INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 1, p. 507-517, 2019

RESEARCH PAPER OPEN ACCESS

Relationship of light intensity and quality with callus biomass and antioxidant potential in *Ajuga bracteosa*

Nizam Ud Din[1], Huma Ali[1,2], Syeda Kokab Shah[3], Syeda Faryal Israr[3], Amir Ali[4], Sher Mohammad[4], Raham Sher Khan[1], Mubarak Ali Khan*[1]

[1]*Department of Biotechnology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan (AWKUM), Mardan, Pakistan*

[2]*Department of Biotechnology, Bacha Khan University, Charsadda, KP, Pakistan*

[3]*Department of Environmental Sciences, University of Peshawar, KP, Pakistan*

[4]*Biotechnology Lab, Agricultural Research Institute (ARI), Tarnab, Peshawar, Pakistan*

Key words: Light, Biomass, Dark, Callus, *Ajuga,* Antioxidant

http://dx.doi.org/10.12692/ijb/15.1.507-517 Article published on July 30, 2019

Abstract

In the current study, effects of different light regimes and spectral lights were evaluated on the callus induction frequency, callus biomass and free radical scavenging activity in *Ajuga Bracteosa*. The hypocotyl explants derived from wild grown plants were used for callus culture establishment on MS solid media fortified with 1.0mg/L TDZ in combination with 0.5mg/L NAA. Among the different light regimes applied in this study, higher growth characteristics in callus cultures (maximum callus induction frequency: 90%) and biomass formation (5.6g/L FBM) were observed in the explants grown under continuous dark for two weeks culture period followed by transference into light (16h light & 8 h dark) for two weeks. The monochromatic lights were found most effective in terms of enhancement of biomass and antioxidant activity. Yellow light was found to influence maximum callus biomass (FW: 28g/L). Comparatively higher antioxidant activity (88%) was observed in yellow light grown callus tissues, followed by red light (80%). Hence, the findings of the current study emphasized the important role of different photoperiod regimes and monochromatic lights on callus induction, biomass accumulation and antioxidant activity in *A. bracteosa*.

* **Corresponding Author:** Mubarak Ali Khan ✉ makhan@awkum.edu.pk

Introduction

Ajuga bracteosa Wall. Ex. Benth commonly known as Kori Booti in Pakistan (due to its bitter taste) and 'Jan-i-adam' in Kashmir of family Labiatae is a highly valued endangered medicinal plant (Gautam *et al.,* 2011). There are 40-50 species in *Ajuga* genus, which are mostly found in temperate and subtropical regions of Pakistan, China, Kashmir to Bhutan, Malaysia and western Himalayas (Khare, 2007). In Pakistan *Ajuga bracteosa* is distributed in northern hilly areas (Chandel and Bagai, 2010). It has pink and white color flowers which are hermaphrodite (Pal and Pawar, 2011). It is used as a phyto-medicine for the treatment of different diseases, like rheumatism, gout, amenorrhoea and palsy (Jan *et al.,* 2014). The leaves of *A. bracteosa* are used for curing of measles, headache, stomach acidity and pimples (Sharma *et al.,* 2004). The powder of leaves of this plant is also useful against burn and boils, hypertension, sore throat, jaundice and as blood purifier. Additionally, the anti-cancerous, anti-inflammatory and anti-malarial activities of the plant extracts are also reported for *A. bracteosa* (Jan *et al.,* 2014). The biological activities of the extracts are due to the presence of different compounds present in different parts of *A. bracteosa*. From its aerial parts, different compounds such as, γ-sitosterol, β-sitosterol, tetracosanoic acid and tri acontanyldocosanoate have been isolated (Chopra and Nayar, 1956). Within the different valuable bioactive compounds isolated from the bark of *A. bracteosa* is a clerodanediterpenoid which is useful for curing jaundice and sore throat in experimental animal models (Rastogi and Mehrotra, 1990). *A. bracteosa* market demand has been intensively increased not only in Pakistan but also in other neighboring countries in the recent years. Therefore, *A. bracteosa* is in danger of extinction in Pakistan due to many factors including habitat destruction, illegal collection and lack of proper protection and cultivation procedures (Saeed *et al.,* 2017). Considering its paramount medicinal significance, research programs should be implemented on the conservation and sustainable utilization of *A. bracteosa*.

Establishment of the *in vitro* plant cultures is necessary for the conservation and continuous production of healthy plant material with sustainable metabolite profiles (Khan *et al.,* 2013). Cell cultures, especially callus cultures can produce a handful of secondary metabolites (Nikolaeva *et al*., 2009;Khan *et al*., 2019a ;Khan *et al*., 2019b). Callus cultures can be exploited further to provide inoculum for the establishment and development of cell suspension as a source of explant for plantlet regeneration for induction of somatic embryos and adventitious root cultures (Abbasi *et al.,* 2016) Callus cultures are usually affected by various *in vitro* conditions including but not limited to explant type, plant growth regulators, nutrient supply, carbon source and other environmental conditions (Khan *et al.,* 2016). A number of physical and environmental factors have been studied to check the extensive production of metabolites (Nagella and murthy, 2011). Strategies are applied during *in vitro* cultures to enhance the production of these valuable metabolites. Light has been studied extensively for its effects on *in vitro* growth and secondary metabolites accumulation in plants. Light is an important parameter that affects plant cell cultures in an array of ways ranging from its effect on growth and development (Shin *et al.,* 2006) to primary and secondary metabolism (Shohael *et al.,* 2006). The present research work was conducted to investigate the effects of plant growth regulators under the influence of different light regimes on callus growth parameters and evaluation of antioxidant potential in the regenerated callus tissues.

