Effect of Methyl Jasmonate on Microtuber Induction and Bioactive Compound Accumulation in Potato (*Solanum tuberosum* L.) *In Vitro*

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Abstract

Microtuber production in vitro is an important method for the quick increase and preservation of potato germplasm as well as the enhancement in accumulation of bioactive compounds. This study was conducted to determine the effects of methyl jasmonate (MeJA) on microtuber induction and the biosynthesis of some selected phytochemicals in potatoes (*Solanum tuberosum* L.) cultured under controlled in vitro conditions.

Surface-disinfected plant materials derived from potato tubers were established on Murashige and Skoog (MS) basal medium. Nodal stem segments were cultured on media with four concentrations of methyl jasmonate (MeJA) (0, 0.5, 1.0, and 2.0 mg L^{-1}) after shoot establishment and multiplication on MS medium supplemented with 0.5 mg L^{-1} benzyl adenine (BA). In this study, the effects of MeJA on microtuber development, biomass accumulation, and some selected biochemical constituents were also subsequently evaluated.

Potato culture response was highly dependent on the MeJA concentration. Of the treatments tested, 1.0 mg L^{-1} MeJA proved to be the most stimulatory and therefore significantly increased the number of microtubers, microtuber diameter, fresh biomass, and dry matter accumulation. It also increased starch and protein contents and lutein and xanthophylls accumulation over untreated control cultures. These results show that MeJA positively controls both the development of the storage organ and metabolic activity during in vitro culture.

The results of this study indicate that methyl jasmonate is an effective elicitor in improving micro tuberization efficiency and enhancing the accumulation of biologically important compounds in potato. The optimum concentration for maximization of both growth performance and biochemical characteristics under the experimental conditions employed was 1.0 mg L^{-1} MeJA.

Keywords: Solanum tuberosum L.; methyl jasmonate; micro tuberization; in vitro; lutein; xanthine; bioactive compounds.

Introduction

Potato (*Solanum tuberosum* L.) is considered one of the major food crops due to its high productivity, nutritional value, and wide adaptability to different environmental conditions. The crop supplies large amounts of carbohydrates as well as vitamins, minerals, and antioxidant compounds that contribute significantly to human nutrition as well as the agricultural economies. In Iraq, potato cultivation is a major component of vegetable production, and it plays a critical role in sustaining a steady supply of fresh produce to local markets.[2 ,1]

The dependence of commercial potato production on seed tubers has led to several challenges. These include the progressive build-up of pathogens over successive cycles of propagation and the relatively low multiplication

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rate of planting material. Such constraints have promoted the adoption of in vitro technologies in the programmers for potato propagation and breeding. Tissue culture systems, besides providing pathogen-free planting materials, offer highly controlled experimental environments for studying developmental responses and metabolic pathway manipulations toward desirable agronomic and physiological traits.[4 ,3]

Microtuberisation is one of the most vital applications of potato tissue culture due to its importance in germplasm conservation, seed production systems, and large-scale distribution of disease-free propagules. The efficiency of microtubere formation is governed by complex interactions among genotype, culture conditions, nutrient availability, and plant signaling molecules that regulate developmental pathways leading to storage organ formation.

In the array of signaling molecules probed in the last few years, methyl jasmonate (MeJA) has been established as a key regulator of plant development and adaptive responses. Synthesized from α-linolenic acid via the octadecanoid pathway, MeJA is involved in the coordination of growth and defense responses as well as metabolic reprogramming as a signal for these processes. It also regulates cell differentiation, organogenesis, stress signaling, and resource allocation within plant tissues–6] .[8

The application of MeJA as an elicitor in plant biotechnology has received considerable attention due to its secondary metabolism-stimulating properties. Treatment with MeJA often enhances the accumulation of compounds with nutritional, pharmaceutical, and protective values in cultured plant tissues. Such responses usually involve transcriptional activation of biosynthetic genes and increased metabolic flux toward specialized metabolites, including phenolic compounds, flavonoids, tocopherols, and related phytochemicals.[11–9]

Despite all studies carried out on the physiological and biochemical activities of jasmonates, their functions in the regulation of microtuber formation and metabolite accumulation in potato remain obscure. Also, there is no information available about the effect of MeJA on the accumulation of lutein and xanthophylls during culture in vitro. These results would help provide new protocols to integrate efficient microtuber production with better biochemical quality. Thus, the present work was undertaken to study the effect of different concentrations of methyl jasmonate on microtuber induction and the accumulation of some selected bioactive compounds in potato under in vitro conditions.

