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Influence of Salicylic Acid and Dimethylsulfoxide on Flowering in *Lemna gibba* G3

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Lemna gibba G3 開花(꽃눈분화)에 대한 Salicylic Acid와 Dimethylsulfoxide의 影響

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ABSTRACT

The reversal effect of salicylic acid(SA) on inhibition of flowering in *Lemna gibba* G3 grown on NH_4^+-free 1/2H medium under continuous light is modified by PO_4^{2-} and Ca^{2+} levels. Dimethylsulfoxide(DMSO) either depresses the SA effect in NH_4^+-free 1/2H medium or amplifies it in E medium. The dual action of DMSO determined by relative levels of macro- and micronutrient components is discussed.

INTRODUCTION

Flowering in *Lemna gibba* G3, the long-day plant, can be induced under strict short day conditions by salicylic acid (SA) which is identified as the active substance in honeydew produced by the aphids feeding on *Xanthium strumarium* (Cleland and Ajami, 1974). When grown on E medium (Hoagland type medium), the plants show a qualitative long-day flowering response (Cleland and Briggs, 1967; Cleland and Tanaka, 1979). Treatment with SA causes a shift in the critical daylength of about 2 hours, from just under 10 hours to just under 8 hours (Cleland and Tanaka, 1979).

Composition changes in culture medium can greatly affect the floral response in *Lemna gibba* G3. Even under continuous light, the flowering is strongly inhibited in ammonium-free half-strength Hutner's medium (NH_4^+-free 1/2H), while the addition of SA demonstrates its promotive effect on flowering (Tanaka *et al.*, 1979). Mechanism of SA action on flowering remains to be revealed. SA must be present in the medium to show its promotive effect, indicating that the action of SA on flowering is probably exerted either during the uptake process or very soon thereafter (Ben-Tal and Cleland, 1982; Cleland and Ben-Tal, 1982). The same authors suggested that SA may be acting at the

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level of the cell membrane. SA is known to cause rapid changes in ion permeability of barley roots (Glass, 1973). Dimethylsulfoxide (DMSO) acts selectively on plant cell membranes, rendering it more permeable to small molecules (Delmer, 1979).

In the present study, attempts are made to see how the effects of SA on flowering in *L. gibba* G3 are modified by DMSO which affects cell membrane property and also by some inorganic ions taken up through the cell membrane. Effect of DMSO on flowering, for the first time, will be discussed in conjunction with its dual effect determined by medium composition.

MATERIALS AND METHODS

Two different culture media were used in this study which were E medium and NH_4^+-free 1/2H medium both supplemented with 1% (w/v) sucrose. NH_4^+-free 1/2H medium was made with 2.5 mM KNO_3 substituted for NH_4NO_3 in half-strength H medium. The media were prepared with double distilled water and were sterilized by autoclaving for 15 min at 1.1 $kg \cdot cm^{-2}$.

Vegetative stock cultures, started with ten 4-frond colonies of *Lemna gibba* G3, were grown axenically in 500 ml Erlenmeyer flasks with 200 ml E medium. The cultures were maintained under 9-hr light, 15-hr dark cycles at 25±1°C during the light period and at 21±1°C in the dark. The illuminance from a mixture of cool white fluorescent and incandescent light was kept at 3300 lux at plant level.

Experimental cultures were started with a 4-frond colony grown on 50ml of either E or NH_4^+-free 1/2H medium in 125 ml Erlenmeyer flasks and were kept in growth chambers under the illuminance of 7500~8000 lux at 28±1°C in the light period and at 24±1°C in the dark.

SA and DMSO were added to the medium prior to autoclaving. *Lemna* colonies were rinsed three times with sterile double distilled water when transferred between control medium and culture medium containing SA and/or DMSO. The flowering and growth were evaluated by flowering percentage (FL%) and total frond number (TF) as described elsewhere (Maeng, 1976; Maeng and Khudairi, 1973).

