METHYL JASMONATE INCREASES ACTIVITIES OF ALDEHYDE OXIDASE AND AUXIN CONTENTS IN MAIZE (*Zea mays*)

D. Han[1], Y. Oh[1,2] and W. J. Park[1,*]

[1] Department of Molecular Biology & Institute of Nanosensor and Biotechnology, Dankook University, Yongin-si, Gyeonggi-do 448-701, South Korea
[2] Department of Molecular Ecology, Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Straße 8, D-07745 Jena, Germany
[*]Corresponding author's e-mail: parkwj@dku.edu

ABSTRACT

Methyl jasmonate (MeJA) affects diverse developmental processes, although it has been extensively investigated as a defence hormone. MeJA likely controls plant development through cooperation with other hormones, especially with auxin. In this study, we found that exogenously applied MeJA increased aldehyde oxidase (AO) activities in the roots and leaves of maize (*Zea mays*). In addition, MeJA increased the level of free IAA in leaves, suggesting that MeJA increased the contents of free IAA by regulating AO activities.

Key words: Aldehyde oxidase; indole-3-acetic acid (IAA); methyl jasmonate; maize (*Zea mays*).

INTRODUCTION

Jasmonates have been intensively investigated as plant defence hormones; however, they show diverse effects on plant development (Wasternack 2007). Adventitious root formation (Fattorini *et al.* 2009) and inhibition of root elongation are the examples of jasmonate-promoted processes (Staswick *et al.* 1992). Jasmonates control plant development may be through interacting with other hormones during signal transduction or affecting homeostasis of other hormones (Hoffmann *et al.* 2011). Because jasmonates affect plant developmental processes, e.g., root formation, which are primarily regulated by auxin (Overvoorde *et al.* 2010), the interaction between jasmonates and auxin has attracted a great deal of attention. Cuéllar Pérez and Goossens (2013) pointed out the similarity between the signalling of jasmonates and auxin and discussed their interaction in a recent review. On the other hand, evidences on homeostatic interaction between jasmonates and auxin are still available only a few. Methyl jasmonate (MeJA) stimulates the expression of *ASA1*, whose product catalyzes the initial step of auxin biosynthesis (Sun *et al.* 2009), indicating the stimulation of auxin biosynthesis by jasmonates in *Arabidopsis*. This idea is further supported by Hentrich *et al.* (2013) showing that jasmonic acid increased the expression of *YUCCA8* and *YUCCA9* and the level of IAA in parallel. During the pathogeneses of the clubroot disease in Chinese cabbage, nitrilase activity is induced by jasmonic acid (Grsic *et al.* 1999). However, similar evidences on the influence of jasmonates on auxin biosynthesis are still unavailable for monocot plants. Therefore, we focused on the effects of methyl jasmonate on auxin contents in a monocot plant,

maize, in which auxin biosynthesis is either Trp-independent (Wright *et al.* 1991) or Trp-dependent (Glawischnig *et al.* 2000) under the the given physiological conditions. The final steps of Trp-dependent auxin biosynthesis are branched and catalyzed either by nitrilase, EC 3.5.5.1 (Park *et al.* 2003) or aldehyde oxidase, EC 1.2.3.1 (Kriechbaumer *et al.* 2006). In maize, there are two aldehyde oxidase genes, *ZmAO1* and *ZmAO2* (Koshiba *et al.* 1996). Aldehyde oxidase has been suggested to play roles in the biosynthesis of auxin and abscisic acid (ABA). We investigated the effect of MeJA on the activities of aldehyde oxidase and measured the level of auxin in maize.

MATERIALS AND METHODS

Plant materials and physiological treatments: Maize (*Zea mays* cv. Golden Cross Bantum 70) seeds were soaked in distilled water and then grown for 3 d at 26°C in paper rolls following Hetz *et al.* (1996). For root samples, MeJA was included in the growth solution. For leaf samples, maize seeds were planted on soil and grown for 10 d. Leaf discs (6 mm in diameter) were then isolated and floated on 5 mL of buffer (5 mM potassium phosphate, pH 6.8) for 18 h in the absence or presence of the indicated concentrations of MeJA.

Detection of aldehyde oxidase activities: The aldehyde oxidase activities were examined by zymograms (Seo *et al.* 1998). Briefly, proteins were extracted and resolved by 7.5% native PAGE. The gels were then soaked and equilibrated in a developing buffer (100 mM potassium phosphate, pH 7.5, 0.1 mM phenazine methosulfate, 0.4 mM 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyletrazolium bromide), after which they were transferred to a fresh

buffer containing 1 mM hypoxanthine as a substrate for xanthine dehydrogenase (XD). XD activity was used as a control in this work. After the development of XD activities, 1 mM indole-3-carboxyaldehyde was added to the solution as the substrate for aldehyde oxidase.

