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Effect of salicylic acid and its analogues on stomatal closing in *Commelina communis* L.

Joon-Sang Lee

Dept. of Biology, Sangji University, Wonju, Gangwon, 220-702, Korea
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Abstract

ABA and SA showed different effect on stomatal closing on same condition. The addition of 1 mM salicylic acid to fully opened stomata resulted in a significant reductionn of 22 % in stomatal aperture. However, 1 mM ABA reduced 73 % of stomatal aperture. The light absorption spectra of the salicylic acid solution showed that SA was degraded within 1 hour. Therefore, SA solution was resupplied to the detached epidermis every 30 min. during incubation and it was found that even at 10 μM SA induced stomatal closing significantly. Its effect was also greatly pH dependent. The reduction of stomatal aperture caused by 1 mM SA was most effective at lower pH (pH 7.2, 5 %; pH 6.2, 40 %; pH 5.2, 78 %). Therefore, if SA was properly treated to the epidermal strips in the medium, the effects of SA on stomatal closing were similar with those of ABA.

Key Words: stomata, abscisic acid, salicylic acid, *Commelina communis* L.

1. Introduction

Salicylic acid (SA) is a naturally occuring plant phenolic compound which has been shown to affect such diverse processes as flowering, seed germination and stomatal behaviour (Malamy and Klessig, 1992). Several studies have shown that SA inhibited stomatal opening even at low concentration (Bhatia *et al.*, 1986; Larque- Saavedra, 1978, 1979; Manthe *et al.*, 1992). Manthe *et al.* (1992) reported that 1μM SA inhibited stomatal opening by 67 % at pH 5.0 in detached epidermis of *Commelina*.

The magnitude of SA and ABA induced stomatal closure was similar. 1 μM ABA inhibited stomatal opening by around 19 % (Ral *et al.*, 1986) and by 72 % (McAinsh *et al.*, 1990). The role of ABA in inhibition of stomatal opening is thought to involve an increase in the permeability of the plasma membrane of guard cells to calcium ions. The Ca^{2+} entering guard cells then acts as a second messenger to regulate the ion fluxes that determine guard cell turgor (MaAinsh *et al.*, 1990). A subsequent look at plant systems showed that 500 μM SA inhibited phosphate uptake by 54 % (Glass, 1973) and potassium absorption in a pH- and concentration dependent manner in oat roots (Harper and Balke, 1981). SA also caused the collapse of the transmembrane electrochemical potential of mitochondria and ATP dependent proton gradient of the tonoplast-enriched vesicles in pea (Macri *et al.*, 1986). These reports suggest that SA can induce stomatal closing by affecting ion fluxes or membrane permeability. However, it is not known whether SA can increase the concentration of guard cell cytosolic free calcium which triggers the intracellular machinary responsible for stomatal

closure as observed that 100 μM SA stimulated stomatal opening at pH 7.0 in *Commelina*.

This contradictory report could be due to the properties of SA as it is known that SA is metabolized rapidly and its effect is pH dependent (Raskin, 1992).

Several of SA analogues has been identified, but there has been no previous reports about their effects on stomatal closing. Therefore, in this study benzoic acid (BA) and *p*-hydroxybenzoic acid (hBA) were used to determine their effect on stomatal closing. Optimum conditions for stomatal closing by SA were also investigated.

2. Materials and methods

Commelina communis L. was grown from seed in John Innes No. 2 potting compost in a heated greenhouse (minimum temperature 20 °C) with supplementary light to give a photoperiod of 16 h and a photon flux density of 100 μmol $m^{-2}s^{-1}$. Five week old plants were transferred to a controlled environment room at a temperature of 25±1 °C and 16 h photoperiod with a photon flux density of 100 μmol $m^{-2}s^{-1}$. They were kept under these conditions for 3 days before use (for the adaptation after transfer). At all stages of development the plants were kept free from water stress by periodic watering.

