Generation of Alkaloid-containing Idioblasts During Cellular Morphogenesis of *Peganum harmala* L. Cell Suspension Cultures

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**Abstract:** Viable heterotrophic cells growing in suspension cultures are heterogeneous in shape and size. Spherical, oval and elongated cell shapes were produced in suspension cultures of *Peganum harmala* grown in culture media supplemented with 2 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D). Spherical and oval cells grow through diffuse growth, while elongated cells grow by tip growth. Low alkaloid production rates were formed in these high growth rate heterogeneous cell populations. On subculturing suspended cells previously growing in liquid medium supplemented with 2,4-D to fresh medium without 2,4-D, a population of high alkaloid-producer idioblast cells were produced. Idioblasts were elliptical cells that have large central vacuole composed of β-carboline alkaloids and serotonin. They are growing through tip growth. The study discussed the development and maintenance of high quality cells growing in suspension culture that may produce efficient *in vitro* system for high alkaloid production.

**Key words:** Cell shape, cell viability, 2,4-dichlorophenoxyacetic acid, fluorescein diacetate, growth type, serotonin

**Introduction**

Microscopic examination of plant cells growing in suspension cultures is essential for both quantitative and qualitative determination of cell growth (Ibaraki and Kenji, 2001). It is possible to count cell numbers directly, to measure the size of single cells and cell aggregates and to estimate the growth rate (Gran and Eison et al., 1989). The color of cultured cells is directly related to their quality, especially in suspension cultures producing pigments and in cell cultures accumulated colored metabolites (Smith et al., 1993, 1995). Cell growth characteristics are also very important indices for cell suspension quality (Ibaraki and Kurata, 1997; Stirn et al., 1994). The distribution patterns of cell aggregate size in suspension culture vary significantly between cell lines and also as a consequence of culture age and culture conditions (Kieran et al., 1997). The aggregate size and its distribution pattern may be used as an index for cell quality (Satou et al., 1986).

Cell suspension cultures are industrially attractive because of their rapid growth, high metabolic rate and ease of manipulation (Kuchan, 1995; Verpoorte et al., 1993; DiCosmo and Misawa, 1995). However, plant cell cultures have some disadvantages, such as low and unstable productivity derived from heterogeneity of plant cells (Banthose, 1994). Additionally, in many cases the metabolite is accumulated intracellularly in the cell vacuole (Ferreira and Dulce, 1997). Quality evaluations of viable cells imply the selection of cell lines with desirable properties, which is necessary for cell quality maintenance (Adamse, 1991). The selection of high-producing cell lines is an important strategy in developing methods for the industrial production of useful secondary metabolites by cultured plant cells (Kieran et al., 1997). The growth characteristics of cultured cells in a suspension culture often reflect the cell status (Ibaraki and Kurata, 1997; Stirn et al., 1994).
In this study we found that cell shape and size of *Peganum harmala* L. cells were changed during alkaloid accumulation, therefore, cell shape may be used as an index for cell line selection. The present study aims at investigating cell shape, cell size, aggregate size, cell viability and accumulation of serotonin and β-carboline alkaloids in *P. harmala* L. cells growing in suspension cultures in order to relate specific cell shapes or sizes to definite metabolic rates.

**Materials and Methods**

*Cell Suspension Cultures*

Seeds of *Peganum harmala* were collected from Wadi El- Arabaime St. Catherine, Sinai, Egypt in July 2002. The seeds were stored dry at room temperature (30-35°C) until used. Seeds were surface sterilized with quick dip in 70% ethanol for 1 min then with 50% (v/v) solution of commercial bleach (sodium hypochlorite) for 20 min, followed by three washes in sterile distilled water. Ten replicates of 10 seeds each were germinated on MS medium (Murashige and Skoog, 1962) prepared from modified basal salt mixture (M8900, Sigma Chemical Co.) supplemented with half-strength of ammonium nitrate and potassium nitrate (total nitrogen is 30 mM). The seeds were germinated at 28±2°C in subdued light. Ten-day-old aseptically germinated seedlings were used as experimental material for callus initiation and subsequent cell suspension cultures. Media used in all experiments were supplemented with 3% sucrose and solidified with 0.8% Agar (LAB M Plant Tissue Culture Agar MC29, Amersham). Culture media were autoclaved for 15 min at 121°C and its pH was adjusted to 5.8 before autoclaving.

