# $Ca^{2+}$ Regulators affect the Gravitropism and Ethylene Production Induced by Malformin A1 in Maize Root

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Received November 8, 2006 / Accepted December 18, 2006

Treatment of malformin A1 is known to increase ethylene production 130% at 4 hr and 56% at 8 hr after treatment in maize root compared to untreated plants. The ethylene production by malformin A1 was maximum level at 4 hr and slowly decreased up to 8 hr. Calcium ion regulators such as A23187 (calcium ionophore) and verapamil (calcium channel blocker) stimulated ethylene production. Treatment of both calcium ion regulators increased about 30% of ethylene production at 4 hr, and 20% at 8 hr. Both calcium ion regulators did not stimulate malformin A1-induced ethylene production at 4 hr as malformin A1 itself did. However, the treatment of calcium ion regulators with malformin A1 maintains the ethylene production for 8 hr. These results suggested that the proper concentration of calcium might need to confer the effect of malformin A1 on the ethylene production. Malformin A1 suppressed the gravitropic curvature of maize root about 58% at 4 hr and 42% at 8 hr compared to control plant. Verapamil inhibited the gravitropic curvature about 54% at 4 hr and 23% at 8 hr compared to control, respectively. But A23187 could not. In addition, verapamil showed more inhibition in malformin A1-induced gravitropic curvature than A23187 in malformin A1 induced. These data suggested that calcium ion regulators affect the malformin A1-induced ethylene production and gravitropic curvature, and give the evidence that calcium ion play an important role in gravitropic curvature in maize root.

**Key words** – malformin A1, calcium ion regulators, ethylene, gravitropic curvature, maize root

## Introduction

The malformins are fungal toxin produced by *Aspergillus niger* and have simple structure such as a cyclicpentapetide. It has been known that the malformins were composed of 9 kinds, and malformin A1 is one of the most active compounds in its physiological effects [8]. The crude extract of malformins showed several physiological effects such as reduction of shoot and root growth and leaf abscission [4]. Furthermore, Curtis and John [5] suggested that the action of malformins might be mediated with ethylene production.

We reported that malformin A1 regulated the ethylene production in mungbean hypocotyls [9] and in the root of maize [12]. According to these data, we found that the action of malformin A1 on the ethylene production was opposite in hypocotyls and roots. That is, higher concentration of malformin A1 inhibited ethylene production in mungbean hypocotyls, but not in roots of maize. And malformin A1 regulated the ethylene production via stimulation or inhibition of ACC oxidase in both organs.

Gravitropism in roots could be occurred via 3 steps such as the perception of gravity in root cap, signal transduction from root cap to the elongation zone and response in elongation zone to make curvature. It has been known that calcium ions play a key role as a second messenger in the step of signal transduction [3]. Calcium ions could be found in the statocyte in columella of root tip, and they showed differential distribution in the gravitropic response. Further, the calcium chelating agent, EDTA, regulated the gravitropic curvature. Calcium ion was correlated with the auxin transport [11]. Therefore, it is considered that calcium ions are one of the key factors during the gravitropism of maize root.

The regulation of gravitropic response in maize roots was one of the malformin A1's function. Kim et al. [10] suggested that malformin A1-pretreated roots showed the inhibition of positive gravitropic curvature due to the inhibition of the lateral transport of IAA across the roots from the upper side to the lower side because of an increased level of ethylene by the pretreatment of malformin A1.

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In the current study, we examined the effect of malformin A1 on the gravitropic curvature and ethylene production according to the role of calcium ions in the gravity signal transduction step of gravitropism using calcium ion regulators such as calcium ionophore and calcium channel blocker in the primary roots of maize.

## Material and Methods

### Plant Material

Maize kernels (*Zea mays* L., Golden cross Bantam 70) were soaked overnight in tap water, and germinated in the plastic tray with wet paper towel in vertical position. The trays were kept in a dark at 25℃. We used two-day old seedling with the primary roots that were about 15 to 20 mm long.

