Note

Induction of Flowering by Inducers of Systemic Acquired Resistance in the *Lemna* Plant

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Flowering is a common process in plant reproduction, and the plant defense response is necessary for plant survival. Both are essential to the preservation of species. Although much research has been devoted to these distinct physiological responses, their underlying mechanisms are not fully understood. Recently, detailed studies of the *FT* and *Hd3a* genes encoding a primary candidate for florigen, a mobile flowering signal, made great progress in understanding photoperiodic flowering mechanisms, but non-photoperiodic flowering mechanisms induced by chemical inducers and other external stimuli have not yet been elucidated in detail.

Salicylic acid (SA) is known to induce flowering. SA also induces systemic acquired resistance (SAR), an important plant defense mechanism. Based on the finding that flowering and SAR share a common inducer, SA, it is reasonable to propose that flowering and SAR share common induction mechanisms. As a first step, we tested this hypothesis.

Previous studies have found that SA binds to and inactivates catalase, resulting in increased levels of reactive oxygen species (ROS), including hydrogen peroxide, which induces SAR. Hence we examined the effect on flower induction in *Lemna paucicostata* 151, a short-day plant, and *Lemna gibba* G3, a long-day plant, under conditions that generated ROS, as follows: Two glass cups were prepared, one filled with 5 ml of 5 N KOH solution and the other with 10 ml of culture medium. A three-frond colony and a four-frond colony of *L. paucicostata* 151 and *L. gibba* G3 respectively was cultured in the glass cup containing the culture medium. The cups were put together in an airtight bottle and incubated at 25 ± 1 °C for 7 d (*L. paucicostata* 151) or 12 d (*L. gibba* G3) under continuous light (168 µmol/m²/s) at plant level. These conditions exposed *L. paucicostata* 151 and *L. gibba* G3 to photooxydative stress caused by the combinatory effect of CO₂ deprivation and high-intensity illumination. The culture media were 1/10 strength liquid E medium supplemented with 1% (w/v) sucrose and 1 µM benzyladeneine, and 1/2 strength NH₄⁺-free Hutner liquid medium supplemented with 1% (w/v) sucrose in the culturing of *L. paucicostata* 151 and *L. gibba* G3 respectively. In 1/2 strength NH₄⁺-free Hutner liquid medium, *L. gibba* G3 generally does not flower even under long-day condition without treatment.

The results of the experiment showed that the percentages of fronds with flowers (FL%) in *L. paucicostata* 151 and *L. gibba* G3 were 15.3 ± 1.8% and 16.0 ± 3.3% respectively when cultured under photooxydative stress (+KOH), while no flowering of *L. paucicostata* 151 was induced, and the FL% of *L. gibba* G3 was 2.7 ± 2.5% when cultured without photooxydative stress (−KOH). The results were expressed as the mean ± SD (standard deviation) obtained from each experiment, with three replications. These results indicate that photooxydation-mediated ROS generation promoted flower induction in both *Lemna* species, suggesting that chemical inducers of SAR promote the flowering in the plants.

SAR is induced by 3-allyloxy-1,2-benzisothiazole-3(2H)-one-1,1-dioxide (probenazole; PBZ) and its active metabolite 1,2-benzisothiazole-3(2H)-one-1,1-dioxide (BIT), and by benzo (1,2,3)thiadiazole-7-carbothionic acid S-methyl ester (BTH) in tobacco and *Arabidopsis*. To determine the effects of these SAR inducers on flowering, *L. paucicostata* 151 and *L. gibba* G3 were grown in a culture medium containing several concentrations of PBZ, BIT, or BTH for 5 d and 11 d respectively under continuous light (84 µmol/m²/s). We found that all three induced flowering in both species (Figs. 1 and 2). A higher percentage of fronds with flowers (FL%) was observed under treatment with the higher concentration of PBZ and BIT in both species. BIT was more effective than PBZ or BIT in flower induction, and showed prominent flowering effects even at a low concentration level (1 µM) in both species.
Methyl salicylate (Me-SA) is also known to be a critical volatile plant SAR-inducer.\(^{17}\) Hence we examined the effect of the Me-SA on the flowering of *Lemna*, as follows: A three-frond colony and a four-frond colony of *L. paucicostata* 151 and *L. gibba* G3 respectively were cultured in a glass cup containing 10 ml of the culture medium. The glass cup and a microtube containing 1 µl of several concentrations of Me-SA dissolved in anhydrous methanol were put together in an airtight bottle and incubated at 25 ± 1°C for 7 d (*L. paucicostata* 151) or 12 d (*L. gibba* G3) under continuous light (84 µmol/m²/s) at plant level. As shown in Fig. 3, the higher concentration of Me-SA obviously promoted flowering in both *Lemna* species.

In the present study, we found that SAR in plants and flowering in *Lemna* share common inducers. However, since we did not find the direct evidence as to whether the SAR of *Lemna* plant is in fact induced by PBZ, BIT, BTH, or Me-SA, the molecular level of SAR development remains to be examined by expression analysis of PR genes as markers for SAR development.\(^{13–16}\)

Although SAR development was not the focus, expression of PR genes has been observed during normal flower formation in tobacco.\(^{18}\) Thus, the next step is to determine whether the SA-mediated signaling pathway in *Lemna* plant is activated by flower-inducing activity in *L. paucicostata* 151.\(^ {19,20}\) If we can induce SAR in the *Lemna* plant by such flower-induction-related chemicals, we will be able to hypothesize that SAR and flowering share common induction mechanisms.

### References


