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# Effects of Abscisic Acid on the Epidermal Structure and Ontogeny of Stomata in *Orostachys malacophyllus* Leaves

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## 둥근바위솔(*Orostachys malacophyllus*) 잎의 表皮構造와 氣孔發生에 미치는 Abscisic Acid의 影響

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## ABSTRACT

The effects of abscisic acid(ABA) spraying for 12 weeks on the stomatal types and frequencies of *O. malacophyllus* leaves were summarized as follows. ABA inhibited the growth of *O. malacophyllus*. The prominent effect of ABA on the epidermal structure was the promotion of senescence such as thickness of cell walls, smooth sinuosity of cell walls, and large size of epidermal cells. The stomatal frequency was decreased to 23 % by 10 $\mu$g ml$^{-1}$ ABA and to 48 % by 100 $\mu$g ml$^{-1}$, and also the stomatal size was more or less smaller than that of control. The developing secondary stomatal mother cell was not found in both 10 and 100 $\mu$g ml$^{-1}$ ABA, but the arrested secondary stomatal mother cell was rarely found in 10 $\mu$g ml$^{-1}$ ABA. The formation of normal stomatal types such as helico-cumesogenous and aniso-cumesogenous was found in both 10 and 100 $\mu$g ml$^{-1}$ ABA asin well as control. Also nine abnormal stomatal types were found, and the frequencies were promoted to 6% by 10 $\mu$g ml$^{-1}$ ABA and to 17 % by 100 $\mu$g ml$^{-1}$ ABA. Among these abnomal stomata, four types such as aborted stomata, single-aborted guard cells, arrested stomata, and modified stomatal complexes were found in control as well as in 10 and 100 $\mu$g ml$^{-1}$ ABA, but five types such as wrenched stomata, unequal stomata, wavy guard cells, guard cells overlapped by guard cells, and dissolved cell wall stomata were found in both 10 and 100 $\mu$g ml$^{-1}$ ABA. The modified stomata complexes were abnomal stomatal types which were newly found and also were varied in types.

## INTRODUCTION

ABA was generally regarded as a depressor or an inhibitor of physiological and biochemical events in plants(Walton, 1980). Most of the reports suggested that exogonous ABA inhibited the germination(Eagles and Wareing, 1963; Schopfer *et al.*, 1979; Wareing and Saundery, 1971) and growth (Sloger and Caldwell, 1970; Harrison and Kaufman, 1980; Kim and Sung, 1986), and promoted the dormancy(Sondheimer *et al.*, 1969; Webb

and Wareing, 1972), abscission(Sagee *et al.*, 1980), and senescence effects such as the loss of chlorophyll, protein, and RNA(Addicott, 1983; Mondal *et al.*, 1983; Kim and Sung, 1986) of a large range of species. And also it was proposed that stomatal closure was regarded as being due to the inhibition of influx and to the acceleration of efflux of $K^+$ from guard cells by ABA(MacRobbic, 1980; Zvilich *et al.*, 1982). Recently, Kim and Sung(1986) reported that ABA inhibited the stomatal frequency and respiration rate of tobacco. However, the effects of ABA on the stomatal ontogeny, types, and frequency were not well understood. The purpose of the study was to investigate the effects of ABA on the epidermal structure, ontogeny of the secondary stomatal mother cell, and stomatal frequency and types in the leaves of *O. malacophyllus* belonging to Crassulaceae.

## MATERIALS AND METHODS

The materials of *Orostachys malacophyllus* belonging to Crassulaceae were collected from the field growing under the natural conditions at the area of Chinju and then were normally grown on the sand-culture of Hoagland solution in the polyethylene pots for 12 weeks from March to May(23±℃).

