Inducing Expression of the BAS1 Gene via the SAG12 Promoter to Delay Flower and Plant Senescence in Transgenic Petunia hybrida

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Abstract

Background: Brassinosteroids (BRs) are essential hormones that play crucial roles in plant growth, reproduction and response to abiotic and biotic stress. Results: In transgenic Petunia hybrida , resulting in short stature, dark green leaves, and slowed aging.We demonstrate that the exogenous expression of the SAG12-BAS1 gene results in delayed senescence of flowers. SAG12-BAS1 transgenic lines, grown in the vegetative state, exhibited a range of phenotypic changes, including dark green leaves, short stature, delayed senescence, increased flower bud counts, branching, reduced internode lengths, and delayed flowering. SAG12-BAS1 transgenic expression increased the activity of protective enzymes, reduced malondialdehyde content, and increased chlorophyll content and soluble sugar accumulation in plants. Expression of senescence genes was increased in the transgenic Petunia hybrida compared to wild-type plants. Conclusions: Our finding suggests that BAS1 could be used as a potential candidate gene regulate plant flower senescence and prolong flower longevity.

Background

The *BAS1* gene encodes a cytochrome P450 monooxygenase with hydroxylase activity, which catalyzes the hydroxyl hydroxylation of brassinosteroid (BR) C–26, leading to the reduction or loss of BR physiological activity [1,2]. As adult plants, BR mutants exhibit essentially the opposite phenotype of mutants lacking the red light receptor, phyB. The BR mutants are dark green, slow-growing, and have blade shrinkage and short stems. In addition, *BAS1* mutants exhibit delayed senescence[3]. Overexpression of the transgenic *BAS1* gene can significantly reduce the BR content in plants, resulting in shrinking leaves, slowed leaf aging, and short stems[4]. The leaf senescence induced by BAS1 overexpression is very similar to the phenotype resulting from overexpression of cytokinin in tobacco plants. To observe cytokinin-induced phenotypic changes, scientists used heat shock[5,6,7], hypothermia induction, copper induction, wounding[8], and light induction[9]to drive cytokinin expression.

Isopentenyltransferase (ipt) catalyzes the rate-limiting step for cytokinin synthesis. Gan and Amasino[10] demonstrated that a specific developmental response could be elicited through precise control of ipt expression. In the Gan study, the SAG12 promoter activated ipt expression only at the onset of senescence. This activation of ipt expression resulted in inhibition of the senescence process. Inhibition of leaf senescence by ipt expression led to attenuation of the senescence-specific promoter, thus preventing cytokinin overproduction that would interfere with other aspects of development. In *SAG12-ipt* tobacco, leaf senescence was effectively controlled without other developmental abnormalities[10]. Subsequently, this strategy was successfully used in rice[11], cauliflower[12], *Petunia hybrida*[13], lettuce[14], and *Brassica chinensis*[15]. For example, leaf senescence was retarded in mature 60 day old lettuce plants that exhibited normal morphology with no significant differences in head diameter or the fresh weight of leaves and roots.

Using the senescence-specific SAG12 promoter to drive *BAS1* expression in tobacco, Yao [16] found that *SAG12-BAS1* transgenic and wild-type plant growth were similar. However, transgenic tobacco leaves exhibited delayed aging, manifested mainly as dark green plant leaves, increased chlorophyll content, increased protective enzymatic activity, and increased cytokinin content. The *SAG12-BAS1* gene experiments laid the foundation for the study of flower senescence.

Flower senescence represents the last stage of floral development and results in wilting or abscission of whole flowers or flower parts[17]. The length of the flowering period is related to many factors, including temperature, nutrition, and other external environmental factors. However, these strategies are external to the plants and are time-consuming and laborious. Genetic engineering can rapidly incorporate stable new genetic material resulting in prolonged flowering. Leaves from cytokinin overproducing *BAS1* transgenic plants display prolonged chlorophyll retention and delayed senescence[16]. In the present study, a plasmid, containing the *SAG12-BAS1* gene was inserted into *Agrobacterium* strains, and, subsequently, was used to transform *Petunia hybrida* leaf discs, resulting in delayed senescence. These transgenic plants were used to develop senescent *Petunia hybrida* for horticultural purposes.

Methods

Plant materials

All *Petunia hybrida* plants (Shanghai 'Ivyu' Gardening Co., Ltd.)were grown in a greenhouse at the Institute of Agro-Bioengineering, Guizhou University, Guiyang (China), The PCR primers were synthesized by Shanghai Yingjun Biotechnology Co., Ltd. Other chemical reagents used are domestic or imported analytically pure.

Plasmids constructions

All DNA manipulations were performed essentially as described by Green and Sambrook[18]. Binary plasmid pSH737 contained a replication origin of wide-range host, one kanamycin resistance gene, *NPTII* (neomycin phosphotransferase I, from Tn903) for bacterial selection, and one chimeric gene bearing GUS reporter gene (from *Escherichia coli)* with an neomycin phosphotransferase II (*NPTII,* from Tn5) selectable gene for plant expression and selection respectively (Supplementary Figure 1). The chimeric gene GUS was driven by the cauliflower mosaic virus 35S promoter (CaMV 35S). Plasmid pSH–737 was provided by the Institute of Agro-Bioengineering (Guizhou University). Plasmid pSH737-SAG12-BAS1 was transferred into *Agrobacterium tumefaciens strain* LBA4404[19]. Colonies resistant to kanamycin were selected to ensure the presence of all plasmids by enzyme digestion and PCR amplification.