Epidermal strips from the fully expanded leaves were obtained by the method of Lee and Bowling (1992). The strips were cut into 5 x 10 mm pieces and placed in 5 cm petri dishes containing 10 mM MES buffer(2-[N-morpholino] ethan sulphonic acid) adjusted to pH 6.2 with KOH in which 50 mM KCl was dissolved. The dishes were incubated in a water bath for 3 h at 25 ± 1 °C under photon flux density of 160μ mol $m^{-2}s^{-1}$. Carbon dioxide free air was obtained by passing room air through a cylinder of soda lime and 2M KOH solution. When the stomata were fully open, SA and its analogues, BA and hBA (Sigma Chemical Co., Poole, U.K.) were added to the medium and the epidermal strips were incubated for a further hour. Stomatal apertures were measured under a microscope (Leitz Labovert, Wetzlar, Germany) at a magnification of x 400 with a calibrated ocular micrometer disc. For the absorption spectra measurments SA, BA and hBA solutions (1 mM) were incubated using the same method as epidermal strips, but no air was given. 1 ml aliquots were taken from the incubation solutions at regular time intervals and the absorption spectra was measured at 190-540 nm by PYE Unicam SP 1800 Ultraviolet Spectraphotometer.

3. Results and discussion

There have been a number of reports which have suggested that salicylic acid can inhibit stomatal opening(Bhatia *et al.*, 1986; Larque-Saavedra, 1978, 1979; Manthe *et al.*, 1992). It is, however, not clear whether under same conditions, SA may also stimulate stomatal closure. Fig. 1. shows the different effects of ABA and SA on stomatal closing. 1 mM ABA reduced 73% of stomatal aperture. However, the addition of 1 mM SA to fully opened stomata of epidermal strips resulted in a significant reduction of 22% ($P>0.05$) in stomatal aperture after 1 hour of incubation in the medium(Fig. 2).

Larque-Saavedra (1979) reported that 1 mM SA took 75 min. to close totally the stomata of *Commelina* which were previously open. However, this may have been due to the low pH of the incubation medium used (pH 4.5). Since the vacuolar pH of guard cells is normally within the range of pH 5.19-5.60, depending on their degree of opening (Penny and Bowling, 1974), it is that the pH used by

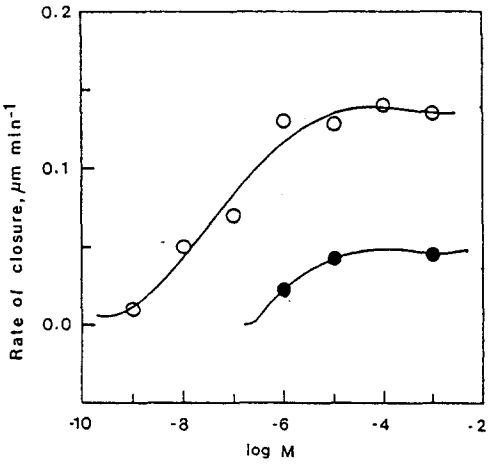

Fig. 1. The effects of ABA and SA on stomatal closing in isolated epidermis of *Commelina communis* L. Samples were incubated in 10 mM MES-KOH buffer (pH 6.2) puls 50 mM KCl. Each point is the mean of three replicate experiments and 80 stomatal apertures were measured. Open circles, ABA; closed circles, SA.

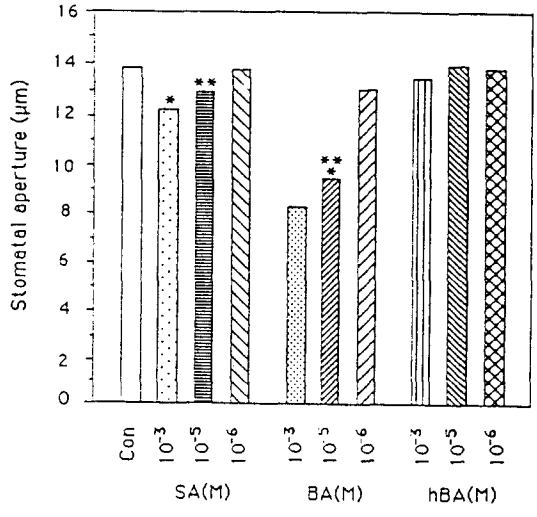

Fig. 2. Responses of stomata to SA, BA and hBA. Means of 80 measurements of individual stomata (Least Significant Difference (L.S.D.); *=1.5, **=2.0, ***=1.1 at P=0.05).

