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# Effects of Abscisic Acid on Some Physiological Responses of the Leaves in *Nicotiana tabacum* L.

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# 담배 (*Nicotiana tabacum* L.) 잎의 몇가지 生理的 反應에 미치는 Abscisic Acid의 影響

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## ABSTRACT

The plants of *Nicotiana tabacum* L. cv. NC2326 were germinated in 10 cm D×20 cm H polyethylene pot, and sand-cultured with Hoagland solution near by the window of laboratory room (26±5°C). The growing plants were sprayed with various concentrations of ABA around 9 : 00 a.m. once in every two days for 12 weeks in summer. As the results, frequency of stomatal number, stomatal opening, chlorophyll content, respiration rate, and protein content in the leaves were decreased with the increasing of concentrations of ABA, respectively. The plant growth was inhibited by exogenous ABA, but leaf abscission was not found during the experimental period. The ratio of three to one in chlorophyll a to b was not altered by exogenous ABA. All the stomata were closed within three minutes by 100 $\mu$g ml$^{-1}$ ABA and within seven minutes by 1—10 $\mu$g ml$^{-1}$ ABA after the spraying of ABA, and then reopended after a few hours in 1—10 $\mu$g ml$^{-1}$ ABA and after 24 hours in 100 $\mu$g ml$^{-1}$ ABA. The polar movement of chloroplast within the guard cells was found in the higher concentrations of 10 and 100 $\mu$g ml$^{-1}$ ABA, but not found in the lower concentrations than 1 $\mu$g ml$^{-1}$ ABA. During the night and weak light, it was found that the inhibition of respiration rate by the higher concentration of ABA was owing to firstly the stomatal closure by the spraying of ABA and secondly the decrease of stomatal frequency by the inhibition of stomatal development with exogenous ABA for the long period of 12 weeks. In the band number of leaf protein by the electrophoresis, most of the protein bands were disappeared by the higher concentration of 100 $\mu$g ml$^{-1}$ ABA, but were not altered by the lower concentration of ABA in comparison with control.

## INTRODUCTION

Early many workers reported that ABA(abscisic acid) acted with two physiological responses such as dormancy regulators(Eagles and Wareing, 1963; Wareing and Saundery, 1971) and senescence(El-Antably *et al.*, 1967; Smith *et al.*, 1968; Addicott and Lyon, 1969; Nooden, 1980). Up to the present, the vigorous studies on ABA were attained in stomatal regulation(Jones and Mansfield, 1970; Cummins *et al.*, 1971; Horton, 1971; Mansfield and Jones, 1971; Loveys *et al.*, 1972; Beardsell and Cohon, 1975; Raghavendra *et al.*, 1976), water stress(Hiron and Wright, 1973; Dörffling *et al.*, 1974; Jordan *et al.*, 1975; Davies, 1978; Ackerson, 1980), ionic relations of stomatal membrane(Schnabl, 1978; Hartung *et al.*, 1980; MacRobbie, 1980), and release of malate during stomatal closure(Van Kirk and Raschke, 1978). Partial studies of ABA were reported that ABA in plant affected as the decrease of protein(Donald, 1975; Hames and Richwood, 1983) as well as inhibitor of biochemistry and physiological events(Walton, 1980), effected on root growth(Moore, 1978), and occurred phloem unloading(Tanner, 1980). Recently sensitive plant (strawberry) to ABA was investigated(Kubik and Antoszewski, 1983).

However, physiological effects to the tobacco leaves by exogenous ABA were rarely studied. The purpose of this study was to investigate the effects of chlorophyll, protein, stomatal opening and closing, polor movement of chloroplast, and respiration by the spraying of ABA on the leaves in *Nicotiana tabacum* L. cv. NC2326 for 12 weeks.

## MATERIALS AND METHODS

**Plant materials.** *Nicotiana tabacum* L. cv. NC2326 seeds were obtained from Dr. Kim, Joon-Chul. The tobacco plants were grown by sand-culture of Hoagland solution in 10 cm D×20 cm H polyethylene pots. The plants were respectively sparyed by about 15 ml of 0.1, 1, 10, and 100$\mu$g $ml^{-1}$ ABA(Sigma) around 9:00 a.m. once in every two days after germination. Control was sprayed by only distilled water. After 12 weeks, the young and fully expanded second leaves from tip were harvested, washed with distilled water, blotted water with filter paper, and then used for each analysis. All the material plants were grown near by the window of laboratory room(26±5°C).