Plants transformation and transgenic plant detection

Petunia hybrida leaves were prepared for transformation using a leaf disk transformation procedure[20]. For co-cultivation, *Agrobacterium* containing pSH737-SAG12-BAS1 was suspended in MS liquid medium, and the OD_{600} was adjusted to 0.6, the *Petunia hybrida* leaves were dipped into the *Agrobacterium* solution for 8 min and dried on sterilized filter paper before they were placed on the co-cultivation medium (MS+1.0 mg L^{-1} ZT) for 2 d at (25±2)°C in dark. Then, the leaves were transferred onto the selective regeneration medium (MS + 2.0 mg L^{-1} ZT+100 mg L^{-1} Timentin +100 mg L^{-1} Kanamycin). Regenerated shoots were transferred to fresh medium biweekly. When the shoots were 3–5 cm, they were separated from the calli and transferred onto rooting medium (1/2 MS +100 mg L^{-1} Timentin +100 mg L^{-1} Kanamycin), rooted shoots were transplanted into pots. according to previously described methods[21]Extraction of *Petunia hybrida* DNA and GUS histochemical staining of *Petunia hybrida* plants was conducted, and the presence of the *BAS1* gene was confirmed with PCR. Positive plants were moved to a soilless growing medium and acclimated to greenhouse conditions. Transgenic plants were grown until WT plants displayed senescence of the lower leaves. Transgenic plants were subsequently monitored for delayed leaf and flower senescence.

Plant growth conditions and morphological analysis of the transgenic phenotype

*Petunia hybrida*s were used for this experiment because this is an ornamental plant with distinct vegetative and generative growth stages that are controlled by the photoperiod. Transgenic *BAS1 Petunia hybrida* lines and WT cultivars were transplanted to 10 cm pots and maintained in a growth chamber at 25 °C during the day (16 h) and 20 °C at night. Plants were allowed to acclimate to the growth chamber conditions for two weeks and then exposed to either vegetative growth conditions or generative growth conditions. Ten plants from each WT and transgenic line were allowed to progress to flower senescence to determine flower longevity. Plant development and morphological changes were observed and recorded.

Flower Senescence Evaluations and Senescence of excised leaves and flowers

Flower senescence was visually rated during natural senescence and following pollination[22]. One day before anthesis (flower opening), flower corollas were slit with a sharp razor blade, and anthers were removed to prevent self-pollination. On the day of anthesis, five flowers from different transgenic plants were either self-pollinated or left unpollinated. At the same time every two days, flowers were evaluated

for corolla wilting, the first visual symptom of senescence. Corolla wilting is represented as the mean time until flower wilting for three replicates. To determine senescence characteristics of transgenic *Petunia hybrida* lines, excised leaves from transgenic lines and wild-type plants (WT) were surface sterilized with 0.6 % sodium hypochlorite for 60 s, rinsed five times with sterile water, and then placed on moist filter paper in a 10 cm petri dish. Each Petri dish contained two excised leaves, one from each transgenic line and one from each non-transformed WT line. The plates were placed in the continuous dark at 25 °C and checked daily over a 7 d period for evidence of leaf senescence. The unopened flowers were exposed to Hollan's solution in a 1.5 ml centrifuge tube.

Measurement of SOD, CAT, and POD activities and MDA, chlorophyll, and soluble sugar content

Superoxide Dismutase (SOD), Peroxidase (POD), and Catalase (CAT) activities were spectrophotometrically measured (721 Visible Spectrophotometer). Extraction of SOD, POD, and CAT was performed using 0.5–1.0 g of leaf tissue (fresh weight, 5th branch at the base end, 5th leaf piece same site, Fully open during sampling) ground in phosphate buffer and homogenized in 5 ml of extraction buffer containing 0.05 M phosphate buffer (pH 7.8) and 1 % polyvinyl pyrrolidone. The homogenate was centrifuged at 10,000 g for 10 min at 4 °C. The resulting supernatant was collected for analysis of enzyme activity following the manufacturer's instruction (Suzhou Comin Biotechnology Co., Ltd).

Maleic Dialdehyde (MDA) content was determined using the thiobarbituric acid (TBA)-based colorimetric method, as described byHeath and Packer[23]. Chlorophyll concentration was assayed prior to the start of dark conditions and after a significant loss of chlorophyll was detected in the WT tissue. Each transgenic and WT line was tested in triplicate and the experiment was repeated three times. Specific chlorophyll concentration was determined using WT and transgenic leaves obtained from each treatment plate of the previously described chlorophyll concentration study. Leaves were blotted dry and 100 mg of tissue from each sample was placed in a 1.5 ml microcentrifuge tube. The samples were re-suspended in 80 % acetone, ground with a disposable pestle, and incubated in darkness for 30 min. Total chlorophyll (g L^{-1}) was determined using absorbance at 645 and 663 nm according to the equation: 20.2 A645 + 8.02 A663[24].

Determination of soluble sugar content was conducted as described [25]. Fresh plant leaves (0.5 g) were placed in 80 % alcohol (4 ml) and carefully ground into a homogenate. The homogenate was transferred into a centrifuge tube and incubated at 80 °C. The homogenate was stirred for 30 min and then centrifuged for 10 min (6000 g). The supernatant was transferred to a 10 ml graduated test tube with 2 ml of 80 % alcohol. Activated carbon (0.5 g) was added to the supernatant and decolorized in a water bath at 80 °C for 30 min. The volume was adjusted to 10 ml and the sample was filtered (diluted 10-fold or 20-fold). The sugar extract (1 ml) was transferred to a clean tube, 5 ml of anthraquinone reagent was added, and the mixture was boiled for 10 min. After cooling, absorbance was measured at a wavelength

of 625 nm. The sugar percentage in each sample was calculated based on a standard curve. The standard curve was generated as follows. A standard glucose solution was serially diluted to final concentrations of 0, 5, 10, 20, 40, 60, and 80(g /L^{-3}). Absorbance was measured as described above, and then linear regression was used to calculate the sugar content in each sample. The sugar percentage was calculated using the following equation:

$$\text{soluble sugar content (\%)} = (C \times V) / (W \times 10^{-6}) \times 100\ \%$$

where V is the volume of the diluted plant sample in mL, C is the sugar content of the extract (g /L^{-3}), and W is the fresh weight of the plant tissue (g).

