

Biosynthetic potential of *in vitro* grown callus cells of *Cassia senna* L. var. *senna*

Cassia senna L. (Caesalpiniaceae) is an important medicinal plant, which has been widely used in Ayurvedic, Unani and Alternative Systems of Medicine¹ due to its purgative action. The drug mainly comprises of dried leaves and pods. The active chemical components of the plant are anthraquinone glycosides – sennosides, especially sennosides A and B, which are responsible for the purgative action². The plant has been put in the priority list of National as well as State Medicinal Plant Board for development. It is one of the principal herbal drugs having export potential for developed countries. India is the major supplier of the leaves and pods (shells) as well as senna glycosides to the world market. Approximately 75% of the senna produced in India is exported. Annual demand of the plant in 2001–2002 was reported to be 6462.5 tonnes, which is expected to go up to 11677.5 tonnes by the year 2006. To fulfil the demand and to maintain the quality of the raw material used, there is need to have an alternative system for the production of desired material and to conserve quality germplasm. In the latter half of the past century plant tissue culture has come up as a potential alternative method to fulfil this requirement. The use of plant tissue culture for production of secondary metabolites holds promise for rapid production of plant metabolites of pharmaceutical importance^{3,4}. It has been established that under appropriate culture conditions, *in vitro*-grown cells/tissue genetically inherit the biosynthetic potential of *in situ* cells and can produce a good amount of secondary metabolites⁵. In the present study, a protocol for tissue culture of *C. senna* is established in different morphogenetic media and *in vitro*-grown tissues/cells were analysed for their biosynthetic potential.

Authenticated seeds of *C. senna*, obtained from the National Research Centre for Medicinal and Aromatic Plants (NRCMAP), Gujarat, India, were thoroughly washed and surface-sterilized with 0.1% HgCl₂ for 2 min. The surface-sterilized seeds were inoculated for germination in axenic conditions on double-distilled water supplemented with 1% sucrose and gelled with 0.8% agar. Cotyledonary leaves of aseptically grown seven-day-old seedlings were used for the

experiment. These were cut into desired size (1 cm) and inoculated onto the Murashige and Skoog 1962 (MS)⁶ medium, supplemented with different concentrations and combinations of growth regulators such as benzyl adenine (BA), adenine sulphate as well as complex nitrogenous supplement namely coconut milk (CM) for induction of shoot differentiation (Table 1). The cultures were incubated under controlled conditions at 24 ± 2°C temperature, 16 : 8 (light : dark) photoperiod and 70–80% relative humidity. The compact non-morphogenic callus mass proliferated during shoot regeneration phase was analysed for total sennoside content (hydroxyanthracene derivatives (HAD), calculated as sennoside B) using the method described in *British Pharmacopoeia*⁷ and *Indian Pharmacopoeia*⁸.

Explants in different nutrient media showed varied morphogenetic response like swelling and enlargement of the explant (Figure 1), formation of morphogenic nodule and semi-organized compact cell mass (Figures 2 and 3). The extent of response varied with the different morphogenetic medium. During culture, it was observed that along with the morphogenic nodules, compact mass of non-morphogenic cells also proliferated. In some of the combinations, like lower concentrations of BA (below 14 µM), the non-morphogenic compact cell masses were more than the morphogenic nodules. This non-morphogenic cell mass, which proliferated along with morphogenic nodules, can be established as a

good source of sennosides, the major chemical components responsible for the therapeutic activity of the plant. Keeping this in view, a primary investigation was carried out to assess the biosynthetic potential of this non-morphogenic compact brown cell mass and the factors influencing the biosynthesis. The non-morphogenic cell masses were harvested and dried in hot air oven at 45–50°C. The dry cell mass thus obtained was analysed for sennoside content (total HAD calculated as sennoside B).

Analysis of the above cell masses showed the presence of sennosides in all the samples tested (Table 1), indicating that these *in vitro* cultured cells of callus inherited the same genetic makeup as the cells of mother plant, and hence have the potential to synthesize secondary metabolites. However, the sennoside content in the cultured cells varied with the variation in the medium composition and cell differentiation response. It was maximum (0.13%) in the medium sup-



Figure 1. Swelling and enlargement of explant after three weeks of inoculation. *a*, Enlarged explant; *b*, Inoculated explant.