**Chlorophyll content.** 200 mg of the leaves were minced with a razor blade and gently ground in a tissue grinder with 15 ml of 80 % acetone for 10 min. The ground solution was centrifuged at 3,000 rpm for 20 min. and the supernatant was used for analysis. The chlorophyll content of the supernatant was measured by UV-190(Shimadzu Co.) at 645 nm and 663 nm(Holden, 1965).

**Protein extracts.** The protein solution was extracted by the method of Hames and Richwood(1983). One of the leaves was minced with a razor blade and gently ground in a

tissue grinder with 3 ml of 1 M sucrose, 0.056 M 2-mercaptoethanol, and Tris-HCl buffer (pH 8.5) in an ice box for 10 min. The ground solution was centrifuged at 17,000 rpm for one hour. After dialysis by dialysis-sack 250-7U (Sigma) with Tris-HCl buffer (pH 7.5), the protein solution was centrifuged at 17,000 rpm for one hour, and the supernatant solution was used as soluble protein extract. This protein extract was stored at −20°C and was used for the quantitative determination.

**Quantitative determination of protein.** The protein extract was precipitated with 10 % trichloroacetic acid, washed with ethanol, dried, and dissolved in 1 M NaOH (Donald, 1975). Total soluble protein was determined by the method of Lowry *et al.* (1951).

**Electrophoresis.** The protein extracts were dissociated into its polypeptide subunits by an equal volume of a solution containing 2% (w/v) SDS (sodium dodecyl sulphate), 40 % (w/v) sucrose, and 0.01 % (w/v) bromophenol blue. The subunits were electrophoretically separated in an SDS-polyacrylamide rod gel (7.5 %). For SDS-Tris glycine buffer system (pH 8.3), 2 mA constant current per rod gel was supplied until the sample was entered into the resolving gel, and then 3 mA constant current per 9 cm rod was supplied for about 4 hours. The rod gel was stained with coomassie brilliant blue, destained with destain solution of 30 % acetic acid, and stored in 7.5 % acetic acid. The electrophoresis used here was followed to the method of Hames and Richwood (1983).

**Respiration rate.** The fresh leaves were collected four times a day such as 2:00~4:00 a.m., 8:00~10:00 a.m., 2:00~4:00 p.m., and 8:00~10:00 p.m., and were cut out 20 discs from physiologically similar leaves with an 8 mm borer. 300 mg of each sample were transferred immediately to 15 ml Warburg respirometer flasks. 3 ml of 0.5 M phosphate buffer (pH 5.5) was added to each flasks. A center well was contained 0.3 ml solutien of 10 % KOH (w/v), and a filter paper was wicked in it to absorb $CO_2$. The flasks were equilibrated for 15 min. before starting $O_2$ uptake of leaf sample. Oxygen uptake was measured by each nanometer of Warburg Apparatus (Braun-V-166, B. Braun Melsungen AG Co.). The respiration rate was expressed with the quantity of oxygen uptake.

**Closure and frequency of stomata.** Sampling laminae were ensured by making paradermal hand section with razor blades. The staining of samples was established with safranin, and then mounted in the glycerine water on a slide glass. They were observed and microphotographed with the Olympus Universal Research Microscope of Vanox Model AD-1. The stomatal frequency was calculated by counting stomata in 1 $mm^2$ on the epidermal surface of leaf sample under the light microscope.

## RESULTS

**Growth.** ABA inhibited the growth of leaf and stem. However, during the period of experiment, there was not abscission phenomenon of the leaves. The growth of tobacco was inhibited with increasing ABA concentrations from 1 to 100 $\mu g\ ml^{-1}$, but was not

**Table 1.** Mean size of the fully expanded leaf and the plant height of *N. tabacum* cv. NC2326 sprayed with various concentrations of ABA for 12 weeks

| ABA ($\mu$g ml$^{-1}$) | Leaf(cm) | | Plant height (cm) |
|---|---|---|---|
| | Length | Breadth | |
| 0 | 13.2 | 7.0 | 23.8 |
| 0.1 | 13.5 | 7.2 | 23.7 |
| 1 | 12.5 | 6.5 | 19.3 |
| 10 | 11.7 | 6.2 | 14.2 |
| 100 | 9.8 | 5.0 | 8.5 |