RESULTS AND DISCUSSION

The flowering in *Lemna gibba* G3 is completely inhibited even under continuous light when grown on NH_4^+-free 1/2H medium. SA reverses this inhibition (Tanaka *et al.*, 1979). The concentrations of inorganic ions, phosphate, calcium and magnesium, in the medium were changed to study how these affected the reversal effect of SA on the flowering (Figs. 1-3).

Fig. 1 shows SA effect when the level of phosphate in NH_4^+-free 1/2H medium (1150

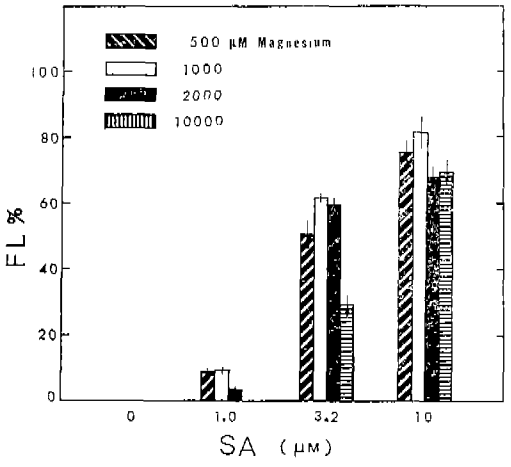

Fig. 3. Influence of magnesium and SA on flowering in *L. gibba* grown on NH_4^+-free 1/2H medium under continuous light. SA was added to the medium at the start of the experiment. FL% was evaluated at the end of day 11. Results are expressed as means ± S.E.

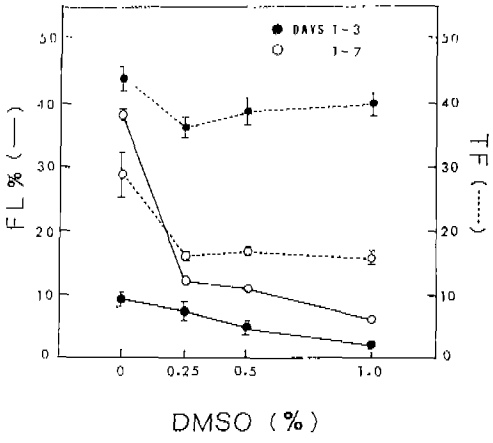

Fig. 4. Effect of DMSO at various concentrations interacted with SA (10μM) on flowering in *L. gibba* G3 grown on NH_4^+-free 1/2H medium. Culture schedule consisted of 7 days of continuous light. DMSO and SA were present in the medium during days 1~3(•) or days 1~7 (○). FL% and total frond number(TF) were evaluated at the end of culture schedule. Results are expressed as means ± S.E.

ingly promoted at each level of calcium. When calcium was maintained at a lower level in the medium, SA could not overcome the inhibition of flowering at its concentration of 1.0 μM. Higher levels of SA, however, could do.

Fig. 3 shows that inhibition of flowering could not be overcome by increasing the level of magnesium up to 10-fold. And higher levels suppressed flowering.

There have been indications that effects of calcium and phosphate ions on flowering are closely related to each other (Hillman, 1962; Ives and Posner, 1982; Posner, 1969). It was reported that depletion of calcium retarded accumulation of phosphate ion into cytoplasm (Foote and Hanson, 1964). A low level of calcium might have mimicked phosphate deficiency effect and thus indirectly depressed the SA promotive action on the flowering.

To extend the discussion that SA action on the flowering in *L. gibba* G3 would be linked to modification of cell membrane permeability and uptake of substances involved, *Lemna* was treated with dimethylsulfoxide (DMSO) which was known to alter the permeability properties of many cell types (Delmer, 1979; Franz and Van Bruggen, 1967).