2. Materials and Methods

Plant Material and Explant Preparation

The experiment was carried out in the Plant Biology Laboratory, Medicinal and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences, University of Baghdad, Iraq, from 15th March to 25th December 2024. Certified seed tubers of potato (*Solanum tuberosum* L., cv. Industrial) were used as the source of plant material. These seed tubers had been stored at 4 °C for 90 days to break dormancy and then further incubated in darkness at 23–25 °C until healthy vegetative sprouts attained a length of 1–2 cm. This is done according to the method described by [15].

Surface Sterilization and Establishment of In Vitro Cultures

The developed sprouts were separated from the tubers, and their basal cut ends were sealed with sterile paraffin wax. This was to stop the sterilizing solution from getting to the inside tissues. The explants were surface sterilized with 6% sodium hypochlorite solution for 15 min with constant shaking, and then three rinses were given with sterile distilled water to remove entrapped residual disinfectant.[16,17]

The sterilized explants were cultured on MS medium supplemented with 5.0 mg L^{-1} benzyl adenine (BA) after removing the paraffin-coated basal portion. The pH of the culture medium was adjusted to 5.7 before autoclave at 121° C and 103 kPa for 20 min. All aseptic manipulations were carried out under the laminar airflow of the cabinet. The cultures

were incubated at 24 ± 2° C under 16-h photoperiod with the light intensity of about 1000 lux.

Methyl Jasmonate Treatments

Cultures were maintained for 28 days, and meristematic shoot tips (0.2–0.5 mm) were excised from actively growing shoots under the dissecting microscope and placed on the same Murashige and Skoog (MS) medium to give putatively virus-free plantlets. After shoot establishment and multiplication, the regenerated plantlets were transferred to MS medium supplemented with methyl jasmonate (MeJA) at concentrations of 0, 0.5, 1.0, and 2.0 mg L^{-1}.

Methyl jasmonate (MeJA) was purchased from Duchefa Biochemie. It was first dissolved in a small volume of absolute ethanol to make a stock solution and then the volume was made up with sterile distilled water. The required final concentrations were obtained by adding appropriate aliquots of the stock solution to the culture medium [10].

Microtuber Induction

For microtuber induction, the sucrose concentration of the *Murashige and Skoog (MS)* culture medium was increased to 80 g L^{-1}. Following autoclaving, 50 mL of the culture medium was dispensed aseptically into each culture vessel, and two plantlets were established per vessel. Each treatment consisted of ten replicates.

Cultures were initially incubated at 18 ± 2° C under a 16-h light/8-h dark photoperiod for two weeks to promote plant establishment. Subsequently, the cultures were transferred to complete darkness and maintained for an additional ten weeks to induce microtuber formation.

Growth and Biochemical Measurements

At the end of the culture period, the microtubers were harvested and evaluated for microtuber number, diameter (cm), fresh weight (g), and dry weight (g). Starch content was determined according to the method described by Moreels [20], whereas protein content was estimated using the procedure described in [21].

HPLC Analysis of Lutein and Xanthine

The concentrations of lutein and *xanthine* were determined using high-performance liquid chromatography (*HPLC*) (SYKAM, Germany). Chromatographic separation was performed on a C18 octadecylsilane (*ODS*) column (250 × 4.6 mm) using an isocratic mobile phase consisting of water (pH 7.0) and methanol (10:90, v/v) at a flow rate of 1.2 mL min^{-1}. Detection was carried out using a UV–Vis detector at 254 nm. Compound identification and quantification were achieved by comparing the retention times and peak areas of sample peaks with those of authentic reference standards according to the procedures described in [22,23]. The concentrations of lutein and *xanthine* were calculated from the corresponding external standard calibration curves (Figs. 1 and 2).

$$\textit{Sample concentration} = \frac{\textit{Standard concentration x sapmle area}}{\textit{Standard area}} \; x \; \textit{Dilution times}$$

