Acta Agrobotanica

DOI: 10.5586/aa.1784

Publication history
Received: 2018-10-29
Accepted: 2019-08-03
Published: 2019-09-30

Handling editor
Marzena Parzymies, Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Poland

Authors' contributions
MSI and ZI conducted the laboratory work and wrote the manuscript; MIA conceived, designed the experiments, and edited the manuscript

Funding
This work was supported by Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, New Delhi (grant No. SB/SO/BB-002/2012) to Dr. Mohammad Israil Ansari.

Competing interests
No competing interests have been declared.

Citation
Iqbal MS, Iqbal Z, Ansari MI. Enhancement of total antioxidants and flavonoid (quercetin) by methyl jasmonate elicitation in tissue cultures of onion (*Allium cepa* L.). Acta Agrobot. 2019;72(3):1784. https://doi.org/10.5586/aa.1784

ORIGINAL RESEARCH PAPER

Enhancement of total antioxidants and flavonoid (quercetin) by methyl jasmonate elicitation in tissue cultures of onion (*Allium cepa* L.)

Mohammed Shariq Iqbal[1], Zahra Iqbal[2], Mohammad Israil Ansari[2]*

[1] Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow 226 028, India
[2] Department of Botany, University of Lucknow, Lucknow 226 007, India

* Corresponding author. Email: ansari_mi@lkouniv.ac.in

Abstract
The onion (*Allium cepa*) is a vegetable used extensively all over the world both for culinary purposes as well as in medicine. Its medicinal values are due to the high levels of biologically-active compounds present within the bulb. There are various phytochemicals of therapeutic importance found in *A. cepa*. Quercetin, a flavonoid, is one of these phytochemicals and it is a potent antioxidant. *Allium cepa* is a dietary supplement and is beneficial for diverse ailments, thus justifying its status as a valuable medicinal plant. Due to its medicinal significance, elicitation of total antioxidants and quercetin levels have been attempted to enhance their production in tissue callus cultures. This study reports in vitro enhancement of total antioxidants and quercetin in *A. cepa* using methyl jasmonate as an elicitor. A reverse phase-high performance liquid chromatography (RP-HPLC) method was used with an isocratic system and a flow rate of 1.0 mL min^{-1} and a mobile phase of acetonitrile: 1% v/v acetic acid (60%:40% v/v). The detection wavelength was 362 nm and the retention time 8.79 minutes. Total antioxidant and quercetin contents were maximal with 100 µM of methyl jasmonate in leaf tissue callus cultures at 84.61 ±6.03% and 0.81 ±0.03 mg g^{-1} dry cell weight, respectively. They decreased with further increases of methyl jasmonate at 200 µM. The increase in total antioxidant and quercetin contents were 2.3- and 13.9-fold, respectively. The optimization of methyl jasmonate as an elicitor, as well as the determination of a suitable concentration in *A. cepa* in callus cultures, will be helpful for enhanced production of various other secondary metabolites of therapeutic significance. This could be beneficial for the pharmaceutical and neutraceutical industries for herbal drug formulations.

Keywords
elicitors; micropropagation; phytochemicals; secondary metabolites; therapeutic

Introduction

Allium cepa L. (onion) is one of the most consumed vegetables used both for culinary purposes and as a herbal medicine. It is cultivated throughout the world. Several investigations have reported that *A. cepa* provides an extensive range of beneficial therapeutic properties for human health, viz. antioxidant [1,2], antimutagenic [3], and anticholesterolaemic capacities [4]. Phytochemical screening of *A. cepa* has shown that it is a rich source of nutritive phytochemicals such as flavonoids, fructans, and organo-sulfur compounds, which are diverse but highly valuable phytochemicals. Although the major constituents of interest in *A. cepa* are sulfur compounds and flavonoids, numerous additional components of intact *A. cepa*, e.g., lectins (most abundant), adenosine, prostaglandins, vitamins B1, B2, B6, C, and E, biotin, nicotinic acid, fructan, pectin,

fatty acids, phospholipids, glycolipids, and many essential amino acids, have also all been extensively investigated over several decades for various therapeutic properties [5–7]. Due to its several important, pharmaceutically significant phytochemicals, *A. cepa* is used for the treatment of numerous diseases and disorders [8]. In ayurvedic formulations, it is used extensively in healing of wounds, cardiovascular disorders, hyperglycemia, and for stomach tumors [9]. It is also claimed to relieve headaches, erectile dysfunction, snakebites, hair damage, and coughs [10]. *Allium cepa* extract gels are used extensively in antiblemishing effects and are also used in the deterrence of presternal hypertrophic scar fortification [9]. It has been reported that regular consumption of *Allium* vegetables can reduce the risk of gastric disorders and prostate cancer [11,12]. The effect of *A. cepa* on the growth of gram-positive and gram-negative bacteria and several fungi has confirmed its antimicrobial activity [13]. It possesses several phenolics and flavonoid phytochemicals which are reported to hold potential anticholesterol, antiinflammatory, anticancer, toxigenicity of oils, and antioxidant properties [14]. By inhibiting fatty acid synthase, *A. cepa* has revealed inhibitory effects on the propagation of cancerous cells and lipocytes [12]. During hot and humid summers, *A. cepa* checks fast dehydration of the body due to extreme heat [15]. Its daily consumption can restore zinc deficiency in the body. Zinc is a vital cofactor for more than 300 metalloenzymes, which are involved in the metabolism of macro- and micronutrients at the cellular level [16,17].

The antioxidant properties of *A. cepa* are well known. The free radical scavenging property of ROS/RNS (reactive oxygen species / reactive nitrogen species) in *A. cepa* makes this vegetable an excellent dietary supplement in daily use [14]. The antioxidant property is due to various phytochemicals present in it, one of which is quercetin. Quercetin (3,3′,4′,5,7-pentahydroxyflavone), which belongs to the category of flavonols that cannot be produced in the human body, is abundantly produced by many plants and notably by *A. cepa* [18]. Quercetin is a type of plant-derived aglycone that has been used as a dietary supplement and is valuable for protection against various diseases. Some of its beneficial properties include anticancerous, cardiovascular fortification, antiviral, antitumor, anti-inflammatory activity, antiulcer, antiallergy, antihypertensive, antidiabetic, antigastroprotective, and immunomodulatory [18,19].

