Effect of Light and Dark Conditions on Biomass Accumulation and Secondary Metabolite Production in Suspension Cultures of *Artemisia amygdalina* Decne

***Mohammad Yaseen Mir, Azra N. Kamili, Qazi P. Hassan[1] and Sumira Tyub**
Centre of Research for Development, University of Kashmir, Srinagar J&K India
[1] Indian Institute of Integrative Medicine (CSIR) Sanat Nagar, Srinagar
**Corresponding authors Email address: yaseencord36@gmail.com*

ABSTRACT

Light and dark periods play a significant role in morphogenetic response of plants particularly in synthesis of secondary metabolites. In this study effect of continuous light and continuous darkness on accumulation of dry biomass and production of secondary metabolites in suspension cultures of *Artemisia amygdalina* was investigated as both continuous light and continuous darkness induces stress in suspension culture systems. The light grown culture systems followed a prolonged log phase of 40 days. The maximum accumulation of dry biomass 9.7 g/l was assessed on day 35 in case of dark grown suspension cultures. The light grown cultures as compared to dark grown cultures showed enhanced production of phenolic content (8.2 mg /g DW) on day 40. However dark grown cultures showed higher production of flavonoids (2.5 mg/g DW) as compared to continuous light grown suspension cultures.

Key Words: *Artemisia amygdalina, continuous light, continuous light, suspension cultures phenolic content, flavonoid content.*

INTRODUCTION

Suspension cultures are of commercial importance in pharmaceutical industries as they are viable systems for the production of secondary metabolites. These culture systems synthesize secondary metabolites over extended periods in amounts comparable to those found in whole plants (Ali *et al*., 2015). The suspension culture systems are reliable alternatives for studying the physiological processes at molecular and cellular levels as they show high rate of cellular growth besides provide abundant material for analysis (Caretto *et al.,* 2011). *Artemisia amygdaliana* D. belonging to Asteraceae family is an endemic species of Kashmir valley. Because of anthropogenic activities it has become critically endangered (Dar *et al*., 2006). The plant extract is used locally for the treatment of epilepsy, piles, nervous disorders, cough, cold, fever, and pain (Khan *et al*., 2013). The women folk of the valley use it for amenorrhea and dysmenorrhoea (Dar *et al*., 2006). Economically important compounds like essential oils were also reported from this plant. And there are few reports regarding its regeneration in *in vitro* conditions. As artemisinin was reported to produce from the callus cultures of *A. amygdalina* (Rasool *et al.*, 2013) this plant species can be used as an alternative source of artemisinin which is produced till now only from *Artemisia annua* L.

Light plays a significant role in secondary metabolism of plants. Both stimulatory and inhibitory effect of light on the synthesis of

secondary metabolites are reported in many medicinal plants (Tabata *et al.,* 1974; Zhang *et al*., 2002). However such kind of study is not so far reported in *A. amygdalina*. Moreover it is reported that phenolic and flavonoid content of *A. amygdalina* plays a significant role in its antioxiditative activities. Under this fact the current study is intended to analyze the effect of light on dry biomass accumulation, total phenolic and flavonoid content and their correlation in suspension cultures of *Artemisia amygdalina* in comparison to dark.

MATERIAL & METHODS

Collection and selection of plant material

The whole plants of *A. amygdalina* were collected from Gurez valley, Kashmir (34.6333°N 74.8333°E). These were indentified at Centre for Biodiversity and Taxonomy, University of Kashmir under Voucher specimen No. 2642- (KASH).

Establishment of suspension cultures

The leaf explants inoculated on Murashige and Skoog (1962) (MS) medium augmented with 10 µM BAP and 2.5 µM NAA showed best response for callus formation. Under this line for the establishment of suspension culture, proliferated friable callus (3 week old) was transferred to 100 ml Erlenmeyer flasks containing MS basal medium supplemented with BAP 10 µM in combination of NAA 2.5 µM. The cultures were kept in shaker (26°C, 125 rpm). The data recording and analysis of the growth kinetics was assessed within the interim of 5 days for period of 45 days. The two experimental lines were set up one in continuous dark conditions and another in continuous light conditions.

Determination of secondary metabolites in suspension cultures

For the analysis of biomass accumulation the samples were collected from the medium and dried in oven (60^{o}C, 24 h) for dry weight (DW) exploration. The extraction of the samples was done as per methodology adopted by Ali *et al*., (2013) with slight modification. The dried samples were ground in a course grinder and powdered. The 100 mg powder was then extracted with 10 ml of methanol (90 % v/v). After that samples were sonicated four times with resting phase of 20 minutes and further subjected to centrifugation for 20 minutes at 7000 rpm. Subsequently supernatant was collected and stored at -20^{o}C or analyzed immediately.