Analysis of related gene expression in leaves of *Petunia hybrida*

Total RNA was isolated from *Petunia hybrida* samples (Fully open during sampling*Petunia hybrida* by grinding previously frozen tissue in a mortar with RNAiso reagent (TaKaRa, Tokyo, Japan). The cDNA was synthesized according to standard procedures. Quantitative RT-PCR was carried out using an ABI 7500 Real-Time PCR system (Applied Biosystems, USA). Detection of qRT-PCR products was performed via staining with a QuantiNova SYBR Green PCR kit (Cat. 208054; Qiagen, Germany). Relative levels of transcripts were determined by normalizing expression against *Actin* transcript levels. Experiments were performed in at least three times. The primer sequences are shown in (Supplementary Table S2).

Statistical analysis

The study was performed independently three times and each result shown in the figures was expressed as the mean ± standard deviation (SD). Excel 2017 software (Microsoft, Redmond, USA) and SPSS Statistics 22.0 software (SPSS Inc., Chicago, IL, USA) were used for statistical analyses. Samples were compared using one-way ANOVA followed by Duncan's multiple range posthoc test. Samples were considered significantly different at $P<0.05$.

Results

Agrobacterium-mediated transformation of transgenic *Petunia hybrida*

GUS histochemical staining of wild-type and transgenic *Petunia hybrida* plant leaves at the 3–5 leaf stage showed that GUS activity was not detected in wild-type *Petunia hybrida* plants, whereas GUS activity was detected in Kan resistant *Petunia hybrida* plants (Fig. 1a). Target bands were not amplified in

wild-type *Petunia hybrida* plants, but the expected 419 nucleotide bands were observed in transgenic plants (Fig. 1b). This demonstrated that the exogenous *BAS1* gene had been successfully integrated into the genomes of Kan resistant *Petunia hybrida* plants, and 29 strains of *SAG12-BAS1* gene *Petunia hybrida* were obtained.

Morphological analysis of transgenic *Petunia hybrida* plants expressing *PSAG12-BAS1*

Wild-type and transgenic *Petunia hybrida* were grown under the same conditions, and three phenotypes of transgenic *Petunia hybrida* were observed (Fig.2a). The transgenic plant, Phenotype 1, and the wild-type phenotypes were similar(Experiment requires phenotype 1 follow-up experiment, Such as TP12,TP28,TP38, etc.), and the expression of the senescence promoter was normal. The *SAG12-BAS1* transgenic plants (Phenotype 2and Phenotype 3) exhibited shorter stature, slower growth, darker green leaves, and delayed aging compared to wild-type (WT) plants. Phenotype 3exhibited the darkest green leaves. The promoters in Phenotype 2and Phenotype 3 may not have been strictly controlled, similar to ipt overexpression phenotypes in transgenic plants (Fig.2a) [4]. Analysis of the expression of *BAS1* gene in three transgenic plants is shown in Figure 2b. Expression of the *BAS1* gene was similar in the Phenotype 1 and Phenotype 2 plants, while *BAS1* gene expression was higher in Phenotype 3 (Fig.2b).

Leaf and flower senescence in *SAG12-BAS1* transgenic *Petunia hybrida*

Leaf senescence in *SAG12-BAS1* transgenic *Petunia hybrida* demonstrated slight leaf yellowing at 5 d, whereas large parts of WT *Petunia hybrida* leaves were yellow at 5 d (Supplementary Figure S2). Similarly, the duration of flowering for *SAG12-BAS1* transgenic *Petunia hybrida* was 12 d, whereas WT *Petunia hybrida* only flowered for 5 d (Fig. 3). After 70 d of growth, plants with the transgenic *SAG12-BAS1* gene were completely covered with dark green leaves, whereas WT *Petunia hybrida* had yellow leaves and no new leaf growth (Fig.3b). After 105 d, WT *Petunia hybrida* plants had pronounced yellowing of the central part of the leaf, whereas much less yellowing was present in transgenic plants (Fig. 3b). 120 day after transplantation, WT *Petunia hybrida* were almost dead with a few yellow leaves, whereas transgenic *Petunia hybrida* had normal growth and development. Thus, senescence in transgenic plants was delayed by more than 10–15 d compared to WT plants. Transgenic *Petunia hybrida* had more branches, shorter stem length, and more flowers compared to WT *Petunia hybrida* (Supplementary Table 1 and Supplementary Figure S3 and S4).

Changes in SOD, CAT, and POD activities and MDA, chlorophyll, and soluble sugar content in transgenic *Petunia hybrida* lines

Chlorophyll and soluble sugar content in transgenic *Petunia hybrida* were average higher than wild-type by 42.89 % and 116.8 %, respectively (Fig.4) ($P < 0.05$).

