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Note

Effect of Jasmonates and Related Compounds on Seed Germination of *Orobanche minor* Smith and *Striga hermonthica* (Del.) Benth

Koichi YONEYAMA,† Masaru OGASAWARA, Yasutomo TAKEUCHI, Makoto KONNAI, Yukihiro SUGIMOTO,* Hideharu SETO,** and Shigeo YOSHIDA**

Weed Science Center, Utsunomiya University, Utsunomiya 321-8505, Japan
**Arid Land Research Center, Tottori University, Tottori 680-0001, Japan*
***The Institute of Physical and Chemical Research (RIKEN), Wako 351-0198, Japan*

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Jasmonates and related compounds were found to elicit the seed germination of the important root parasites, clover broomrape (*Orobanche minor* Smith) and witchweed [*Striga hermonthica* (Del.) Benth]. The stimulation of seed germination by the esters was more effective than by the corresponding free acids, and methyl jasmonate (MJA) was the most active stimulant among the compounds tested.

Key words: cucurbic acid; jasmonic acid; *Orobanche minor* Smith; seed germination; *Striga hermonthica* (Del.) Benth

Broomrapes (*Orobanche* spp.) and witchweeds (*Striga* spp.) are the two most important root parasitic weeds that cause considerable yield reduction to various crops in tropical and semi-tropical regions.[1] The seeds of these parasites have special germination requirements, including pretreatment (conditioning) in a warm, moist environment for several days and subsequent exposure to an exogenous germination stimulant.[2,3] Once germinated, these parasites will die within a week unless they attach to the roots of host plants. Therefore, inducing seed germination in the absence of host plants, termed "suicidal germination," is a promising strategy for depleting seed reserves in the soil. However, natural *Striga* and *Orobanche* germination stimulants such as strigol,[4,5] sorgolactone,[6] alectrol,[7] and dihydrosorgoleone[8] are unstable in soil, and no useful and economic suicidal germination stimulants based on these molecules have been obtained.[3] Under these circumstances, germination stimulants structurally unrelated to the natural ones are important molecular probes to understand the germination mechanism of the parasites, and also to develop useful and economic germination stimulants. We recently found that the fungal metabolites, cotylenins and fusicoccins, which have diverse physiological effects on plants,[9] could elicit the seed germination of *Orobanche minor* Smith and *Striga hermonthica* (Del.) Benth.[10] This suggests that other compounds with different chemistry may induce germination as well.

In the search for new germination stimulants, various compounds were first examined for their effects on *O. minor* germination. In general, *Orobanche* spp. appeared to have stricter germination requirements than *Striga* spp., and the compounds which elicited *Orobanche* germination also induced *Striga* germination. So far, except for cotylenins and fusicoccins, only strigol-related compounds have been reported to induce *Orobanche* germination.[3] In contrast, in addition to cotylenins, fusicoccins, and strigol-related compounds, *Striga* germination is induced by other chemicals including ethylene, cytokinins, and auxins. Although various plant-growth regulators (gibberellins, cytokinins, auxins, abscisic acid, ethylene, and brassinosteroids) have been found not to induce *Orobanche* germination,[3,11] jasomonic acid (JA, Fig. 1) had not been examined for its effect on the seed germination of these parasites. Therefore, JA was included in the assay and found to induce the germination of *O. minor*. JA and related compounds were then examined for their effects on the seed germination of *O. minor* and *S. hermonthica*.