The plants were respectively sprayed with about 15 ml of 10 and 100 $\mu$g $ml^{-1}$ ABA(Sigmma) water solution around 9:00 a.m. once in two days. Control was sprayed with only distilled water. The epidermal peels of sample on the last day of 12 weeks were taken from the young and mature leaves 3 hours after being sprayed with ABA by making the paradermal hand section for the observation of stomata and epidermal structure. They were stained with safranin, and then mounted in the glycerine water solution. They were observed and microphotographed with the Olympus Universal Research Microscope of Vanox Model AD-1. The terminology used here was followed Jeong and Sung(1985 a, b).

## RESULTS

The growth of *O. malacophyllus* leaf was inhibited by the exogenous ABA even though the data was not shown.

The epidermal cells of control as well as 10 and 100 $\mu$g $ml^{-1}$ ABA were polygonal, isodiametric, sometimes elongated in vein, and irregularly arranged. The epidermal cell size of 100 $\mu$g $ml^{-1}$ ABA was more large. The cell walls were considerably thickened and not deeply sinuous in compaison with that of control and 10 $\mu$g $ml^{-1}$ ABA(Fig. 1).

The number of stomata per square millimeter and the stomatal size in both 10 and 100 $\mu$g $ml^{-1}$ ABA were smaller than those of control. The stomatal number of each sample was a little greater on the lower surface than on the upper surface, but the stomatal number in two surfaces of leaves was not shown to be very different. The

**Table 1.** Distribution and size of stomata on the upper and lower surfaces of the leaves in *O. malacophyllus* non-sprayed or sprayed with 10 and 100 $\mu$g ml$^{-1}$ ABA

| ABA, $\mu$g ml$^{-1}$ | Stomatal number/㎟ | | Stomatal size, $\mu$m | | | |
|---|---|---|---|---|---|---|
| | | | Upper | | Lower | |
| | Upper | Lower | Length | Width | Length | Width |
| 0 | 33.2±0.30 | 36.7±0.24 | 26.2±0.15 | 20.4±0.11 | 32.0±0.12 | 19.7±0.17 |
| 10 | 25.4±0.27 | 28.3±0.23 | 23.9±0.13 | 19.7±0.18 | 28.1±0.11 | 18.8±0.20 |
| 100 | 20.4±0.22 | 23.3±0.20 | 22.7±0.11 | 19.8±0.18 | 26.2±0.18 | 19.1±0.18 |

* Total of 54 samples were examined for each treatment; 3 samples/segment×6 segments/leaf×3 leaves.

stomatal number was decreased to 23 % by 10 $\mu$g ml$^{-1}$ ABA and to 38% by 100 $\mu$g ml$^{-1}$ ABA in comparison with that of control(Fig. 1, Table 1). And also the developing secondary stomatal mother cell was not found in both 10 and 100 $\mu$g ml$^{-1}$ ABA. The arrested secondary stomatal mother cell was rearely found in 10 $\mu$g ml$^{-1}$ ABA.

Generally, the normal stomatal types such as helico-eumesogenous(Fig. 2 A,B,C) and aniso-eumesogenous(Fig. 2 D) were found in contol. Besides, they were found by 10 and 100 $\mu$g ml$^{-1}$ ABA though the figure was not shown(Fig. 2). Abnormal types of stomata were found more in ABA spraying than in control. And also nine abnormal stomatal types were found in control and in 10 and 100 $\mu$g ml$^{-1}$ ABA. The abnormal types were mainly single-aborted guard cells, aborted stomata, arrested stomata, and modified stomatal complexs(Fig. 3 B,C,D,E,I), but the five abnormal stomatal types such as wrenched stomata, unequal stomata, wavy guard cells, guard cells overlapped by guard cells, and dissolved cell wall stomata were found in both 10 and 100 $\mu$g ml$^{-1}$ ABA(Fig. 3 F, G, H, J). The frequency of total abnormal stomatal types was increased to 6 % by 10 $\mu$g ml$^{-1}$ ABA and to 17 % by 100 $\mu$g ml$^{-1}$ ABA in comparison with that of control. Among the nine abnormal stomatal types, three types such as aborted stomata, single-aborted guard cells, and modified stomatal complexes were observed to be higher in frequency. Two types such as wrenched stomata and unequal stomata exhibited the common frequency, but dissolved cell wall stomata, arrested stomata, wavy guard cells, and guard cells overlapped by guard cell showed the lower occurrence. And also the types of modified stomata were varied.