Transgenic *Petunia hybrida* SOD, POD, and CAT activities were average greater than WT *Petunia hybrida* by 82.74 %, 131.8 %, and 135 %, respectively. The MDA content of transgenic *Petunia hybrida* was lower compared to WT *Petunia hybrida* by 46% (Fig. 5). Thus, activities of protective enzymes were enhanced in the plant, including enhanced reactive oxygen scavenging and prevention of membrane lipid peroxidation, and MDA accumulation was reduced. Expression of the *SAG12-BAS1* gene enhanced the antioxidant capacity of *Petunia hybrida* plants, thereby reducing the degree to which plant membranes were damaged while improving the stress tolerance of plants.

Changes in *BAS1* gene expression in transgenic lines and related gene expression in plant flowers

Gene expression analysis showed that expression of the PhARR2 gene that regulates cytokinin and the average expression of *Petunia hybrida hybrida* cytokinin receptor histidine protein kinase (PhHK) were significantly increased by 2.35 times and 2.41 times, respectively, in transgenic plants compared to WT plants. Expression of *Petunia hybrida* PhGA2ox1 and PhGA2ox3 were significantly higher (by 11 times and 3 times, respectively) in transgenic plants compared to WT plants *($P < 0.01$)*.. The auxin response factor (PHARF4) gene expression level in transgenic plants decreased by 0.74 times compared to WT plants. The expression of ERF, a gene involved in regulating ethylene synthesis, decreased by 0.5 times in transgenic *Petunia hybrida* compared to WT plants. The homeodomain leucine zipper transcription factor in *Petunia hybrida* (PhHD-Zip) decreased by 0.25 times in senescing transgenic *Petunia hybrida* compared to WT plants (Fig. 6).

Discussion

Our results show transgenic and WT *Petunia hybrida* plant morphology, physiology, biochemical metabolism, and the regulation of genes involved in senescence. *BAS1,* part of C–26-hydroxylase, is an important brassinosteroid inactivating gene[26–29], which affects the content of active brassinosteroids in plants. The overexpression of plant brassinosteroids affects plant phenotype, but the mechanism of *BAS1* action on plant senescence is not completely understood.

Our experimental results show that the *BAS1* gene of *Petunia hybrida* can affect senescence signals and activity of the protective enzymes SOD, POD, and CAT in leaves. SOD, POD, and CAT activities in

transgenic *Petunia hybrida* leaves increased by 82.74 %, 131.80 %, and 135 %, respectively, compared to WT *Petunia hybrida* . During plant senescence, the expression ofthe *SAG12-BAS1* gene improved the activity of protective enzymes and enhanced oxygen scavenging activity in the plant. In contrast, MDA content was 46 % lower in transgenic *Petunia hybrida* compared to WT, which suggests that the cell membranes of transgenic *Petunia hybrida* were not damaged. In addition, the chlorophyll and soluble sugar content in transgenic *Petunia hybrida* leaves were higher than in WT plants.

Overexpression of Type A members of the Arabidopsis response regulator (ARR) gene family, including ARR4, ARR5, ARR6, ARR7, can inhibit ARR6 transcription, and stable overexpression of ARR8 in transgenic plants inhibits the cytokinin response, suggesting that type A ARR can negatively regulate the cytokinin pathway[30]. Additional research indicates that multiple mutants of A-ARR are suppressed in response to exogenous cytokinins[31]. ARR15 is highly cytotoxic in response to cytokinin because His-Asp phosphorylation inhibits negative feedback regulation[32]. In the present study, expression of PHARR4 in transgenic plants was increased to 2.35 times that of WT plants, indicating that ARR4 appears to positively regulate cytokinin levels.

The cytokinin receptor histidine kinases, AHK2, AHK3, and CREI/AHK4/WOODEN LEG (WOL), bind to cytokinins and autophosphorylate[33]. These receptor histidine kinases then transfer the phosphate group from a histidine residue, which is conserved in the kinase domain, to an aspartate residue, which is conserved in the signal receiving region. Phosphoric acid groups are transferred to cytoplasmic Arabidopsis histidine-phosphotransfer proteins (AHPs) and these AHPs subsequently enter the nucleus and transfer the phosphate groups to a series of ARR. This regulates the downstream cytokinin response and results in a series of biochemical effects that regulate plant growth and development[34]. The experimental results show that the average expression of PHAHK is 2.41 times higher in the transgenic *Petunia hybrida* compared to the wild-type.

Gibberellins (GA) affect growth and developmental processes in plants, and gibberellin 2-oxidase (GA2ox) is an enzyme involved in the degradation of GA. Thus, plant overexpression of GA2ox results in reduced GA, and the associated phenotypic changes include dwarf phenotypes, pollen tube reduction, increased flowering time, and seed sterility in wheat, rice, tobacco, Paspalum notatum, and Solanum. Expression of GA2ox can reduce the height of plants and prolong flowering time[35]. The reduction of endogenous GA3 concentration by GA2ox expression can not only cause plant dwarfing, but also change the time of seed dormancy and chlorophyll concentration. The results show that the average expression of PHGA2ox1 and PHGA2ox3 increased by 11 and 3 times, respectively, in transgenic *Petunia hybrida* compared to wild-type.

Auxin plays an important role in the growth and development of plants. Auxin signal transduction models demonstrate that under low concentrations of auxin, Aux/IAA inhibitors bind to the ARF transcription factor and inhibit the activity of ARF. When the concentration of auxin increases, the combination of the auxin receptor and auxin enhances the binding capacity of Aux/IAA. This results in ubiquitination of Aux/IAA, and degradation by the 26S proteasome. Subsequently, the ARF transcription factor becomes

active, which activates or inhibits the expression of downstream genes, while Aux/IAA acts as a downstream gene and feedback inhibits auxin signaling pathways. This study indicated that expression of the PHARF4 gene in transgenic *Petunia hybrida* was down-regulated compared to wild-type *Petunia hybrida* plants, which suggests that the ARF transcription factor inhibits the auxin signal transduction pathway.

