ストリゴラクトン生産制御設計のための基本構造となるアバミン

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著者名	北畑,信隆 伊藤,晋作 加藤,敦隆 上野,琴巳 中野,雄司 米山,香織 米山,弘一 浅見,忠男
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Note

Abamine as a basis for new designs of regulators of strigolactone production

Nobutaka KITAHATA[#], Shinsaku ITO[#], Atsutaka KATO[#], Kotomi UENO, Takeshi NAKANO[†], Kaori YONEYAMA[††], Koichi YONEYAMA[††] and Tadao ASAMI*

Department of Applied Biological Chemistry, The University of Tokyo, 1–1–1 Yayoi, Bunkyo, Tokyo, 113–8657 Japan
[†] *Advanced Science Institute, RIKEN, 2–1 Hirosawa, Wako, Saitama, 351–0198 Japan*
[††] *Weed Science Center, Utsunomiya University, 350 Mine-machi, Utsunomiya, 320–8505 Japan*

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Nine-*cis*-epoxycarotenoid dioxygenase (NCED), belonging to the family of carotenoid-cleaving dioxygenases (CCDs), is the rate-limiting step in the abscisic acid (ABA) biosynthetic pathway in plants. Abamine is the first reported abscisic acid biosynthesis inhibitor to target NCED. Its utility has also been considered in the inhibition of other enzymes. As CCDs other than NCED are also involved in the strigolactone biosynthetic pathway, abamine may affect strigolactone biosynthesis. In this study, we found that abamine decreases the level of strigolactones in several plant species and reduces the germination rate of *Orobanche minor* seeds co-grown with tobacco. Taken together, these data suggest that abamine may be used as a scaffold for the development of specific regulators of strigolactone production. © Pesticide Science Society of Japan

Keywords: strigolactone, parasitic weed, rice, abamine, abamineSG, *Orobanche minor*.

Introduction

Strigolactones were first identified as seed germination stimulants for parasitic weeds such as *Orobanche* spp. and *Striga* spp from the root exudates of host plants,[1] and since then have been recognized as a group of terpenoid lactones. Strigolactones also induce hyphal branching in arbuscular mycorrhizal fungi that form symbiotic associations with the roots of more than 80% of land plants.[2] More recently, it has been reported that strigolactones and their metabolites inhibit shoot branching.[3,4] Genetic analyses performed on a series of branching mutants, such as *more axillary growth* (*max*) mutants of *Arabidpsis*, *ramosus* (*rms*) mutants of pea, the *dwarf* (*d*) or *high tillering dwarf* (*htd*) mutants of rice and the *decreased apical dominance* (*dad*) mutants of petunia, revealed that strigolactone biosynthesis is mediated by two carotenoid cleavage dioxygenases, CCD7 (also known as MAX3, RMS5 D17/HTD1, DAD3) and CCD8 (also known as MAX4, RMS1, D10, DAD1), cytochrome P450 monooxygenase (MAX1), and DWARF27, a novel iron-containing protein;[5,6] however, several biosynthetic steps still need to be identified.[7]

Biosynthetic inhibitors regulate the endogenous levels of biologically active substances throughout various developmental stages and sites. In addition, a mutation or targeted knockout of an individual gene in sets of paralogous genes encoding these biosynthetic inhibitors has few phenotypic effects as they are able to overcome such gene redundancy in many cases; therefore, the use of specific biosynthetic inhibitors in the study of plant biology is an alternative and valuable way to determine the physiological functions of endogenous substances. As was seen in the case of brassinosteroid biosynthesis inhibitors,[8–11] specific strigolactone biosynthesis inhibitors proved useful in functional studies of strigolactone biosynthesis and in assessments of the effect of strigolactone in plants. Moreover, strigolactone biosynthesis inhibitors may be useful in understanding the regulation of germination and infestation in parasitic weeds. Accordingly, our study focused on identifying novel strigolactone biosynthesis inhibitors.

Materials and Methods

1. Chemicals

Abamine was synthesized using the method outlined by Han *et al.*[12,13] GR24, a synthetic mimic of strigolactones, was synthesized as previously described.[14] In this study, GR24 with the same relative stereochemistry as (±)-strigol was used.