Larque-Saavdra resulted in severe damage to the epidermal and subsidiary cells (Weyers and Meidner, 1990). Certainly, at low pH Harper and Balke (1981) found that SA was a more effective inhibitor of potassium uptake. This suggests that the protonated form of SA is more active than its charged form and that SA inhibits stomatal opening by affecting potassium uptake. However, it is not known whether SA also stimulates potassium efflux, thereby affecting stomatal closure. 1 mM BA stimulated stomatal closing by 46 %, but hBA had no

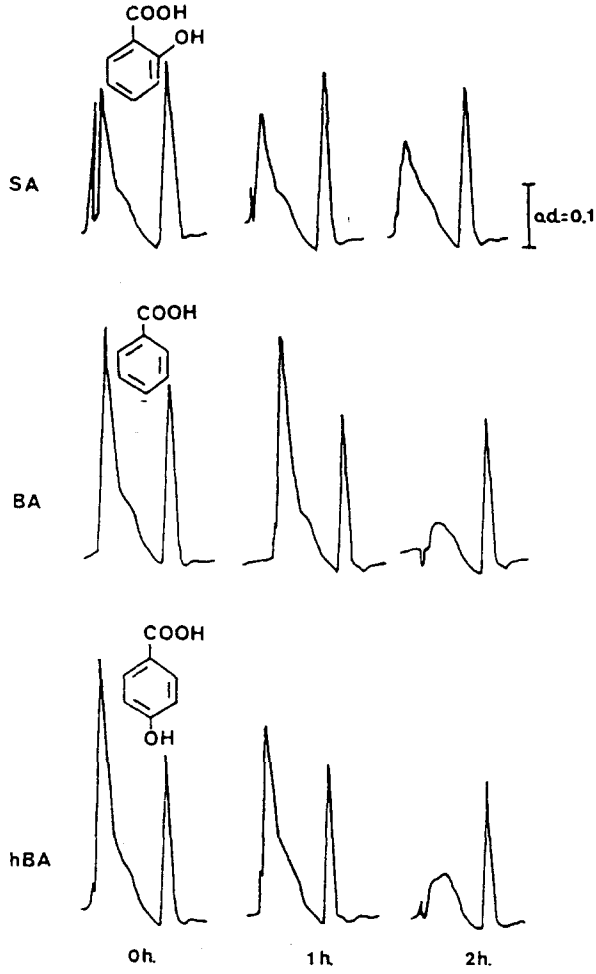

Fig. 3. Absorption spectra of 1 mM SA, BA and hBA solution. Wavelength range was at 190-540 nm (o.d.=optical density).

significant effect (Fig. 2). BA is known as a precursor of SA. Plants have been found to produce SA when they are flowering and during pathogen attact. It is possible that SA synthesis during flowering or pathogen attact could induce stomatal closure.

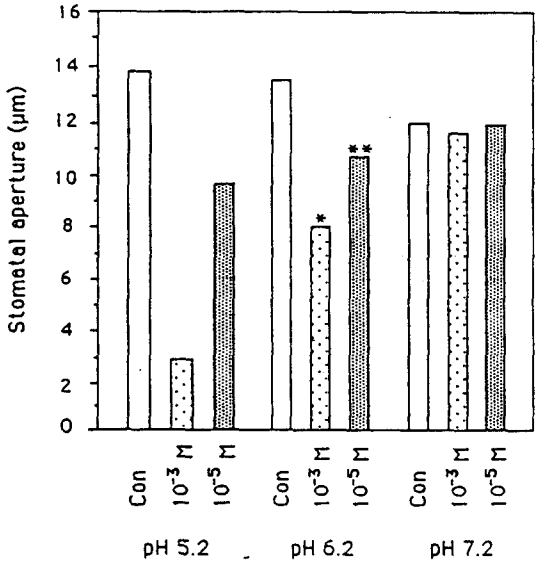

Fig. 4. The effects of pH on the response of stomata to SA. Means of 80 measurements of individual stomata (L.S.D.; *=1.4, **=1.6 at P=0.05).

Fig. 3 shows that SA, BA and hBA were degraded within 1 hour. SA is known to be metabolized rapidly. Levels of SA in excised tobacco leaves fed with SA through the cut petioles increased rapidly and reached a maximum after 3 h. Thereafter, SA levels decreased by 80% (Raskin, 1992). Futhermore, metabolic inactivation of SA may result from additional hydroxylation of the aromatic ring (Raskin, 1992). Light could stimulate its degradation. Therefore, to maintain the activity of SA in the incubation medium, SA was resupplied every 30 min. while epidermis was floated on the medium and different pH was used to incubate epidermis to see SA relation with pH (Fig. 4). After resupplying the SA solution, the effect of SA on stomatal closing had been significantly increased and even at 10 μM(pH 6.2). At pH 5.2 its effect was maximum, but at pH 7.2 there was no effect of SA on stomatal closing. That is why Ral *et al.* (1986) found stomatal opening by SA as they used pH 7.0 in conclusion this study suggests that SA is very sensitive to light and its effect on stomatal closing is dependent on the pH of the incubation medium. Moreover, when SA was properly treated to the epidermal strips in medium, the magnitude of SA and ABA induced stomatal closure was similar.