Combined with 10 μM SA, an optimal level for overcoming the inhibition of flowering in *L. gibba* grown on NH_4^+-free 1/2H medium under continuous light, DMSO was added to the medium either for the first 3 days or throughout the whole 7-day period (Fig. 4). For 3-day treatment group, the plant colonies were rinsed with distilled water and trans-

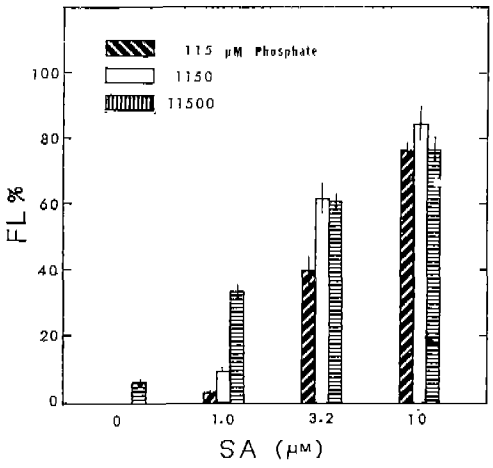

Fig. 1. Influence of phosphate and SA on flowering in *L. gibba* G3 grown on NH_4^+-free 1/2H medium under continuous light. SA was added to the medium at the start of the experiment. Flowering percentage (FL%) was analyzed at the end of day 11. Results are expressed as means ± S.E.

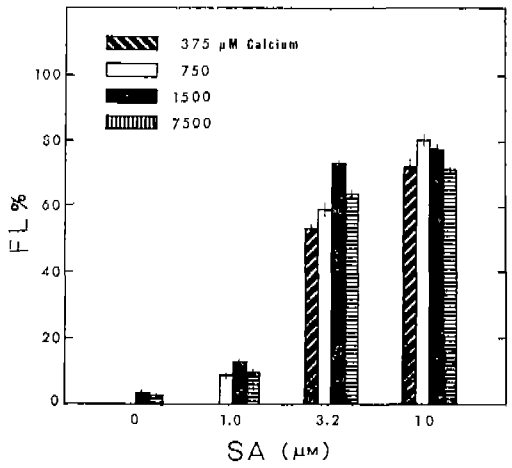

Fig. 2. Influence of calcium and SA on flowering in *L. gibba* G3 grown on NH_4^+-free 1/2H medium under continuous light. SA was added to the medium at the start of the experiment. FL% was evaluated at the end of day 11. Results areexpressed as means ± S.E.

μM) was changed. In the absence of SA, the flowering in *L. gibba* was kept completely inhibited with standard level of phosphate in the medium. The inhibition, however, was partially overcome by increasing phosphate level 10-fold. FL% was detected to be 6.5%. When plants were treated with a low level of SA (1 μM), the increase in phosphate level amplified the SA effect up to 10-fold. With a high concentration of SA (3.2 μM) in the medium, the flowering was saturated at 1150 μM or higher level of phosphate. In the presence of highest level of SA examined (10 μM), the FL% became leveled off even at a lower phosphate concentration (115 μM). The flowering greatly varied with phosphate level when a lower concentration of SA was present in the medium, while with a higher SA level the flowering was promoted to 80% even with a lower phosphate supply, and the increase in phosphate level could not further enhance the flowering in *Lemna*. Based on the observation, it is shown that the promotive effect of SA on the flowering in *L. gibba* might be somewhat connected with availability of phosphate ion. The present results partially support the discussion by Ben-Tal and Cleland in 1982.

NH_4^+-free 1/2H medium contains 750 μM calcium. The SA effect on flowering promotion was shown to be modified by various calcium levels in the medium (Fig. 2). In control medium with no SA, standard or lower level of calcium ion could not evoke flowering. If the level was increased 2- to 10-fold, flowering occurred at 3 to 4%. The effect of higher level calcium which could induce the flowering even with no SA present in the medium was similar to that of phosphate. As SA concentration increased, the flowering was accord-

ferred to control medium at the end of treatment with SA and DMSO for 3 days. With SA alone present in the medium, FL% was analyzed to be 9.7%, which was gradually reduced as DMSO increased. Flowering was still very much inhibited, not only because the 3-day treatment period was insufficient for SA to exert its promotive effect, but also because of rinse effect (Halaban and Hillman, 1970). If SA and DMSO were present in the medium throughout the period, effect of DMSO was profoundly amplified. SA alone evoked the flowering to a level of 38%. Addition of 0.25% DMSO greatly diminished the SA effect, showing only 12% flowering. The inhibitory effect of DMSO against SA action was observed to be saturated at its 0.25% level. Both for 3- and 7-day treatment periods, DMSO at its concentrations higher than 0.25% showed a similar effect.

