the donor and the recipient were fully matched, the HLA A*, B* typing further confirmed them to be both the haplotypes matched (full house match) (Table 5). Thus under Indian conditions, matching for HLA DRB1*, HLA A* and B* and perfect match of 6 out of 6 allele is sufficient enough in the case of sibling donation[9]. (www.bmtinfonet.org.newsletters.issue53.perfectdonor.usa.html).

A tissue-matching laboratory needs to establish inhouse reference standards, run reference standards with every new batch of reagents, confirm in repeat typings and confirm with commercially available reference reagents as and when required. In the absence of clinical laboratories coming forth to establish this kind of state-of-the-art facilities due to various reasons such as the high cost involvement, lack of understanding, lack of technical manpower, etc. a partnership between research institution/universities and the local hospitals aspiring to offer this kind of state-of-the-art therapy is the need of the hour and will be highly beneficial to the families concerned.

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Green hairy root cultures of *Solanum khasianum* Clarke – a new route to *in vitro* solasodine production

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Solasodine is recognized as a potential alternative to diosgonin. Both share the characteristic that they can be converted to 16-dehydro-pregnenolone acetate; the first step in steroid synthesis. Effort has been ongoing in the production of solasodine using cell and lately hairy root cultures of various *Solanum* species. As seen in our pilot experiments, some of the hairy root lines of *Solanum khasianum* Clarke show enhancement of solasodine production compared to non-transformed roots. Light and temperature as physical factors of growth are found to have an important role in controlling greening, growth and secondary product formation in these hairy root cultures. This is similar to the greening observed in tubers of *Solanum tuberosum* (potato), leading to stimulation of solanine production. Enrichment of the nutrient medium with CO_2 further increases growth and secondary metabolite production in the hairy root cultures of *S. khasianum*. Thus by manipulating environmental and nutritional conditions, solasodine production can be maximized in hairy root cultures of *S. khasianum*.

SEVERAL morphological changes are observed in hairy root cultures when exposed to light, viz. change in biomass as a direct effect of light[1], and greening of root clones[2]. The physiological effects of light are thought to be due to the total amount of energy (intensity of light) that has been incident on an organ or tissue rather than the daylength of phytochrome-controlled process[3]. Root formation in green protocorms of *Cymbidium* is reported to occur only at high intensity of light[3], while light stimulates greening in *Solanum tuberosum* tubers at low temperature[4]. Ramaswamy *et al.*[5] suggested that total glycoalkaloids in potato are formed within the chloroplast. Therefore, as a result of greening, the total glycoalkaloid can be increased in potato. Chloroplast may become starch-containing amyloplasts when tubers are grown in the dark. Given appropriate stimuli of light, amyloplasts may be converted to chloroplasts and vice versa[3]. Hence it is thought interesting to stimulate greening in the hairy root cultures of *Solanum khasianum* using light and to study its effect on solasodine production. Green roots developed under light conditions could be used for increased secondary metabolite synthesis[6–11]. In plants, sugars favour the expression of enzymes in connection with biosynthesis and storage of reserves, while repressing the expression of enzymes involved in

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photosynthesis[12]. There is an inevitable requirement of carbohydrate in the medium as a source of energy, i.e. carbon substrate, the most widely used carbon substrate being sucrose. Sucrose concentration is known to affect a range of culture parameters such as growth, primary metabolism and yield of secondary products. Sucrose also has a physical role as an osmotically active solute, osmotic stress being known to exert considerable influence on productivity of plant cell cultures[13]. In general, specific growth rate is considered as a function of sucrose concentration[14]. Thus any study on greening of hairy roots would require understanding the effect of medium sucrose as a metabolic resource and regulator on developmental and growth processes. Hairy root cultures utilize both sugars and CO_2 in the medium and headspace. CO_2 uptake by excised root tissue had been shown to occur both in the presence[15] and the absence of light[16]. The negative geotrophic nature of the hairy roots allows good contact between the gas and the aerial roots. We report here the relationship among light, temperature, chlorophyll content and sucrose on growth and solasodine production both in the presence and absence of CO_2.