Material and methods

Plant material, sterilization, and preparation of explants

The wild grown plantlets of *A. bracteosa* were collected from Swat area of Khyber Pakhtunkhwa and used for callus induction. The plants were then sterilized to free explants from contamination; those can be maintained in aseptic environment. For this purpose, the plants were washed with tap water for 25 minutes followed by surface sterilization with 2% solution of sodium hypochlorite for 20-30 minutes. Then it was treated through 0.5% of mercuric chloride for 2-3 minutes. The plants were then washed through distilled water 5 times for 5 minutes. The young hypocotyl area was removed from the surface sterilize plantlets and was

cut into suitable size of 0.3 cm for making explants to be used in the experiments.

Optimization of different light regimes and plant growth regulators (PGRs) for callus induction and culture characteristics

Callus cultures were initiated from the surface sterilized hypocotyl explants (~0.3 cm), inoculated in each 100-ml flask containing 30ml of MS (Murashige and Skoog, 1962) medium containing 3% sucrose and 0.8% (w/v) agar and supplemented with varying concentrations of PGRs. The PGRs used were, Thidiazuron (TDZ), Indole-3-butyric acid (IBA), 2,4-dichlorophen-oxyacetic acid (2,4-D) and Naphthalene acetic acid (NAA) at varying levels (0.5, 1.0 and 1.5mg/L) or in combinations for callus induction and growth parameters in explants. Among all the PGRs treatments, NAA (0.5mg/L) + TDZ (1.0mg/L) were the optimum PGRs combination and was selected for subsequent experiments on to check the effects of different photoperiod regimes on callus growth characteristics. The different photoperiods employed in this study are listed in table 1. The pH of the media was adjusted at 5.6-5.8 before autoclaving and the cultures were placed at 25±2°C in a growth room having 16/24 hours of light, 35-45μmol/m²/s of irradiance, and approximately 70% relative humidity. Data was recorded after 4 weeks of culture cultivation as callus induction frequency calculated by number of responding explants divided by total number of cultivated explants into 100 and callus fresh and dry weight (g/L). The dry weight of callus was gravimetrically determined after drying at 60°C for 48 h.

Effects of different monochromatic colored lights on callus induction and culture growth characteristics

Three to four hypocotyl explants were cultivated on MS medium, fortified with TDZ (1.0mg/L) + NAA (0.5mg/L) under different spectral lights. For assessment of the effects of different monochromatic lights on callus formation, different spectral light sources were selected. The different spectral light sources were consisting of yellow, blue, green, red, and violet color tube rods. For control treatment cool-white light was provided. After four weeks of culture cultivation, data on growth parameters in callus cultures was collected as callus induction frequency (%), fresh weight (g/L) and dry weight (g/L).

Free radical scavenging activity

Antioxidant potential of the callus cultures, established in response to the effects of different light sources was determined according to the method of Abbasi *et al.,* (2010) through DPPH° free radical scavenging method. The dried plant extract (10mg) from each sample was dissolved in 4ml of methanol and was then added to a methanolic solution of DPPH (1, 1-diphenyl-2-picrylhydrazyl; 1 mM, 0.5ml). The mixture was then vortexed and the solution was read through spectrophotometer at 517 nm. The less absorbance showed higher scavenging activity. Ascorbic acid was then used as a standard antioxidant. The DPPH scavenging activity at various concentrations was checked by using formula,

Antioxidant activity of the sample = [(Ac1 - As2 / Ac1) × 100],

Where Ac1 = Absorbance of control and As2 = Absorbance of the sample.

Table 1. Application of various photoperiods in combination with PGRs from L1 to L9 for *in-vitro* callus induction of *A. bracteosa*.

Treatments	MS + PGRs (mg/L)	Light regimes	Period of Incubation
L1	TDZ (1.0mg/L) + NAA (0.5mg/L)	Darkness	24-hrs dark (4weeks)
L2	TDZ (1.0mg/L) + NAA (0.5mg/L)	Light	24-hrs light (4weeks)
L3	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	16-hrs light, 8-hrs dark (4weeks)
L4	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	3 weeks dark followed by 1 week light (16/8-hrs)
L5	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	2 weeks dark followed by 2 weeks light (16/8-hrs)
L6	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	1 week dark followed by 3 weeks light (16/8-hrs)
L7	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	3 weeks light (16/8-hrs) followed by 1 week dark
L8	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	2 weeks light (16/8-hrs) followed by 2 week dark
L9	TDZ (1.0mg/L) + NAA (0.5mg/L)	Photoperiod	1 week light (16/8-hrs) followed by 3 weeks dark

Results

Impacts of different photoperiod regimes on callus induction frequency and biomass formation

In this study, hypocotyl explants of *A. bracteosa* grown *in vitro* under continuous dark for two weeks followed by two weeks light (L5), resulted in the maximum callus induction (90%) and biomass formation (5.6g/L FBM and 3.2g/L DBM) on solid MS medium supplemented with TDZ (1.0mg/L) + NAA (0.5mg/L).