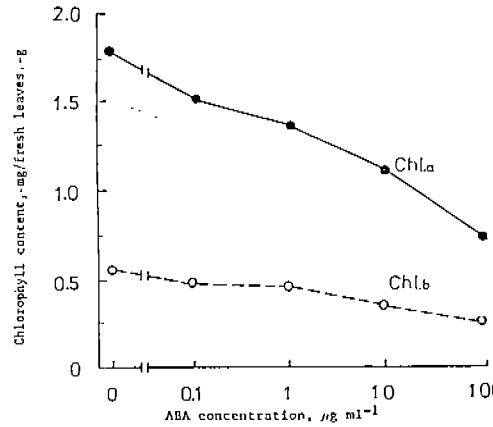


**Fig. 1.** Effect of ABA on the chlorophyll content of *N. tabacum* cv. NC2326 leaf sprayed with various concentrations of exogenous ABA for 12 weeks.

effected by the lower concentration of 0.1 $\mu$g ml$^{-1}$ ABA.

Mean sizes of the fully expanded leaf and the plant height appeared as in Table 1. Both the leaf length and the plant height were decreased 6 % and 19 % by 1 $\mu$g ml$^{-1}$, 12 % and 40 % by 10 $\mu$g ml$^{-1}$, and 25 % and 65 % by 100 $\mu$g ml$^{-1}$ ABA in comparison with control, respectively.

**Chlorophyll content.** Total chlorophyll content was decreased with increasing ABA concentrations from 0.1 to 100 $\mu$g ml$^{-1}$(Fig. 1). The chlorophyll content of control showed

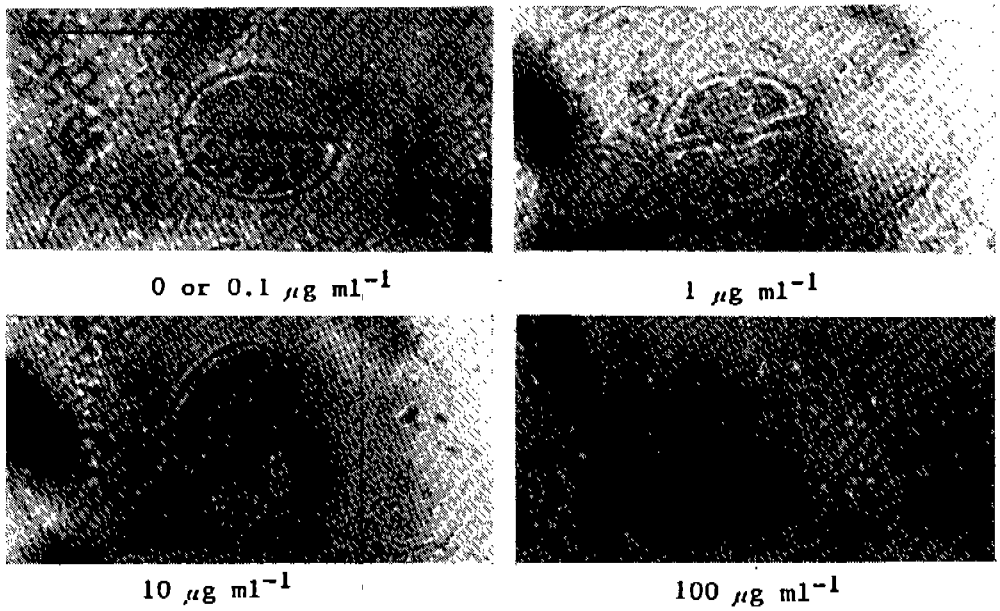


**Fig. 2.** None polar movement(upper) and polar movement(lower: ↔) of chloroplasts in guard cells after the spraying of ABA concentrations of *N. tabacum* cv. NC2326 leaf for 12 weeks.

about 2.41 mg per 1 g sample. Total chlorophyll content was decreased 17 % by 0.1 $\mu$g ml$^{-1}$, 24 % by 1 $\mu$g ml$^{-1}$, 39 % by 10 $\mu$g ml$^{-1}$, and 55 % by 100 $\mu$g ml$^{-1}$ ABA in comparison with control, respectively. The content of each chlorophyll a and b was decreased with increasing ABA concentration, but the ratio of three to one in chlorophyll a and b was not changed by various ABA concentrations as shown in Fig. 1.

