# Influence of light on diosgenin and sterol production in *Dioscorea deltoidea* callus

**G. A. Ravishankar* and S. Grewal**
Plant Tissue Culture Section, Regional Research Laboratory, Jammu Tawi 180 001, India
*Present address: Autotrophic Cell Culture Discipline, Central Food Technological Research Institute, Mysore 570 013, India

***Dioscorea deltoidea*** **callus exposed to blue or red or white light was superior to dark-incubated cultures in terms of growth of tissues and diosgenin and sterol production on absolute basis. Blue light enhanced diosgenin whereas red light inhibited the same. Moreover, blue light could reverse the inhibitory effect of red light on diosgenin and sterol production. The maximum increase in production of diosgenin and sterol was 2.0-fold and 1.5-fold respectively in a treatment with 15-day exposure to blue light followed by a dark period.**

THERE is increasing interest in industrial production of secondary metabolites by plant tissue and cell culture methods[1]. Pharmaceutical compounds produced by these methods command greater attention than other chemicals[2]. Extensively studied parameters for growth of cultured cells and metabolite production are tissue or cell characteristics, media composition, temperature, dissolved oxygen, pH and conductivity. Secondary metabolite production is also influenced by quality and quantity of light[3]. In this communication we report the influence of quality of light on diosgenin and sterol production in callus cultures of *Dioscorea deltoidea*.

Seedlings of *D. deltoidea* collected from the Kashmir valley were germinated aseptically. The callus was initiated from hypocotyls of 10-day-old seedlings on modified MS medium[4] supplemented with 1 mg/l 2,4-D and 3% sucrose and maintained on the same medium by regular subcultures of 45 days interval in continuous light of 3000 lux. The stock cultures were incubated in the dark for 15 days to minimize the carryover effect. Callus masses weighing 300 ± 10 mg were transferred on to the MS medium and incubated under different light conditions for 30 days. One set of treatment had continuous light for 30 days, viz. blue (475–500 nm), red (680–720 nm), white light and dark period. The other sets had 15 days of one type of light followed by 15 days of the other, viz. red/blue, blue/red, dark/blue, dark/red, red/dark and blue/dark. In all the experiments cultures were exposed to light intensity of 2000 lux.

The growth of callus was measured after 30 days in terms of fresh and dry weight. Dry weight was recorded after drying the tissue in hot air oven at 60°C to constant weight. The harvested tissues were dried and powdered. One gram dry powder was soxhlet-extracted[5] with petroleum ether (30–50°C) for 24 h. The extract was evaporated and examined for free sterols[6]. The residual cells were hydrolysed by refluxing

**Table 1.** Effect of light on diosgenin and sterol production in cultured tissues of *D. deltoidea*.

| Light | Callus[a] | | Diosgenin | | Sterol | |
|---|---|---|---|---|---|---|
| | Fresh weight[b] mg/culture | Dry weight mg/culture | % | mg/culture | % | µg/culture |
| 1. White (Control) | 3869 (±225) | 182 (±11) | 0.56 (±0.32) | 1019 (±68) | 0.81 (±0.028) | 1978 (±124) |
| 2. Dark | 2345 (±150) | 116 (±9) | 0.42 (±0.021) | 490 (±36) | 0.80 (±0.032) | 929 (±48) |
| 3. Blue | 4830 (±363) | 233 (±16) | 0.66 (±0.028) | 1526 (±110) | 0.82 (±0.019) | 1908 (±95) |
| 4. Red | 5315 (±421) | 250 (±15) | 0.51 (±0.032) | 1275 (±98) | 0.74 (±0.032) | 1847 (±125) |
| 5. Red/blue | 4982 (±390) | 250 (±17) | 0.63 (±0.035) | 1572 (±125) | 0.87 (±0.03) | 2175 (±150) |
| 6. Blue/red | 5756 (±442) | 240 (±15) | 0.37 (±0.017) | 880 (±62) | 0.66 (±0.035) | 1586 (±85) |
| 7. Dark/blue | 3136 (±223) | 157 (±8) | 0.98 (±0.050) | 1532 (±132) | 1.54 (±0.065) | 2418 (±115) |
| 8. Dark/red | 3187 (±195) | 172 (±11) | 0.66 (±0.042) | 1147 (±98) | 0.77 (±0.073) | 1324 (±114) |
| 9. Red/dark | 4230 (±412) | 285 (±14) | 0.45 (±0.012) | 1271 (±92) | 0.96 (±0.054) | 2747 (±213) |
| 10. Blue/dark | 3602 (±267) | 322 (±22) | 0.64 (±0.039) | 2060 (±152) | 0.95 (±0.048) | 3052 (±242) |