## DISCUSSION

The growth of *O. malacophyllus* leaf was inhibited by the exogenous ABA even though

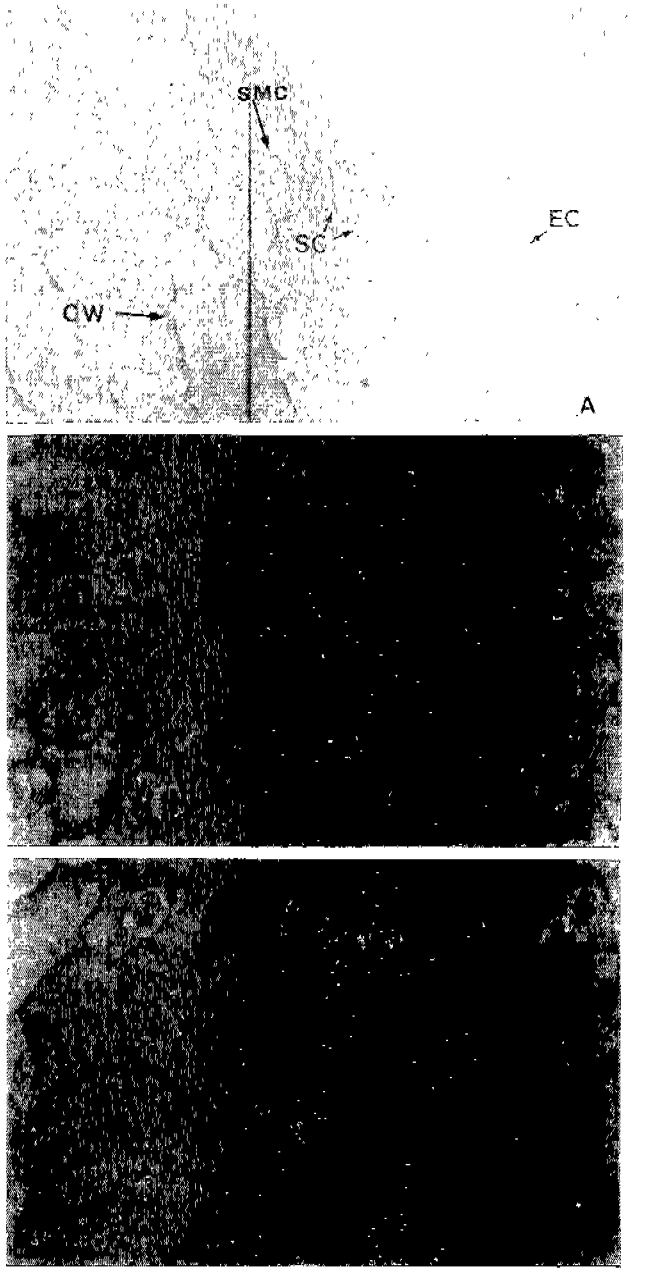


**Fig. 1.** Epidermal structure and stomatal distribution of the leaves in *O. malacophyllus*(SC, Subsidiary cell; EC, Epidermal cell; CW, Cell wall; SMC, Stomatal mother cell).
A, Control(polygonal and isodiametric epidermal cells, sinuous cell wall); B, 10 $\mu$g ml$^{-1}$ ABA(polygonal and isodiametric epidermal cells, sinuous cell wall); C, 100 $\mu$g ml$^{-1}$ ABA(polygonal and isodiametric epidermal cells, not markedly but thicken cell wall, smoothly sinuous cell wall, large epidermal cells).