In the present study the polar movement of chloroplasts within the guard cells was not found by the lower concentrations of 0.1 and 1 $\mu$g ml$^{-1}$ ABA, but was observed by the higher concentrations of 10 and 100 $\mu$g ml$^{-1}$ ABA (Fig. 2). The polar movement was not found in the leaves of control.

**Content and band patterns of protein.** The effect of ABA on protein content was given in Fig. 4. The protein content of control showed about 360 $\mu$g per 100 $\mu$l sample solution of protein extracts. The protein content was decreased with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA. The decrease rate was 10 % by 1 $\mu$g ml$^{-1}$, 38 % by 10 $\mu$g ml$^{-1}$, and 52 % by 100 $\mu$g ml$^{-1}$ ABA in comparison with control, respectively. However, the content was not effected by 0.1 $\mu$g ml$^{-1}$ ABA. The band patterns of protein by the electrophoresis were given in Fig. 4.

The protein patterns of each 100 $\mu$l sample extract contained about 170~360 $\mu$g protein content (Fig. 3) were developed into almost

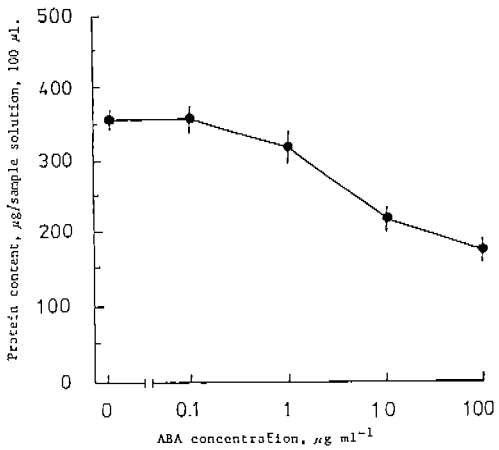


**Fig. 3.** The protein content of *N. tabacum* cv. NC2326 leaf sprayed with various concentrations of exogenous ABA for 12 weeks.

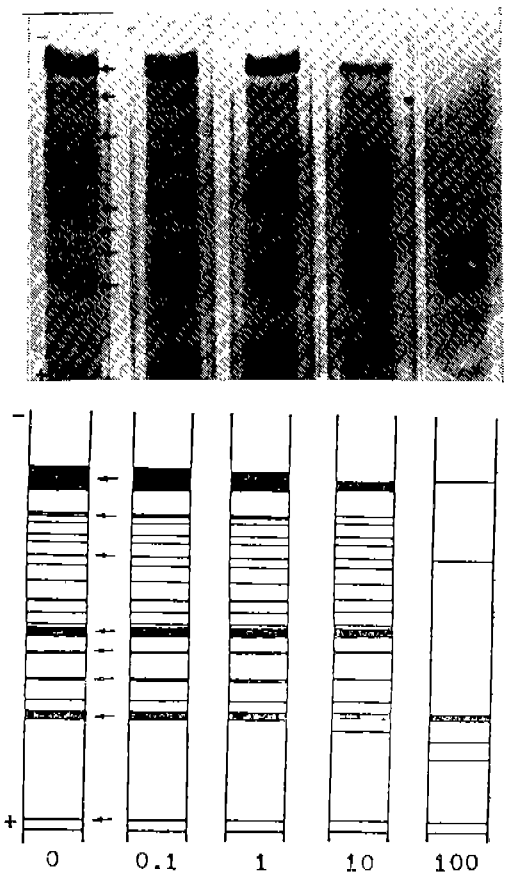


**Fig. 4.** Protein patterns of the extracts in *N. tabacum* cv. NC2326 leaf sprayed with various concentrations of exogenous ABA for 12 weeks (upper; photograph, lower; idiogram).

