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FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Development of a Rosette Gall in *Aster scaber* Thunberg

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CONTENTS

SUMMARY IN KOREAN

참취에 발생하는 충영들은 독특한 형태적 특성을 나타내었다. 이러한 충영들은 mautre stage에 비록 왜성인 형태를 갖추었지만, 장미꽃 모양의 기관(rosette gall) 으로 발달한 완전한 소형식물체의 모습을 하고 있었다. 더군다나, last stage에는 rosette gall은 꽃모양의 기관으로 발달하였다. 참취 식물체에 로제트 모양의 충영이 형성된다는 것은 1944년 Shiji에 의해 보고가 되었으나, 단지 곤충학적 조사를 제외하고는 다음과 같은 연구는 수반되지는 않았다. 충영의 발생에 있어서 전분화과정과 그 조절인자들을 이해하기 위하여 본 연구에서는 참취에 발생하는 rosette gall의 식물학적 특성 (Part I), rosette gall을 형성하는 인자 (Part Ⅱ), rosette gall 분화과정 (Part Ⅲ)과 rosette gall 발생에 포함되는 유전자들 (Part Ⅳ)을 조사하였다.

Mature stage의 참취 식물체의 rosette gall의 기본적 구조는 반구형의 혹모양과 ectopic 소엽이 발생하였고 꽃모양의 기관으로 구성되었다. rosette gall 들은 폭이 0.3 - 2.0 cm 이고, 높이는 0.3 - 1.0 cm 로 다양한 크기를 나타냈다. 대표적인 참취 식물체의 rosette gall 들은 왜성의 작은 식물체이나 엽병, 절간 줄기, 화서 또는 뿌리는 발생하지 않았다. 그러나 소수의 rosette gall 들은 잎 엽병들이 발생하는 것이 관찰되었다. 또한 좀처럼 보기드문 현상이지만, rosette gall은 화경과 *de novo* 패턴의 뿌리 발생이 관찰되었다. rosette gall 은 모식물체의 모든 부분, 즉 잎의 앞면과 뒷면, 엽병, 줄기, 절, 정단부위 및 뿌리에서도 발생하는 것이 관찰되었다. 보통의 식물 기관들은 잎맥 부근의 분열조직세포로부터 분화하지만, 잎에서 발생한 rosette gall 인 경우에는 잎의 잎맥 사이의 비분열조직세포로부터 항시 발생하는 것을 관찰할 수 있었다. 비기관 특이적인 rosette gall 형성뿐만 아니라 비분열조직에서의 rosette gall 형성은 비록 rosette gall 분화 원인과 분화정도에 관계없이 식물세포의 고유한 전분화능력에 대한 직접적인 증거를 보여주는 예라고 할 수 있다.

참취 식물체에 발생하는 rosette gall을 유도하는 원인 곤충은 파리목 혹파리과의 나도쑥혹파리 (*Dasineura asteriae* Shinji) 로 동정 되었다. 4월부터 5월 사이

에 성충들은 교미를 하였으며, 토양으로부터 출아한 참취 식물체의 근생잎 표면에 산란을 하는 것이 관찰이 되었다. 암컷 성충들은 참취 식물체의 어린잎에 산란을 하였으며, 그 알들은 2-4 일 후 부화하였다. 부화된 유충들은 정착할 곳을 찾아 움직였으며, 유충들이 정착한 참취 식물체 기관의 표면은 차후 rosette gall로 발생하였다. 유충들은 rosette gall의 gall조직내에 유충방(larval chamber)을 만들었으며, 그곳에서 생존하였다. rosette gall의 성장과 함께 이 유충방들은 ectopic 소엽들로 둘러싸여 졌으며, 이후 ectopic 소엽들이 발생한 rosette gall 내부의 유충방들은 floral bud 로 발달하였다. 하나의 rosette gall 내부에는 2개 이상의 유충들이 각각의 유충방에 서식하는 것이 관찰이 되었으며, 각 각의 유충들은 floral bud 로 둘러싸여 졌다. 유충들은 rosette gall에 서식하며 유충시기를 보냈으며, 늦가을이 되면 유충들은 노화된 rosette gall 에서 나와 토양으로 내려갔다. 유충은 토양에서 번데기가 되고 월동하였으며, 이듬해 봄 성충으로 우화하였다.

참취 식물체에 발생한 rosette gall은 4단계의 발달과정을 거쳤다. Rosette gall 발생초기는 모식물체의 어떤한 기관 표면에서라도 작은 gall 조직들이 발생하였다. 둘째단계에서는 gall 조직으로부터 ectopic 소엽들이 분화하였으며, 셋째단계에서는 ectopic 잎들은 성장하였으며, rosette 모양의 작은 소형식물체로 발달하였다. 그리고 이 소형식물체는 꽃기관으로 분화하는 마직만 단계를 가졌다. Rosette gall의 전분화과정은 식물세포의 탈분화와 재분화를 형성하는 식물조직배양계에서의 체세포 배분화 단계를 거쳐 식물 재분화 단계로 이루어지는 발달과정과 유사하였다. rosette gall 분화는 *D. asteriae* 유충의 조절에 의해 발생하는 것으로 나타났다. 초기 rosette gall 발생은 곤충의 유충이 정착한 참취 식물체의 조직 표면에서 gall 분화가 시작이 되었으며, 성충이 산란한곳에서는 gall 분화가 발생하지는 않았다. 따라서 참취 식물조직상에 초기 gall 형성은 곤충의 유충에 의해서 나타나는 것이었으며, 성충이나 그 알들에 의해서 gall 들이 형성되는 것은 아니었다. Rosette gall의 ectopic 잎과 꽃기관이 발생하는 기간 동안 유충들은 gall 조직내에서 서식하였으며, 이것은 분화된 모식물세포로의 탈분화 과정과 탈분화된 gall 조직이 ectopic 소엽으로 발생하는 재분화 과정, ectopic 잎의 정형화 및 성장 그리고 ectopic 꽃기관의 발생을 포함하는 rosette gall의 모든 분화 과정을 곤충

의 유충이 조절 한다는 것을 시사한다고 할 수 있다. 왜성의 rosette gall들은 또한 유충들의 거주지라 할 수 있다.

유전자 수준에서 rosette gall의 발생을 이해하기 위하여 기주식물인 참취의 cDNA library 와 rosette gall의 식물호르몬 유전자들을 분석하였다. 총 2306개의 cDNA 들을 참취 식물체로부터 얻었으며, 1843개의 unigene 들을 분석한 결과 잠재적 기능이 알려진 유전자는 45% 였으며, 기능을 알 수 없는 유전자는 13%, 그리고 no hit 유전자는 42% 이었다. 그 중 잠재적 기능이 알려진 유전자 중 식물호르몬 대사체계에 관여하는 유전자는 28개가 있었다. 식물호르몬인 옥신, 시토키닌, 지베렐린의 생합성 단계에 관여하는 유전자인 NIT, IPT, GA3ox 를 선발하여 발현 분석을 수행하였다. NIT는 rosette gall의 ectopic 소엽 조직에서 발현이 높았으며, gall 부분의 조직은 모식물체의 잎조직과 발현 양상은 유사하였다. IPT는 모식물체의 잎 조직에 비하여 rosette gall에서 과발현하는 양상을 나타냈다. 그러나 ectopic 소엽 조직과 gall 부분의 조직 간에는 발현 양상은 유사하였다. 시토키닌은 식물의 뿌리에서 합성되는데, rosette gall에서 IPT 의 높은 발현양상은 놀라운 결과라고 추정이 되어진다. NIT와 IPT의 과발현 양상은 옥신과 시토키닌의 합성량을 조절함으로써 rosette gall의 형태를 결정하는 것으로 추정이 된다. NIT와 IPT의 발현 양상과는 대조적으로 GA3ox는 rosette gall의 ectopic 소엽 조직에서는 발현 양상이 낮았으나, gall 부분의 조직은 모식물체의 잎조직과 발현양상이 유사하였다. 따라서 왜성 rosette gall의 내부 소엽 조직에서의 GA3ox의 낮은 발현양상은 지베렐린 생합성 단계에 참하는 효소를 암호화하는 지베렐린 3 β 수산화 효소의 감소에 의한 결과라고 추정이 된다. 잠재적 기능을 알 수 없는 유전자인 REA28은 흥미롭게도 GA3ox의 발현 양상과는 대조를 이루었다. RAE 28은 rosette gall의 내부 소엽 조직에서 발현이 매우 높았으며, gall 부분 조직에서는 발현이 현저히 낮았다. REA28은 450 bp의 ORF를 갖고 있었으며, 150 amino acid 로 번역되었다. 또한 intron 영역이 존재하지 않았으며, 그리고 regulatory element 로 작용할 것으로 추정이 되었다. Rosette gall 조직에서의 REA 28의 과발현 양상은 GA3ox 의 발현 억제를 수반함으로써 이는 아마도 지베렐린의 합성을 저해함과 동시에 왜성의 rosette gall 발달을 조절한다고 추정이 된다.

ABBREVIATIONS

SEM	Scanning electron microscopy
EST	Expressed sequence tag
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
PCR	Polymerase chain reaction
RACE	Rapid amplification of cDNA ends
RT-PCR	Reverse transcription polymerase chain reaction
DDRT-PCR	Differential display Reverse transcription polymerase chain reaction
SQRT-PCR	Semi-quantitative reverse transcription polymerase chain reaction
CK	Cytokinin
GAs	Gibberellins
IPT	Isopentenyltansferase
NIT	Nitrilase
GA3ox	Gibberellin 3β-Hydroxylases
ARP	Auxin-repressed protein
CCoAOMt	Caffeoyl-CoA 3-O-methyltransferase
UGT	UGT-glycosyltransferase

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SUMMARY

The plant galls occurring in *Aster scaber* Thunberg had unique morphological characteristics. Their apparent shape at mature stage resembled a miniature plant which showed a well-organized rosette-like morphology (rosette gall), although they showed extreme dwarfism. Furthermore, the rosette gall formed a flower-like organ at the later stage of development. The rosette gall of *A. scaber* was reported long time ago by Shinji in 1944, however there are no subsequent studies on it until now except only a few entomological survey. In this study, the rosette gall of *A. scaber* was investigated in relation with botanical characteristics (Part I), inducer organism (Part II), developmental process (Part III) and genes involved in its development (Part IV) to understand the overall process and regulatory factors in the plant gall development.

The basic structure of mature rosette gall of *A. scaber* consisted of hemispherical gall basement, ectopic leaves and flower-like organ(s). The sizes of normal mature rosette galls were 0.3 - 2.0cm in diameter and 0.3 - 1.0 cm in height, respectively. Typical rosette galls of *A. scaber* showed dwarfism and did not develope petiole, internodal stem, inflorescence or root. However, some of the rosette galls developed leaf petioles. Even though rarely, the rosette gall formed floral stalk and developed *de novo* roots. The rosette gall was developed from any organ of the mother plant including leaf (adaxial and/or abaxial surface), petiole, stem, node, shoot apex and even root. In case of the leaf, the rosette galls were usually developed from the non-meristemic cells between leaf veins unlike ordinary plant organs which differentiated mostly from the meristemic cells near the veins. Together with the non-organ specificity of rosette gall formation, the non-merstemic developments of rosette galls showed direct evidence for the intrinsic totipotency of the plant cells regardless of their origin and degree of differentiation.

The inducer organism of rosette gall in *A. scaber* was identified as an insect, *Dasineura asteriae* Shinji (Diptera Cecidomyiidae). In Jeju, Korea the adult insects

mated and layed eggs from April to May when the radical leaves of *A. scaber* emerged from the soil surface. The female layed eggs on the surface of young leaves of the host plant. The egg hatched in 2-4 days, and the larva moved around and settled down at a certain position on the surface of plant organs where the rosette gall was later developed. Thereafter the larva made a chamber within the gall tissue of the rosette gall and resided in the chamber. The larval chamber became surrounded by ectopic leaflets with growth of the rosette gall, and later the chamber developed an additional organ resembling floral bud inside of the rosette gall leaflets. Two or more larvae were often observed in a single rosette gall, and in this case each larva occupied a floral bud. The larva stayed within the rosette gall throughout its larval stages. When the rosette gall aged to senescence in the late of autumn, the larva came out of the rosette gall and entered into the underground soil. The larva developed to a pupa and hibernated during winter time in the soil until next spring.

The developmental process of rosette gall in *A. scaber* was devided into four phases. The rosette gall initiated with forming a tiny gall tissue on the surface of any organ in the mother plant. In the second phase, the ectopic leaves were differentiated from the gall tissue. In the third phase, the ectopic leaves grew and were organized into a rosette shape miniature plant. At last a floral organ was developed from the center of the rosette gall. The overall process of rosette gall development showed close similarity with the plant regeneration *via* somatic embryogenesis in the laboratory tissue culture which consisted of dedifferentiation and redifferentiation of plant cells. The development of rosette galls seemed to be regulated by the larva of *D. asteriae*. At the beginning of the rosette gall development, the initial gall was formed from the point of plant surface where insect larva settled down, not from the point of oviposition. Therefore the gall formation was appeared to be initiated by the insect larva, neither by egg nor adult female. The larva stayed inside the rosette gall throughout the development of ectopic leaves and floral organs. This indicated that the larva controlled all the processes of the rosette gall development including dedifferentiation of differentiated mother plant cells, redifferentiation of gall cells to

ectopic leaves, growth and organization of ectopic leaves, and development of ectopic flowers. The dwarfism of the rosette galls also seemed to be related with the larval residence.

For the understanding of rosette gall development at the gene level, the cDNA libray of the host plant, A. scaber and phytohormonal genes of rosette gall were analyzed. The clones of 2306 cDNA from the host plant were clustered into 1843 unigenes which consisted of 45% of known function, 13% of unknown function and 42% of no hit genes, respectively. The library contained 28 genes which putatively related with phytohormone metabolism. Of these three hormonal genes of NIT, IPT and GA3ox encoding key step enzymes in the synthesis of auxin, cytokinin and gibberellin, respectively, were cloned and their expressions were examined. NIT was highly expressed in the ectopic leaves of the rosette gall while its expression in the gall part was similar to mother leaf. In case of IPT, it was highly expressed not only in the ectopic leaves but also in the gall tissue by the similar level. The high expression of IPT over the whole rosette gall was very surprising because cytokinin is known to be synthesized in roots in plants. The high expressions of NIT and IPT suggested that the compact morphology of rosette gall might be related with the levels of auxin and cytokinin. In contrast to NIT and IPT, GA3ox was suppressed in the ectopic leaf of rosette gall while its expression in the gall part were similar to mother leaf. Thus the dwarfism of the rosette gall seemed to be resulted from the lack of gibberellin due to the suppression of GA3ox in the inner leaf because gibberellin 3beta-hydroxylase encoded by this gene mediates the final step in the synthesis of active gibberellin. Interestingly, the expression of an unknown gene denoted as GAS was high in the ectopic leaf of rosette gall and low in the gall part which showed inversed relationship with the expression of GA3ox. The length of this gene was relatively short, 450bp (150 amino acid), and had no introns which supposed to play as a regulatory element. The coincident suppression of GA3ox with the expression of GAS suggested that this gene might control the dwarfism of rosette gall, probably via inhibition of GA synthesis.

PART Ⅰ. Botanical Characteristics of the Rosette Gall

Abstract

The plant galls occurring in *A. scaber* took a special interest because they grew to miniature plants showing a unique well-organized rosette-like morphology (rosette gall) although they showed extreme dwarfism. The normal sizes of mature rosette galls were 0.3 - 2.0cm in diameter and 0.3 - 1.0 cm in height, respectively. One of the most distinctive feature of these secondary ectopic mini-plants was to develope even a floral organ unlike other ordinary plant galls forming only an amorphous gall tissue. The typical rosette galls of *A. scaber* did not develope petiole, internodal stem, inflorescence or root. However, some of the rosette galls developed petioles and/or floral axes. Furthermore, the rosette galls showed sometimes *de novo* development of roots even though this was rarely observed under the open field condition.

The rosette galls were usually formed on a leaf surface of the host plant. In this case, the rosette galls are developed from the non-meristemic cells between leaf veins unlike ordinary plant organs which differentiated mostly from the meristemic cells near the veins. In addition, the rosette galls could be developed from any organ of the host plant including leaf (adaxial and/or abaxial surface), petiole, stem, node, shoot apex and even root under the natural environment. The developments of rosette galls from the non-merstemic cells of various differentiated organs of *A. scaber* provided direct evidences for the intrinsic totipotency of the host plant cells regardless of their origin and degree of differentiation.

Introduction

Aster scaber Thunberg, a perennial herb of the Asteracease family, is widespread and cultivated as culinary vegetables in Korea (Kim, 1996). The leaf is 9 to 24 cm long, 6-1 cm wide, heart shaped with serial edge, and both leaf surfaces pubaceous. The roots are thick and sort. *A. scaber* flowers are develop from August to October, and the seeds are ripen from September to November. The flower is hermaphrodite, white color. The involurce is hemisphere, 4-5m long, 7-9 mm wide (Kim, 1996). The root and aerial part of *A. scaber* have been used traditional medicine to detoxify, ameliorate pains, stimulate blood circulation, and reduce throat inflammation (Chung and Lee 2001). Presents of 17 Aster genus in Korea which are *A. koraiensis*, *A. puinnatifidus, A. incisus, A, yomena, A. pekinensis, A, hispidus, A. maackii, A, tataricus, A. ageratoides, A. fastigiatus, A. scaber, A. cilious, A. altaicus* var. uchiyamae. *A. spathylifolius, A. tripolium, A. glehni. A. associatus* (Kim, 1996).

Gall formation is defined as pathologically developed cells, tissues or organs of plants that have arisen by hypertrophy (increase in cell size; cell enlargement) and/or hyperplasia (increase in cell number; cell proliferation) as a result of stimulation from various organisms including fungi, bacteria, viruses, nematodes, mites and insects (Mani 1964). Morphological criteria and mode of gall formation are used to classify galls. Küster (1911) has classified gall into two groups: organoid and histioid, the latter being further subdivided into kataplasmatic galls and prosoplasmatic galls (Dreger-Jauffret and Shorthouse 1992). According to Dreger-Jauffret and Shorthouse (1992), a more empirical classification used terms such as filz, pit, pouch, roll, covering, mark, bud and rosette gall.

The Rosette galls observed on *Aster scaber* plants growing in Jeju island, located southernmost of Korean peninsula. These galls are form on the surface of leaves and all

organs of *A. scaber* and have a rose-like structure. The rosette gall of *A. scaber* was reported long time ago by Shinji in 1944, however, detail botanical illustrated of rosette gall is not recoded. Therefore, in this study, we described here the botanical characters of the rosette galls of *A. scaber*.

Materials and Methods

Collecting plants of *A. scaber* and rosette galls

The rosette galls used in this study were collected from the area of the deciduous forest plants or grass field at 300 to 530 m above sea level in Jeju island, Korea. The GPS sites of rosette gall collections were 33°25′47"N/126°33′22"E (near Gwaneum temple), 33°26′13"N/126°34′04"E (near Jeju College of Technology), 33°27′09"N/126°44′12"E (Seonheul-ri) and 33°22′01"N/126°22′11"E (near Sogil-ri).

When the plant samples were collected, the rosette gall was not separated from the mother plant, instead the intact rosette gall was collected together with a mother plant. After collection, the plants of *A. scaber* bearing rosette galls were put in a polyethylene film zipper bag with some soils, brought to laboratory in a few hours, and transplanted in pot or greenhouse for propagation. The rosette galls were collected at the juvenile stage or at the early adult stage of mother plant not to disturb the development of rosette galls. The rosette galls were collected over the year of 1996 to 2008.

Propagation and observation of rosette galls

The amount of available rosette gall was very limited in the wild and the collected rosette galls were propagated in a greenhouse located in the Ara campus of Cheju National University. For mass propagation, the plant of *A. scaber* bearing rosette galls were collected from the field and cultivated in a greenhouse covered with transparent polyethylene film. The collected plants were divided into two groups, one group was directly planted in the ground soil in greenhouse. The soil in the greenhouse was The Jeju series in which volcanic ashy integrated to Red-Yellow soils (http://asis.rda.go.kr/soil_intro//soilseries/j/JEJUe.htm).

The other group of plants were washed to remove soil and parasites prior to transplantation, and then planted into a sterilized soil mixture of peat moss and

vermiculite (7:3)) in a transparent plastic chamber (50 x 50 x 50cm l/w/h). Top side of a chamber were covered with a double layer of white nylon material instead of plastic plate for aeration and water supply. About twenty plants were planted in each chamber and the chamber was placed in a greenhouse. The greenhouse was maintained under the ambient condition including light, humidity, temperature, aeration without additional fertilization. Water was supplied by spray nozzle if necessary.

The rosette gall was observed visually or under stereo micropscope (OLYMPUS SZX-ILLB100, JAPAN). The *in situ* photograph of the gall was taken by a digital camera (Cannon EOS 20D). Some of the rosettes galls were preserved in a FAA solution (50% ethanol, 5% glacial acetic acid and 3.7% formaldehyde (v/v)) and used for anatomical study under microscopes.

Results

Structure of rosette gall in *A. scaber*

The plant gall observed in *Aster scaber* had a rosette-like shape which resembled a dwarf miniature plant as shown in Figure 1 (B) and a floret-like structure appeared in the innermost region of the rosette as the gall mature (Figure 1. C). The sizes of normal mature rosette galls were 0.3 - 2.0cm in diameter and 0.3 - 1.0 cm in height, respectively. The basic structure of mature rosette gall consisted of hemispherical gall basement, rosette-like organization of ectopic leaves and floret-like organ. The floret-like organ consisted of several petals which distinguished from leaflets of rosette gall and it was not further developed to a fertile flower. The usual rosette galls of *A. scaber* did not develop any other plant organs. However, a flower stalk was developed from a certain rosette gall although it was rare and did not grew to produce normal flower (Figure 1. D). Furthermore, some rosette galls developed a root-like structure at the gall basement (Figure. 1. E).

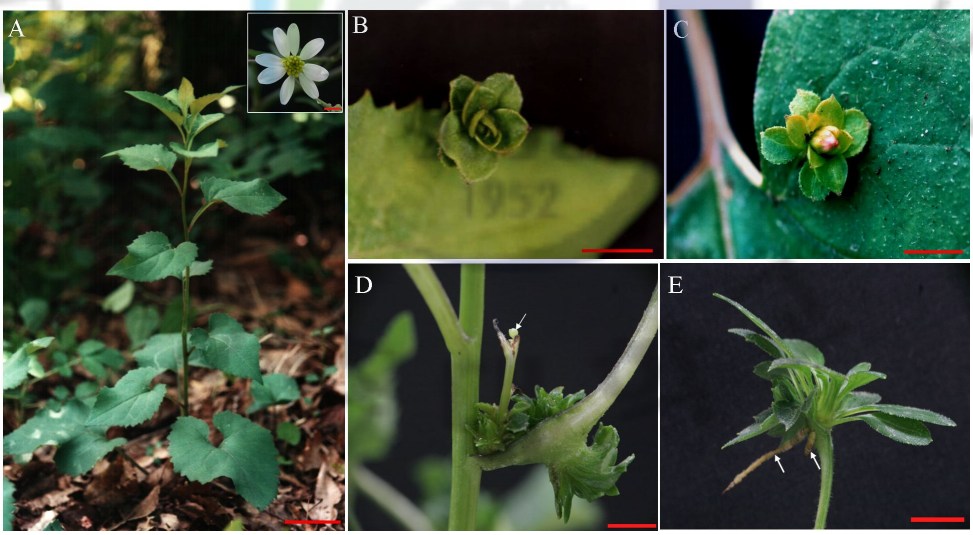

Figure 1. The rosette galls observed in *Aster scaber* Thunberg. A: a wild type plant and flower of *A. scaber*. B: a typical rosette gall on a mother leaf, C: a rosette gall with floral organ at mature stage, D: an unusual type of rosette gall developing a floral stalk, E: another rare type of rosette gall developing ectopic roots. Scale bar is 1cm.

Wide variations of the rosette galls were observed in morphology of ectopic leaf, in structure of the rosette, and in color of the floret-like organ. Most of the rosette gall showed extreme dwarfism and petioles were not developed in the ectopic leaves. However, some of them developed petioles as in Figure 2. (A). The leaflets of rosette gall usually developed separately, however leaflets fused by two or more were often found and an extreme example was shown in Figure 2. (B). In addition to the fusion between ectopic leaves the fusion was also observed between whole rosette galls as shown in Figure 2. (C). Many rosette galls formed single floret-like organ, sometimes several floret organs might be appeared in a rosette gall as in Figure 2. (D). The color of wild type flower was white (Figure 2. E), however that of floret-like organ in rosette gall varied from white (Figure 2. F) to yellow-green (Figure 2. G) and pink (Figure 2. H). The diversity in color was observed not only at the initial stage of floret-like organ development but also at the mature stage.