While the flowering in *Lemna gibba* G3 grown on NH^+_4-free 1/2H medium is completely inhibited, the plants when cultured on E medium show a qualitative long-day flowering response. Fig. 5 presents DMSO effect on flowering in the plants grown on E medium under continuous light. Culture schedule consisted of 7 days of continuous light followed by 4 cycles of 9-hr light, 15-hr dark. At the beginning of day 3, the plants were transferred to a medium containing DMSO (0~1.0%). After 24-hr or 48-hr incubation in DMSO-containing medium, plants were rinsed with distilled water and transferred back to their old medium. Due to transfer and rinse effect, the overall FL% was rather low. Plants grown in control medium without transfer or rinse showed 20.3% flowering. The flowering

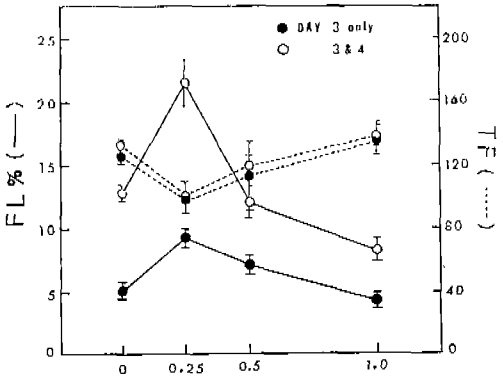

Fig. 5. Effect of DMSO on flowering in *L. gibba* G3 cultured on E medium. Culture schedule consisted of 7 days of continuous light followed by 4 cycles of 9-hr light, 15-hr dark. DMSO treatment given at the beginning of day 3 lasted for 24 (•) or for 48 hrs (○). Plants were transferred back to their old medium after DMSO treatment. FL% and TF were analyzed at the end of culture period. Results are expressed as means ± S.E.

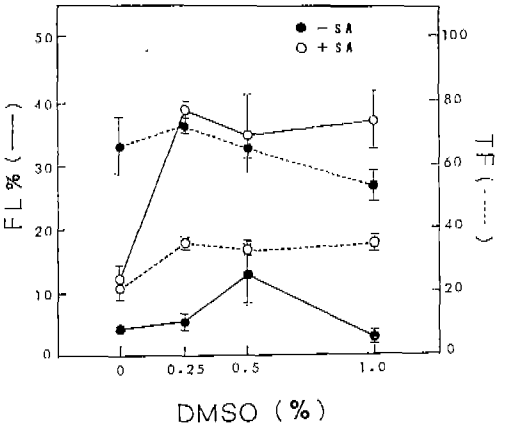

Fig. 6. Effect of DMSO on flowering in *L. gibba* G3 grown on E medium with (○) or without (•) 3.2 μM SA. DMSO was present in the medium throughout the culture period. Culture schedule consisted of 8 cycles of 12-hr light, 12-hr dark followed by 3 days of continuous light. FL% and TF were analyzed at the end of culture period. Results are expressed as means ± S.E.

was considerably enhanced by 0.25% DMSO treatment. At higher levels, the promotive effect of DMSO was disappeared, or moreover the flowering was rather inhibited.

Lemna gibba G3 was cultured on E medium under 12-hr light, 12-hr dark cycles which is close to the critical photoperiod for flowering (Fig. 6). FL% was 4.1% when neither DMSO nor SA was added to the medium. Addition of 0.5% DMSO alone enhanced flowering 3-fold, and higher level of DMSO decreased flowering. When SA alone was present in the medium, FL% showed 12.3% and TF was greatly decreased. DMSO treatment along with SA profoundly promoted flowering. The effects of both substances combined were shown to be synergistic.