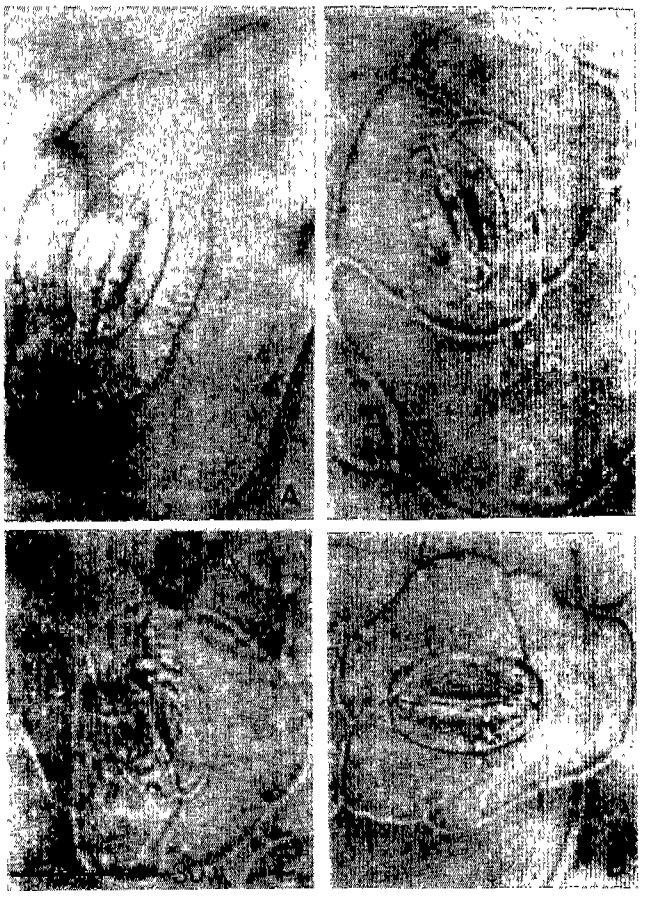

**Fig. 2.** Two normal stomatal types of helico-cumesogenous types(A, B, C) and aniso-cumesogenous type(D) were found in control. They were also found by 10 and 100 $\mu$g ml$^{-1}$ ABA spraying, though the figures did not show.

the data was not shown. It was agreed with Kim and Sung's report(1986) that ABA inhibited the growth of leaf and stem in tobacco. Newton(1977) reported that ABA inhibited frond cell expansion in duck-weed, and reduced the increase of both width and length in developing frond. We thought that the growth inhibition of O. *malacophyllus* leaf was the result by the loss of chloroplasts and protein by the exogenous ABA. Recently, Kim and Sung(1986) reported that ABA caused the loss of chloroplasts and protein in tobacco leaf.

The senescece phenomena such as the thickness of cell wall, smooth sinuosity, and large size of epidermal cell were found in 100 $\mu$g ml$^{-1}$ ABA. From this results, it was speculated that ABA affected the cell division and development of epidermal structure.


**Fig. 3.** Abnormal stomatal types discovered in control(AS, SAG, ARS, MSC) and leaves sprayed with 10 and 100 $\mu$g ml$^{-1}$ ABA(SAG, AS, MSC, WS, GCOGC, ARS, WGC, US, DCWS). A, Stomatal mother cell(SMC) and Normal stomata(NS); B-C, Single-aborted guard cells(SAG); D, Aborted stomata(AS); E, Modified stomata complex(MSC); F, Wrenched stomata(WS) and Guard cells overlapped by guard cells(GCOGC); G, Unequal stomata(US); H, Dissolved cell wall stomata(DCWS); I, Arrested stomata(ARS); J, Wavy guard cells(WGC).

Addicott(1983) described that ABA inhibited the cell division and promoted the senescence of many plants. Generally it has been well known that the cell wall of plants was thickened during the aging of plants. In this study we speculated that the senescence phenomena of epidermal structure was caused by the inhibition of cell division and loss of chloroplasts and protein by the exogenous ABA.