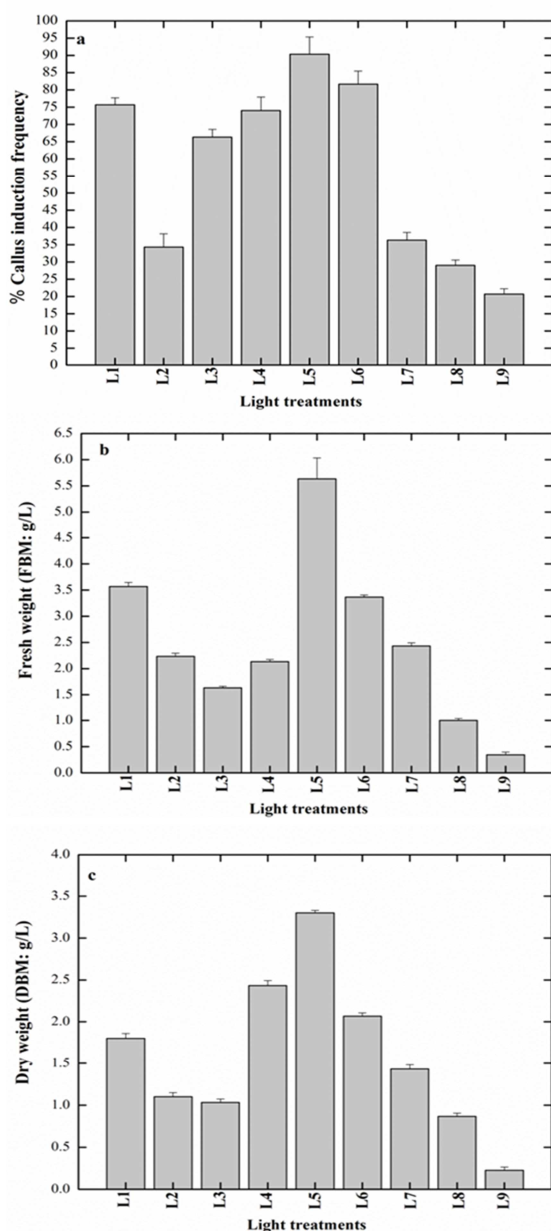

Fig. 1. Callus organogenesis in *A. bracteosa* as affected by different light treatments after 4 weeks culture period at significance level of P<0.05. (a): Callus induction frequency, (b): Fresh weight, (c): Dry weight.

It was further observed that when the culture cultivation period of explants in continuous dark exceeds the two weeks period, a gradual decrease in the callus growth parameters was observed. It can be seen in Fig 1a,b,c that three weeks dark pre incubation followed by one week light exposure caused a decrease in the induction frequency (80%) and biomass accumulation (3.0g/L FBM; 1.5g/L DBM) respectively. Incubation of hypocotyl explants under continuous dark for four weeks (L1) produced considerable values in the callus growth parameters i.e. callus induction frequency of (75%) and biomass formation (3.5g/L FBM and 1.8g/L DBM) respectively, on the same hormonal treatment. At control treatment (16 h light & 8h dark; L3) the explants were induced to form 65.3% callus of 1.6g/L FBM (Fig 1,b). Further, the pre incubation of culture flasks into continuous light followed by transference of flasks into dark condition (L7, L8 and L9) did not enhance the callus induction frequency as well as the biomass formation. Among all the light regimes very less values of callus induction (20%) and biomass formation (0.5g/L fresh weight) were observed in explants grown in L9 i.e one week light followed by three weeks dark.

Impacts of different photoperiod regimes on antioxidant potential in callus cultures

For determination of the antioxidant potential in the callus cultures raised *in vitro* under the effects of different photoperiod regimes, DPPH free radical scavenging assay was employed. Significant variations were observed in the callus cultures in response to the different light regimes. As indicated in Fig. 2, Maximum antioxidant activity (82%) was observed in the callus cultures grown under continuous dark (L1). It was followed by L5, where in 76% free radical scavenging activity was determined in the callus cultures raised *in vitro* by cultivation of explants in continuous dark for two weeks followed by transference into light treatment for two weeks (16 h light & 8 h dark). Continuous light luminance (L2) was found less inducer of the antioxidant potential in callus cultures. Among all the light regimes very less antioxidant activity (22%) was recorded in the callus cultures under continuous light (L2).

Over all light treatments were less effective in induction of antioxidant potential in callus cultures in present study (Fig 2). Pre exposure of the culture flaks to light followed by dark treatment resulted in lower antioxidant activity when compared with pre-incubation of flasks in dark followed by light treatment. Cultures grown in control photoperiod (L3: 16h light & 8 h dark) resulted in a moderate antioxidant response (45%) in the callus cultures (Fig 2). Thus it is evident that like the *in vitro* growth patterns in the callus induction and biomass formation, the antioxidant potential was also strictly influenced by the application of different photoperiod regimes in current study.