At present, 25% of all the recommended therapeutic formulations that are in use in industrially developed countries comprise compounds that are semisynthetic, directly or indirectly derived from plants [20]. These hold a massive market value in western countries, as most prescribed drugs contain phytochemicals that are valued at more than US$30 billion in the USA alone [21]. Many plants that contain valuable compounds of therapeutic importance are difficult to cultivate, or because of overharvesting, they are in danger of extinction [22]. Moreover, plant-derived phytochemicals that are chemically synthesized are not often economically achievable due to their extremely complex structures or the particular stereo-chemical necessities of the complex compounds [20]. Thus, the biotechnological production of economically valuable and therapeutically important secondary metabolites by plant cell, tissue, or organ culture techniques is an unconventional method for their extraction [20]. Plant cell, tissue, or organ cultures have had only restricted commercial success. This is due to the unexplained experiential nature of choosing high-yielding varieties, established culture techniques and a limited understanding of the metabolic pathways of secondary metabolite syntheses and their regulation [23,24]. A number of biotechnological methodologies have been postulated and investigated for enriched production of secondary metabolites from potentially significant plants [25]. Techniques available involve screening out a high-yielding cell line, precursor feeding, media alteration, large-scale refinement in the bioreactor system, elicitation, hairy-root culture, immobilization of plant cells, and biotransformation [26–29]. Cell or tissue culture systems from several plants have been demonstrated but even so, they are often incapable of producing adequate quantities of the essential secondary metabolites [25,30]. Nevertheless, in several instances the manufacture of secondary phytochemicals could be improved further by the application of undifferentiated cell treatment / callus cultures with elicitors such as salicylic acid, chitosan, methyl jasmonate, or by the use heavy metals [31–33]. The use of elicitors could therefore enhance the level of secondary metabolites which are useful for therapeutic purposes. These secondary metabolites often play an important role in defense systems of different organisms. Humans use secondary metabolites as medicines, flavorings, and recreational drugs. In vitro production of such secondary metabolites in plant cells

through callus cultures has been reported for various medicinal plants, and bioreactors are the key step for their commercial production. The present investigation was therefore designed to enhance the level of antioxidants in the form of flavonoid (quercetin) and others present in *A. cepa* with consideration to the previous findings described above. This could overproduce antioxidants present in the plant useful for various therapeutic applications and nutritional value. Hence, in the present investigation, a high-yielding cultivar of *A. cepa*, 'Agrifound light red' cultivar, was used as it is grown extensively in India. However, only limited information is presently available about its nutritional value. The investigation is based on the elicitation of total antioxidants and a flavonoid (quercetin) by methyl jasmonate in order to hyper-produce the secondary metabolite in callus culture of leaf tissue, the basal plate and roots of the plant. Enhanced production of quercetin in *A. cepa* could be useful in the neutraceutical, pharmaceutical, and agricultural industries.

Fig. 1 Sprouting of the 'Agrifound light red' cultivar of *A. cepa* in moist conditions.

Material and methods

Explant preparation

Bulbs of the 'Agrifound light red' cultivar of *A. cepa* were kept in moist conditions for the regeneration of leaves and roots (Fig. 1). After sprouting, the leaves, basal plate and roots were used in the study. These plant parts were washed with soap solution for 1 minute and rinsed several times with running tap water. They were then treated with 0.1% $HgCl_2$ for 30 seconds under laminar air flow and rinsed several times with sterile water. Next, they were treated with 70% ethanol for 30 seconds and again rinsed five times with sterile water. All the explant preparations were performed according to the protocol of Bandekar and Lele [34], with some modifications. Murashige and Skoog [35] basal medium (MS) with 30 g L^{-1} sucrose was used in this study.

The sterile plant parts were each trimmed to 2 cm under laminar air flow and transferred for callus formation on to agar-solidified MS medium supplemented with 2,4-D (1 mg L^{-1}) (see Fig. 3). The calli obtained after 4 weeks were transferred to MS medium supplemented with methyl jasmonate at different concentrations in triplicate (viz. 0 µM 10 µM, 20 µM, 50 µM, 100 µM, and 200 µM) and maintained for further use. The culture bottles were kept at a light intensity of 150 lux, provided by cool fluorescent lamps with a photoperiod 16/8-hour day/night and maintained at 25 ±2°C until healthy calli were formed.

Extraction and analysis

Twenty g of each of the callus cultures obtained from the plant parts (leaves, basal plate, roots) were taken for drying overnight at 50°C for the extraction of quercetin. The dry samples were extracted in methanol (100 mL) for 2 hours using a Soxhlet apparatus (Borosil). The extract so obtained was reduced to a dried residue using a rotary evaporator (IKA RV 10 DS96). Hydrolysis of the dried extract obtained (1.2 g) was performed by refluxing with 7% H_2SO_4 (100 mL) for 30 min. The reaction mixture was then filtered and the filtrate obtained extracted into two portions of ethyl acetate (each 25 mL) using a separating funnel. The residue so obtained was prepared for further analysis (1 mg mL^{-1} stock in methanol).

Antioxidant activity assayed by the DPPH method

Antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) for a free radical scavenging assay. The Brand-Williams et al. [36] protocol was followed with minor modifications. The reaction mixture consisted of 3.0 mL absolute ethanol, 0.3 mL

DPPH (0.5 mM in ethanol), and 0.5 mL sample. The reaction mixture was kept at room temperature for 60 minutes to complete the reaction. The change in color was measured at 517 nm using spectrophotometer (Shimadzu UV-1800). A blank was prepared by mixing 3.3 mL of ethanol and 0.5 mL of sample. For control solutions, ethanol (3.5 mL) and DPPH (0.3 mL) were used. The antioxidant activity was calculated according to the formula of Mensor et al. [37]:

$AA\ (\%) = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\}$.