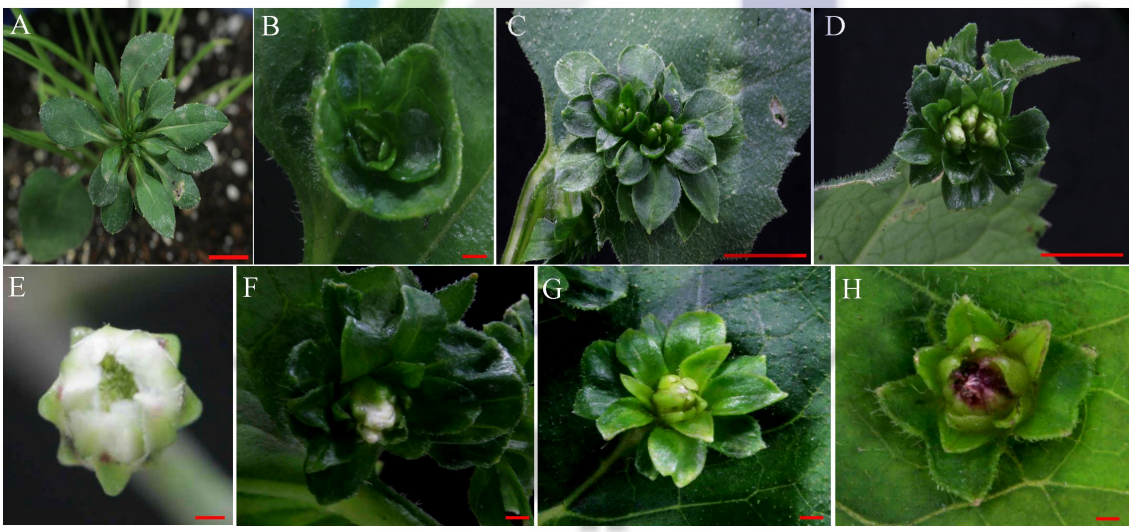

Figure 2. Diversities of the rosette gall of *A. scaber* in morphology and in color of ectopic flower. A: a rosette gall developed petioles (Scale bar is 1cm), B: A fused ectopic leaf in a rosette gall (Scale bar is 0.1cm), C: a rosette gall fused by two (Scale bar is 1cm), D: Three ectopic flowers in a single rosette (Scale bar is 1cm); Color of flower bud: wild type (E, white), white (F), yellow-green (G), pink (H) flower bud of rosette gall at mature stage. Scale bars are 0.1cm (E, F, G, H).

Locations of rosette galls within a mother plant

Many of the rosette galls were found on the leaf of *A. scaber* as in Figure 3. (A). The rosette galls were formed not only on the adaxial surface of leaf but also on the abaxial side as well as on the edge. The rosette galls also occurred on the petiole as shown in Figure 3. (B). Single leaf might have two or more rosette galls, and several rosette galls often appeared simultaneously on the adaxial/abaxial surface, the edge in a leaf and the petiole. In addition to leaf, the rosette gall was observed in the other plant organs including shoot apex (Figure 3. C), node (Figure 3. D), internodal stem (Figure 3. E). Sometimes the rosette gall was developed even from root (Figure 3. F). The formation of the rosette gall was not confined to a specific organ and any part of a plant including leaf, petiole, shoot apex, node, stem and root appeared to develop the rosette gall in *A. scaber*.

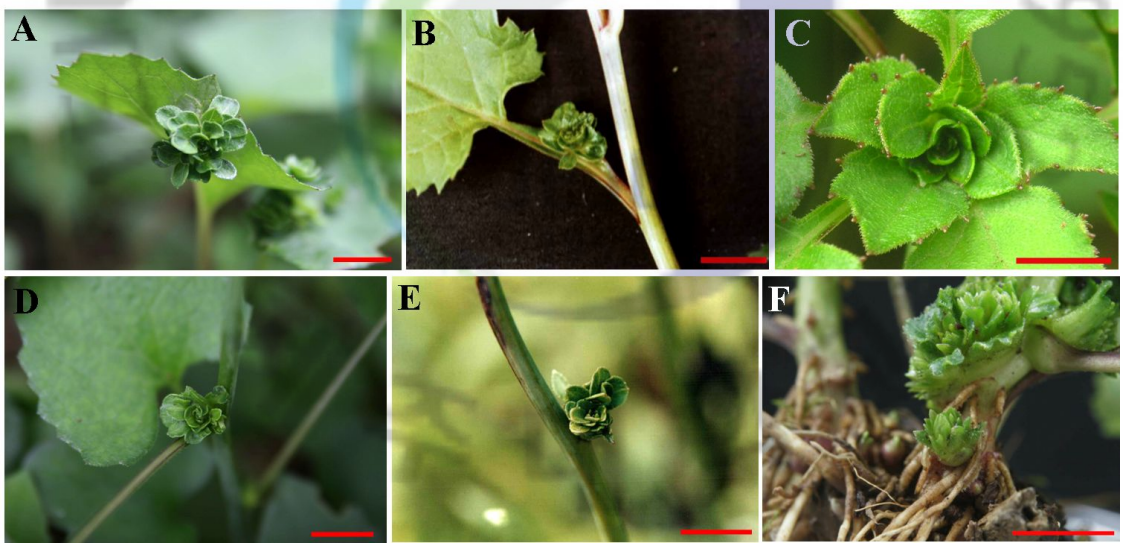

Figure 3. The organs of mother plant to develop rosette galls. A: adaxial and/or abaxial leaf surface, B: petiole, C: shoot apex, D: node, E: internodal stem (arrow), F: root (arrow). Scale bar is 1 cm.

The development of ectopic leaflets in a rosette gall was unidirectional and all the leaflets grew to the same direction opposite to gall basement (Figure 4. A). The ectopic leaflets of a rosette gall might appear on either adaxial or abaxial side of a mother leaf, none of them, however, was developed from the gall basement (Figure 4. B). On the other hand, the location of each gall basement showed the exact site of rosette gall development in a mother leaf. Some of the rosette gall basements were located on the secondary veins and many of them at the region between main veins of a leaf as shown in Figure 4. (B). This indicated that the rosette gall might be developed from non-veinous leaf cells unlike ordinary plant organs which differentiated from the meristemic cells.

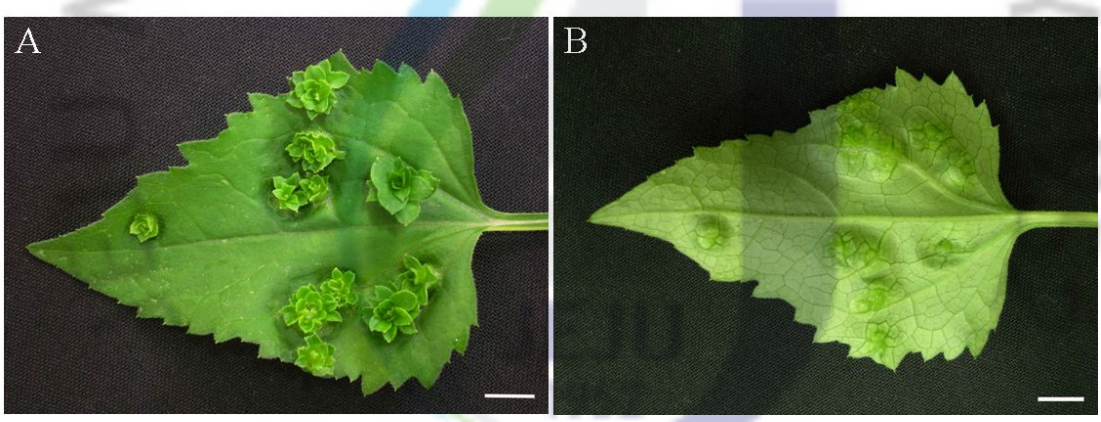

Figure 4. Unidirectional growths of ectopic leaflets in rosette galls and locations of rosette gall formation in a mother leaf. Top-view of ectopic leaflets showing unidirectional growth of rosette galls (A), Bottom-view of gall basements showing the developmental point of rosette galls in a mother leaf (B); Scale bar = 1cm.

Discussion

Plant galls were known and used extensively in medicine, industry, and even as human food for over 1000 years in China, India, and Europe. In 1679, the first scientific studied of galls were those of Malpighi of Italy. Since then thousands of galls and causative organism have been described from around the world. In the 1900s, many cecidologists studied for plant gall; curiou object of natural history, Pathological aspects, Morphogenetic problems of galls, origin and evolution of the complex interspecific interrelationships, and nature and factors underlying neoplasm. Recently, every modern scientific technique is emploied in gall studies (Mani 1992).

Galls occur on all plants from algae and fungi to higher plants. Insects-induced galls occur on gymnosperms and angiosperms. Dicotytedons have over 92% of all types of plant galls while monocotyledons account for about 6% and gymnosperms 2% (Mani 1964). Certain angiosperm families serve as host to gall-inducer. Galls are most commonly found on member of the Compositae, Rosaceae and Fagaceae families. Gall most frequently form on leaf blades (80%), with the remainder induced on petioles, stems, buds, fruits, and roots.

Küster (1911) has classified gall into two groups: organoid and histioid, the latter being further subdivided into kataplasmatic galls and prosoplasmatic galls (Dreger-Jauffret and Shorthouse 1992). Organoid galls result form the proliferation of organ or are a modification of organ in which the attacked organs remain recognizable. Examples of organoid galls are abnormally shaped leaves, Fasciations (ribbon-like stems resulting from fusion of apices), chloranthy (greening of petals), and witches' brooms (many buds developing at one spot). Parasitic fungi (Uredinaceae, Ustilaginacea, Exoascaceae, etc.), mites, aphids, and virusare the incitants in organoid galls (Mani 1964,). Histioid galls are complex characterization and derived from a proliferation on modified cells that lead to a new organ. Like organoids, hyperplasia and hypertrophy occur in histioid galls, but unlike organoids,

some degree of de- and re-differentiation occurs in histioids. These galls are produced mainly by Diptera (Cecidomyiidae) and Hymenoptera (Cynipidae) (Mani 1964, Larew 1982, Dreger-Jauffret and Shorthouse, 1992).

According to Dreger-Jauffret and Shorthouse (1992), a more empirical classification used terms such as filz, pit, pouch, roll, covering, mark, bud and rosette gall. Filz galls are characterized by the hairy epidermal outgrowths. Most filz galls are induced on the underside of the leaf blade and in this type of gall the inducer is situated externally. Pit galls are characterized by slight arching of the leaf blade and sometime surrounded by a swelling. The simplest of these galls are hardly visible and are called "rudimentary galls." Pouch galls developed first as a bulging of the leaf blade and forming an invaginated pouch on one side and a prominent bulge on the other. These galls are provoked mostly by Acarina, aphids, ceccidomyiids, and psyllids. Roll and fold galls may present different degrees of complexity and there are turned-over leaf blades. There is usually an opening to the outside referred to as an ostiole. Thysanoptera, aphids, psylleds, and cecidomyiids are mostly responsible for this type galls. Covering galls often present a greater degree of differentiation than the previous form. In this type of gall inducer, acting externally at first, provokes a strong hypertrophy of the surrounding tissue. These type galls are mostly caused by Acarina, aphids, coccids, cecidomyiids, and cynipids. Mark galls are arise mostly on stems, but also can be found on leaves. The larvae are completely enclosed within plant tissues from the beginning of gall development. In this case the egg is deposited inside the tissue (tenthredinids) or the lavar bores into the tissue (tenthredinids) or the larva bores into the tissue (tephritids and cecidomyiids). Bud and rosette galls arevery complex and cause enlargements of buds or a multiplication of the leaves resulting in rosette galls. Most bud galls develop during the spring. They are usually globose, but some are flask shaped. Often the gall insect uses the special potential of the vegetative point causing an arrest of the elongation of the internodes and a multiplication of the leaves resulting in rosette galls, which resemble a miniature cabbage or pine cone.

Morphological characteristics of the rosette gall in *A. scaber*

Rosette galls of histioid with prosoplasmic gall type were observed on vegetative organs of *A scaber* growing on volcanic soil in Jeju island. Basic structure of gall on *A. scaber* were hemispherical gall, and its developing to well-organize rosette-like structure. Also, galls seem to be floral-like structure developed in autumn. Floral-like structures appear as the inner most organ of the gall develop in the mature stages at approximately the same time as normal flower

The typical rosette gall on *A. saber*, when juvenile stage rosette shown dwarfism form and its did not developing fused leaflets shape, petiole, internodal stem, inflorescence, and roots. Ectopic leaflets of rosette galls were developed plantlets on *A. scaber*. It is resemble leaflets in appearance to leaf of mother plant. A comparison of the rosette galls shows a progressive increase in the relative number of mature leaflets. However, galls founded fused leaflets together with galls, especially of the outer most leaflets, but most rosette leaflets remain singly. Interestedly, sometime galls founded rare types which are developed leaf petioles, floral axes, and de novo developmental type of roots on gall. When mature stage rosette galls were developed floral-like structure with stalk, petal-like, however, its did not fertile flower. The rosette galls have a various form, and with colors which are white, yellow-green, and pink. In contrast, normal flower of A. scaberwas only white colors.

Most rosette galls are induced from bud tissue and consist of sworls of overlapping scales or leaves develop. The rosette galls reported are rosette gall of Acacia leucophloea(Raman & Ananthakishnan 1983), rose rosette (Doudrick et al. 1986), apical rosette galls of Solidago altissima (Raman et al. 1995), rosette gall on Salix sp (Dreger-Jauffret and Shorthouse 1992). The morphology of these rosette galls are quite different from those observed in Jeju Aster. Rose rosettes show a form of witche`s broom, Solidago galls are formed on the apical galls, similar to witche`s broom. Acacia galls are artichoke galls form on axillary buds. Salix galls are large leaf rosette form on shoot-tip. In Japan, similar rosettes galls were reported

early (Shinji, 1944). Leaflet rosette developing from bud leaves and stem found on *A. scaber*. However, none of these rosette galls are reported to develop flowers of flower-like structures.

Botanical significance of the rosette gall

Plant galls are variously formed as a result of specific interaction with organisms and their unique patterns of differentiation on tissue of host (Mani 1964, Raman 2008). Many bud galls are reported to alter the host plant morphology, developed in the apical meristemic tissue on the host plant, and generally significantly different morphology (Silva et al 1996, Westphal 1977, Dreger-Jauffret 1977, Jauffret et al 1977, Meyer 1969, Raman and Ananthakrishnan 1983). Rosette galls were shown morphological diversity including fused leafletsform, leaf petioles develop form, floral stalk form, roots-like develop form, and ectopic flowers with various colors develop on galls. These galls were rare types and observed under the open field condition. Therefore, these significances of developmental and morphological diversity in rosette galls are suggest unique patterns with variously developmental, morphological characters on *A. scaber*in Jeju island, Korea.

The rosette gall formed on almost all part of *A. scaber* including leaf, petiole, stem, node, shoot apex and even root. However, galls were most frequently on adaxial surface and/or abaxial surface of leaf on *A. scaber*. In addition, rosette galls were developed form meristemic cell in leaf veins, however, galls developed in secondary leaf veins. In this case, rosette galls were usually developed from non-meristemic cells between leaf veins. Therefore, these results with the non-organ specificity of rosette gall formation, the non-merstemic developments of rosette galls showed direct evidence for the intrinsic totipotency of the plant cells regardless of their origin and degree of differentiation.

PART Ⅱ. The Insect Inducing the Rosette Gall

Abstract

The rosette gall of *A. scaber* appeared to be induced by an insect which was identified as *Dasineura asteriae* Shinji (Diptera Cecidomyiidae). Under the natural condition of Jeju, Korea the adult insects mated and layed eggs from April to May when the radical leaves of *A. scaber* emerged from the soil surface. The female layed eggs on the surface of young leaves of the host plant. The egg hatched in 2-4 days, and the larva moved around and settled down at a certain position on the surface of plant organs where the rosette gall was later developed. Thereafter the larva made a chamber within the gall tissue of the rosette gall and lived in the chamber. The larval chamber became surrounded by ectopic leaflets with growth of the rosette gall, and later the chamber developed an additional organ resembling floral bud inside of the rosette gall leaflets. Two or more larvae were often observed in a single rosette gall, and in this case each larva occupied a floral bud. The larva resided within the rosette gall throughout its larval stages. After the rosette gall aged to senescence in the late of autumn (November), the larva came out of the rosette gall and entered into the underground soil. The larva developed to a pupa and hibernated during winter time in the soil until next spring.

Introduction

Plant galls are induced by specific interactions between plants and a wide variety of organisms, including insects, mites, nematodes, fungi, viruses and bacteria (Mani 1964; Meyer 1987). Gall forming insects account for about 2% of all described insect species, with approximately 13,000 sepecies known. About 13,000 gall-forming insects have been recorded in seven orders of insects: Thysanoptera, Hemiptera, Homoptera, Lepidoptera, Coleoptera, Diptera and Hymenoptera. Due to the specific interaction between the plant and the gall-forming insects, one of a variety of gall types is formed (Weies *et al*., 1988; Dreger-Jauffret and Shorthouse, 1992; Williams, 1994).

A number of insects, representative of several genera, cause the formation of rosette-like galls. Induced in this group are organism from the genera *Asphondylia, Bayeria, Dasineura, Gallacoccus, Marcolabis, Rhopalomyia*, the *Thilak* that stimulate rosette-like gall formation in specific species in the genera Baccharis, Euphorbia, Aster, shorea, Populus, Solidago, Acacia, and Rosa respectively (Gagné and Boldt, 1995; Solinus and Pecora, 1984; Shinji, 1944; Anthony, 1977; Skuhrav *et al*., 1997; Raman and Abrahamson, 1985; Raman and Anthakrishnan, 1983; Amrine and Hindal, 1988).

Among gall midge species (Diptera: Cecidomyiidae) is gall formation on the plant and with approximately 5500 described species (Hsrris 1994, Gagné 2004). They are remarkable in that each essentially species specific induces a structurally distinct gall (Rohfritsch 2008, Yukawa *et al* 2005). In korean Peninsula and surrounding islands, a total of 52 gall-inducing species of Cecidomyiidae have been recorded (Paik *et al.,* 2004). Therefore, In this study, to identification of rosette gall inducer on *A. scaber*, and there describe the life cycle as recorded in Jeju island.

Materials and Methods

Insect collection

Adults, pupae were collected on insect collection pots. Eggs were collected on leaf surface of *A. scaber* after insects laying on insect collection pots. Larval were collected from leaf surface and rosette galls.

Insect identification

Adults, eggs, larval and pupae specimens collected in this study were preserved in 70% ethanol or live for morphological studies. Adults female specimens preserved in 70% ethanol and were subsequently mounted on slide for identification. The identified specimens were confirmed by Dr. Makoto Tokuda at kysuhu Univiersiy in Japan.

Mass rearing insect

For rearing insects, collection and their life cycle observation were a design of rearing pots (Figure 4). *A. scaber* and rosette galls were collected from grasses field in Seonheul-ri, Jeju-city, Jeju-do (33°27′09" N, 126°44′12" E) on 2002 between 28 October and 2 November. Roots of *A. scaber* were washed, transplants into sterilized soil (combined peat moss and vermiculite (7:3)) on rearing pots (50 x 50 x 50cm l/w/h (Figure A), 30 x 25 x 25cm l/w/h (Figure B)). About 400 rosette galls were put onto sterilized soil on rearing pots (Figure 5 A). About 800 larval were put onto sterilized soil on rearing pots (Figure 5 B, 18 x 18 x 25 l/w/h (Figure 5 C)). Rearing pots were covered with a double layer of white nylon material. Pots were placed in nature, greenhouse and laboratory.

Microscopy analysis

Developmental stage of rosette gall and larval live samples were 70um sectioned using an Vibratome Series 1000(USA). The sample were observed by light microscopy(OLYMPUS BX 60-F3, JAPAN). Insect morphological characters were observed by Stereomicroscope (OLYMPUS SZX-ILLB100, JAPAN). Larval in gall were examined with a SEM (Hitachi, Japan).

Figure 4. Rosette galls growth on *A. scaber* in rearing pot.

Photographed 2003. 8 June. Pot size is 50cm x50cm x50cm

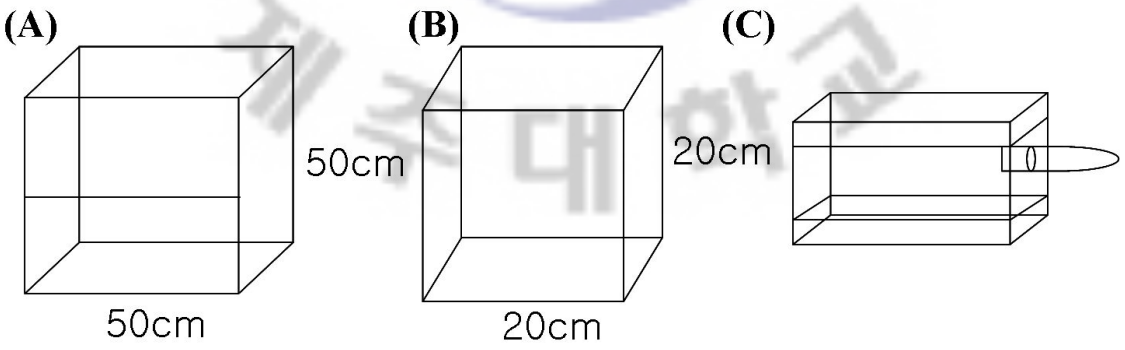

Figure 5 . For insect collection and rosette gall growth port

A. pot size is 50 x 50 x 50cm l/w/h, B. pot Size is 30 x 25 x 25cm l/w/h, C, size C, pot size is 18 x 18 x 25 l/w/h

Results

Morphology of insect in each development stage

To investigated of insect morphology by light microscopy and Stereomicroscope. The female *of Dasineura assteriae* Shinji were collection on rearing container at 2003 17 March in laboratory. The body is reddish yellow color; the female is 2.53 ± 0.51 mm long (n = 5), 2 wings, 6 long legs, Antenna with 18 flagellomeres on Head. The male *of Dasineura assteriae* Shinji were collection on rearing container at 2003 17 March in laboratory. The body is light yellow color; the male is 2.82 ± 0.16 mm long (n = 5), 2 wings, 6 long legs, Antenna with 17 flagellomeres on Head (Figure 6 A).

Eggs (Figure 6 E) of are about 1 - 1.5 mm long, light orange, slightly elongate, with rounded ends, and have a smooth. The female *D. sateriae* observed were layed eggs (Figure 6 D) in the end of body with ovipositor. The ovipositor is too short, deep pink color, elongate-protrusible for laying (Figure 6 C).

Larva (Figure 6 F, G, H) showed developmental stage of larva of *D. sineura*. Larva grown to after hatched from egg until before pupate in lived gall were observed. The larvae were observed in the gall from early May to November. Size is about 1.0 ~ 3.0 mm long, Cylindrical and Slightly sharp at both ends, Reddish yellow color, no foot, very small head, head capsule with integrity and ossification weak, tentacle with one pair and short, eight segments in abdomen

Pupa were collected from rearing container. they are about 3.0 mm in length, brown color, very small head, Cylindrical and Slightly sharp at both ends, tentacle with one pair and short, eight segments in abdomen (Figure 6 I, J). A cocoon were light brown color (Figure 6 K)

Identification of the insect inducing the rosette gall of *A. scaber*

The female of gall midge were collection on rearing port (Figure 5 C) in laboratory. The gall midge insects were identified by Dr. Maokoto Tokuda at kysuhu

University in Japan. This identification result that the gall midge was *Dasineura asteriae* Shinji of the family cecidomyiidae of the order diptera (Figure 6 B).

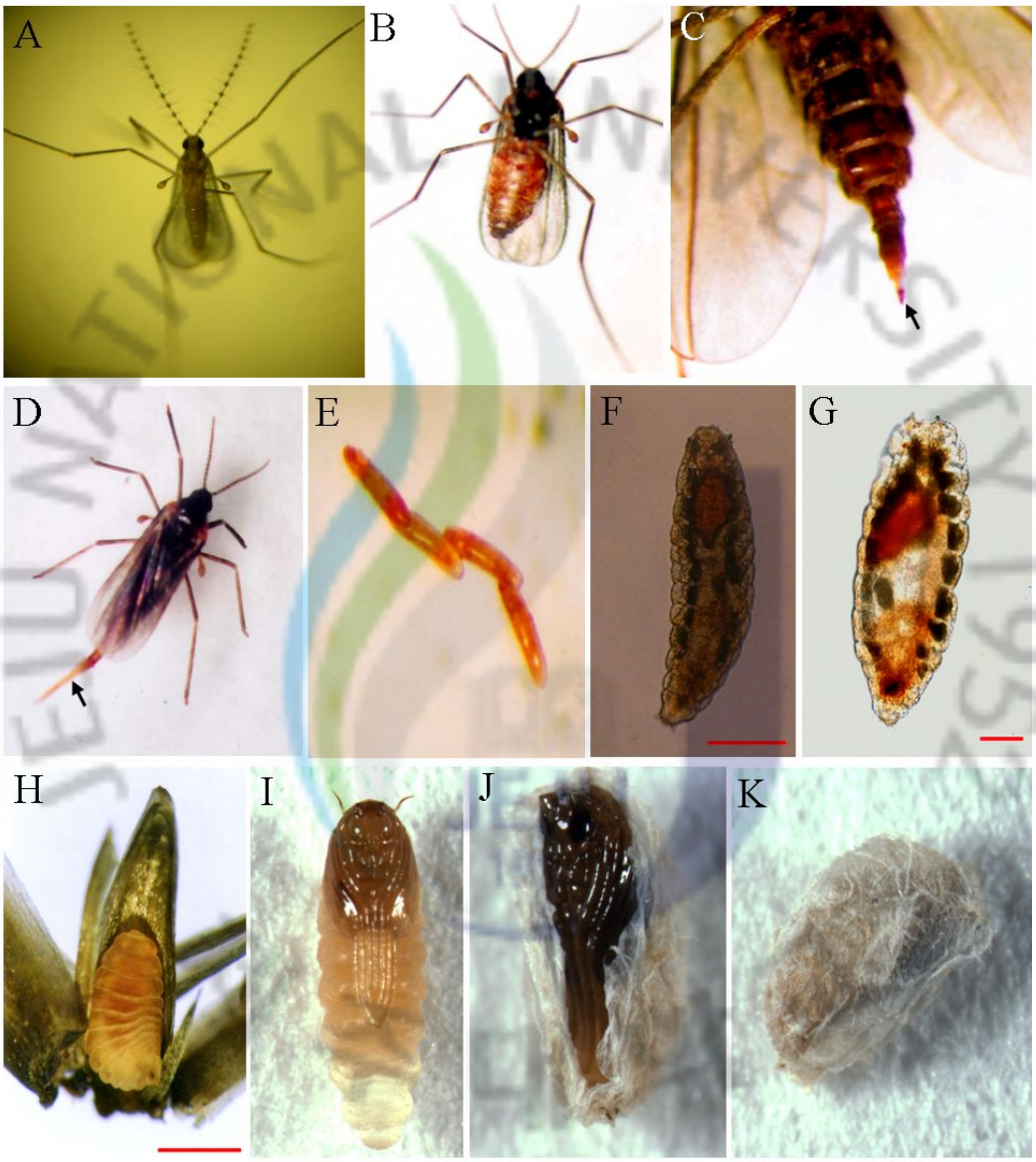

Figure 6. The insect (*Dasineura asteriae* Shinji) inducing each developmental stage.
A, Adult male. B, Adult female. C, A female with ovipositor (arrow). D, A female laying from elongated ovipositor. E, Laid eggs. F, First-instar larva on April 19th (Scale bar is 50um). G, A larva on June 5th (Scale bar is 150um). H,A larva on November 15th (Scale bar is 1cm). I, A pupa. J, A pupa in cocoon. K, A cocoon.