Callus was initiated from whole aseptically-germinated seedlings on agar solidified MS medium supplemented with 2 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Cell suspension cultures were initiated from third callus cycle. Cells were subcultured every three weeks by inoculating 5 mL of stationary cells (5×10^6 cell mL^-1) into 50 mL of fresh liquid medium in 250 mL Erlenmeyer flasks incubated on an orbital shaker at 120 rpm. Cultures were incubated at 28°C±1 under cool white fluorescent light in a 16 h photoperiod at 110 Wm^-2.

*Determination of Cell Viability*

Cell viability was assayed using fluorescein diacetate (FDA), which was prepared as 0.5% (w/v) in acetone and stored at 4°C (Widholm, 1972). Dilute FDA solution was prepared by adding 0.1 mL FDA stock solution to 5 mL liquid MS culture medium and stored on ice (used within 1 h). Aliquots of 0.01 mL from cell suspension of each sample was mixed with 0.01 dilute FDA solutions on a microscope slide and covered by a cover slip. After 2-5 min the slide was viewed under a Carl Zeiss Axiolab fluorescent microscope. The percentage of viable cells was determined. For each individual sample, 10^3 cells were scored.

*Morphometry*

Viable cells stained with fluorescein diacetate (FDA) was examined under the fluorescent microscope in order to investigate growth rate, cell shape, cell size (mean diameter (μm) × mean length (μm) and type of cell growth (diffuse or tip growth). The examined cells are heterogeneous plant cells growing in suspension cultures for three weeks. Cell length and width were determined from the central section of the cells. The ratio of cell length to cell width was used as a measure of cell elongation. Cells are considered elongating, when the cell length exceeds twice the width (Vissenberg et al., 2000). For each treatment 30 individual cells were scored. Experiments were repeated three times.

*Types of Cell Growth*

Polar growth of plant cells occurs by two main processes: diffuse and tip growth. Diffuse growth is characterized by cell extension that is dispersed over the entire cell surface. It is driven by turgor pressure and constrained by cellulose microfibrils arranged parallel to one another but transverse to the
axis of growth (Martin et al., 2001). The site of expansion in tip-growing cells is focused to the domed apex, which is filled with secretory vesicles. The microtubules in tip-growing cells occur as axial arrays parallel to the growth axis and are absent from the apical dome (Kopf et al., 1998). Actin filaments are arranged as long bundles along the length of the cell.

Four main characters were investigated in viable cells stained with fluorescien diacetate to determine the type of cell growth under the fluorescent microscope: 1) observation of cell morphogenesis to distinguish cells that have uniform expansion (diffuse growth) from cell expansion limited to one region of the cell (tip growth), 2) arrangement of microtubules as bundles along the longitudinal axis of the cell (tip growth), 3) during diffuse growth, the microtubules were arranged parallel to each other and transverse to the axis of growth, 4) observation of a an organelle-rich zone in the sub-apical region of cells growing through tip growth.

Alkaloid Extraction

Aromatic and dihydro-beta-carboline alkaloids as well as serotonin (5-hydroxytryptamine 5-HT), the indole alkaloid accumulated in Peganum harmala L. suspension cultures were determined. Fresh plant cells were extracted with methanol for 5 min in a homogenizer. The homogenate was left overnight at room temperature and then filtered. The filtrate was evaporated to dryness in a rotary evaporator at 40°C and the residue was dissolved in 3 ml methanol. The extract thus obtained was used immediately for analysis or stored freeze until used.

High Performance Liquid Chromatographic (HPLC) Analysis

Determination of β-carboline alkaloids and serotonin were done with a Hewlett Packard HP 1090 Liquid Chromatography supplemented with an automatic injection device (Kartal et al., 2003). Samples (25 μL) soluble in HPLC grade methanol were applied to a 250×4 mm RP8 (7 μm) column combined with a pre-column RP8 (5 μm). Samples were eluted with methanol: water: formic acid (166:34:1) (v/v/v) buffered with triethylamine at pH 8.5. The flow rate was 1 mL min and the column pressure was 400 psi. β-carboline alkaloids were detected at 330 nm, while serotonin at 302 nm. The alkaloids were identified and quantified by comparison with authentic standards separated at the same conditions.

