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Research Article

CORN MINT ROOTS-PROSPEROUS RESOURCE OF METABOLITES, A COMPARATIVE STUDY OF *IN VITRO* AND *IN VIVO* PRODUCED PLANTS

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ABSTRACT

Mentha arvensis L. is well known important medicinal and aromatic plant widely used in several indigenous systems of medicine for various therapeutic benefits viz. analgesic, anesthetic, antiseptic, astringent, carminative, decongestant, expectorant, nervier, stimulant, inflammatory disease, ulcer and stomach problems. A regeneration protocol was optimized for more rapidly propagation from nodal explants supplemented with NAA 0.4 mg/l and BAP 2.0 mg/l for shoot multiplication and IAA 1.0 mg/l for root production. Different plant parts obtained from *in vivo* and *in vitro* conditions were analyzed and compared for biochemical estimation. Various primary metabolites such as chlorophyll, proteins, starch, sugars and phenols from different plant parts of corn mint were evaluated. Maximum amount of carbohydrates and phenol was obtained from *in vitro* derived roots. It concluded that roots are rich source of primary metabolites which are easily produced by *in vitro* technique compare *in vivo* position plant. Carbohydrates are an ideal source of energy as they can be converted more readily into glucose, the form of sugar. This crop is generally injured by a number of diseases and pests, causing a loss of growth of plant. Plant tissue culture skill offers to means to produced economically important metabolites at superior level in restricted atmosphere.

Keywords: *Mentha Arvensis L., In Vitro Regeneration, PGRs, Biochemical, Primary and Secondary Metabolites*

INTRODUCTION

Mentha arvensis L. is a perennial herb belongs to Lamiaceae and commonly known as Corn mint. It was introduced into India in 1952 from Japan. The percentages properties of various constitute vary considerably according to genetic makeup, geographical and ecological conditions and stages of growth (Bariya and Pandya, 2011). Corn mint is widely used in production of confectionary, soaps, detergents, cosmetics, perfumery, mouth fresheners, cough drops, tobacco goods, medicated oil tooth pastes, analgesic balms, lotions, shampoos, chewing gums, candies. Mint volatile oils composition is largely well known in literature (Clark, 1998). Corn mint have been utilized traditionally for the treatment of much digestive tract disease due to its carminative, antiemetic, spasmodic, analgesic, and anti-inflammatory attributes (Moreno *et al.,* 2002; Gulluce *et al.,* 2007). Chemical reactions taking place in plant cells notably of food substances which provide the energy required by the cells and biosynthetic reactions leading to the formation of compounds needed by the plant cells. As a result of these metabolic reactions various products are formed, out of which some products are further needed in growth and development of cell are called primary metabolites. These active substances are present in storage organs of plants as roots, leaves, flowers, bark, seeds, etc. The plant synthesizes sugar, amino acids, nucleic acids, protein etc. The present work has taken up to evaluate the biochemical profile of *in vivo* and *in vitro* produced materials of *Mentha arvensis* L. Tissue culture technique could play an important role in the production of metabolites substances (Bariya and Pandya, 2014). There are numerous reports describing the production of diverse metabolites through different plant parts selection and or addition of precursor in to the production medium.

MATERIALS AND METHODS

Plant materials for *Mentha arvensis* L. for tissue culture studies were grown and maintained in the Botanical Garden of Gujarat University. Nodal explants were surface sterilized with 0.1% Mercury chloride, 3% Sodium hypochlorite following by washing of sterile double distilled water for one minutes each to remove the traces of $HgCl_2$ and Sodium hypochlorite. Sterile nodal explants were excised in to pieces of 1 to 2 cm and carefully inoculated on MS basal medium (Murashige and Skoog's, 1962) revised and slightly modified with sucrose (3.5 %) was used as carbon source and agar agar (0.8 %) as a gelling agent supplemented different combination of BAP (0 to 2 mg/l), NAA (0 to 1.8 mg/l). The pH of the medium was adjusted to 5.8 before the addition of agar and autoclaved at 121^0 C (1.06 kg/cm^2) for 20 minutes. The cultures were incubated at 25 ± 2^0 C, for 16-18 hours photoperiod. Each treatment consisted of 5 replicates and was periodically carried out at 4 weeks interval for further shoot multiplication. Regenerated shoots (more than 5 cm in length) will well developed with roots were removed without any traces of agar, transferred to grown in plastic pots filled with sterile sand, soil and vermiculate (2:1:1) kept in the controlled atmosphere room (26 ºc ± 2ºc, 55 ± 5%, Humidity and 2000 Lux). The hardened plants were acclimatized in the green house and then finally they were transferred to pots containing normal garden soil rite after one month.

Different plant parts like Leaf, Stem, Root were evaluated quantitatively to estimate the total content of soluble sugars, starch, proteins, phenols and chlorophyll following the established methods for Protein content (Bradford, 1976), Total Sugar (Nelson, 1944), Starch (Chinoy, 1939), Phenols (Bray and Thorpe, 1954) and Chlorophyll (Mahadevan, 1974).

