3 Biotech (2020) 10:482
https://doi.org/10.1007/s13205-020-02470-4

ORIGINAL ARTICLE

Salicylic acid mediated up regulation of carvone biosynthesis during growth phase in cell suspension cultures of *Anethum graveolens*

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Received: 24 July 2020 / Accepted: 5 October 2020 / Published online: 21 October 2020

Abstract
The study illustrates the system for enhanced production of a medicinally important unexplored compound, carvone occurring naturally in *Anethum graveolens*. The effect of salicylic acid (SA) on biomass yield, carvone biosynthesis, growth and major enzymatic antioxidant parameters in *A. graveolens* was evaluated. The effects of different combinations of benzyl adenine (BA) and 1-Naphthalene acetic acid (NAA) were tested. Murashige and Skoog (MS) medium comprising 1.76 µM BA + 3.24 µM NAA was the best for friable callus induction. The friable callus was used for the initiation of cell suspension culture. MS salts in combination with 4.4 µM BA and 2.6 µM NAA, 3% sucrose was appropriate for cell growth and bioactive compound accumulation. The cell suspension cultures were then treated with SA (0.1, 0.75 and 1.5 mM) as an elicitor for four weeks. An up regulation of enzymatic antioxidants, ascorbate peroxidase (APX); superoxide dismutase (SOD) and catalase (CAT) activity with increasing concentrations of SA whereas a reduction in guaiacol peroxidase (GPX) activity was recorded at the end of the growth phase. The results also showed that higher concentrations of SA significantly increased malondialdehyde (MDA) and Proline content. Cell suspension culture was then subjected to extraction and isolation. The quantification of carvone through HPLC analysis revealed highest amount of carvone (0.063%) in cell suspension culture treated with 0.1 mM concentration of SA whereas higher concentration 0.75 mM SA showed reduction in amount (0.035%) of carvone. SA elicited cell suspension culture offered an effective and favorable *in vitro* method to improve the production of carvone for its potential use in pharmaceuticals.

Keywords *Anethum graveolens* · Cell suspension cultures · Salicylic acid · Carvone · HPLC

Introduction

Anethum graveolens is an annual aromatic herb belonging to the family Apiaceae. Being one of the most important culinary herbs it's been extensively used as a flavoring agent and spice from approximately 2000 years. There is a history of its use as an essential home remedy to treat infections related to the digestive system (Kaur and Arora, 2010; Jana and Shekhawat 2010a). *A. graveolens* possesses a broad range of naturally occurring secondary metabolites endowed with a wide variety of structural diversity and biological activities (Raghawan 2006; Dhir et al. 2014). The herb has gained renewed interest as it contains higher levels of carvone, fundamentally a monoterpene it is the most important constituent, synthesized in maximum proportion and responsible for imparting the plant it's most important medicinal properties including its potential antiviral activity (De Carvalho et al. 2006; Coppens and Cesa 2014). It has now been widely used as a commercial product in food, cosmetic and pharmaceutical industry. Carvone is also used as flavor additive, fragrance enhancer, potato sprouting inhibitor, building block (Score et al. 1997), antimicrobial agent (Aggwart et al. 2002), anti tumor substance (Carter et al. 2000), insecticidal agent against fruit flies (Franzois 1997) and biochemical environmental indicator (Supuka and Berta 1998). Considering various applications of carvone and the mounting demand for this active component, the production of carvone needs to be improved using biotechnological approaches in an efficient way. In nature, the bioactive compound occurs

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in very small quantities therefore the production needs to be enhanced by in vitro culture techniques.

Plant cell culture is an effective feasible system to improve the production of secondary compounds of biological significance. In vitro cultivation of plant cells provides an additional advantage of manipulation of culture conditions (Jana and Shekhawat 2010b; Shekhawat et al. 2009, 2010). Biosynthetic pathways responsible for the synthesis of secondary metabolites are often inducible by exogenous addition of elicitor and precursor feeding leading to stimulation of secondary products accumulation (Barber et al. 2000; Gupta and Sharma 2014; Vats 2018). Hence elicitation has been considered as an effective strategy for enhanced accumulation of secondary metabolites.

Salicylic acid (SA), a potent signaling molecule in plants, plays different regulatory roles in growth, metabolism, development, interaction and biotic and abiotic stresses (Senaratna et al. 2000). These responses vary depending on the concentration and time duration of SA treatment. Although moderate doses of SA are known to improve the antioxidant activities and stimulate stress tolerance; higher concentrations lead to activation of the cell death pathway and develop sensitivity to stress (Yang et al. 2004). Furthermore, it is well recognized that stress tolerance is strictly associated with the antioxidant enzymatic activities, such as superoxide dismutase, ascorbate peroxidase and guaiacol peroxidase. The antioxidant activities in plants get activated in response to damage caused by the generation of ROS during stress conditions (Gupta and Huang 2014; Rao and Shekhawat 2016; Dwivedi et al. 2016). These biochemical estimations suggest a view about the developmental changes occurring during the growth phase of plant cell suspension cultures (Jana and Shekhawat 2010c; Jana and Shekhawat 2012a, b). Several studies report SA induced elicitation in plants; taxol synthesis in *Taxus chinensis* cell cultures (Wang et al. 2004); hypericin and pseudohypericin in *Hypericum perforatum* cell suspension cultures (Gadzovska et al. 2013); ginsenoside accumulation in *Panax ginseng* (Ali et al. 2006); Elutherosides in *Eleutherococcus koreanum* (Lee et al. 2015); Gymnemic acid in *Gymnema sylvestre* (Bhuvaneswari et al. 2014); Withanolides in *Withania somnifera* (Sivanandan et al. 2012); bioactive compounds in *Orostachys cartilaginous* (Wen et al. 2019) cell suspension cultures. However, insufficient information is available on the accumulation of carvone and its anti-oxidative mechanisms upon SA treatment. Meanwhile, no report could be traced regarding elicitation of carvone. The article gives detailed insights to the effect of SA on carvone production in cell suspension cultures of *A. graveolens* at shake flask scale.

