Response to Plant Hormones of Senescence-related Genes for *Cucumis sativus* L. in Cotyledon Development

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This study was carried out to discover the response of cucumber (*Cucumis sativus* L.) senescence-associated genes (SAGs) to several plant hormones in detached and developing cotyledon. Accordingly, a collection of cucumber SAGs were examined to characterize their gene expression response through semi-quantitative RT-PCR. Cotyledons were excised at day 14 after seed sowing from plantlets, then incubated in 100 μM each of IAA or zeatin solution for up to 4 days in light and darkness. They were collected at 2-day intervals and used for total RNA extraction and subjected to RT-PCR. Gene expression levels of several cucumber SAGs were significantly changed during the incubation period. More than five cucumber SAGs involving SAG 60 responded to the IAA and zeatin treatment. In the ethylene response study, cotyledons were exposed up to 10 days by ethylene gas. Most of the cucumber SAGs did not show immediate response to ethylene in green cotyledon. The exceptions were PCK, SAG 158, and SAG 288 genes, which responded after 1 day of exposure to green cotyledon, while ICL and SAG 281 revealed strong responses after 10 days of being exposed to yellowing cotyledon. These results suggest that several cucumber SAGs react actively in response to starvation or senescence against exogenously applied stimulus. This induced senescence response is able to understand the SAGs role in lipids and amino acids metabolism partly and function in organ senescence during development.

Key words: Cotyledon, cucumber, gene expression, plant hormones, senescence

Introduction

Plant organ senescence is referred to a sequence of biochemical and physiological events that constitute the final stage of development [9]. Also, senescence has been studied in many plant species [7, 19, 20, 28, 29]. Although senescence occurs in an age-dependent mode in many species, the initiation and progression of senescence can be modulated by a variety of environmental factors (exogenous and endogenous stimulus), such as temperature, mineral deficiency, drought conditions, and pathogen infections following nutrients, hormone and light stress. It is known that endogenous factors such as plant growth regulators, reproduction and cellular differentiation also influence senescence. Among these endogenous factors, various plant hormones such as auxin, cytokinin, ethylene and jasmonic acid etc., have been characterized most comprehensively at the molecular and physiological level [17, 21, 32, 35, 39]. More recently, the roles of an additional plant hormone strigolactones (SLs) were intensely discussed during leaf senescence development [41]. Another potent senescence inducing factor is presence or absence of light. The interaction between light and senescence is complex and many reports have been published describing both its senescence-inhibiting and promoting qualities [2, 22]. The plant hormones such as auxin and cytokinin, regulate many aspects of plant growth and development [28, 33]. Furthermore, gas form ethylene, which organ senescence and fruit mature motivating plant hormone [26, 31, 34] is another factor to SAGs study in this research to find out the genes reaction.

Here, we demonstrated that cucumber senescence-related genes reaction to the exogenously applied indole-3-acetic acid (IAA), zeatin and ethylene for finding of the artificial stresses or induced senescence responses using detached and developing cucumber cotyledons. In this report, we discuss the selected cucumber SAGs biological function during cotyledon senescence and the reactions against the...
plant hormones in induced cotyledon senescence.

Materials and Methods

Plant materials and chemicals

Seeds of cucumber were imbibed in sterile water at 4°C for 12 hr and sown in wet vermiculite. The resultant plants were maintained in a growth chamber (Lab-Line Biotronette) at 25°C and 70% humidity under continuous illumination. Plant hormone was prepared as described in the manufacture’s protocol and suggestion as 100 μM of working solution (Sigma). Cucumber cotyledons were detached at day 14 from seed sown and the detached cotyledons were incubated in a Petri-dish containing sterile distilled water, IAA or zeatin solution. The Ethephon (Dongbu Fine Chemical, Korea) was used to 14 day old cucumber seedling with 20% KOH to induce ethylene gas within the plant growth chamber following manufacturer’s instruction and previous report [6]. Then, cotyledon was collected rapidly at 1, 5 and 10 day after treatment of Ethephon, then they were frozen in liquid nitrogen to isolate total RNA.