Some studies have shown that ERF1 can regulate the expression of ACS3, ACO, and ACO2 in plants, and thereby enhance the biosynthesis of ethylene in transgenic plants[36–39]. This suggests that transcription termination factors may play an important role in regulating plant ethylene biosynthesis[40,41]. The homeodomain-leucine Zipper (HD-Zip) is a type of transcription factor that is unique to higher plants and belongs to a class of homeodomain (HD) transcription factors [42]. For conserved HD, the HD carboxyl terminal is tightly linked to the leucine zipper domain (LZ). Research suggests that these transcription factors mainly regulate the development of plants, including the development of vascular tissues and trichome[43]. These factors are also involved in the regulation of external signals that regulate the growth of plants. Overexpression of these factors leads to a series of phenotypic variations such as black lobes, leaf leveling, life cycle shortening, and early flowering. Results show that PHERF gene expression was 50 % lower in transgenic *Petunia hybrida* compared to the wild-type. The homeodomain leucine zipper transcription factor, *Petunia hybrida* (PHHD-Zip), is involved in the regulation of biosynthesis of ethylene. The results showed that PHHD-Zip expression was reduced by 25 % in transgenic *Petunia hybrida* compared to the wild-type.

Conclusions

In conclusion, the results obtained in this study show that *SAG12-BAS1* transgenic lines grown in the vegetative state exhibit a range of phenotypic changes, including increased flower bud counts, branching and increased internode lengths, a delay in flowering, increasing activity of protective enzymes, and the expression of BAS1 related genes. The regulation of aging-related genes may increase cell division, decrease ethylene content, and prolong the senescence of *Petunia hybrida*

Abbreviations

RT-PCR Reverse transcriptase polymerase chain reaction

GUS Beta-glucuronidase

MDA Malondialdehyde

SOD Superoxide dismutase

POD Peroxidase

CAT Catalase

PhARF4 *Petunia hybrida* putative ARF4 protein

PhARR2 *Petunia hybrida* mRNA for type-A response regulator 2

PhHK *Petunia hybrida* mRNA for cytokinin receptor histidine protein kinase PhGA2ox1 *Petunia hybrida* Gibberellin 2-oxidase

PhGA2ox3 *Petunia hybrida* Gibberellin 2-oxidase3

PhERF *Petunia hybrida* ethylene-responsive-element-binding factor 2

PHZIP *Petunia hybrida* A *Petunia hybrida* Homeodomain-Leucine Zipper Protein

Declarations

Author contribution statement

XZY and LTL conceived and designed the research. XZY conducted the experiments, contributed analytical tools, and analyzed data. XZY wrote the manuscript. LTL and DGZ supervised the studies and revised the manuscript, All authors read and approved the manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Declarations

Consent for publication

Competing Interests

The authors declare that they have no competing interests.

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Figures

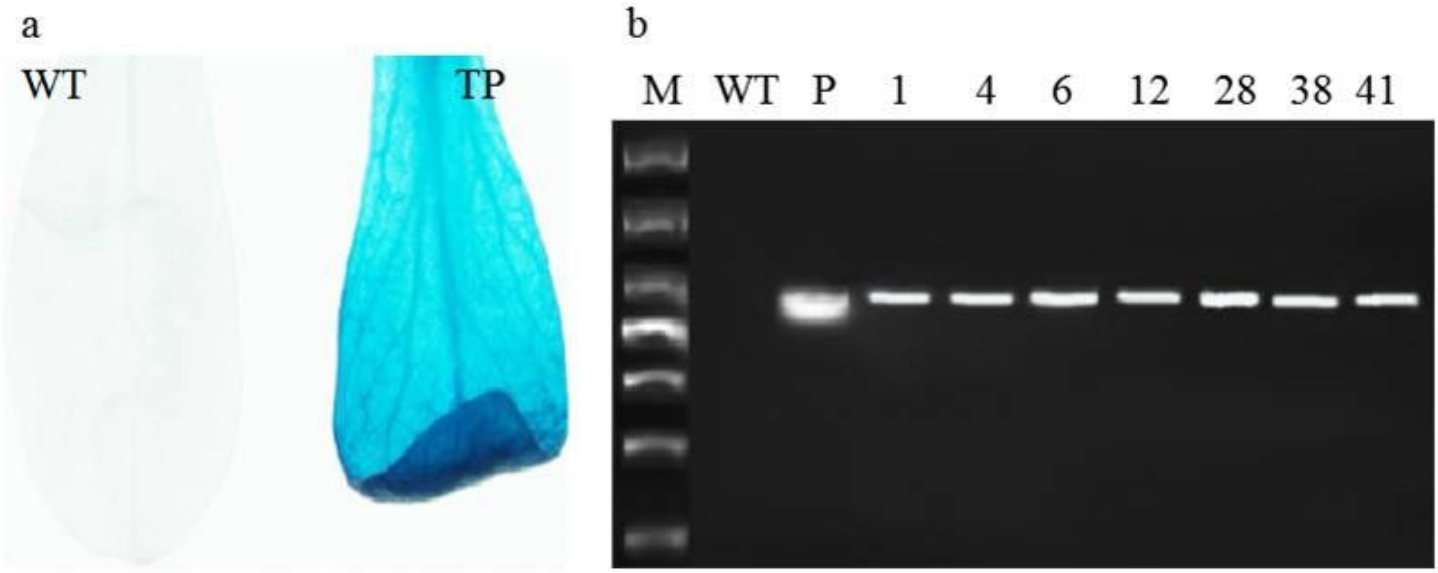

Figure 1

GUS staining in Petunia hybrida plant leaves at the 3-5 leaf-stage and PCR analysis. (a) GUS expression in Petunia hybrida . (b) PCR analysis showing the presence of the expected 419 bp fragment from putative transgenic Petunia hybrida lines. After resistance selection and PCR identification, we got a total of 29 strains of transgenic plants. And the three lines TP-12, TP-28 and TP-38 were used to analyze the drought tolerance. M: DL 1000 Marker; P: Plasmids; WT: Wildtype plants.