Materials and methods

Collection of plant material and callus induction

Murashige and Skoog medium (Murashige and Skoog 1962) was used as the basal medium including 3% (w/v) sucrose and 0.7% (w/v) agar (chemicals for MS medium preparations were procured from HiMedia Laboratories, Merck, Germany). Phytohormones (HiMedia) from previously prepared stock solutions were added to the MS medium and pH (ELICO LI 120 pH meter) of the medium was maintained to 5.8 ± 2 by using 1 M KOH (Loba Chemie, Mumbai, India) / 1 M HCl (Fishers Scientific Co.). Leaf tissues from plantlets developed in vitro (Bulchandani and Shekhawat 2018) were utilized as explants for callus induction. The leaves were sliced into small pieces and inoculated on culture medium supplemented with different concentrations of BA and NAA. The inoculated flasks were incubated in thermostatically controlled culture rooms with a temperature of 26 ± 2 °C, a 12 h photoperiod provided by white fluorescent tubes (Philips, India Ltd.) with 40–50 µmole m^{-2} s^{-2} of light intensity; 55–60% RH.

Suspension culture development

Mature callus (0.5–1 g) obtained from the multiplication medium that comprised of MS augmented with a combination of 4.4 µM BA and 2.6 µM NAA was used as initial inoculums for the development of cell suspension cultures of *A. graveolens.* Three different concentrations of SA (molecular weight = 138.12; HiMedia, 250 g): 0.1 mM, 0.75 mM, 1.5 mM were incorporated into the MS liquid multiplication medium. The suspension cultures were incubated in an orbital shaker (Infors HT CH-4103 Bottmingen) with continuous agitation at a rate of 110 rpm. The samples from suspension cultures were collected after every seven days to study the biomass yield and packed cell volume.

Determination of lipid peroxidation

Lipid peroxidation was determined according to the procedure given by De Vos et al. (1989). The LP level was estimated by determining MDA using the extinction coefficient of 155 mM^{-1} cm^{-1}. 0.5 g of freshly isolated suspension cells from 7, 14, 21 and 28 days old elicited suspension cultures of SA were subjected to homogenization in 10 ml of thiobarbituric acid and trichloroacetic acid. The absorbance of the supernatant was recorded at 532 and 600 nm.

Determination of proline accumulation

Estimation of proline was carried out by the method given by Bates et al. (1973). 0.5 g of sample was homogenized

in 5 ml of sulfosalicylic acid. After centrifugation (Sigma Centrifuge, Sigma Aldrich) at 3000 × g for the duration of 20 min, the supernatant was reacted with glacial acetic acid (2 ml). After incubation at 100 °C for 60 min in a water bath, the reaction was terminated on ice bath. The mixture was spin for 1 min after subsequent addition of 4 ml toluene. The absorbance of chromatophores was recorded at 520 nm.

Enzymatic antioxidant assay

For the extraction of anti-oxidative enzymes, 0.5 g of freshly isolated suspension cultures were homogenized in 5 ml of pre chilled 50 mM phosphate buffer. The homogenate was subjected to centrifugation at 12,000 rpm for 10 min at 4℃. The supernatant so obtained was utilized for enzymatic estimation.

The SOD (superoxide dismutase) (EC 1.15.1.1) activity was estimated by the method given by Beauchamp and Fridovich (1971). The reduction of NBT by superoxide radicals to blue colored formazan was measured at 560 nm. The activity of Catalase enzyme CAT (EC 1.11.1.6) was determined by adopting the protocol given by Aebi (1974). The estimation was based on the disappearance of H_2O_2 in the presence of the enzyme source which was followed at 505 nm.

Ascorbate peroxidase activity, APX (EC 1.11.1.11) was calculated by the procedure suggested by Chen and Asada (1989). The rate of oxidation of ascorbic acid was assayed by observing the absorbance at 290 nm. The Guaiacol peroxidase enzyme activity, GPX (EC 1.11.1.7) was estimated by recording the absorbance at 436 nm according to the method given by Putter (1974).

Extraction procedure

The extraction of Carvone was achieved by the method given by Ahmed et al. (1980) with some minor modifications. Air-dried samples of suspension cultures were defatted with petroleum ether. Followed by treatment with KOH, the extracts were refluxed on steam bath for 90 min. Thereafter, they were extracted thrice with ethanol. After filtration, the samples were dried over anhydrous sodium sulfite; the extracts were dissolved in ethanol (2 ml) and stored at – 4 °C for HPLC analysis.

Quantitative estimation

Samples prepared by above-described procedure were further analyzed by HPLC by HPLC instrument (Perkin Elmer Series 200). The samples were passed through C18 column (150 × 4.6 mm), 0.5 micron. The samples were detected by PDA detector. The solvent system comprised of methanol and 1 ml TFA in 1000 ml filtered degas HPLC water. The quantitative estimation of Carvone production in cell suspension cultures of *A. graveolens* treated with or without SA was calculated using the formula:

$$\text{Quantity of Carvone } (\%) = \frac{\text{Teat area of sample}}{\text{Test area of standard}} \times \frac{\text{Amount of standard } (mg)}{\text{Dilution}} \times \frac{\text{Dilution } (ml)}{\text{Amount of sample } (mg)} \times \frac{\text{Potency of standard}}{100} \times 100$$

For calibration, the analytical grade standard of carvone (with 99% purity) were procured from Sigma (22,060–1 ml), Sigma Aldrich, USA.