Life of insect in the rosette gall.

Mating of adult *D. asiteriae* with male and female on the net of rearing port at 5 April 2003.(Figure 7. A). Adult *D. asteriae* emerge from the soil generally at the mid May. There is only one generation per year. After mating the female searched for a suitable oviposition site. After mating, the female (Figure 7. B) searched for a suitable oviposintion site and the layed eggs (Figure 7. C). Eggs hatched after about 2-4 days, depending on the temperature. After hatching, the larvae moved away from oviposintion site and within a few hours, found the settle down place to start gall initiation. A larva body was in closed contact with the epidermis of the leaf (Figure 7. D).

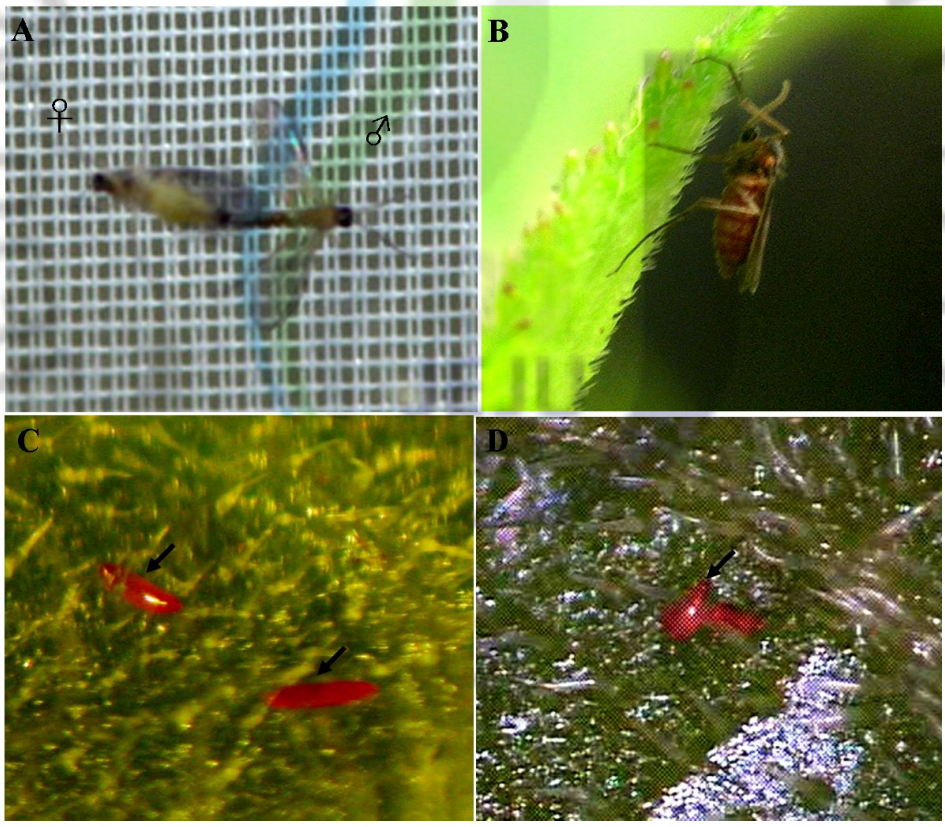

Figure 7. Oviposition of the insect female and gall initiation on the surface of leaf
A, Mating of a female with a male. B, Adult female on leaf. C, Close-up of eggs (arrows) on the leaf surface. D, Larva settle-down (arrow). It body is close contact with the epidemis.

To investigated growing larva in gall chamber by light microscopy and SEM. The gall were complete rosette shape plantlets developed, the larva growing in enters the gall chamber (Figure 8 A). Only one larva will develop per chamber. There might feeds on the nutritive layer (Figure 8 B). When removed larva in chamber, leaflets were well-developed surrounded chamber on gall (Figure 8 C). To further examine the rosette gall, dissected leaflets on rosette (Figure 8 D). Also, larva were observe inside the larval chamber in rosette gall (Figure 8 E). Larval chamber were observed vacant structure, also, leaflets showed surround chamber on gall (Figure 8. F). This is contrast with shoot apical meristem of normal plants that shows dome-shaped morphology. At the June, the gall is made up of leaflets that contained mostly mature and a few immature leaf structures and gall development occurred until late October. At that time the gall occurs pseudoflowers in one gall (Figure 8. G) and also flower during the normal plant. One more larva were observed live in the each chamber on rosette gall (Figure 8. H. I). In last October to early December, larvae were an escape from senescent rosette gall. The larva developed to a pupa and during winter time in the soil.

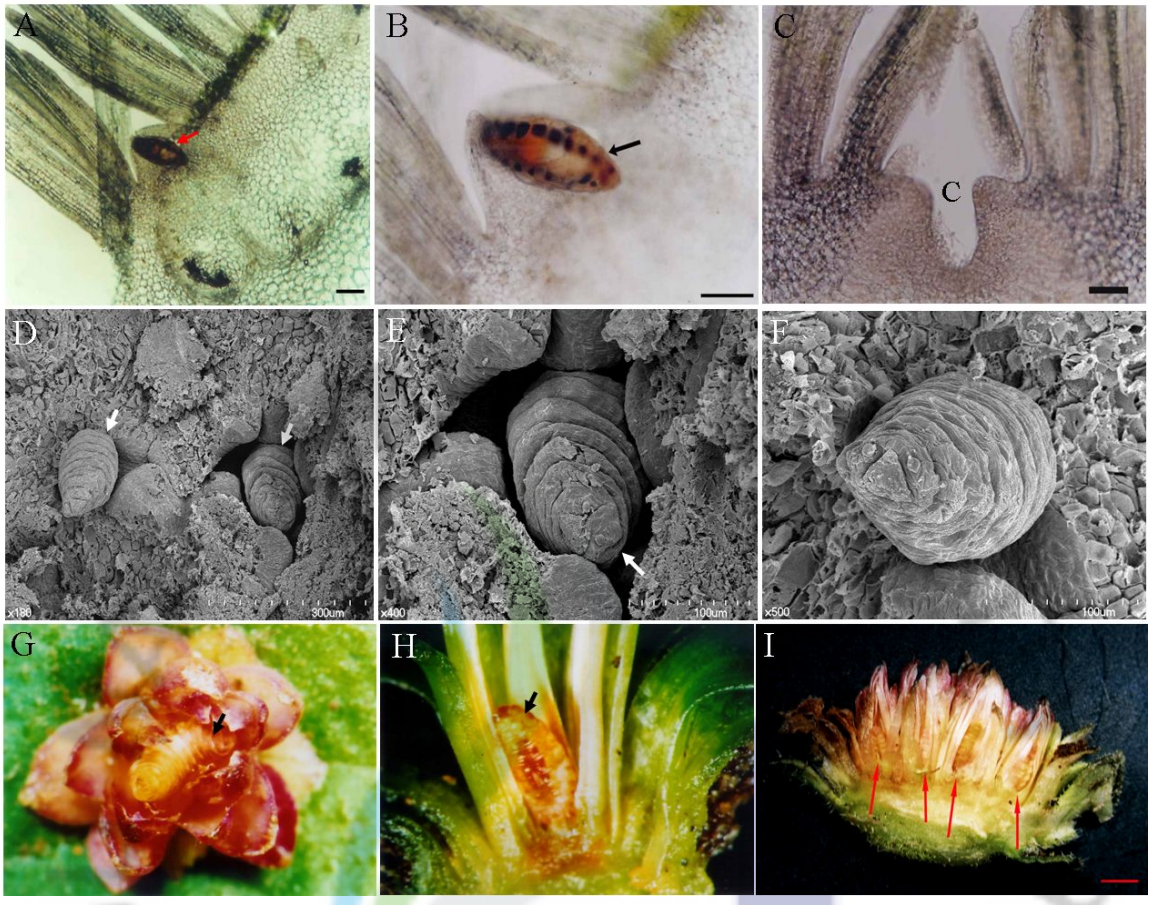

Figure 8. Larvae in the rosette gall at the immature (A − F) and the mature (G − I) stage of the rosette. A, Immature stage of gall development showing the larva (LV) within its gall chamber (arrow). Leaflets (L) grown on gall tissue. Scale bar is 150um. B, Close-up of the larva (arrow) in chamber. Growing leaflets (L) were covered the larva. Scale bar is 100 um, C, Empty larval chamber (C) of an immature stage gall, Scale bar is 150 um. D, Scanning electron micrography of larvae (arrows) in each gall chamber (x180). E, Close-up of the larva (arrow) in chamber (x400). The larva located between leaflets. F, Scanning electron micrography of larva (x500). The larva has been removed to observe the chamber. (x600). G, A larva in the center of rosette gall in mature stage. At mature stage the larva (arrow) stay inside of the floral bud. The floral bud was artificially opened. H, Dissection through a mature stage of rosette gall with larva. The larva (arrow) is contact with the gall tissue. I. Dissection through a mature stage of rosette gall with larval chamber. Each larva (arrows) occupies one flower bud when two or more larvae reside in a rosette gall. Scale bar is 1mm.

Discussion

The gall midge inducing the rosette gall in *A. scaber*

The gall midges (Diptera: Cecidomyiidae) are one of most abundant groups among galling arthropods (Yukawa 2005, Gagné 1989). Several gall inducer (*Lasioptera astericola* Shinji, *Lasioptera gibaushi* Shinji, *Asteralobia doellingeriae* Kovalev, *Asteralobia asteris* Kovalev, *Dasineura asteriae* Shinji, *Lasioptera* Sp., Unidentified) known to cause gall formation on *A. scaber*, their species that cause formation of irregular swelling on the stem, drop-shaped gall on the flower, subglobular gall on the flower, cluster-shaped gall on the leaf and the stem, conical gall on the leaf, and subglobular gall on the flower and the leaf edge seems to be member of the Cecidomyiidae (Shinji, 1944, Yukawa 1971, Yukawa and Masuda 1996, Tokuda *et al* 2003).

The rosette gall of *A. scaber* appeared to be induced by an insect which was identified as *Dasineura asteriae* Shinji (Diptera Cecidomyiidae). This study presents evidence for the influence *Dasineura asteriae* larvae have rosette gall on tissues of *Aster scaber* plant in Jeju, Korea.

Development of the insect with rosette gall development

Most gall midge of adult is relatively briefly, highly host-specific, often no longer than a few hour or days and the priorities are mate location by males and then host location by ovipositing females. Eggs are generally deposited singly on host plants and hatch within a few days or weeks, followed by larvae development (Harris, 1994). Gall midge larvae remain settle-down place on host plant, their attack stimulates growth and induces plant tissue differentiation which the larva chamber (Rohfritsch 2008).

Although the rosette gall inducer on *A. scaber* have been reported in Japan (Shinji, 1944, Yukawa, 1971, Yukawa and Masuda 1996). However, detail progressed

have been not recorded includes full metamorphosis of their insects. Therefore, the present describe on the details of developmental progress of insects with rosette gall development.

A females of *D. asteriae* lay eggs on the surface of host plant. The newly hatched larvae search for the settle-down place to initiated gall. Larvae of *D. asteriae* induce rosette galls on *A. scaber*, its only known host. Larvae develop in deeply concave on the surrounding larval chamber with ectopic leaflets, at the base of the gall on the host plant throughout spring, summer. Each of these larval chambers is occupied by one larva. In autumn, larvae more grown and larval chamber developed an additional organ resembling floral bud inside of the rosette gall leaflets. Two or more larvae were often observed in a single rosette gall, and in this case each larva occupied a floral bud.

Summary of D. asteriae life cycle

D. asteriae life cycle is one generation per year includes full metamorphosis of egg, larva, pupa and adult. The rosette gall of A. scaber is forms in response to the activities of a single larva of *D. asteriae.* Adults emerge from overwintering gall in April to May. After mating, adult females lay eggs in the organ of Aster plant. A larva, hatches from an eggs 2 - 4 days after oviposition. A larva moved and settled down, after 1 week, large trichomes surrounded larvae settled down region. After 1 week, galls developed to rosette type. During the time that gall grows, the larva grown into the rosette gall. In autumn, rosette galls developed, size are more 1cm by the time gall have a floret organ, also larvae more grown about 3 mm in gall chamber. Late autumn, the larvae came out of the rosette gall and entered into the underground soil. The larva developed to a pupate in spring

PART Ⅲ. Process of the Rosette Gall Development

Abstract

The mature rosette gall of *A. scaber* divided into three basic structures which consisted of ectopic leaves, floral organs and hemispherical gall at the basement. The rosette gall initiated with forming a tiny gall tissue on the surface of any organ in *A. scaber*. In the second phase, the ectopic leaves were differentiated from the gall tissue. In the third phase, the ectopic leaves grew and were organized into a rosette shape miniature plant. At last a floral organ was developed from the center of the rosette gall. The overall process of the rosette gall development included the dedifferentiation of a gall from the differentiated organs of the mother plant, the redifferentiation of ectopic leaves from the dedifferentiated gall, the growth and organization of the ectopic leaves to form a rosette shape, and the floral development from the mini-plants. This process showed close similarity with the plant regeneration via somatic embryogenesis in the laboratory tissue culture system. The first two steps in the development of rosette gall, dedifferentiation and redifferentiation, had a special significance because these implied the reversibility of cell fate. Therefore, the rosette gall in *A. scaber* was considered to be an excellent model system for the research on the plant development

The development of rosette galls appeared to be precisely regulated by the insect of D. asteriae. At the beginning of the rosette gall development, the initial gall was formed from the point of plant surface where insect larva settled down, not from the point of oviposition. Therefore the gall initiation appeared to be induced by the insect larva, not by egg or adult female. After the gall induction the larva stayed inside the rosette gall throughout the development of ectopic leaves and floral organs. This indicated that the larva controlled all the processes of the rosette gall development including dedifferentiation of differentiated mother plant cells, redifferentiation of gall cells to ectopic leaves, growth and organization of ectopic leaves, and development of

ectopic flowers. The extreme dwarfism in the typical rosette galls implied that the larva inhibited the developments of stems and petioles as well as auxiliary buds and roots. The unidirectional development of ectopic leaves and floral organs of the rosette gall seemed also to be controlled by the insect because the ectopic leaves and floral organs were developed from the insect-residing surface while only a small gall tissue was developed at the opposite side.

Introduction

Plant grow and development as a result of cell division and differentiation of tissues ordinarily occurs in meristems (Steeves and Sussex, 1989; Doonan and Hunt, 1996). Plant organogenesis *in vitro* is process during which *de nove* organs, such as shoots, bud and roots from cultured tissues (Thorpe, 1980).

Plant galls are atypical growth from dedifferentiation on tissue and organs of plant induced by insects. Galls occur as the results that various morphological characters from interspecific association between a plant and an insect (Roheritsch and Shorthouse, 1984; Roheritsch, 1992). Gall morphology is influenced by two genotypes, that of the insect, which provides the stimulus, and that of the plant, which determines the growth response (Weis and Abrahamson, 1986).

According to Rohfritsch (1992), cecidogenesis (insect-induced plant gall formation) and development can be divided four basic stages: initiation, growth and differentiation, maturation, and dehiscence. Gall initiation is either associated with oviposition by female insects (sawflies and cynipids) or with the activity of first-instar larvae (gall midge, aphid). These interactions are the most important stage in gall development and their can be modified host plant tissue (Rohfritsch 1992). Initiate gall development is physiological and cytological changes in the cell by insect feeding activities (Harris 1994). The gall growth and development stage occurs when the biomass of the gall is vastly increased by cellular hyperplasy and phypertrophy (Rohfritsch 1992). The gall rate of increase is response to the feeding activity of the insect (Mani 1992). Larva feeding activity is primarily response for shaping the larva chamber of inner gall and differentiation cells in feeding site to become a nutritive tissue (Rohfritsch 1992). The maturation stage of gall occurs while the larva is most active feeding phase and a period in life when consumes the largest amount of food. Differerntiation of plant cells occurs, causing the development of tissue layer, such as a sclerenchyma sheath forms outside the nutritive layer, separates the gall

into two regions referred to as an inner and outer gall (Rohfritsch 1992). Gall dehiscence or opening occurs toward the end of the maturation phase and is a period of major physiological and chemical change in the gall tissue. All of dehiscence process are strictly correlated with the development of the gall inhabitants and facilitate their escape. In some species larvae emerge fully grown and pupate on plant or in soil. In other larvae pupates in the gall on the host plant (Rohfritsch 1992).

Dasiineura asteriae was reported rosette gall formation on *A. sacer* in Japan (Shinji, 1944). However, the rosette gall developmental progress on *A. scaber* were no records. In this study, we present the first record of gall formation by the *D. asteriae* in Jeju, Korea, and then we describe the morphological character gall forming processes of recorded.

Materials and Methods

Process analysis of rosette gall development

Developmental stage of rosette galls used in this study were collection in the greenhouse at Cheju National University. Developmental progress of rosette gall, phase division, phase identification criteria were observed visually in situ and under Stereomicroscope (OLYMPUS SZX-ILLB100, JAPAN). Some of the rosettes galls were preserved permanently FAA solutin (50% ethanol, 5% glacial acetic acid and 3.7% formaldehyde (v/v)) for future observation. The photograph of the gall in situ was mad with KODA film ASA100 and digital camera Cannon EOS 20D.

Insect-plant interaction analysis

All the galls and insects used in this study were collected in the greenhouse at Cheju National University. To know the insect life cycle and developing progress of galls, they were cultivated in a greenhouse. After emergence, a male and female gall midge were placed together to mate. The fertilized females were released on Aster plants in rearing potted and in a greenhouse. Galls were developed toward rosette type or ceased patterns on A. scaber observed. These galls were collected and investigated for existence, larvae at each phase in gall tissue under microscopy (OLYMPUS BX 60-F3, JAPAN). This stage of gall samples were 100um sectioned using an Vibratome Series 1000(USA).

After oviposition, the females were removed. Leaves with eggs were observed daily for gall initiation. Larvae were removed from gall initiation stage and observed daily for gall growth under stero microscopy (OLYMPUS SZX-ILLB100, JAPAN).

Histological analysis

Rosette gall materials for histological analysis were fixed overnight at room temperature in a solution of 50% ethanol, 5% glacial acetic acid and 3.7% formaldehyde (v/v). The samples were then dehydrated by sequential 30-minute incubations in 50%, 60%, 70%, 80%, 90%, 95% and 99.5% (v/v) ethanol, followed by two incubations of 1 hour each in 100% (v/v) ethanol.

The dehydrated samples were set in Technovit 7100 resin (Heraeus Kulzer, Wehrheim/Ts., Germany) at room temperature, once in 50% (v/v) resin and twice in 100% resin. Serial 3 to 4-m thick sections of the plant tissues were cut with a rotary microtome (MICROM International, Walldorf, Germany), and stained with 0.5% Toluidine Blue for 30 seconds. Photographs were taken from the inverted microscope Axiovert (Carl Zeiss, Germany).

Scanning electron microscopy

The rosette gall samples used for scanning electron microscopy were prepared in the same way as those for the histological analysis until 100% ethanol step. The material was then critical point dried in liquid CO2, coated with gold and palladium at 10-20 nm thickness, and examined at an acceleration voltage of 10-20 kV using a scanning electron microscope (Hitachi, Japan).

Results

Rosette galls were observed dynamic developmental progress on the Aster plant. They can be development and organization through redifferentiation from organogenesis of dedifferentiated gall tissue. To examine morphological character of rosette galls was by scanning electron microscopy (SEM) analysis and histological analysis.

Gall Initiation (dedifferentiation)

Initiation stage of gall were observed in a lot of hair-like organs developed on adaxial leaf face of *A. scaber* (Figure 9 A). *A. scaber* which is induced by larvae of *D. asterie* developed concave structure in induced area about 7 days later. In SEM analysis, trichomes shown different morphological character in the initiation stage of gall region (Figure 9 C) and normal region of adaxial side of leaf face (Figure 9 D). These organs resembled morphology of trichome of control leaf with increase in their length. We do not know an exact role of accumulation of trichomes at this site, but it seems to be related with protection of early developing larva inside the rosette gall. This study was collaboration with Dr. Chan Man HA at Fletcher laboratory, UC berkeley in USA.

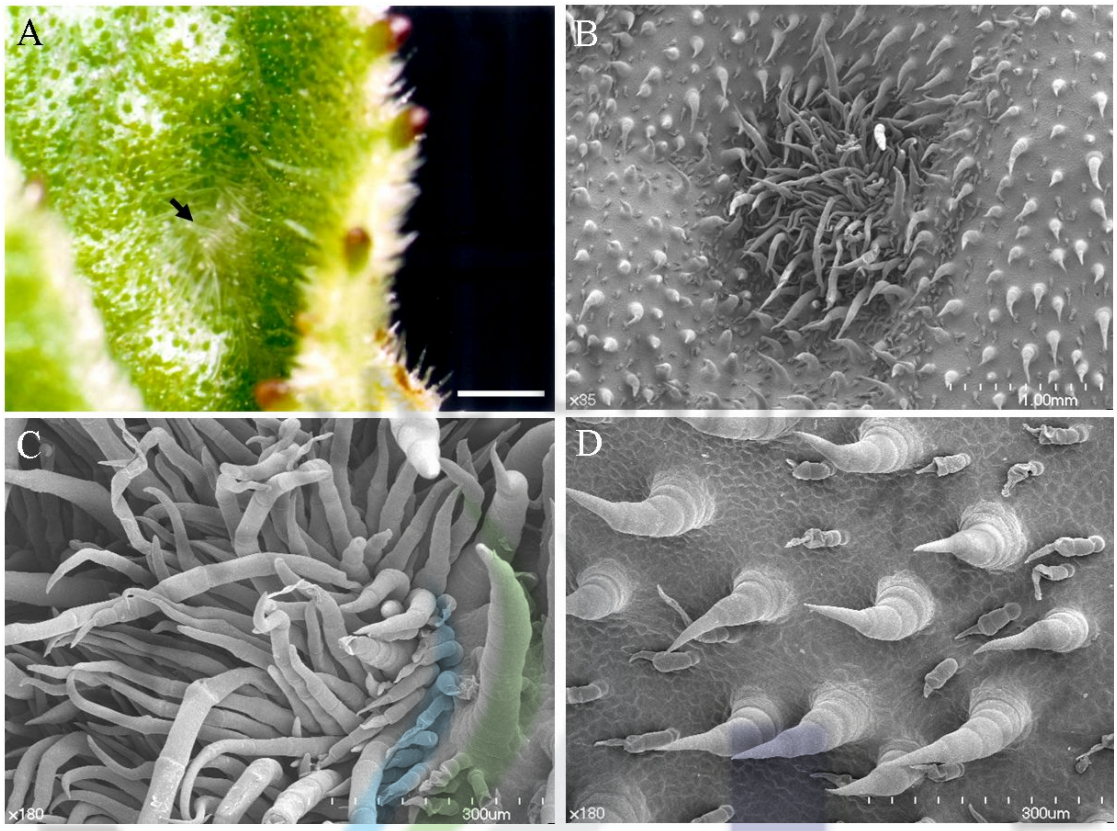

Figure 9. Gall initiation stage on leaf of *A. scaber*.

A, Initiation stage of gall on adaxial leaf surface of *A. scaber*. Trichomes (arrow) were observed brightly white and large grown on adaxial leaf (Scale bar is 1 mm). B, Scanning electron micrography of initiation stage of gall on adaxial leaf (x35). C, Close-up of trichomes in gall (x180). C; Trichomes on normal adaxial region (x180). Trichomes were large, intensive grown in gall than normal adaxial leaf.

Leaflet development (redifferentiation)

Figure 10 is show that ectopic leaflets development progress of rosette gall on leaf of *A. scaber*. The spherical type gall were growing and developing on the leaf of *A. scaber* (Figure 10 A). At this time, galls on adaxial and abxial leaf surface of *A. scaber* were initiation leaflets developing on sphere type with covered trichomes. Rosette leaflets were development through early heart stage (Figure 10 B) to late heart stage(Figure 10 C). In this time, rosette gall was leaflets developmental stage (Figure 10 D). After initiated leaflets of galls, about 1 month, the developed galls were observed visually small dwarf plantles of redifferentiation of the dedifferentiated cells to the secondary ectopic leaf of *A. scaber* (Figure 10 E, F). The galls can be developed to nearly a complete plant *in situ* although it is dwarf, rosette-like and rootless.