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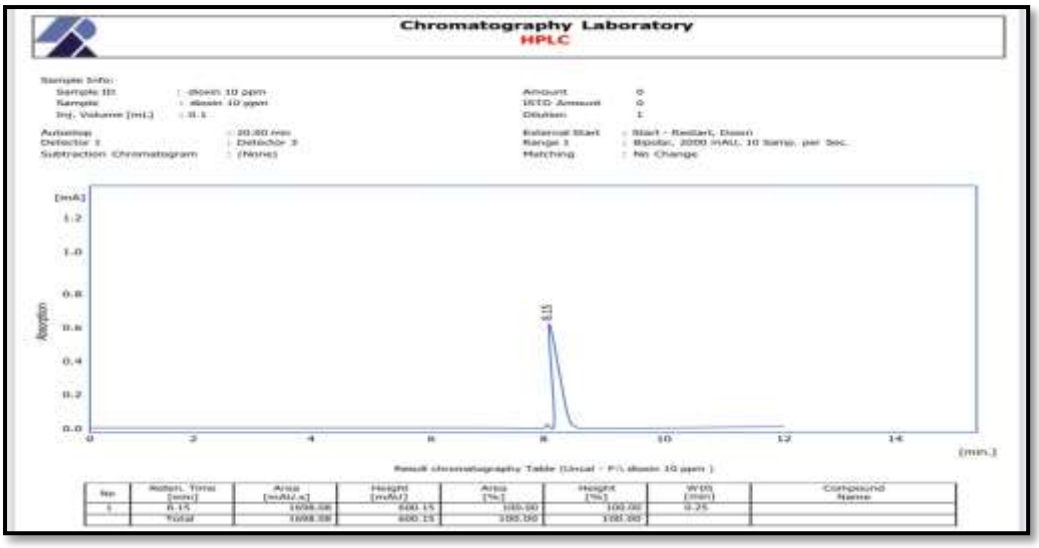

Figure 1. Calibration curve of lutein.

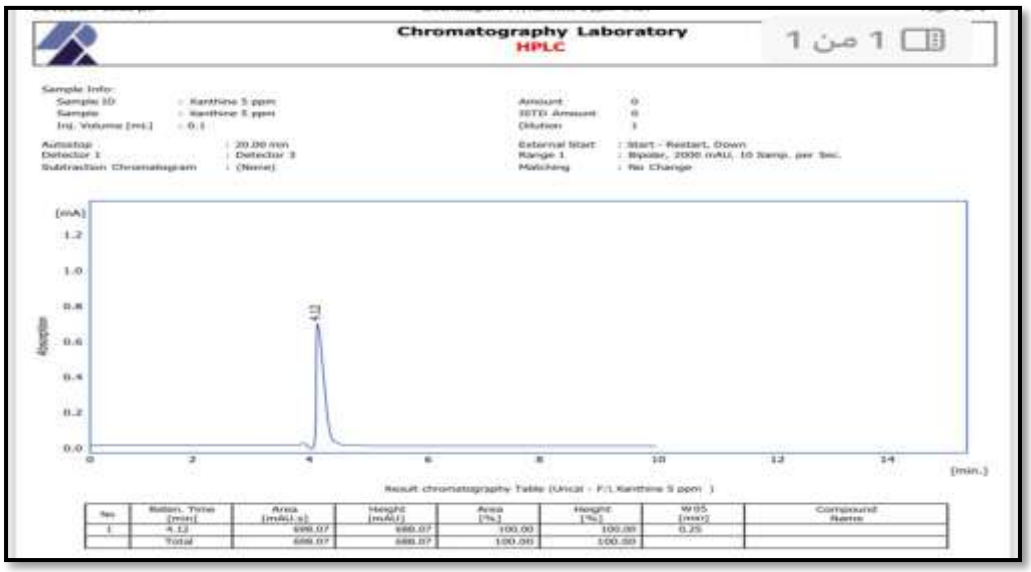

Figure 2 Calibration curve of xanthine.

2.3 Statistical design

The experiment was arranged as a factorial treatment structure within a Completely Randomized Design (*CRD*) with ten replicates per treatment. Data were subjected to analysis of variance (*ANOVA*) using *GenStat* software.

When significant treatment effects were detected, means were compared using the Least Significant Difference (*LSD*) test at $P \leq 0.05$ [24].

3.Results and Discussion

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3.1. Effect of Methyl Jasmonate on Microtuber Number and Diameter

Microtuber development was significantly affected by methyl jasmonate (*MeJA*) treatments (Table 1). The application of 1.0 mg L^{-1} *MeJA* produced the highest microtuber number (5.00 microtubers per explant) and the largest microtuber diameter (0.800 cm), whereas the control treatment recorded the lowest values for both parameters.

3.2 Effect of Methyl Jasmonate on Protein and Starch Content

Methyl jasmonate (MeJA) supplementation significantly affected the biochemical composition of potato microtubers (Table 2). The application of 1.0 mg L^{-1} *MeJA* resulted in the highest protein and starch contents, reaching 1.380% and 58.30%, respectively, whereas the control treatment recorded the lowest values for both parameters.