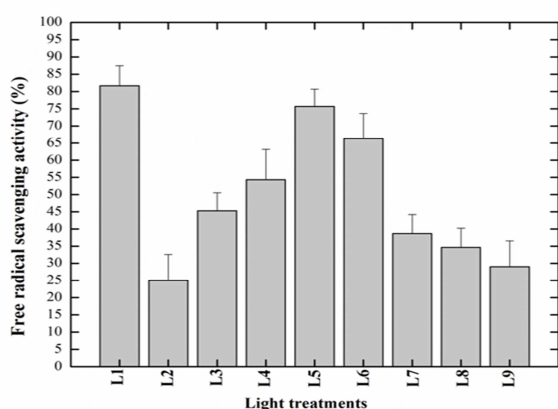

Fig. 2. Percent free radical scavenging activity in the callus cultures as affected by different photoperiod treatments at 4 weeks culture stage at significance level of P<0.05.

Impacts of different colored monochromatic lights on callus induction frequency and biomass formation

In this part of research studies we checked the effects of different colored lights on callus induction, biomass formation and antioxidant potential in *A. bracteosa*. Hypocotyl explants were exploited on MS medium containing 1.0mg/L TDZ in combination with 0.5mg/L NAA under different colored light sources. Highest callus induction frequency (90%) was observed in cultured flaks grown under yellow color. In control treatment (white color), the callus induction frequency observed was 75% (Fig. 3a). By applying green color light 32% callus induction frequency was observed. When blue color light was applied it gave 65% callus induction frequency. By applying red color light the callus induction frequency observed was good 81%.

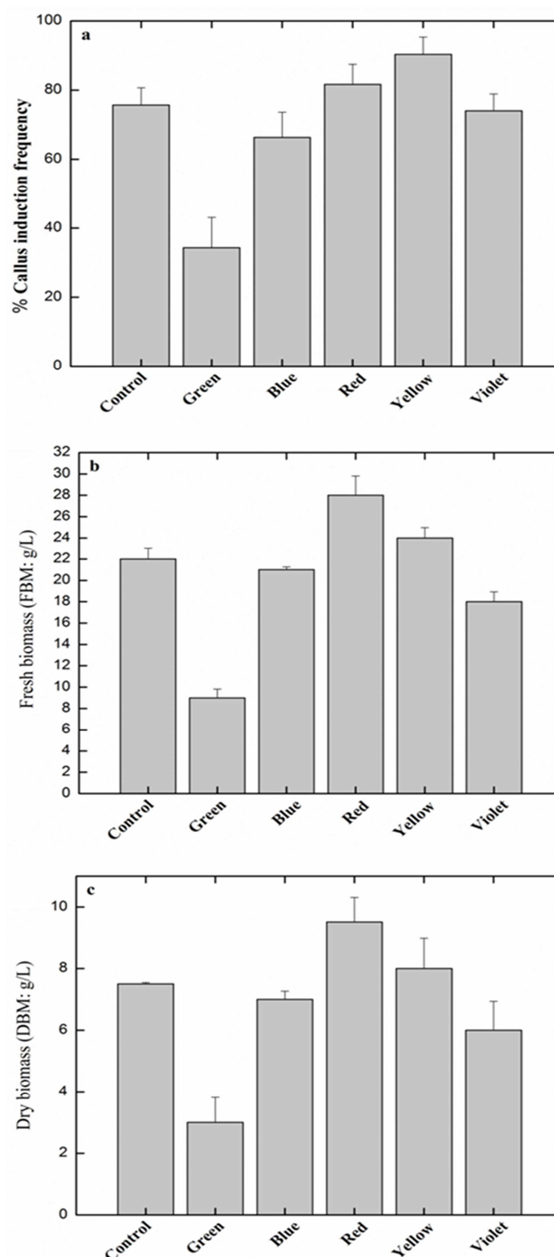

Fig. 3. Callus organogenesis in *A. bracteosa* as affected by different colored spectral lights after 4 weeks culture period at significance level of P<0.05. (a): Callus induction frequency, (b): Fresh weight, (c): Dry weight.

And in last when violet color light was applied it gave about 73% callus induction frequency. Fresh callus biomass (FBM) was checked by applying different color lights. In control treatment the fresh callus biomass observed was 22g/L. By applying green color light 9g/L fresh callus biomass was observed.

When blue color light was applied it gave 21g/L fresh callus biomass (Fig. 3b). By applying red color light the fresh callus biomass observed was the best 28g/L. Dry callus biomass (DBM) was also determined in callus cultures grown under different colored lights. By applying red color light the dry callus biomass observed was the best 9.5g/L (Fig. 3c). When yellow color light was applied the result obtained was good that was 8g/L dry callus biomass. However, under violet color light was applied it gave about 6g/L dry callus biomass.