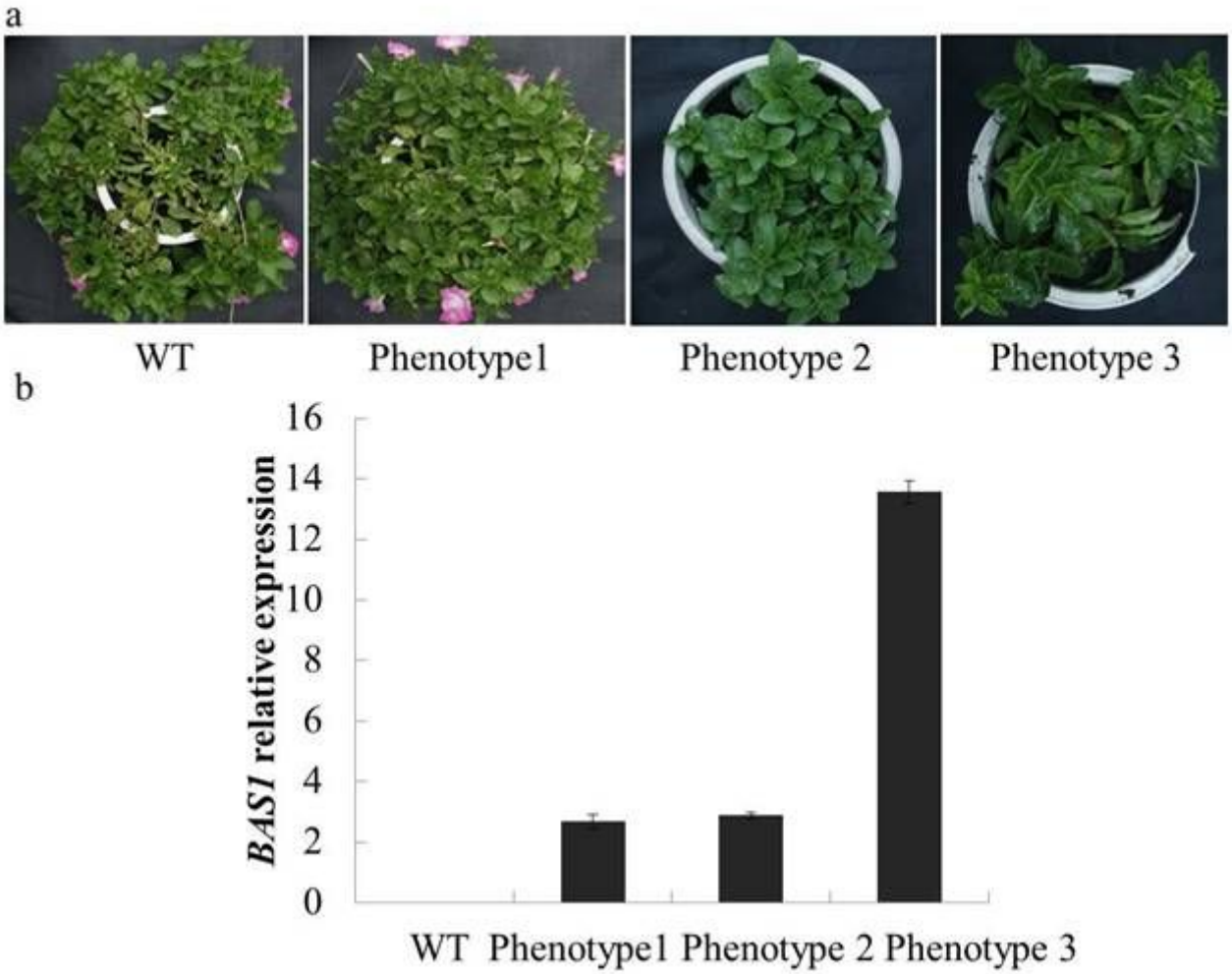

Figure 2

The phenotype of transgenic Petunia hybridas. (a) Obtained three transgenic phenotypes; (b) Expression of the BAS1 gene from Wildtype plants (WT) and transgenic phenotypes (Phenotype 1, Phenotype 2, Phenotype 3). Experimental needs to select wild type and transgenes(Phenotype 1) with similar phenotypes for subsequent experiments.

