density intrusive material was deposited in the upper crust to the north of the Narmada. Upper crustal deposition did not take place to the south of Narmada.

During phase II, activity along the south fault started during the Jurassic–Cretaceous period. The mafic intrusion in the upper crust caused due to this activity was limited to the region south of Narmada and east of Barwani–Sukta fault.

Phase III coincided with the passage of India over the reunion plume during late Cretaceous. The plume head at that time was situated to the south of Narmada region. Due to tectonic disturbances close to the west coast, material from the plume head got emplaced at the base of the crust and extended to east to a large distance. This caused underplating in large parts of the crust. Subsequent extrusion from the plume led to the Deccan Trap activity and its deposition. Around the same time some eruption might also have taken place from the upper crustal mafic body to the south of Narmada zone.

The reinterpreted results of the seismic data across NSL, lead to the following conclusions. (1) The Barwani–Sukta fault divides the Narmada zone into two parts. While the SW part is a graben, the NE part is a basement uplift. (2) The upper crust east of the Barwani–Sukta fault shows a horst feature between the Narmada north and south faults, indicating that the Narmada zone is a ridge between two pockets of mafic intrusion of different ages. (3) Upwarp of the Moho and intra crustal layers in the Narmada zone suggest that the two faults involve deep-seated tectonics. (4) On the basis of present-day crustal structure, at least three major phases of tectonic activity during the Archaean–Proterozoic, Jurassic–Cretaceous and late Cretaceous can be identified in the Narmada region.

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Photoregulation of adventitious and axillary shoot proliferation in menthol mint, *Mentha arvensis*

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A direct and indirect methodology for efficient shoot proliferation and regeneration from various explants of *Mentha arvensis* has been developed by studying the interactive effect of plant growth regulator and light colour. Nodal explants cultured on Murashige and Skoog's (MS) medium supplemented with 10 μM thidiazuron and incubated under red light, proliferated an average of 50 shoots after 30 days, whereas internodal explants cultured in modified MS medium supplemented with 40 μM 6-benzylaminopurine and 0.5 μM α-naphthaleneacetic acid and exposed to red light, regenerated an average of 200 shoots after 60 days. Among the six cultivars tested, internodal explants of cv. Gomti regenerated about 90 shoots after 20 days of culture. Under the above conditions, leaf explants were observed to regenerate an average of 60 shoots after 60 days of culture, which was preceded by callus development. The protocol standardized for high efficiency shoot proliferation and regeneration in *M. arvensis* from nodal, internodal and leaf explants is suitable for micropropagation, genetic transformation and for obtaining somaclonal variants in this essential oil-yielding crop plant.

MENTHA ARVENSIS, a member of Lamiaceae, is a source of several single monoterpenes, including menthol and their mixtures that are extensively used in flavour, fragrance,

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cosmetics, pharmaceutical and agrochemical industries[1,2]. Since *Mentha* species are largely cultivated by the use of vegetatively produced root suckers as planting material[3], successive use of the same material carries with it any associated bacterial, fungal and/or viral infections to the next crop. Rapid and convenient regeneration and micropropagation procedures are required for cleaning of planting material free of infection and for transformation of genome with homologous or heterologous genes for the incorporation of desired characters such as disease and pest resistance and modification of monoterpene pathway towards more economic production of essential oil of better quality[4]. In recent years, several protocols have been described for micropropagation via shoot proliferation and regeneration from stem[5–7] and leaf[8,9] explants in *Mentha* species. However, these have certain disadvantages such as callus intervention and/or low-level shoot regeneration efficiency to varying extent. The present study describes experiments specifically designed for standardization of efficient protocols for regeneration and proliferation of shoots at high efficiency from nodal, internodal and leaf explants of *M. arvensis*.