The determination of phenolics was done by utilizing Folin Ciocalteu's reagent as per the methodology of Malick and Singh (1980). The absorbance of the samples was taken at 765 nm and calibrated against gallic acid.

The slightly modified colorimetry method of Chang *et al*., 2002 was utilized for the determination total flavonoid content. The absorbance of the samples was determined at 415 nm rutin.

Statistical analysis

Completely randomized design (CRD) was setup for carrying out the experiments and statistics was carried out by means of one way analysis of variance (ANOVA). Moreover Duncan's multiple range test was used for the determination of significant differences (P<0.05) for multiple comparisons.

RESULTS AND DISCUSSION

The *A. amygdalina* suspension cultures grown under light showed longer lag and log phases of 10 and 40 days respectively as compared to

cultures grown in dark. The light grown culture system is found to have lower profiles of dry biomass within log phase when compared to dark grown culture systems. Starting with the inoculum of 1.5 g/l more than double increment in biomass accumulation was analyzed on day 10 in case of dark grown cultures. Moreover in case of light grown cultures more than double increase in dry biomass accumulation was assessed on day 15. The maximum accumulation of dry biomass (9.7 g/l) was assessed on day 35 in case of dark grown cultures (Table 1). Furthermore, in case of continuous light grown suspension cultures the maximum accumulation of dry biomass (8.7 g/l) was assessed on day 40 (Table 1). It is pertinent to mention here that both the culture systems showed significant correlation in dry biomass accumulation within stationary phases.

Effect of dark and light conditions on total flavonoid production

The profiles of total phenolic content in *A. amygdalina* suspension cultures under dark and light conditions was assessed to have different growth phases. In case of light grown suspension cultures the maximum phenolic content (8.2 mg /g DW) was analyzed on day 40 (Fig.1) whereas in case dark grown suspension cultures the maximum phenolic content (6.4 mg /g DW) was analyzed on day 30 (Fig 1). Further, it is pertinent to mention here that light stimulated the production of higher levels of total phenolic content than to dark conditions. This may due to the fact that continuous light creates stress conditions within suspension cultures and hence stimulates signal transduction cascade for increment in production of phenolic compounds. Our result is in line with Ali *et al*., 2014 who reported increased production of phenolic content within suspension cultures of *A. absinthium* in response to light. Abbasi *et al*., 2007 also reported that light stimulation increased production of caffeic acid derivatives in hairy root cultures of *Echinacea purpurea*. Further, Beckwith *et al*., 2004 studied the relationship between anthocyanin production and light quantity in *Pennisetum setaceum*. They reported high light environments play a significant role in anthocyanin pigmentation. Similarly Zhang *et al*., 2002 reported that integration of jasmonic acid and light irradiation resulted in increment in anthocyanin biosynthesis within *Vitis vinifera* suspension cultures. All these studies show significant correlation with our studies.

Table 1. Table showing dry biomass under light and dark conditions

Culture days	DBM under light conditions (g/l)	DBM under dark grown conditions (g/l)
0	1.5	1.5
5	2.1	2.8
10	2.8	3.7
15	3.4	4.9
20	5.1	6.3
25	6.4	7.2
30	7.4	8.8
35	8.2	9.7
40	8.7	9.4
45	8.1	9.1

Effect of dark and light conditions on total flavonoid producation

In our study it was analyzed that dark grown cell biomass in suspension cultures resulted in higher production of flavonoids as compared to continuous light grown suspension cultures. The maximum flavonoid content (2.5 mg/g DW) (Fig. 2) in case of dark grown suspension cultures was found on day 40 (stationary phase). However in case light grown suspension cultures maximum flavonoid content (1.9 mg/g DW) (Fig. 2) was scored on day 35 (log phase). The reason for less flavonoid production under continuous light conditions may be because of transformation efficacy of some secondary metabolites. According to Poutaraud *et al*., 2001 under continuous light condition there occurs increased transformation efficiency of hypericin and as a result there is decrease in the accumulation of hypericin pigment in *Hypericum perforatum*. Interestingly, Walker *et al*., 2002 reported enhanced production of hypericin in dark grown suspension cultures of *H. perforatum* which is completely corroborating with our findings. The secondary metabolites undergo photo-block in continuous light conditions resulting in photoconversion.