Seed germination assays were conducted as reported,[10,11] except that smaller glass Petri dishes (*i.d.* 4.5 cm) were used in this study. Each test compound was dissolved in acetone. An aliquot (0.1 ml) of the respective test solution was added to each Petri dish lined with a filter paper. The solvent was allowed to evaporate before conditioned seeds were placed on the filter paper and treated with distilled water (0.7 ml). Racemic mixtures of JA and related compounds were prepared as described elsewhere.[12,13] Seeds treated with 10^{-8} M (±)-strigol, which had been synthesized as described in the literature,[14,15] or distilled water was used as the controls. Each treatment was replicated three times and conducted at least twice. The data presented in the tables are from single typical experiments, because the germination percentages may have varied from test to test.[16]

In general, the response by seeds of both parasites to germination stimulants including (±)-strigol was rather low when compared to that reported earlier.[10,11] This low germination response could have been due to the difference in size of the Petri dishes, or simply due to a season-dependent variation. Seasonal variations in response to germination stimulants have been reported for both *Striga* and *Orobanche* spp.[16] Among the JA derivatives (**1–3**), methyl jasmonate (MJA, **1**) at 10^{-4} M

† To whom correspondence should be addressed. Fax: +81-28-636-7662; E-mail: yoneyama@cc.utsunomiya-u.ac.jp
Abbreviations: CA, cucurbic acid; JA, jasmonic acid; MCA, methyl cucurbate; MJA, methyl jasmonate

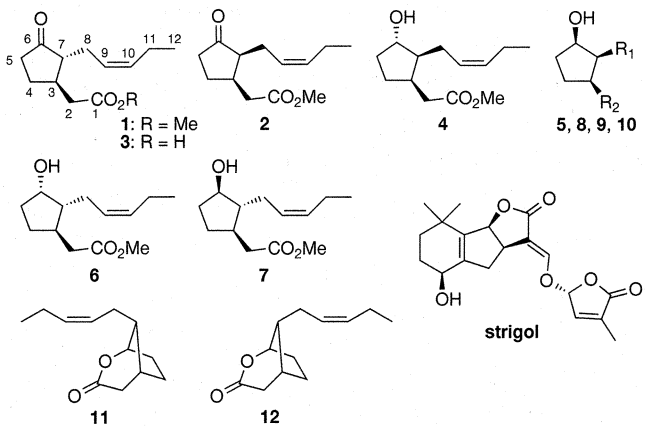

Fig. 1. Structure of Jasmonic Acid (JA), Cucurbic Acid (CA), Their Related Compounds and Strigol.

5, R_1=(*Z*)-3-pentenyl, R_2=CH_2CO_2Me; **8**, R_1=(*Z*)-3-pentenyl, R_2=CH_2CO_2H; **9**, R_1=(*Z*)-3-pentenyl, R_2=CH_2CH_2OH; **10**, R_1=pentyl, R_2=CH_2CO_2Me.

induced the highest germination of *O. minor* (Table 1). The activity of 7-*epi*-MJA (**2**) was less than 50% that of MJA. Furthermore, JA (**3**) was only 1/10 as active as MJA, suggesting that the ester function was preferable for high activity and/or, presumably, for penetration into the seeds. The structural and stereochemical requirements for germination-stimulative activity can be seen in more detail with the cucurbic acid (CA) derivatives (**4**–**12**), of which the most active stimulant was 6-*epi*-9,10-dihydro-MCA (**10**). The ester, 6-*epi*-MCA (**5**), was about 2-fold as active as the free acid, 6-*epi*-CA (**8**). This difference in activity is relatively small when compared to that observed between MJA and JA. On the other hand, reduction of the carboxyl group to the alcohol, as in **9**, resulted in a significant loss of activity. This implies that the carboxyl group played an important role in stimulating germination. When comparing the activities of compounds **4**–**7** which have different relative configurations at the 3, 6 and 7 positions on the cyclopentane ring, the 6,7-*cis* configuration seems to have been preferable for high activity. For example, (3,6,7-all *cis*) 6-*epi*-MCA (**5**) was the most active. MCA (**4**) and 6,7-di-*epi*-MCA (**7**), the two 6,7-*trans* derivatives, were 1/6 and 1/10 as active as 6-*epi*-MCA (**5**), respectively. In contrast, the relative stereochemical relationship between the pentenyl side chain and the acetate moiety appears to have been of lesser importance; (3,7-*trans*) 7-*epi*-MCA (**6**) was 1/2 as active as (3,7-*cis*) 6-*epi*-MCA (**5**). In addition, with the cucurbic acid derivatives, the unsaturated pentenyl group at the 7 position seems to have reduced activity. The compound carrying an saturated alkyl side chain (6-*epi*-9,10-dihydro-MCA, **10**) was more active than 6-*epi*-MCA (**5**). The germination stimulative activity of the CA lactones (**11** and **12**) suggests that these two compounds may have interacted directly with the receptor(s) of germination stimulants, since 6-*epi*-CA lactone (**11**), which would afford the moderately active 6-*epi*-CA (**8**) after hydrolysis, was totally inactive, whereas 6,7-di-*epi*-CA lactone (**12**) was much more active than 6,7-di-*epi*-MCA (**7**). It is thus apparent that MJA (**1**) and 6-*epi*-9,10-dihydro-MCA (**10**) were the most active among the JA- and CA-related compounds, respectively. Furthermore, the high activity of 6-*epi*-9,10-dihydro-MCA (**10**) suggests that proper modification of the pentenyl side chain may further increase the activity, not only in CA derivatives but also in JA derivatives. At 10^{-5} M, these compounds induced only negligible germination.

