Ethylene Action Inhibitors Suppressed Clomeprop Metabolite [2-(2, 4-Dichloro-*m*-Tolyloxy) Propionic Acid]-Induced Electrolyte Leakage from Radish Roots

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Abstract: Involvement of ethylene was investigated in the physiological mechanism of clomeprop [2-(2, 4-dichloro-*m*-tolyloxy) propionanilide]- or its hydrolytic metabolite DMPA [2-(2, 4-dichloro-*m*-tolyloxy) propionic acid]-induced electrolyte leakage from radish roots. The treatment of DMPA to radish roots caused increase of electrolyte leakage from the roots, suppression of the root growth and stimulation of ethylene production. Although ethylene action inhibitors, 2,5-norbornadiene (NBD) and *cis*-propenylphosphonic acid (PPOH), did not suppress ethylene evolution from the DMPA-treated radish roots for the first 24 hrs, these compounds suppressed the electrolyte leakage and to some extent restored the growth of lateral roots. Evan's blue dye tests revealed that death of the root cells occurred 12 hr after the DMPA treatment. This indicated that the increase in electrolyte leakage preceded the death of the root cells. These results suggest that the ethylene induced by DMPA is attributable to the leakage of electrolyte from the radish roots.

Key words: clomeprop, ethylene, electrolyte

leakage, PPOH, NBD

Introduction

An auxin-type herbicide, clomeprop [2-(2, 4-dichloro-*m*-tolyloxy) propionanilide] is used for controlling broad-leaved weeds in rice fields⁹⁾. Clomeprop is hydrolyzed at the acylamide bond by plant arylacylamidase and converted to a hydrolytic metabolite 2-(2, 4-dichloro-*m*-tolyloxy) propionic acid (DMPA)¹⁸⁾. In a previous study, we proposed that clomeprop itself has no auxinic or herbicidal activity and that these activities are derived from DMPA^{11,12)}.

The auxin-type herbicides 2, 4-D, picloram and dicamba are known to increase ethylene biosynthesis in susceptible plants by inducing 1-aminocyclopropane-1-carboxylic acid (ACC) synthase^{4~6,15)}. Grossmann and Kwiatkowski³⁾ reported that there was a positive correlation between the phytotoxicity of auxin-type herbicides and ethylene production. However, little is known about the role of ethylene in the auxin-type herbicide induced death of susceptible plants⁷⁾. This study was conducted to examine the involvement of ethylene in clomeprop- and DMPA-induced electrolyte leakage from radish roots. Two competitive ethylene binding inhibitors, 2,5-norbornadiene (NBD) and *cis*-propenylphosphonic acid (PPOH), were utilized to inhibit the action of ethylene.

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Materials and Methods

Plant materials

Seeds of a radish (*Raphanus sativus* L. var. *hortensis* cv. Shogoin) were germinated on vermiculite moistened with distilled water and grown for 3 days in a growth chamber at 30/25°C for day (500 $\mu\text{E}/\text{m}^2 \cdot \text{s}$, 12 hr)/night, respectively.

Electrolyte leakage measurement

Roots of the seedlings were soaked for 90 min in 5 mM potassium phosphate buffer (pH 5.5) containing 5×10^{-5} M DMPA with 0.5% dimethyl sulfoxide (DMSO) and 0.1% Tween 20 and then washed with distilled water. The effect of ethylene on the DMPA-caused electrolyte leakage was examined by NBD or PPOH treatment. For the post-treatment of NBD, ten DMPA-treated seedlings were transferred to small glass beakers each containing 15 ml of distilled water. Two beakers with the plants were placed in a 1,000 ml beaker, tightly sealed with Parafilm™, and 20 μl of liquid NBD was injected into the beaker with a syringe. For the post-treatment of PPOH, the roots of DMPA-treated seedlings were soaked in 15 ml of 1×10^{-7} M PPOH solution in small beakers. The beakers were placed in a 1,000 ml beaker and sealed in the same manner as for NBD treatment. After treatment by the inhibitors, plants were placed in a growth chamber. To determine electrolyte leakage, conductivity in the root-bathing solution in the beakers was measured with a conductivity meter (Model AOC-10, DENKI KAGAKU Co., LTD). Number of lateral roots between 0 to 2 cm from the base of the main root was also measured.

Ethylene evolution

1. Ethylene evolution from whole plants

After the DMPA-, DMPA plus NBD- or DMPA plus PPOH-treatment, 1 ml of gas

sample was taken from each 1,000 ml beaker by a syringe. Ethylene evolution was analyzed by the method described^{10,11}.