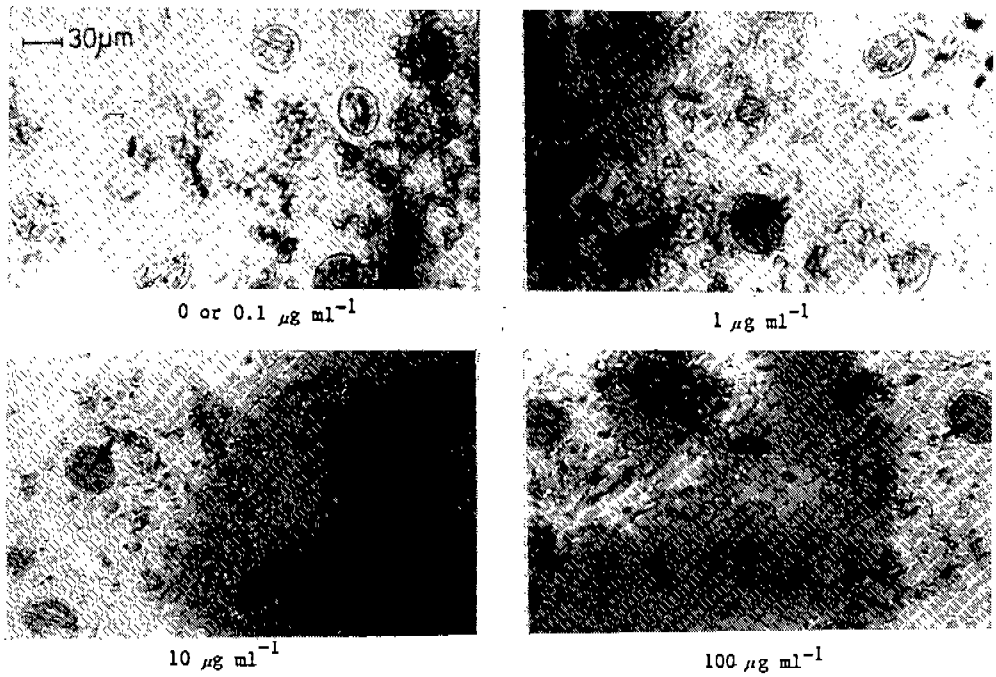


**Fig. 5.** The stomatal closure(←) of *N. tabacum* cv. NC2326 leaf within 3~7 minutes after the spraying of exogenous ABA.

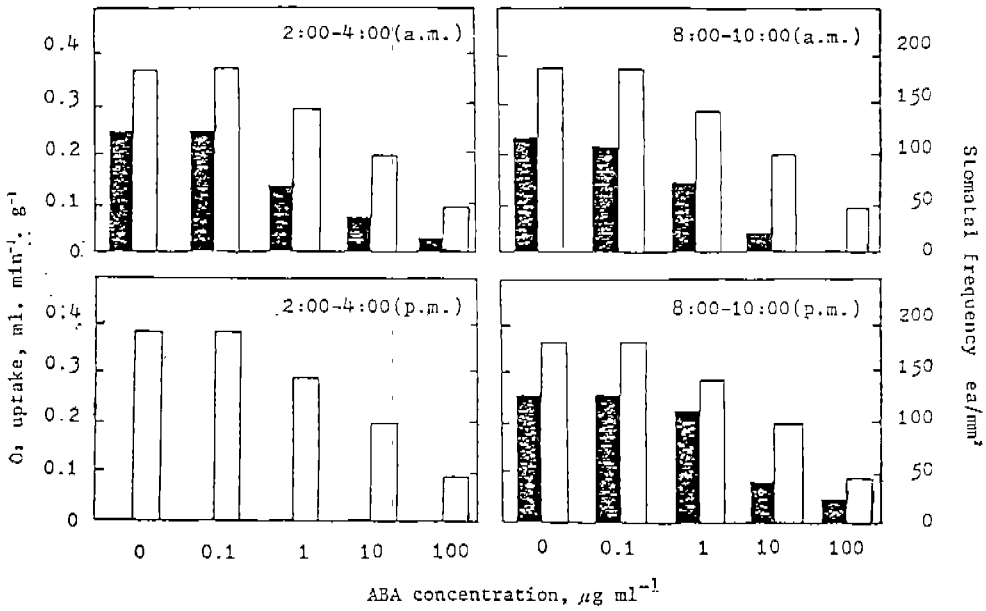


**Fig. 6.** Effects of ABA on the stomatal frequency and respiration rate of *N. tabacum* cv. NC2326 leaf sprayed with various concentrations of exogenous ABA for 12 weeks (■ : $O_2$ uptake, □ : Stomatal frequency) Correlation coefficient ($r=0.963$) and significance ($p<0.1\%$) between respiration rate and stomatal frequency.