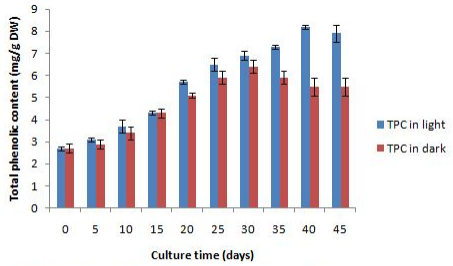

Fig 1. Total phenolic content (TPC) under continuous light and dark conditions

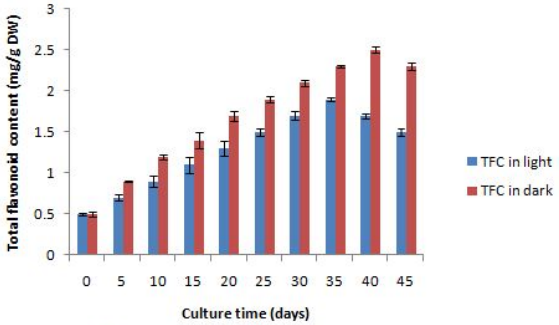

Fig 2. Total flavonoid content (TFC) under continuous light and dark conditions

CONCLUSION

The cultures grown under dark conditions show more flavonoid production where as cultures grown under light show enhanced production of phenolic compounds.

ACKNOWLEDGEMENTS

The authors highly acknowledge help provided by Prof Bashir Ahmad Ganai, Centre of Research for Development, University of Kashmir. The authors also acknowledge the comments from two anonymous reviewers which improved the quality of the manuscript.

REFERENCES

Abbasi, B. H., Tian, C. L., Murch, S. J., Saxena, P. K. and Liu, C. J. 2007. Light-enhanced caffeic acid derivatives biosynthesis in hairy root cultures of *Echinacea purpurea*. *Plant Cell Rep*, **26**: 1367–1372.

Ali, M. and Abbasi, B. H. 2014. Light-induced fluctuations in biomass accumulation, secondary metabolites production and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. *Journal of Photochemistry and Photobiology B Biology*, **140C**:223-227

Ali, M., Abbasi, B. H. and Ali, G. S. 2015. Elicitation of antioxidant secondary metabolites with jasmonates and gibberellic acid in cell suspension cultures of *Artemisia absinthium* L. *Plant Cell Tiss Organ Cult*, **120**: 1099–1106.

Ali, M., Abbasi, B. H. and Ihsan-ul-haq. 2013. Production of commercially important secondary metabolites and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. *Ind. Crops Prod*, **49**: 400–406.

Beckwith, A. G., Zhang, Y., Seeram, N. P., Cameron, A. C. and Nair, M. G, 2004. Relationship of light quantity and anthocyanin production in Pennisetum setaceum cvs. Rubrum and Red Riding Hood. *J. Agric. Food Chem*, **52**: 456–461.

Caretto, S., Quarta, A., Durante, M., Nisi, R., De Paolis, A., Blando, F. and Mita, G. 2011. Methyl jasmonate and miconazole differently affect arteminisin production and gene expression in *Artemisia annua* suspension cultures. *Plant Biology*, **13**: 51–58.

Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analaysis*, **10**:178-182.

Dar, A. R., Dar, G. H. and Reshi, Z. 2006. Conservation of *Artemisia amygdalina*-A critically endangered endemic plant species of Kashmir Himalaya. *Endangered Species Update*, **23**: 34-39.

Khan, M., Ganai, B. A., Ghazanfar, K., Akbar, S., Malik, A. H. and Masood, A. 2013. Evaluation of *Artemisia amygdalina* D. for anti-Inflammatory and immunomodulatory potential. *Hindawi Publishing Corporation ISRN Inflammation*. Article ID 483646, pages 5.

Mallick, C. P. and Singh, M. B. 1980. Plant enzymology and Histoenzymology. *Kalyani publishers, New Delhi. pp*, 286.

Poutaraud, A., Di Gregorio, F., Tin, V. C. F. and Girardin, P. 2000. Effect of light on hypericins contents in fresh flowering top parts and in an extract of St. John's wort (*Hypericum perforatum*). *Planta Med*, **67**: 254–259.

Rasool, R., Ganaie, B.A., Kamili, A. N., Akbar, S. and Masood, A. 2013. Synergistic effect of auxins and cytokinins on propagation of *Artemisia amygdalina* (Asteracae), a critically endangered plant of Kashmr. *Pak. J. Bot*, **45**(2): 629-634.

Tabata, M., Mizukami, H., Hiraoka, N. and Konoshima, M. 1974. Pigment formation in callus cultures of *Lithospermum erythrorhizon*, *Phytochemistry*, **13**: 927– 932.

Walker, Bais, H. P. and Vivanco, J. M. 2002. Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort), *Phytochemistry*, **60**: 289–293.

Zhong, J. J. Seki, T., Kinoshita, S. I. and Yoshida, T. 1991. Effect of light irradiation on anthocyanin production by suspended culture of *Perilla frutescens*. *Biotechnol.Bioeng*, **38:** 653–658.