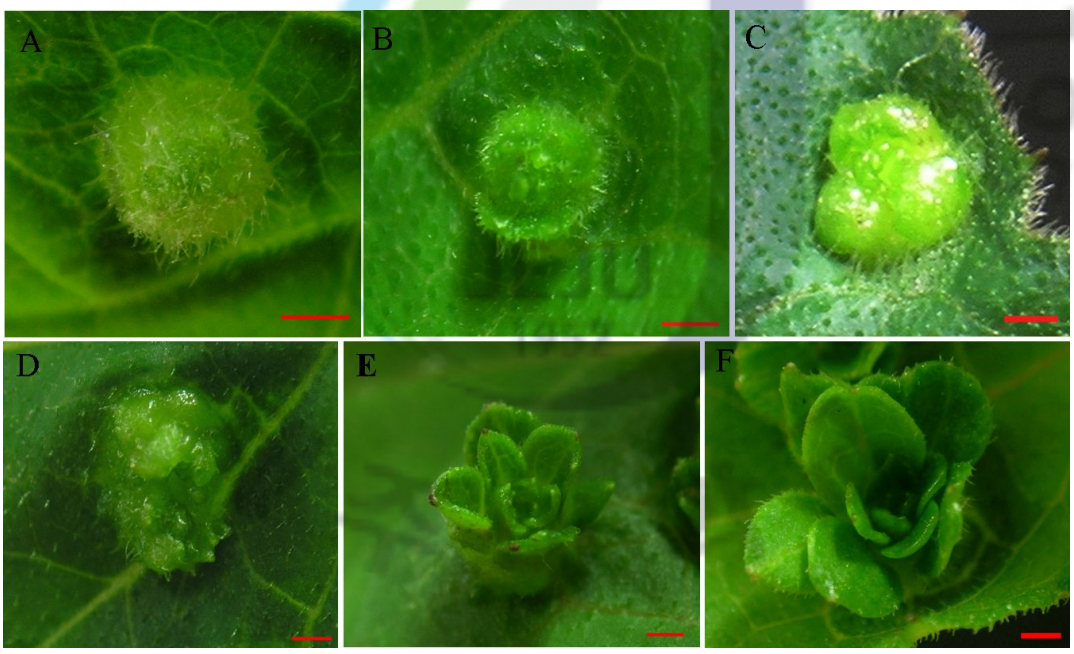

Figure 10. Leaflet development progress of rosette gall

A, Globular stage on the leaf surface. B, Early heart stage on the leaf surface, C, Late heart stage on the leaf surface, D, Early leaflet development stage on the leaf surface, E, A rosette shape plant on the leaf surface, F, Growing stage of rosette gall on the leaf surface. Scale bars are 1 mm.

To examine the phenotypic effect in a cellular level, we did section analysis. In a transverse section of initiation leaflets developmental stage of rosette gall, we could observe a development of leaf organ with spiral phyllotaxy (Figure 11 A, C). When we sectioned inside the rosette gall in serial order, we could see sectioned larvae in a central region of larval chamber in rosette gall (Figure 11 B, D). We could also examine a lot of trichome development on abaxial side of developing leaf. The vascular tissue and epidermis is present in the leaflets of gall (Figure 11 C)

Presence of larva was evident in a longitudinal section of rosette gall. Leaf primordia were developed around this larva. Most of the cells of the nutritive layer were large, and contain prominent nuclei and separated from the underlying tissue by sheath of flattened cells (Figure 11 E, F). The gall leaflets proximal to the developing larva are similar in appearance to leaf primordia in a normal plant apex. The cells of the primordia were also similar in appearance to cells of the nutritive layer. We could not observe dome-shaped SAM structure of normal plant in rosette gall region. However, plant cells under insect were stained strongly with toluidine blue staining dye, implicating the presence of meristemic-like cells surrounded larval chamber region (Figure 11 F). The rosette gall was protruded into abaxial side.

In SEM micrograph, galls were observed young developing leaflets organs until about 2 weeks later from caved-in structure. Furthermore, aggregate of trichomes were shown at the abaxial side of developing leaf of gall (Figure 11 G, H). When removed larval, larva chamber were observed vacant structure, also, leaflets showed surround chamber on gall (Figure 11. I). This is contrast with shoot apical meristem of normal plants that shows dome-shaped morphology.

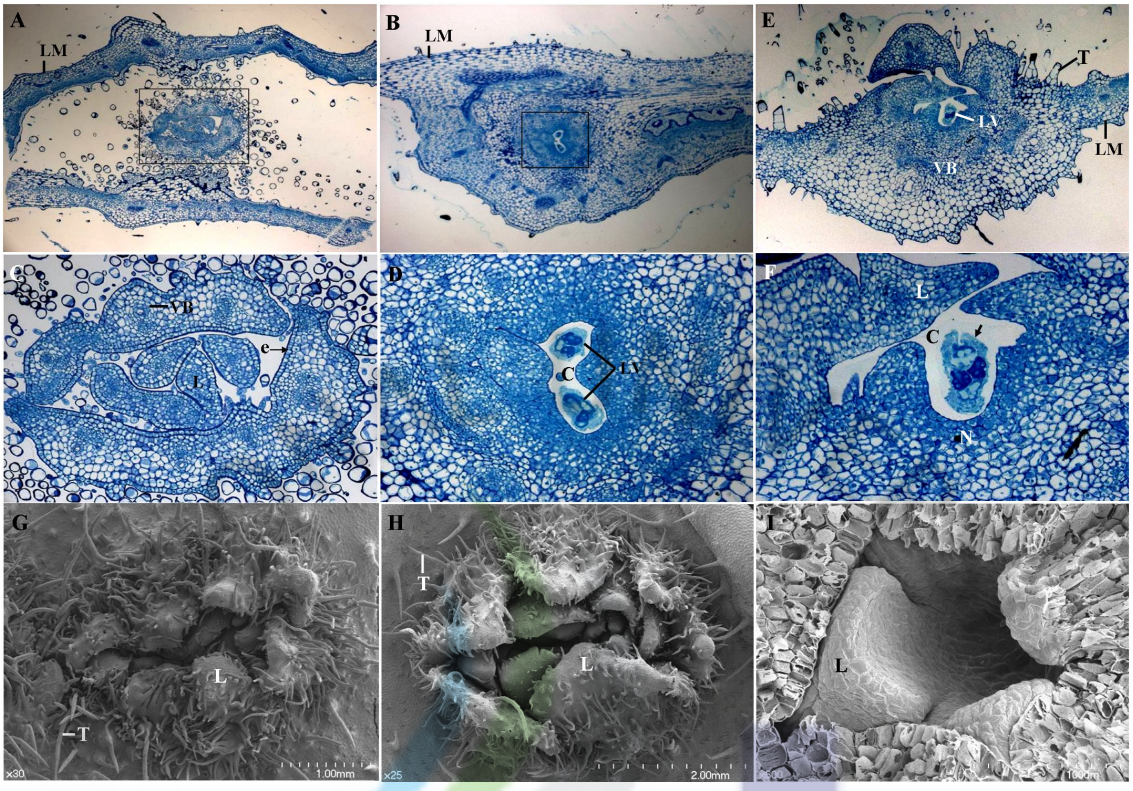

Figure 11. Early leaflets developmental stage of rosette gall

A and B, transverse section of early leaflets developmental stage on mother leaf (LM) of *A. scaber*. C and D, close-up of early leaflet (L) development (black box) in A and B. Serial section in inside of rosette gall, C, leaflet (L) showing with spiral phyllotaxy pattern. Outer-leaflets (OL) and leaflets (L)showing with vascular bundles (VB) and epidermis (e), a lot of trichome (T) on adaxial side of leaf. E and F, Longitudinal section of early leaflet developmental stage. E, Rosette gall on *A. scaber* leaf showing early leaflets (L), nutritive layer (N), larva (LV), vascular bundles (VB), trichome (T) and unaltered mother leaf portion (LM). F, Nutritive layer (N) surrounding the larva (LV) within larval chamber (C). Fixed with FAA and stained with toluidine blue. G, SEM photograph of early leaflets developmental stage of rosette gall (x30), H, SEM photograph of leaflets developmental stage of rosette gall (x25). Rosette gall showing developing leaflets (L) organ and trichomes (T) at abaxial side of leaflets organ on *A. scaber* leaf. I, Scanning electron micrography of larval chamber. The larva has been removed to observe the chamber (x600).

Organization into rosette shape

SEM analysis, complete leaf organs were developed in a spiral phyllotaxy (Figure 12 A). Some ectopic galls were further developed to make an inflorescence shoot-like organ. In order to examine further phenotypic effects, we examined morphology of epidermal cells from ectopic developing leaf organs. Control leaf had epidermal cell of relatively uniform size in its adaxial side (Figure 12 D). In contrast, abaxial side had epidermal cells with relatively nonuniform size (Figure 12 E). Furthermore, abaxial epidermis exhibited many developing guard cells, whereas adaxial epidermis did not. Ectopic leaf in rosette gall region also showed same epidermal cell morphology with control leaf in their adaxial (Figure 12 B) and abaxial side (Figure 12 C), respectively.

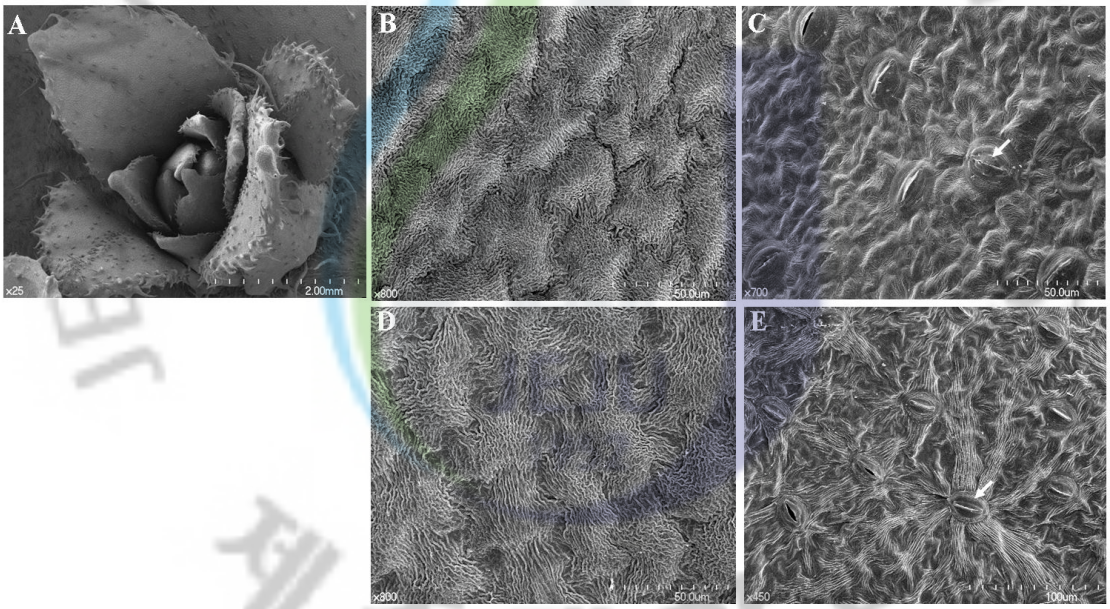

Figure 12. Comparison between normal leaf of A. scaber and etopic leaf of rosette gall. A, SEM photograph of rosette-shape plantlet stage (x25). Rosette gall showing the morphology of ectopic developing leaf organs. B, Epidermal cell morphology of adaxial side on etopic leaf of rosette gall (x800). C, Abaxial side showing developing guard cells (arrow) on etopic leaf of rosette gall (x700). D, Epidermal cell morphology of adaxial side on normal leaf of A. scaber (x800). E, Abaxial side showing developing guard cells (arrow) on normal leaf of A. scaber (x450).

Transverse section of leaflets development stage and rosette shape plantlets. In serial section, we could clearly see developing leaf organ in spiral phyllotaxy (Figure 13). Sectioned larva was evident with different staining pattern with plant cells in the innermost position of rosette gall. Larva occupied possible shoot apical meristem (SAM) region in rosette gall. Rosette galls were developed on adaxial as well as abaxial side of developing leaf.

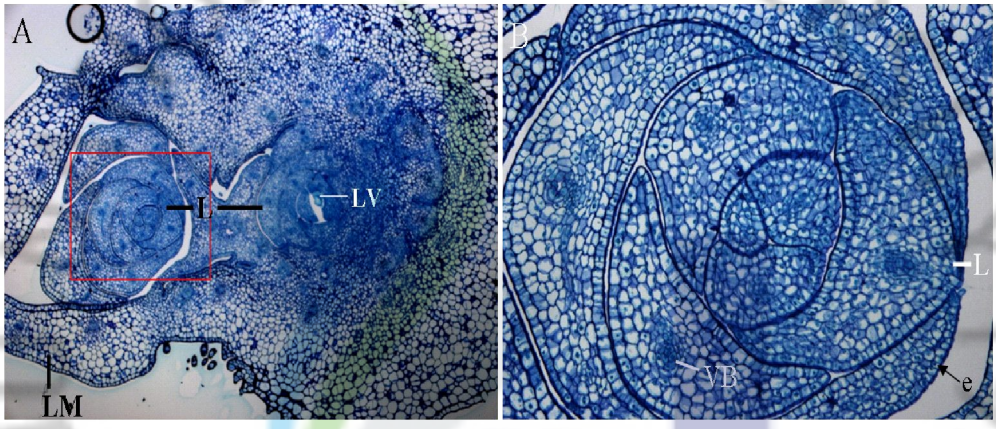

Figure 13. Leaflets developmental stage of rosette gall

A, Tansverse section of growing stage of rosette gall. Rosette gall on *A. scaber* leaf showing numerous leaflets (L), larva (LV) and unaltered mother leaf portion (LM). B, Close-up of red box in figure A. Leaflets (L) showing developing organ in spiral phyllotaxy, vascular bundles (VB) and epidermis (e). Fixed with FAA and stained with toluidine blue.

Flower-like organ formation

In early fall, the rosettes continue to grow by increasing the size and numbers of leaflets on the *A. scaber*. Organization of rosette galls outcome can be progress towards flower-like organ formation (Figure 14), although it is no more evidence, the other normal flowers. At maturation each rosette gall formed one to several pseudofloral buds with petaloid scales. The rosette pseudoflowers differed from the flowers of the host plant, but developed synchronously, lacking reproductive structures.

Figure 14. Mature stage of rosette gall

Rosette leaflets showing numerous petal likes structure, change colors in the edge of the leaflets. Florets like organs of rosette gall showing with white color.

Effect of the insect in the rosette gall development

To investigated of effect of rosette gall development by larva of *D. asteriae*. Figure 18 is shows that gall were dedifferntiation of modified epidermal cells of the leaf induced the larva of which lives and feed activity in gall. 1st instar larva were freshly removed from the trichomes development gall on May (Figure 15 A). After 8 days, gall grown on alive larva in gall were observed (Figure 15 B, C), however, in contrast, the larva is removed from a trichomes developmental stage of rosette gall grown on young leaf surface, the growth of that gall ceases to remain with a puddle-like, circle on leaf surface(Figure 15 D).

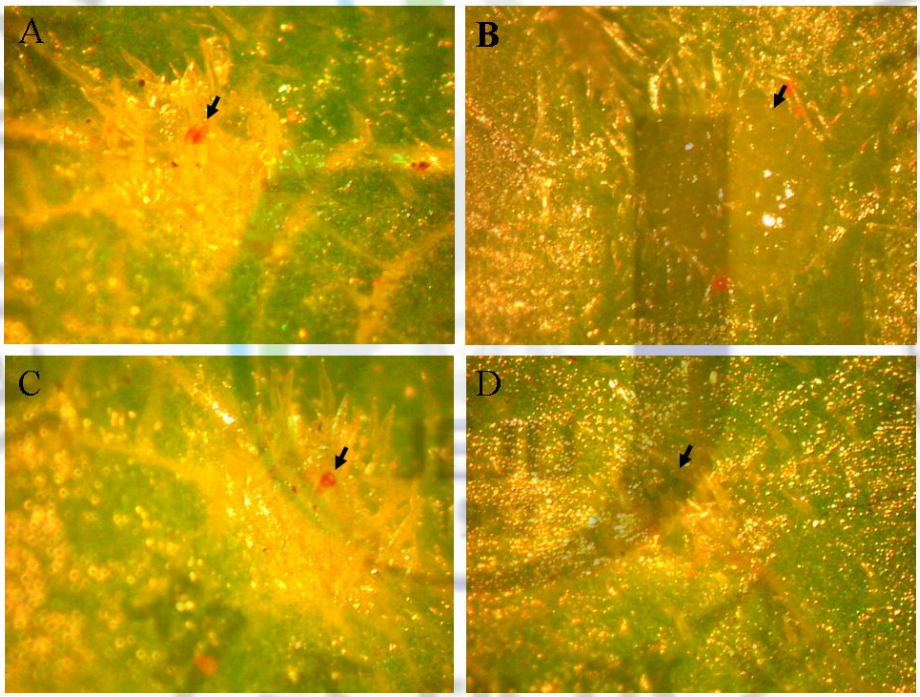

Figure 15. Effect of remove of larva of *D. asteriae* in on leaf surface of *A. scaber*.
A and C, Tirchomes surrounded larva (arrow) in gall. B, After 8 days, the larva was still lived in gall, in contact with dedifferentiated of modified epidermal of the cell of leaf by larva feed activity. D, Gall removed a larva after 8 days. Gall ceases to remain with a puddle-like, circle (arrow) on the leaf surface.

In field, gall differentiation were observed on the same leaf of *A. scaber,* about 16 days, under natural condition (Figure 16 A, B). Gall growth type (red box) were observed larva in gall tissue and leaflets surrounded the larva (Figure 16 E). However, galls growth cease type(yellow and blue box) were observed without larva in chamber, remained a sign of puddle-like in gall tissue (Figure 16 C, D). Gall growth cease type was may larvae transfer new settled down region or feeding by predators. Such as, figure 44, 45, indicated that gall induced by larva of *D. asteriae*.

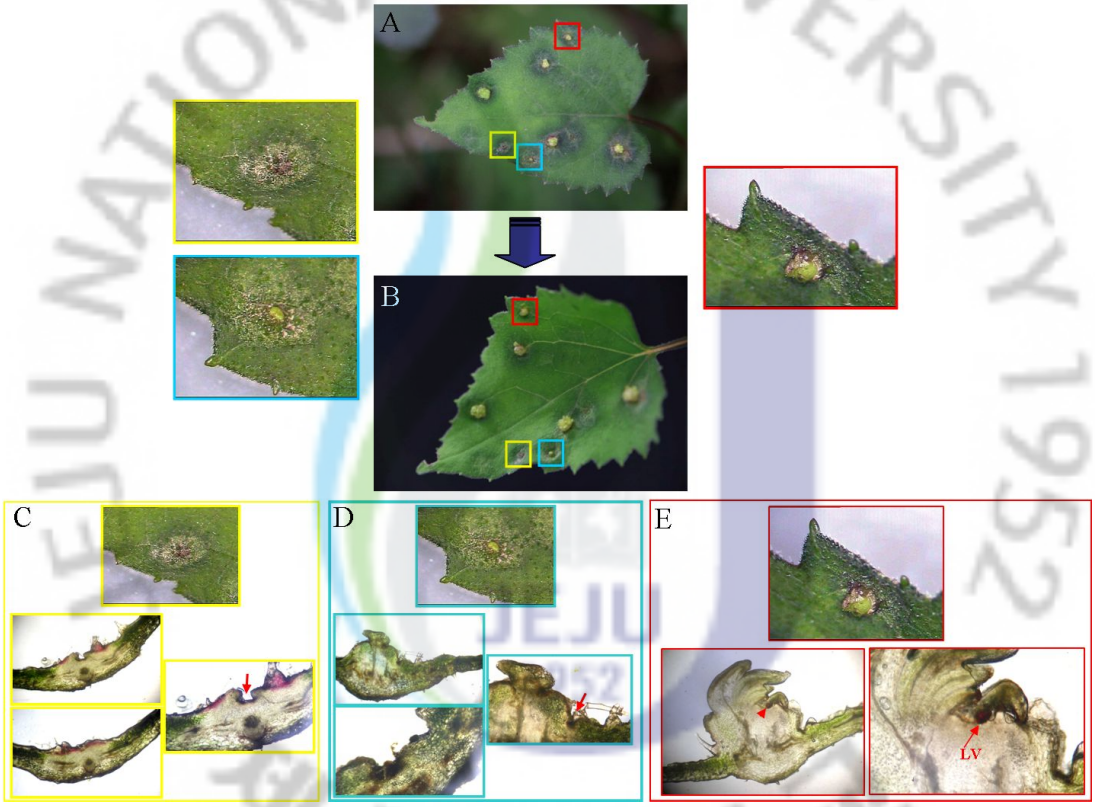

Figure 16. Different morphology of rosette galls growing on adaxial leaf surface of *A. scaber*. A: Rosette gall development stage, Photographed 14 June 2008, B: Rosette gall development stage, Photographed 1 July 2008, C: Gall growth cease type on the leaf (yellow box) showing the larva chamber (arrow), Photographed 1 July 2008, 100㎛ Vibratome section., D: Gall growth cease type on the leaf (blue box) showing the larva chamber (arrow), Photographed 1 July 2008, 100㎛ Vibratome section., E: Gall development on the leaf (red box) showing the larva (LV, arrow), 100㎛ Vibratome section.

Discussion

Overall process of rosette gall development

Gall forming insects are highly host and organ specific, that is, each species of gall inducer galls a closely related group of host species. (Dreger-Jauffret and Shorthouse, 1992). Galls of cynipids and cecidomyiids are classified as prosoplasmic gall which a have pass through dedifferentiation and redifferentiation (Roheritsch and Shorthouse, 1984; Dreger-Jauffret and Shorthouse, 1992; Williams 1994).

In Aster rosette gall, also development can be divided into redifferentiation from dedifferentiation. Figure 17 showed overall progress of rosette gall development on *A. scaber* including life cycle of *D. asteriae*, which four stage are 1) initiation (dedifferentiation), 2) Leaflets development (redifferentiation), 3) organization into rosette shape miniature plant, 4) maturation (psudoflower development). During initiation stage, gall developed from the leaf vein or non-meristemic cells between veins unlike ordinary plant organs which differentiated from the meristemic cells. Leaflets developmental stage of rosette gall was redifferentiation (Leaflet development) which *de nove* developmental process including organogenesis. Also, rosette shape miniature plant developmental stage, gall showed development of ectopic leaf organ with spiral phyllotaxy, presence of nutrient cells, leaf primordia in gall. During maturing stage, leaflets developed to change morphology with ectopic floral buds with petaloid scales.

Figure 17. Overall process of rosette gall development on *A. scaber*, including the life cycle of *Dasineura asteriae*. Rosette gall development progress can be divided into four stages: 1) initiation (dedifferentiation), 2) Leaflets development (redifferentiation), 3) organization into rosette shape miniature plant, 4) maturation (pseudoflower development).

Formation of nutritive tissue has been termed "metaplasia" (Meyer 1952). During mataplasy, meristemic and slightly differentiated cell cease to differentiated and remain in or return to a meristemic state. They then differentiate to become nutritive cell (Larew 1982). Cell influenced by the insect cease host normal pattern of differentiationand become cytological rejuvenated (Rohfritsch and Shorthouse 1982). The nutritive cells are cytological characterized by cytoplasmic richness and a vacuolar fragmentation, nuclear and nucleolar hypertrophy, richness in ribosomes, often grouped in polysomes, week differentiation of the plastids, strong development of dictyosomes, presence of autophagic vacuoles, accumulation of nuclear and ribosomal RNA, strong concentration of soluble proteins that are continually replenished, strong hydrolase activity (acid phosphatase, amino-peptidase, invertase), and absence of starch in the nutritive cells nearest the gall insect (Bronner and Meyer 1976, Bronner 1977).

The other prosoplasmic gall, ork cynipid gall development also with three gall development phases: initiation, growth, and maturation (stone, 2002). Cynipids are able to change the natural growth patterns of their host plants to such an extent that the galls have been described as new plant organs. However, very little is know about at actual mechanisms employed by gall wasps to control the growth of such novel structure (Schönrogge *et al.,* 1998). Some of gall-inducing gall midges and cynipids show an unusual ability to induce differentiated tissue to revert to a meristemic state and resume cell-division activity. Physiology of gall is broadly similar to that of endosperm or meristematic tissues (Raman 2007). Also, stone (2002) note cynipid gall induction for require active meristematic or other wise totipotent cells (Atkinson *et al.,* 2002). Although specific requirements in terms of degree of host tissue differentiation tolerated and nutritional state required unknown (Stone, 2002). The aphid and the other galling insect manipulate latent plant development programs to produce modified atavistic plant morphogenesis rather than

creating *de novo* forms (Stern, 1995). For that reason, rosette gall of *A. scaber* suggested that gall initiation and development is turn over of origin cell from reprogram of host cell by insect.

Regulation of rosette gall development by the insect

Gall-inducing insects are specialist plant feeders with most species confined to one specific host plant. Gall develop as a result of interactions between the inducing insect and plant, wherein the insects gain control and redirect the growth and physiology of attacked organs to the insects` advantage (Shorthouse *et al.,* 2005). An active and feeding gall inducer must be present in the gall system for the completion of cecidogenetic events. Cell proliferation ceases once the gall inducer is withdrawn or killed (Rohfritsch and Shorthouse, 1982).

Most cecidomyiid galls that have been studied to date are "cover galls", in which larvae first feed on epidermal cells and then become covered by the gall tissues (e.g., *Didymomyia reaumuriana* (Low); *Geocrypta galii* (Loew)), but some are "mark galls", in which larvae first enter the host tissues and only then induce gall formation (e.g., Lasioptera Meigen spp.) (Rohfritsch 1987, Rohfritsch 1988, Rohfritsch 1992, Dorchin et al., 2002).