Spectral scanning

Spectral scanning of quercetin for maximum absorbance was performed by spectrophotometry (Shimadzu UV-1800). The Duan [38] protocol was used with minor modifications where ethanol was used as a solvent.

RP-HPLC analysis

The study was conducted using an isocratic system of reverse phase-high performance liquid chromatography (UFLC Shimadzu), UV-Vis detector (SPD-20A), and pump (LC-20AD). A C-18 column (250 × 4.6 mm; 5 µm particle diameter) (Shiseido) was used for the analysis. Lab Solution Lite software (Shimadzu) was used for data acquisition. An authentic standard of quercetin dihydrate extra pure grade (Sisco Research Laboratories Pvt. Ltd.) and HPLC-grade acetonitrile, methanol, water (MERCK), and acetic acid each were used for the analysis.

The RP-HPLC tests were executed for quercetin by following the protocol of Lee et al. [39], with some alterations. Quercetin was estimated by an isocratic system with a flow rate of 1.0 mL min^{-1} and mobile phase acetonitrile: 1% v/v acetic acid (60%:40% v/v); detection was at 362 nm and the retention time 8.79 minutes. The mobile phase was filtered (0.22 µm syringe filter) and degassed using a bath sonicator. Standard stock solutions of quercetin (1 mg mL^{-1}) were prepared using the mobile phase for the preparation of the calibration curve with a concentration range from 0.005–0.2 mg mL^{-1}. Each quercetin standard and unknown sample were run in triplicate. To draw the calibration graph, peak area was plotted alongside the corresponding concentrations and a linear relationship was obtained.

Statistical analysis

All data are expressed as means ± standard deviation. Two-way ANOVA was performed on the data for the different plant parts and concentrations of methyl jasmonate used in the callus cultures. Linear regression analyses were also performed for total antioxidant activity and quercetin content in the callus cultures using the tests available in Microsoft Excel software.

Results

A total assay of antioxidant activity using DPPH was attempted. The results showed a good effect of methyl jasmonate in elevating the level of total antioxidant in the callus cultures of *A. cepa*. The maximum antioxidant level was in calli of leaf tissues with 84.61 ±6.03% at 100 µM of methyl jasmonate used, whereas the minimum was observed in calli of roots with 36.51 ±3.78% at 200 µM of methyl jasmonate. A linear elevation of total antioxidant was observed from control calli up to 100 µM of added methyl jasmonate, but further increases reduced the total antioxidant activity (Fig. 2). This may be due to inhibitory effects of methyl jasmonate that reduced the formation of secondary metabolites at higher concentrations. The increase in total antioxidant activity was by almost 2.31-fold in calli treated with methyl jasmonate at the 100 µM of concentration.

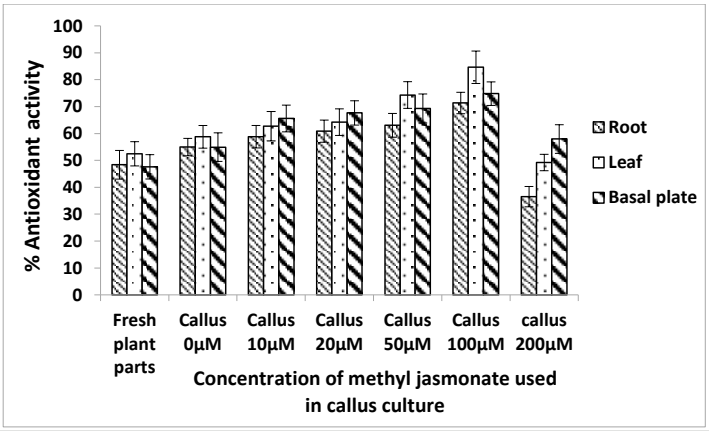

Fig. 2 The effect of methyl jasmonate on total antioxidant in callus cultures of root, leaf, and basal plate tissues of *A. cepa*. Two-way ANOVA of methyl jasmonate concentration used and calli of plant parts showed significant effects $p < 0.05$.

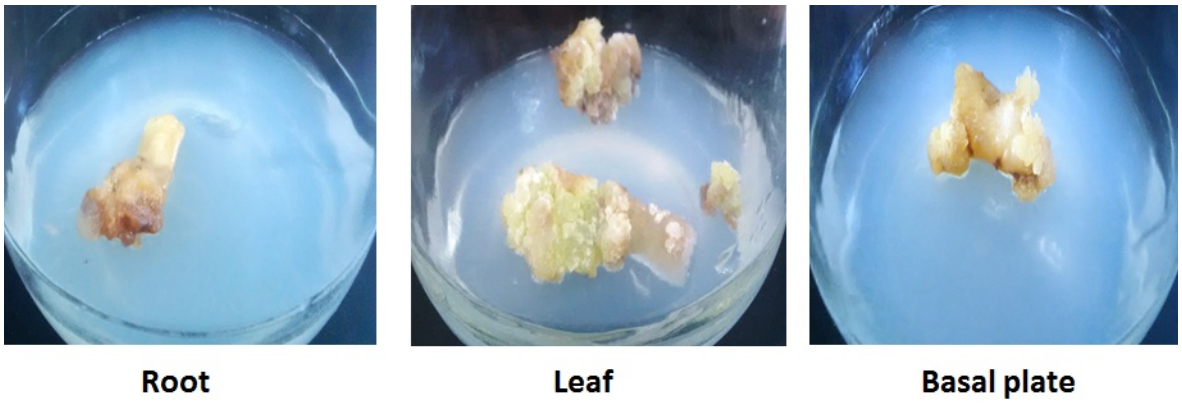

Fig. 3 Callus proliferation on MS medium supplemented with methyl jasmonate after harvesting proliferated friable biomass from medium supplemented with 2,4-D (1 mg L^{-1}) for callus formation.