Table 1. Number and diameter of potato microtubers under different methyl jasmonate concentrations.

MeJA ($mg.l^{-1}$)	Number of Micro tubers	Diameter (cm)
0	1.00	0.400
0.5	3.00	0.533
1.00	5.00	0.800
2.00	4.00	0.533
LSD 0.05	1.913	0.2469

Table 2. Protein and starch accumulation in potato microtubers treated with methyl jasmonate concentrations.

MeJA ($mg.l^{-1}$)	Protein%	Starch%
0	0.980	53.34
0.5	1.200	55.79
1.00	1.380	58.30
2.00	1.260	54.24
LSD 0.05	0.022	1.613

3.3 Effect of Methyl Jasmonate on Xanthine and Lutein Accumulation

Significant differences in the accumulation of *xanthine* and lutein were observed among the *methyl jasmonate (MeJA)* treatments (Table 3). The application of 1.0 mg L^{-1} *MeJA* resulted in the highest concentrations of *xanthine* and lutein, reaching 28.00 and 24.33 mg g^{-1}, respectively, compared with the control treatment (Figs. 3 and 4).

3.4 Effect of Methyl Jasmonate on Fresh and Dry Weight of Microtubers

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Fresh and dry weights of potato microtubers were significantly affected by *methyl jasmonate (MeJA)* application (Table 4). The application of 1.0 mg L^{-1} *MeJA* resulted in the highest fresh and dry weights, reaching 0.569 and 0.457 g, respectively, whereas the control treatment recorded the lowest biomass accumulation.

Table 3. Lutein and xanthine accumulation in potato microtubers treated with methyl jasmonate concentrations.

MeJA ($mg.l^{-1}$)	Xanthine ($mg.g^{-1}$)	Lutein ($mg.g^{-1}$)
0	22.83	14.67
0.5	23.38	18.97
1.00	28.00	24.33
2.00	27.43	21.03
LSD 0.05	1.901	2.336

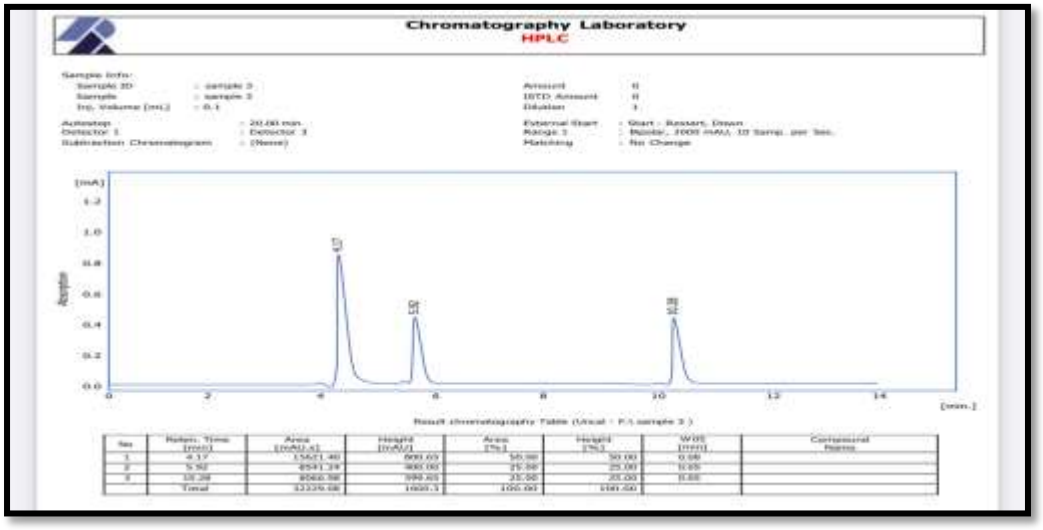

Figure 3. Xanthine accumulation in potato microtubers at 1.0 mg L^{-1} MeJA.

Table 4. Fresh and dry weight of potato microtubers under different methyl jasmonate concentrations.