Total RNA preparation and evaluation

An RNAeasy plant mini kit (Qiagen GmBH) was used to extract total RNA from cucumber cotyledon samples and treated with DNase I (Qiagen GmBH) to remove any genomic DNA contamination following manufacturer’s protocol. Three pairs of cotyledon were used from classified plant hormone-treated samples for individual extraction procedures. Twice of final elution of total RNA was carried out in equal volumes (50 μl) of diethylpyrocarbonate (DEPC) treated sterile H2O. The quality of total RNA was examined by the 1.2% agarose gel electrophoresis in 0.5x Tris/Borate/EDTA (TBE) buffer and quantity was estimated through Biophotometer at 260 nm (Eppendorf, Germany). Total RNA extraction and estimation were carried out three times in each experiment.

Reverse transcription (RT) reaction and PCR for cucumber genes

An equal volume (10 μl) of total RNA solution was used to produce the first-strand of complimentary DNA (cDNA) by reverse transcription (RT) reaction. The poly(A)+ RNA was primed by the oligo (dT)′s primer using an AccuPower RT PreMix (Bioneer, Korea) following standard procedure. Polymerase chain reaction (PCR) was performed for 30 cycles using MyCycler (BioRad, USA) with an AccuPower PCR PreMix Kit (Bioneer, Korea) by gene-specific primers (5′-3′), as described in a previous report [19]. All primers were manufactured through Bioneer Co (Daejeon, Korea). An equal volume (10 μl) of RT-PCR products were fractionated on 1.2% (w/v) agarose gel in 0.5× TBE buffer for 60 min at 100 V, and the gel was digitally photographed and processed. These RT-PCR experiments were repeated at least three times independently under the same conditions.

Results and Discussion

Genes and bioinformatics analysis

Cucumber SAGs were presented in Table 1, which had

| Clone No | Possible Identity (in silico) | ID+ (%) | L/C (aa)** | Source |
|----------|-------------------------------|---------|------------|--------|
| SAG 60   | Cytochrome P450 monooxygenase (QQ475074) | 67      | 104/153    | Zea mays (AF465265) |
| SAG 66   | Metallothionein-like protein type 3 (QQ467332) | 36      | 37/65      | Musa acuminate (Q40256) |
| SAG 67   | Cucumber plastid genome sequence | 96      | 124/128    | Cucumis sativus (DQ119058) |
| SAG 87   | Polyubiquitin, UBQ14 (QQ487330) | 100    | 190/190    | Antirrhinum majus (S25164) |
| SAG 153  | Superoxide dismutase [Cu-Zn] (EU407180) | 90     | 132/152    | Ipomoea batatas (Q07796) |
| SAG 158  | Matrix metalloproteinase 21.2-like protein | 62     | 18/29      | Arabidopsis thaliana (BAB01942) |
| SAG 281  | Matrix metalloproteinase (AJ133571) | 100    | 134/134    | Cucumis sativus (CA876364) |
| SAG 288  | Putative stress-induced protein (QQ475073) | 82     | 191/232    | Solanum commersonii (CAJ192689) |
| SAG 338  | Proline dehydrogenase (EU407181) | 63     | 136/214    | Arabidopsis thaliana (NP_189701) |
| SAG 1-9  | GA-like protein (light regulated Lrl1) (QQ475076) | 58    | 47/80      | Pisum sativum (T06776) |
| SAG 3-5  | O-acetylserine (thiol) lyase (QQ475075) | 90     | 110/120    | Citrullus vulgaris (D28777) |
| SAG 11-6 | Peroxidase 21 (ATP2a) (FJ397624) | 78     | 174/223    | Arabidopsis thaliana (NP_181250) |

+, ID Implies that the percentage of amino acid sequence identity in a continuous region of a particular length (overlap).

**Amino acids numbers are matched length compared (L/C).
been isolated from previous study [19]. However, we have cloned more than hundreds of cDNA clones which should be further identified the possible biological functions from the bioinformatics study through National Center for Biotechnology Information (NCBI) (Data not shown). Most of the cDNA clones were registered in the GenBank through NCBI, except SAG 67 and 158, which are in progress for registration.

**Total RNA changes during plant hormones treatment**

Freshly extracted total RNA showed that small accumulation of transcripts in detached cotyledons in all circumstances; water, IAA and zeatin (Fig. 1). However, there were no significant differences between water-treated and IAA/zeatin-treated cotyledons. Furthermore, total RNA change was not significant in ethylene exposed developing cucumber cotyledon up to 10 day exposure (Fig. 4).