2. Plant materials

A rice cultivar, Shiokari, was used in all experiments. Rice seeds were sterilized in 2.5% sodium hypochlorite solution containing 0.01% Tween-20 for 30 min, rinsed with sterile water and then incubated for two days in sterile water at 25°C in the dark. Germinated seeds were transferred into hydroponic culture media[4] solidified with 0.6% agar and cultured at 25°C under fluorescent white light with a photoperiod of 14-hr light/8-hr dark for 6 days. Seedlings were then individually transferred to glass vials containing 12 ml of a sterilized hydroponic culture solution supplement with or without chemicals and grown for 6 days. For strigolactone analysis, one-week-old seedlings were transferred to glass vials containing 12 ml of a sterilized hydroponic culture solution. After 5 days, seedlings were transferred to new glass vials containing culture solutions both with and without chemicals for 1 day. Sorghum was grown according to the method described in the literature.[15] Tobacco (*Nicotiana tobacum* L. cv. Samsun NN)

[#] These authors contributed equally to this work.
* To whom correspondence should be addressed.
E-mail: asami@pgr1.ch.a.u-tokyo.ac.jp
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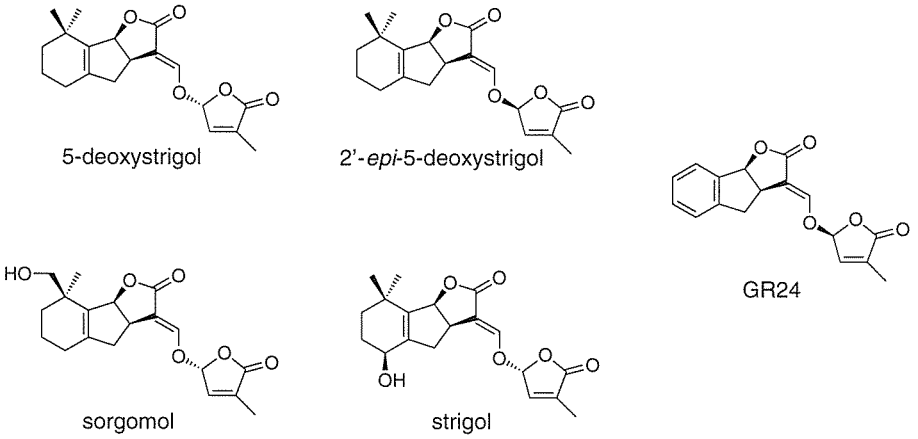

Fig. 1. Chemical structures of several strigolactones.

seeds were sown on 0.8% agar-solidified medium containing 1/2 Murashige and Skoog salts and 1.5% sucrose (w/v) in Agripots (Kirin Brewery Co., Tokyo, Japan) with or without chemicals. The seeds were then stratified for 3 days at 4°C before being transferred to a growth chamber at 28°C with a photoperiod consisting of 16-hr light (240 μmol photon/m^2/s) and 8-hr dark for 8 days.

3. Orobanche germination assay

Seeds of *O. minor* were surface-sterilized with a 5% bleach solution for 3 min before being thoroughly rinsed with 250 ml sterilized distilled water. For conditioning, the sterilized seeds were placed on moistened filter paper in sealed Petri dishes and incubated at 20°C in the dark for 7 days. Plants were then grown in a growth chamber at 28°C with a photoperiod consisting of 16-hr light (240 μmol photon/m^2/s) and 8-hr dark for 8 days.

Approximately 100 conditioned seeds were suspended in 300 ml sterilized water before plating onto 0.8% agar medium containing 1/2 Murashige and Skoog salts and 1.5% sucrose (w/v) in Agripots (Kirin Brewery Co., Tokyo, Japan) with or without chemicals. They were then solidified so that the seeds were evenly distributed in the medium. Ten tobacco plants were transformed onto the plates including the conditioned seeds prepared previously and placed in a growth chamber at 28°C with a photoperiod consisting of 16-hr light (240 μmol photon/m^2/s) and 8-hr dark. After incubation for 8 days, the number seeds that had germinated were counted.