The nodal segments excised from potted plants of six cultivars (Gomti, Shivalik, MAS-1, Himalaya, MAH-3 and Kalka) of *M. arvensis* were sterilized by dipping into Teepol detergent solution (2% v/v, Sigma Chemicals, St. Louis, USA) for 5 min followed by thorough washing under running water for 3–4 h. Surface disinfestation was carried out by treating the nodes for 10 min in 0.5% sodium hypochlorite (4% w/v commercial bleach, Qualigens Fine Chemicals, India) followed by rinsing with sterile distilled water. The explants were then prepared by trimming at the edges prior to inoculation in Murashige and Skoog's (MS) medium[10] supplemented with 10 μM 6-benzylaminopurine (BAP) and solidified with 0.6% agar, pH had been adjusted to 5.8 prior to autoclaving for 20 min at 105 kPa and 121°C. The cultures were incubated under 16 h photoperiod (60 μmol m^{-2} s^{-1}) at 25 ± 2°C for 30 days. Shoots proliferated from nodal segments were rooted in plant growth regulator (PGR)-free MS medium. The nodal, internodal and leaf explants prepared from such *in vitro* raised shoots were used to optimize culture conditions for proliferation and regeneration in *M. arvensis* cv. Gomti. The interactive effect of light colour and cytokinin on axillary bud proliferation from nodal explants was evaluated on MS medium supplemented with or without thidiazuron (TDZ) or BAP at 0, 2, 10 or 20 μM concentrations; blue, green, yellow or red light was obtained by combining respective filters with cool white fluorescent tubes (Philips, TL-40W/54, 6500 K). In the preliminary test for shoot regeneration from internodal explant, α-naphthaleneacetic acid (NAA) at 0.2 to 1.0 μM concentration in combination with BAP at 10 to 80 μM concentration was supplemented in MB medium (MS salts + B5 vitamins[11]). It was observed that NAA at 0.5 μM in combination with BAP (10–80 μM) was more efficient in regenerating shoots than NAA alone, either at lower or higher concentrations (data not included). A comparative study of the effect of red and white light on shoot regeneration response was carried out by incubating the internodal (10 mm) and leaf (5 × 5 mm) explants cultured in MB medium containing 0.5 μM NAA and 10, 20, 40, 60 and 80 μM BAP under respective light conditions. To compare the intervarietal differences in response to shoot regeneration, internodal segments of 6 cultivars of *M. arvensis* were cultured in MB medium supplemented with 40 μM BAP and 0.5 μM NAA and exposed to red light. In all the experiments, cultures were incubated under respective light conditions at 25 ± 2°C with 16 h photoperiod. The regenerated and proliferated *in vitro* shoots were carefully separated and transferred to PGR-free MS medium to permit elongation and rooting. After 15 days, rooted shoots were transferred to distilled water for 7 days for acclimatization and were transferred to pots containing 1 : 1 (v/v) mixture of soil and compost and kept under green-house conditions. The experiments were arranged in randomized complete blocks and replicated 30 times. Analysis of variance was done by *F*-test and the differences between means tested using CD (critical difference) at 1%. For histological observations, the regenerating and proliferating explants were fixed in 18 : 1 : 1 mixture of 50% (v/v) ethanol, glacialacetic acid and formaline, dehydrated in ethanol and ethanol–xylene series before embedding in mixture of paraffin : bee wax (8 : 1). Twenty μm thick sections were cut on a rotary microtome (Spencer make) and the sections were stained with saffranin and fast green.

The effects of differentially coloured visible light on axillary bud proliferation were examined with and without BAP or TDZ supplementation. It was observed that while the incubation of explants under light passed through blue or green filter lowered the rate of shoot proliferation, exposure to light passed through yellow or red filter significantly improved the shoot proliferation. On an average, 23 and 52 shoots proliferated per nodal explant under light passed through red filter on MS medium containing 10 μM BAP and TDZ, respectively (Table 1). Histological studies of sections of proliferating nodal explants revealed darkly-stained vascularized areas arising directly from the original vascular bundle. The internodal explants of *M. arvensis* cv. Gomti cultured on MB medium containing 40 μM BAP and 0.5 μM NAA and incubated under red light, regenerated about 90 shoots after 20 days and 200 shoots after 60 days of culture (Table 2). Microscopic examination of thin sections of regenerating internodal explants revealed darkly-stained vascularized areas in the cortical region and regeneration of shoot buds. This showed clear evidence of non-involvement of any callus in the shoot bud initiation events in the internodal explant. Leaf explants cultured on MB medium containing 60 μM BAP and 0.5 μM NAA formed little callus at the cut ends and regenerated on an

average 20 shoots per explant after 60 days of culture. Observations of sections of leaf explants showed that callus originated from palisade cells and regeneration of shoots from the callus. Replacement of white light with red light in the same regeneration medium significantly enhanced shoot regeneration, resulting in 60 shoots per explants after 60 days of culture (Table 2). The internodal explants from all the six cultivars of *M. arvensis* cultured on MB medium supplemented with 40 µM BAP and 0.5 µM NAA and incubated under red light regenerated about 50–90 shoots after 20 days. Among the cultivars tested, Gomti and Himalaya were found to be superior in regenerating shoots from internodal explants compared to other cultivars (Table 3). The proliferated and regenerated shoots were individually separated, rooted on MS medium and transferred to greenhouse for acclimatization.