**Response to IAA and zeatin with light or dark in detached cotyledon**

Several cucumber SAGs showed that three distinct patterns of gene expression in detached cotyledons; constitutively expressed, light-dependent expression and zeatin/IAA regulated. Initially, we examined actin gene’s expression pattern to confirm the constitutive positive control elements (Fig. 2, Actin). Neither auxin nor zeatin-treatment affected the actin gene expression to cucumber cotyledons both in the light and in the dark conditions. Otherwise, cucumber ICL gene rapidly responded to zeatin whatever the conditions of light and darkness, but the gene expression strongly depends on darkness to auxin treatment (Fig. 2, ICL). In particular, zeatin stimulates the ICL expression more than three times (Fig. 2, ICL). However, IAA showed higher effect only in the dark that implies the different mechanism of the signal path by two hormones. The PEPCK gene is for gluconeogenesis in oil-seed germination [18] showed similar patterns of gene expression to ICL in water-treated control (Fig. 2A, ICL and PCK). The zeatin removed the light-dependent repression factor for PEPCK expression at the early stage (L2), but there was no change in the dark. IAA showed similar response to zeatin, but responded slowly in the light (Fig. 2C, PCK ). From these observation, we could presume that catabolic mobilization of demolishing membranous or internal lipids following renewal of glyoxylate cycle and gluconeogenesis to provide energy for induced senescence possibly for final stage of natural senescence [37].

The cytochrome P450 mono-oxygenase encoding SAG 60 (named as CsCYP72A) mediate a wide range of oxidative reactions involved in the biosynthesis of plant secondary metabolites, including the phenylpropanoids and phytoalexins. A precursor of IAA, indole-3-acetaldoxime (IAOx) was synthesized from tryptophan by the cytochrome P450s in Arabidopsis [15]. Recently, auxin and light responsive cy...
tochrome P450s also suggested that their key roles in the biosynthesis of various metabolites in Withania somnifera [30]. Interestingly, cucumber CsCYP72A was up regulated by IAA treatment in all circumstances from the day 2 (Fig. 2C, 60) and these are quite well matched with their observation.

Genes encoding metal-binding proteins were the major component of senescence [4]. Cucumber metallothionein-like type 3 protein coding SAG 66 (named as CsMTP3) is extremely active in developing cotyledon. Gene expression has been reported in Brassica napus leaves, post-harvest flower of broccoli (Brassica oleraceae), and leaves of senescing sweet potato (Ipomoea batatas) [5, 14, 24]. Furthermore, protein coding GhMT3a gene showed strong expression against various external stresses not only to drought, salinity, low temperature, but also to abscisic acid (ABA), ethylene and reactive oxygen species (ROS) in cotton seedlings [40]. CsMTP3 expression was totally blocked by zeatin and IAA in this experiment (Fig. 2B and 2C, 66). In particular, IAA effect was the utmost to the CsMTP3 gene. Therefore, it would be confirmed that IAA plays a role for strong antagonist in senescence development.

The SAG 67 was identified as a part of cucumber plastid genome. The SAG 67 gene transcripts were detected in water-treated controls and it is independent to light (Fig. 2A, 67). It implies that SAG 67 is a housekeeping gene for chloroplast or cytosolic metabolism rather than for photosynthesis. Zeatin showed a positive effect in light condition but not the case in the dark (Fig. 2B, 67). IAA suppressed Sen 67 expression progressively in all circumstances (Fig. 2C, 67). Unfortunately, we do not have further information about biological function of the SAG 67 sequence. It will be find out in the future work.

A polyubiquitin encoding SAG 87 (named as CsUBQ14) has been reported in earlier reports in natural senescing cotyledons and petals [19, 20]. Polyubiquitin conjugate of key pro-and anti-apoptotic molecules have characterized ubiquitin as an essential regulatory modification targeting proteins for proteosomal degradation. A complete homology of amino acids sequences among plant polyubiquitins imply that the significance in plant development. Our investigation showed that CsUBQ14 was not affected to the hormones (Fig. 2, 87). Therefore, it is not the case of the regulatory involvement to this gene by the hormones but may play a housekeeping role as protease in overall stages of cotyledon development [19].

We have found an increase of superoxide dismutase (Cu-Zn)-like (SOD) encoding SAG 153 (named as CsSOD) expression in senescing cucumber cotyledons [19]. However, the CsSOD expression was not affected significantly in all situations, but slightly decreased by the hormones (Fig. 2, 153). Otherwise, there was no effect to the CsSOD gene expression by ethylene treatment (Fig. 4B). As we well know, the SOD plays a central role to remove toxic ROS during development both normal and stress environments for protection against such highly reactive superoxide radicals [36]. Therefore, we could presume that a pattern of CsSOD gene expression as a constitutive mode during whole development of cotyledon.