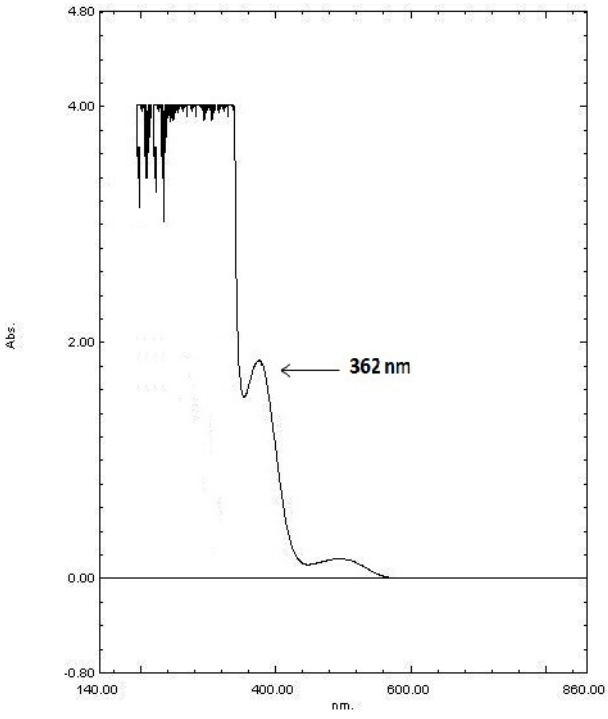

Fig. 4 UV–visible spectral scan of the quercetin standard.

During further investigation, elicitation of flavanol (quercetin) was attempted in *A. cepa* through in vitro tissue culture (Fig. 3). The estimation of quercetin was by RP-HPLC and was confirmed by spectral scanning (Shimadzu UV-1800). The result is shown in Fig. 4. The maximum absorbance for quercetin was observed at 362 nm.

As the solubility of quercetin is good in methanol, it was used as a solvent [18]. Before quantitative analysis of quercetin in extracts of *A. cepa*, a linear calibration curve of quercetin from standards was prepared by RP-HPLC. The concentration ranged from 0.005 to 0.2 mg mL^{-1}. The mean coefficient of determination (R^2) for quercetin was 0.995, whereas the % *RSD* (relative standard deviation) was 21.74% (Fig. 5). Linearity, selectivity, accuracy, precision, and robustness of data were appropriately taken into consideration for the justification and reproducibility of the procedure.

When RP-HPLC analysis of fresh plant parts and callus extract was performed, it was found that leaf tissue has maximum concentration of quercetin (0.09 ±0.01 mg g^{-1} dry cell weight), whereas the minimum was observed in root cultures (0.06 ±0.01 mg g^{-1} dry cell weight). When elicitor treatment with methyl jasmonate was given to the calli, the quercetin content increased linearly with the increase in methyl jasmonate concentration, but there was

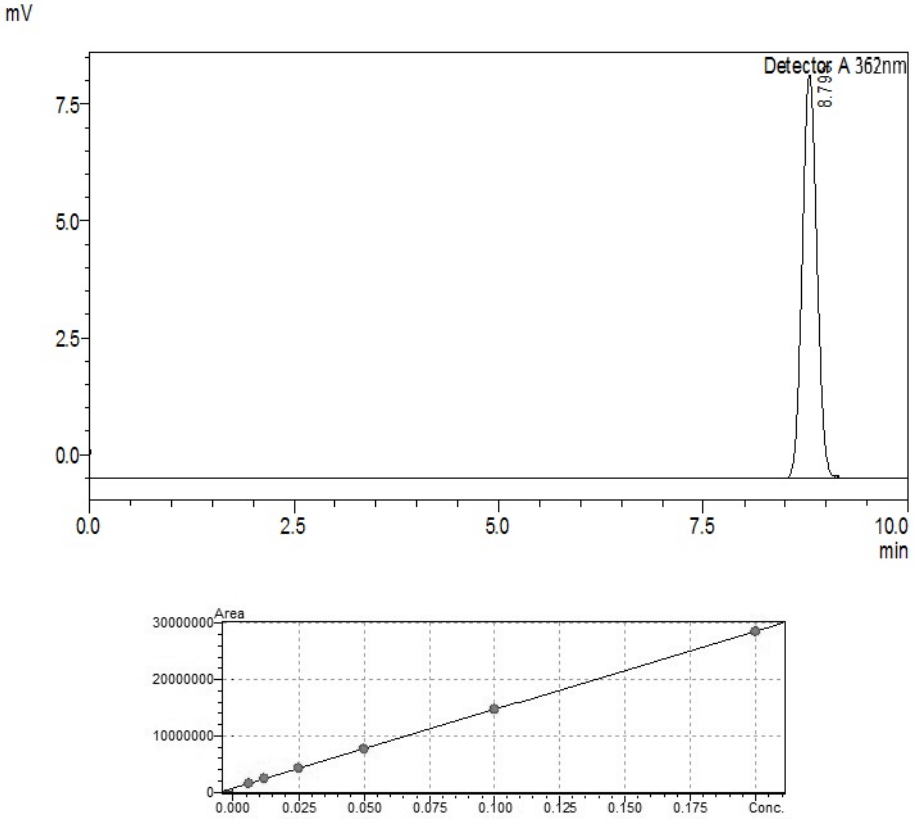

Fig. 5 Chromatogram and standard curve of quercetin (λ_{max} – 362 nm) with a retention time of 8.79 minutes.