Impacts of different monochromatic lights on antioxidant potential in callus cultures

The data on antioxidant potential in callus cultures raised *in vitro* under different monochromatic lights, showed that almost every colored light resulted in a significantly higher antioxidant activity in callus cultures (Fig. 4). In control treatment the callus cultures were observed to detoxify 68% of the free radicals through DPPH assay. Green color light resulted in 70% of antioxidant activity followed by blue color light (72%). An increase in the antioxidant activity was observed when the callus cultures were grown under red light which resulted in 80% activity. When yellow color light was applied the result obtained was the highest which was about 88%. Further under violet light the callus cultures were observed to have a free radical scavenging activity of 68% (Fig. 4).

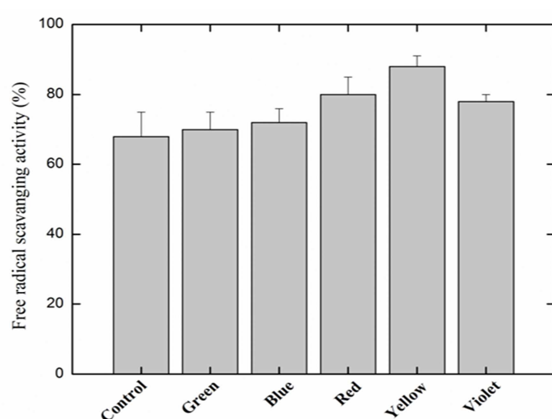

Fig. 4. Percent free radical scavenging activity in the callus cultures as affected by different colored spectral lights at 4 weeks culture stage at significance level of $P<0.05$.

A different response in the antioxidant potential was observed in the callus cultures under colored lights when compared with the callus grown under different photoperiod regimes. The application of different intensities of light and quality are thus useful in induction of the antioxidant potential in medicinal plants through *in vitro* cultures such as callus culture in the medicinally important plant *A. bracteosa* in the present study.

Discussion

Ajuga bracteosa is one of the vital pharmacological plant species with broad spectrum of health promoting attributes. It is an endangered plant species that needs to be properly maintained and utilized through advance technologies. Plant cell culture technology provides an efficient and alternative platform for conservation (ex-situ) of endangered medicinal plant species, where the conventional propagation procedures are limited (Ganaie *et al.,* 2016, Ibrar and Hussain, 2009). In the current study, effects of different light regimes and several monochromatic spectral lights were evaluated on callus induction frequency, callus biomass and free radical scavenging activity in *A. bracteosa*. A total of 9 different treatments on hypocotyl explant of *A. Bracteosa* were carried out on MS solid media containing TDZ (1.0mg/L) + NAA (0.5mg/L) for different incubation periods under different light regimes. Additionally the effect of different colors of light i.e. control (white color), green, blue, red, yellow and violet were also tested in this study. Among the different light regimes applied in this study we observed higher growth characteristics in callus cultures i.e higher induction frequency and biomass formation in the explants grown under continuous dark for two weeks followed by transference into light (16h light & 8 h dark) for two weeks. Moreover very poor growth response in cultured flaks was observed in presence of continuous light treatment. Light interacts with plant growth regulating substances during morphogenesis and thus the interplay between them can directly affect the cell division, growth and biomass formation (Khan *et al.,* 2013). Several studies have shown the promoting effects of light intensity, photoperiod or spectral quality on callus