The size and number of control stomata were similar to those in the report of Jeong and Sung(1985 a). However, the stomatal size was decreased by 10 and 100 $\mu$g ml$^{-1}$ ABA and the number of stomata was decreased to 23 % by 10 $\mu$g ml$^{-1}$ ABA and to 48% by 100 $\mu$g ml$^{-1}$ ABA(Table 1). And also the developing secondary stomatal mother cells were not found in 10 and 100 $\mu$g ml$^{-1}$ ABA, but the arrested secondary stomatal mother cells were rarely found in only 10 $\mu$g ml$^{-1}$ ABA in comparison with those of control. We thought that ABA inhibited cell division and growth, and then the stomatal differentiation and ontogeny. Although the correlation between ABA and stomtal frequency as well as ontogeny has yet been investigated extensively, Kim and Sung(1986) reported that ABA decreased the stomatal frequency, and inhibited the development of the secondary stomatal mother cell in tobacco. Otherwise Stebbins *et al.*,(1967) reported that 2-mercaptoethanol reduced the number of stomatal complexs, and suppressed the cell division which formed the stomatal complex in *Hordeum vulgare*.

According to Jeong and Sung(1985 a), the normal stomatal types of *O. malacophyllus* were helico-eumesogenous, sometimes aniso-eumesogenous. In this experiment the normal stomatal types such as helico-eumesogenous and aniso-eumesogenous were found in control as well as in 10 and 100 $\mu$g ml$^{-1}$ ABA. It was thought that the appearance of the normal stomatal types in ABA spraying was according to the unbalance spraying for ABA inhibition of the leaf surface by ABA spraying. And also Jeong and Sung(1985 b) reported that the abnormal stomata rarely found in the normal leaves of *O. malacophyllus* were thee types such as single-aborted guard cells, aborted stomata, and arrested stomata. However, in this study, besides these abnormal stomata, six abnormal stomatal types such as modified stomatal complexes, unequal stomata, dissolved cell wall stomata, wavy guard cells, wrenched stomata, and guard cells overlapped by the guard cells were found. Among the abnormal stomata found, the aborted stomata, single-aborted guard cells, arrested stomata, and modified stomatal complexes were found in control as well as 10 and 100 $\mu$g ml$^{-1}$ ABA, and the others were found in both 10 and 100 $\mu$g ml$^{-1}$ ABA. Three types such as aborted stomata, single-aborted guard cells, and modified stomatal complexes were observed to be higher in frequency. And also two types such as wrenched stomata and unequal stomata exhibited the common frequency, but the dissolved cell wall stomata, arrested stomata, wavy guard cells, guard cells overlapped by the guard cells showed the lower occurrence. The frequencies of total abnormal stomatal types were increased to 6 % by 10 $\mu$g ml$^{-1}$ ABA and to 17 % by 100 $\mu$g ml$^{-1}$ ABA in comparison