The strains of *Agrobacterium rhizogenes* used were A4 containing pRi A4 and LBA 9402 containing pRi 1855 and the binary vector pBIN 19 containing the reporter gene *npt*II (gifted by Dr Yuke, National Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology, The Chinese Academy of Science, Shanghai). The leaves of *in vitro*-grown plants were co-cultivated for 2 days on MS basal medium[17] with liquid suspension cultures of *A. rhizogenes* showing an O.D. of 0.6 at 600 nm in YMB medium[18]. The leaves were then transferred to solid MS basal medium containing 250 mg/l of cefotaxime. The hairy root cultures mediated by A4 were maintained as clones A–E, while the hairy root lines obtained as a result of co-cultivation by LBA 9402 were selected on MS medium containing 50 mg/l kanamycin and were marked as clones 1–5. The root cultures were confirmed to be transformed by opine analysis[19]. The cultures were maintained on MSB at 2000 lux, 14/10 h of photoperiod, 25°C. Next, 100 mg of hairy root was inoculated into 100 ml of a liquid MS medium (without the addition of any phytohormones) in 250 ml flasks and incubated in a rotary shaker at 100 rpm. In two separate experiments, different light regimes such as 2000, 200 and 0 lux and temperature regimes such as 15, 25 and 35°C were maintained for a period of six weeks. The controls in each of the experiments were 0 lux (dark) and 25°C. The hairy root cultures were maintained at 25°C and 14/10 h of photoperiod while varying light. As 2000 lux was seen to favour growth and production, the cultures were then maintained at 2000 lux and 14/10 h of photoperiod while varying the temperature. By varying the concentration of sucrose, 1, 3 and 5% sucrose was added to the medium and the various clones were incubated for six weeks at 25°C and 2000 lux with 14/10 h of light and dark photoperiod. The control was 3% sucrose. Chlorophyll was estimated using the method of Arnon[20] and calculated using the formula:

$$\text{mg total chlorophyll/g tissue} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{100 \times W}.$$

Hairy root cultures were grown in the upper tier of specially designed two-tier shake flasks containing liquid MSB medium at 25°C and 2000 lux for six weeks. Varying concentrations of CO_2 was supplied to the roots through a glass tube that extends from the lower to the upper tier (Table 1). The lower tier contained varying concentrations of $KHCO_3$ (3 M) and K_2CO_3 (3 M). Growth index and solasodine production were estimated in the roots at the end of six weeks.

Solasodine was extracted and estimated by the method of Bhatt and Bhatt[21], spectophotometrically. The concentration of solasodine was calculated from a standard curve prepared by solasodine (Sigma). Solasodine content in the cultured hairy roots was expressed as mg/g dry weight. Fresh weight shows the biomass achieved at the end of the incubation period. It was determined by blotting the tissue on sterile blotting paper and weighing them. Growth was thus expressed as growth index (GI)[22].

$$\text{GI} = \frac{\text{Final fresh weight of biomass} - \text{Initial fresh weight of innoculum}}{\text{Initial fresh weight of innoculum}}$$

Hairy root cultures of *S. khasianum* showed increased chlorophyll formation and greening, on increasing the light intensity to 2000 lux (Figure 1 *a*). This implies that the transformed root cultures of *S. khasianum* exhibit a properly developed chlorophyll biosynthesis pathway. The increase in chlorophyll production was concomitant with an increase in solasodine production (Figure 1 *a*). This suggests that the synthesis of photosynthetic pigment and glycoalkaloid accumulation might have similar receptors and/or signal transduction pathways and that they are biochemically related. Greening was absent in roots grown in dark conditions. Bright light (2000 lux) promotes maximum solasodine production and greening, with maximum greening during the stationary phase when sucrose in the medium is depleted. On subculturing these roots in fresh medium, the green pigmentation was lost and it reappeared