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References

Bhatia, D.S., V. Jindal and C.P. Malke, 1986, Effect of salicylic acid and tannic acid on stomatal aperture and some enzyme changes in isolated epidermal peelings of *Euphorbia hirta* L., Biochem. Physiol. Pflanzen., 181, 262-264.

Glass, A.D.M., 1973, Influence of phenolic acids on ion uptake: I. Inhibition of phosphate uptake, Plant Physiol., 51, 1037-1041.

Harper, J.R. and N.E. Balke, 1981, Characterization of the inhibition of K^+ absorption in oat roots by salicylic acid. Plant Physiol., 68, 1349-1353.

Larque-Saavedra, A., 1978, The antitranspirant effect of acetylsalicylic acid on

Phaseouls vulgaris. Physiol. Plant., 43, 126-128.

Larque-Saavedra, A., 1979, Stomatal closure in response to acetylsalicylic acid treatment, Z. Pfanzenphysiol. Bd., 93, 371-375.

Lee, J.S. and D.J.F. Bowling, 1992, Effect of the mesophyll on stomatal opening in *Commelina communis*, J. Exp. Bot., 43, 951-957.

Macri, F., A. Vianello and S. Pennazio, 1986, Salicylate-collapsed membrane potential in pea stem mitochondria, Physiol. Plant., 67, 136-140.

Malamy, J. and D.F. Klessig, 1992, Salicylic caid and plant disease resistance, The Plant J., 2, 643-654.

Manthe, B., M. Schulz and H. Schnable, 1992, Effects of salicylic acid on growth and stomatal movements of *Vicia faba* L.: Evidence for salicylic acid metabolization. J. Chem. Ecology, 18, 1525-1539.

McAinsh, M.R., C. Brownlee and A.M. Hetherington, 1990, Abscisic acid induced elevation of cytoplasmic calcium precedes stomatal closure in *Commelina communis*, Nature(London), 343, 186-188.

Penny, M.G. and D.J.F. Bowling, 1974, A study of potassium gradients in the epidermis of intact leaves of *Commelina communis* L. in reaction to stomatal opening, Planta, 119, 17-25.

Ral, V.K., S.S. Sharma and S. Sharma, 1986, Reversal of ABA-induced stomatal closure by phenolic compounds, J. Exp. Bot., 37, 129-134.

Raskin, I., 1992, Role of salicylic acid in plants, Ann. Rev. Plant Physiol., 43, 439-463.

Weyers, J.D.B. and H. Meidner, 1990, Methods in stomatal research, Longman Scientific Technical, Harlow, England, 155pp.

닭의장풀의 기공닫힘에 미치는 살리실릭산과 그 유사물의 효과

이 준 상

상지대학교 이공대학 생물학과

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ABA와 SA는 같은 조건하에서 기공 닫힘에 대하여 다른 효과를 나타냈다. 활짝 열린 기공에 1 mM Salicylic acid(SA)를 첨가 하였을 때, 열린 기공 크기의 22%가 감소하였다. 그러나, 1 mM ABA는 열린 기공 크기를 73%나 감소시켰다. SA 용액의 광흡수 스펙트라 조사 결과, SA가 1시간 내에 분해 되었다. 따라서, 분리표피 배양 동안에 SA 용액을 30분 마다 갈아 주었다. 그 결과 심지어 10 μM SA도 기공 닫힘을 크게 유도 하였으며, 기공 닫힘에 대한 SA의 효과는 pH에 크게 의존적이었다. 1 mM SA에 의하여 유도된 기공 크기의 감소는 낮은 pH (pH 7.2, 5%; pH 6.2, 40 %; pH 5.2, 78%)에서 가장 효과적이었다. 따라서, SA를 배양액에 있는 분리 표피에 적절히 처리하면 기공 닫힘에 대한 효과는 ABA와 거의 같다.