organogenesis, somatic embryogenesis and secondary metabolites production in a variety of medicinal plants (Ali *et al.,* 2018; Khan *et al.,* 2017; Mohammad *et al.,* 2019). Similar to our study, it has been reported that continuous light irradiance can cause an intense stress condition in explants that can eventually results in lesser biomass formation (Khan *et al.,* 2017). Conversely, Ali and Abbasi (2014) reported a comparative elevated response in callus biomass under light than in dark grown cultures. In a similar study, Wu, C.H. *et al.* (2007) observed 68.5% callus induction under 16-hrs light and 8-hrs dark, while 51.6% callus induction frequency was observed under 12-hrs light and 12-hrs dark in the adventitious root cultures of *Echinacea purpurea*. The different colored lights also induced considerable induction and biomass formation of callus in explants. Yellow light was found to influence maximum callus induction frequency. However, red light resulted in the maximum accumulation of biomass in callus cultures. It can be inferred from this study that red-light might activated the gene expression and regulation of certain growth related factors in plant cell for instance, activation of phytochromes system is the main factor involved in the higher induction, promotion and biomass formation of callus in hypocotyl explants of *A. bracteosa*. In a similar fashion, H. Fazal *et al.,* (2016) observed callus growth in *Prunella vulgaris* L. under various spectral lights and concluded maximum callogenic response of (95%) induced by green light. In another study, Ali *et al* (2019) also recorded maximum production of biomass and medicinal metabolites through adventitious roots in *Ajuga bracteosa* under different spectral lights. Callus culture has also been known to be the essential step for the development of plantlet either through somatic embryogenesis or directly through culture (Ahmad *et al.,* 2014). Contrary Tariq *et al.* (2014) detected enhanced callus growth (90%) in *A. absinthium* upon subjection of white light followed by green light (82%) and dark incubation resulted in (70%). There has been a direct effect of light system on growth and development of plant including production of important medicinal compounds. The antioxidant activity of the callus cultured samples raised *in-vitro* under different light treatments were subjected through the DPPH free radical scavenging method as described in (Khan *et al.,* 2013). The control exhibited 68% of free radical scavenging activity. Comparatively higher antioxidant activity of 88% was observed under yellow light color, followed by 80% in red light. In a similar study, as compared to control, green and yellow lights have incremented the higher potential in the somatic embryos of *Eleutherococcus senticosus* (A. Shohael *et al.,* 2006). Comparatively, blue color light resulted in 72% of antioxidant activity. Senger (1982) observed a major role of blue light in formation of chlorophyll, stomata opening and chloroplast development, furthermore promotion of lettuce seedling growth. Evans (2001) observed enhanced growth with blue light while decreased growth with white light because of photochemical alterations of the culture medium. By applying green light color we observed free radical scavenging activity of 70%.Whereas the lowest antioxidant activity (68%) was found by applying violet light color. The reason behind the observed conflicting results by various researchers may be due to the differences in the intensities and sources of light and variation of light perceptions among the plant species (Tariq *et al.,* 2014). Though many reports have established the physiological and morphological effects of light color and quality, rather the responses varied considerably upon species of plants (da Silva *et al.,* 1997). In this study, the maximum dry biomass (9.5g/L) was observed by applying red color light after 4weeks. Under red light a higher amount of biomass and artemisinin were observed in hairy root cultures of *Artemisia annua* (Wang, Y *et al.,* 2001). Ghasemzadeh *et al.,* (2010) observed improved flavonoids synthesis in lower light intensities (310µmol m^{-2} s^{-1}) as compared to higher light pulses (790µmol m^{-2} s^{-1}) irradiated cultures of Haliabara species. However upon subjection to lower light intensities the antioxidant activity was greater in the leaves of Halia bara and Haliabentong when determined through 1,1-diphenyl-2 picrylhydrazyl (DPPH) method. The application of different intensities of light and quality enhanced the antioxidant potential in the callus cultures of *A. bracteosa* in the present study. It was evident that like the *in vitro* growth patterns in the callus

induction and biomass formation, the antioxidant potential was also strictly influenced by the application of different photoperiod regimes and spectral lights in current study. These results assertively related the impact of light on plant cellular growth and antioxidant potential. In a similar study, as compared to dark grown cultures of *Catharanthus roseus*, the light grown cultures accumulated lesser amount of ajmalicine which is an antihypertensive activity alkaloid (L. Almagro *et al.,* 2011).

Conclusion

Conclusively, the *Ajuga bracteosa* is a valuable medicinal plant species. The current study involves the establishment of callus culture and also investigated the effects of photoperiod regimes as well as distinct monochromatic lights on callus culture of *A. bracteosa*. The hypocotyl explants were used for callus culture establishment on MS media fortified with 1.0mg/L TDZ in combination with 0.5mg/L NAA. The monochromatic lights were found most effective in terms of enhancement of biomass and antioxidant activity. The highest callogenic response and biomass production was found in cultures maintained under yellow and red lights respectively. The Yellow light was found profitable in enhancement of DPPH activity as compared to other lights and control. Hence, the findings of the current study emphasized the important role of different photoperiod regimes and monochromatic lights in callus production, biomass accumulation and antioxidant activity of *A. bracteosa*.

References

Abbasi BH, Ali H, Yucesan B, Saeed S, Rehman K, Khan MA. 2016. Evaluation of biochemical markers during somatic embryogenesis in *Silybum marianum* L. 3 Biotech **6,** 71. https://doi.org/10.1007/s13205-016-0366-1

Abbasi BH, Khan MA, Mahmood T, Ahmad M, Chaudhary MF, Khan MA. 2010. Shoot regeneration and free-radical scavenging activity in *Silybum marianum* L. Plant Cell, Tissue and Organ Culture (PCTOC) **101,** 371-376. https://doi.org /10.1007/s11240-010-9692-x

Ahmad N, Abbasi BH, Fazal H, Khan MA. Afridi MS. 2014. Effect of reverse photoperiod on *in vitro* regeneration and piperine production in *Piper nigrum* L. Comptes Rendus Biologies **337(1),** pp.19-28. http://dx.doi.org/10.1016/j.crvi.2013.10.011

Ali H, Khan MA, Kayani WK, Dilshad R, Rani R, Khan RS, Khan T. 2019. Production of biomass and medicinal metabolites through adventitious roots in Ajuga bracteosa under different spectral lights. Journal of Photochemistry and Photobiology B: Biology **193**. 109-117 https://doi.org%2F10.1016%2Fj.jphotobiol.2019.02.010

Ali H, Khan MA, Ullah N, Khan RS. 2018. Impacts of hormonal elicitors and photoperiod regimes on elicitation of bioactive secondary volatiles in cell cultures of Ajuga bracteosa. Journal of Photochemistry and Photobiology B: Biology **183,** 242-250. https://doi.org/10.1016/j.crvi.2013.10.011

Ali M, Abbasi BH. 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. https://doi.org/10.1016/j.jphotobiol.2014.