with that of control. Therefore, we speculated that ABA promoted the frequency of abnormal stomata and the development of new abnormal stomatal types. Generally, the aborted stomata and single-aborted guard cells rarely appeared in the many species of Crassulaceae(Jeong and Sung, 1985 b). The frequency of this types was gradually increased during the aging of stomata(Jeong and Sung, 1985 b). And also the dissolved cell wall stomata was observed at the position between guard cells and subsidiary cell(Jeong and Sung, 1985 b). In the present study it seemed that the appearance of abnormal types such as aborted stomata, single-aborted guard cells, and dissolved cell wall stomata was due to the non-function of the guard cell which caused by the loss of chloroplasts and degeneration of nucleus in guard cells and efflux of $K^+$ from the guard cell into the epidermal cell. Kim and Sung(1986) reported that ABA promoted the loss and polar movement of chloroplasts in the guard cell of tobacco. It was reported that ABA affected $K^+$ and solute transport between guard cells and epidermal cells(Zvilich *et al.*,1982). And also the arrested stomatal type was formed by the high vacuolation of nucleus at each stage of stomatal development(Inamdar, 1970; Jeong and Sung, 1985 a). We thought that ABA promoted the high vacuolation and the degeneration of nucleus at each stage of stomatal development even though we did not show them. The modified stomatal complexes were the type which was found newly in Crassulaceae. It seemed that the appearance of modified stomatal complexes were the results of environmental adaption by the water stress of the exogenous ABA. According to many workers, it was known that ABA increased the water stress of plants and the stomata of plants closed and the function of stomata lost(Lovey and Kriedemann, 1973; Bengtson *et al.*, 1977; Eavis and Taylor, 1979). Generally during the ontogenic pathway, the guard mother cells are divided. In the case of unequal guard cells, it was reported that the guard mother cell was divided into unequal two cells during the ontogenic pathway(Jeong and Sung, 1985 b). In the case of wavy guard cells, it was reported that after the guard mother cell was divided into two guard cells, the back wall of one or two guard cells was divided into the wavy shape(Jeong and Sung, 1985 b). In the case of wrenched stomata, Jeong and Sung(1985 b) proposed that the wrenched stomata were formed by the results of abnormal arrangement with an irregular fashion during the maturation. In the present study we speculated that the appearance of types such as unequal stomata, wavy guard cells, wrenched stomata, and guard cells overlapped by the guard cells was the results of inhibition of stomatal meristemoid and ontogeny by the exogenous ABA. Although Stebins *et al.* (1967) reported that 2-mercaptoethanol treatment on barley leaves interfered with the spindle formation and changed the orientation of the division of some guard cells, and Kaufman *et al.* (1967) reported that gibberellic acid caused the infrequent aberrent transverse divisions of guard mother cells of the developing stomata, we could not know whether ABA inhibited some stage of abnormal stomatal meristemoid and ontogeny. Accordingly we think that the effect of ABA on the epidemal structure and ontogeny of stomata remains a

subject to be studied at the time to come.

## 摘 要

돌나물科(Crassulaceae)에 屬하는 둥근바위솔(*Orostochys malacophyllus*)을 晋州 近郊에서 採集하여 Hoagland 培養液으로 生長시키면서 ABA 10과 100 $\mu$g ml$^{-1}$를 約 15 ml씩 12週동안 2日에 한번씩 葉에 噴霧하였다. ABA에 의하여 生長抑制는 물론, 細胞壁의 肥厚, 완만한 波狀形, 그리고 表皮細胞의 巨大한 크기와 같은 老化現像이 發見되었다. ABA에 依한 氣孔의 크기는 control보다 多少 작았고 그 類度는 ABA 10$\mu$g ml$^{-1}$에서 約 23% 및 ABA 100 $\mu$g ml$^{-1}$에서 約 48% 減少하였다. 二次氣孔母細胞의 發生은 ABA 10 과 100 $\mu$g ml$^{-1}$에서 發見되지 않았으나, 發生이 靜止된 二次氣孔母細胞인 arrested stomata가 ABA 10$\mu$g ml$^{-1}$에서 드물게 發見되었다. 또한 ABA가 正常的인 氣孔形成에 影響을 주는지는 알수 없었지만 非正常氣孔의 發生과 頻度를 促進시키는 것으로 나타났으며 ABA에 依한 非正常氣孔은 control보다 ABA 10$\mu$g ml$^{-1}$에서 約 6%, ABA 100 $\mu$g ml$^{-1}$에서 約 17% 더 많이 發見되었다. ABA를 處理한 葉에서 發見된 非正常氣孔의 類型은 single-aborted guard cells, aborted stomata, modified stomatal complexes, wrenched stomata, guard cells overlapped by guard cell, unequal stomata, dissolved cell wall stomata, arrested stomata, wavy guard cells 等 總 9種類이었고 그 중 aborted stomata, single-aborted guard cells, arrested stomata, modified stomatal complexes는 control에서도 간혹 發見되었다. Modified stomatal complexes는 새로이 發見된 非正常氣孔이고 그 類型은 多樣했다.

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