Table 1. Concentrations of CO_2, $KHCO_3$ and K_2CO_3 used in the study

% CO_2	$KHCO_3$ (3 M) (%)	K_2CO_3 (3 M) (%)
0.5	50	50
1	62	38
2	73	27
2.5	85	15

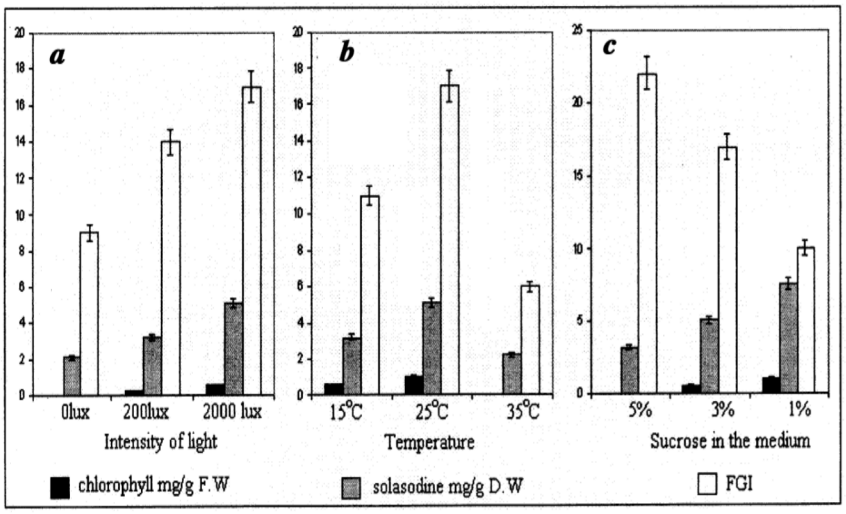

Figure 1. Relationship between chlorophyll formation, solasodine production and FGI. ***a***, Under varying light regimes, ***b***, Varying temperature regimes, ***c***, Varying sucrose concentration. Bars show average of six flasks.

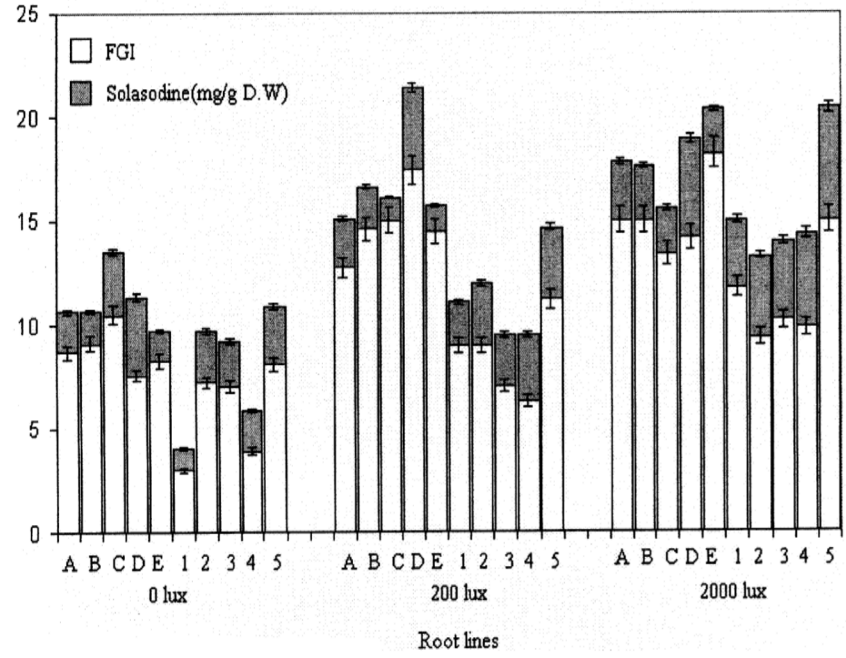

Figure 2. Effect of different light regimes on growth and solasodine production in hairy root cultures of *Solanum khasianum*. Bars show average of six flasks (two replicates of three flasks each).

