

EFFECT OF METHYL JASMONATE ON ARABIDOPSIS PROTEINS

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ABSTRACT

In this study, wild *Arabidopsis thaliana* seeds were grown on MS medium containing 40µM methyl jasmonate (MeJA) to determine if this compound could induce synthesis of jasmonate-induced proteins (JIPs) other than previously reported 27, 29 and 31kDa (kilodalton) vegetative storage proteins (VSPs) in Arabidopsis. Total proteins were extracted from twelve-day old MeJA-treated and untreated seedlings. Since seedlings are small, whole seedling was used for protein extraction. Using SDS-polyacrylamide gel electrophoresis protein samples were separated. Gels were stained with both silver salts and Coomassie blue R250, respectively. Comparison of protein banding patterns of MeJA-treated and untreated wild Arabidopsis seedlings revealed that MeJA induced synthesis of four proteins with molecular masses of approximately 23, 27, 65 and >66 kDa. The identity of one of these, the 27kDa protein, was determined using Western blot analysis. The 27kDa protein crossreacted with an antibody developed for Arabidopsis 29kDa VSP. The 23 and 65kDa proteins could be related to barley JIPs, JIP23 and JIP66. In Arabidopsis, the MeJA-dependent increase in these proteins may suggest that MeJA may act as growth regulator and play a role in the regulation of the amount of nitrogen that partitions into proteins, possibly under stress conditions.

Key Words: Methyl Jasmonate, *Arabidopsis thaliana*, Jasmonate Induced Proteins

METİL JASMONATIN ARABIDOPSIS PROTEİNLERİ ÜZERİNE ETKİSİ

ÖZET

Bu çalışmada, metil jasmonatın (MeJA) *Arabidopsis thaliana*'da daha önceden bildirilen 27, 29, ve 31 kDa (kilodalton) vejetatif depo proteinlerinden başka jasmonat teşvikli proteinlerin sentezini teşvik edip etmediğini belirlemek için yabancı Arabidopsis tohumları 40µM MeJA içeren MS besiyerinde büyütülmüştür. Toplam proteinler MeJA ile muamele edilmiş ve muamele edilmemiş 12 günlük fidelerden ekstre edilmiştir. Fideler küçük olduğu için protein ekstraksiyonunda fidelerin tamamı kullanılmıştır. SDS-poliakrilamid jel elektroforezi ile protein örnekleri ayrılmıştır. Jeller sırasıyla gümüş tuzları ve Coomassie blue R250 boyanmıştır. MeJA ile muamele edilmiş ve muamele edilmemiş yabancı Arabidopsis fidelerinin protein bantlaşma şekillerinin karşılaştırılması MeJA'nın moleküler ağırlıkları yaklaşık 23, 27, 65 ve >66 kDa olan dört proteinin sentezini teşvik ettiğini açığa çıkarmıştır. Bunlardan birisi olan 27kDa proteinin kimliği Western blot analizi ile belirlenmiştir. 27kDa protein Arabidopsis 29kDa vejetatif depo proteini için geliştirilen antibadi ile karşılıklı tepkimeye girmiştir. 23 ve 65kDa proteinler arpa JIP'leri JIP23 ve JIP66 ile ilişkili olabilir. Arabidopsis'de bu proteinlerdeki MeJA'a bağlı artış, MeJA'ın olası stres durumlarında proteinlere katılan azot miktarının düzenlenmesinde rol sahip olabileceği önerisini ortaya koymaktadır.

Anahtar Kelimeler: Metil Jasmonat, *Arabidopsis thaliana*, Jasmonat Teşvikli Proteinler

1. INTRODUCTION

Jasmonic acid (JA) and its methyl ester (MeJA) are known to induce accumulation of specific proteins (Jasmonate Induced Proteins, JIPs) in a large number of plant species, both monocotyledons and dicotyledons. The first report on jasmonate-induced proteins was presented in 1986 at the UCLA Conference on molecular biology of plant growth control [1]. Jasmonate-induced proteins accumulate in the leaf tissues of MeJA-treated plant species, but they have not been detected in MeJA-treated roots or untreated leaves of barley plants. There is a great variability in the number and relative molecular masses of JIPs in various plant species, and even

different barley cultivars can differ in the pattern of JIPs they produce [2]. At least six groups of jasmonate-induced proteins can be distinguished: proteinase inhibitors [3, 4], thionins [5], proline-rich proteins [6], phenylalanine ammonia-lyase [7], ribosome-inactivating proteins [8] and vegetative storage proteins (VSPs) [9].