4. Strigolactone analysis

Strigolactone analysis in rice was performed according to Ito *et al.*[16] Briefly, hydroponic culture media (10 ml) was extracted with ethyl acetate twice after adding d1-2′-*epi*-5DS (200 pg) as an internal standard. The organic layer was dried under nitrogen gas and dissolved in 1 ml ethyl acetate:*n*-hexane (15 : 85). The resuspended solutions were then loaded onto a Sep-Pak Silica 1 ml cartridge (Waters), washed twice with the same solution, eluted with ethyl acetate : *n*-hexane (35 : 65) three times and concentrated *in vacuo*. The roots were homogenized in acetone containing d1-2′-*epi*-5DS (200 pg). The filtrates were dried under nitrogen gas and dissolved in water. The solutions were extracted with ethyl acetate twice, dried and dissolved in 10% acetone. The extracts were then loaded onto Oasis HLB 3 ml cartridges (Waters), washed twice with water, eluted twice with acetone and dried under nitrogen gas. The concentrates were dissolved in 1 ml ethyl acetate : *n*-hexane (15 : 85) and loaded onto a Sep-Pak Silica 1 ml cartridge, washed, eluted and concentrated in the same way. The 2′-*epi*-5DS-containing fractions from culture media and roots were dissolved in 50% acetonitrile and subjected to LC/MS-MS analysis (ABI4000). Strigolactones in sorghum were analyzed according to the method reported by Yoneyama *et al.*[15]

Results and Discussion

Nine-*cis*-epoxycarotenoid dioxygenases (NCEDs) belongs to the family of carotenoid-cleaving dioxygenases (CCDs) and is the rate-limiting step in the abscisic acid (ABA) biosynthetic pathway of plants.[17] Abamine (ABM) is the first reported abscisic acid biosynthesis inhibitor that targets nine-*cis*-epoxycarotenoid dioxygenases (NCED),[12,13] and it has been suggested that abamine may also target other CCDs.[18] Interestingly, AbamineSG (ABMSG) was developed as an abscisic acid biosynthesis inhibitor based on the structural and functional characteristics of abamine, but was found to be a more specific and potent NCED inhibitor than abamine.[18] Recently Ropez-Reaz *et al.* reported that abamineSG reduced the level of strigolactone in tomato plants and concluded that this reduction may be due to the inhibition of abscisic acid biosynthesis;[19] however, the potency of abamine for the regulation of strigolactone production is yet to be determined. As CCD7 and CCD8 are involved in the strigolactone biosynthetic pathway and share similar functions and sequences with all CCDs, abamine might affect strigolactone biosynthesis by inhibiting CCD7, CCD8 or other related enzymes in addition to NCED, and has potential as a new scaffold for regulators of strigolactone biosynthesis. In this context, we evaluated the potency of abamine in the regulation of strigolactone production.

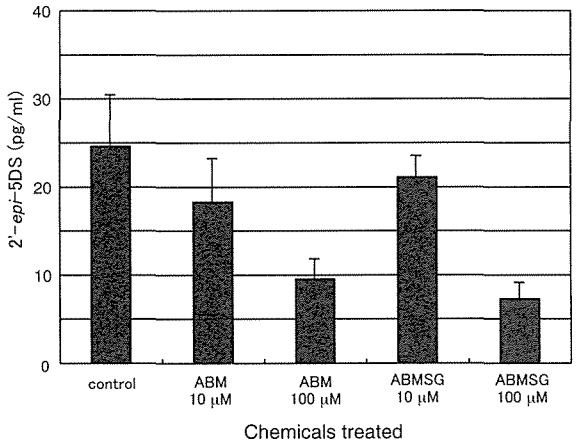

Fig. 2. 2′-*epi*-5DS levels in root exudates of chemicals-treated rice seedlings determined by LC-MS/MS.

1. Analyzing strigolactone levels in chemical-treated rice

The levels of 2′-*epi*-5-deoxystrigol (2′-*epi*-5-DS), one of the strigolactones identified in rice, were analyzed in root exudates to determine whether abamine regulates strigolactone production. As levels of strigolactones in root exudates of rice seedlings increase when inorganic phosphate is depleted in the culture medium,[4] the levels of 2′-*epi*-5-deoxystrigol (2′-*epi*-5-DS) were analyzed under conditions of phosphate deficiency.

Abamine was shown to reduce the levels of 2′-*epi*-5DS in root exudates to the same extent as abamineSG (Fig. 2). Considering that abamineSG is a more potent NCED inhibitor than abamine, these results suggested that abamine may inhibit enzymes other than NCED and reduce the levels of strigolactones in plants. Furthermore, 30 μM abamine completely inhibited the production of strigol in roots of rice, while 100 μM abamine inhibited only 60% of the production of 2′-*epi*-5DS (Fig. 2): the reason for this difference is not clear. Further investigations are required to determine the mechanism of how abamine regulates strigolactone production. As parasitic weeds are responsible for large-scale crop devastation throughout the world, we investigated the potential of abamine for regulating parasitic weed infestation.