The rosette gall of *A. scaber* appeared to be induced by an insect which was identified as *Dasineura asteriae* Shinji (Diptera Cecidomyiidae). Early rosette gall development was dedifferntiation of modificatied epidermal cells on leaf by larva. In contrast, gall growth cease type was larva removed. Therefor, the direction of development and growth of ectopic rosette leaves seemed to be regulated by the insect because the ectopic leaves developed only from the insect-residing surface, the larva of insect make chamber in the gall tissue and live in the chamber during overall process of rosette gall development.

Significance of the rosette gall in plant development

In plant cell, totipotency is the ability of a single cell to develop into a new organism (Skoog and Miller, 1957; Steward *et al*., 1958; Murashige and Skoog, 1962). Totipotency involves two major developmental processes such as dedifferentiation and redifferentiation (Melissa 2006). *In vitro* plant regeneration *via* somatic embryogenesis from proliferation of dedifferentiated plant cell cultures.

Natural phenomenon process of rosette gall development resembled very closely that of *in vitro* plant regeneration *via* somatic embryogenesis. Natural phenomenon process of rosette gall development resembled very closely that of *in vitro* plant regeneration *via* somatic embryogenesis. Therefore, specially significant of rosette gall development in *A. scaber* is evaluated to have many advantages as a model system for the research on the plant development. As Mani (1964) puts it "gall biology is a profitable of investment both applied and basic, and should put more emphasis on granting"

PART Ⅳ. Genes Involved in the Rosette Gall Development

Abstract

A cDNA libray was constructed from the whole plant of A. scaber with radical leaves at the juvenile stage to obtain basic information of the gene expression in the host plant. The sequences of 2306 cDNA clones were determined and their homology was analyzed with NCBI database. The cDNAs clustered into 1843unigenes, which consisted of 45% of known function, 13% of unknown function and 42% of no hit genes, respectively. The library contained 28 genes related with phytohormone metabolism which were assumed to be important in the rosette gall development.

For the understanding of rosette gall development in relation with hormonal controls, the genes involved in the metabolism of phytohormones were cloned and their expressions were examined. Three genes of NIT, IPT and GA3ox encoding key step enzymes in the synthesis of auxin, cytokinin and gibberellin, respectively, were cloned. NIT was highly expressed in the inner and outer leaves of the rosette gall and its expression in the gall tissue was similar to mother leaf. Therefore, auxin was supposed to be actively synthesized in the ectopic leaves of rosette gall. In case of IPT, it was highly expressed not only in the inner and outer leaves of the rosette gall but also in the gall tissue by the similar level. The delocalized high expression of IPT over the whole rosette gall might contribute to the compact morphology and retarded senescence of the rosette gall by providing high cytokinin.

In contrast to NIT and IPT, GA3ox was suppressed in the inner leaf of rosette gall while its expression in outer leaf and gall tissue were similar to mother leaf. The dwarfism of the rosette gall might be resulted from the lack of gibberellin due to the suppression of GA3ox in the inner leaf because gibberellin 3beta-hydroxylase encoded by this gene mediates the final step in the synthesis of active gibberellin. On the other

hand, an unknown gene denoted as GAS was highly expressed in the inner leaf of rosette gall whereas the expressions in outer leaf and gall tissue were similar to mother leaf. The length of this gene was relatively short, 450bp (150 amino acid), and had no introns. Therefore, GAS was supposed to play as a regulatory element in the rosette gall development, probably via the down-regulation of GA3ox because the expression of GA3ox was coincidently suppressed with the expression of GAS, and vice versa.

Introduction

Plant growth and development are both by continued growth and organogenesis, its regulation by many environmental influences and endogenous signals that, together with intrinsic genetic program, determine plant form (Jager 2005, Gray 2004). Basis to this process are control by several growth regulation factors, which are phytohormones includes auxin, cytokinin, the gibberellins (GAs), abscisic acid (ABS), ethylene, the brasinosteroids (BRs), and jasmonatic acid (JA), each of which acts at low concentrations to regulate many aspects of plant growth and development (Gray 2004).

Plant gall formation is complex physiological and biochemical responses between plants and a wide variety of organisms, including insects, mites, nematodes, fungi, viruses and bacteria (Mani 1964, Meyer 1987). The formation of plant galls illustrates the plasticity of plant tissues and the ability of cells to be reprogrammed to form unusual structures (Shorthouse and Rohfritsch 1992).

Gall inducers are common, the mechanism by which insects induce galls remains largely unknown. Possible mechanisms include mechanical damage, plant hormone analogs, and genetic manipulation (Hori 1992, Price 1992). Genetic manipulation is involved in the process of gall induction by the bacterium *Agrobacterium tumefaciens*. The bacterium inserts a portion of a Ti plasmid into the genome of some of the cells of its host, which eventually leads to abnormal growth (Davey *et al*. 1994). The formation of root-knot galls by plant parasitic nematodes as a *Meloidogyne* species also includes alteration of plant gene expression (Opperman and Conkling 1994, Huang 2003). Although it has been suggested that such manipulation may also occur in the case of gall inducers (Cornell 1983), no evidence for such a mechanism has been found (Price 1992).

Numerous reported have been implicated in plant gall formation. Plant growth hormones such as auxin, cytokinin, Gaibberellins are involved in gall development and differentiation (Hori 1992, Bayer 1976, Mapes 2000, Stone 2003). Several secondary

metabolites accumulates in plant galls (Hori 1992, Bayer 1994, Stone 2003). However, little is known about biochemical changes that may regulation events during gall formation (Bayer 1994). Also, the molecular basis of gall induction remains unknown in all insect inducing gall (Stone, 2003). Therefor, this study, to understand for rosette gall differentiation on gene level.

Materials and Methods

Construction of cDNA library of *A. scaber*

The whole plants of *Aster scaber* Thunb. (Asteraceae) were grown on greenhouse in Cheju National University, Jeju-do. The whole plants of *Aster scaber* was used for cDNA library construction and gene cloning (Figure 18). For RNA isolation, collected plants and rosette galls were immediately frozen in liquid nitrogen and all sample was kept at -80℃ until use for RNA preparation. Total RNA was extracted using easy-BLUE reagent (Intron biotech) according to the manufacturer's instruction.

cDNA library was constructed using ZAP express cDNA Synthesis Kit and ZAP express cDNA Gigapack III Gold Cloning Kit (Stratagene, USA) according to the manufacturer's instruction. Double-stranded cDNA was synthesized from approximately 10 ㎍ of poly(A) RNA and then modified to ligate into vector. The cDNA fragments larger than about 500 bp were collected by fractionation using drip column and ligated to Uni-Zap XR vector. The ligated product was packaged *in vitro*, and amplified.

E. coli XL1-Blue (MRF') was used as a hosts for λ phage and subcloned plasmid. *E. coli* SOLR was used as a host for in vivo excision of Uni-Zap clones into plasmid clones. pBluescript SK (-) was used for the construction of sublibrary of plasmid. pGEM-T Easy (Promega) vector was used for subcloning of PCR product.

EST Sequencing

The phage library was converted to phagemid by mass excision according to the protocol described by Stratagene. The obtained phagemid library was transformed into SOLR cell and plated onto LB agar containing ampicillin (100 ㎍/ml), isopropyl-β-D-thiogalactopyranoside (IPTG) and X-gal, and incubated at 37℃ overnight. The white colonies were randomly picked into 96 well blocks containing 1.3 ml of LB-ampicillin medium and cultured at 37℃ overnight. Plasmid extractions were performed in a 96-well format using Wizard SV 96 Plasmid DNA Purification

System (Promega). The sequencing reactions were performed using BigDye™ Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems) and T3 primer. The reaction products were analyzed using an ABI 3700 DNA Sequencer (Applied Biosystems).

Figure 18. Young stage of *A. scaber* used for cDNA library construction

Construction of subtractive library

Subtractive library used the protocol elaborated by Wang and Brown (1991) and Buchanan-Wollaston and Ainsworth (1997) with some modification. Double-stranded cDNA from the target(Rosette gall) and the driver (normal leaf) were separately digested with restriction enzymes RsaI and AluI. EcoRI linkers were added to the tester cDNA and HindIII linkers to the driver cDNA (HindIII linker, 5'-ATCGTCAAGCTTCAAGTTAGCATCG-3', 5'-GCTAACTTGAAGCTTGACGAT-3'; EcoRI linker, 5'-TAGTCCGAATTCAAGCAAGAGCACA-3', 5'-CTCTTGCTTGAATT CGGACTA-3'). Ligated cDNA fragments were amplified for 30 cycles using Taq polymerase (Takara, Korea) in the presence of 0.4 M primer and 0.2mM dNTPs under conditions described by the manufacturer. Target cDNA fragments were amplified using the HindIII primer(5'-ATCGTCAAGCTTCAAGTTAGCATCG-3') and the driver cDNA was amplified using a Biotin-21-dUTP and EcoRI primer (5'-TAGTCCGAATTCAAGCAAGAGCACA-3'). PCR amaplification paramaters:

1-min denaturation at 94℃, 1-min annealing at 53℃, 2-min elongation at 72℃. PCR-amplified driver cDNA (25㎍) was mixed with 1.25㎍ of amplified target fragments. The DNA was precipitated with ethanol, the pellet dissolved in 10 ㎕ HE buffer (10 mM Hepes pH 7.3, 1 mM EDTA) and boiled for 3 min. 10㎕ of 2×hybridisation buffer (1.5 M NaCl, 50 mM Hepes pH 7.3, 0.2% SDS) was added, the solution overlaid with 20 ㎕ mineral oil and boiled for a further 3 min. The mixture was allowed to hybridise at 65℃ for 20 h. After hybridization, the oil was removed and the salt concentration of the DNA solution adjusted by the addition of 80㎕ water and 100㎕ of 2×binding buffer (10mMTris Cl pH 7.5, 2MNaCl, 1 mMEDTA). The biotinylylated DNA was then removed from the solution by binding to 100㎕ of Streptavidin magnetic beads as described by the manufacturer (Promega, USA). Unbound DNA was removed after the paramagnetic beads were attracted to the side of the tube with a magnet. The subtracted DNA was mixed with a further 12.5㎍ of driver DNA and the hybridization repeated as before with the exception that the incubation at 65℃ was carried out for 2 h only, before removal of biotinylated DNA by streptavidin magnetic beads. A small amount of the subtracted DNA was then amplified by PCR using the HindIII primer. After each round, subtracted cDNA was PCR-amplified as described and used in the next round of subtractive hybridization. After three rounds, cDNA fragments were cloned into pGEM-T Easy vector (Promega, USA).

The DNA inserts from the selected clones were amplified by PCR. The PCR products (100 ng) were dotted onto nylon membranes (Roche, DK) according to Short protocol (Ausubel *et al*., 1997). Nonsubtracted cDNA probes from target and driver were ^{32}P-labeled using random primer labelin kit (Roche, DK). Hybridizations were performed at 42℃ in hybridization solution containing formamide overnight. Membranes were washed twice with 2×SSC, 0.1% SDS at room temperature for 20 min, twice with 0.1×SSC, 0.1% SDS at 65℃ for 15min, then exposed to X-ray film at -70℃ for 12-48 h.

Differential display RT-PCR

Leaves and leaflets developmental stage of rosette gall samples were ground

with liquid nitrogen (Figure 19). Total RNA was extracted using Tri-zol (Intron biotech. Korea) according to the manufacturer's instruction. The first step is first-strand cDNA synthesis, which is performed using the dT-ACP1 primer (GeneFishingTM DEG kits, Seegene, Korea), 20 U of RNase inhibitor (Promega, USA), and 200 U of M-MLV (Promega, USA). For the amplification, the RT reactions were conducted according to the protocol of GeneFishingTM DEG kits. The cDNAs are then subjected to second-strand cDNA synthesis by random PCR amplification using dT-ACP2 and one of 40 arbitrary ACPs (GeneFishingTM DEG kits, Seegene, Korea) as primers. This PCRs were conducted according to the protocol of GeneFishingTM DEG kits. The amplified products were cloned into the pGEM -T Easy vector of the pGEM - T Easy Vector System Ⅰ (Promega, USA) and transformed into JM109 competent cells (Promeg, USA). The colonies were grown for 16-18 hr at 37℃ on Luria broth agar plates containing ampicillin, X-gal (5-bromo 4-chloro 3-indoyl-b-D-galactopyranoside), and isopropyl-b-Dthiogalactopyranoside were used for blue/white colony selection. The plasmids were extracted and the inserts were subjected to dideoxy chain termination sequencing (Applied Biosystems, Model 377). The identity of each product was confirmed by sequence homology analysis using the Basic Local Alignment Search Tool at NCBI.

Figure 19. Leaflets developmental stage of rosette galls on leaf surface of *A. scaber* used for RNA isolation in differential display RT-PCR and gene cloning

First strand cDNA synthesis and degenerate RT-PCR

First strand cDNA was synthesized from mRNA of leaflets developmental stage of rosette gall of *A. scaber*. Approximately 200 ng of mRNA was reverse transcribed with ImProm-II™ Reverse Transcriptase (Promega) and oligo(dT) primer according to the manufacturer's instruction. The first strand cDNA was used as template for PCR amplification. For cloning of isopentenyltransferase gene, Nitrilase and Knotted-1 gene, degenerate primers were designed on the basis of the conserved amino acid sequences of previously reported genes by Block maker and CODEHOP program (http://bioinformatics.weizmann.ac.il/blocks/blockmkr/www/make_blocks.html). Degenerate primer sets were described in Table 1.

The PCR amplification was carried out in T-Personal Thermal Cycler (Biometra) with 20 ㎕ reaction mixture containing 2 ㎕ of cDNA, 20 pmol of each degenerate forward and reverse primers, 2.5 unit of i-MAX™ II DNA polymerase (Intron biotech), 2 ㎕ of 10 mM dNTP mix, 2 ㎕ of PCR buffer, and sterilized water. The PCR conditions consisted of an initial denaturation step of 5 min at 94℃, followed by 35 cycles of 40 sec at 94℃, 40 sec at 60 or 63℃, and 60 sec at 72℃, with a final extension step of 10 min at 72℃. The PCR product was visualized on 1.2 % agarose gel stained with ethidium bromide.

Table 1. Primer sequences for degenerate RT-PCR

Target gene	Primer sequence
IPT	F: 5`-GGTGGTGGTGATCATGGGNGCNAC-3` R: 5`-GCACGGGCAGCTGCACRTCANCCCA-3`
NIT	F: 5'-CCGAGGCCTTCATCGGNGGNTAYCC-3 R: 5'-GGGCATCCTGTTCTCCCARCADAT-

Rapid amplification of cDNA ends, cloning and sequence analysis

Full-length of cDNA sequences was obtained by both of 5' and 3' RACE PCR using Gene-RACE Kit (Invitrorgen, USA). The Gene specific primer sequence for RACE PCR were shown in Table 2. The PCR amplification was carried out as follow; initial denaturation step of 5 min at 94℃, 35 cycles of 40 sec at 94℃, 40 sec at 60 or 63℃, and 60 sec or 90sec at 72℃, a final extension step of 10 min at 72℃. The first PCR products were amplified with nested primer and 1 ㎕ of the first PCR products were used as templates. The PCR product was separated on 1% agarose gel and stained with ethidium bromide.

The PCR product was visualized on 1.2 % agarose gel stained with ethidium bromide, and amplified cDNA fragment was cloned into the pGEM-T Easy vector (Promega, USA). Recombinant bacteria was identified by white/blue screening and confirmed by PCR. Plasmid containing the insert was purified with DNA-spin™ Plasmid purification kit (Intron biotech, Korea). Clone was sequenced with the T7 forward and SP6 reverse primers. Nucleotide sequence was analyzed using the BLASTX program with default parameter in Genbank (http://www.ncbi.nlm.nih.gov/BLAST/). Translation and protein analysis were performed using the ExPASy tools (http://us.expasy.org/tools/). CAP3 sequence assembly program was used for overlapping of cDNAs sequences (http://pbil.univ-lyon1.fr/cap3.php). The deduced amino acid sequences of cloned genes and homologous genes in NCBI were aligned with ClustalW and GeneDoc program, and identities between compared proteins were also estimated by these program.

Northern blot analysis

Northern blot analysis was performed against total RNA preparations. 15 micrograms of total RNA separated on 1.2% denaturing agarose gel, and then trnasferred to possitively charge nylon membrane (Schleicher & Schull, USA). According to the instruction manual (BD Bioscience, USA), hybridization was carried out using α-^{32}P labeled DNA probe that was made by PCR (Sambrook and

Russel, 2001) as previously mentioned in 'DNA isolation PCR amplification' section. The membrane was washed using standard saline solution and exposed to X-ray film at -70℃.

Semi-quantitative RT-PCR

The expression of cloned genes were analyzed by one-step semi-quantitative RT-PCR using Maxime RT-PCR PreMix Kit (Intron biotech, Korea). The template RNA for RT-PCR was prepared as described above and 100 ng of total RNA was used. The RT-PCR reactions were performed as follow; reverse transcription reaction at 45℃ for 30 min, inactivation of RTase at 94℃ for 5 min, 30 cycles of 94℃ for 30 sec, 53℃ for 30 sec and 72℃ for 50 sec, a final extension step at 72℃ for 10 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA cloned from *A. scaber* was used as control with same condition as described above. After amplification, the RT-PCR products were separated on 1.4% agarose gel and stained with ethidium bromide. The primer sequences used for RT-PCR were listed in Table 3.

Table 2. Primer sets used for 5` & 3` RACE PCR of rosette gall of *A. scaber*

Primer	Sequence	Description
IPT-F	5`-ATGGGGGCCACTGGAACCGGGAAA-3`	gene specific primer for IPT 5`-RACE PCR
IPT-NF	5`-TCATGGCGTGCCACACCATCTCCTT-3`	gene specific nested primer IPT for 5`-RACE PCR
IPT-R	5`-CACGGGCAGCTGCACGTCAACCCA-3`	gene specific primer for IPT 3`-RACE PCR
IPT-NR	5`-ATGAATGAGTTAGATCCACCAGCGAT-3`	gene specific nested primer for IPT 3`-RACE PCR
GA3ox-R	5`-ACGAGGAAAGAATTGCCTGATTCATA -3`	gene specific primer for GA3ox 5`-RACE PCR
GA3ox-NR	5`-TGGGTGTGTTTCAAGTCATTAACCA -3`	gene specific nested primer for GA3ox 5`-RACE PCR
REA5-R	5`-GACCCGTTCCAAAGAGTGTTATCG-3`	gene specific primer for CCoAOMT 5`-RACE PCR
RE25-NR	5`-TTTCACCTAGGAACCCATCGAG-3`	gene specific nested primer for UDP-glycosyltransferase 5`-RACE PCR
RE25-F	5`-TCCTAGGTGAAAGGGGTCGAGTAG-3`	gene specific primer for UDP-glycosyltransferase 3`-RACE PCR
RE28-R	5`-CTGCCTAGTGACCACGTACGAAAC-3`	gene specific primer for no hits gene 5`-RACE PCR
RE28-NR	5`-CTATAGGAAAGCCACCCATACCAC-3`	gene specific nested primer for no hits gene 5`-RACE PCR
RE28-F	5`-GGGGGTGTAGGTATGGGATATCAA-3`	gene specific primer for no hits gene 3`-RACE PCR
GeneRACE 5` Primer	5′-CGACTGGAGCACGAGGACACTGA-3′	
GeneRACE 5` nested Primer	5′-GGACACTGACATGGACTGAAGGAGTA-3′	
GeneRACE 3` Primer	5′-GCTGTCAACGATACGCTACGTAACG-3′	
GeneRACE 3` nested Primer	5′-CGCTACGTAACGGCATGACAGTG-3′	

Table 3. Primer sequences used for semi-quantitative RT-PCR analysis

Gene	Primer sequence	Product size (bp)
NIT	F: 5`-GTAGTTCCTGGCCCTGAAGTAGACA-3` R: 5`-GTGTCGTAGACAGGGATGGTTGAT-3`	227
IPT	F: 5`-TCATGGCGTGCCACACCATCTCCTT-3` R: 5`-CTAGTAACTTACAACTGCCATCG- 3	742
GA3ox	F: 5`-GCACGAGGAAAGAATTGCCTGA-3` R: 5`-ATGATCCAAGTATTAGCCACATTA-3`	483
NAM	F: 5`-CTCATTTCTTTCTACCTTTACCC-3` R: 5`-CATTCCCACAAGCGATTTCTCTT-3`	261
REA28	F: 5`-ATGACAAGGTTGTGTTTTA-3` R: 5`-CTCCAAAAGTTCCTACACCACCAG-3	304
GAPDH	F: 5`-ATGGCCTTCACTATGGCCAATTC-3` R: 5`-TTCCCAGCTCCATCTCTGTCCA-3`	518

Results

Construction and analysis of cDNA library of *A. scaber*

The cDNA library was constructed from young stage of *A. scaber*. Procedures for cDNA library construction and ESTs generation was shown in Figure 20. The primary titer of cDNA library was 5.0×10^6 pfu/ml. When randomly selected recombinent phages were digested with Xho I and EcoR I, the insert size of cDNA fragments were larger than about 2.0 kb. A total of 2306 cDNA were randomly selected from the cDNA library and partially sequenced. The average length of EST sequences following vector and low-quality sequence trimming was 446bp bp.

The 2036 ESTs were analyzed with CAP3 sequence assembly program to identify unigenes. The initial ESTs were clustered into 1843 unigenes consisting of 232 contigs containing more than two ESTs and 1611 singletons (Figure 21 A). To assign the functions of 1843 unigenes, homology search was carried out with BLASTX program. According to the analytic results, 1843 unigenes could be categorized into three groups. Group I consisted of 695 unigenes that were homologous to the protein sequences with known or putative function in the public databases. Group II consisted of 249 unigenes that were homologous to protein with unknown function. Group III consisted of 763 unigenes that had no significant match to any protein sequences in NCBI database (Figure 21 B).

EST related to phytohormones such as plant growth and differentiation genes were screen on the base of putative function in the 695 unigenes. In the EST set, 28 unigenes were homologous with phytohormone related genes, whereas cytokinin synthase and indole acetic acid (IAA) synthase were not found. The putative phytohormone related gene were shown in Table 4.

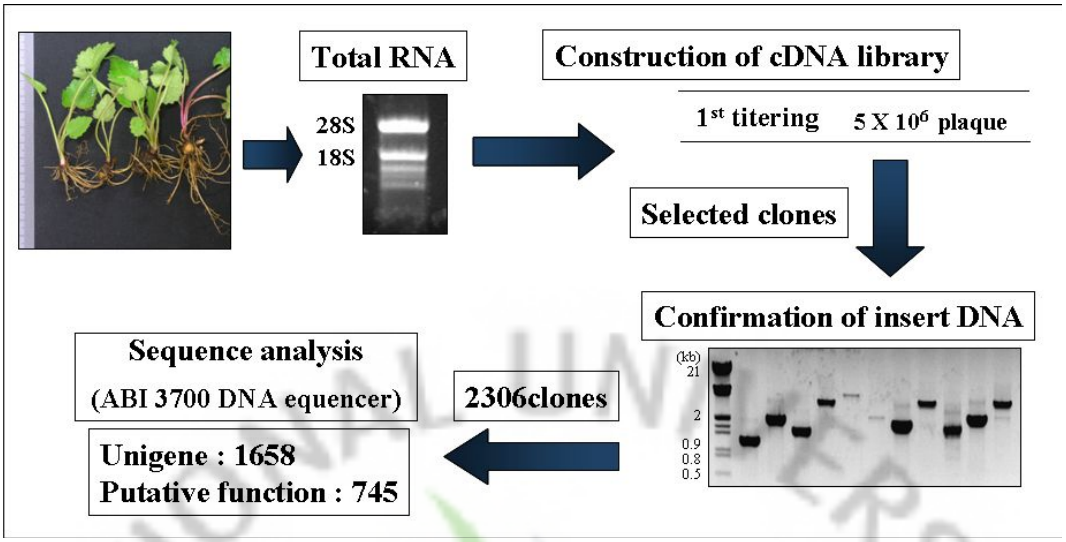

Figure 20. Procedure for cDNA library construction and ESTs generation from *A. scaber*.

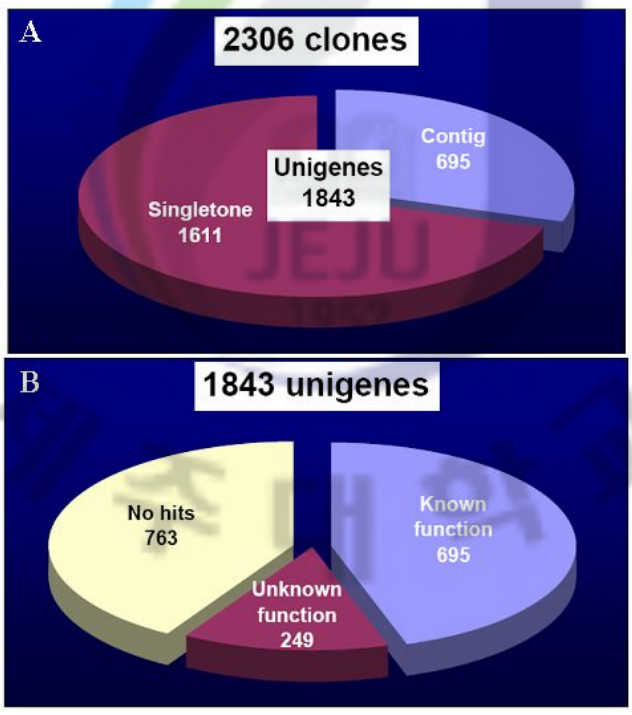

Figure 21. Graphical representation for clustering of 2036 clones (A) and putative identification of unigenes (B). ESTs clustering was carried out with CAP3 program and homology search was performed with BLASTX program in NCBI.