MeJA induces the accumulation of two VSP polypeptides in soybean that are believed to function in temporary nutrient storage in leaves and other organs [10]. The JA-dependent increase in peptides in soybean occurred at concentrations of JA below those required for growth inhibition or induction of senescence. The suggestion was made that changes in the level of specific peptides may be representative of the phytohormonal activity of JA [11]. Soybean VSP antiserum cross-reacted with two polypeptides of similar size in several plant species, including Arabidopsis, when plants were grown with high levels of nitrogen. Staswick *et al.*[12], showed that the cross reacting proteins in Arabidopsis were inducible by MeJA as they are in soybean. Also, Feys *et al.*[13], reported that both coronatine and MeJA increased the level of two proteins of approximately 29 and 31kDa, detected by SDS-polyacrylamide gel electrophoresis in wild Arabidopsis but not in the coronatine insensitive mutant, *coi1*. However, in these studies researchers concentrated on only 27, 29 and 31kDa VSPs that were homologous to soybean VSPs. This study aimed to determine any other Jasmonate induced proteins of Arabidopsis, except for previously reported 27, 29 and 31 kDa proteins, in the presence of MeJA.

2. MATERIALS AND METHODS

2.1. Plant and Growth Conditions

Arabidopsis thaliana ecotype Columbia seeds were kindly obtained from Nottingham Arabidopsis Stock Centre (NASC). Seeds were soaked in water overnight in a microfuge tube at room temperature. The water was discarded and replaced by 1 ml 20% (v/v) NaOCl (sodium hypochloride solution) for 15 min. After that, seeds were rinsed three times with sterile distilled water. Surface-sterilised seeds were added to 4 ml cooled sterile 0.6% (w/v) agarose and this was dispersed onto MS medium with/without 40 μ M MeJA according to Feys *et al* (13). The plates were left in a flow hood to dry for 15 min. To break dormancy and synchronise germination, the plates were kept at 4°C for 3 days and then placed in a growth cabinet in continuous light (150 μ E m⁻² sec⁻¹) at 21 \pm 2°C.

2.2. Protein Extraction

Twelve-day old Arabidopsis seedlings grown on MS medium containing 40 μ M MeJA and MS medium without MeJA were washed briefly in distilled water. Approximately 3 seedlings from each group were transferred to eppendorf tubes and homogenised in extraction buffer (0.1 M Tris, pH 6.8; 10% SDS; 5% β -mercaptoethanol and 25 μ g/ml leupeptine) and then held on ice for 5 minutes. Insoluble material was removed by centrifugation using a desktop centrifuge at 12000xg for 5 minutes. Proteins were precipitated with 3 volumes of cold acetone. After 1hr at -20°C , precipitated proteins were collected by centrifugation. Protein pellets were washed with cold acetone and resuspended in Laemmli buffer (containing Tris-HCl, 62.5 mM, 10% SDS; 10% glycerol, 5% mercaptoethanol, 0.001% bromophenol blue), [14]. The protein content of sample extracts was determined using the methods of Bradford [15]. Prior to electrophoresis protein samples were heated to 90°C for 5 minutes and separated on 12% (w/v) SDS-polyacrylamide gels according to Sambrook *et al.*[16] at 100V for 2.5 hr. The gels were stained first with silver salts according to Blum *et al.* [17], and then with Coomassie blue R250 according to Sambrook *et al.* [16]. After silver staining all protein bands in the gel were in brown color. However, after staining the same gel with Coomassie blue the color of some bands changed to blue. This yielded more distinctive protein banding pattern between MeJA-treated and untreated seedlings so that some of differently synthesized proteins were easily visualized.