It is noted that DMSO is acting on flowering in *L. gibba* in two different ways opposite to each other; depression of promotive effect of SA when added to NH_4^+-free 1/2H medium, or, on the other hand, amplification of SA effect to have further promotion of flowering when present in E medium. Comparison of composition of two media shows that macronutrient levels are much higher in E than in NH_4^+-free 1/2H, while micronutrient concentrations are higher in NH_4^+-free 1/2H.

The composition of E medium was modified by changing levels of macronutrients, KH_2PO_4, KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$, and/or micronutrients, $FeCl_3 \cdot 6H_2O$, H_3BO_3, $ZnSO_4 \cdot 7H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $MnCl_2 \cdot 4H_2O$, tartaric acid. Plants were grown on E

Table 1. Modification of DMSO effect on flowering in *L. gibba* G3 by various levels of macro- and micronutrients of E medium. Culture schedule consisted of 8 cycles of 12-hr light, 12-hr dark followed by 3 days of continuous light. Concentrations of macro- and micronutrients are expressed as fraction of E medium. FL% and TF values are means ± S.E.

Concentrations of		DMSO(%)	FL%	TF
macronutrients	micronutrients			
1	1	0	4.10±0.46	66.32±10.33
		0.25	5.43±1.26	73.33±15.06
		0.50	13.26±5.19	65.33± 4.49
		1.00	2.47±0.14	53.66± 5.90
1	0.2	0	3.59±1.15	102.00±21.16
		0.25	24.23±4.09	97.66± 3.85
		0.50	24.66±6.30	98.66± 9.17
		1.00	25.92±3.39	72.00±14.52
0.2	1	0	3.64±0.73	64.00± 0.94
		0.25	5.58±0.57	59.66± 4.02
		0.50	6.43±1.68	67.33± 3.85
		1.00	1.10±0.77	60.00± 2.82
0.2	0.2	0	0	126.00± 5.71
		0.25	0.82±0.08	121.00±11.43
		0.50	2.38±0.46	111.66± 2.49
		1.02	17.84±3.87	89.66± 8.80

medium thus modified, and DMSO effect on flowering was analyzed (Table 1). If micronutrient components were adjusted at fifth-strength to bring a ratio of macro- to micronutrients high, DMSO effect was so amplified that the flowering increased up to more than 10-fold. On the other hand, when macronutrient level was brought down at fifth-strength to keep the ratio low, the promotive effect of DMSO almost diminished. Lowering both macro- and micronutrient components at fifth-strength exhibited little flowering at 0.25 and 0.5% DMSO. But 1.0% DMSO could induce flowering to 17.8%, indicating that a minimum level of DMSO for induction of flowering was elevated if both macro- and micronutrients were maintained low. The minimum DMSO concentration to induce significant level of flowering was 0.5% in control E medium.

Flower induction in *L. gibba* by raising phosphate or calcium levels in NH_4^+-free 1/2H medium containing relatively low levels of macronutrients (Figs. 1 and 2) can be explained in part in connection with the results presented in Table 1. It is concluded that direction of DMSO action on the flowering in *L. gibba* was largely dependent on relative levels of macro- and micronutrient components, i.e. if macro- to micronutrient ratio is kept high DMSO effect is promotive, while the ratio is maintained low the effect turns to be very inhibitory.

摘 要

長日植物인 *Lemna gibba* G3는 NH_4^+를 제거하고 1/2로 희석한 Hutner 배양액(NH_4^+-free 1/2H)에서 생장할 때에는 비록 連續光하에서도 開花(꽃눈분화)가 완전 억제된다. 이러한 開花抑制를 극복하여 開花를 誘導하는 salicylic acid(SA)의 효과는 배양액 내의 PO_4^{2-} 및 Ca^{2+} 농도에 의하여 변형된다. 또한, 細胞膜 透過性에 영향을 미치는 dimethylsulfoxide(DMSO)는 NH_4^+-free 1/2H 배양액 내에서는 上述한 SA의 효과를 억제하나, E 배양액내에서는 오히려 이 효과를 증대시킨다. 이러한 DMSO의 二頂作用은 배양액내의 大量 및 微量營養素사이의 相對的 水準에 따라 결정된다.

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