We tested the potency of abamine in the regulation of strigolactone production in sorghum, which was chosen as it is an important crop in Africa and suffers from heavy infestation of *Striga*. In this experiment, 1 μM abamine was used and the levels of sorgomol and 5DS, not 2′-*epi*-5DS, were determined. Both the level of sorgomol and 5DS in root exudates remained unchanged (Fig. 3A). Surprisingly, however, the levels of both strigolactones were clearly reduced in the roots (Fig. 3B). These results show that abamine is effective in reducing the levels of strigolactones in sorghum and suggests that abamine treatment could possibly reduce the level of a variety of strigolactones in many plant species. As such, abamine could also be used to treat parasitic weed infestations.

2. Orobanche germination assay

As strigolactones are not only branching inhibitors but also seed germination stimulants for the parasitic weeds *Striga* spp. and *Orobanche* spp.,[1] a germination assay using *Orobanche minor* seeds was used to determine the ability of regulators of strigolactone production to control parasitic weed germination. In this test we used tobacco as the host plant because tobacco produces several kinds of strigolactones[20,21] and our bioassay system is a simple system to see the direct interaction between host plants and parasitic seeds. In agreement with the results of 2′-*epi*-5DS analysis in rice, the culture medium of abamine-treated tobacco seedlings was shown to have less germination-stimulating activity than control plants (Fig. 4). The reduced germination-stimulating activity of abamine-treated tobacco was further shown to

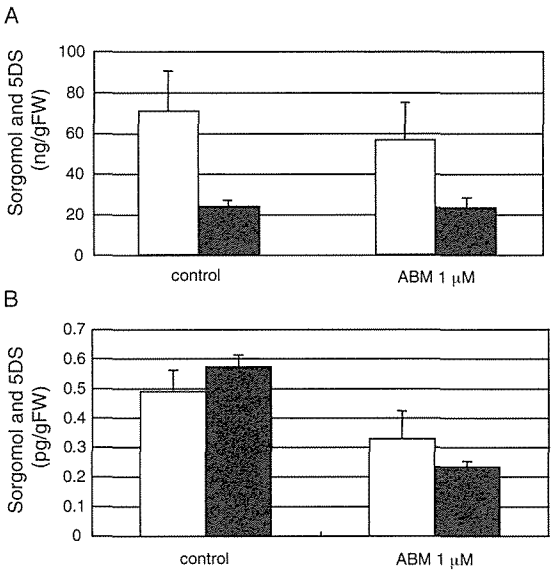

Fig. 3. Effect of abamine on strigolactone production in sorghum. The amounts of strigolactone sorgomol (open bar) and 5DS (closed bar) in root exudates (A) or roots (B) of abamine-treated sorghum seedlings determined by LC-MS/MS.

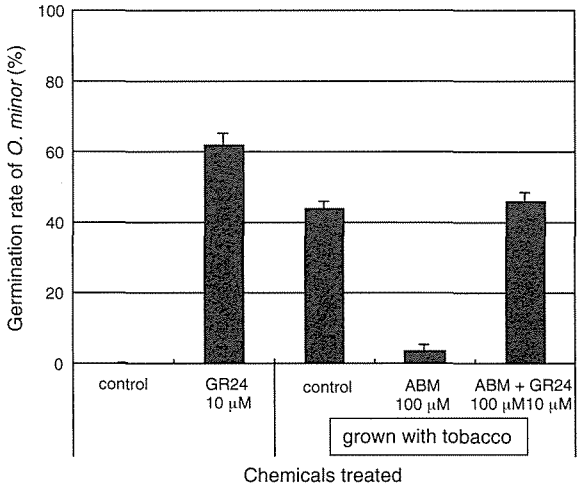

Fig. 4. Stimulation of germination of *O. minor* seeds grown with tobacco seedlings in agar culture medium.