Statistical Analysis

Mean length and width (μm) of the alkaloid-producer cells of various shapes and sizes grown on 2,4-D media and 2,4-D-free media, were reported as the mean of 30 cells for each treatment. Experiments were repeated three times and means and standard error of the means were calculated. All data were subjected to an analysis of variance (ANOVA). Significantly different parameters were subjected to Tukey's HSD test (Zar, 1984; Lentner and Bishop, 1986) in order to test the significance (p = 0.05) of cell growth parameters on selection of high-alkaloid producer cell lines and evaluation of cell quality.

Results

The relationship between cell size (diameter: length μm) and cell shape of plant cells growing in suspension cultures supplemented with 2 mg L^{-1} 2,4-D are showing in Table 1-2 and Fig. 1-2. As cells grow in suspension cultures for three weeks, they perform four phases of cell growth including: lag, log, stationary and decline phases. Cells entered the stationary growth phase were considered appropriate for scaling up growth and alkaloid production. They were also suitable for subculturing into fresh growth media. Cells examined in this study are harvested in the early stationary phase of growth after 21 days growth in liquid medium at 120 rpm on an orbital shaker.
Table 1: Various cell shapes of heterotrophic *Peganum harmala* L. cells growing in suspension cultures

<table>
<thead>
<tr>
<th>Cell shape</th>
<th>Type of cell growth</th>
<th>Abundance during growth phases</th>
<th>Average cell size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td>MS medium with 2,4-D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oval</td>
<td>Diffuse growth</td>
<td>Log growth phase</td>
<td>Small to medium-size cells</td>
</tr>
<tr>
<td>Spherical</td>
<td>Diffuse growth</td>
<td>Log growth phase</td>
<td>Small cells</td>
</tr>
<tr>
<td>Elongated</td>
<td>Tip growth</td>
<td>Late log phase and stationary growth phase</td>
<td>Large elongated and tubular cells</td>
</tr>
<tr>
<td>Irregular</td>
<td>MS medium without 2,4-D</td>
<td>Log growth phase</td>
<td>Curved cells like the crescent</td>
</tr>
<tr>
<td>C-Shape</td>
<td>Tip growth</td>
<td>Log growth phase</td>
<td>Elongated and tubular cells like the letter S.</td>
</tr>
<tr>
<td>S-Shape</td>
<td>Tip growth</td>
<td>Log growth phase</td>
<td>Large cells swollen in the middle with tapering ends, contain opaque metabolites in central vacuole, absorb purple color</td>
</tr>
<tr>
<td>Elliptical</td>
<td>Tip growth</td>
<td>Log and stationary phase</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean cell diameter and mean cell length of various cell shapes of heterotrophic *Peganum harmala* L. cells growing in suspension cultures

<table>
<thead>
<tr>
<th>Cell shape</th>
<th>Mean cell diameter (μm±SE)</th>
<th>Mean cell length (μm±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oval</td>
<td>6.33±0.26a</td>
<td>12.19±0.53d</td>
</tr>
<tr>
<td>Spherical</td>
<td>5.62±0.21b</td>
<td>5.62±0.21b</td>
</tr>
<tr>
<td>Elongated</td>
<td>10.22±0.64c</td>
<td>22.82±1.35d</td>
</tr>
<tr>
<td>Elliptical</td>
<td>11.25±1.25d</td>
<td>50.84±0.84c</td>
</tr>
<tr>
<td>Irregular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Shape</td>
<td>6.18±0.49a</td>
<td>26.95±1.70g</td>
</tr>
<tr>
<td>S-Shape</td>
<td>6.94±0.74a</td>
<td>43.89±1.21h</td>
</tr>
</tbody>
</table>

Numbers followed by the same letters are not significantly different at p = 0.000 (as analyzed by an analysis of variance (ANOVA) followed by Tukey’s HSD test).