[a] Data represent an average of 5 replicate cultures (30 day old). Figures in parentheses are standard errors.
[b] Initial inoculum 300 ± 30 mg fresh tissue. [c] In treatments 5–10 each phase was 15 days (details in text).

with 10% HCl for 2 h, cooled and filtered. The residue was washed first with water and then with 0.1 N NaOH and finally with water to neutrality. The residue was then dried at 60 C, powdered and soxhlet-extracted with $CHCl_3$ for 48 h. The $CHCl_3$ extract was evaporated and the diosgenin and bound sterols were quantified by comparing O.D. values with the standard curve constructed by preparative TLC method[6].

Exposure of *D. deltoidea* callus to any kind of light, i.e. blue, red or white was superior to dark incubated cultures for growth of tissues and production of diosgenin and sterols on culture basis (Table 1). Cultures grown under blue or red light alone reached higher growth values than those grown under white light. Continuous blue light was beneficial for diosgenin production and did not affect sterol content markedly over the control (treatment 3). Increase in production of diosgenin over the control under blue light was by a factor of 1.17 and 1.5 on percentage and absolute basis respectively. Red light inhibited diosgenin and sterol percentage but on absolute basis the production was at par with control (treatment 4).

Similar stimulatory effect of blue light on anthocyanin synthesis in *Haplopapus gracilis* was reported by Reinert *et al.*[7] They also found red light to have no effect on anthocyanin synthesis. It is interesting to note that in the treatments with blue light (Table 1) at second phase (i.e. treatments 5,7) or blue light followed by dark phase (treatment 10) there was increase in diosgenin percentage by 1.1- 1.75-, 1.28-folds respectively. Similarly, percentage of sterols increased in these treatment over the control. It was clear from treatment 5 that blue light could reverse the inhibitory effect of red light on diosgenin and sterol production. Kadkade and Andrade[8] showed that fluorescent light influences diosgenin synthesis in *Dioscorea* spp. over the cultures grown in dark. Both enhancement[9] and inhibitive[10] effects of red light on growth have been reported from *Pelargonium zenale* and *Daucus carota* respectively. It is clear from this study that quality of light influences the diosgenin production and blue light influences diosgenin synthesis more markedly than red light.

This study establishes that enhancement of growth of *D. deltoidea* and diosgenin as well as sterol production is possible by exposing culture to blue light which can be adopted in large scale production systems.


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# Cardol: The antifilarial principle from *Anacardium occidentale*

**M. Suresh and R. Kaleysa Raj**
Department of Biochemistry, University of Kerala, Trivandrum 695 581, India

**Cardol, a phenolic compound isolated from cashew nut shell, showed pronounced antifilarial activity. The hydroxyl groups and the alkyl side chain were found to be necessary for activity. Compared to diethylcarbamazine that was active at a concentration of 350 ppm($LC_{100}$), cardol was active at as low a concentration as 3.5 ppm ($LC_{100}$). Cardol given orally was tolerated up to a concentration of 5 g/kg body weight in laboratory rats.**

FILARIASIS is a major tropical disease. More than 400 million people in the world are infected by filarial parasites. *Wuchereria bancrofti* and *Brugia malayi*, the two common filarial parasites causing filariasis in the Asiatic region are estimated to infect around 800 million people[1]. Although the mainstay of therapy and control of filariasis is the drug 1-diethylcarbamyl-4-methyl-piperazine (DEC), by itself it does not provide a complete cure and is also reported to have harmful side effects[2]. Consequently considerable research has been taken up to develop an alternative to DEC[3–5]. The anthelmintic activities of many indigenous materials have already been reported[6–8]. In a recent study, *Anacardium occidentale* was found to be the most active among 28 medicinal plants screened for antifilarial activity. Among the compounds reported from cashew nut shell liquid, our studies showed the antifilarial activity to cardol. Investigations on the isolation, detection of activity and certain structural studies form the subject matter of this paper.

The filarial parasite of cattle was used as the test organism. This was specially selected for the *in vitro*