) VATITHHGWDPSK---GHYTTDARRKNG-----------QWLRFDD--
UBP3      (278) FAVVVHVGSGPNH---GHYVSLVKSHNH-----------WLFFDD--
UBP4      (273) FAVVVHVGSGPNH---GHYVSLVKSHNH-----------WLFFDD--
UBP12     (371) HSVLVHSGG-VHG---GHYYAFIRPTLS-----------DQWYKFDD--
UBP13     (371) HSVLVHSGG-VHG---GHYYAFIRPTLS-----------DQWYKFDD--
UBP26     (239) SAVLIHKGSAVNS---GHYVAHIKDEKTG-----------LWWEFDD--
UBP14     (514) FGIVSHMGT-SVHC--GHYVAHILKE-G-----------RWVIFND--
UBP6      (383) VAVLTHKGR-SADS--GHYVAWVKQESG-----------KWIQYDDDN
UBP7      (379) VSVLTHKGR-SADS--GHYVAWVKQESG-----------KWVQYDDAN
UBP1      (880) AGLVEHLGA-MSR---GHYVSYIRGGHKERRDSDTKEPNSSIWYHASD--
UBP2      (731) AGLVEHSGT-MRG---GHYVAYVRGGQR-VKETDS---SSTAWYNVSD--
UBP27     (353) VTVVEHFGR-TGS---GHYTVYRSVRVFSQEEEEEDCDEDLSWFSISD--
UBP22     (302) FAVVTHKGM-LES---GHYVTYLRLKGL-----------WYRCDD--
UBP20     (247) YALVEHFGY-SVA--YGHYSSYVRSAPK-----------IWHHFDD--
UBP21     (253) YAFVEHIGI-RAT--FGHYSSYVRSAPE-----------TWHNFDD--
UBP23     (250) YGVLVHYGR-SSH--SGHYACFVRTSSG-----------MWYSLDD--
UBP25     (258) FGIIVHSGF-SPE--SGHYYAYVKDSLG-----------RWYCCND--
UBP15     (286) YAVIVHLDT-LNASFSGHYISYVKDLRG-----------NWYRIDD--
UBP16     (289) YGVIVHLDV-MNAAFSGHYVCYIRNQN-----------KWYKADD--
UBP17     (287) YAVVVHLDA-MSTLFSGHYVCYIKTLDG-----------DWFKIDD--
UBP18     (289) YAVIVHLDM-LNASFFGHYICYIKDFCG-----------NWYRIDD--
UBP19     (289) YAVIVHLDM-LNASFFGHYICYVKDFRG-----------NWYRIDD--