Table 1. Percentage Germination for Seeds of *O. minor* after Exposure to Solutions of Jasmonate and Its Related Compounds

		Germination ± SE (%)*	
No.	Compounds**	10^{-4} M	10^{-5} M
1	MJA	67±2.3	3±0.6
2	7-*epi*-MJA	29±2.7	2±0.5
3	JA	6±0.8	1±0.2
4	MCA	7±1.0	0
5	6-*epi*-MCA	42±2.3	1±0.3
6	7-*epi*-MCA	22±1.8	0
7	6,7-di-*epi*-MCA	4±0.8	0
8	6-*epi*-CA	20±1.4	0
9	6-*epi*-CA-ol***	2±0.5	0
10	6-*epi*-9,10-dihydro-MCA	58±2.0	3±0.3
11	6-*epi*-CA lactone	0	0
12	6,7-di-*epi*-CA lactone	39±2.3	0
	(±)-strigol****	(68±2.0)	—
	control	0	0

* Mean±SE (n=3).
** Racemic mixture.
*** Alcohol derivative of 6-*epi*-CA.
**** (±)-Strigol was tested at 10^{-8} M.

Table 2. Percentage Germination for Seeds of *S. hermonthica* after Exposure to Solutions of Jasmonate and Its Related Compounds

		Germination ± SE (%)*	
No.	Compound**	10^{-3} M	10^{-4} M
1	MJA	50±1.2	12±2.3
3	JA	26±1.9	6±2.1
10	6-*epi*-9,10-dihydro-MCA	22±1.8	6±1.3
	(±)-strigol***	(44±3.6)	—
	control	0	0

* Mean±SE (n=3).
** Racemic mixture.
*** (±)-Strigol was tested at 10^{-8} M.

MJA (**1**), JA (**3**) and 6-*epi*-9,10-dihydro-MCA (**10**) were then examined for their effects on *S. hermonthica* germination (Table 2). At 10^{-4} M, all the compounds elicited negligible germination (6–12%). At 10^{-3} M, MJA (**1**), JA (**3**) and 6-*epi*-9,10-dihydro-MCA (**10**) induced 22–50% germination. The relative order of activity is somewhat different from that observed with *O. minor* seeds, JA (**3**) being as active as 6-*epi*-9,10-dihydro-MCA (**10**).

JA and MJA display various physiological effects in plants, and they inhibit or promote seed germination depending on the plant species.[17,18] This study has shown that JA and related compounds could induce seed germination of the root parasitic weeds, *O. minor* and *S. hermonthica*, which have special germination requirements. Although the activity of JA and related compounds was rather weak, proper modification of their structures, in particular, of the ester and alkenyl moieties,[13] may lead to more active stimulants. Furthermore, the activity of individual enantiomers as germination stimulants should be examined. The stereochemistry of JA and related compounds appears to have had a pronounced effect on their activity.

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