### Measurement of Gravitropic Curvature

Roots were pretreated with malformin A1 or other chemicals for 1 hr vertically in solution. The pretreated roots were transferred to the humidified chamber (>95% RH) and placed in the horizontal position to give a gravistimulation. We measured the pattern of gravitropic curvature for 8 hr, using a time-lapse video cassette recorder (Samsung, STLU-36D, Korea) equipped with a CCD camera (Samsung Aerospace, SAC-410NDX, Korea). The time-lapse series pictures were analyzed with an Image Tool Program.

### Measurement of Ethylene Production

Root segments, including the root tip (10 mm), were incubated in a 0.5 mL potassium phosphate buffer (0.05 M, pH 6.8) at 27℃ in the dark in 25-mL vials sealed with silicon stoppers. A 1-mL gas sample was withdrawn every 2 hr and analyzed with a gas chromatograph (Hewlett Packard, HP5890 series II, USA) that was equipped with an aluminum column and a flame-ionization detector at 180℃. The vials were held in the dark at 27℃ in a shaking incubator.

## Results and Discussion

### Effect of calcium regulators on the malformin A1-induced ethylene production

We reported that malformin A1 stimulated ethylene production in maize roots. Further, malformin A1 regulated the ACC oxidase (ACO) activity to stimulate ethylene production [12].

Based on these previous results, we examined the effect of calcium ions regulators, A23187 (calcium ionophore) and verapamil (calcium channel blocker), on the ethylene production in the presence of malformin A1. Malformin A1 stimulated ethylene production at 4 hr and 8 hr as we expected (Fig. 1). The stimulation rates of ethylene production by 10 uM malformin A1 were about 130% at 4 hr, and 56% at 8 hr (Fig. 1). This result means that the stimulation of ethylene production by malformin A1 was maximum level at 4 hr and slowly decreased until 8 hr. The stimulation of ethylene production by malformin A1 did not maintain for 8 hr. We have chosen the proper concentrations of calcium ions regulators to show their maximum effects in ethylene production (data not shown). A23187 and verapamil stimulated ethylene production at 1 nM and 0.1 mM concentration, respectively (Fig. 1). Treatment of A23187 and verapamil resulted in about 35% and 32% increase of ethylene production at 4 hr, respectively. Both calcium ion regulators showed about 20% stimulation at 8 hr (Fig. 1). Although the stimulation levels of ethylene production by A23187 and verapamil were lower than by malformin A1, we could not observe the significant difference in the stimulation of ethylene production at 4 hr and 8 hr as we could see in $10^{-5}$ M malformin A1 treatment.

One of possible explanation is that the action of malformin A1 might be different from the calcium ions regulators to stimulate the ethylene production. Previous report

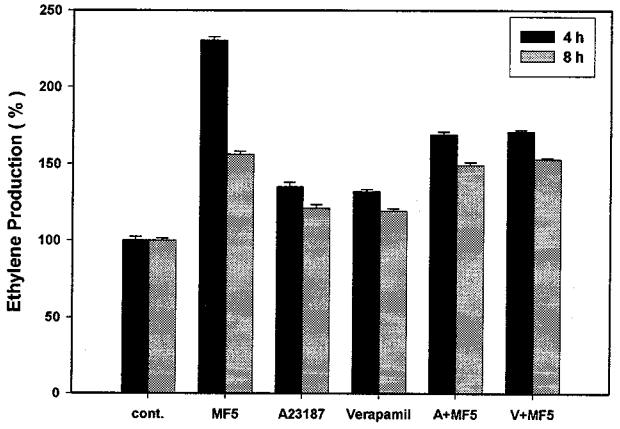


Fig. 1. Ethylene production by malformin A1 and calcium regulators, A23187 and verapamil at 4 hr and 8 hr. Ethylene productions were measured in the presence of $10^{-5}$ M malformin A1, 1 nM A23187 and 0.1 mM verapamil at 4 hr and 8 hr as described in Material and Methods. Values were calculated based on control as 100% (n=8). MF5; $10^{-5}$ M malformin A1A + MF5; A23187 + $10^{-5}$ M malformin A1, V + MF5; verapamil + $10^{-5}$ M malformin A1.