Statistic analysis

All the data is presented as mean ± S.E. The effects of different concentration of SA on biochemical parameters and carvone content were analyzed statistically by Sigma plot (Version: 12). The level of significance was determined by ANNOVA by SPSS software (Version: 16) at $p \leq 0.05$.

Results and discussion

Explant and callus induction

Callus was induced from leaf explants of *A. graveolens.* MS medium devoid of plant growth hormones did not show callus initiation from explants. Callus growth initiation was observed on MS basal medium augmented with BA and NAA in combination. The growth of calli was observed from the incised edges of explants (Fig. 1.a). Lower concentration of BA gave rise to compact nodular callus whereas higher concentrations of BA in combination with NAA induced green friable callus (Fig. 1.b, c). The highest frequency of leaf disc showing callus formation (77%) was with BA (1.76 µM) and NAA (3.24 µM), respectively (Table 1). Similar results of callus proliferation on MS medium fortified with a combination of auxin and cytokinin have been reported in *Ceropegia bulbosa* (Dhir and Shekhawat 2013; 2014), *Salvadora persica* (Mathur et al. 2002a, b; Mathur et al. 2008).

Determination of fresh weight, dry weight and PCV in cell suspension cultures

Cell suspension cultures of *A. graveolens* were initiated from healthy friable callus. The suspension cultures were maintained in MS liquid augmented with a combination of BA (4.4 µM); NAA (2.6 µM) and 3% sucrose (Fig. 1.d).

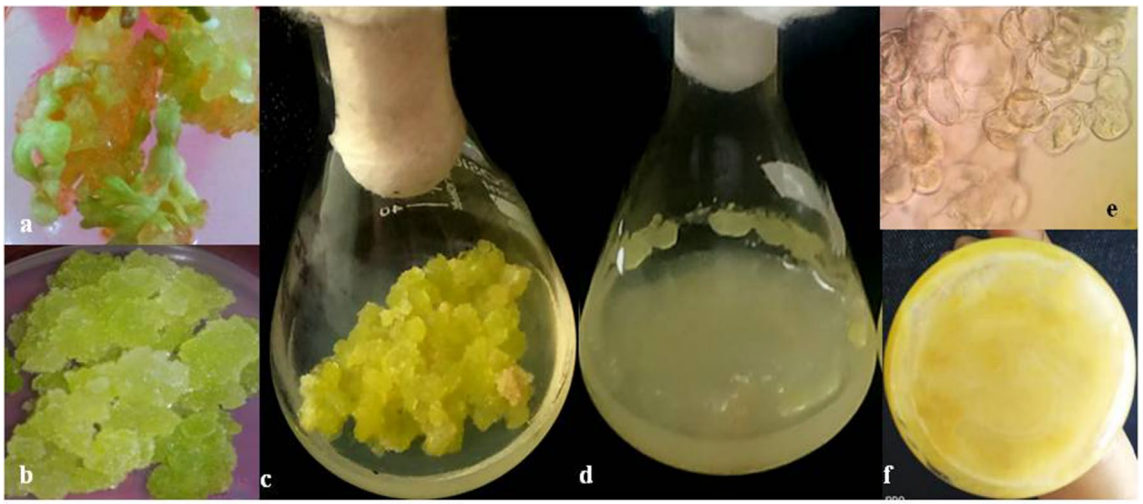

Fig. 1 Establishment of callus and cell suspension cultures of *A. graveolens*. **a** Initiation of callus from leaf explants **b** mature callus in MS + BA at 1.76 µM + NAA at 3.24 µM **c** sub cultured callus for seeding cell suspension d suspension culture of *A. graveolens* in flask **e** photomicrograph of cell suspension growth during exponential phase (10X) **f** cell aggregates at the bottom of flask after third subculture

Table 1 Effects of different combinations of BA and NAA on callus induction from leaf explants

S. no	PGR's (µM)		% Callogenesis	Nature of callus
	BA	NAA		
1	1.32	–	–	–
2	1.76	–	58 ± 0.0462^{c}	Green compact
3	1.76	3.24	77.70 ± 0.0267^{a}	Green friable
4	2.2	–	$69. \pm 0.0300^{b}$	Green compact
5	2.2	5.4	44 ± 0.0300^{d}	Green friable
6	2.2	8.1	72 ± 0.0300^{ab}	Green friable
7	3.08	2.7	72 ± 0.0737^{ab}	Green friable
8	3.96	2.7	30 ± 0.0533^{e}	Green compact
9	4.4	2.7	69.3 ± 0.0567^{b}	Multiple shoots
10	5.28	2.7	30 ± 0.0267^{e}	Green compact

Results recorded after 3 weeks of culture. Data represents mean ± SE. Means having the same letter in each column do not differ significantly at $P \leq 0.05$ (DMRT)