Table 4. Putative phytohormone related genes isolated from cDNA library

Putative identification [Origin]	% identity	E-value
Auxin response factor 1 [*Arabidopsis thaliana*]	77	1.8E-37
Auxin:hydrogen symporter [*Arabidopsis thaliana*]	72	9.0E-08
Auxin-induced-related / indole-3-acetic acid induced-related-like [*Oryza sativa*]	74	1.7E-52
Auxin-regulated protein [*Lycopersicon esculentum*]	80	4.0E-16
Auxin-regulated protein [*Zinnia elegans*]	98	1.1E-21
Brassinosteroid-regulated protein BRU1 precursor	86	8.7E-40
Class II ethylene responsive element binding factor-like protein [*Nicotiana benthamiana*]	74	2.6E-15
Cytokinin-regulated kinase 1 [*Nicotiana tabacum*]	48	5.8E-33
Gibberellic acid insensitive phloem [*Cucurbita maxima*]	52	1.8E-11
Gibberellin 2-oxidase No.1 [*Lactuca sativa*]	76	4.4E-48
Gibberellin 3beta-hydroxylase [*Lactuca sativa*]	78	4.7E-66
IBR5 (Indole-3-Butyric acid Response 5) [*Arabidopsis thaliana*]	75	1.6E-50
1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) [*Diospyros kaki*]	72	1.9E-63
CYP71A25 [*Arabidopsis thaliana*]	70	1.6E-17
CYP72A11 [*Arabidopsis thaliana*]	42	3.2E-21
CYP76C2 [*Arabidopsis thaliana*]	54	5.3E-21
CYP82C1p [*Glycine max*]	45	8.5E-33
CYP82C1p [*Glycine max*]	51	1.1E-32
CYP83D1p [*Glycine max*]	52	1.5E-39
CYP96A10 [*Arabidopsis thaliana*]	53	3.4E-37
CYP96A9 [*Arabidopsis thaliana*]	38	1.1E-10
Cytochrome P450 [*Helianthus tuberosus*]	72	5.7E-58
Cytochrome P450 [*Solanum tuberosum*]	63	1.8E-24
Cytochrome P450 [*Tussilago farfara*]	85	1.3E-41
Cytochrome P450 like_TBP [*Nicotiana tabacum*]	63	1.3E-19
Cytochrome P-450 protein [*Catharanthus roseus*]	46	6.1E-17
Cytochrome P450-dependent fatty acid hydroxylase [*Nicotiana tabacum*]	56	5.2E-44
P450 mono-oxygenase [*Stevia rebaudiana*]	55	5.2E-48

Cloning and expression of phytohormone genes

For the understanding of rosette gall development in relation with hormonal controls, the genes involved in the metabolism of phytohormones were cloned and their expressions were examined. Three genes of NIT, IPT and GA3ox encoding key step enzymes in the synthesis of auxin, cytokinin and gibberellin, respectively, were cloned.

Isopentenyltransferase (IPT) A cDNA encoding IPT was cloned and sequenced from rosette gall of *A. scaber* by RT-PCR using degenerate oligonucleotide primers based on the highly conserved sequences of *A. thaliana* IPT isozymes (AtIPT1, AtIPT3, AtIPT4, AtIPT5, AtIPT6, AtIPT7 and AtIPT8). The terminal sequences of cDNA were obtained by 5‘-RACE and 3‘-RACE PCR methods. A 1509-bp full-length cDNA contained a 207-bp 5’ noncoding region, a 987-bp open reading frame encoding a molecular mass of 36.3 kDa protein, 8.43 PI with 329 amino acids, and a 310-bp of 3‘ non-coding region (Appendix Figure 25).

The deduced amino acid sequence of rosette gall IPT showed 46%, 49%, 53%, 43%, 60%, 42%, 53% and 46% identity to those of IPT isozymes from *A. thaliana*, AtIPT1, AtIPT1, AtIPT2, AtIPT4, AtIPT5, AtIPT6, AtIPT7 and AtIPT8, respectively (Appendix Figure 26). Rosette gall IPT contains GATGTGKS (amino acids number 38 - 45) sequence known as the ATP/GTP binding motif which is universally observed in ATP-consuming enzymes including ATP-binding cassette transporters (Takei *et al*. 2001, Kakimoto, 2001).

In the phylogenetic tree (Appendix Figure 27), the IPT from rosette gall of *A. scaber* forms a cluster with *A. thaliana* AIPTs (AtIPT1, AtIPT3, AtIPT4, AtIPT5, AtIPT6, AtIPT7 and AtIPT8), but a separate cluster with other IPT enzymes including *A. thaliana* tRNA IPT (AtIPT2 and AtIPT9) and IPTs from plant-pathogenic bacteria. The rosette gall of *A. scaber* IPT appear to be evolutionary more related to AtIPT3 than to AtIPT1, AtIPT4, AtIPT5, AtIPT6, AtIPT7 and AtIPT8 isoforms.

Nitrilase (NIT) The partial cDNA sequence of NIT was isolated from rosette gall of *A. scaber* by RT-PCR using degenerate oligonucleotide primers based on the highly conserved sequences of *A. thaliana* NIT isozymes. The nucleotide and amino acid sequences was shown in appendix Figure 28. The length of partial cDNA was 387 bp and encoding 113 amino acids.

The partial amino acid sequence of rosette gall NIT showed 77%, 79%, 78%, and 73% identity to those of NIT isozymes from *A. thaliana*, AtINIT1, AtINIT2, AtINIT3, AtINIT4, respectively (Appendix Figure 29). Sequences were aligned using ClustalW and GeneDoc program. Black and gray shading boxes showed identical and similar amino acids, respectively.

GA3β-Hydroxylase The partial gene encoding GA3β-Hydroxylase was isolated by EST sequencing in cDNA of *A. scaber*. The length of partial cDNA was 483 bp and this partial sequences was homologous with GA3β-Hydroxylaseox. Thus, base on this sequences, primers for 5' RACE were designed and 5' end cDNA was amplified. The amplified product of rosette gall of *A. scaber* were 655 bp in length. The complete sequences of this gene was acquired by overlapping of 5' and partial cDNA sequences, and designated as GA3β-Hydroxylase. The nucleotide and amino acid sequences was shown in appendix Figure 30. The length of partial cDNA of rosette gall of *A. scaber* of GA3β-Hydroxylase was 670bp bp encoding 176 amino acids.

The amino acid sequence of the putative partial GA3β-Hydroxylase gene cloned from rosette gall of *A. scaber* is 75% identical to its homologus from *Lactuca sativa*, 60% identical to the *Prunus subhirtella*, 58% identical to the *Nicotiana tabacum* and 49% identical to the *Arabidopsis thaliana* (Appendix Figure 31). Sequences were aligned using ClustalW and GeneDoc program. Black and gray shading boxes showed identical and similar amino acids, respectively.

Expression analysis of phytohormone genes To investigated expression pattern of phytohormone related genes of IPT, NIT, GA3ox in rosette gall by Northern blot analysis and Semi-quantitative RT-PCR. Expression patterns of IPT, NIT and GA3ox in tissues of rosette gall (Figure 22 A) were analyzed using semi-quantitative RT-PCR using gene specific primer. IPT were observed highly expression in inner-leaflets tissue, outer-leaflet tissues and hemisphere gall tissues than mother leaf tissue. NIT expressed in inner-leaflet tissues and outer-leaflet tissue. GA3ox were observed low expressed in inner-leaflet tissue, similar expressed pattern shown in mother leaf tissue and the other tissues of rosette gall (Figure 22 B). Also, IPT were observed highly expressed pattern more than NIT, GA3ox in rosette gall tissues. Expression pattern of IPT in developmental stage of rosette gall was investigated by northern blot analysis (Figure 22 C). Northern blot analysis used total RNA from leaves of *A. scaber*, early stage of rosette galls, and growing stage of rosette galls. The expression pattern of rosette gall of IPT was over-expressed in rosette galls than leaves of *A. scaber*.

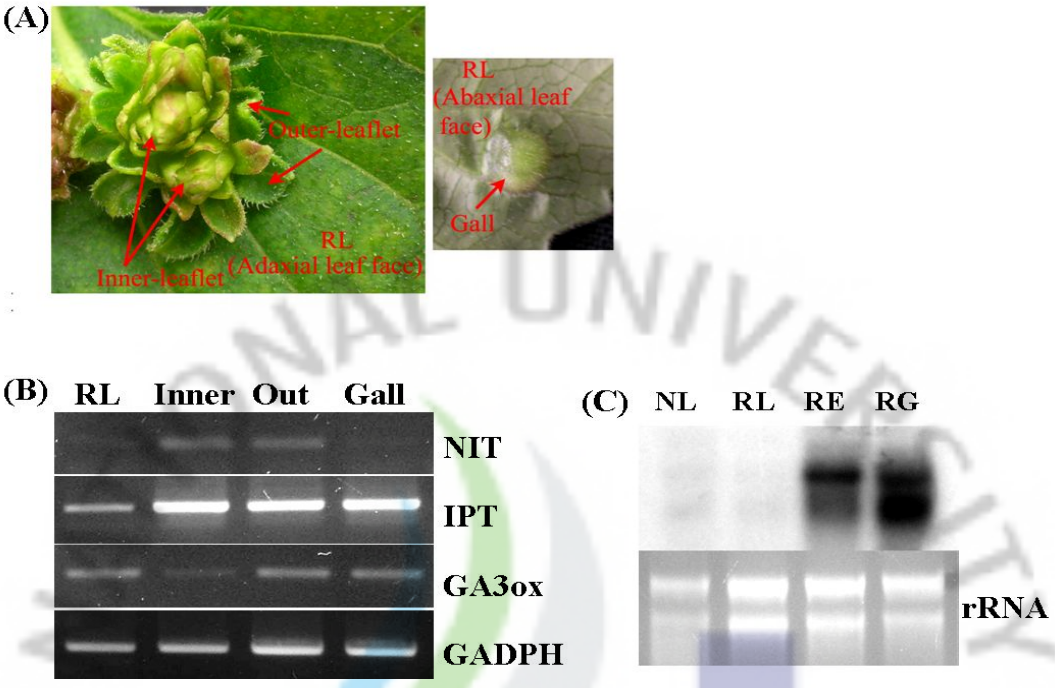

Figure 22. Expression of phytohormone related genes in rosette gall tissue on A. scaber leaf. A, Rosette gall on A. scaber leaf. B, Expression analysis of phytohormone related genes by semi-quantitative RT-PCR. RT-PCR was performed using mRNA from the appropriate part and gene-specific primers for NIT, IPT, GA3ox, respectively. Reaction products were resolved by agarose gel electrophoresis and stained with ethidium bromide. GADPH was used as a control. RL; Leaves nearby rosette galls growth, Inner; Inner-leaflets of rosette-like galls, Out; Outer-leaflets of rosette galls, Gall; Gall was hemisphere type on abaxial leaf. C, Expression analysis of IPT by Northern blot. Total RNA (15ug) from different tissue was loaded in 1.2% formaldehyde gel, transferred to nylon membrane and hybridized with 32P-labeled cDNA probe. NL; normal leaves of A. scaber, RL; Leaves nearby rosette galls growth, RE early stage of rosette galls, RG; growing stage of rosette galls.

Cloning and expression of differential expression genes in rosette gall

Subtractive hybridzation To identify genes in rosette gall, subtracted cDNA libraries were prepared using maturing stage of rosette galls and normal leaves of *A. Scaber* at August. After the three cycles of subtractive enrichment and amplification, the subtracted cDNA was cloned into pGEM-Easy vector and transformed into *E. coli* strain JM109. Resultant colonies were picked and plasmid DNA isolated. The inserts in the plasmids were amplified by PCR using M13F and M13R primers and the sizes of the inserts estimated by gel electrophoresis. The majority of the clones analysed64 had inserts between 200 and 500 bp, the small insert size being due to the initial digestion of the target cDNA with RsaI and AluI. A total of about 210 randomly picked colonies from libraries were their expression in rosette gall and normal leaf was analyzed by reverse northern dot-blot. Sequenced were about 60 clones of differentially expressed patterns showed significant changes in their expression.

About 210 randomly picked clones from subtracted cDNA library were analyzed by reverse northern blot. Consequently, 56 differentially expressed clones were selected and sequenced. The sequences were compared against GenBank database using BLASTX. Among these clones, 31 cDNAs were homologous with known genes, including auxin-repressed protein like-protein, protease Do-like 1, orf107a and senescence-associated protein. The others 25 cDNAs were no homologous with any gene in Genbank (Table 5).

Table 5. Putative identification of subtracted cDNA clones

Clone	putative identification	origin
R3	Auxin-repressed protein like-protien	*Malus* x *domestica*
R132	Protease Do-like 1, Chloroplase precursor	*Arabidopsis thaliana*
R62, R95, R96, R161, R199	orf107a	*Arabidopsis thaliana*
R2, R24, R29, R43, R59, R108, R111, R116, R145, R146, R162, R163, R164, R165, R166, R169, R179, R181, R182, R187, R192, R196, R203, R206	Putative senescence-associated protein	*Pisum sativum*
R16, R25, R46, R55, R56, R61, R70, R76, R86, R87, R88, R89, R97, R106, R115, R121, R122, R142, R143, R147, R172, R179, R198	No hit	

DDRT-PCR To explore differential expression genes from rosette gall development, RNAs extracted from leaves of *A. scaber* and leaflets development rosette galls(Figure 47) were subjected to RT-PCR using a combination of 40 arbitrary primers and two anchored oligo(dT) primers (dT-ACP1 and dT-ACP2). 13 differentially expressed DNA bands were identified between leaves and leaflets developmental stage of rosette galls. Among these 13 DNA bands were purified from agarose gels and cloned into pGEM-T easy vector. The clones were sequenced, and the sequence similarities and characterization of these differential expression genes are summarized in Table 6.

Table 6. Putative identification of cDNA clones by differential display RT-PCR.

clone	length (bp)	putative identification	origin	% identity[a]	E-value
REA5	693	S-adenosyl-L methionine:trans-caffeoyl-CoA 3-O-methyltransferase	*Zinnia elegans*	94	3.00E-63
REA8	790	lipoxygenase	*Nicotiana attenuata*	76	8.00e-12
REA25	781	UDP-glycosyltransferase	*Stevia rebaudiana*	75	3.00E-86
REA28	468	no hits			
REA31	670	protein phosphatase 2C	*Medicago sativa*	70	4.00E-58
REA34	528	HSP like protein	*Arabidopsis thaliana*	48	1e-07
RLA6	494	Unknown protein	*Vitis vinifera*	44	0.017
RLA6	55	xylose isomerase	*Hordeum vulgare*	78	5e-46
RLA9	797	COLD SHOCK DOMAIN PROTEIN 1	*Arabidopsis thaliana*	48	2.00E-18
RLA29	444	photosystem II CP47 protein	*Liriodendron tulipifera*	97	4.00E-21
RLA30	502	plastidic aldolase NPALDP1	*Nicotiana paniculata*	94	2.00E-41
RLA32	485	kinesin-like calmodulin binding protein	*Solanum tuberosum*	82	4.00E-20
RLA40	824	Unknown protein	*Arabidopsis thaliana*	68	2.00E-37

a percent identity at amino acid level.

Auxin-repressed protein (ARP) A cDNA encoding Auxin-repressed protein (ARP) was cloned and sequenced from rosette gall of *A. scaber* by subtractive hyberization and differential screening. The length of partial cDNA was 383 bp and this partial sequences was homologous with ARP. The terminal sequences of cDNA were obtained by 5‘-RACE and 3‘-RACE PCR methods. The nucleotide and amino acid sequencess were shown in Appendix Figure 32. A 702-bp full-length cDNA contained a 49-bp 5’ noncoding region, a 348-bp open reading frame encoding a molecular mass of 12.5 kDa protein, 9.69 PI with 116 amino acids, and a 305-bp of 3‘ non-coding region. The conserved domain search in NCBI indicated that contained ARP domain.

S-adenosyl-Lmethionine:trans-caffeoyl-CoA3-O-methyltransferase(CCoAoMt)

Differentially expressed gene in rosette gall of *A. scaber*, CCoAoMt, was isolated by modified DDRT-PCR. Nucleotide sequence analysis of 693bp of partial cDNA and comparison with the GenBank data base indicated it to be a CCoAoMt. Based on this nucleotide sequences, primer for 5' RACE was designed and 5' end cDNA was amplified. The amplified product of 5' end cDNA was 600 bp in length. The complete sequences of this gene was acquired by overlapping of 5' and 3' end sequences and designated as CCoAMT. The nucleotide and amino acid sequencess were shown in Appendix Figure 33. The full-length cDNA was 980 bp long, contained a 22-bp 5’ noncoding region, an ORF of 732 bp encoding a polypeptide of 244 amino acids with a theoretically molecular weight of 27.5 kDa and isoelectric point of 5.29, and a 164-bp of 3‘ non-coding region. The conserved domain search in NCBI indicated that contained S-adenosylmethionine-dependent methyltransferases superfamily(AdoMet-MTase) domain.

UDP-Glycosyltransferase Differentially expressed gene in rosette gall of A. scaber, UDP-Glycosyltransferase, was isolated by modified DDRT-PCR. Nucleotide sequence analysis of 781bp of partial cDNA and comparison with the GenBank data base indicated it to be a UDP-glycosyltransferase. The terminal sequences of cDNA were obtained by 5‘-RACE and 3‘-RACE PCR methods. The amplified product of 5' end cDNA was 1066bp in length. The complete sequences of this gene was acquired by overlapping of 5' and 3' end sequences and designated as UDP-Glycosyltransferase. The nucleotide and amino acid sequencess were shown in Appendix Figure 34. The full-length cDNA was 1624 bp long, contained a 96-bp 5’ noncoding region, an ORF of 1383 bp encoding a polypeptide of 460 amino acids with a theoretically molecular weight of 51.9 kDa and isoelectric point of 5.61 and a 145-bp of 3‘ non-coding region. The conserved domain search in NCBI indicated that contained Glycosyltransferase_GTB_type Superfamily domain.

Expression patterns of differential expression clones Expression pattern of differential expression clones was in investigated by Northern blot analysis. The differential expressed clones of ARP, CCoAMt, UDP-glycosyltransferase from subtractive hybridization, DDRT-PCR was investigated by Northern blot analysis. Norther blot analysis used total RNA form leaves of *A. scaber* and roette galls. Expression of ARP genes was over expressed in rosette gall than in leaves of *A. scaber* (Figure 23 A) Also, CCoAMt gene was over expressed in rosette gall than in leaves of *A. scaber* (Figure 23 B). UDP-glycosyltransferase expression was over expressed in rosette gall than in leaves of *A. scaber* and higher expressed in leaflets development stage of rosette galls more than early stage of rosette galls (Figure 23 C).

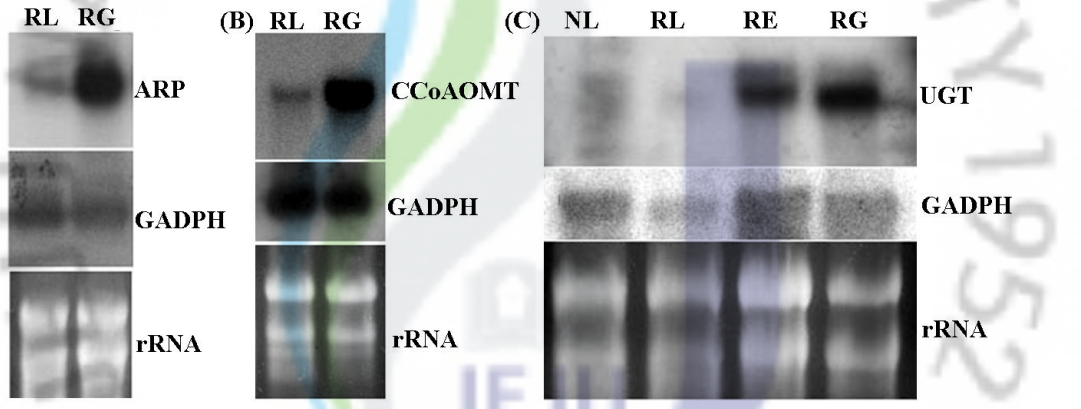

Figure 23. Northern blot analysis of differential expressed genes in rosette gall.
A, Auxin-repressed protein (ARP). B, CoA 3-O-methyltransferase (CCoAOMT). C, UDP-glycosyltransferase (UGT). Total RNA (15ug) from different tissue was loaded in 1.2% formaldehyde gel, transferred to nylon membrane and hybridized with 32P-labeled cDNA probe. Hybridization to GADPH was used as a loading control. NL; normal leaves of A. scaber, RL; Leaves nearby rosette galls growth, RE early stage of rosette galls, RG; growing stage of rosette galls.

Cloning and expression of an unknown gene in rosette

REA28 **D**ifferential expressed gene in rosette gall of *A. scaber*, REA28, was isolated by DDRT-PCR (Appendix Figure 35). Nucleotide sequence analysis of 468bp of partial cDNA and comparison with the GenBank data base indicated it to be a no hits gene. Based on this nucleotide sequences, primer for 5' RACE was designed and 5' end cDNA was amplified. The amplified product of 5' end cDNA was 570 bp in length. The complete sequences of this gene was acquired by overlapping of 5' and 3' end sequences and designated as REA28. The nucleotide and amino acid sequencess were shown in Appendix Figure 36. The full-length cDNA was 711 bp long, contained a 62-bp 5' noncoding region, an ORF of 450 bp encoding a polypeptide of 150 amino acids with a theoretically molecular weight of 13.9 kDa and isoelectric point of 7.89, and a 196-bp of 3' non-coding region. The putative Signal peptide sequences were found in the sequences at N-terminal. The BLAST analysis of the cDNA sequence showed no homology to any known genes in GneBank and ExPASy.

Expression pattern of Unknown gene Expression pattern of Novel gens in rosette gall developmental stage, each tissue of rosette gall, each organ in normal plant of *A. scaber* was in investigated by Northern blot analysis and Semi-quantitative RT-PCR.

Expression pattern of REA28 gene in developmental stage of rosette gall and each organ of *A. scaber* was investigated by northern blot analysis. Northern blot analysis used total RNA from leaves, initiation of leaflets stage, leaflets developmental stage of rosette galls of *A. scaber,* flower buds, Leaves, Petioles, stems and Roots. Developmental stage of rosette galls was specific expressed in rosette gall and more than expressed in initiation leaflets developmental stage of rosette gall (Figure 24 A). Also this gene showed that was specifically expressed in rosette galls rather than other parts of normal plant including flower buds, leaves, petioles, stems, roots in maturing stage of *A. scaber* (Figure 24 B).

To investigated on the transcription level of REA28 genes in each development

stage (May to September) of rosette gall by Semi-quantitative RT-PCR using gene-specific primers. The expression pattern of R28 was specific expression in rosette gall rather than leaves of *A. scaber* (Figure 24 C). In rosette gall tissue, this gene was over expressed in inner-leaflets than outer-leaflets of rosette-like galls (Figure 34 D).

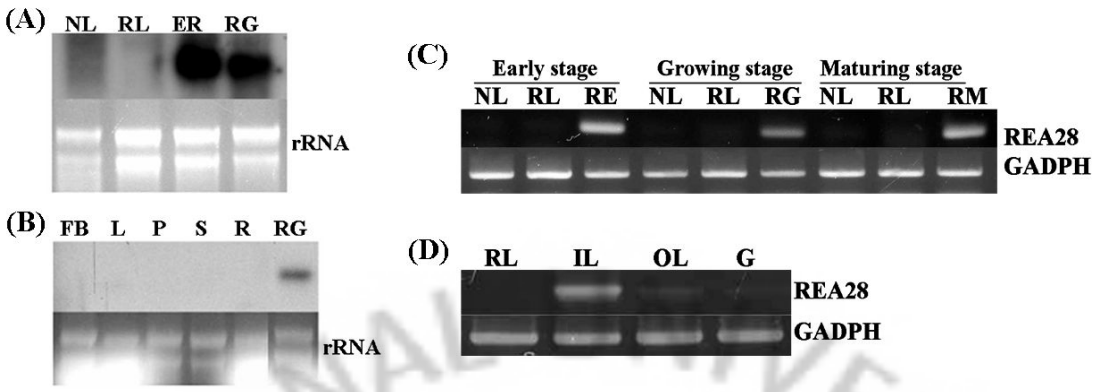

Figure 24. Expression of clone REA28.

A, Northern blot analysis of REA 28. Expression pattern of REA 28 were over-expression in rosette gall tissues. NL; normal leaves of A. scaber, RL; Leaves nearby rosette galls growth, RE early stage of rosette galls, RG; growing stage of rosette galls. B, Northern blot analysis of REA 28 in various organs of A. scaber. REA 28 was specific expression in rosette gall tissue than normal organs of A. scaber. Northern blot analysis using total RNA (15ug) from different tissue was loaded in 1.2% formaldehyde gel, transferred to nylon membrane and hybridized with 32P-labeled REA28 cDNA probe. FB; Flower-buds, L; Leaves, P; Petioles, S, Stems, R, Roots, RG; Growing stage of rosette galls. C, Semi-quantitative RT-PCR analysis of REA 28 in developmental stage of rosette gall. NL; Normal leaf of A. scaber, RL Leaves nearby rosette galls growth, RE; early stage of rosette gall (early May), RM; Growing stage of rosette gall (mid-May), RM Maturing stage of rosette gall (mid-September). D, Expression analysis of REA 28 in rosette gall tissues by semi-quantitative RT-PCR. RT-PCR was performed using mRNA from the appropriate part and gene-specific primers for REA28. Reaction products were resolved by agarose gel electrophoresis and stained with ethidium bromide. GADPH was used as a control. RL; Leaves nearby rosette galls growth, IL; Inner-leaflet tissues of rosette gall, OL; Outer-leaflet tissues of rosette gall, G; Gall was hemisphere type on abaxial leaf.