suggested that malformin A1 activated the ACC oxidase to increase the ethylene production [12]. However, calcium ions regulators might increase the ethylene production through another system such as second messenger system, not through ACC oxidase activity. Another explanation is that calcium ions regulators might act on ACC oxidase as malformin A1 did. However, their action on ACC oxidase might maintain for 8 hr unlike malformin A1. Gallardo et al. [6] reported that calcium ions were involved in the regulation of ACC oxidase activity from seed embryonic axes. Therefore, we need to check the action of calcium ions regulators on ACC oxidase activity in time course to understand the role of calcium ions in ethylene production in maize root. From these results, we suggested that calcium ions might play a role in regulation of ethylene production in maize roots. In future, we need further experiments to figure out the role of calcium ions in malformin A1-induced ethylene production in roots.

To understand the role of calcium ions regulators in malformin A1-induced ethylene production, we measured the ethylene production in the presence of malformin A1 with 1 nM A23187 or 0.1 mM verapamil (Fig. 1). Treatment of A23187 and verapamil did not increase the malformin A1 induced-ethylene production at 4 hr. However, calcium ion regulators maintained the malformin A1-induced ethylene production up to 8 hr (Fig. 1). We could not observe the significant difference of ethylene production at 4 hr and at 8 hr by treatment of calcium ion. These results suggested that calcium ions regulators maintained the stimulation of ethylene production for 8 hr, although we do not know the exact mechanism yet.

Previously we examined the effect of calcium ion on the ethylene production in maize root. However, we could not find any consistent effect of calcium ions in ethylene production according to the various calcium ion concentrations (data not shown). These results suggested that calcium ion concentration in the maize root cell might be enough to regulate the ethylene production in this system.

Although we expected that calcium ionophore, A23187 and calcium channel blocker, verapamil showed opposite effect on the malformin A1-induced ethylene production, both of calcium ion regulators showed the same effect on ethylene production with malformin A1. A23187 causes opening of the calcium channel and resulted in the increase of the intracellular calcium ion concentration. Verapamil inhibits the activity of calcium channel, and resulted in the decrease of intracellular calcium ion concentrations. A23187 and verapamil showed the same effect in the malformin A1-induced ethylene production at 4 hr and 8 hr. The possible explanation is that the proper level of calcium ion might be very important role in the ethylene production in the presence of malformin A1, and malformin A1 might act on physiological effect via the second messenger system in maize roots. And the action of malformin A1 on ethylene production might need the regulation of calcium ion in maize root. Further experiments will focus on the action of calcium ion regulators on two regulation enzymes activity of ethylene production such as ACC oxidase and ACC synthase.

### Effect of calcium regulators on the malformin A1-induced gravitropic curvature

It has been known that malformin A1 regulated gravitropic curvature via the regulation of IAA lateral transport by malformin A1-induced ethylene production [10]. Gravitropic curvature in plants is the result of asymmetric growth. To elicit the gravitropic curvature, the gravity signal should be perceived in columella cell of root tip. The signal moves to the elongation zone to show the gravitropic response. It has been known that gravity signals are mediated through calcium ions in cytosol, which is normally maintained at very low concentrations such as a few micromolar levels by calcium transport systems in membrane [1]. Further calcium ions also have a role in the auxin redistribution as a second messenger and lead to the gravitropic response [7]. In this study the pretreatment of malformin A1 suppressed the gravitropic curvature about 58% and 42% of control at 4 hr and 8 hr, respectively (Fig. 2). We also examined the role of calcium ions in malformin A1-induced gravitropic curvature by treatments of two kinds of calcium ions regulators.