Fig. 1: Mean cell size (diameter × length (μm)) of various *Peganum harmala* L. cell shapes growing in suspension cultures in the presence of 2 mg L⁻¹ of 2,4-dichloroacetic acid

Cell Shape and Size

Viable *Peganum harmala* cells growing in the presence of 2 mg L⁻¹ 2,4-D examined with fluorescent microscope showed various cell shapes, which include oval, spherical and elongated cells (Fig. 2A). Some irregular cell shapes are also observed in the late exponential and early stationary phases including C-shape, S-shape (Table 1). The mean diameter of cells is more or less similar in both spherical and oval cells (Table 2, Fig. 1 and 2A). Mean cell diameter of elongated and elliptical cells is
double the diameter of spherical or oval cells (Table 2 and Fig. 1). Mean cell length is different from various cell shapes and range from moderate to large in the order spherical < oval < elongated (Table 2). Generally, the increase in cell sizes was due to both length and diameter of the cells. An expansion in cell diameter was associated with cell elongation, which increased cell length through polar growth. The smallest cell size observed was the spherical cells (Table 2).

Cells grown on 2,4-D free liquid medium showed distinctive idioblast cells (Fig. 2B). Idioblasts are specialized plant cells that contain a distinctive chemical composition compared with surrounding cells. Idioblast cells of *P. harmala* producing alkaloids are elliptical in shape which are elongated and swollen from the middle due to the presence of large central cell vacuoles that accumulate great amount of β-carboline alkaloids and the indole alkaloid serotonin and red pigments intercellularly in their cell vacuoles (Fig. 3). Their characteristic opaque appearance (Fig. 2B) that absorbs purple color is also characteristic reaction revealed by irradiating serotonin by long wave UV light.

Analysis of variance followed by the BSD Tukey test showed that *P. harmala* cell suspension culture grown in liquid media in the presence or absence of 2 mg L⁻¹ 2,4-D produced various cell shapes that differ in size too (Table 2 and 3). Although spherical, oval, elongated (Fig. 2A) and elliptical cells are different in shape and size; elliptical cells are significantly distinct (Fig. 2B) due to
their swollen middle-shape and their characteristic tapering ends. Moreover, high alkaloids accumulated in the central vacuoles of elliptical cells show markedly opaque appearance in the UV light because they absorb purple color.

**Cell Viability**

Percentage of viable cells are high in cells grown in MS medium supplemented with 2,4-D or without 2,4-D (about 90%). The fluorescent green color of fluorescein, which was liberated as a reaction of the non-fluorescent stain, fluorescein diacetate with cellular esterase was accumulated within viable cells (Fig. 2A). Elliptical cells grown in the absence of 2,4-D showed opaque color vacuoles as large amount of metabolites (serotonin) are accumulated in their central vacuole (Fig. 2B).

**Growth Type**

The type of cell growth was examined for all studied cells using images examined by the fluorescent microscope by the characters indicated in the materials and methods part. Spherical and oval cells were developed through diffuse growth, while elongated and elliptical cells were growing through tip growth (Table 1). Photomicrographs of spherical cells show clearly the arrangements of cellulose microfibrils that are arranged parallel to one another but transverse to the axis of growth (Fig. 2A). On the other hand, irregular cell shapes (C-shape and S-shape) were growing through tip growth (Table 1).

**Aggregate Size**

Generally, P. harmala L. cells grown in suspension cultures are grow as single cells or as small cell aggregates. Spherical and oval cells tend to grow as small cell aggregates (Fig. 2A). Elongated and elliptical cells grow as single cells (Fig. 2B). Kidney-shape cells and irregular shape cells such as C-shape S-shape cells were grown as single cells.

**Cell Homogeneity/ Heterogeneity**

P. harmala L. cell suspension cultures grown in MS medium supplemented with 2 mg L⁻¹ 2,4-D show a population of heterogeneous low-alkaloid producer cells composed of various cell shapes and sizes (Fig. 2A). On subculturing suspended cells previously growing on MS liquid medium supplemented with 2 mg L⁻¹ 2,4-D on fresh medium without 2,4-D, a homogenous population dominated by high alkaloid-producing idioblast cells was produced (Fig. 2B). High-alkaloid idioblasts were large elliptical cells, which are elongated and swollen from the middle due to the presence of large central cell vacuoles that contains β-carboline alkaloids and the indole alkaloid serotonin. The central cell vacuole absorbs purple color and showed characteristic appearance. Viable cell counts of P. harmala L. cells grown in suspension cultures in the presence of 2 mg L⁻¹ 2,4-D are growing in dense population of cells of various shapes and sizes (Fig. 2A). This high count of heterogeneous cells gives high growth rate at the stationary phase of growth. Elliptical cells grown in a medium without growth regulators produce much lower growth rate resulted in less cell count at all growth phases (Fig. 2B).
Metabolite Productivity