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                    1251                                                1300
UBP9      (760) ------SHVSSVNESEIRNSAA----------------------------
UBP10     (760) ------SHVSSVNESEIKNSAA----------------------------
UBP11     (753) ------SRVSAVNESEIKTSAA----------------------------
UBP5      (790) ------SHISHINEDDVKSGAA----------------------------
UBP8      (567) ------SHVHQISQEKIKTSAA----------------------------
UBP24     (333) ------ASVTPIGTKLVLHDQA----------------------------
UBP3      (309) ------ENVEMIEESAVQTFFGSSQEYSSN--------------------
UBP4      (304) ------ESVEIIEESAVQTFFGSSQEYSSN--------------------
UBP12     (403) ------ERVTKEDLKRALEEQYGGEEELPQTNPGFNNN------------
UBP13     (403) ------ERVTKEDVKRALEEQYGGEEELPQNNPGFNN-------------
UBP26     (272) ------EHVSELGKRPCNEASSSTPQSESNGTASSGNITDGIQSGSSDCR
UBP14     (545) -------DKVGISTDPPKDMG------------------------------
UBP6      (417) PSMQREEDITKLSGGGDWHMA------------------------------
UBP7      (413) TSLQRGEDIIKLSGGGDWHMA------------------------------
UBP1      (924) ------SQVRPASLEEVLRSEA----------------------------
UBP2      (771) ------AYVRQVSLEKVLHSEA----------------------------
UBP27     (397) ------SEVCRVSESDVLGAEA----------------------------
UBP22     (332) ------AWINEVEEEVVRGCEC----------------------------
UBP20     (279) ------SKVTRIDEDMVLSQDS----------------------------
UBP21     (285) ------SKVTRISEERVLSRPA----------------------------