in the stationary phase (6 weeks later). Therefore, the ability of cultures to form chloroplast was seen to depend on the presence of non-dividing cells or hairy root cultures in the stationary phase. The sections of older roots located in the centre of the root bunch showed maximum greening. Bright light (2000 lux)-treated roots also showed maximum biomass accumulation (Figure 2). The increased growth of green transformed roots may be due to the enhanced availability of photosynthetically derived carbohydrate. The coordinated action of light and cytokinins on chloroplast has been described earlier[23,24]. Temperature also showed a marked effect on greening, growth and solasodine production. Hairy roots grown at 25°C at 2000 lux showed enhanced greening, growth and secondary

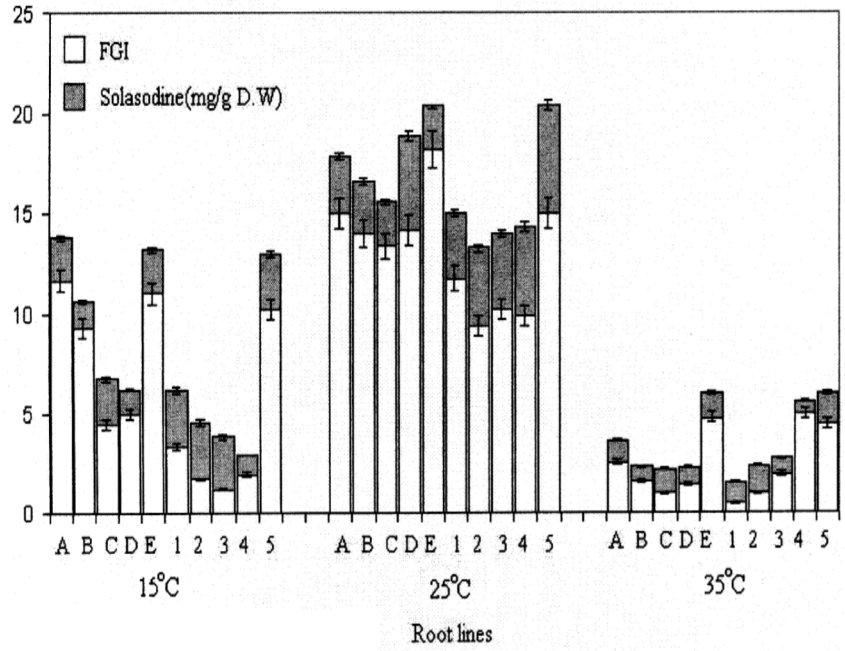

Figure 3. Effect of different temperature regimes on growth and solasodine production in hairy root cultures of *S. khasianum*. Bars show average of six flasks (two replicates of three flasks each).