Alkaloid content (μg g⁻¹ FW) of various *Peganum harmala* cells grown in suspension cultures differ with the addition or omission of the synthetic auxin 2,4-D. Five alkaloids were determined in the extracts of *Peganum* cells grown in liquid culture media supplemented with 2 mg L⁻¹ 2,4-D or growing in 2,4-D-free media. The alkaloids determined were the four β-carboline alkaloids: harmine, harmaline, harmol and harmalol and the indole alkaloid: serotonin. Cells grown in liquid media supplemented with 2 mg L⁻¹ 2,4-D produced lower amounts of alkaloids compared with those grown in 2,4-D free media (Fig. 3). Total alkaloid content of cells growing in the presence of 2,4-D was 9.07 μg g⁻¹ FW, while cells growing in 2,4-D free media produced total alkaloid content of 82.70 μg g⁻¹ FW. Harmaline, harmol and harmalol content of 2,4-D free media were more than ten-fold over that of 2,4-D containing media. Serotonin content of cells extracted from 2,4-D free media was ten-fold the quantity of cells grown in liquid media supplemented with 2,4-D (Fig. 3).

Discussion

The prime rational for culturing plant cells is the belief that knowledge of the properties of cells isolated from the plant will provide essential information regarding the properties and interrelationships of cells within the plant (Krikorian and Berquain, 1969). For many decades scientists have recognized the enormous potential of the culture of isolated individual plant cells as an experimental system for biochemical, physiological and cytotoxic studies of cellular processes in plants (Kieran et al., 1997). Average eukaryotic cells are about 20 μm. Plant cells growing in suspension cultures are usually heterogeneous in shape and size. Moderate plant cells growing in *in vitro* cultures are about 40-55 μm.

Since the 1990's *Peganum harmala* became a useful model system for investigating alkaloid manipulation in *in vitro* culture systems (Berlin et al., 1994). Tracing viable cell growth of *P. harmala* alkaloid-producing cells was the main scope of the present study. Three main outcomes were achieved from this study. Firstly, cytological examination of suspended plant cells in order to determine various parameters of cell growth. Secondly, *in vitro* screening and selection of high-alkaloid producing cell lines, which reveal the presence of distinct high-alkaloid containing idioblast cells. The third outcome was the indication of possible involvement of serotonin or its direct precursor tryptophan in the developments of specific cell shapes of cultured cells.
A plant cell normally adapts distinctive cell shape in order to perform a specialized function (Martin et al., 2001). *P. harmala* cells growing in vitro in the presence of 2 mg L\(^{-1}\) 2,4-D were spherical, oval and elongated cells as observed in the late exponential and early stationary phases. It is obvious that most cells were spherical or small oval and starts to develop into larger oval cells then elongate during cell division processes. Cells investigated in this study were either grown on 2 mg L\(^{-1}\) 2,4-D and subcultured on the same 2,4-D liquid media or subcultured on 2,4-D free liquid media. Therefore, it is reasonable to consider the primary effect of 2,4-D on either cultures. The plant growth regulator 2,4-D as an exogenous supplement to suspension culture media usually induce complex physiological alterations including permeability of cell walls and increase in pore size of cell wall (Hensel et al., 2002). The effect of 2,4-D on plant cell morphology has been analyzed by Yang et al. (1999) in suspension cultures of *Morus* cells. They described only minor cell elongation at low 2,4-D concentration (0.001-0.2 mg L\(^{-1}\)), significant cell elongation (0.5-2 mg L\(^{-1}\)) at higher concentration and that 2,4-D promoted cell elongation by water uptake. The auxin 1-naphthaleneacetic acid stimulates cell elongation of *Nicotiana tabacum* cell cultures at lower concentration than 2,4-D, while 2,4-D promotes cell division but not cell elongation (Campanoni and Nick, 2005). Since idioblast cells containing high alkaloid concentration are elliptical cells growing in liquid 2,4-D free media, the synthetic auxin 2,4-D may not influence directly cell elongation. However, the production of elliptical cells, which contains large central vacuoles are indication of the possible primary effect of 2,4-D, from which the cells were originated, on cell permeability due to the increase in cell wall pore size (Hensel et al., 2002). This observation may be applied to cultures subcultured numerous times on 2,4-D liquid media, which have larger and more elongated cells every subculture.