2.3. Western Blotting

After the proteins were separated by SDS-PAGE, the gel was placed on top of a nitrocellulose membrane. The gel and its attached nitrocellulose filter are sandwiched between 8 pieces of Whatmann 3MM paper that have been soaked in a transfer buffer containing 30mM glycine, 48mM Tris-base (pH 8.3), 0.037% SDS, 20% methanol. The sandwich is then placed between graphite plate electrodes, with the nitrocellulose filter on the anodic side. In order to transfer proteins, a current of $\sim 0.8\text{-}1$ mA/cm² of gel was applied for a period of 1.5-2 hours using the Novablot apparatus (Pharmacia). The filter was stained in Ponceau S (2% Ponceau S, 30% trichloroacetic acid, 30% sulfosalicylic acid) and the positions of proteins used as molecular-weight standard were marked with a waterproof pen. After several washes with distilled water, the nitrocellulose filter was incubated in blocking solution (5% (w/v) nonfat dried milk, 0.01% antifoam A, 0.02% sodium azide in phosphate-buffered saline) for 1-2 hours. The filter was then incubated overnight in blocking solution with 0.05% (v/v) Arabidopsis VSP antibody

(gift from Benedetti). The filter was then removed and then washed three times for 10 minutes in TBS (pH 7.4) (8g NaCl, 0.2g KCl, 3g Tris-base in 1L water) and transferred to a solution containing 150mM NaCl, 50mM Tris-Cl (pH 7.5) and incubated for 10 minutes. The filter was then placed in a tray containing phosphate-free, azide free blocking solution (5% (w/v) nonfat dried milk, 150mM NaCl, 50mM Tris-Cl, pH 7.5. The second antibody which was goat-antirabbit IgG peroxidase was added (0.05% v/v) and they were incubated for 1-2 hours on a platform rotary shaker. After that, the filter was transferred to 200ml of 150mM NaCl, 50mM Tris-Cl (pH 7.5) and incubated for three times for ten minutes each.

For the final step, the filter was stained in a solution containing 6mg of diaminobenzidine tetrahydrochloride, 9ml of 0.01M Tris-Cl (pH 7.6), 1ml of 0.3% CoCl₂ and 10µl of H₂O₂ until bands of desired intensity appeared. The filter was then washed briefly with distilled water and transferred to PBS (8g of NaCl, 0.2g of KCl, 1.44g of Na₂HPO₄, and 0.24g of KH₂PO₄ in 800ml of distilled water).

3. RESULTS

The changes in soluble proteins in wild Arabidopsis seedlings after 12 days of treatment were analyzed. Figure 1A demonstrates induction of synthesis of ~23, ~27, ~65 and >66 kDa proteins in MeJA-treated Arabidopsis seedlings. The MeJA-dependent increase in the four peptides was reproduced in over five separate experiments and these proteins were not detected in untreated wild type seedlings (Figure 1A).

In order to test whether the ~27kDa MeJA-induced protein is related to the 29kDa protein reported by Benedetti *et al* [18], I have used an antibody raised against the 29kDa protein to probe Western blots from gels containing the ~27kDa protein. A positive cross-reaction with the ~27kDa protein was observed (Figure 1B).

A) SDS-gel electrophoresis of proteins extracted from untreated (A, B) and MeJA-treated (C, D) wild Arabidopsis seedlings. The arrows show polypeptides induced by MeJA, M indicates molecular weight standarts in kilodalton.**B)** Western blot analysis of specific proteins induced by MeJA in Arabidopsis seedlings. Proteins of MeJA-treated and untreated wild seedlings probed with antiserum against the 29 kDa Arabidopsis VSP show high levels of a ~27kDa VSP-like protein in MeJA-treated wild Arabidopsis seedlings (C, D) but not in untreated seedlings (A, B). The minor bands are

thought to be non-specific cross-reactions. Molecular weight markers in line M are the same as for Figure 1A and their position is indicated on the blot.