Quantification of IAA: IAA was quantified by GC-MS as previously reported (Seo *et al.* 2009). Briefly, the methanol extracts of leaf discs were concentrated, diluted in distilled water and then pre-separated using a C18 cartridge (Waters) at acidic pH. The samples were then purified by HPLC (600E, Waters; Apollo C18, 5 μm RP column, Alltech) using a system equipped with a fluorescence detector (486, Waters). The peak containing IAA was subsequently collected, concentrated and subjected to GC-MS analysis (CP 3000, Saturn 2200, Varian) after derivatization with (trimethylsilyl) diazomethane. For quantification, known amounts of [^{2}H$_5$]-IAA (Cambridge Isotopes) were added as the internal standard before the methanol extraction of the samples.

RESULTS AND DISCUSSION

During maize growth, we applied various concentrations (10^{-5} – 10^{-3} M) of methyl jasmonate (MeJA) to the incubation solution through the paper rolls for 3 d and then extracted the total protein from the root tip (0 – 1 cm). Maize root revealed aldehyde oxidase (AO) activities in the zymogram test (Fig. 1, lower bands). The signals appeared in the zymogram are the precipitation of insoluble colourful products that are converted from the specific substrates by the AO enzyme resolved on a gel. Therefore, the zymogram bands show the AO activities. The AO activities increased proportional to concentrations of MeJA, when the concentrations were 10^{-4} M or higher. In the same protein extract from the primary root tip, the activity of xanthine dehydrogenase was not heavily influenced by MeJA (Fig. 1, upper bands).

We also tested the effect of MeJA on AO activities in maize leaves (Fig. 2). Leaves of 10-d-old maize seedlings were harvested and discs (6 mm in diameter) were isolated using a paper punch. The leaf discs were then floated on 5 mL of buffer solution (5 mM potassium phosphate, pH 6.8) in the absence or presence of 1 mM MeJA, at which concentration AO activities increased in the primary root tips. MeJA increased the AO activity also in the maize leaf discs (Fig. 2A, lower),

while the xanthine dehydrogenase activity remained unchanged (Fig. 2A, upper). Quantification of the band intensity of the zymogram revealed that MeJA increased the AO activity to about 150% of that of the control (Fig. 2B). These results show that MeJA increased the activity of maize AO in both the roots and leaves. However, the molecular mechanism responsible for the increased AO activity by MeJA is still unclear. Induction of AO gene expression was not consistent and differential dependent on the tissues used for RT-PCR analyses (data not shown).

The functions of AO were verified in ABA biosynthesis (Seki *et al.* 2007), and suggested in auxin biosynthesis (Krichbaumer *et al.* 2006). We examined possible correlations between the increased AO activities and the level of free IAA by MeJA in the leaf discs (Fig. 2C). After incubation in the presence or absence of 10^{-3} M MeJA, IAA was extracted from the leaf discs with methanol and quantified by GC-MS using deuterium-labeled IAA as the internal standard. Comparison of the fragmentation peaks from the endogenous and standard IAA revealed that MeJA increased the free IAA contents by about 1.4 times.

The observed increase in the activities of AO and free IAA contents by MeJA suggests that AO may play a role in auxin biosynthesis and that MeJA is one of the positive regulators of AO. This is the first report that MeJA positively regulates auxin contents and AO activities in monocot plants. In dicot plants, a positive influences of MeJA on auxin biosynthesis have been reported (Sun *et al.* 2009; Hentrich *et al.* 2013) including the induction of anthranilate synthase 1-coding gene (*ASA1*) by MeJA in *Arabidopsis* (Sun *et al.* 2009). While ASA1 is involved in the very early stage of auxin biosynthesis, even before tryptophan synthesis, AO catalyzes the final step of auxin biosynthesis. Interestingly, MeJA inhibits the expression of nitrilase, which is the key enzyme of another branch of Trp-dependent auxin biosynthetic pathway (Kriechbaumer *et al.* 2007). This suggests that MeJA can play important roles in the regulation of auxin biosynthesis at diverse steps of auxin biosynthesis even in a pathway-specific manner.