Discussion

Profile of gene expression in the host plant of *A. scaber*

The sequences of 2306 cDNA clones were determined and their homology was analyzed with NCBI database. The cDNAs clustered into 1843 unigenes, which consisted of 45% of known function, 13% of unknown function and 42% of no hit genes, respectively. The library contained 28 genes related with phytohormone metabolism which were assumed to be important in the rosette gall development.

Phytohormone genes in the rosette gall

Plant hormone is a molecule that at micromolar or low concentrations acts as a messenger between plant cells (Cleland, 1999). There are important roles in plant morphogenesis and architectural establishment which are auxins, cytokinins, the gibberellins (GAs), etc. Plant hormone biosynthesis is closely associated to primary and secondary metabolism. NIT, IPT and GA3ox encoding key step enzymes in the synthesis of auxin, cytokinin and gibberellin, respectively. Plant nitrilases to convert indole-3-acetonitrile (IAN) into the plant growth hormone indole-3-acetic acid (IAA) has key enzyme in auxin biosynthesis (Piotrowski 2008). Cytokinin synthesis (Zeatin) is catalyzed by adenosine phosphate-isopentenyltransferase (IPT), which transfers the prenyl group of dimethylallyl diphosphate (DMAPP) to the N6 position of adenine nucleotides (ATP, ADP or AMP; Sakakibara 2006). GA3ox is enzyme that catalyze the final steps in the synthesis of bioactive GAs (Sun and Gubler, 2004).

In plant, antagonistic action of auxin and cytokinin, high cytokinin to auxin ratio promotes shoot development in plant cell culture. These processes are directed by the relative concentrations of the plant hormones cytokinin and auxin (Skoog and Miller 1957, Steward 1970), such that a high cytokinin to auxin ratio promotes shoot development, whereas a low cytokinin to auxin ratio promotes root development. Cytokinin and gibberellins are mutually antagonistic to each other.

These are including on plant developmental progress such as root and shoot elongation, cell differentiation, and shoot regeneration in plant culture, and meristem activity (Weiss and Ori, 2007).

The mechanism of gall induction is understood are bacterial, such as crown gall induced by *Agrobacterium*spp. and the root nodules induced by nitrogen-fixing *Rhzibium* and *Frankia* spp., (Stone and Schonrogge 2003). The molecular basis of crown gall involves the transfer and integration of the T-DNA region of *A. tumerfasciens* Ti plasmid into the genome of recipient plant cells, followed by expression of bacterial genes (Davey et al 1994). Nodulation (Nod) factors are lipo-chitooligosaccharide signal molecules produced by bacterial such as *Rhzibium* and *Frankia* during the initiation of nodule formation on the root of legumes (D`Haeze and Holsters 2002, Stone and Schonrogge 2003).

In this study, for the understanding of rosette gall development in relation with hormonal controls, the genes involved in the metabolism of phytohormones were cloned and their expressions were examined. Three genes of NIT, IPT and GA3ox encoding key step enzymes in the synthesis of auxin, cytokinin and gibberellin, respectively, were cloned. From SQRT-PCR analysis (Figure 22 B), NIT gene showed highly expressed in the inner and outer leaves of the rosette gall and its expression in the gall tissue was similar to mother leaf. IPT gene showed highly expressed not only in the inner and outer leaves of the rosette gall but also in the gall tissue by the similar level. In contrast to NIT and IPT, GA3ox was suppressed in the inner leaf of rosette gall while its expression in outer leaf and gall tissue were similar to mother leaf. From Northern blot analysis (Figure 22 C), IPT gene, also higher expression in rosette gall than normal tissue of A.scaber. Therefore, auxin was supposed to be actively synthesized in the ectopic leaves of rosette gall. The delocalized high expression of IPT over the whole rosette gall might contribute to the compact morphology and retarded senescence of the rosette gall by providing high cytokinin. The dwarfism of the rosette gall might be resulted from the lack of

gibberellin due to the suppression of GA3ox in the inner leaf because gibberellin 3beta-hydroxylase encoded by this gene mediates the final step in the synthesis of active gibberellin. These results suggest that rosette gall formation and development might manipulate regulation of endogenous hormones in *A. scaber* various pathways, modified hormone levels by signal factors or stimulation of insect.

IPT was the first identification of CK biosynthesis enzyme from *A. tumdfaciens*, a crown gall-forming bacterium (Akiyoshi et al 1984, Barry et al 1984). Also, IPT was identification from phytopathogen of *Rhodococcus fascians*, a leafy gall (fasciation) forming bacterium (Crespi et al 1992).

Infection of Arbidopsis (*Arabidopsis thaliana*) and tobaco (*Nicotinana tabacum*) with *R. fascians* induced abnormal flower, multiple rosettes, and small and serrated leaves (Vereecke et al. 2000, de O. Manes et al. 2004, Depuydt et al 2008). The fasciation phenotype is induced many plant species and results from the activation of axillary meristems and *de novo* meristem formation (de O. Manes et al. 2001, Simón-Mateo et al., 2006). These morphological changes of the aerial plant part with *R. fascians* showed to induce genes involved in abscisic acid (ABA), GA catabolism, CK metabolism and transcription factors of class-I *KNOTTED*-likes homebox (KNOX) family (Simón-Mateo et al. 2006, Depuydt et al 2008).
Transcription factors of the class-I KNOTTED-like homeobox (KNOX) family, SHOOT MERISTEMLESS [STM], BREVIPEDICELLUS [BP]/KNOTTED-LIKE1 [KNAT1], KNAT2, and KNAT6 are crucial for function and maintenance of meristems (Hake et al., 2004). KNOX gene expression is also involved in leaf shape determination (Chuck et al. 1996, Byrne et al, 2001, Frugis et al. 2001, Hake et al. 2004, Müller et al., 2006, Depuydt et al 2008).

Morphological phenotype of rosette galls were showed various form, which are ectopic leaflets development, fused leaflets, inflorescence, floral-like structure, and

de nove developed roots (show in Part I, Part III). Also, phytohormone genes, NIT, IPT, GA3ox were expressed in rosette gall. Therefore, it possible that rosette gall development on *A. scaber* is may be suggested involved to genes of KNOX family with regulation of plant hormones.

Although numerous study have been reported to understanding of the differentiation and development of insect galls, the gall-inducing signal molecules involved in insect-plant interactions remain obscure (Bayer, 1994). In all cecidogensis (gall formation) morphogenetic changes occur concomitantly with physiological and biochemical modifications at the cellular level. The most commonly proposed signals are known plant growth substances, such as indole acetic acid (IAA) and other auxins, zeatin and other cytokinins, gibberellins are intimately involved gall development and differentiation (McCalla, 1962; Byers, 1976; Hori, 1992; Miles, 1999; Mapes and Davies, 2001). However, littles known about biochemical change that may mechanisms during gall formations (Bayer, 1994; Bayer et al., 1994; Stone and Schönrogge 2003).

According to Mani (1964), the insect saliva or ovipostional fluid contains either a plant hormone analogue that directly stimulates plant cells, or a compound that indirectly affects cell growth by triggering hormone production. Also, plant growth regulators are associated with hypertropic and phyerplasic response of many gall tissues. Auxin, indole-3-acetic acid (IAA) have been found in insects saliva and salivary grand including phloem-feeding aphids, some other homopterans, larvae of the midge *Janetiella* sp. near *J. colouradensis,* fly *Eurosta solidaginis,* and their inducing galls (Byers 1976, Hori 1992, Mapes and Davies 2001). This growth regulator suggested may play main role in gall induction by insect saliva (Hori 1992). Cytokinin or related compounds that promote plant cell division and have been found in insect saliva or salivary grand associated with oviposition and gall initiation (Schultz 2002). Leitch (1994) was found isopentenyladenine,

isopentenyladenosine, isopentenyladenine ribotide, and isopentenyladenine-9-glucoside in galls induced by *Pontania proxima.* Van Staden and Davey (1978) were found zeatin, zeatin riboside, and zeatin glucoside in gall tissues formed by a chalcid wasp on *Erythrina latissima* (Mapes and Davies 2001). Ohkawa (1974) isolated compounds active in the tobacco callus bioassay from the oriental chestnut gall wasp (*Dryocosmus kuriphilus*), and presented evidence for the presence of zeatin in the larvae (Mapes and Davies 2001). McDermott *et al.* (1996) was found high levels of isopentenyladenosine in hackberry (*Celtis occidentalis*)gall tissues than in control leaf tissues. Mapes and Davies (2001) isolated zeatin, zeatin riboside, isopentenyladenine, and isopentenyladenosine in ball gall of *Solidago altissima,* and in larva of *E. solidaginis*. Other plant hormone, gibberellin-like substance werefound high levels in pinyon (*Pinus edulis*) gall induced by larva of the midge *Janetiella* sp. near *J. colouradensis* (Byers 1976).

Differential expression genes in rosette gall

Plant gall differ form the normal host plant tissue with regard to their morphology, histology, and biochemical composition (Bayer et al, 1994). Most gall tissue are contains high levels of phenolic compounds, flavonoids and nutritive cells with high concentration of polysaccharides, lipid, sugars and amino acid than normal host tissue (Bronner, 1992; Hori, 1992; Bayer, 1994; Nyman and Julkunen-Tiitto, 2000; Stone and Schönrogge 2003). These compounds are asscociated with chemical defecnes to wounding herbivory, as stimulants to insect feeding (Hartley, 1998; Nyman and Julkunen-Tiitto, 2000).

From subtractive cloning, 56 clones showed Known or no hits in the current databases (Table 5). These clones might be play roles in the gall formation and development were differentially expressed by *D. asteriae* and *A. scaber* interaction.

From DDRT-PCR, 13 clones showed known, unknown or no hits in the current

databases (Table 6). Among these genes, 6 clones showed expressed in rosette gall, 7 clones showed expressed in mother leaf. These clones could probably be involved in gall formation and defense response were by *D. asteriae* and *A. scaber* interaction

Differential expression genes in rosette gall (Figure 23), ARP, CCoAOMt and UDP-glycosyltransferse was expressed in gall tissue than normal leaves of *A. scaber*. ARP, UDP-glycosyltransferase and CCoAOMT expressed might response of the host plant by insect simulation. Caffeoyl coenzyme A O-methyltransferase (CCoAOMT) converts caffeoyl coenzyme A into feruloyl coenzyme A which is essential for the biosynthesis of monolignols (Ye *et al.*, 1999). The presence of lignin in the cell wall increases the mechanical strength of the plant, enables solute conductance, and plays a role in resistance against biotic and abiotic stress (Chen et al.,1999).

A novel gene expressed in the rosette gall

Clones REA28 showed specifically expressed in the rosette gall (Figure 24). Inner leaf tissue in rosette gall, GA3ox expression pattern showed low expression, on the other hand, clone REA 28 showed highly expression by SQRT-PCR analysis. Also, this gene showed specific expression in rosette gall than various tissue of host plant by northern blot analysis. In this case, these results suggest that might play as a regulatory element in the process of rosette gall development. Also, the coincident suppression of GA3ox with the expression of clone REA28 suggested that this gene might control the dwarfism of rosette gall, probably via inhibition of GA synthesis.

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Appendix

```
001 GGACACTGACATGGACTGAAGGAGTAGAAAGACTGGAGCACGAGGACACTGACATGGACT     60
0061 GAAGGAGTAGAAAATATTCAACTCAAACCTGTTTCCTTCAATTAATTCAACTCAGCCAAG   120
0121 AGCAGCACAACAACCCAGCACACCACATCCAACCACCTTCTATATATTAACTTTTAAGAT   180
0181 TGGTTGTATATATAACAAAGAACCAAAATGATGATGTGCAAACAAGCTCAAGCACCAGT    240
                                  M  M  M  C  K  Q  A  Q  A  P  V      11
0241 AATGCAAATACCGACTGGAGGAATGGACTTATCGGTGCTCCGATGCCATCCACATAAGCA   300
M  Q  I  P  T  G  G  M  D  L  S  V  L  R  C  H  P  H  K  Q      31
0301 AAAGGTGGTGGTTGTAATGGGAGCCACTGGAACCGGGAAATCAAGACTTTCTATTGACCT   360
K  V  V  V  V  M  G  A  T  G  T  G  K  S  R  L  S  I  D  L      51
0361 TGCCACCCGTGTGCCGGCTGAAATAATCAATTCTGACAAAATTCAACTATATGAAGGTTT   420
A  T  R  V  P  A  E  I  I  N  S  D  K  I  Q  L  Y  E  G  L      71
0421 AGATATCGTAACGAATAAAATAACTGAAGAAGAATGTCATGGCGTGCCACACCATCTCCT   480
D  I  V  T  N  K  I  T  E  E  E  C  H  G  V  P  H  H  L  L      91
0481 TGGAATAGTTGATCCCGAGGCGGATTTTACTGCTGGGAACTTCGTCACCACGGCTTCACT   540
G  I  V  D  P  E  A  D  F  T  A  G  N  F  V  T  T  A  S  L     111
0541 TGCCATGAAATCAATAGCTGGGAGAGGGAAGCTGCCCATAATCGCTGGTGGATCTAACTC   600
A  M  K  S  I  A  G  R  G  K  L  P  I  I  A  G  G  S  N  S     131
0601 ATTCATTGAAGCATTAGTTGATGATAATAACTACGAGTTTCGATCAAGGTATGATGTTTG   660
F  I  E  A  L  V  D  D  N  N  Y  E  F  R  S  R  Y  D  V  C     151
0661 CTTCTTATGGGTTGATGTTGCAATGCGGGTTCTTAACCAATTCGTATCTGACCGTGTTGA   720
F  L  W  V  D  V  A  M  R  V  L  N  Q  F  V  S  D  R  V  D     171
0721 TCGCATGGTGGCTGCTGGGATGGTGGAAGAAGTTAGAAACATGTATCACCCCAAAGCCGA   780
R  M  V  A  A  G  M  V  E  E  V  R  N  M  Y  H  P  K  A  D     191
0781 CTATTCAAAAGGGATTCGTAGAGCCATTGGAGTGCCAGAATTCGACTCATATTTTCGTTC   840
Y  S  K  G  I  R  R  A  I  G  V  P  E  F  D  S  Y  F  R  S     211
0841 CCAATATTCATCTTCCACTGATGAAAAAACTCGCTCCAAATTACTAGAAGCCGCTATTAA   900
Q  Y  S  S  S  T  D  E  K  T  R  S  K  L  L  E  A  A  I  N     231
0901 TGAAACAAAGATTAACACATGCAAGCTTGCCTATAAGCAACTTAAAAAAATTCACCGGTT   960
E  T  K  I  N  T  C  K  L  A  Y  K  Q  L  K  K  I  H  R  L     251
0961 ACGAAATGTTAAAGGGTGGCATATTCATCGGTTAGATGCTACTGCAGTCTTTCAGAAAAA  1020
R  N  V  K  G  W  H  I  H  R  L  D  A  T  A  V  F  Q  K  K     271
1021 GGGTTGTGAAGCCGATGAGGCGTGGGCAAAGTTGGTGGCCCAACCTGCGTCAGCTATTGT  1080
G  C  E  A  D  E  A  W  A  K  L  V  A  Q  P  A  S  A  I  V     291
1081 AAACGAGTTTCTTTACAGTTTTGGTGATTCTCGGGCCTTTGCTGTGGCAGTTGACGGTGG  1140
N  E  F  L  Y  S  F  G  D  S  R  A  F  A  V  A  V  D  G  G     311
1141 CGGAAGAGGGATTAGAGAGGCTGAAATGGGGGCGGCGATGGCAGTTGTAAGTTACTAGGA  1200
G  R  G  I  R  E  A  E  M  G  A  A  M  A  V  V  S  Y  *        329
1201 TTTGAATTGAAAATGAACTAGAGTTAACAAGGTAATATTGGGATAGACAAAATTTTGAAC  1260
1261 AGAAGCATATAAGGTAGCTAAGTGATTTTATAAGTTTGCCCCTTGACCTTATTTTGACAT  1320
1321 GCTTTGGTGGTGCATGTTTACAAGGGCTTTATGGTTTCTGATACCATAGTATATTTTTAC  1380
1381 GTCTTTAACTTGTAGTAGTTTAACTTTTTCTTTTCCTTTTGTAACAAACTATATTCTATA  1440
1441 TTGAAAGAATAATACGCCCTTATATAAAAAAAAAAAAAAAAAAAAAACACTGTCATGCCGT  1500
1501 TACGTAGCG                                                      1509
```

Figure 25 . Nucleotide and deduced amino acid sequence of the rosette gall of IPT cDNA. The nucleotide and deduced amino acid residues are numbered in left and right margins, respectively. Underline is TATA box. The termination cordon is marked by an asterisk.

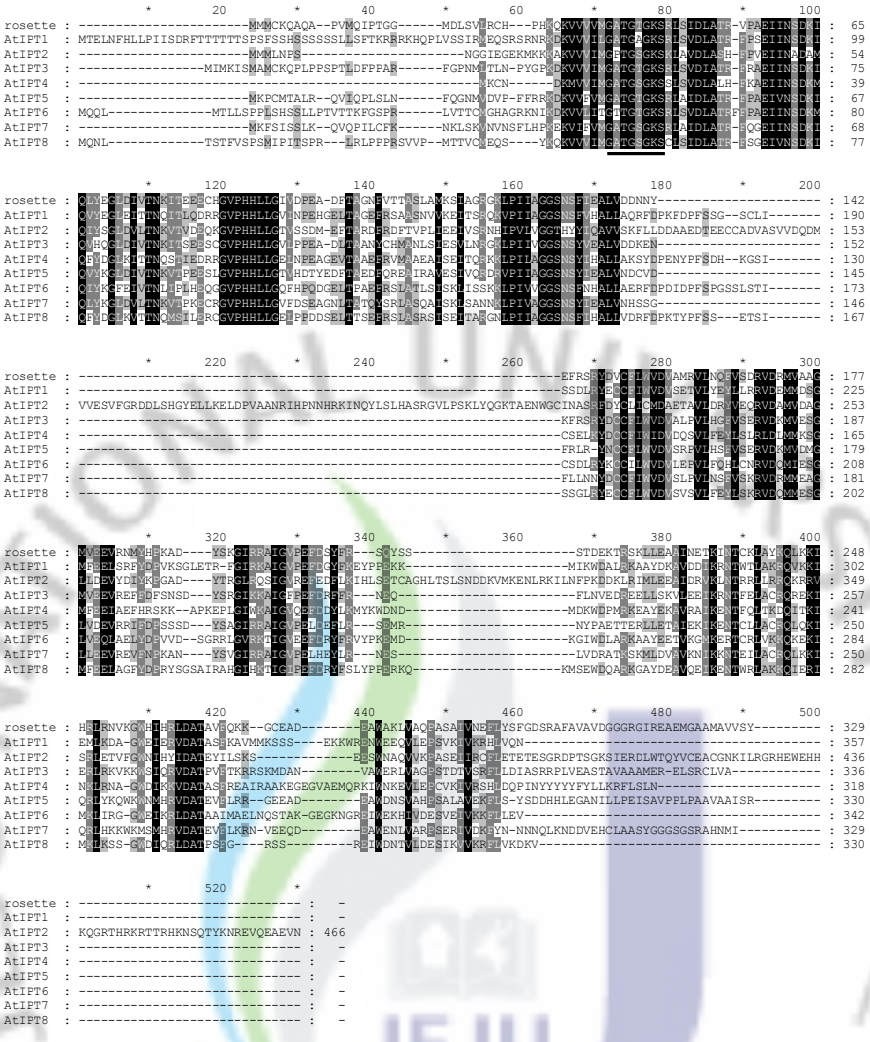
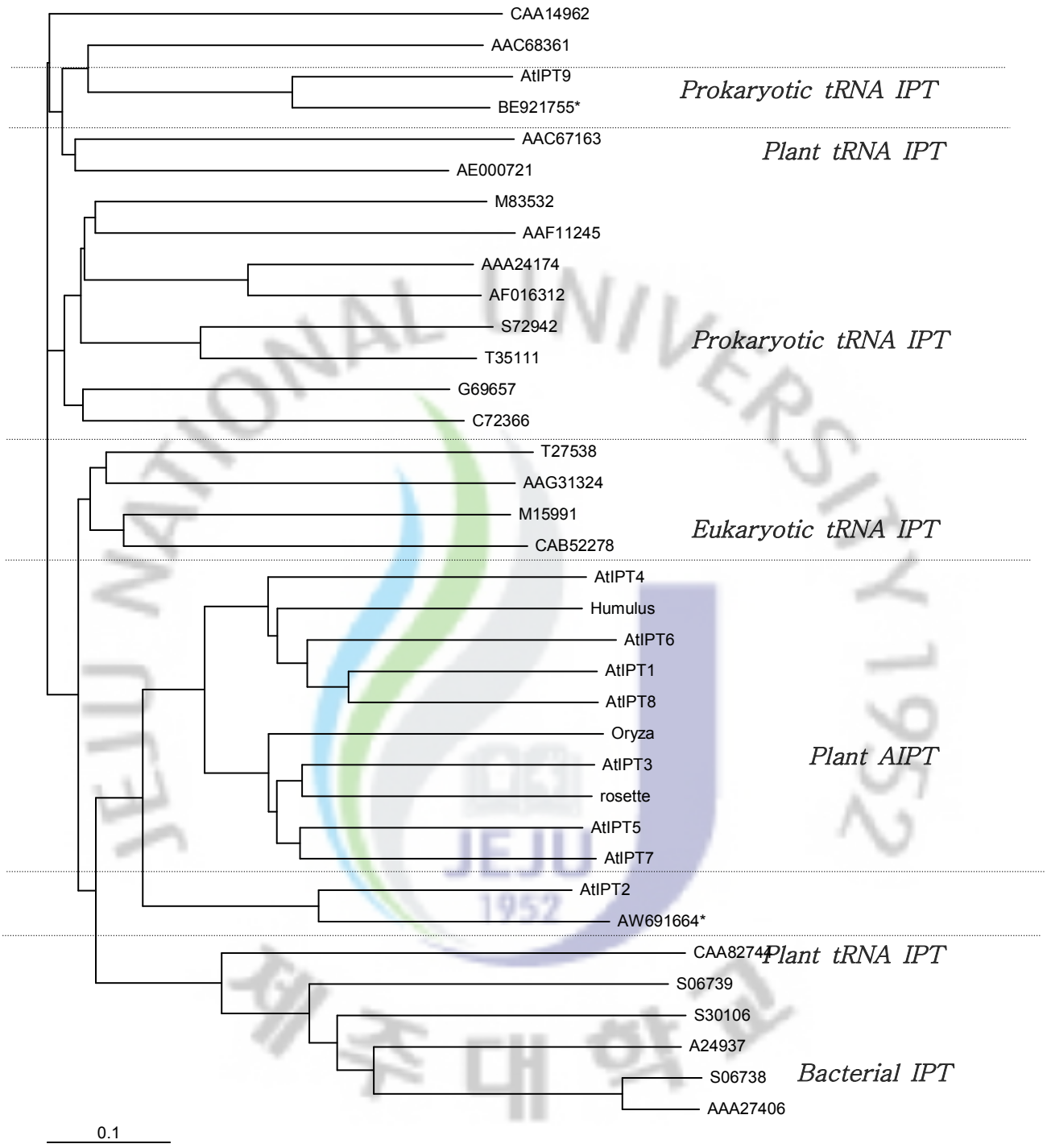


Figure 26. Comparison of deduced amino acid sequences of rosette gall IPT and *A. thaliana* AIPT isozymes (AtIPT1, AtIPT2 AtIPT3, AtIPT4, AtIPT5, AtIPT6, AtIPT7, AtIPT8). Underline indicates an ATP/GTP binding motif. Numbers in parentheses indicate the numbers of amino acid residues present of each gene product.


CAA14962
AAC68361
AtIPT9
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Prokaryotic tRNA IPT
AAC67163
AE000721
Plant tRNA IPT
M83532
AAF11245
AAA24174
AF016312
S72942
T35111
G69657
C72366
Prokaryotic tRNA IPT
T27538
AAG31324
M15991
CAB52278
Eukaryotic tRNA IPT
AtIPT4
Humulus
AtIPT6
AtIPT1
AtIPT8
Oryza
AtIPT3
rosette
AtIPT5
AtIPT7
Plant AIPT
AtIPT2
AW691664*
CAA82744
S06739
S30106
A24937
S06738
AAA27406
Plant tRNA IPT
Bacterial IPT
0.1

Figure 27. Phylogenetic tree of the IPT enzymes. Multiple sequence alignment performed by CLUSTAL W (1.8). *Aquifex aeolicus* (AE000721), *Agrobacterium rhizogenes* pRiA4 (S06738), *Agrobacterium tumefaciens*(M83532), *Agrobacterium tumefaciens* pTiC58 (AAA27406), *Agrobacterium vitis plasmid* pTiS4(S30106), *A. thaliana* AtIPT1 (AB061400/AB062607), *A. thaliana* AtIPT2 (AB062609), *A. thaliana* AtIPT3 (AB061401/AB062610), *A. thaliana* AtIPT4 (AB061402/AB062611), *A. thaliana* AtIPT5 (AB061403/AB062608), *A. thaliana* AtIPT6 (AB061404/AB062612), *A. thaliana* AtIPT7 (AB061405/AB062613), *A. thaliana* AtIPT8 (AB061406/AB062614), *A. thaliana* AtIPT9 (AB062615), *Humulus lupulus* (AAS94327), *Oryza sativa* (AAT77921), *Borrelia burgdorferi* (AAC67163), *Bacillus subtilis* (G69657), *Caenorhabditis elegans* (T27538), *Chlamydia trachomatis* (AAC68361), *Deinococcus radiodurans* (AAF11245), *E. coli* (AAA24174), *Homo sapiens* (AAG31324), *Medicago truncatula* AW691664*, *Mycobacterium leprae* (S72942), *Pseudomonas putida* (AF016312), *P. solanacearum* (S06739), *P. syringae* pCK1 (A24937), *Rhodococcus fascians* pFiD188 (CAA82744), *Richettsia prowazekii* (CAA14962), *Solanum tuberosum* BE921755*, *Saccharomyces cerevisiae* (M15991), *Streptomyces coelicolor* (T35111), *Schizosaccharomyces pombe* (CAB52278), *Thermotoga maritima* (C72366). The two EST clones are indicated by asterisk.

```
GGGTTGGCGTTCATAACGAAGCTGGTCAGGGACTGTTTCCGCAGATATCATGCTTCTGCC   60
G  L  A  F  I  T  K  L  V  R  D  C  F  R  R  Y  H  A  S  A     20
ATTGTAGTTCCTGGCCCTGAAGTAGACAAGCTGGCGGAGATGGCTAGGAAAAACAAAGTG  120
I  V  V  P  G  P  E  V  D  K  L  A  E  M  A  R  K  N  K  V     40
TACTTGGTGATGGGAGCGATGGAGAAGGATGGGTATACACTTTATTGCACAGCCCTTTTC  180
Y  L  V  M  G  A  M  E  K  D  G  Y  T  L  Y  C  T  A  L  F     60
CTCAGTTCCGAAGGTCGCTTTTTGGGCAAGCACCGTAAAGTCATGCCCACATCTCTGGAA  240
L  S  S  E  G  R  F  L  G  K  H  R  K  V  M  P  T  S  L  E     80
CGTTGCATCTGGGGTTACGGAGATGGATCAACCATCCCTGTCTACGACACTCCGCTTGGC  300
R  C  I  W  G  Y  G  D  G  S  T  I  P  V  Y  D  T  P  L  G     100
AAACTCGGTGCTGCTATATGCTGGGAGAACAGGATGCCC                       339
K  L  G  A  A  I  C  W  E  N  R  M  P                         113
```

Figure 28. Nucleotide and deduced amino acid sequence of the rosette gall of partial NIT cDNA.