As shape of *P. harmala* cells may change during alkaloid accumulation, cell shape should be considered as an index for cell line selection of high-alkaloid-producing cells. Elliptical cell shape was dominant in the exponential growth phase of 2,4-D free liquid media. They accumulate large amounts of \(\beta\)-carboline and serotonin alkaloids intercellularly in their large cell vacuoles. Growing in auxin free media omit the possibility of having shape change as a result of exogenous auxins. However, tryptophan the precursor of serotonin produce auxin (Azmitia, 2001), which may stimulate changes in cell shape and this may involve in the production of elongated *P. harmala* cells at some stages of growth in auxin-free media. Alternatively, serotonin itself may be the causative agent of elliptical cells formation following its high accumulation in high-alkaloid idioblast cells of *P. harmala*. The effects of serotonin on cell morphology have long been recognized from previous studies with animal cells. Serotonin regulates cell proliferation, migration and maturation in a variety of cell types including platelets, endothelial cells, fibroblasts and neurons (Azmitia, 2001). Serotonin has been known to constrict blood vessels (Leven et al., 1983) and induce shape changes in skeletal muscles (Dodson et al., 2004). The biological mechanism used by serotonin to change cell morphology and induce proliferation is directly target the cytoskeleton with its main components the microtubules and the microfilaments (Tian and Lagnado, 1975). Cultural manipulation that induced the accumulation of beta-carboline alkaloids only and not the serotonin, fail to induce the production of such elliptical idioblasts. Similarly, there is no information in the literature concerning any apparent effect of harmine, harmaline, harmol or harmalol on plant cell morphogenesis.

Generally, research studies imply that alkaloid biosynthesis and accumulation involve a highly regulated process that includes the expression of pathways within organs, within specific cells, or within organelles inside those cells (Kutchai, 1995). Low alkaloid yield of callus and cell suspension cultures of *P. harmala* suggests that their biosynthesis is under a rigid-cell, tissue, development- and environment-specific control. Similar low indole alkaloid accumulation levels were noticed for *in vitro* cultures of *Catharanthus roseus*, due to the need for specific cells and specific cellular organelles for alkaloid accumulation (De Luca, 1993).

Cellular morphogenesis is usually induced during cell growth of plant cells in suspension cultures. Alkaloid containing idioblast cells of *P. harmala* showed different growth type than spherical and oval cells. Spherical and oval cells were developed through diffuse growth, while elongated and elliptical
cells were growing through tip growth. The majority of differentiating cells in intact plants show diffuse growth (Lyndon, 1990), while tip growth is observed only in some specialized plant cells like pollen tubes and root hairs. Cultured cells become polarized upon the addition of growth regulators in culture media and start to elongate from an initial spherical shapes (Vissenberg et al., 2000). The induction of polar growth that transforms diffusely growing Nicotiana tabacum cells into elongated cells occurs where localized auxin efflux starts a signal cascade that triggers molecules that reorient microtubules. These then guide cellulose deposition in the cell wall and leads to cell elongation (Vissenberg et al., 2001). Again as elliptical cells were grown in 2,4-D free media, the possible trigger of their cell elongation may be from the metabolites accumulated in their vacuoles.

Conclusions

In conclusion, the amount of total alkaloids accumulated in P. harmala cells growing in 2,4-D free liquid culture media was higher than cells growing in suspension cultures supplemented with a moderate exogenous level of 2,4-D. Generation of high alkaloid idioblasts by omitting 2,4-D is a simple manipulating scheme and a simple observable index of alkaloid accumulation in P. harmala cell cultures. Tracing such specialized idioblast cells during various cultural and biochemical exploitation techniques should be an inspiring task for further studies. The possible role of metabolites accumulated in cell vacuoles for cellular morphogenesis is an interesting assignment that may require further investigation.

References