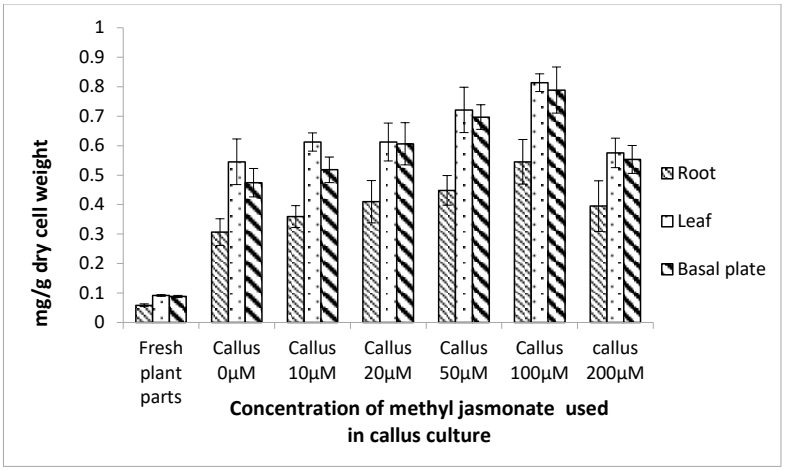

Fig. 6 The effect of methyl jasmonate on quercetin content in callus cultures of root, leaf, and basal plate tissues of *A. cepa*. Two-way ANOVA showed significant ($p < 0.05$) variation between quercetin content of plant parts and different concentrations of methyl jasmonate used.

a decrease observed at higher concentration. The quercetin content was found to be maximal in calli of leaf tissue with 0.81 ±0.03 mg g^{-1} dry cell weight at a concentration of methyl jasmonate of 100 µM. The minimum was found in calli of roots with 0.31 ±0.04 mg g^{-1} dry cell weight in the control treatment (0 µM methyl jasmonate). The detailed results are given in Fig. 6.

In order to elucidate the effect of methyl jasmonate as an elicitor, regression analyses were performed with the assumption that there was a concentration-dependent effect of methyl jasmonate on total antioxidant activity and quercetin content. A strong positive effect was observed with R^2 values of 0.52, 0.7, 1, and 0.91 for root, leaf, and basal plate of *A. cepa*, respectively (Fig. 7). Further increasing the concentration of methyl jasmonate

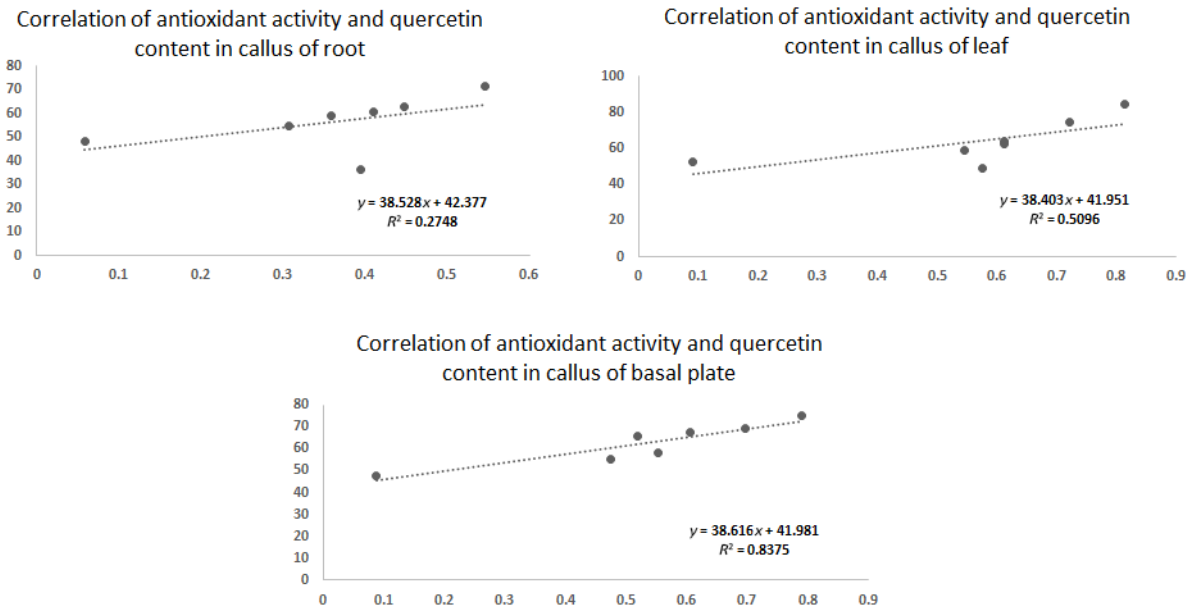

Fig. 7 Regression analyses of total antioxidant activity and quercetin content in callus cultures of *A. cepa*.

reduced the formation of quercetin and total antioxidant activity at a concentration of 200 μM of methyl jasmonate. It was also noted that the total antioxidant level was raised up by 2.31-fold, whilst flavonoid (quercetin) production reached a 13.94-fold increase at a concentration of 100 μM of methyl jasmonate.

Discussion

In the present study, an elicitation technique was applied to enhance the level of antioxidants in *A. cepa*. Several studies have been made on various plants by other workers in order to elicit antioxidants using methyl jasmonate as an elicitor. In the our study treatment with methyl jasmonate enhanced the level of antioxidants in *A. cepa*. The strong positive effect clearly indicates the role of methyl jasmonate in the elicitation of quercetin with an increase in total antioxidant activity in a concentration-dependent manner. The inferences of our results are in good agreement with those of Ghasemnezhad and Javaherdashti [40] where treatment with methyl jasmonate elevated the antioxidant level in raspberry fruit. In a study by Kim et al. [41], these authors also reported the effect of methyl jasmonate on *Lactuca sativa* L. in enhancing the levels of phenolics, carotenoids and antioxidants in this important horticultural crop. The enhancement of antioxidants by methyl jasmonate led us to investigate the effect further by studying the quercetin content in the callus cultures. Prior to our RP-HPLC investigation, spectrophotometric scanning was attempted in order to confirm the maximum absorbance wavelength for quercetin (λ_{max} – 362 nm). The results obtained were in good accordance with the report of Sharma and Janmeda [42], where the scanning of flavonoid and phytochemical has been compared with standard quercetin in leaf extracts of *Euphorbia neriifolia*. The RP-HPLC analysis was also in agreement with the report of Jing et al. [43] where different concentrations of methyl jasmonate were used to elicit flavonoids. It was observed that a concentration of 100 μM methyl jasmonate was most effective for elicitation of antioxidants for *A. cepa* in callus cultures. Furthermore, it was found that the content of flavonoid was maximal (52.8 mg g^{-1} dry weight) at 100 μM, which was greater than previously reported values for root cultures (42.7 and 48.6 mg g^{-1} dry weight) [44,45]. The undesirable consequence of methyl jasmonate at the upper concentration used (200 μM) on cell growth and various metabolite formation has also been reported in cell suspension cultures of *Gymnema sylvestre* R. Br. [46]. In our study, it was noted that the increase in quercetin content was almost 13.9-fold in callus cultures treated