Almagro L, Sabater-Jara AB, Belchí-Navarro S, Fernández-Pérez F, Bru R, Pedreño MA. 2011. Effect of UV light on secondary metabolite biosynthesis in plant cell cultures elicited with cyclodextrins and methyl jasmonate. In Plants and Environment. InTech.

Chandel S, Bagai U. 2010. Antiplasmodial activity of Ajuga bracteosa against Plasmodium berghei infected BALB/c mice.

Chopra RN, Nayar SL. 1956. Glossary of Indian medicinal plants, Council of Scientific And Industrial Research; New Delhi.

da Silva MM, Debergh PC. 1997. The effect of light quality on the morphogenesis of in vitro cultures of *Azorina vidalii* (Wats.) Feer. Plant cell, tissue and organ culture **51(3),** pp. 187-193. https://doi.org /10.1023/A:1005988621036

Evans P, Halliwell B. 2001. Micronutrients: oxidant/antioxidant status. British Journal of Nutrition **85(S2)**, pp. S67-S74. https://doi.org /10.1049/ BJN2000296

Fazal H, Abbasi BH, Ahmad N, Ali M. 2016. Elicitation of medicinally important antioxidant secondary metabolites with silver and gold nanoparticles in callus cultures of Prunella vulgaris L. Applied biochemistry and biotechnology **180,** 1076-1092. https://doi.org/10.1007/s12010-016-2153-1

Ganaie H, Ali M, Ganai B, Kaur J, Ahmad M. 2016. GC-MS analysis and evaluation of mutagenic and antimutagenic activity of ethyl acetate extract of Ajuga bracteosa Wall ex. Benth: An endemic medicinal plant of Kashmir Himalaya, India. Journal of Clinical Toxicology **6,** 288. 10.4172/2161

Gautam R, Jachak SM, Saklani A. 2011. Anti-inflammatory effect of Ajuga bracteosa Wall Ex Benth. mediated through cyclooxygenase (COX) inhibition. Journal of ethnopharmacology **133,** 928-930. DOI: 10.1016/j.jep.2010.11.003.

Ghasemzadeh A, Jaafar HZ, Rahmat A. 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiberofficinale Roscoe). Molecules **15(6),** pp. 4324-4333. https://doi.org/10.3390/molecules1506

Hare C. 2007. Indian Medicinal Plants-An Illustrated Dictionary. 1st Indian Reprint Springer (India) Pvt. Ltd., New Delhi, India **28.**

Ibrar M, Hussain F. 2009. Ethnobotanical studies of plants of Charkotli hills, Batkhela district, Malakand, Pakistan. Frontiers of Biology in China **4,** 539. https://doi.org/10.1007/s11515-009-0045-2

Jan M, Singh S, Kaloo ZA, Maqbool F. 2014. Callus induction and multiple shoot regeneration in Ajuga bracteosa Wall. ex Benth. An important medicinal plant growing in Kashmir Himalaya. J Sci Innov Res **3,** 319-324.

Kazmi A, Khan MA, Ali H. 2019a. Biotechnological approaches for production of bioactive secondary metabolites in Nigella sativa: an up-to-date review. International Journal of Secondary Metabolite **6(2),** 172-195. https://doi.org/10.21448

Kazmi A, Khan MA, Mohammad S, Ali A, Kamil A, Arif M, Ali H. 2019b. Elicitation directed growth and production of steviol glycosides in the adventitious roots of *Stevia rebaudiana* Bertoni. Industrial Crops and Products **139,** 111530. www.DOI.org%2F10.1016%2Fj.indcrop.2019.111530

Khan MA, Abbasi BH, Ahmed N, Ali H. 2013. Effects of light regimes on in vitro seed germination and silymarin content in Silybum marianum. Industrial Crops and Products **46,** 105-110. http://dx.doi.org/10.1016/j.indcrop.2012.12.035

Khan MA, Abbasi BH, Ahmed N, Ali H. 2013. Effects of light regimes on in vitro seed germination and silymarin content in Silybum marianum. Industrial Crops and Products **46,** 105-110. DOI: 10.1016/j.indcrop.2012.12.035

Khan MA, Abbasi BH, Ali H, Ali M, Adil M, Hussain I. 2015a. Temporal variations in metabolite profiles at different growth phases during somatic embryogenesis of *Silybum marianum* L. Plant Cell, Tissue and Organ Culture (PCTOC) **120,** 127-139**.**DOI:10.1007/s11240-014-0587-0

Khan MA, Abbasi BH, Shah NA, Yücesan B, Ali H. 2015b. Analysis of metabolic variations throughout growth and development of adventitious roots in *Silybum marianum* L.(Milk thistle), a medicinal plant. Plant Cell, Tissue and Organ Culture (PCTOC) **123,** 501-510. DOI: 10.1007/s11240-015

Khan MA, Khan T, Ali H. 2019a. Plant cell culture strategies for the production of terpenes as green solvents. Ind. Appl. Green Solvents **50,** 1–20. https://doi.org/10. 21741/9781644900239-1.