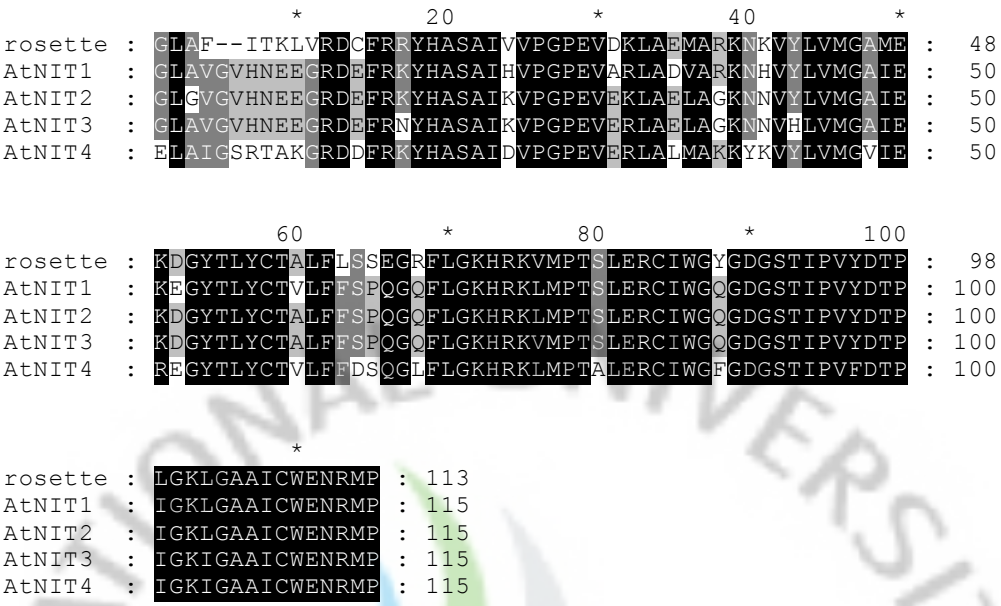

Figure 29. Alignment of the deduced amino acid sequences of rosette gall NIT and *A. thaliana* NIT isozymes (AtNIT1, AtNIT2 AtNIT3, AtNIT4). Genbank accession number of *Arabidopsis thaliana* NIT: ATNIT1(NP_851011.1), ATNIT2(NP_190016.1), ATNIT3(NP_190018.1), ATNIT4(NP_197622.1) Sequences were aligned using ClustalW and GeneDoc program. Black and gray shading boxes showed identical and similar amino acids, respectively.

```
GAAAAACATATCTTCTCCCTTCTTCTATCACAAGTATTATTAACATATAACCACCATTTC    60
CCTTGTGCTATAACTACTTTAATCTTATTCTTATTACTATCATATTACTAGACCTCTTTT    120
TCTTTAAACCTCACCTCCAATATGTCTTTAAAACTACTTACCAATGAAAAGCATCATTTG    180
                      M  S  L  K  L  L  T  N  E  K  H  H  L      13
GACCTAAATTCAATGAAAGAATTGCCTGATTCATATGCATGGTCACTAATGGATGATGAT    240
D  L  N  S  M  K  E  L  P  D  S  Y  A  W  S  L  M  D  D  D      33
GGCTACCCATCCACTAATTGTATTTCAGAGCCAGTTCCTTTGATTAACCTCAAAGATCCT    300
G  Y  P  S  T  N  C  I  S  E  P  V  P  L  I  N  L  K  D  P      53
AATGCCATCAAACACATTGGCCATGCATGCAAAACATGGGGTGTGTTTCAAGTCATTAAC    360
N  A  I  K  H  I  G  H  A  C  K  T  W  G  V  F  Q  V  I  N      73
CATGACATCCCTACCGCGGTTCTTGATGACATGGAGGCGTCCGGAAGAACGCTATTTTCT    420
H  D  I  P  T  A  V  L  D  D  M  E  A  S  G  R  T  L  F  S      93
CTCCCTATTCAACAAAAACTTAAAGCGGCTCGTTCTCCCGATGGTGTTTCGGGTTATGGT    480
L  P  I  Q  Q  K  L  K  A  A  R  S  P  D  G  V  S  G  Y  G     113
GTTGCTAGGATATCTTCTTTTTTCCCAAAACTTATGTGGTCTGAAGGTTTCACCATCATT    540
V  A  R  I  S  S  F  F  P  K  L  M  W  S  E  G  F  T  I  I     133
GGATCACCATATGAACATGCTCAAAAACTATGGCCACAAGGTTACAAAAACTTTTGTGAT    600
G  S  P  Y  E  H  A  Q  K  L  W  P  Q  G  Y  K  N  F  C  D     153
GTAATTGAAGAGTACAAGAAGGAAATGAACAAATTAGCCAATAGGCTAATGTGG          654
V  I  E  E  Y  K  K  E  M  N  K  L  A  N  R  L  M  W           171
```

Figure 30. Nucleotide and deduced amino acid sequence of the rosette gall of partial GA3ox cDNA.

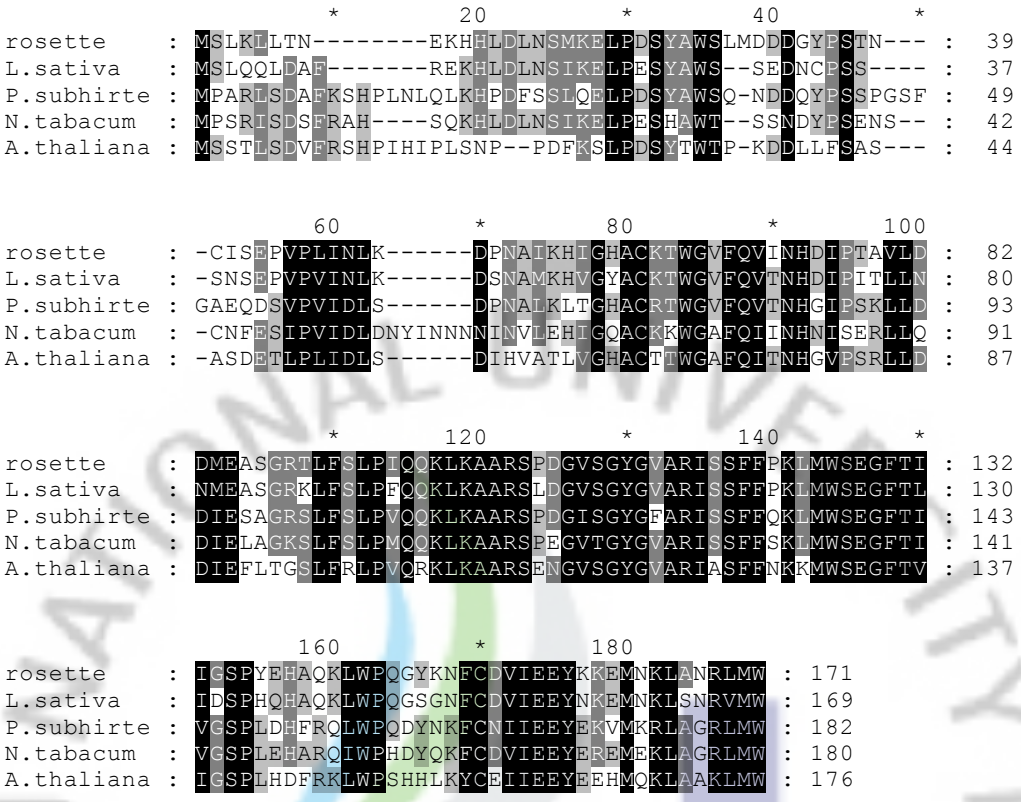

Figure 31. Alignment of the deduced amino acid sequences of partial GA3ox of rosette gall cDNA. The deduced amino acid of partial rosette gall of *A. scaber* from *Lactuca sativa*(BAA37130.1), *Prunus subhirtella*(BAD91162.1), *Nicotiana tabacum*(BAA89316.1) and *Arabidopsis thaliana*(AAC83647.1) are shown. Sequences were aligned using ClustalW and GeneDoc program. Black and gray shading boxes showed identical and similar amino acids, respectively.

```
ACAATCAAACCAATTACTATTATTATTACTACACAAATCAAAACAAGCCATGGTTCCCAT     60
                                                  M  V  P  I      4
AGATCAGTTTTGGGATGAAGTTGTTGCTGGACCTCAGCCTCAACGTGGTATTGGCGAGCT    120
 D  Q  F  W  D  E  V  V  A  G  P  Q  P  Q  R  G  I  G  E  L     24
CCGCAGAGTTGTCACCGTGACTGACAAAGGAGAAGGAAGCAGTAAGTTCCAGAAGTCTGT    180
R  R  V  V  T  V  T  D  K  G  E  G  S  S  K  F  Q  K  S  V     44
ATCGATGCCACCAACTCCGACGACACCAGGAACGCCGACAACACCATCGCCGACGGCGGC    240
 S  M  P  P  T  P  T  T  P  G  T  P  T  T  P  S  P  T  A  A     64
ACGTAAAGATAATGTGTGGAGGAGTGTTTTTAATCCAGGAAGCAACTTAGCCACTAAAGG    300
 R  K  D  N  V  W  R  S  V  F  N  P  G  S  N  L  A  T  K  G     84
CATTGGATCCAACTTCTTTGATAAGCCAGCAACCACCGTTGGATCTCCCACTGTTTATGA    360
 I  G  S  N  F  F  D  K  P  A  T  T  V  G  S  P  T  V  Y  D    104
CTGGCTATACAGTGGGGATACAAGGAGCAAGCATCGTTAATTAAGTTACTAAAGTCGTGG    420
 W  L  Y  S  G  D  T  R  S  K  H  R  *                        116
TCACATGATACATGGTGATCGAATGTAAATATTTGTTCGTAGATCGCAACGTGGTGGTGT    480
TTTCGTGTACGTGATGACTATTTGATGTGTTTTTTGTTTGAAAAAAGAGTGTATGTAGTT    540
GATCTGATAAAGACAATCATCAGCCGTTAGATCGATACTATGAACGATGGATATGAGCTA    600
TATACACCACTGTATTGTATTACAATGTACTGTTCAAGTGGACCTTAATATATTATTATA    660
ATCTTCTATCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA                     702
```

Figure 32. Nucleotide and deduced amino acid sequence of the rosette gall ARP cDNA. The nucleotide and amino acid residues are numbered in right margins, respectively. The termination cordon is marked by an asterisk.

```
CATGGACTGAAGGAGTAGAAAAATGGCAACAGTCGGAGAAACTCAACCCGCAAAACACCA   60
                      M  A  T  V  G  E  T  Q  P  A  K  H  Q     13
AGAAGTTGGCCACAAGAGTCTCCTTCAAAGCGATGCGCTTTACCAATACATTCTTGAAAC   120
 E  V  G  H  K  S  L  L  Q  S  D  A  L  Y  Q  Y  I  L  E  T    33
TAGTGTTTACCCGAGAGAGCCCGAATCCATGAAAGAGCTACGTGAGGTCACTGCTAAACA   180
 S  V  Y  P  R  E  P  E  S  M  K  E  L  R  E  V  T  A  K  H    53
CCCTTGGAATCTTATGACAACATCAGCTGACGAAGGGCAATTCTTGAACTTGCTTCTCAA   240
 P  W  N  L  M  T  T  S  A  D  E  G  Q  F  L  N  L  L  L  K    73
GCTCATAAATGCTAAGAACACAATGGAGATCGGTGTTTACACCGGCTATTCGCTTCTTTC   300
 L  I  N  A  K  N  T  M  E  I  G  V  Y  T  G  Y  S  L  L  S    93
TACTGCCCTCGCTCTTCCAGAGGATGGAAAGATATTGGCTTTGGACATAAACCGTGAGAA   360
 T  A  L  A  L  P  E  D  G  K  I  L  A  L  D  I  N  R  E  N   113
TTACGAAATTGGTCTTCCCATTATTGAGAAAGCCGGTGTTGCCCACAAGATCGACTTCAG   420
 Y  E  I  G  L  P  I  I  E  K  A  G  V  A  H  K  I  D  F  R   133
AGAAGGCCCTGCTCTTCCTCTTCTCGACCAAATGGTTGACGATGTGAAGTTCCATGGATC   480
 E  G  P  A  L  P  L  L  D  Q  M  V  D  D  V  K  F  H  G  S   153
CTTTGATTTTATTTTTGTGGATGCTGATAAAGACAACTACCTTAACTACCACAAGAGATT   540
 F  D  F  I  F  V  D  A  D  K  D  N  Y  L  N  Y  H  K  R  L   173
AATTGACCTAGTCAAAATCGGGGGAGTCATTGGCTACGATAACACTCTTTGGAACGGGTC   600
 I  D  L  V  K  I  G  G  V  I  G  Y  D  N  T  L  W  N  G  S   193
TTTGGTGGCCCCTGCTGATGCACCACTGAGGAAGTATGTCAGGTATTACAGAGATTTCGT   660
 L  V  A  P  A  D  A  P  L  R  K  Y  V  R  Y  Y  R  D  F  V   213
GCTAGAGCTTAACAAAGCCTTGGCTGTCGACCCGAGAGTTGAGATTTGTCAACTTCCCGT   720
 L  E  L  N  K  A  L  A  V  D  P  R  V  E  I  C  Q  L  P  V   233
TGGTGATGGAATCACTTTGTGCCGTCGTATAAGCTAATCACATATCGTGGGTTACTTGCA   780
 G  D  G  I  T  L  C  R  R  I  S  *                           244
ATTCAATATTATGGGACCAAAGTGCAGGATACTTGTAAAAGAACATATGTACAATTTCGT   840
TTATGTGTTGACATATGTTAATGTAACTTTTCTTGATTTTAATAAAAAAAAAAAAAAAAA   900
AAAAAAAAAAAAAAAAAAAA                                           921
```

Figure 33. Nucleotide and deduced amino acid sequence of the rosette gall CCoAOMT cDNA. The nucleotide and amino acid residues are numbered in right margins, respectively. The termination cordon is marked by an asterisk.

```
GAAAAAAAGACTGACCGGGGCATATTTATTTTGCTGCAAGAATACAACAAGATCAGCCAA    60
ATTAATTGCCATGGAGAAGCAATCAACGACCATCGTTAGCCGGAACCGGAGAATAATCTT   120
          M  E  K  Q  S  T  T  I  V  S  R  N  R  R  I  I  L      17
GTTTCCTTGGTCATTTCAGGGCCACCTAAACCCAATGCTTCAGCTTGCCAATATTCTATA   180
F  P  W  S  F  Q  G  H  L  N  P  M  L  Q  L  A  N  I  L  Y      37
TTCCAAAGGCTTCAGTATCACCATCCTTCATACCAATTTCAATGCACCCAAAACCTCCAA   240
S  K  G  F  S  I  T  I  L  H  T  N  F  N  A  P  K  T  S  N      57
TTACCCTCACTTCACTTTCCGATCAATCCTTGATAACGATCCTGAAAGCCAACGCTTATT   300
Y  P  H  F  T  F  R  S  I  L  D  N  D  P  E  S  Q  R  L  L      77
GTCGTCAGAAGGCATCCTCGGTTTTGCTAAGAATTTCATATTCAACCAAGATCGTGGAGA   360
S  S  E  G  I  L  G  F  A  K  N  F  I  F  N  Q  D  R  G  D      97
TGCATTACGCCAAGAGCTGGAACTGTTGTTAGCTTCAGGAAAAGACGAACCCGTATCGTG   420
A  L  R  Q  E  L  E  L  L  L  A  S  G  K  D  E  P  V  S  C     117
TTTGATCACTGATGCGCTCTGGCACTTTACGCAATCTGTGGCTGATAGCCTTAACCTCCC   480
L  I  T  D  A  L  W  H  F  T  Q  S  V  A  D  S  L  N  L  P     137
AAGGCTTGTTTTATGGACAAGCAGCTTGTTCTCTATGGTTGTTCATGATTCAATATCACT   540
R  L  V  L  W  T  S  S  L  F  S  M  V  V  H  D  S  I  S  L     157
TCTTGATGATCGTGGTTTCTTCAAACTTGACAATAGTCATTTGGATGAGCAAGTTGAAGA   600
L  D  D  R  G  F  F  K  L  D  N  S  H  L  D  E  Q  V  E  E     177
GTTTCCTTTCCTAAAAGTGAAAGACATGTTGAAGACGGGAATCAAAGGCAAGAAAGATCC   660
F  P  F  L  K  V  K  D  M  L  K  T  G  I  K  G  K  K  D  P     197
ACTCATCGAATTTCTCGTGGGTCCTGCGCTGAAACAAACAAAAGTATCATCAGGAATCAT   720
L  I  E  F  L  V  G  P  A  L  K  Q  T  K  V  S  S  G  I  I     217
CTCGAACTCATTCAAGGAACTTGAAGGACCCGAGCTAGAAACGGTTCTTAATGACTTCCC   780
S  N  S  F  K  E  L  E  G  P  E  L  E  T  V  L  N  D  F  P     237
GGTACCAAGTTTCTTGACACCATTCGCCAAACATTTCACAGCGGCATCGAGCAGCTTACT   840
V  P  S  F  L  T  P  F  A  K  H  F  T  A  A  S  S  S  L  L     257
AGAACATGATCGATCTTTTTTCCCATGGTTAGACCAACAACCGGCCAATTCTGTACTGTA   900
E  H  D  R  S  F  F  P  W  L  D  Q  Q  P  A  N  S  V  L  Y     277
TGTTAGTTTTGGTAGCATTAGTCAACTGGAGGAGAAAGATTTCTTTGAAATAGCTCATGG   960
V  S  F  G  S  I  S  Q  L  E  E  K  D  F  F  E  I  A  H  G     297
GTTGGTCGATAGCAAGCAGTCATTCTTATGGGTGGTCCGGCTTGATACTCCGGAACTTGT  1020
L  V  D  S  K  Q  S  F  L  W  V  V  R  L  D  T  P  E  L  V     317
TAATGGCTCAAAATGGCTCGAATCTTTGCCTGATGGGTTCCTAGGTGAAAGGGGTCGAGT  1080
N  G  S  K  W  L  E  S  L  P  D  G  F  L  G  E  R  G  R  V     337
AGTAAAATGGGCCCCTCAGCAAGAAGTGCTAGCTCATAAATCAATCGGAGCATTTTGGAC  1140
V  K  W  A  P  Q  Q  E  V  L  A  H  K  S  I  G  A  F  W  T     357
TCATAATGGATGGAACTCGACAATGGAAAGCATTTGTGAAGGTGTTCCTATGATTTCTTC  1200
```

```
H  N  G  W  N  S  T  M  E  S  I  C  E  G  V  P  M  I  S  S     377
GCCTATGATGGCTGACCAACCACTAAATGCGAGATACATGAGCGATGTTGCGAAGGTGAG  1260
P  M  M  A  D  Q  P  L  N  A  R  Y  M  S  D  V  A  K  V  R     397
GGCGTATTTGGAGAATGGGTTGCAAAGACAAGTGATAGCAAGTGCGATAAGAAGAGTAAT  1320
A  Y  L  E  N  G  L  Q  R  Q  V  I  A  S  A  I  R  R  V  M     417
GGTGGATGAAGAAGGGAAAGAGATTAGAGAGAATGCTAGAGTTTTGAAACAAAAGTTAGA  1380
V  D  E  E  G  K  E  I  R  E  N  A  R  V  L  K  Q  K  L  D     437
TGTTTCTTTAGATAAGGGTGGCTCTTCCTATGAATCATTGAACTCACTTGTTGATCATAT  1440
V  S  L  D  K  G  G  S  S  Y  E  S  L  N  S  L  V  D  H  I     457
TTCTTCGTTTTAACCGCCTATTATATATGGTGTTCAAATGTATTGTGTAACGACGAGTCC  1500
S  S  F  *                                                     460
GTTTCAACGCTTCATTCAAAGAGTCCTAAAGCAAATTTTGTTTTGGGGCTTTACATTTAA  1560
GATGCACTTTAATTTACCTTTCAAAAAAAAAAAAAAAA                        1598
```

Figure 34. Nucleotide and deduced amino acid sequence of the rosette gall UGT cDNA. The nucleotide and deduced amino acid residues are numbered in left and right margins, respectively. The termination cordon is marked by an asterisk.

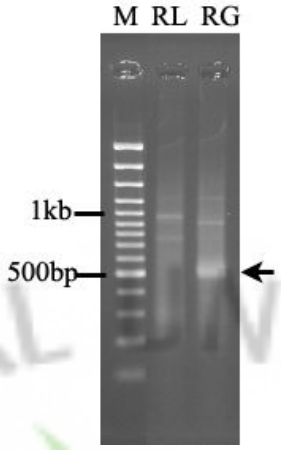

Figure 35. An agarose gel electrophoresis of amplified cDNA products for the differentially expression genes in leaf and rosette gall using a arbitrary ACP28 and dT-ACP2. Total RNAs from leaves and rosette-like galls were used as starting materials for the synthesis of first-strand cDNAs. M: size maker, RL: Leaves nearby rosette-like galls growth. RG: leaflets developmental stage of rosette galls.

```
  1 GAAAATCAACTTCTCGTCTATTAAGGTAAAGTCGAACCAAAACAAAGAGAGAAAAACAAA
 61 AAATGACAAGGTTGTGTTTTATGTTAGTAGCAACTCTAGCAGTAGTGGTTTATACTACTA
    M  T  R  L  C  F  M  L  V  A  T  L  A  V  V  V  Y  T  T  T  20
121 CTGCTAGAGACATTCCGAAACAGAACAAAGATACTATTGGTCTTACGGACCAAAAGAACG
    A  R  D  I  P  K  Q  N  K  D  T  I  G  L  T  D  Q  K  N  V  40
181 TTTATACTTTTGGTGGAACCGGAGGGTTCAATGGTATTGGAAACAATGGTCTACCCATGG
    Y  T  F  G  G  T  G  G  F  N  G  I  G  N  N  G  L  P  M  G  60
241 GAGGGATGGGAACCGGGGTTGGTGTAGGTGGTGATTTTGGCGGTGCAAATGGAGTCGGGG
    G  M  G  T  G  V  G  V  G  G  D  F  G  G  A  N  G  V  G  G  80
301 GTGTAGGTATGGGATATCAATATGGTGGACCGGGTGGGCCGGCTGGTGGTGTAGGAACTT
    V  G  M  G  Y  Q  Y  G  G  P  G  G  P  A  G  G  V  G  T  F 100
361 TTGGAGGTCTTGGAAATGGTTTTGGTGGATTACCAACTCTCGGTGGTGGTGGACTTGGTG
    G  G  L  G  N  G  F  G  G  L  P  T  L  G  G  G  G  L  G  G 120
421 GTGGTGGTCCTGGTGTTGGTGGTGTGTTTCCTATTGGTGGTATGGGTGGCTTTCCTATAG
    G  G  P  G  V  G  G  V  F  P  I  G  G  M  G  G  F  P  I  G 140
481 GTGGTGGTGCTGGTGGTGCACTCCCTCATCCTTAAAGGTTGTTTCGTACGTGGTCACTAG
    G  G  A  G  G  A  L  P  H  P  *                             150
541 GCAGTCGCTTCTTTACTATGAATAGGTTAACTAGTGTAAGATGGTATGTTTTATGTGTTT
601 GTGTTAGTAGATCATATCTTCGGTTTTAAGAATATGTTATGTTTGTTATATCATGTTGTA
661 TTTCTGGAATATTTAAACTTCCATTTATTTCTAAAAAAAAAAAAAAAAAAA
```

Figure 36. Nucleotide and deduced amino acid sequence of the rosette gall REA28 cDNA. The putative signal peptide is in bold type. The nucleotide and deduced amino acid residues are numbered in left and right margins, respectively. The stop codon is shown by an asterisk.