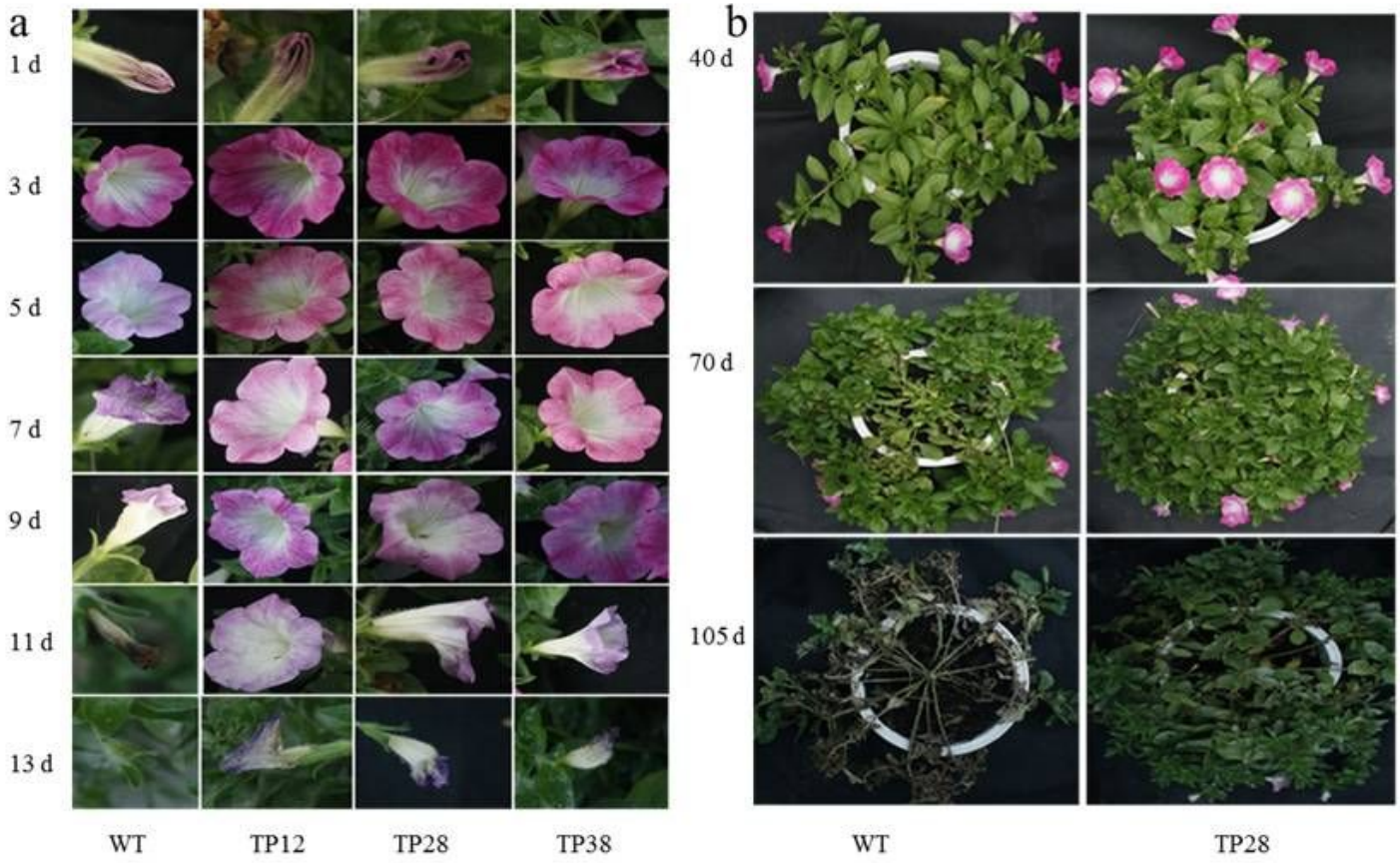

Figure 3

Transgenic and wild-type growth processes. (a) Chronology of wild-type and transgenic Petunia hybrida flower development and senescence. Growth status records of wild-type and transgenic line (Phenotype 1:TP12, TP28, TP38) flowers at different times; (b) Transgenic (TP28) and wild-type plant growth process. The time (days) is indicated on the left of each panel.