UBP23     (282) ------NRVVQVSEKTVFNQKA----------------------------
UBP25     (290) ------SFVSLSTLQEVLSEKA----------------------------
UBP15     (320) ------SEIHPVPMTQVMSEGA----------------------------
UBP16     (322) ------STVVTSDVERILTKGA----------------------------
UBP17     (321) ------SNVFPVQLETVLLEGA----------------------------
UBP18     (323) ------SEIESVELEDVLSQRA----------------------------
UBP19     (323) ------SEVEKVELEDVLSQRA----------------------------
```

SUPPLEMENTAL FIGURE 1 (continued)

```
                1301                                                1350
UBP9      (776) ----------YVLFYR------------------------------
UBP10     (776) ----------YVLFYR------------------------------
UBP11     (769) ----------YVLFYQ------------------------------
UBP5      (806) ----------YVLFYR------------------------------
UBP8      (583) ----------YVLFYK------------------------------
UBP24     (349) ----------YVLFY-------------------------------
UBP3      (333) ---------TDHGYILFYE---------------------------
UBP4      (328) ---------TDHGYILLYE---------------------------
UBP12     (435) --PPFKFTKYSNAYMLVYIRESD------------------------
UBP13     (434) --PPFKFTKYSNAYMLVYIRESD------------------------
UBP26     (316) SAIKSEVFSSSDAYMLMYSQSLEEQYFWISTDWLRLWADTTLPPALDNTP
UBP14     (559) ----------YVYFFQ------------------------------
UBP6      (438) ----------YITMYK------------------------------