The studies described above define a protocol for high-efficiency shoot proliferation from excised nodes, internodes and leaves of *M. arvensis*. Kukreja *et al.*[6] described a method for obtaining 30–40 shoots via callus formation in 60 days, thus rendering the protocol unsuitable for maintaining genetic integrity, since it led to the development of somaclones. On the other hand, the direct proliferation procedure standardized by Rech and Pires[5], produced only 2–4 shoots per explant over a period of 4–8 weeks. In the present work, the supplementation with TDZ in medium was more effective in proliferating shoots compared to an alternate cytokinin screened. It has been demonstrated that addition of TDZ in place of BAP in MS medium increased shoot differentiation and proliferation in the cultures of certain medicinal plant species like *Cinnamomum*[12] and *Saussurea*[13]. In the study of adventi-

Table 1. Effect of differentially coloured visible light in the presence of different concentrations and types of cytokinins supplemented in MS medium on the proliferation ability of nodal explants in *Mentha arvensis* cv Gomti

Plant growth regulator		Mean number of shoots formed per explant under differentially coloured filter					
Compound	Concentration (µM)	Nil	Blue	Green	Yellow	Red	Mean
Nil	0	1.2	1.5	0.8	1.4	1.5	1.3
BAP	2	3.7	1.7	1.7	5.5	5.4	3.6
BAP	10	6.5	4.5	5.9	12.4	23.0	10.4
BAP	20	3.1	3.1	3.1	5.1	5.4	4.0
TDZ	2	6.2	3.9	3.5	6.4	14.4	6.9
TDZ	10	10.0	7.1	3.4	17.7	52.1	18.1
TDZ	20	3.1	3.7	2.1	6.7	15.4	6.2
Mean		4.8	3.6	2.9	7.9	16.7	7.2

CD values at 1% between: Two main factors (filters) = 0.9; Two sub factors (PGR) = 1.1; Sub factors at fixed level of main factors = 2.6; Main factors at fixed level of sub factors = 2.5.

Table 2. Regeneration and shoot proliferation responses of internodal and leaf explants of *Mentha arvensis* cv Gomti, growing on MB medium (MS salts + B5 vitamins) containing 0.5 µM NAA and different concentrations of BAP as affected by the red colour light

Colour of the filter	Concentration of BAP in the medium (µM)	Internodal explants that regenerated into shoots (%)	Mean number of shoots regenerated per internodal explant	Leaf explants that regenerated into shoots (%)	Mean number of shoots regenerated per leaf explant
Nil	10	54.9	11.9	18.9	11.6
	20	74.8	27.9	38.4	14.9
	40	90.3	90.4	58.8	17.0
	60	77.3	37.6	79.0	22.6
	80	54.2	8.2	28.5	10.9
Red	10	75.9	30.9	19.5	28.4
	20	83.9	45.4	40.6	46.0
	40	99.1	198.6	62.1	52.1
	60	77.0	47.3	85.5	62.0
	80	53.1	62.8	30.8	36.0
Mean		66.6	38.0	46.2	30.1
CD 1%		4.8	6.9	3.4	3.6

Table 3. Relative shoot regeneration responses of the internodal explants of different cultivars of *Mentha arvensis* cultured on MB medium (MS salts + B5 vitamins) containing 40 μM BAP and 0.5 μM NAA and exposed to red light

M. arvensis variety	Gomti	Shivalik	MAS-1	Kalka	Himalaya	MAH-3	Mean	CD 1%
Percentage of explants that regenerated shoots	92.2	86.7	58.8	80.2	73.1	71.6	80.3	5.8
Mean number of shoots regenerated per explant	92.8	78.8	52.0	79.2	85.8	80.2	78.1	7.2

tious shoot regeneration, callus formation was avoided by increasing the proportion of BAP : NAA through use of higher concentration of BAP and lower concentration of NAA. Earlier, BAP and NAA have been effectively used to culture internodal[14,15] and leaf[16–18] segments of several medicinal plants. It has been reported that plants sense many aspects of light, including its wavelength, duration, intensity and direction[19]. Irradiation of plants with light at various wavelengths is known to markedly affect expression of genes encoding proteins related to photosynthesis, morphogenesis and organogenesis[20]. In the present study, incubation of cultured explants under red light almost doubled the shoot proliferation and regeneration response within the same period of culture. As observed in the present study, the potentiating effects of red light on axillary shoot proliferation has been previously demonstrated in several dicotyledonous plant species[21–23]. The enhancement effect of red light in proliferating shoots has been attributed to phytochrome action in *Pelargonium* cultures[24] or to enhanced protein synthesis and cell division in *Crepis* cultures[25]. A direct relationship between phytochrome and cell division in the *in vitro* cultures has been earlier postulated and evidenced[26]. The adventitious bud formation and improvement in shoot regeneration response on exposure to red light during culture period in several plants have been reported earlier[24,27].

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