A matrix metalloproteinase (MMP) 21.2 encoding SAG 158 (named as CsMMP21) has shown that the gene is expressed from the early stage of cotyledon senescence (stage I) in cucumber [19]. CsMMP21 gene’s transcript could not be detected in green tissues until 3-4 weeks after germination, but it shows strong activation at the beginning of senescence. In this study, we found that CsMMP21 rapidly reacted to zeatin after 2 days of incubation in light and darkness (Fig. 2B, 158) then, increased at day 4. The CsMMP21 also reacted to IAA as inconsistent mode whose cannot be made any conclusion in this report. The SAG 281 (Cs1-MMP) encodes a pre-pro-enzyme of zinc metalloprotease, especially the matrix metalloprotease family. MMP has been identified in higher plants [11, 23, 43]. Here, our RT-PCR again revealed that its strong responses were observed in the zeatin- and IAA-treated cotyledons (Fig. 2, 281). However, the pattern of response is slightly different between zeatin and IAA. Zeatin activated Cs1-MMP expression independently in the light from day 4 cotyledons, (281, L4 and D4). Otherwise, tremendous effect of IAA can be seen only in the dark from day 2 cotyledon. Light did not affect to zeatin-treat cotyledons, but repressed the gene expression in IAA-treated cotyledon up to 8 days in the light although gene expression was maintained in the dark consistently (Data not shown). Therefore, it is clear that the Cs1-MMP has different regulatory factors for the two hormones. The role of the Cs1-MMP enzyme is unclear in plant organ senescence, but Cs1-MMP is expressed de novo at the end stage of senescence, in cucumber cotyledon and male flowers [19, 20]. Protease is probably not involved in nutrient remobilization during senescence, but it suggested a strong connection in programmed cell death (PCD) from tomato Sl2-MMP and Sl3-MMP through extracellular pro-
teolytic cascade [7, 43].

Recent database search revealed that cucumber SAG 288 encodes putative stress-induced protein, therefore we do not know exact biological function in senescing cotyledon. However, SAG 288 DNA sequences shared some homology with Arabidopsis pentose phosphate translocator (formerly CsPPT, Data not shown). Interestingly, SAG 288 expressed a weak level in green cotyledon (Fig. 2A, 288, 14C). We also found that a weak expression in control experiment (Fig. 2A, 288) but a strong up-regulation in zeatin-treatment particularly in the light (Fig. 2B, 288). However, there was no effect by the IAA treatment (Fig. 2C, 288). Unfortunately, we could not find further information about the role of SAG 288 reaction against plant hormone and exogenous stress from available databases.

SAG 338 codes an enzyme for proline dehydrogenase 2 (named as CsPDH2) for abscisic acid (ABA) synthesis during the maturation of plant organs which osmotic stress inducible proline oxidase [27]. The CsPDH2 gene is highly active in cotyledon senescence [19]. Effect of zeatin and IAA was minor to CsPDH2 which zeatin showed no effects but IAA suppress the gene expression in the light, from L2 to L4 (Fig. 2C, 338). The PDH is involved in drought-induced proline metabolism and turnover in many plant species has led to the hypothesis that further increases in proline accumulation would promote drought tolerance [3]. Although, the PDH enzymes could be classified into stress related group in plant development but we found a negligible change of the CsPDH2 gene expression from organ detachment stress and exogenous hormone application.

A gibberellin-stimulated (GA-like protein) SAG 1-9 (named as Cs-Lir1) is responsive to light regulation for synthesis of MGDG [42]. The Cs-Lir1 gene is strongly expressed in green cotyledon then gradually decreases until the end of senescence [19]. It implies that the changes of endogenous GA level in senescing organs. Furthermore, Cs-Lir1 also revealed a dark response (down-regulation) that shows clearly at D4 stages (Fig. 2A, 1-9). Furthermore, both zeatin and IAA completely shut down the Cs-Lir1 gene in the dark and some suppression in the light too (Fig. 2B and C, 1-9). This results apparently show that the Cs-Lir1 gene expression is under regulation of light condition. There is not sufficient report about Cs-Lir1 gene action in relation with senescence and stress to this point.