UBP7      (434) ----------YIVMYK------------------------------
UBP1      (940) ----------YILFYE------------------------------
UBP2      (787) ----------YILFYE------------------------------
UBP27     (413) ----------SLLFYE------------------------------
UBP22     (348) ----------YMLFY-------------------------------
UBP20     (295) ----------YILFYA------------------------------
UBP21     (301) ----------YILFYA------------------------------
UBP23     (298) ----------YMLFYV------------------------------
UBP25     (306) ----------YILFFS------------------------------
UBP15     (336) ----------YMLFYM------------------------------
UBP16     (338) ----------YMLFYA------------------------------
UBP17     (337) ----------YMLLYA------------------------------
UBP18     (339) ----------YMLLYS------------------------------
UBP19     (339) ----------YMLLYSSEESQDEKKTDTLNTESNQDGSVESSGVGTN
```

SUPPLEMENTAL FIGURE 1 (continued)

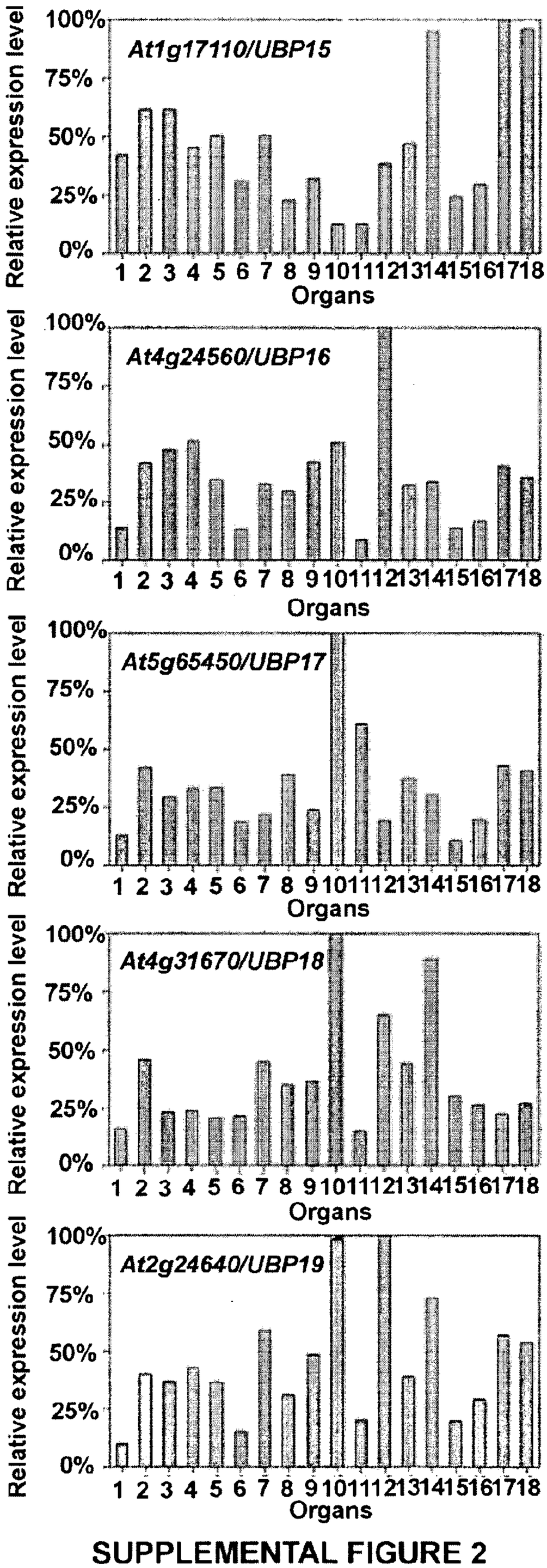
```
                    1351                                                1400
UBP9      (782) --------------------------------------------------
UBP10     (782) --------------------------------------------------
UBP11     (775) --------------------------------------------------
UBP5      (812) --------------------------------------------------
UBP8      (589) --------------------------------------------------
UBP24     (354) --------------------------------------------------