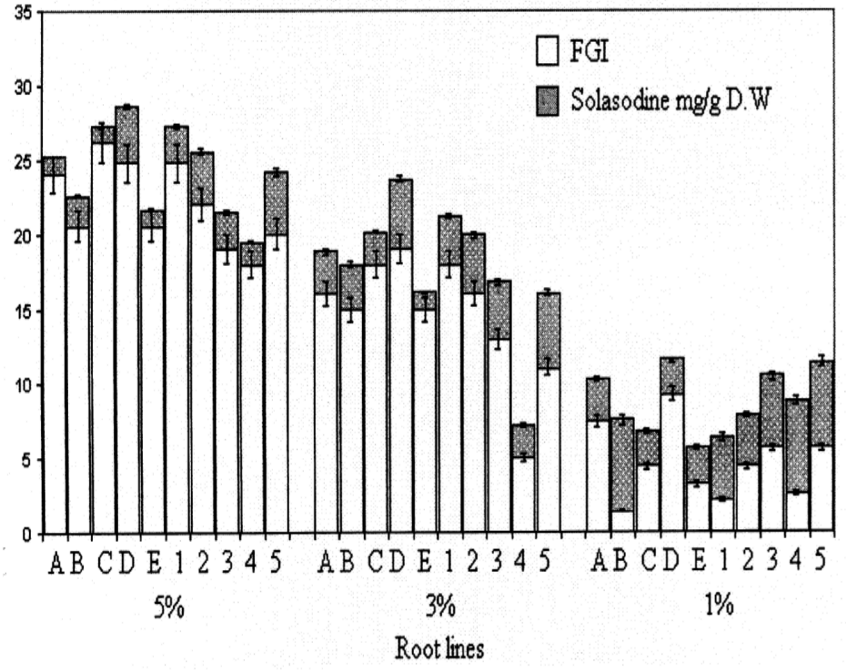

Figure 4. Effect of sucrose on growth and solasodine production in hairy root cultures at 5, 3 and 1% sucrose. Bars show average of six flasks (two replicates of three flasks each).

metabolism followed by those at 15°C, while those at 35°C reduced greening, growth and solasodine production (Figures 1 *b* and 3).

Carbon metabolism of hairy root cultures is complex and regulated by many environmental, biochemical and physiological factors. Sucrose can be identified as initiat-

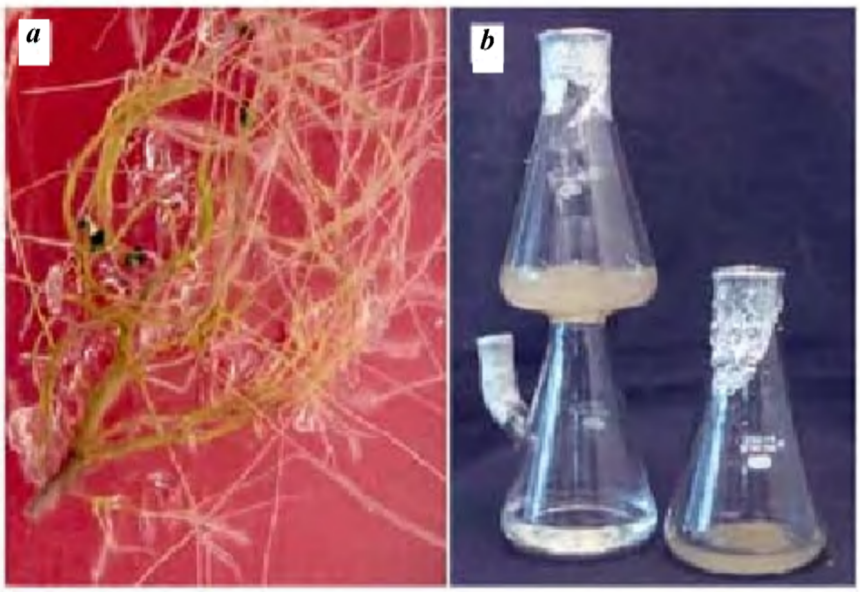

Figure 5. *a*, 'Green roots' seen in bright light of 2000 lux, 14/10 h photoperiod, 25°C and 1% sucrose. *b*, Hairy root culture of clone 5 grown in two-tier flask at 1% CO_2 and 3% sucrose showing enhanced growth compared to control.

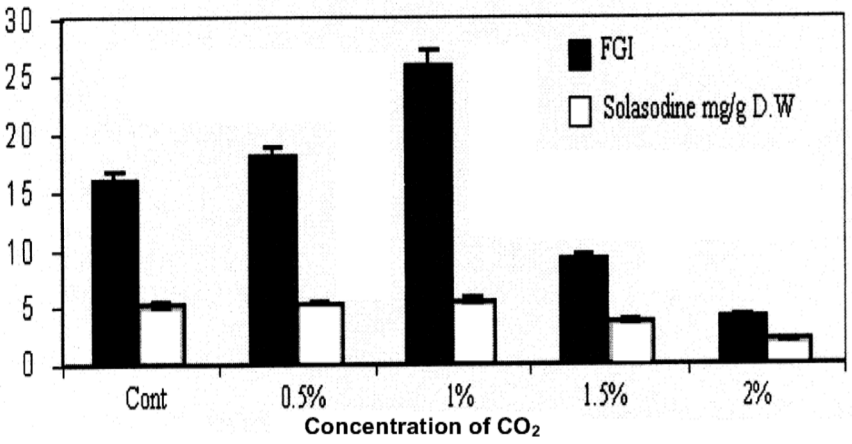

Figure 6. Effect of CO_2 on growth and solasodine production in clone 5 of *S. khasianum*. Bars show average of six flasks (two replicates of three flasks each).