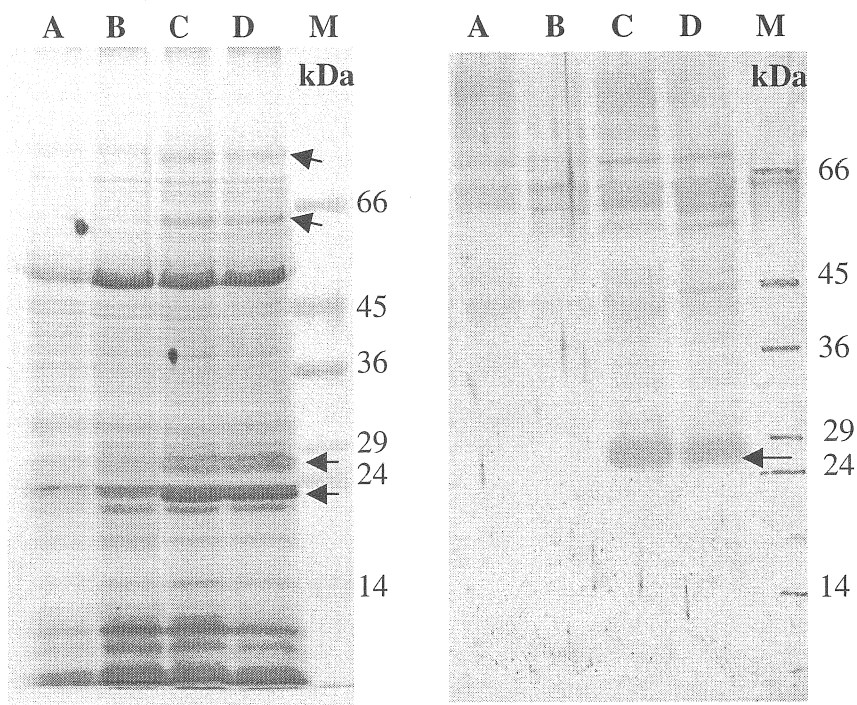


Figure 1. Patterns of MeJA induced proteins in wild Arabidopsis seedlings.

4. DISCUSSION

Jasmonate –induced proteins (JIPs) are synthesized when MeJA is applied exogenously or when tissues are affected by treatments known to induce endogenous MeJA synthesis such as water deficit, wounding, or in diseased tissues [8]. Most plant species treated with MeJA respond with JIP accumulation, but the patterns indicate qualitative and quantitative variations. In this study, it was shown that MeJA is responsible for the accumulation of four new polypeptides including the ~23, ~27 and ~65 and >66kDa proteins by altering gene expression in wild Arabidopsis seedlings. One of these, the 27kDa protein was antigenically related to coronatine- and MeJA-induced 29kDa protein [18] of Arabidopsis. Benedetti *et al.* [18] has shown that the 29kDa protein from Arabidopsis is a VSP: it is detected by antibodies raised against the vegetative storage proteins from soybean and

poplar. Also, Berger *et al.*[19] reported similar results that 29kDa and 30kDa proteins of Arabidopsis cross-react with antibodies against soybean VSP, are abundant in flowers, and accumulate in roots and leaves of plants treated with MeJA. In addition, Staswick *et al.* [12] showed that two proteins (27 and 29kDa) accumulated which were similar in size to the soybean VSPs when wild type Arabidopsis plants were sprayed with MeJA. The accumulation of three VSPs with molecular masses of 27kDa, 29kDa and 94kDa was observed when soybean plants were sprayed with low levels of atmospheric MeJA [20]. It was suggested that MeJA may either alter nitrogen utilization or increase the overall rate of amino nitrogen storage thus, modify nitrogen partitioning in soybean.

The MeJA-induced 27kDa protein detected in this study is probably the same as the ~29kDa VSP like protein reported by Benedetti *et al.*[18]. The discrepancy in apparent molecular weight is possibly due to the protein structure of VSP, since the VSP is usually glycosylated [21], its molecular weight could not be determined precisely. A more accurate figure for the molecular weight of this protein can be obtained by electrospray mass spectrometry.

In this study, except for ~27kDa protein, synthesis of ~23, ~65 and >66kDa proteins in MeJA-treated wild Arabidopsis seedlings was also demonstrated. There is no other reports on MeJA-induced synthesis of these ~23, ~65 and >66kDa proteins for Arabidopsis. These proteins are likely JIPs. However, further studies are required to test whether two of these proteins are related to the most abundant barley JIPs: JIP23 and JIP66.

Functions of JIPs are still not known, but they are usually considered as “stress-related proteins”[8]. After MeJA treatment, Arabidopsis seedlings also exhibited stunted growth and anthocyanin accumulation in their leaves (data will be published elsewhere). These are typical phenomena that are usually seen in stressed plants. Therefore, it can be said that MeJA caused stress and consequently induced synthesis of specific polypeptides including ~23, ~65 and >66kDa proteins in Arabidopsis plants.

In conclusion MeJA cause a massive reprogramming of the gene expression program of treated plants. Exogenously applied MeJA plays the role of a stressor that causes typical stress responses like the induction of stress proteins (JIPs).

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