UBP3      (343) --------------------------------------------------
UBP4      (338) --------------------------------------------------
UBP12     (456) --------------------------------------------------
UBP13     (455) --------------------------------------------------
UBP26     (366) LLCSHGKVHASKVNCMKRISELAWIKLESKFNGGPKLGKGDYCRDRTNYG
UBP14     (565) --------------------------------------------------
UBP6      (444) --------------------------------------------------
UBP7      (440) --------------------------------------------------
UBP1      (946) --------------------------------------------------
UBP2      (793) --------------------------------------------------
UBP27     (419) --------------------------------------------------
UBP22     (353) --------------------------------------------------
UBP20     (301) --------------------------------------------------
UBP21     (307) --------------------------------------------------
UBP23     (304) --------------------------------------------------
UBP25     (312) --------------------------------------------------
UBP15     (342) --------------------------------------------------
UBP16     (344) --------------------------------------------------
UBP17     (343) --------------------------------------------------
UBP18     (345) --------------------------------------------------
UBP19     (376) DTSVSSLCNGIISHSEDPEYEKESSLSASVPVSEEGKEVDVKVDTVDSES
```

SUPPLEMENTAL FIGURE 1 (continued)

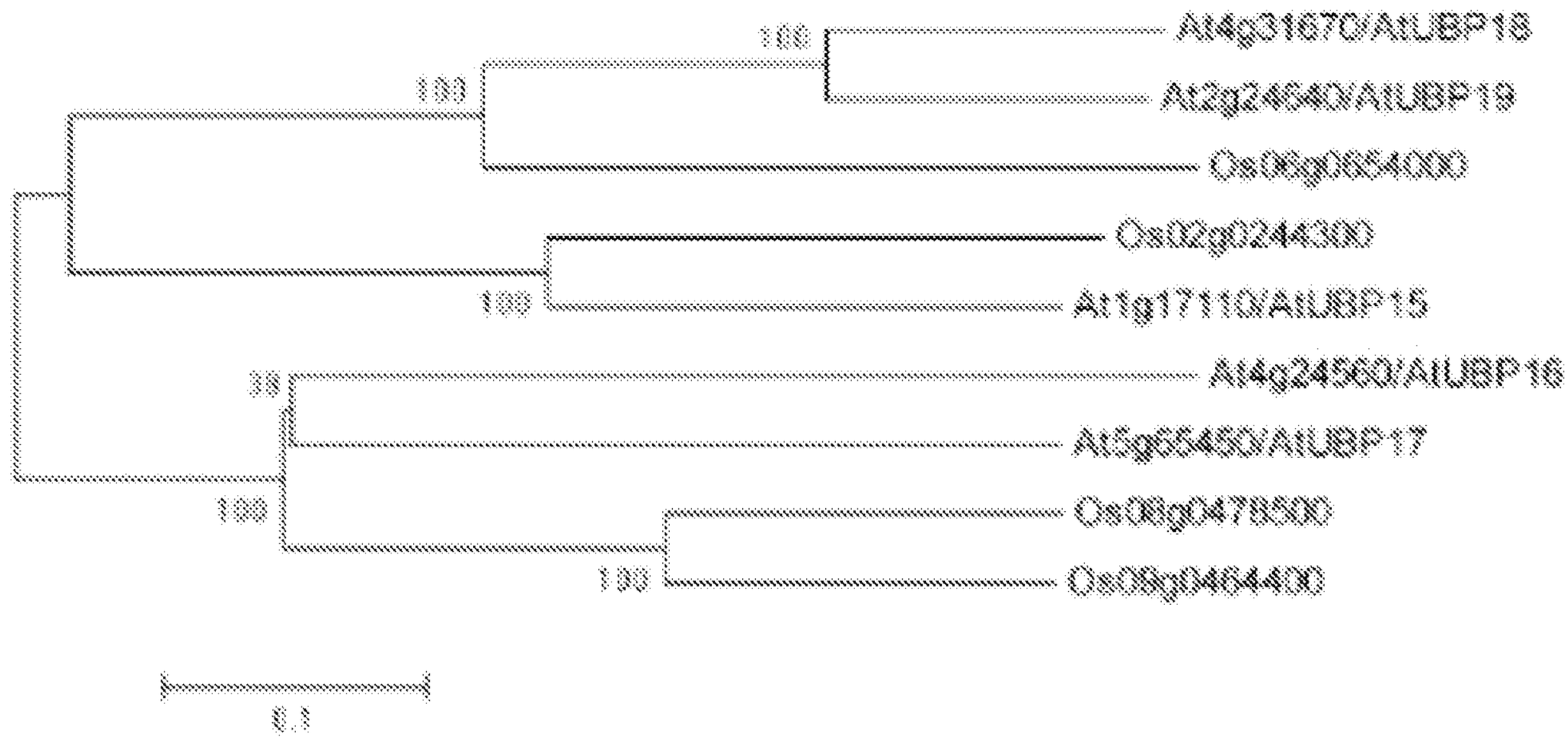