One of the cysteine biosynthesis involving SAG 3-5 (named as CsOAS-TL) was reported in the previous report [19], which suggests the amino acid cysteine may have a special role in mediating cell metabolism during senescence. Cysteine synthesis normally occurs in the plastids, mitochondria, and cytosol by serine acetyltransferase (SAT) and O-acetylserine (thiol) lyase (OAS-TL) through two-step process in plants [38]. Hell et al., have focused on its biosynthesis and have determined that reduced sulfur is channeled from cysteine into many sulfur-containing compounds in food and feed [12]. Furthermore, cysteine biosynthesis is part of the regulatory network that mediates between inorganic sulfur supply and the demand for reduced sulfur during plant growth and in response to environmental changes. In accordance with biosynthesis of cysteine, crucial role of senescence-associated serine and cysteine proteases was proposed in the efficient protein remobilization during leaf senescence of oilseed rapeseed [25]. In this detached experiment, CsOAS-TL gene showed minor response to zeatin and IAA that equally negligible effect in light and darkness (Fig. 2, 3-5). Otherwise, prolonged ethylene exposure revealed that gradual increase of the CsOAS-TL expression in this experiment (Fig. 4B), which could be agreed with the protein remobilization process during organ senescence.

Finally, a peroxidase coding SAG 11-6 (named as CsPOX) was examined to find out hormonal reaction in detached cucumber cotyledon. As a plant-specific oxidoreductase, Class III plant peroxidase (POX) is one of the many types of peroxidases that are widely distributed in animals, plants and microorganisms. POXs exist as isoenzymes in individual plant species and they are involved in various physiological processes such as lignification, auxin catabolism, wound healing, defense and organ senescence [13]. From earlier senescence study in cucumber, a plant peroxidase (33-CPO) activity was promoted by ABA and JA as ethylene but was retarded by IAA, GA and kinetin from excised cotyledon [1]. They also showed induction of the peroxidase isoenzymes by both ethylene and JA following induced senescence in cucumber cotyledon. Here we also showed similar results that CsPOX gene expression was not affected by auxin (Fig. 2A and 2C 11-6), and additionally zeatin repressed the gene action in the dark (Fig. 2B, 11-6). Ethylene treatment also revealed similar conclusions by gradual increase of CsPOX gene expression (Fig. 4, 11-6). Increasing peroxidase activity was correlated with chlorophyll degradation during organ senescence which means ethylene could trigger the intracellular membrane destruc-
This is a probe study to find out the relations between stress and senescence correlation in cucumber cotyledon development. Application of plant hormone is considered as an internal cause of organ senescence. Detachment of plant organ is expected to play a role for rapid external stress which may elicit senescence response. Therefore, these stimuli encouraged senescence development and related genes reactions. Gene’s reaction was diverse to hormones, light and darkness that is interesting to preview the succeeding step of study for finding of specific signal route in cotyledon senescence. Oil seed germination associated ICL and PCK showed fairly similar reaction to hormones that implies corresponding signal routes as suggested in earlier observation [18]. However, the regulatory study has not been done until currently. CsCYP72A (SAG 60) was up regulated by hormone when auxin interrupts immediately, but zeatin affected lately. CsMTP3 (SAG 66) was a dramatic example on hormonal repression as a cucumber SAG. Additional discovery is Cs1-MMP (SAG 281), which exciting senescence specific gene for closing stage of cotyledon development [7]. Although, hormonal reactions was somewhat discrete by two hormones, then Cs1-MMP strongly up regulated by them. A discrepancy is on-set stage of the gene expression, which is the first observation ever in cucumber. Another MMP family CsMMP21 (SAG 158) also revealed that strong up regulation by the hormones but response pattern is rather different from Cs1-MMP. The SAG 288 was surprising because response begins on L2 by zeatin then on D4. The late on-set of gene expression in darkness may be starvation effect by detachment but it should be examined in the following research.

Response to ethylene exposure in developing cotyledon

After 14 days of seed sowing, seedlings of cucumber were exposed directly by ethylene gas within the plant growth chamber rather than detachment of cotyledon because of technical matter of gas form ethylene. Cotyledons were collected after 1, 5 and 10 days of ethylene exposure from seedling. The ethylene effect appeared slow but rapidly that complete yellowing of green cotyledon in 24 hr after 10 days of prolonged exposure (Fig. 3). Total amount of RNA were not changed significantly, but increased small at 5 day exposed cotyledons (Fig. 4A). Following RT-PCR approach under basis of single cotyledon, only two of those cucumber SAG (ICL and 281) showed parallel response with the shifting point of yellowing cotyledon (Fig. 3 and 4B). It clearly implies that ICL and SAG 281 gene are involved in cotyledon senescence at the point of chloroplast (or chlorophyll) destruction together with other senescence-related enzyme coding gene’s action such as various plant peroxidases [1, 8, 10, 16, 26, 34]. As discussed in earlier section, Cs1-MMP may be responsible for closing the death of a cell [43]. However, a key glyoxylate cycle enzyme coding ICL will participate in remobilization of demolishing internal membrane lipids during senescence.