2. Ethylene evolution from radish roots

Root segments (2 cm) were prepared from DMPA-treated plants by cutting to 2 cm below the base of the main roots. Ten root segments were placed in a 20 ml vial containing 1 ml of distilled water. The vial was tightly sealed with Parafilm™ and 1 μl of liquid NBD was injected into the vial. For PPOH treatment, other 10 main root segments of the DMPA-treated seedlings were immersed with 1 ml of 1×10^{-7} M PPOH solution and sealed. One ml gas was sampled from each vial and its ethylene content was analyzed by GC.

All treatments were performed in triplicate and repeated at least twice.

Results and Discussion

Effect of NBD or PPOH on clomeprop -or DMPA-induced electrolyte leakage and growth inhibition

Although root-treated clomeprop and DMPA both significantly increased the electrolyte leakage from radish roots, DMPA was found to be the more active (Fig. 1), causing increase of electrolyte leakage and being detectable at 2 hr after the treatment. In the bathing solution, a large amount of potassium ion was detected (data not shown). We^{11,12} previously reported that DMPA induced ethylene accumulation in radish plants immediately after it was treated. Application of NBD arrested the DMPA-induced ion leakage 12 hr after it had been treated (Fig. 2). NBD itself, however, has no effect on electrolyte leakage from radish roots. PPOH, developed by Kyowa Hakko Kogyo Co., Ltd., is a new highly water-soluble inhibitor of ethylene actions. The mode of PPOH action is assumed to be a direct bind-

Fig. 1. Electrolyte leakage from clomeprop or DMPA-treated radish roots.

Radish roots were immersed with 1×10^{-4} M clomeprop or DMPA for 12 hr.

Fig. 2. Effect of NBD on electrolyte leakage from DMPA-treated radish roots.

Radish roots were soaked with 5×10^{-5} M DMPA for 90 min and then treated with NBD.

Fig. 3. Effect of PPOH on electrolyte leakage from DMPA-treated radish roots.

Radish roots were soaked with 5×10^{-5} M DMPA for 90 min and then treated with PPOH.

ing to the ethylene receptor in a competitive way similar to NBD^{8,14}). Post-treated PPOH delayed DMPA-induced ion leakage (Fig. 3). PPOH alone did not induce the leakage as well as NBD. The suppression of electrolyte leakage by PPOH was observed to be earlier than that by NBD; this may be

because of the direct absorption of PPOH from roots. These results suggest that the DMPA-enhanced electrolyte leakage from radish roots is attributable to DMPA-induced ethylene.

DMPA strongly inhibited root development (Figs. 4 and 5). By NBD or PPOH alone, the growth of their lateral roots was slightly suppressed. However, the number of lateral roots in DMPA treated plants was restored to some extent by addition of NBD or PPOH. The number of lateral roots per plant and electrolyte leakage were negatively correlated. However, the electrolyte leakage occurred prior to the growth inhibition of the radish roots. These results suggest that the growth inhibition of the lateral roots is also caused by DMPA-induced ethylene.

To evaluate the viability of DMPA-treated root cells, Evan's blue dye test of the roots was conducted. Blue dyed root cells were observed more than 12 hr after treatment (data not shown), suggesting that death of the root cells began 12 hr after the treatment. It was believed, therefore, that the DMPA-in-

Fig. 4. Effect of NBD on DMPA-induced inhibition of emergence of lateral roots. Radish roots were immersed with 5×10^{-5} M DMPA for 90 min.

Fig. 5. Effect of PPOH on DMPA-induced inhibition of emergence of lateral roots. Radish roots were soaked with 5×10^{-5} M DMPA for 90 min.

duced electrolyte leakage preceded the death of these cells. The rapid increase of the conductivity in the solution may not be caused by the root cell death.

Effect of NBD or PPOH on DMPA-induced ethylene production

Figure 6 shows the effect of NBD on DMPA-induced ethylene evolution from whole plants and excised roots. There was little difference in the amount of ethylene evolution from whole plants between DMPA- and DMPA plus NBD-treated radish seedling until 24 hr after treatment. Ethylene evolution from excised root of DMPA plus NBD-treated radish was greater than that from DMPA-treated roots for the first 24 hrs, however, it then gradually decreased up to 48 hr. There was no difference in ethylene evolution from whole plants between DMPA- and DMPA plus PPOH-treated radish seedlings (Fig. 7). The amount of ethylene from excised roots of DMPA-treated radish was slightly increased by PPOH for the first 24 hrs, but the evolution was gradually suppressed after 24 hr. At 72 hr after treatment, the

amount of ethylene evolution was, significantly less in PPOH-treated seedlings (data not shown). These data confirmed that neither NBD nor PPOH suppressed DMPA-induced ethylene production until at least 24 hr after treatment. However, these ethylene action inhibitors brought restoration of DMPA-caused damage within 24 hr as shown previously. Decrease of ethylene which was observed in whole plant and excised roots after 24 hr may be due to suppression of an autocatalytic effect to promote ethylene production by the evolved ethylene¹⁴⁾ or inhibition of ACC synthase and/or ACC oxidase by NBD and PPOH¹⁷⁾.