		1401 1450
UBP9	(782)	----------
UBP10	(782)	----------
UBP11	(775)	----------
UBP5	(812)	----------
UBP8	(589)	----------
UBP24	(354)	----------
UBP3	(343)	----------
UBP4	(338)	----------
UBP12	(456)	----------
UBP13	(455)	----------
UBP26	(416)	NLTSLKVSATTTVYQLKMMIWELLGVMKENQELHKGSKVIDQESATLADM
UBP14	(565)	----------
UBP6	(444)	----------
UBP7	(440)	----------
UBP1	(946)	----------
UBP2	(793)	----------
UBP27	(419)	----------
UBP22	(353)	----------
UBP20	(301)	----------
UBP21	(307)	----------
UBP23	(304)	----------
UBP25	(312)	----------
UBP15	(342)	----------
UBP16	(344)	----------
UBP17	(343)	----------
UBP18	(345)	----------
UBP19	(426)	NRSIDMEHDSGTDHQEEEANGKEDPTVENLAVDSSCLDITTPSPSAATEF

SUPPLEMENTAL FIGURE 1 (continued)

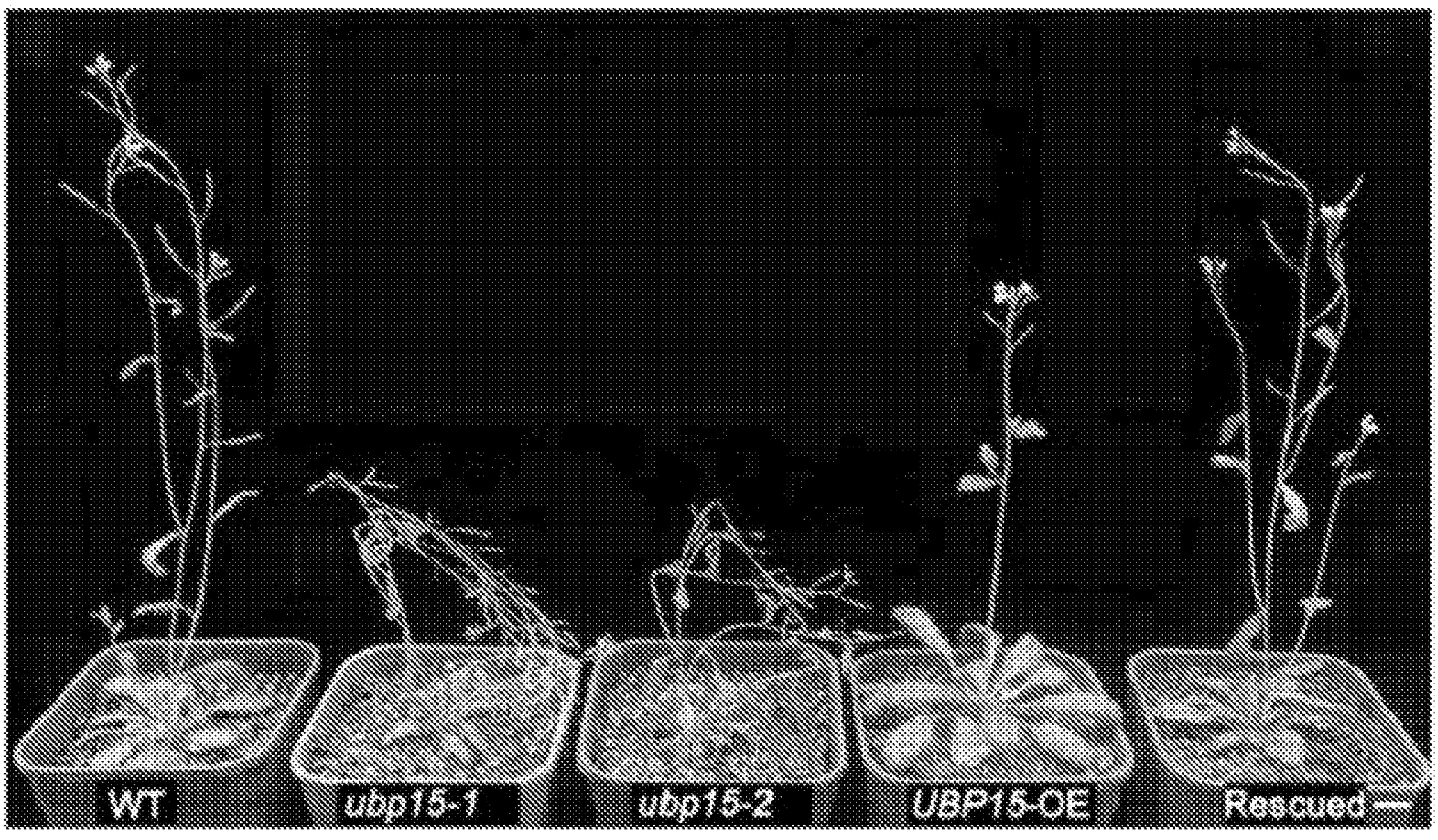
```
                    1451                                                1500
UBP9      (782) --------------------------------------------------
UBP10     (782) --------------------------------------------------
UBP11     (775) --------------------------------------------------
UBP5      (812) --------------------------------------------------
UBP8      (589) --------------------------------------------------
UBP24     (354) --------------------------------------------------
UBP3      (343) --------------------------------------------------
UBP4      (338) --------------------------------------------------