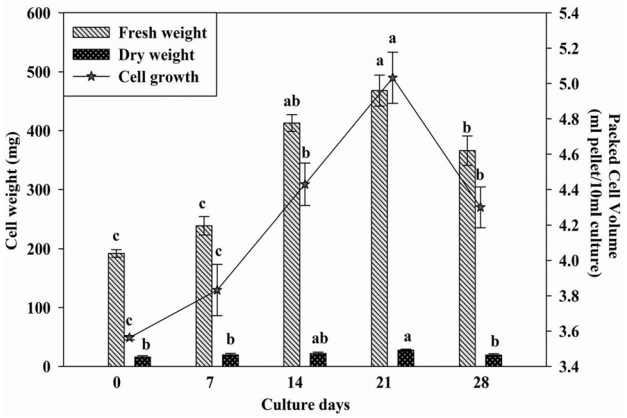

Fig. 2 Growth kinetics of the optimized cell suspension cultures of *A. graveolens*. Data represent mean ± SEM, $n = 3$. Means with same letter do not differ significantly at $P \leq 0.05$

Approximately 1 g of inoculum was sub-cultured from mother cultures for further multiplication of suspension cultures. The advantage of suspension cultures over other techniques is to minimize associated complex factors implicated in the organogenesis processes (Kim et al. 1995; Choi et al. 2000).

The suspension cultures analyzed for a period of four weeks determined that the cell growth exhibited a lag phase up to second week and subsequently the cells entered their exponential phase with a maximum dry weight of 3.49 mg/L on 21st day (Fig. 2 left axis). The production of terpenoids (carvone in the present study) is directly related to cell growth. A stationary phase was observed between 14 and 21 days during which maximum accumulation of carvone content was obtained. The cell cultures chiefly comprised of uniform cells of smaller size dispersed in medium and dense friable cell aggregates at the bottom of the flask (Fig. 1e, f). The cell suspension comprised of distinct types of cells ranging from spherical to elliptical. During the exponential phase of growth, the number of large spherical cells increases in cell cultures (Mathur and Shekhawat 2013). The growth of cell suspension cultures gets affected by the availability of macronutrients in the MS basal medium. Both, PCV and fresh weight of the cell cultures remained unaffected till 7th day

of seeding the suspension. Later, PCV and fresh weight increased gradually with a significant increase in cell suspension cultures upto 21st day which was maximum with 4.95 ml/ 10 ml; insignificant reduction in packed cell volume (4.3 ml/10 ml) was observed on 28th day (Fig. 2 right axis). The initial appearance of culture cells was green which subsequently turned brown while approaching 28th day of the culture period. Similar results were also reported in suspension cultures of *Stevia rebaudiana* which showed higher packed cell volume until 21st day of the growth cycle (Gupta 2014). Browning of the cell suspensions was also observed by Gadzovska et al. (2013) at the end of the elicitor treatment period for each SA concentration tested.

Non enzymatic parameters

The SA treated suspension cultures of *A. graveolens* showed a continuous rise in MDA content irrespective of the concentration of SA. Following 7th day, maximum increase (2.07 folds) was observed at 1.5 mM concentration of SA than in control (Fig. 3a). After two weeks, a significant rise (45%) was noticed in total MDA content at 1.5 mM concentration in SA treated cultures when compared to control. Similarly, after three weeks interval, 1.5 mM SA treated cultures increased twofold of non treated cultures (Fig. 3b, c). The observations of the present study are in corroboration with Ali et al. (2006), which showed that the formation of MDA content increased in the SA exposed roots of *P. ginseng* compared to the control. Through the growth