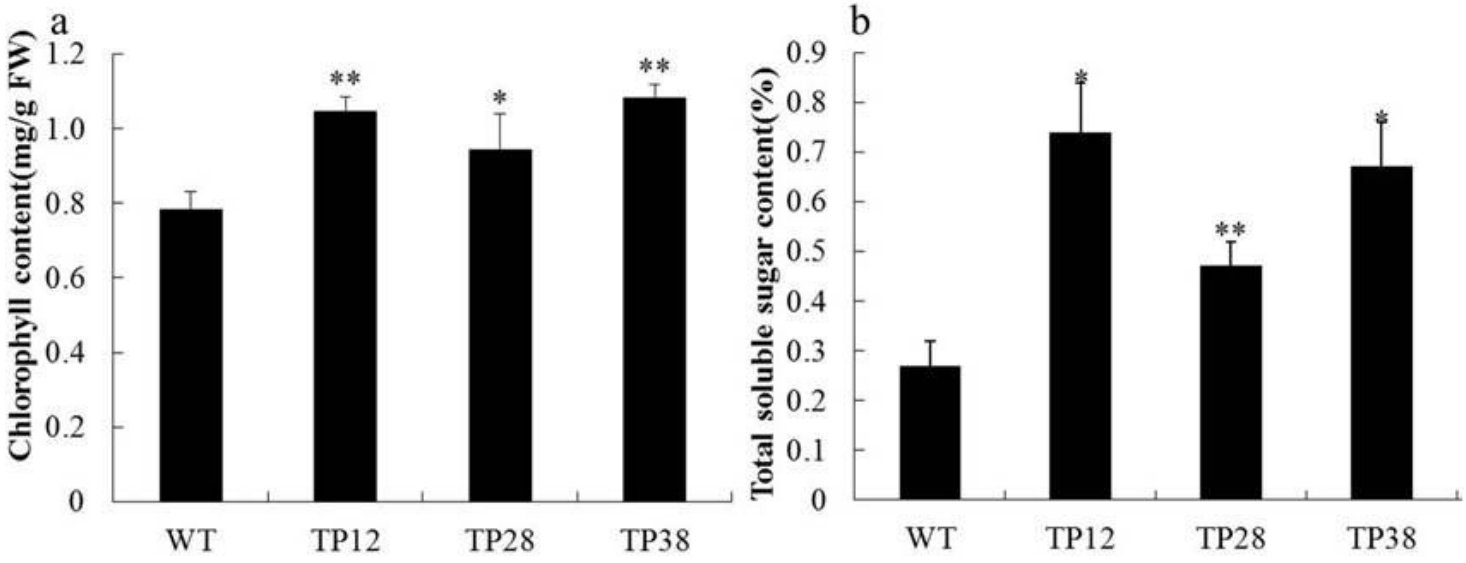

Figure 4

Total chlorophyll content (a) and soluble sugar content (b) measured in leaves from WT and 4 different transgenic plants after 90 days of plant growth. Data are presented as means ± SD (n=3). Asterisks indicate significant differences between wild-type (WT) and transgenic lines (, TP12, TP28, TP38) (*P < 0.05; **P < 0.01).

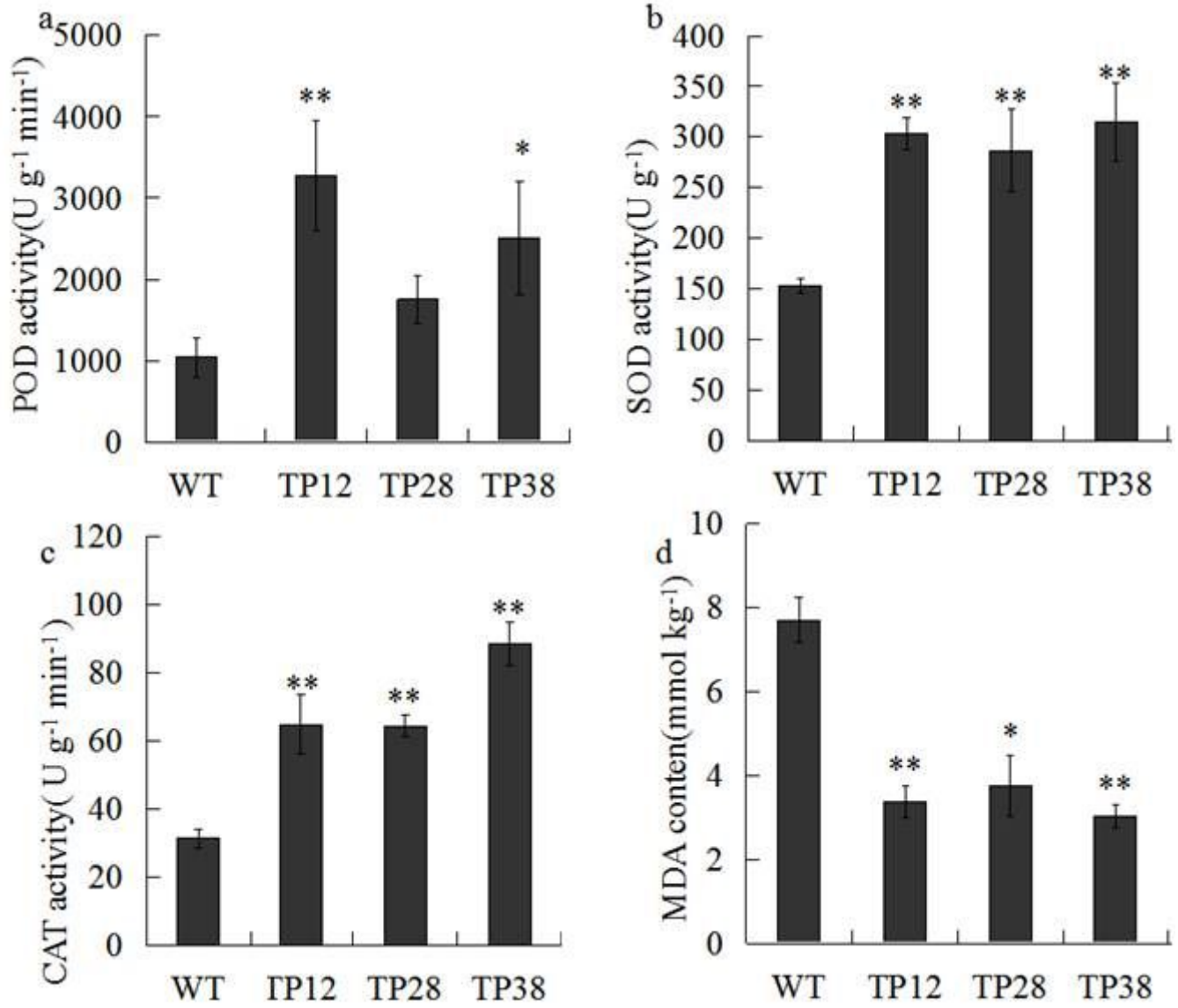

Figure 5

SAG12-BAS1 positively regulated the expression of antioxidant defense enzymes and negatively modulated the accumulation of MDA in Petunia hybrida leaves. Levels of POD (a), SOD (b), and CAT (c) activities and MDA content (d) of wild-type (WT) and transgenic lines (TP12, TP28, TP38). Data are presented as means ± SD (n=3). Asterisks indicate significant differences between WT and the five transgenic lines (*P<0.05; **P<0.01).

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