ing 'feast or famine' responses[25]. The growth index of hairy root cultures was maximum at 5% sucrose and bright light, while secondary metabolism was reduced (Figure 4). Sucrose concentration of 1% in bright light increased the greening of roots and solasodine production, while decreasing growth (Figure 4). It appears that the positive effect of light and greening of roots on solasodine production overrides the disadvantage of reduced growth in cultures grown in bright light, 25°C and 1% sucrose (Figures 1 *c* and 4). Greening is induced by carbohydrate depletion (famine response), whereas increasing sugar concentrations stimulate the feast response[12,26]. A decrease in the sucrose concentration supplied shows an increase in greening of the transformed roots (Figure 1 *c*). Therefore, green roots (Figure 5 *a*) can be said to be photoheterotrophic in nutrition (require both light and sucrose).

CO_2 can affect the growth kinetics of hairy roots, as shown by Dilorio *et al.*[27]. They have proposed that root tissues require certain amount of CO_2 in their environment in order to function normally. It was shown that biomass yield increased 2.5 times for hairy root cultures of *Carthamus tinctorius* grown with 1% CO_2 in the head space. Similarly, in *Beta vulgaris* 1.4 times more growth in 1.5% CO_2 was shown by the same workers. In our experiments

total elimination of sucrose, accompanied with 1% CO_2 enrichment in the nutrient medium and bright light were detrimental to growth. This clearly shows the inevitability of sucrose in the medium for hairy root cultures of *S. khasianum*. Sugars are involved in the differentiation of xylem and phloem elements in cultured cells[3]. We have observed that growth and solasodine production improved at 3% sucrose, 1% CO_2 and bright light (Figures 5 *b* and 6). Concentrations higher than 1% CO_2 were seen to inhibit growth (Figure 5). Other research groups have reported similar decrease in secondary metabolite production in cell cultures as a result of high CO_2 concentration[28]. Therefore, by manipulating environmental and nutritional conditions, optimum growth and solasodine production could be achieved in hairy root cultures of *S. khasianum*. This requires a light intensity of 2000 lux at 14 h photoperiod (14 h of light and 10 h of dark), 25°C, 3% sucrose and 1% CO_2 as seen from our results. These conditions can be used to achieve a scale up of solasodine and hairy root cultures in suitable bioreactors.

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Assessment of habitat loss in Kameng and Sonitpur Elephant Reserves

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The Kameng and Sonitpur Elephant Reserves in north-eastern India are comprised of trans-border subtropical evergreen to tropical moist deciduous forests of Arunachal Pradesh and Assam. The reserves are facing deforestation and habitat loss in recent years. The present study attempts to investigate the loss of habitat in these reserves using temporal satellite imagery of periods 1994, 1999 and 2002. The on-screen visual interpretation of the three-period imagery revealed alarming and continuous habitat loss from 1994 to 2002. The overall habitat loss was found to be 344 km^2 between 1994 and 2002. The average annual rate of deforestation worked out to be 1.38%, which is much higher than the national average. The rate of deforestation was highest between 1999 and 2002. The study indicated that at this rate much of the forests in the study area would be depleted within the next few years. It also showed that moist deciduous forests, which possess highest biodiversity in Assam, are facing maximum deforestation. High deforestation has resulted in high man–elephant conflicts. The study suggests rehabilitation of affected forests in the larger interest of elephants and biodiversity.

THE long-term sustenance of wildlife depends on the sustenance of wildlife habitats. Hence, habitat protection and conservation is vital to any meaningful wildlife conservation

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