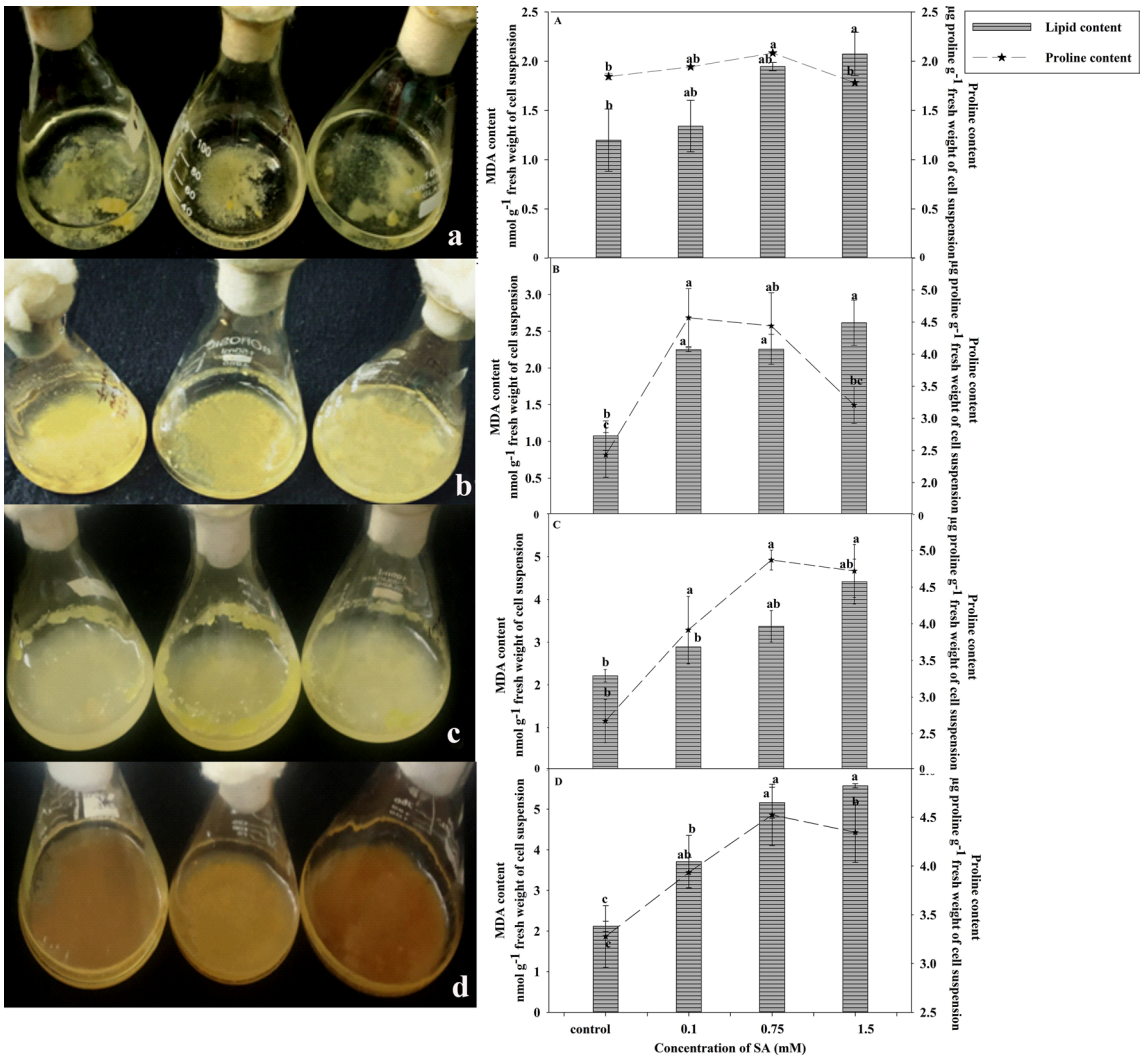

Fig. 3 MDA and proline content in cell suspension cultures of *A. graveolens*. The cell cultures were elicited with 0.1, 0.75 and 1.5 mM SA for a period of 4 weeks **a** Lag phase **b**; **c** exponential growth phase (second and third week) **d** declining phase (after fourth week) of SA elicitation. Control represents non treated cell line. Vertical bar and horizontal line represents mean ± standard error ($n = 3$) and were analyzed by one way ANOVA. The values are significant at 0.01 level

phase, the highest level of lipid peroxidation was observed at 1.5 mM SA concentration during the fourth week which was 2.54 times higher than MDA content during first week (Fig. 3d). ROS generation due to induced stress brings about peroxidation of membrane lipids, leading to membrane damage (Scandalios 1993). In accordance with these early studies, exposure to SA significantly increased the MDA content, an index of lipid peroxidation. Elicitor induced ROS generation has been reported previously by Gomes et al. (2006) which showed enhancement in MDA content at 28th day of the growth cycle. However, lower MDA increases observed in SA-treated suspension cultures during initiation of cell growth suggest a better protection from oxidative damage.

Proline has been reported to improve plant's resistance to oxidative stress by scavenging ROS, by means of increasing anti-oxidative enzyme activity, thereby maintaining redox equilibrium (Zafar et al. 2020). The proline content showed a significant increase at 0.75 mM concentration after 21 days in cell suspension with respect to initial days of the culture period. 58% increase in proline content at 0.75 mM concentration of SA was observed on 21st day with respect to 7th day of the culture period. During the concluding phase of the culture period, the maximum accumulation of proline was observed at 0.75 mM SA concentration (Fig. 3). The elevation in proline accumulation could be directly correlated to the onset of stress conditions for plants. Progressive culture period leads to a reduction in essential nutrients for further development of the cell. According to Misra and Misra (2012), SA induces the activity of γ-glutamyl kinase and pyrroline-5-carboxylate reductase enzymes responsible for proline biosynthesis under stress conditions resulting in an enhanced level of proline. SA has already been reported to cause growth enhancement and stimulate proline accumulation in wheat plants (Shakirova et al. 2003).

Enzymatic parameters

Visible changes in antioxidant enzymes were observed in SA treated cell suspension cultures when compared to control. Through the culture period, significant rise was observed in APX activity at 0.75 mM concentration after two weeks and 0.1 mM concentration after three weeks of the culture period (Fig. 4a). APX activity upregulated by 4.5 folds increase at 0.75 mM concentration of SA with respect to control whereas higher concentrations (1.5 mM) did not support APX activity. Therefore it appeared that APX played the most important role in offering resistance to H_2O_2 production. Similar increase in APX activity in suspension cultures of *Jatropha curcas* treated with Jasmonic acid was reported by Zaragoza et al. (2016). When compared to control, all