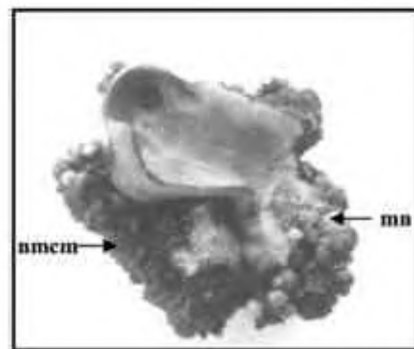


Figure 2. Formation of morphogenic nodule (mn) along with non-morphogenic cell mass (nmcm).

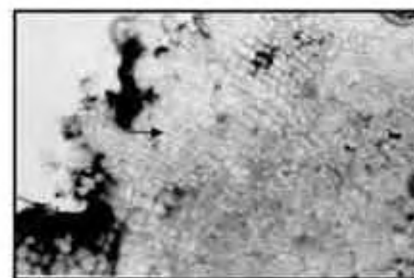


Figure 3. Photomicrograph of non-morphogenic cell mass.

Table 1. Percentage of total hydroxyanthracene derivatives (HAD) calculated as sennoside B in different medium composition

Medium composition	mg% of total HAD
MS + 14 μ M BA	0.1 \pm 0.0003
MS + 16 μ M BA	0.09 \pm 0.0003
MS + 18 μ M BA	0.09 \pm 0.0001
MS + 14 μ M BA + 10% CM	0.08 \pm 0.0001
MS + 16 μ M BA + 10% CM	0.13 \pm 0.0001
MS + 18 μ M BA + 10% CM	0.08 \pm 0.0002
MS + 16 μ M BA + 8 μ M adenine sulphate	0.06 \pm 0.0008
MS + 16 μ M BA + vitamins of B ₅ medium	0.09 \pm 0.002
MS + 16 μ M BA + increased thiamine (10 times)	0.09 \pm 0.0002

*Mean \pm SD ($n = 3$), BA, benzyl adenine; CM, coconut milk.

plemented with 16 μ M BA and 10% CM and was minimum (0.06%) in the medium supplemented with 14 μ M BA and 8 μ M adenine sulphate (Table 1), while in other media composition it varied from 0.08% to 0.1%. This variation in the sennoside content showed that appropriate chemical composition of nutrient medium, plant growth regulators and physical components of the axenic culture play an important role in the biosynthesis of the chemical constituents in the *in vitro*-grown cells⁹. Detailed analysis of the results showed that the medium supporting maximum sennoside biosynthesis also supported maximum shoot differentiation (data not shown here), while the medium supporting minimum organogenesis showed minimum biosynthesis of sennosides (Table 1), however other than the combinations given in Table 1 no other combination gave any cell/organ differentiation response. The above observations indicate that the stage of growth and development in cell culture is also an important factor in the regulation of biosynthetic pathways. In the medium supplemented with 16 μ M BA and 10% CM, along with the shoot-forming nodules, non-morphogenic compact cell mass was also present, which failed to produce shoot primordia. This cell mass though non-morphogenic, might have some degree of differentiation at the cellular level and/or due to co-evolution, it mimics the biochemistry of differentiated cells. Plant cells are totipotent and all of the necessary genetic and physiological potential for the secondary metabolite production is expected to be present in an isolated cell¹⁰. According to this, the cultured cells obtained from any part of the plant are expected to yield secondary metabo-

lites similar to those of the plants grown *in vivo* under suitable culture conditions¹⁰. The failure to do this, in a way is linked to the level and degree of organ differentiation required for the expression of the genes associated with secondary metabolism¹⁰. Our results are in agreement with the results obtained in onion, where it was observed that the differentiation of callus into roots and shoots resulted in the production of onion odour¹¹. Organogenesis has often been used as a means of inducing monoterpene production in tissue cultures, which fail to produce monoterpenes as long as they are maintained in morphologically undifferentiated state, e.g. valepotriate production was increased by the induction of root morphogenesis in callus cultures of *Valeriana officinalis* and positive correlation was observed between the levels of differentiation and valepotriate production. Callus of *Eucalyptus citriodora* produced no essential oil and organogenesis was found to be an essential prerequisite of monoterpene production¹².

The results of the study indicate that the *in vitro* cultured partially organized cells of *C. senna* inherited the biosynthetic potential, which can be exploited for production of sennosides on a large scale under proper growth conditions.

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