UBP12     (456) --------------------------------------------------
UBP13     (455) --------------------------------------------------
UBP26     (466) NIFPGDRLWVRDT-------------------------------------
UBP14     (565) --------------------------------------------------
UBP6      (444) --------------------------------------------------
UBP7      (440) --------------------------------------------------
UBP1      (946) --------------------------------------------------
UBP2      (793) --------------------------------------------------
UBP27     (419) --------------------------------------------------
UBP22     (353) --------------------------------------------------
UBP20     (301) --------------------------------------------------
UBP21     (307) --------------------------------------------------
UBP23     (304) --------------------------------------------------
UBP25     (312) --------------------------------------------------
UBP15     (342) --------------------------------------------------
UBP16     (344) --------------------------------------------------
UBP17     (343) --------------------------------------------------
UBP18     (345) --------------------------------------------------
UBP19     (476) IPQENERSDTESKPLEKEHSDTESNKPLEKEHLDSESKPLEKEHSDTEMID
```



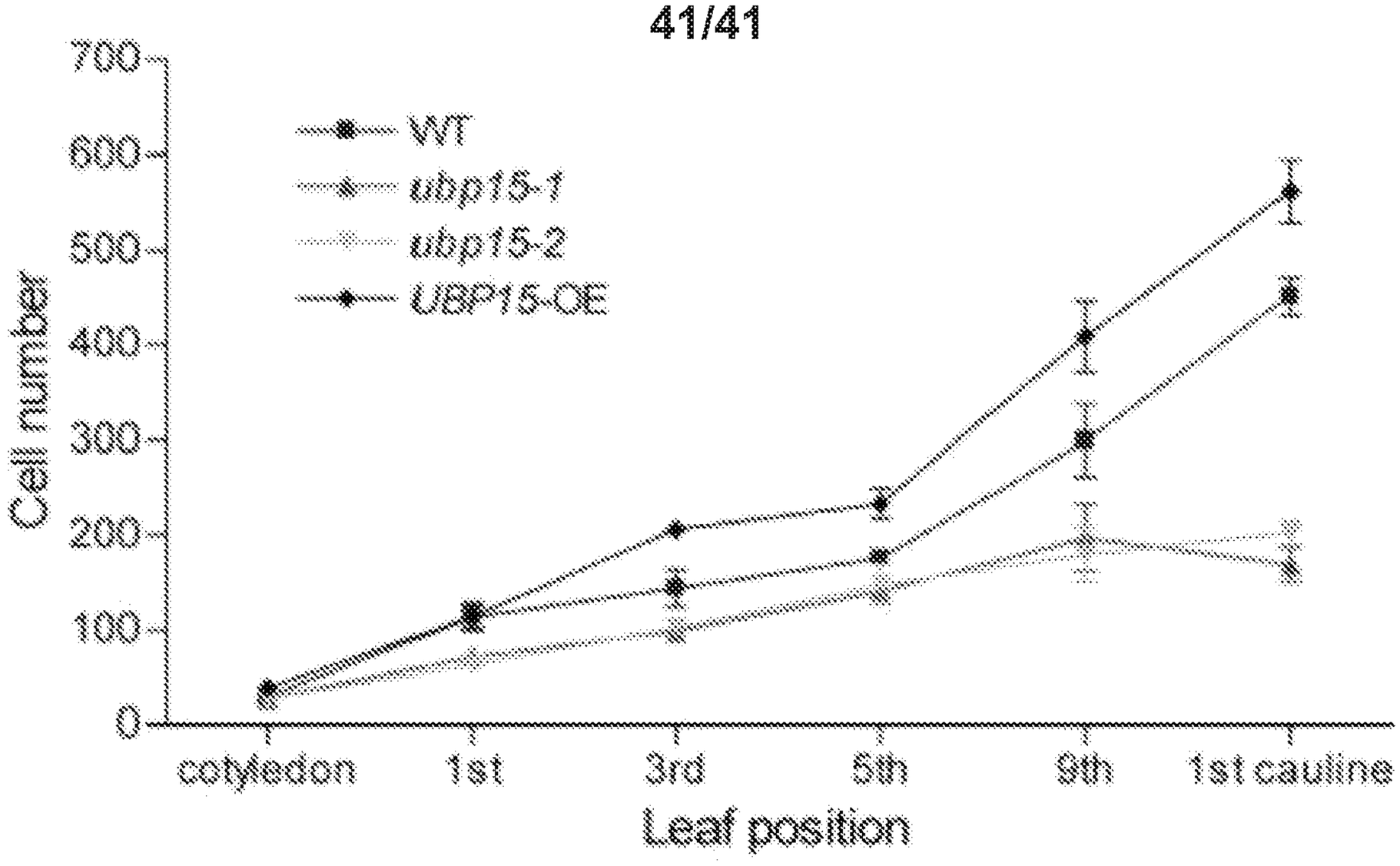
SUPPLEMENTAL FIGURE 2



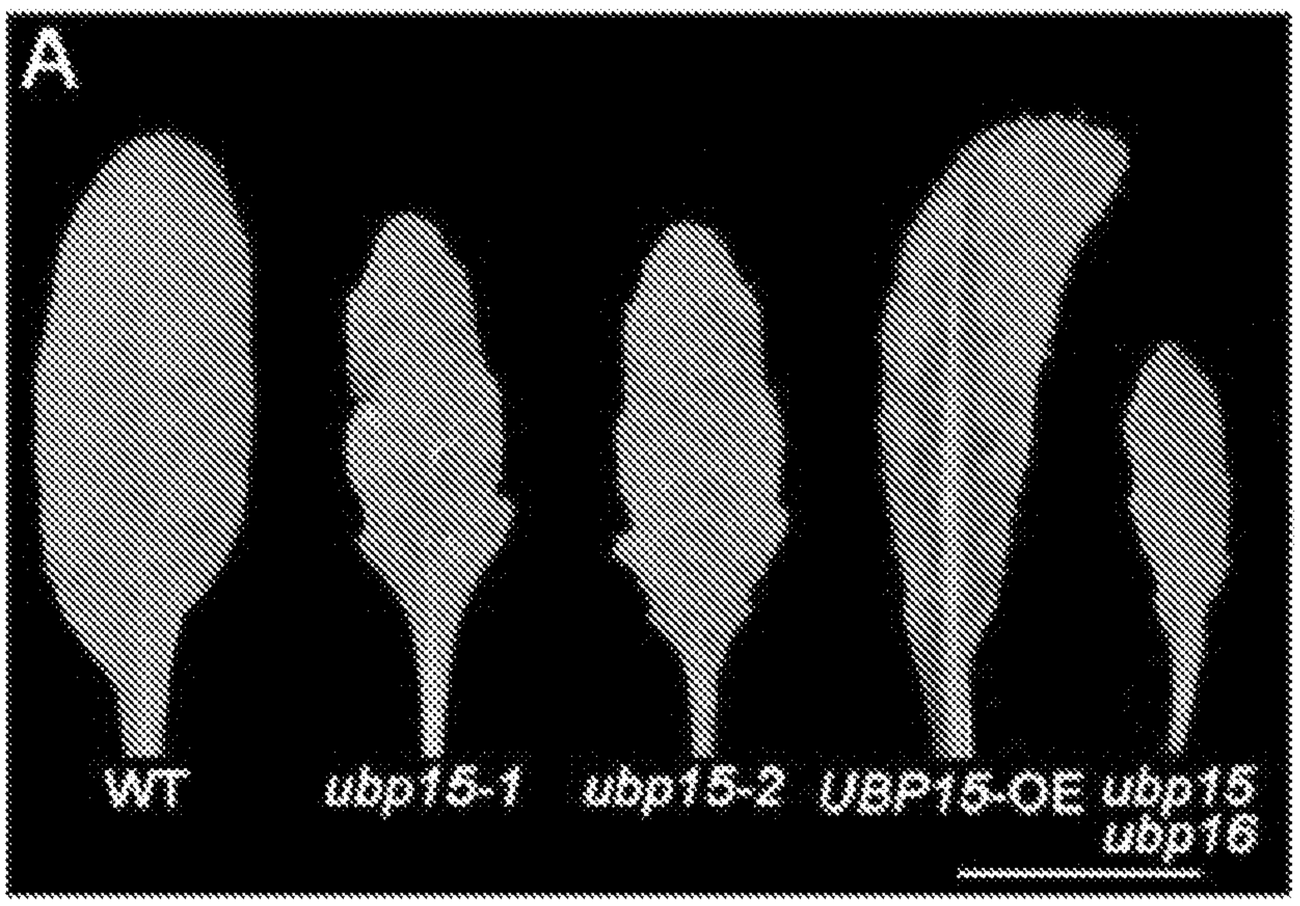
SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE 5



SUPPLEMENTAL FIGURE 6