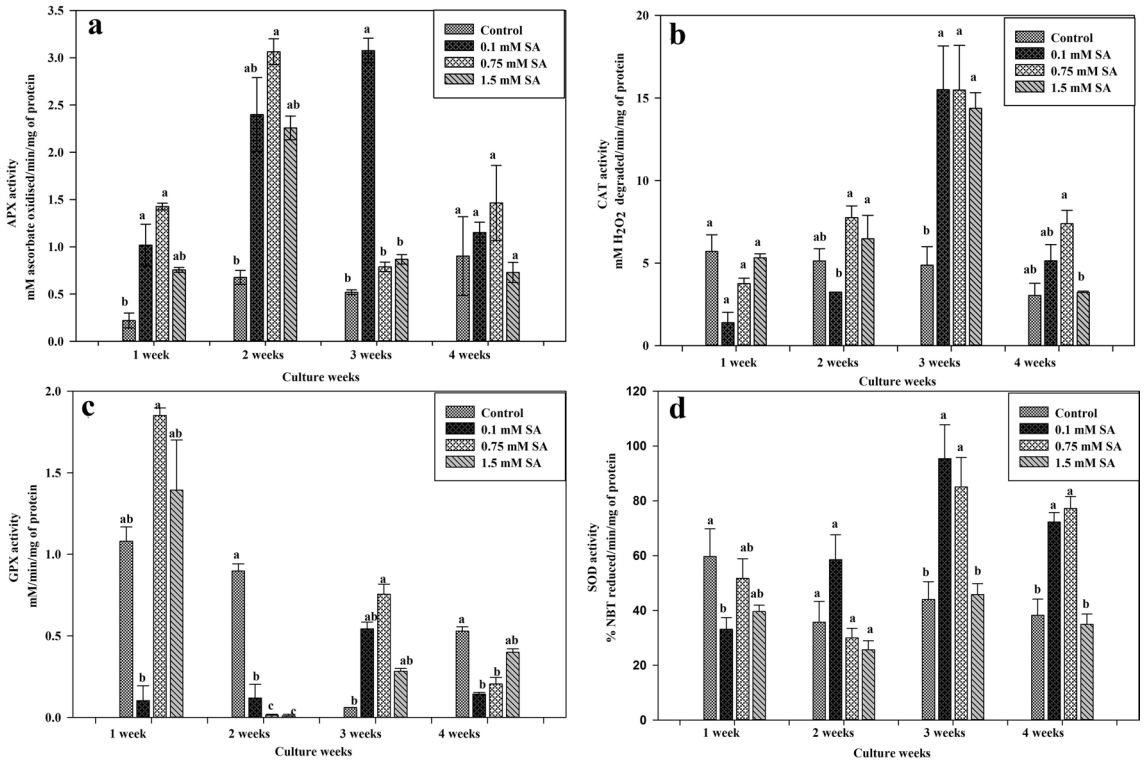

Fig. 4 Antioxidant enzyme APX (**a**), CAT (**b**), GPX (**c**), and SOD (**d**) activity under SA pretreatments (0.1, 0.75 and 1.5 mM), exposure duration (7, 14, 21 and 28 day) in cell suspension cultures of *A. graveolens*. Data represents the mean ± standard error ($n = 3$) and were analyzed by one way ANOVA. The values are significant at 0.01 level

the concentrations of SA improved the activity of Catalase. Among all the concentrations, maximum CAT activity with three folds increase compared to control was observed at 0.1 mM SA concentration (Fig. 4b). In the course of the culture growth period, the highest activity of CAT enzyme was recorded following the third week. SA-mediated stress responses are related to an increase in H_2O_2 generation by inhibition of CAT activity and promotion of peroxidase activity (Krantev et al. 2008). The amount of GPX was found to increase during initial days of culture period but showed a subsequent reduction with progressive culture growth. During the first week, highest GPX activity with 0.8% increase as compared to control was recorded at 0.75 mM SA concentration which continued till the third week of culture period (Fig. 4c). Studies have demonstrated that plants activate their defense systems by adjusting antioxidant molecule levels and inducing anti-oxidative enzymes to counteract oxidative stress (Noctor and Foyer 1998). Antioxidant defense enzymes such as APX, CAT and GPX are systems that are devised to minimize the ROS levels (Mittler 2002).

SOD activity is responsible for the degradation of the superoxide radical ($O_2^{\bullet -}$), producing O_2 and H_2O_2 (Ighodaro and Akinloye 2018). Here, enhancement in SOD concentration was recorded with progressing culture growth up to the third week which declined after the fourth week of the culture period (Fig. 4d). During the growth phase, the maximum SOD activity was noticed at 0.1 mM SA concentration which further got reduced at higher concentrations. In previous reports by Ali et al. (2006), SA induced SOD activity increased in roots of *P. ginseng* to maximum by 56% and retained the elevated levels in remaining SA-treated roots as compared to the control due to the increase of the scavenging capacity for O_2^- and prevents cellular damage.

Effect of SA on Carvone production

In the present investigation, the effect of different concentrations of SA on carvone content in cell suspension cultures of *A. graveolens* was examined on the 21st day of the growth phase. As reported in earlier studies by Bondarev (2001), the suspension cultures (control) collected after 3rd week and before 4th week produced the maximum amount of secondary metabolites (terpenoid) in comparison to initial culture weeks.

The content of carvone in the cell suspension cultures was significantly different among the treatments. The quantity