Pretreatment of A23187 exhibited about the same level of gravitropic curvature by the malformin A1 compared to the control (Fig. 3A). The maximum curvature of by A23187 pretreatments was about 60 degree at 8 hr after treatment.

In addition, the inhibitions of gravitropic curvatures by A23187 with malformin A1 were 73% at 4 hr and 51% at 8 hr compared to the control, respectively (Fig. 3A). These inhibition rates were 10% more than by $10^{-5}$ M malformin A1 at 4 hr and 8 hr (Fig. 2). These results suggested that A23187, calcium ionophore, somewhat inhibited the malformin A1-induced gravitropic curvature.

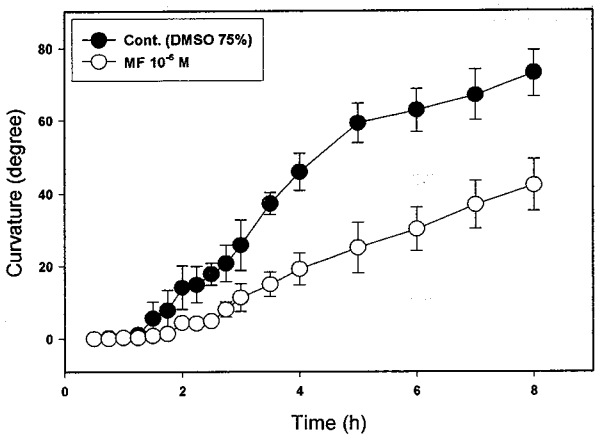


Fig. 2. Gravitropic curvature induced by malformin A1 for 8 hr. Roots were pretreated with $10^{-5}$ M malformin A1 for 1 hr vertically in solution. The pretreated roots were transferred to the humidified chamber (>95% RH) and placed in the horizontal position. Gravitropic curvatures were measured as described in Material and Methods. Symbols are mean value (n = 15).

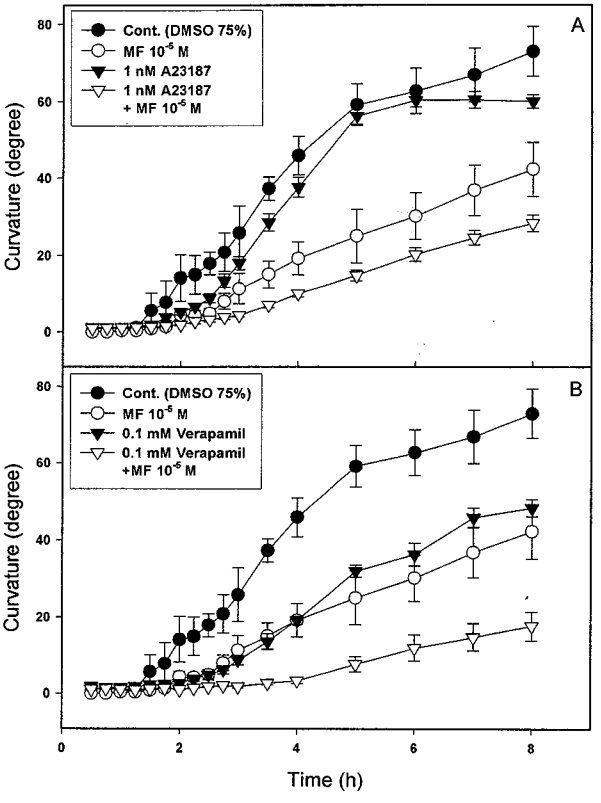


Fig. 3. Gravitropic curvature induced by malformin A1 with calcium ion regulators for 8 hr. Roots were pretreated with A23187 (A) and with verapamil (B) in the presence of $10^{-5}$ M malformin A1 for 1 hr vertically. The pretreated roots were transferred to the humidified chamber (>95% RH) and placed in the horizontal position. Gravitropic curvatures were measured as described in Material and Methods. Symbols are mean value (n = 15).