Khan MA, Khan T, Riaz MS, Ullah N, Ali H, Nadhman A. 2019b. Plant cell nanomaterials interaction: growth, physiology and secondary metabolism. Compr. Anal.Chem **84,** 23–54. https://doi.org/10.1016/bs.coac.2019.04.005

Khan T, Abbasi BH, Khan MA, Azeem M. 2017. Production of biomass and useful compounds through elicitation in adventitious root cultures of *Fagonia indica*. Industrial crops and products **108,** 451-457. DOI: 10.1016/j.indcrop.2017.07.019

Khan T, Abbasi BH, Khan MA, Shinwari ZK. 2016. Differential effects of thidiazuron on production of anticancer phenolic compounds in callus cultures of *Fagonia indica*. Applied biochemistry and biotechnology **179,** 46-58. https://doi.org/ 10.1007/ s12010-016-1978-y

Mohammad S, Khan MA, Ali A, Khan L, Khan MS. 2019. Feasible production of biomass and natural antioxidants through callus cultures in response to varying light intensities in olive (*Olea europaea*. L) cult. Arbosana. Journal of Photochemistry and Photobiology B: Biology **193,** 140-147. https:// doi.org%2F10. 1016%2Fj.jphotobiol. 2019.03.001

Nagella P, Murthy HN. 2011. Effects of macroelements and nitrogen source on biomass accumulation and withanolide-A production from cell suspension cultures of *Withania somnifera* (L.) Dunal. Plant Cell, Tissue and Organ Culture (PCTOC) 104, 119-124. DOI: 10.1007/s11240-010-9799-0

Nikolaeva TN, Zagoskina NV, Zaprometov MN. 2009. Production of phenolic compounds in callus cultures of tea plant under the effect of 2,4-D and NAA. Russian Journal of Plant Physiology **56,** 45-49. https://doi.org/10.1134/S10214437090100

Pala A, Jadona M, Katarea Y, Singoura P, Rajakb H, Chaurasiyaa P, Patila U, Pawar R. 2011. Ajuga bracteosa wall: a review on its ethn opharmacological and phytochemical studies. Der Pharmacia Sinica **2,** 1-10.

Rani R, Khan MA, Kayani WK, Ullah S, Naeem I, Mirza B. 2017. Metabolic signatures altered by in vitro temperature stress in Ajuga bracteosa Wall. ex. Benth. Acta physiologiae plantarum **39,** 97. DOI 10.1007/s11738-017-2394-9

Rastogi R, Merhotra B. 1990. Compendium of Indian Medicinal Plants published by Central Drug Research Institute. Lucknow and National Institute of Sciences Communication and Information Resources, New Delhi **1994,** 395-398.

Saeed S, Ali H, Khan T, Kayani W, Khan MA. 2017. Impacts of methyl jasmonate and phenyl acetic acid on biomass accumulation and antioxidant potential in adventitious roots of Ajuga bracteosa Wall ex Benth., a high valued endangered medicinal plant. Physiology and Molecular Biology of Plants **23,** 229-237.https://doi.org/10.1007/s12298-016-0406-7

Senger H. 1982. The effect of blue light on plants and microorganisms. Photochemistry and Photobiology **35(6),** pp.911-920. https://doi.org /10.1111/j.1751-1097.1982.tb02668.x

Sharma P, Mohan L, Srivastava C. 2004. Larval susceptibility of *Ajuga remota* against anopheline and culicine mosquitos.

Shin KS, Murthy HN, Heo JW, Hahn EJ, Paek KY. 2008. The effect of light quality on the growth and development of in vitro cultured Doritaenopsis plants. Acta Physiologiae Plantarum **30,** 339-343. DOI: 10.1007/s11738-007-0128-0

Shohael A, Ali M, Yu K, Hahn E, Islam R, Paek K. 2006. Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. Process Biochemistry 41. DOI: 10.1016 /j.procbio.2005.12.015

Tariq U, Ali M, Abbasi BH. 2014. Morphogenic and biochemical variations under different spectral lights in callus cultures of *Artemisia absinthium* L. Journal of Photochemistry and Photobiology B: Biology **130,** pp. 264-271. https://doi.org /10.1016 /j.jphotobiol.2013.11.026

Wang **Y,** Zhang **H,** Zhao **B,** Yuan **X.** 2001. Improved growth of Artemisia annua L hairy roots and artemisinin production under red light conditions. Biotechnology Letters **23,** 1971-1973. DOI: 10.1023/A:1013786332363

Wu CH, Murthy HN, Hahn EJ, Paek KY. 2007. Enhanced production of caftaric acid, chlorogenic acid and cichoric acid in suspension cultures of *Echinacea purpurea* by the manipulation of incubation temperature and photoperiod. Biochemical Engineering Journal **36(3),** pp.301-303. DOI: 10.1016/j.bej.2007.02.024

Yousaf R, Khan MA, Ullah N, Khan I, Hayat O, Shehzad MA, Naeem I. 2019. Biosynthesis of anti-leishmanial natural products in callus cultures of *Artemisia scoparia*. Artificial cells, nanomedicine, and biotechnology **47(1),** 1122-1131. https://doi.org/10.1080/21691401.2019.1593856