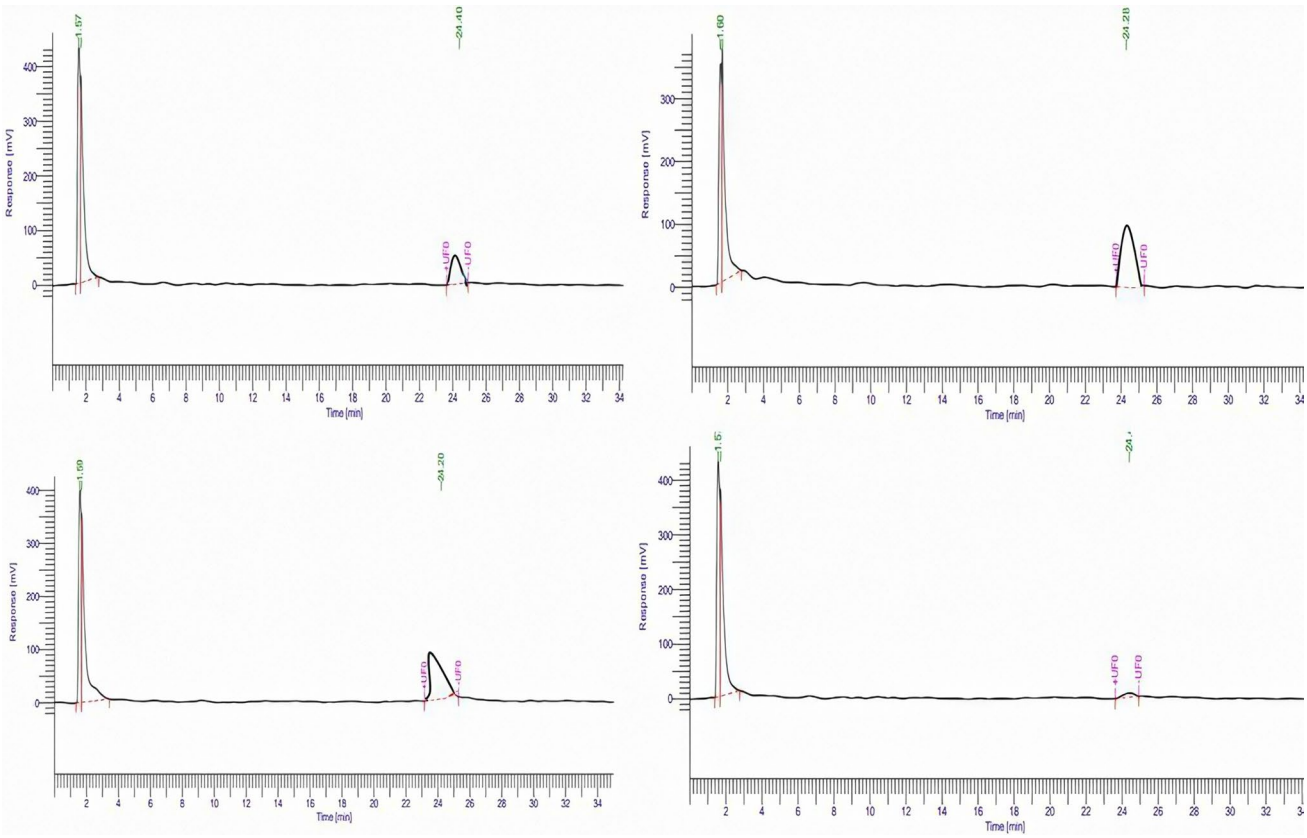

Fig. 5 HPLC chromatogram for cell suspension cultures of *A. graveolens* after 21 days of SA pretreatment: control (**a**) 0.1 mM SA (**b**) 0.75 mM SA (**c**) 1.5 mM SA (**d**). Detection wavelength = 254 nm

of carvone production in suspension cultures treated with SA has been depicted in Fig. 5; 0.1 mM concentration of SA stimulated a threefold increase in carvone content with respect to control cultures (0.063%). Highest APX as well as CAT activity was also recorded at this particular concentration. The results in this regard are supported by Wen et al. (2019) that reported lower concentrations of SA (25–150 µM) result in enhanced production of secondary metabolites in cell cultures of *Orostachys cartilaginous*. According to the study, the total polysaccharide content increased to the maximum with 218.5 mg/g DW at 100 µM SA, phenolic compound 27.2 mg/g DW, and flavonoid content 35.4 mg/g DW. Further, it was observed that the content of carvone reduced to 0.035% concentration at 0.75 mM SA treatment which is 1.8% more than control cultures (Fig. 6a). At 1.5 mM concentration, the carvone content reduced to its minimum and reached below the control value (0.013%) as compared with all the samples collected on the 21st day which shows that only lower concentrations of SA support carvone accumulation in cell suspension cultures of *A. graveolens*. For instance, Kang et al. (2009) reported 3.1 and 6.1 times improved production of ginkgolides A and B, compared with control, in two weeks old SA (0.1 mM) treated cell cultures of *Ginkgo biloba.*

The activated antioxidant defense mechanism at lower SA concentrations establishes the balance in antioxidant activities and nuetralization of free redicals but elevated concentrations and prolonged treatment duration affects the morphology and physiology of the cells. The results suggested a positive correlation between carvone accumulation and antioxidant activities at lower concentration of SA (Fig. 6b). However MDA content showed a negative correlation with metabolite accumulation which suggests that rise in SA concentration above the optimum levels for carvone accumulation leads to a negative impact on antioxidant activities as manifested by increase in lipid peroxidation levels. These results clearly confer that 0.1 mM concentration of SA could be used for the production of carvone on a large scale using cell suspension cultures of *A. graveolens*.