MeJA ($mg.l^{-1}$)	fresh weight (g)	dry weight (g)
0	0.271	0.167
0.5	0.395	0.286
1.00	0.569	0.457
2.00	0.483	0.374
LSD 0.05	0.004	0.005

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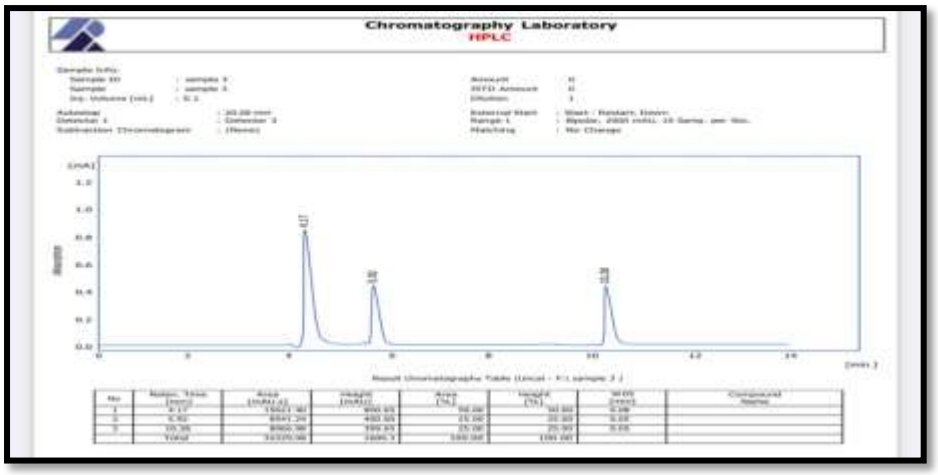

Figure 4. Lutein accumulation in potato microtubers at 1.0 mg L^{-1} MeJA

4. Discussion

The reaction of potato cultures to methyl jasmonate (MeJA) varied with concentration, showing that this signaling molecule has a key regulatory role in microtuber development under vitro conditions. The best results were obtained with 1.0 mg L^{-1} MeJA, especially regarding the number, diameter, fresh weight, and dry weight of microtubers, indicating that this concentration offered the best conditions for the initiation of an organ for storage and its development afterward.

The accelerated development of microtubers observed in this study could be related to the regulatory role of jasmonates in assimilating partitioning and sink establishment. Increased biomass and dry weight would indicate better use of available carbohydrates and their subsequent deposition within developing microtubers. These results support the well-established role of jasmonates in coordinating developmental and metabolic processes associated with storage organ formation.[25]

The increase in starch content further supports the involvement of MeJA in the regulation of carbohydrate metabolism. Enhanced starch accumulation reflects more efficient conversion of assimilated carbon into storage reserves; a process closely related to microtuber growth and maturation. Therefore, the increase in protein content may be attributed to increased metabolic activity and biosynthetic capacity in cultured tissues during microtuber development.

The stimulatory effects seen at moderate MeJA levels were not maintained at the highest concentration tested. This shows that the biological effects of MeJA depend on its concentration; excessive levels may place metabolic constraints that limit growth and reserve accumulation. Such concentration-dependent responses to jasmonates have been reported in many in vitro plant culture systems.

Besides its effects on growth-related characteristics, MeJA significantly boosted the buildup of lutein and xanthine. This finding proves MeJA can steer cellular metabolism toward the biosynthesis of biologically active compounds. Elicitation effects of jasmonates have been ascribed to the activation of regulatory networks that control secondary metabolism, thereby increasing the production

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of compounds with antioxidant, protective, and nutritional functions.[26]

Similar responses have been reported in other studies. Badrhadad et al. [13] showed increased α-tocopherol accumulation in cell cultures of Elaeagnus angustifolia after treatment with MeJA, while Ahmed and Ibraheim [14] observed increased production of anthraquinone and flavonoids in suspension cultures of Emex spinosa. All these facts provide evidence that MeJA is a good effective elicitor in boosting biosynthesis of

. Conclusion5.

These results prove that methyl jasmonate (MeJA) is a promising biotechnological tool for enhancing microtuberization and the accumulation of bioactive compounds in potato (*Solanum tuberosum* L.) cultured in vitro. The most favorable responses in terms of microtuber development and biochemical characteristics were obtained with 1.0 mg L^{-1} MeJA, among the concentrations studied. This, therefore, indicates that proper MeJA supplementation could help in the development of improved in vitro propagation protocols aimed at producing high-quality potato microtubers with enhanced nutritional and physiological attributes.

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secondary metabolites in different plant species.

In summary, this study shows that methyl jasmonate (MeJA) improves microtuber yield and biochemical content in potato (*Solanum tuberosum L.*) cultured in vitro. The best treatment was 1.0 mg L^{-1} MeJA, which shows that proper application of this signaling molecule is a viable approach to microtuber enhancement with concomitant support for bioactive compound accumulation.

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