17~18 bands from control to 10 $\mu$g ml$^{-1}$ ABA instead of almost 6~7 bands by 100 $\mu$g ml$^{-1}$ ABA. The decrease of protein content appeared higher in the higher molecular protein than in the lower molecular protein as in Fig. 4.

**Closure and frequency of stoma.** The closure and frequency of stoma were observed on the leaf epidermis (Figs. 5 and 6). The frequency of stomatal number per square millimeter was decreased with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA, but was not effected by 0.1 $\mu$g ml$^{-1}$ ABA. About 184 stomatal numbers per 1mm$^2$ on the leaf epidermis were distributed in the control. The stomatal frequency was decreased with 29 % by 1 $\mu$g ml$^{-1}$, 47 % by 10 $\mu$g ml$^{-1}$, and 77 % by 100 $\mu$g ml$^{-1}$ ABA in comparison with control, respectively. All the stomata also were closed with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA, but were not closed by the lower concentration of 0.1 $\mu$g ml$^{-1}$ ABA. In the experiment the closing onset occurred within three minutes by 100 $\mu$g ml$^{-1}$ ABA and seven minutes by 1~10 $\mu$g ml$^{-1}$ ABA after the spraying of ABA. The stomatal closure also was reopened after a few hours in 1~10 $\mu$g ml$^{-1}$ ABA and 24 hours in 100 $\mu$g ml$^{-1}$ ABA during the daytime.

**Respiration rate.** The respiration rate was decreased with increasing the higher concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA, but was not effected by the lower concentration of 0.1 $\mu$g ml$^{-1}$ ABA (Fig. 6). The respiration rate of control showed about 0.256 ml $O_2$. min.$^{-1}$g$^{-1}$. The respiration rate was decreased with 46 % by 1 $\mu$g ml$^{-1}$, 73 % by 10 $\mu$g ml$^{-1}$, and 91 % by 100 $\mu$g ml$^{-1}$ ABA in comparison with control, respectively. During one day, the respiration rate was higher during the night than during the daytime.

It was the highest at 8:00~10:00 p.m., but did not occur at 2:00~4:00 p.m. During the night and weak light, the correlation between the decrease of respiration rate and the decrease of stomatal frequency with increasing the concentrations of ABA was given in Fig. 6. The decrease of respiration rate with increasing the concentrations of ABA was consistent with the decrease of stomatal frequency except for 2:00~4:00 p.m. of non-occurring respiration during the daylight.

## DISCUSSION

In this study both the leaf length and the plant height inhibited with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA. Although the material plants were different from our materials, it was agreed with the report (Newton, 1977) that ABA inhibited frond cell expansion in duck-weed and reduced the increase of both width and length. It was also reported that the spraying of various ABA concentrations inhibited the growth of leaf and stem (Smith *et al.*, 1968).

The chlorophyll content was decreased with increasing the concentrations from 0.1 to 100 $\mu$g ml$^{-1}$ ABA. It was agreed with the report (Mondal *et al.*, 1983) that the spraying of ABA with 15 $\mu$g ml$^{-1}$ concentration significantly promoted the loss of chlorophyll.

However, the ratio of three to one in chlorophyll a to b was not changed.

The polar movement of chloroplasts in the guard cells was found by the high concentrations of 10 and 100 $\mu$g ml$^{-1}$ ABA. It was thought that this phenomenon was the result of inhibition of influx and acceleration of efflux from guard cell by the action of ABA (Raschke, 1975; Schnabl, 1978; MacRobbie, 1980), and the result of hyperpolarization of subcellular membranes with simultaneous stimulation of uptake of amino acids in the guard cells by ABA (Hartung *et al.*, 1980; Tanner, 1980).

The protein content was decreased with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA. It was known that exogenous ABA induced the decrease of protein in plants by other report (Mondal *et al.*, 1983). However, most of the band patterns of the leaf protein were disappeared by only the higher concentration of 100 $\mu$g ml$^{-1}$ ABA. Also the decrease of protein content was higher in the higher molecular protein than in the lower molecular protein. It was assumed that the disappearence of bands by the higher concentration of ABA resulted from the inhibition of the synthesis of most enzymes consisted of higher molecular protein.