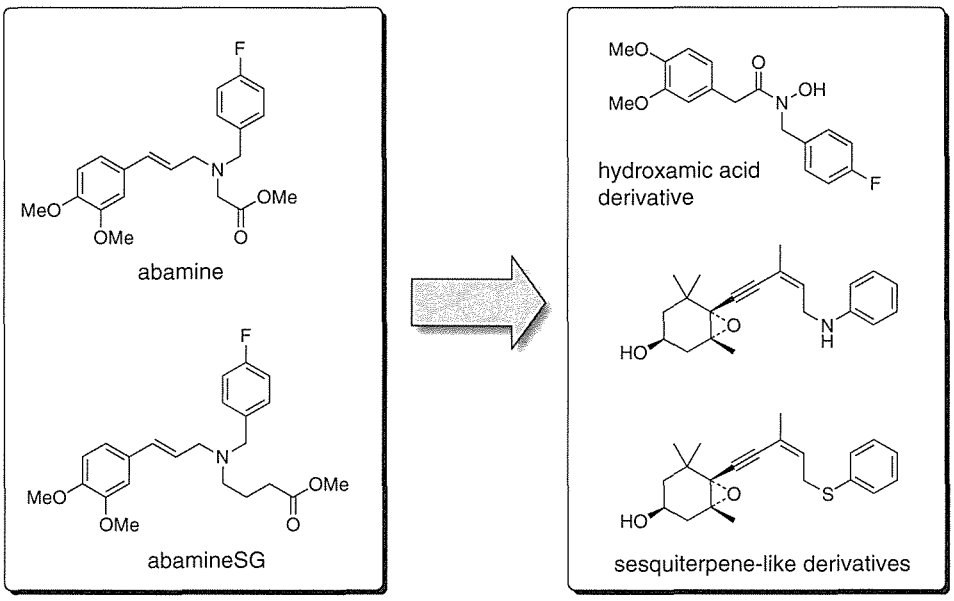

Fig. 5. CCD inhibitors designed from the structure of abamine and abamineSG.

be unrelated to the direct inhibition of *Orobanche* spp. germination, because the co-application of 10 μM GR24 with abamine-treated culture medium did not inhibit *Orobanche* germination (Fig. 4). We have not yet determined the levels of strigolactones in abamine-treated tobacco, but this study suggests that abamine may regulate the production of strigolactones in tobacco and inhibit *Orobanche* germination. These results demonstrate the potential for regulators of strigolactone production to be applied in control measures against parasitic weed germination. Germination of parasitic weeds is initiated by the recognition of strigolactone secreted by the roots of the host plant. Without this recognition, parasitic weeds cannot germinate and remain dormant in the soil. While several methods to control parasitic weeds have been suggested,[22] regulators of strigolactone production could be used as new tools for controlling parasitic weeds.

In this report we showed that abamine regulates strigolactone production, although the precise mechanism remains unclear. While CCD7 or CCD8 are potential abamine targets, *in vitro,* abamine did not inhibit the function of these enzymes. Future elucidation of the SL biosynthesis pathway, such as feedback regulation analyses of SL biosynthetic genes,[23] will be a great help for identifying the target site(s) of abamine.

The data obtained in this research indicate that the structure of abamine may be used as the basis for the development of specific and potent regulators of strigolactone production through the inhibition of its biosynthesis. Hydroxamic acid analogues were designed based on the structure of abamine and abamineSG, inhibit the activity of many CCDs, including AtCCD7, and increase the number of branches in inhibitor-treated *Arabidopsis* at 100 μM.[24] Likewise, sesquiterpene-like inhibitors of an NCED have been designed based on the structure of abamine, as reported by Boyd *et al.*[25] (Fig. 5). In conclusion, the data obtained in this study demonstrate the potential of abamine as a model for designing new inhibitors targeting several types of CCDs.

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ストリゴラクトン生産制御剤設計のための基本構造となるアバミン

北畑信隆，伊藤晋作，加藤敦隆，上野琴巳，
中野雄司，米山香織，米山弘一，浅見忠男

カロテノイド開裂酵素ファミリーに属するNCEDはアブシシン酸生合成経路における律速段階であり，アバミンはこの酵素を阻害する最初の薬剤として知られている．しかしアバミンはNCED以外の酵素を阻害することで有用性を示す可能性があることも報告されている．新しく知られるようになった植物ホルモンであるストリゴラクトンの生合成経路にはカロテノイド開裂酵素が関わっていることが明らかにされたために，われわれはアバミンがこの酵素を阻害する可能性を考えた．そこでアバミン処理した植物について，植物が生産するストリゴラクトン量変化について調べるとともに，宿主植物より滲出されるストリゴラクトンにより発芽促進される寄生雑草の発芽率変化をアバミン処理したタバコと共培養した場合について調べた．その結果アバミンはストリゴラクトン滲出量，寄生雑草発芽率ともに低下させる活性があることを明らかにした．