The specific function of MeJA-induced auxin is still unclear. Accordingly, it is important to further study, whether the IAA produced by MeJA only plays a role in stress-related responses or acts as a general regulator of plant development.
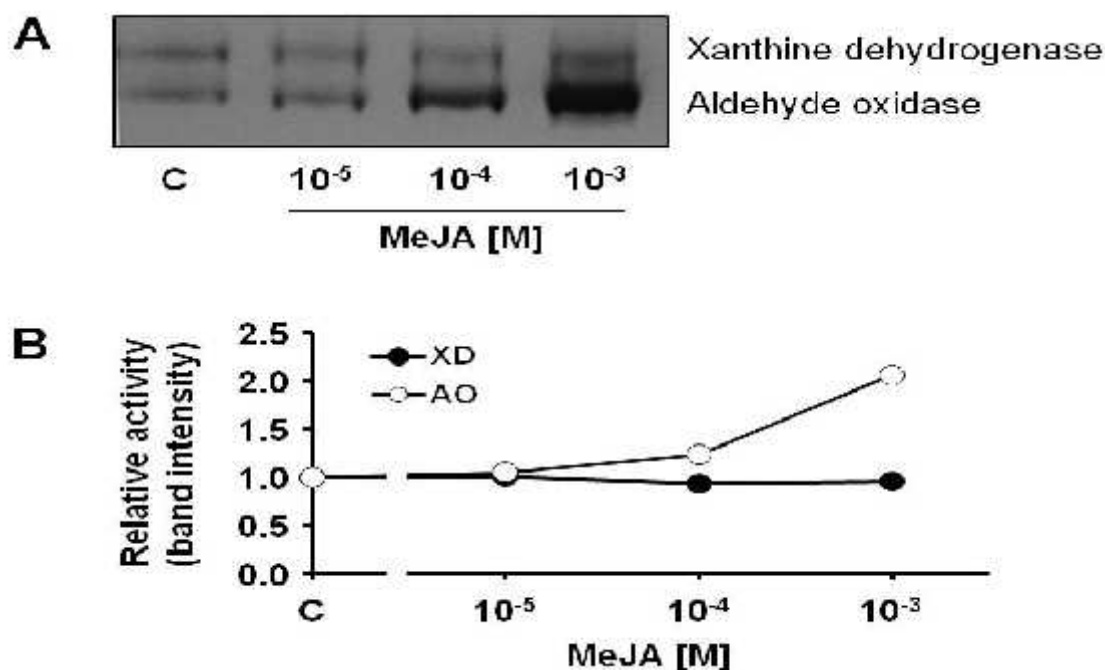
Figure 1. Effects of methyl jasmonate (MeJA) on the activity of aldehyde oxidase in the primary root tip of maize.
A. Zymogram of aldehyde oxidase obtained with 1 mM indole-3-carboxyaldehyde as a substrate after protein extract was resolved by 7.5% native PAGE. Xanthine dehydrogenase activity was developed with 1 mM hypoxanthine and used as the control. B. Quantified bands indicating the aldehyde oxidase activities corresponding to the increasing concentrations of MeJA. Band intensity was quantified using the TINA 2.0 software.
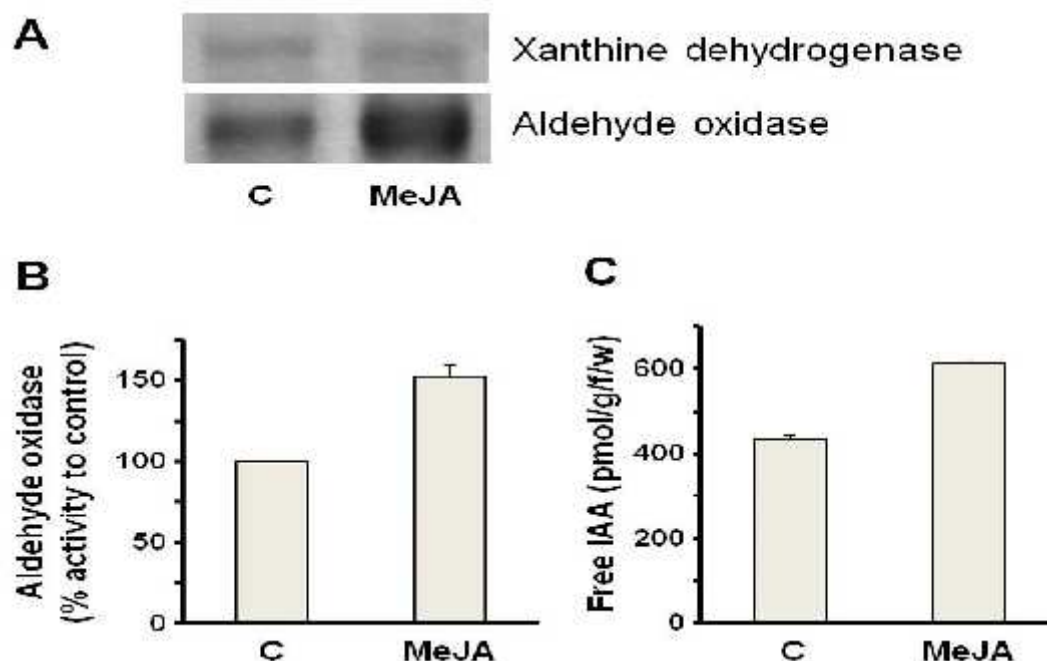
Figure. 2. Effects of MeJA on aldehyde oxidase activity and the level of free IAA in maize leaves.
A. Zymograms of aldehyde oxidase and xanthine dehydrogenase in the presence or absence of MeJA (10^{-3} M). Protein extract was technically divided into two parts and the activity staining was carried out on separated gels. B. Quantified aldehyde oxidase activities. C. The free IAA level determined by GC-MS with or without 10^{-3} M MeJA. Vertical bars indicate the standard deviations.

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