with methyl jasmonate at 100 μM compared to that in the mother plant. This result is in good agreement with a previous study of Bandekar and Lele [34] where the quercetin content was estimated in the fig, *Ficus benghalensis* L. in the mother plant and in callus culture. Their results showed elevated levels of quercetin in callus cultures with 20-fold more in the callus than in mother plant. Many other investigations on plant cell cultures have confirmed methyl jasmonate as an elicitor which can stimulate the production of secondary metabolites. Methyl jasmonate has been acknowledged as an operational elicitor which can increase the production of paclitaxel in *Taxus cuspidata* Siebold & Zucc. and *T. canadensis* Marshall [47], anthocyanin in *Tulipa gesneriana* L. [48], and gymnemic acid in *Gymnema sylvestre* [46]. An appropriate elicitor at a suitable concentration is a vital factor for the improved growth of cells and metabolite yield in the process of plant cell culture [49]. The beneficial effect of methyl jasmonate could be attributed to its structure, where the two chiral carbon atoms in the molecule which possess either the R or S configuration yields four potential isomers. Methyl (+)-epijasmonate, (3*R*, 7*S*)-(+)-methyl 3-oxo-2-[2-(*Z*)-pentenyl] cyclopentane-1-acetate are the distinctive two isomers that demonstrate the prominence of the shape of the molecule by attaching to the receptors and initiating the effects [50]. Thus, due to differential interactions between phytochemical moieties of *A. cepa* and methyl jasmonate used as an elicitor at high concentration, it might be toxic to callus cells leading to their death. This could be a reason for the deprived level of antioxidants in callus cultures at higher concentrations of methyl jasmonate used as an elicitor in this study.

As a result of our study, it can now be suggested that the production of secondary metabolites using methyl jasmonate as an elicitor at a particular dose in *A. cepa* can beneficially enhance the level of secondary metabolites (2–3-fold increase), which could be useful for the neutraceutical and pharmaceutical industries.

Conclusions

Plant tissue culture is an effective alternative technique for the production of various valuable metabolites of therapeutic importance. In the present investigation, tissue cultures of *A. cepa* were made in order to enhance the production of total antioxidants and flavonoids using methyl jasmonate as an elicitor. It was observed that the total antioxidant level was raised by a factor of 2.31-fold, with the maximum in calli of leaf tissue with 84.61 ±6.03%, whilst flavonoid (quercetin) was elevated with an 13.94-fold increase, again maximal in callus of leaf tissues with 0.81 ±0.03 mg g^{-1} dry cell weight at a concentration of 100 μM of methyl jasmonate. It was concluded that methyl jasmonate can be used as an elicitor to enhance the level of antioxidants. Its biosynthetic pathway should be elucidated and it is recommended for future research in order to use it in more efficiently as an elicitor. Notwithstanding, the outcomes of the present investigation will be advantageous for the production of antioxidants from *A. cepa* and will be helpful for the manufacture of beneficial phytocompounds from plant tissue cultures, which could be particularly useful for the neutraceutical and pharmaceutical industries.

References

1. Pérez-Gregorio RM, González-Barreiro C, Rial-Otero R, Simal-Gándara J. Comparison of sanitizing technologies on the quality appearance and antioxidant levels in onion slices. Food Control. 2011;22:2052–2058. https://doi.org/10.1016/j.foodcont.2011.05.028
2. Lu XN, Wang J, AL-Oadiri HM, Ross CF, Powers JR, Tang JM, et al. Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chem. 2011;129:637–644. https://doi.org/10.1016/j.foodchem.2011.04.105
3. Singh BN, Singh BR, Singh RL, Prakash D, Singh DP, Sarma BK, et al. Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent

antioxidant and antimutagenic activities. Food Chem Toxicol. 2009;47:1161–1167. https://doi.org/10.1016/j.fct.2009.02.004