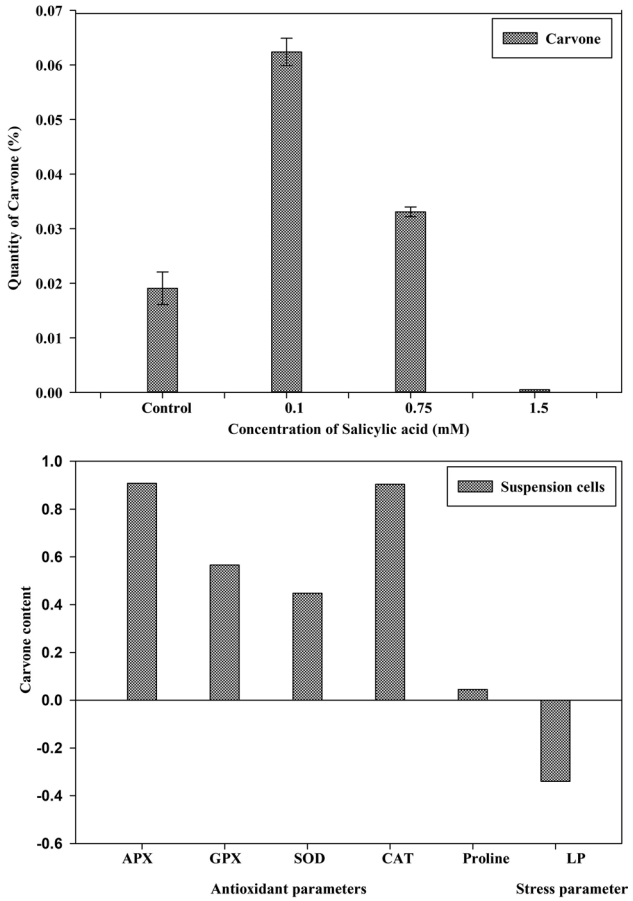

Fig. 6 a Total carvone content in cell suspension cultures of *A. graveolens* after 21 days of SA (0.1, 0.75 and 1.5 mM) pretreatment b Linear correlation of carvone content with antioxidant parameters in cell suspension cultures of *A. graveolens*. The correlation values are significant at 0.01 level

Conclusion

This is the first report on the subject of cell suspension culture studies in *A. graveolens* and analysis of the effect of SA during the growth period. The study demonstrated a feasible approach for SA induced enhanced accumulation of an overlooked therapeutically important compound; carvone in cell suspension cultures of *A. graveolens*. A synergistic correlation was manifested between the enzymatic parameters and carvone production at different levels of SA treatments. Through the growth phase, all the enzymatic antioxidant components maintained higher values except GPX in SA elicited cultures. However, the treatment of suspension cultures with SA as an elicitor showed a decrease in MDA content with increasing concentration of SA. The outcomes suggested that an optimum concentration of SA positively influences the production of carvone (Fig. 7). Present investigation confers SA as a promising elicitor for stimulation of the antioxidant defense system and carvone accumulation. The medicinal properties of carvone have refocused attention towards the improved production of this compound. Use of different elicitors for modification of plant secondary metabolism offers an alternative approach to induce some beneficial changes in the production of phytochemicals. The approach discussed in the present study could be efficiently utilized for exploring the potential for the commercial production of carvone from *A. graveolens* at pilot scale and subsequently commercial scale.

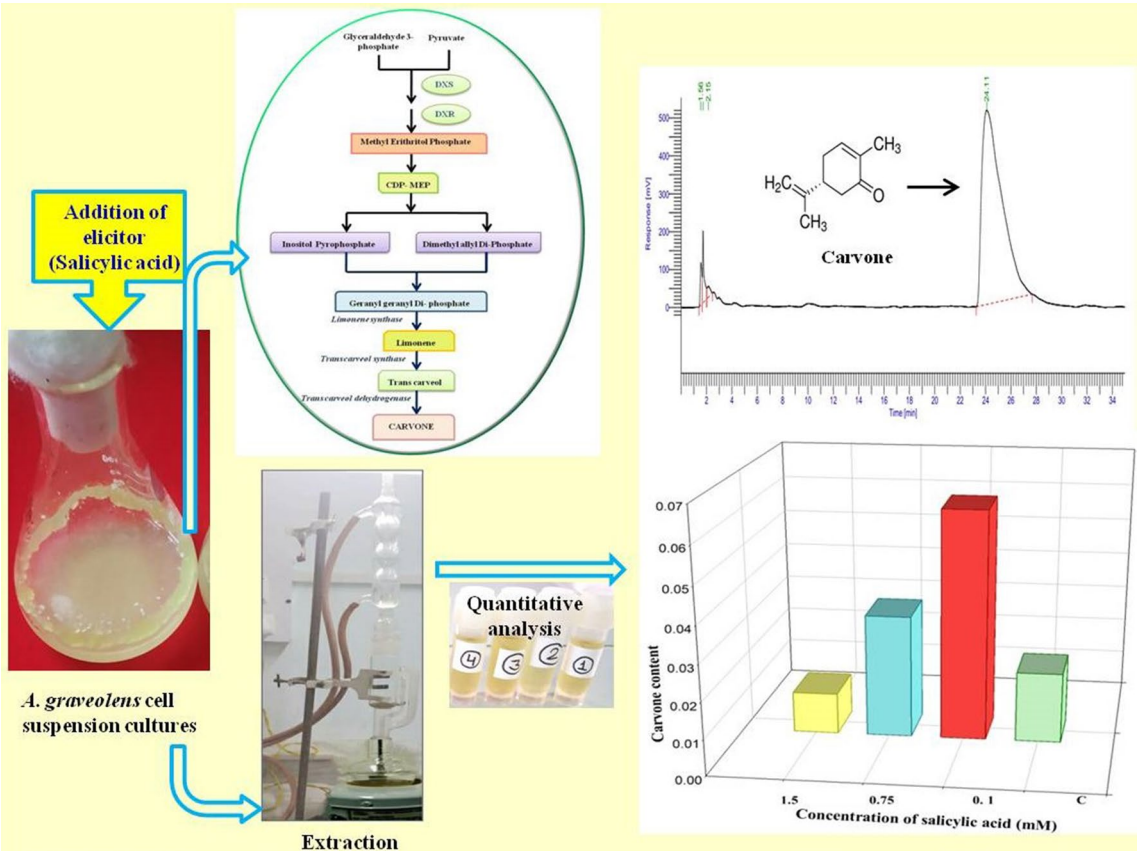

Fig. 7 A descriptive model of targeted approach for elicitor mediated enhanced accumulation of carvone used in the study

Acknowledgements Authors gratefully acknowledge University Grant Commission, New Delhi for providing financial assistance in the form of Centre for Advanced Study scheme.

Compliance with ethical standards

Conflict of interest The authors declare none.

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