Although SAG 66 gene showed very strong expression from 14C to 10 days of ethylene exposure with small increasing (Fig. 4B, 66), it showed clear response against ethylene as shown in cotton seedling development from earlier report [40]. As discussed earlier section about SAG 3-5 for cysteine protease following cysteine synthesis by CsOAS-TL, a prolonged ethylene exposure revealed that gradual increase of the SAG 3-5 gene expression in this experiment (Fig. 4B), which could be agreed with the protein remobilization process during cotyledon senescence [25].

Another interesting observation is the PCK and SAG 288 gene, which responded rapidly to ethylene after 1 day exposure (Fig. 4B). Especially, gradual increase of PCK gene expression means anaplerotic response to senescence inducing ethylene signal for supplying metabolic intermediates.
into tricarboxylic acid cycle [18]. Although the gene expression was not escalating, a rapid reaction of SAG 288 gene could be a stress response against metabolic instability come from exogenous stimulus, but yet to be answered. Similar response appeared in SAG 158 that will drive gradual destruction of cellular compartments such as most of MMP enzyme action during PCD or senescence [43]. SAG 338 showed some increase of the gene expression against ethylene that may be explained to be an osmotic-stress response for proline accumulation as drought tolerance [3].

As mentioned in earlier section, SAG 338 is a stress responsible gene in plant development, and we found some response of the CsPDH2 gene expression (Fig. 4B, 338). Rests of the cucumber SAGs could not be confirmed as reactive to ethylene stimulus in cucumber cotyledon except SAG 11-6 (Fig. 4B). As we mentioned earlier, ethylene treatment revealed a similar conclusion that by gradual increase of SAG 11-6 expression (Fig. 4B, 11-6). Increasing peroxidase activity was correlated with chlorophyll degradation during organ senescence which means ethylene could trigger the intracellular membrane destruction by induced senescence [1].

In conclusion, we were able to confirm hormone-dependent cucumber SAGs such as ICL, PCK, SAG 66, SAG158, SAG281, SAG288, SAG 338, SAG3-5 and SAG11-6, using three different type of plant hormones. They can be categorized into two group of SAGs from this result as destruction SAG and remobilization SAG; the first group would be SAG 66, 158, 281, 338 and 11-6 and, the second group would be ICL, PCK and SAG3-5. Then, we will focus on these genes developmental importance in organ senescence especially about lipid metabolism and transport to other cellular compartment during development.

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초록: 오이 떡잎에서 노쇠화 관련 유전자들의 식물 호르몬에 대한 반응 연구

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본 연구는 발달중인 오이 떡잎에 식물호르몬을 처리하여 나타나는 노쇠화 관련 유전자들(SAG)의 유전자 발현 반응을 탐구하기 위하여 수행되었다. 따라서 선별된 오이의 노쇠화 관련 유전자들에 대하여 그들의 유전자 발현 반응을 특정하기 위하여 역전사-중합효소연쇄반응(RT-PCR)을 통하여 조사되었다. 과종 후 14일 된 오이의 떡잎을 절취한 후에 100 µM IAA 또는 zeatin 용액 위에 두고 빛이 있거나 없는 조건에서 4일째 까지 처리하였다. 떡잎은 2일 간격으로 횟수하여 총RNA추출과 RT-PCR의 서로에 사용하였다. RT-PCR 결과에 따르면 몇몇 오이의 SAG 전사체들은 처리기간 동안에 상당한 변화를 나타냈다. 예를들면 실험에서는 처리 1일 후 반응을 나타낸 PCK, SAG 158과 SAG 288은 제한된 대부분의 오이 SAGs는 즉각 반응을 보이지 않았으나 ICL과 SAG 281은 10일 처리 후 노란 떡잎에서 강한 반응을 나타냈다. 이러한 결과들은 몇몇 오이의 SAGs가 외부적 자극에 대하여 영향 결핍 이나 노쇠화 반응을 나타낼 수 있는 정보를 제공한다.