4. Yin M, Cheng W. Antioxidant activity of several *Allium* members. J Agric Food Chem. 1998;46:4097–4101. https://doi.org/10.1021/jf980344x
5. Pareek S, Sagar NA, Sharma S, Kumar V. Onion (*Allium cepa* L.). In: Yahia EM, editor. Fruit and vegetable phytochemicals: chemistry and human health. 2nd ed. Hoboken, NJ: Wiley Blackwell; 2017. p. 1145–1162. https://doi.org/10.1002/9781119158042.ch58
6. Arora EK, Sharma V, Khurana A, Manchanda A, Sahani DK, Abraham S, et al. Phytochemical analysis and evaluation of antioxidant potential of ethanol extract of *Allium cepa* and ultra-high homoeopathic dilutions available in the market: a comparative study. Indian Journal of Research in Homoeopathy. 2017;11:88–96. https://doi.org/10.4103/ijrh.ijrh_13_17
7. Gazuwa SY, Makanjuola ER, Jaryum KH, Kutshik JR, Mafulul SG. The phytochemical composition of *Allium cepa* / *Allium sativum* and the effects of their aqueous extracts (cooked and raw forms) on the lipid profile and other hepatic biochemical parameters in female albino wistar rats. Asian J Exp Biol Sci. 2013;4(3):406–410.
8. Krishnaswamy K. Traditional Indian spices and their health significance. Asia Pac J Clin Nutr. 2008;17(1):265–268.
9. Gangopadhyay KS, Khan M, Pandit S, Chakrabarti S, Mondal TK, Biswas TK. Pharmacological evaluation and chemical standardization of an ayurvedic formulation for wound healing activity. Int J Low Extrem Wounds. 2014;13:41–49. https://doi.org/10.1177/1534734614520705
10. Park J, Kim J, Kim MK. Onion flesh and onion peel enhance antioxidant status in aged rats. J Nutr Sci Vitaminol (Tokyo). 2007;53:21–29. https://doi.org/10.3177/jnsv.53.21
11. Jenwitheesuk K, Surakunprapha P, Jenwitheesuk K, Kuptarnond C, Prathanee S, Intanoo W. Role of silicone derivative plus onion extract gel in presternal hypertrophic scar protection: a prospective randomized, double blinded, controlled trial. Int Wound J. 2012;9:397–402. https://doi.org/10.1111/j.1742-481X.2011.00898.x
12. Zhou Y, Zhuang W, Hu W, Liu GJ, Wu TX, Wu XT. Consumption of large amounts of *Allium* vegetables reduces risk for gastric cancer in a meta-analysis. Gastroenterology. 2011;141:80–89. https://doi.org/10.1053/j.gastro.2011.03.057
13. Colli JL, Amling CL. Chemoprevention of prostate cancer: what can be recommended to patients? Curr Urol Rep. 2009;10:165–171. https://doi.org/10.1007/s11934-009-0029-4
14. Upadhyay RK. Nutraceutical, pharmaceutical and therapeutic uses of *Allium cepa*: a review. International Journal of Green Pharmacy. 2016;10(1):46–64.
15. Wang Y, Tian WX, Ma XF. Inhibitory effects of onion (*Allium cepa* L.) extract on proliferation of cancer cells and adipocytes via inhibiting fatty acid synthase. Asian Pac J Cancer Prev. 2012;13:5573–5579. https://doi.org/10.7314/APJCP.2012.13.11.5573
16. Gautam S, Platel K, Srinivasan K. Assessment of zinc deficiency and effect of dietary carrot, amchur and onion on zinc status during repletion in zinc-deficient rats. J Sci Food Agric. 2012;92(1):165–170. https://doi.org/10.1002/jsfa.4558
17. Sharma A, Patni B, Shankhdhar D, Shankhdhar SC. Zinc – an indispensable micronutrient. Physiol Mol Biol Plants. 2012;19(1):11–20. https://doi.org/10.1007/s12298-012-0139-1
18. Lakhanpal P, Rai DK. Quercetin: a versatile flavonoid. Internet Journal of Medical Update. 2007;2(2):22–37. https://doi.org/10.4314/ijmu.v2i2.39851
19. Begum AN, Terao J. Protective effect of quercetin against cigarette tar extract-induced impairment of erythrocyte deformability. J Nutr Biochem. 2002;13(5):265–272. https://doi.org/10.1016/S0955-2863(01)00219-4
20. Namdeo AG. Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn Rev. 2007;1(1):69–79.
21. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, et al. Plants and human health in the twenty-first century. Trends Biotechnol. 2002;20(12):522–531. https://doi.org/10.1016/S0167-7799(02)02080-2
22. Rates SMK. Plants as sources of drugs. Toxicon. 2001;39(5):603–613. https://doi.org/10.1016/S0041-0101(00)00154-9
23. Verpoorte R, Memelink J. Engineering secondary metabolite production in plants. Curr Opin Biotechnol. 2002;13:181–187. https://doi.org/10.1016/S0958-1669(02)00308-7