The stomatal frequency from our study was decreased with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA. Although the correlation between ABA and stomatal frequency has not been investigated extensively, we found that the spraying of ABA inhibited the initial generation of stoma, and then inhibited the development of secondary stoma for the long period of 12 weeks. In the present study all the stomata also were closed with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA. The closing onset occurred within approximately 3~7 minutes after the spraying of exogenous ABA. It was agreed with the report (Moore, 1978) that the onset of stomatal closure was quite rapid, occurring within approximately 3~9 minutes after the spraying of exogenous ABA to the cut base of the leaves of such as *Zea mays*, *Rumex obtusifolia* and *Beta vulgaris*. It has been known that ABA caused the guard cells to become leaky with respect to $K^+$ and turgor, and then the stomata closed.

Many workers (Jones and Mansfield, 1970; Cummins *et al.*, 1971; Horton, 1971) reported that ABA acted specifically and reversely on stomatal guard cells by initiating and maintaining stomatal closure, thus preventing death through desiccation. Zvilich *et al.* (1982) presented that ABA affected $K^+$ and solute transport between guard cells and epidermal cells. Also the closed stomata were reopened approximately after a few hours to 24 hours. Recently Kubik and Antoszewski (1983) reported that the stomata closed by ABA were reopened approximately after a few hours to 24 hours as the same tendency of many workers (Hiron and Wright, 1973; Dörffling *et al.*, 1974; Beardsell and Cohen, 1975; Jordan *et al.*, 1975; Aharoni *et al.*, 1977). It was thought that the stomatal reopening from closure during the daytime by ABA was the result from photosynthesis as well as time-delay of a drop in the concentration of ABA.

The respiration rate was decreased with increasing the concentrations from 1 to 100 $\mu$g ml$^{-1}$ ABA. For one day, the respiration rate was higher during the night than the

daytime. It was the highest at 8:00~10:00 p.m., but did not occur at 2:00~4:00 p.m.

In this study the decrease of respiration rate with increasing the concentrations of ABA was consistent with the decrease of stomatal frequency except for 2:00~4:00 p.m. of the daylight. During the daytime at 2:00~4:00 p.m., it was thought that non-occurring respiration without depending on the concentration of ABA was the reason from the activating of photosynthesis under the daylight as same tendency of the report (Sung and Kwon, 1982). Although the accumulation of ABA in response to low water potential allowed tissues to maintain turgor by inducing stomatal closure and resultly to decrease respiration rate, in our experiment it was also thought that the decrease of respiration rate during weak light and night was the results of firstly the stomatal closure by spraying of ABA and secondly the decrease of stomatal frequency during the growth by the spraying of exogenous ABA for the long period because of the significance of 0.1% for the correlation coefficient between stomatal frequency and respiration rate.

## 摘 要

담배 (*Nicotiana tabacum* L.)品種 NC2326 種子를 모래를 넣은 플라스틱花盆에 播種하여 室溫에서 發芽시킨 후, 12週 동안 2日에 한번씩 ABA를 0.1, 1, 10 및 100 $\mu g$ $ml^{-1}$ 濃度別로 葉에 噴霧한 후 몇가지 生理作用을 調査하였다. 葉의 形態, 氣孔의 頻度와 開孔, 葉의 葉綠素含量, 呼吸率 및 蛋白質의 含量은 ABA 1 $\mu g$ $ml^{-1}$부터 100 $\mu g$ $ml^{-1}$까지 濃度增加에 따라 減少하였다. 葉綠素 a와 b의 3 : 1 比率은 ABA의 影響을 받지 않았다. 氣孔은 ABA 處理後 3~7分內에 閉孔되었고, 그 後 最小 數時間부터 24時間 사이에 開孔되었다. 孔邊細胞內 葉綠體의 polar movement는 ABA 10 $\mu g$ $ml^{-1}$와 100 $\mu g$ $ml^{-1}$에서 發見되었고, 그 以下의 濃度에서는 發見되지 않았다. 呼吸作用은 ABA 濃度增加에 따라 抑制되었고, ABA에 依한 呼吸의 抑制原因은 生長中 ABA 處理에 依한 氣孔發生의 抑制로 因한 氣孔의 頻度減少와 氣孔의 閉孔때문인 것으로 나타났다. 電氣泳動에 依한 葉의 蛋白質 패턴은 對照區에 比하여 ABA 100 $\mu g$ $ml^{-1}$에서 뚜렷한 band수의 減少를 나타냈으나 그 以下의 濃度에서는 變化가 없었다.

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