Unlike calcium ionophore, A23187, 0.1 mM verapamil, calcium ions channel blocker, inhibited the gravitropic curvature about 54% and 23% of the control at 4 hr and 8 hr, respectively (Fig. 3B). Verapamil inhibited the $10^{-5}$ M malformin A1-induced gravitropic curvature about 92% at 4 hr and 72% at 8 hr, respectively. These results suggested that verapamil exhibited more inhibition than A23187 in the malformin A1-induced gravitropic curvature. Calcium ions have been suggested to be an important factor for eliciting gravitropic response [2,3]. Especially, cytoplasmic free calcium ions concentrations were part of the gravity transduction mechanism in young Arabidopsis seedlings [13]. And external calcium ions in roots transported from upper to lower side in horizontal roots to produce the gravitropism [14].

According to these reports, we suggested that verapamil, calcium ions channel blocker, might inhibit the calcium transport from endoplasmic reticulum in the columella cell of the root tip, inducing the same concentrations of cytoplasmic calcium ions between upper and lower side in horizontal root. And another possibility is that verapamil might inhibit the transport of external calcium ions between upper and lower side in horizontal roots. This equal distribution of cytoplasmic free calcium ions or external calcium ions between upper and lower side by verapamil might inhibit the gravitropic curvature.

Unlikely verapamil, A23187, calcium ionophore, might act on the opening of the calcium channel, inducing an asymmetrical calcium ions distribution between upper and lower side in horizontal root by the gravity. This asymmetrical calcium ions distribution by gravity resulted in the gravitropic curvature in horizontal root.

From these results, we concluded that verapamil and A23187 inhibited the malformin A1-induced gravitropic curvature, and calcium ions were important role in the gravitropic curvature.

## Acknowledgment

This work was supported by a grant from 2004 Research Fund of Andong National University

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## 초록 : 옥수수 뿌리에서 칼슘 이온 조절제가 malformin A1에 의해 유도된 굴중성과 에틸렌 생합성에 미치는 영향

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malformin A1은 옥수수 뿌리에서 대조구와 비교하여 에틸렌 생성을 4시간에는 130%, 8시간에는 56%를 각각 촉진하였다. 에틸렌 생성량이 4시간을 최대로 하여 8시간까지 서서히 증가율이 감소하였다. 칼슘 이온 조절제인 A23187 (calcium ionophore)과 verapamil (calcium channel blocker)을 각각 단독으로 처리한 경우 모두 대조구와 비교하여 4시간에는 30%, 8시간에는 20% 정도로 에틸렌 생성을 촉진하였다. 칼슘 이온 조절제가 malformin A1에 의해 유도된 에틸렌 생성에 미치는 영향을 조사한 결과 4시간에는 malformin A1만 처리하였을 경우에 관찰되는 수준만큼 촉진되지 않았지만 칼슘 이온 조절제의 촉진 효과는 8시간까지 유지되었다. 이러한 결과는 malformin A1이 에틸렌 생성을 촉진하기 위하여 적절한 칼슘 농도가 필요할 가능성을 제시하고 있다. Malformin A1은 굴중성 굴곡 반응을 대조구와 비교하여 4시간과 8시간에 각각 58%와 42% 정도 억제하였다. A23187과 달리, verapamil은 굴중성 굴곡반응을 4시간과 8시간에 대조구와 비교하여 각각 54%와 23%를 억제하였다. 더 나아가 verapamil은 malformin A1에 의해 유도된 굴중성 굴곡 반응을 A23187보다 더 억제하였다. 이러한 결과는 칼슘이온 조절제가 malformin A1에 의해 유도된 옥수수 뿌리의 굴중성 반응에 영향을 주며, 칼슘 이온이 굴중성 반응에 중요한 역할을 한다는 증거를 제시한다.