24. Sevo'n N, Oksman-Caldentey KM. *Agrobacterium rhizogenes*-mediated transformation: root cultures as a source of alkaloids. Planta Med. 2002;68(10):859–868. https://doi.org/10.1055/s-2002-34924
25. Rao SR, Ravishankar GA. Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv. 2002;20(2):101–153. https://doi.org/10.1016/S0734-9750(02)00007-1
26. Vanishree M, Lee CY, Lo SF, Nalawade SM, Lin CY, Tsay HS. Studies on the production of some important metabolites from medicinal plants by plant tissue cultures. Botanical Bulletin of Academia Sinica. 2004;45:1–22.
27. Namdeo G, Patil S, Fulzele DP. Influence of fungal elicitors on production of ajmalicine by cell cultures of *Catharanthus roseus*. Biotechnol Prog. 2002;18(1):159–162. https://doi.org/10.1021/bp0101280
28. Bhalsingh SB, Maheshwari VL. Plant tissue culture – a potential source of medicinal compounds. J Sci Ind Res (India). 1998;57:703–708.
29. Dornenburg H, Knorr D. Strategies for the improvement of secondary metabolite production in plant cell cultures. Enzyme Microb Technol. 1995;17:674–684. https://doi.org/10.1016/0141-0229(94)00108-4
30. Oksman-Caldentey KM, Hiltunen R. Transgenic crops for improved pharmaceutical products. Field Crops Res. 1996;45:57–69. https://doi.org/10.1016/0378-4290(95)00059-3
31. DiCosmo F, Misawa M. Eliciting secondary metabolism in plant cell cultures. Trends Biotechnol. 1985;3(12):318–322. https://doi.org/10.1016/0167-7799(85)90036-8
32. Ebel J, Cosio EG. Elicitors of plant defense responses. Int Rev Cytol. 1994;148:1–36. https://doi.org/10.1016/S0074-7696(08)62404-3
33. Sánchez-Ramos M, Bahena SM, Romero-Estrada A, Bernabé-Antonio A, Cruz-Sosa F, Gonzálesssz-Christen J, et al. Establishment and phytochemical analysis of a callus culture from *Ageratina pichinchensis* (Asteraceae) and its anti-inflammatory activity. Molecules. 2018;23:1258. https://doi.org/10.3390/molecules23061258
34. Bandekar H, Lele SS. Production of flavonol quercetin from cultured plant cells of banyan (*Ficus benghalensis* L.) Int J Innov Res Sci Eng Technol. 2014;3(5):12150–12157.
35. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15:473–479. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
36. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol. 1995;28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
37. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001;15:127–130. https://doi.org/10.1002/ptr.687
38. Duan Y. Ultraviolet–visible spectrum characterizations of quercetin in aqueous ethanol solution with different pH values. J Chem Pharm Res. 2014;6(9):236–240.
39. Lee FA, Mun FY, Yvonne TTF, Peh KK, Yusrida D. HPLC method for simultaneous quantitative detection of quercetin and curcuminoids in traditional Chinese medicines. J Pharmacopuncture. 2014;17(4):36–49. https://doi.org/10.3831/KPI.2014.17.035
40. Ghasemnezhad M, Javaherdashti M. Effect of methyl jasmonate treatment on antioxidant capacity, internal quality and postharvest life of raspberry fruit. Caspian Journal of Environmental Sciences. 2008;6(1):73–78.
41. Kim HJ, Fonseca JM, Choi JH, Kubota C. Effect of methyl jasmonate on phenolic compounds and carotenoids of romaine lettuce (*Lactuca sativa* L.). J Agric Food Chem. 2007;55(25):10366–10372. https://doi.org/10.1021/jf071927m
42. Sharma V, Janmeda P. Extraction, isolation and identification of flavonoid from *Euphorbia neriifolia* leaves. Arabian Journal of Chemistry. 2017;10(4):509–514. https://doi.org/10.1016/j.arabjc.2014.08.019
43. Jing W, Jie Q, Lingyun Y, Yanhua L. Enhanced production of flavonoids by methyl jasmonate elicitation in cell suspension culture of *Hypericum perforatum*. Bioresour Bioprocess. 2015;2:5. https://doi.org/10.1186/s40643-014-0033-5
44. Cui XH, Murthy HN, Jin YX, Yim YH, Kim JY, Paek KY. Production of adventitious root biomass and secondary metabolites of *Hypericum perforatum* L. in a balloon type airlift reactor. Bioresour Technol. 2011;102:10072–10079. https://doi.org/10.1016/j.biortech.2011.08.044

45. Cui XH, Murthy HN, Paek KY. Pilot-scale culture of *Hypericum perforatum* L. adventitious roots in airlift bioreactors for the production of bioactive compounds. Appl Biochem Biotechnol. 2014;14:1123–1128.

46. Veerashree V, Anuradha C, Kumar V. Elicitor-enhanced production of gymnemic acid in cell suspension cultures of *Gymnema sylvestre* R. Br. Plant Cell Tissue Organ Cult. 2012;108:27–33. https://doi.org/10.1007/s11240-011-0008-6

47. Ketchum RE, Tandon M, Gibson DM, Begley T, Shuler M. Isolation of labeled 9-dihydrobaccatin III and related taxoids from cell cultures of *Taxus canadensis* elicited with methyl jasmonate. J Nat Prod. 1999;62(10):1395–1398. https://doi.org/10.1021/np990201k

48. Saniewski M, Miszczak A, Kawa-Miszczak L, Wegrzynowicz-Lesiak E, Miyamoto K, Ueda J. Effects of methyl jasmonate on anthocyanin accumulation, ethylene production, and CO_2 evolution in uncooled and cooled tulip bulbs. J Plant Growth Regul. 1998;17(1):33–37. https://doi.org/10.1007/PL00007009

49. Vasconsuelo A, Boland R. Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci. 2007;172:861–875. https://doi.org/10.1016/j.plantsci.2007.01.006

50. Wang SY, Bowman L, Ding M. Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus* sp.) and promotes antiproliferation of human cancer cells. Food Chem. 2008;107(3):1261–1269. https://doi.org/10.1016/j.foodchem.2007.09.065

Zwiększenie zawartości antyoksydantów oraz flawonoidów (kwercetyny) poprzez elicytację jasmonianem metylu w kulturach tkankowych cebuli (*Allium cepa* L.)

Streszczenie

Cebula (*Allium cepa* L.) to warzywo o szerokim zastosowaniu (kulinaria, środek leczniczy) i powszechnie wykorzystywane na całym świecie. Właściwości lecznicze cebuli wynikają z wysokiej zawartości związków biologicznie czynnych, m.in. flawonoidów o działaniu antyoksydacyjnym, np. kwercetyny. Celem pracy było zwiększenie produkcji antyoksydantów ogółem i kwercetyny w kulturach tkankowych poprzez elicytację. W pracy udowodniono zwiększenie zawartości antyoksydantów ogółem i kwercetyny w cebuli z zastosowaniem jasmonianu metylu jako elicytora. Wykorzystano RP-HPLC (wysokosprawną chromatografię cieczową z odwróconymi fazami), w przebiegu izokratycznym przy prędkości przepływu 1,0 mL/min z zastosowaniem fazy ruchomej o następującym składzie: acetonitryl w mieszaninie z 1% kwasem octowym (60:40 v/v), przy długości fali 362 nm i czasie retencji 8,79 minuty. Największą zawartością antyoksydantów ogółem oraz kwercetyny charakteryzował się kalus z liści (odpowiednio 84,61 ±6,03% i 0,81 ±0,03 mg/g suchej masy) uzyskany przy zastosowaniu 100 μM jasmonianu metylu). Stwierdzono, że zawartość antyoksydantów ogółem i kwercetyny wyniosła odpowiednio o 2,3% i 13,9%. Optymalizacja wykorzystania jasmonianu metylu jako elicytora oraz określenie jego najbardziej odpowiedniego stężenia dla kultur kalusowych cebuli może być przydatne do produkcji innych metabolitów wtórnych o znaczeniu leczniczym, które mogą być wykorzystanie w przemyśle farmaceutycznym.