Grossmann and Kwiatkowski³⁾ reported a positive correlation between the phytotoxicity of auxin-type herbicides, such as quinclorac and 2, 4-D, and the amount of ethylene production. However, it is not yet clear whether ethylene is directly involved in the action of auxin-type herbicides. Tittle *et al.*¹³⁾ and Grossmann and Kwiatkowski^{2,3)} proposed that cyanide, a coproduct of herbicide-induced ethylene biosynthesis, might be

Fig. 6. Effect of NBD on DMPA-induced ethylene production. Radish roots were immersed with 5×10^{-5} M DMPA for 90 min.

Fig. 7. Effect of PPOH on DMPA-induced ethylene production. Radish roots were soaked with 5×10^{-5} M DMPA for 90 min.

responsible for the phytotoxicity. More cyanide was believed to be formed in DMPA plus NBD- and DMPA plus PPOH-treated radish roots than in roots treated with DMPA alone for at least 24 hr after application because cyanide is produced in stoichiometrically equivalent amounts to ethylene during its biosynthesis. However, post-treatment of NBD and PPOH suppressed the electrolyte leakage and restored the growth of lateral

roots. These results suggest that ethylene plays a more important role in the electrolyte leakage from radish roots than cyanide. In order to confirm this hypothesis, it is necessary to investigate cyanide content and β -cyanoalanine synthase (β -CAS) activity in radish roots, which is a key enzyme to detoxify cyanide^{1,16}. Our results strongly suggest, however, that clomeprop- or DMPA-induced ethylene is attributable to the electrolyte

leakage from radish roots. Destruction of membrane permeability may result in inhibiting the growth of lateral roots.

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クロメプロップ代謝物 (DMPA) によるダイコン幼根からの電解質漏出のエチレン作用阻害剤による軽減

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摘 要

著者らは、クロメプロップ自身はオーキシシン活性を示さず、植物体内でその加水分解物である DMPA に分解された後に初めてオーキシシン結合蛋白質に認識され、オーキシシン活性を示すこと、さらにその DMPA が ACC synthase を誘導することにより蓄積するエチレンによって、ダイコンの形態的变化を引き起こしている可能性が高いことを既に報告した。

本研究では、クロメプロップの更に詳しい作用メカニズムを調べることを目的とし、クロメプロップの活性本体であると考えられている、DMPA の処理後に引き起こされるダイコン幼根からの電解質漏出と生育抑制にエチレンが関与しているかについての検討を行った。

(1) クロメプロップ、DMPA 処理により処理直後から電解質漏出が見られた (Fig. 1)。しかし、エチレン作用の阻害剤である NBD や PPOH の処理によりその漏出は抑えられた (Fig. 2, 3)。また、

DMPA による側根の発生阻害も NBD, PPOH によって抑えられた (Fig. 4, 5)。

(2) 植物体全体からのエチレン放出量と切断根からのエチレン放出量は DMPA 処理直後から顕著に増加した。また、切断根からのエチレン放出量では、処理 24 時間までは NBD や PPOH を後処理することによりわずかに増加し、減少は見られなかった (Fig. 6, 7)。

これらの結果は、NBD や PPOH によりエチレン作用を直接的に抑制すると、2 次的なエチレン生成抑制が起こらない段階で DMPA 処理により現れた電解質漏出や側根の発生阻害が抑えられたことを示している。

(3) エバンス・ブルー染色を行った結果、DMPA 処理後の細胞は 12 時間以降に青色に染まり始めたことから、処理直後から 12 時間までに引き起こされる電解質漏出は細胞死によって引き起こされるものではないと推察される。

以上の結果より、クロメプロップ、DMPA が ACC synthase 活性を増大することによって蓄積するエチレンが両薬剤処理後に引き起こされる電解質漏出に関与しており、その膜の完全性損失の結果として側根の生育が阻害されている可能性が強く示唆された。

キーワード: クロメプロップ, エチレン, 電解質漏出, PPOH, NBD

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