RESULTS AND DISCUSSION

In vitro and *in vivo* plant material of Corn mint was used for all the biochemical estimations like Protein, Sugar, Phenol, Starch, Chlorophyll content. Proteins are the essential constituents of all living organisms on this earth. Total Protein as a biochemical analysis to investigate and compare the properties of *in vitro* and *in vivo* produced was estimated and evaluated. The Protein was recorded high in *in vitro* produced than *in vivo* plant material. Biochemical parameter observed for *in vitro* and *in vivo* leaves, stem and root were promising. *In vitro* leaves contain high amount (0.03638mg/g fw) of Total protein and less amount (0.03610mg/g fw) *in vivo* root sample. It was also recorded maximum protein content in the stem part was also observed *in vitro* produced *Commiphora wightii* (Singh *et al*., 2011). However on contrary to this, the total amount of protein was found to be higher in seeds, Leaves and pods of, *Clitoria ternatea* (L.), *Guazuma ulmifolia* (Lam.) and *Madhuca indica* (Gmel.) (Shekhawat and Vijayvergia, 2010) and *Moringa oleifera* (Talreja, 2011).

Total amount of Starch was high content from *in vitro* produced root (5.767mg/g fw) and less amount in vitro produced leaf (5.620mg/gfw). Vijayvergia and Kumar (2007) was also found in callus of *Nerium oleander* and *Thevetia peruviana* showed higher amount of starch whereas, root and stem contain lower amount of starch content. On contrary to this, fruits of *Momordica charantica* (Ullah *et al*., 2011) contain maximum amount of starch.

Total Sugar estimation was high for *in vitro* produced than *in vivo* plant parts. *In vitro* produced root showed high amount of Total Sugar and *in vivo* root, less amount of Reducing Sugar was recorded than leaves and stem sample. Singh *et al*. (2010) also showed maximum amount of sugar in *in vitro* derived callus of *Commiphora wightii* and *Andrographis paniculata* respectively. And also reported by (Tanveer *et al*., 2010), root part of *Alangium salviifolium* to have appreciable amount of sugar.

In vitro root contained very high amount of Phenol than other sample. Phenols have immunomodulating, anti-tumour, antibacterial activities. Rajore (2002) and Singh *et al*. (2011) also examined the presence of appreciable amount of phenol in *in vitro* callus culture of *Jatropha curcas*, *Cordia gharaf* and *Withania Somnifera*, respectively.

The Total Chlorophyll content was high for *in vitro* leaf sample investigations than *in vivo* leaf samples. The Chlorophyll a content also showed high than Chlorophyll b for *in vitro* than *in vivo* in leaf sample.

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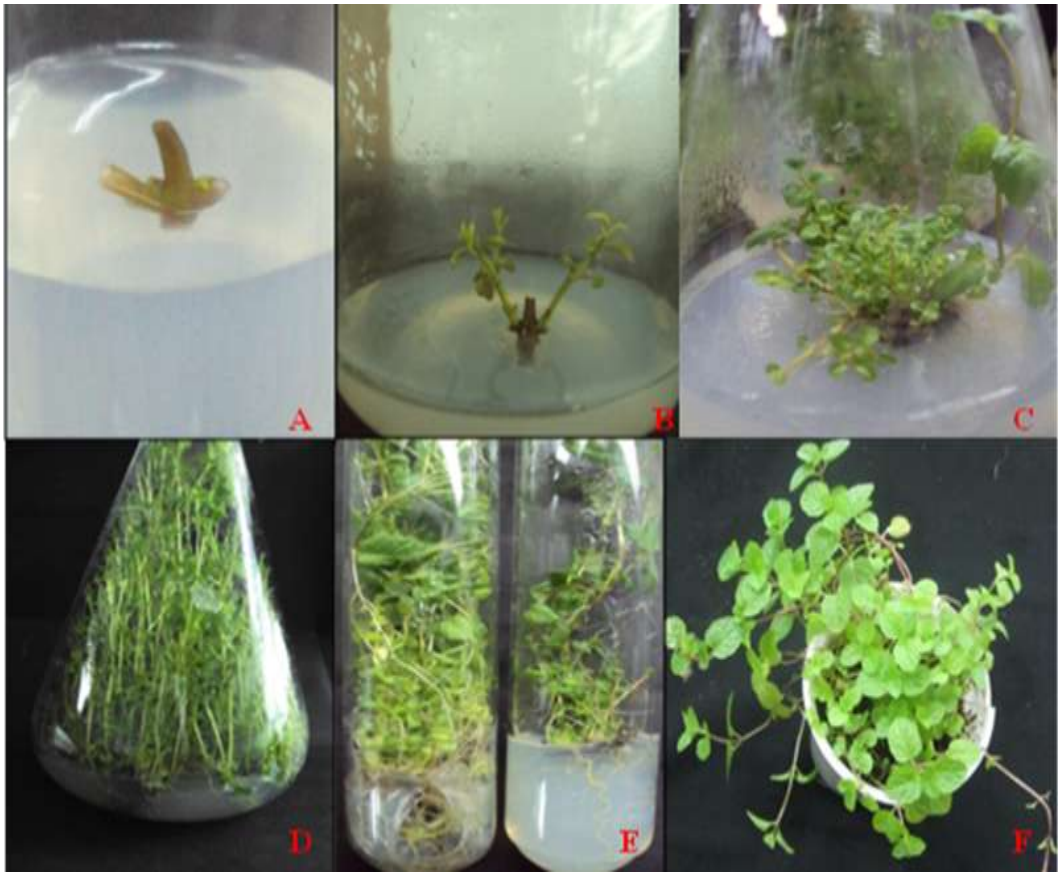
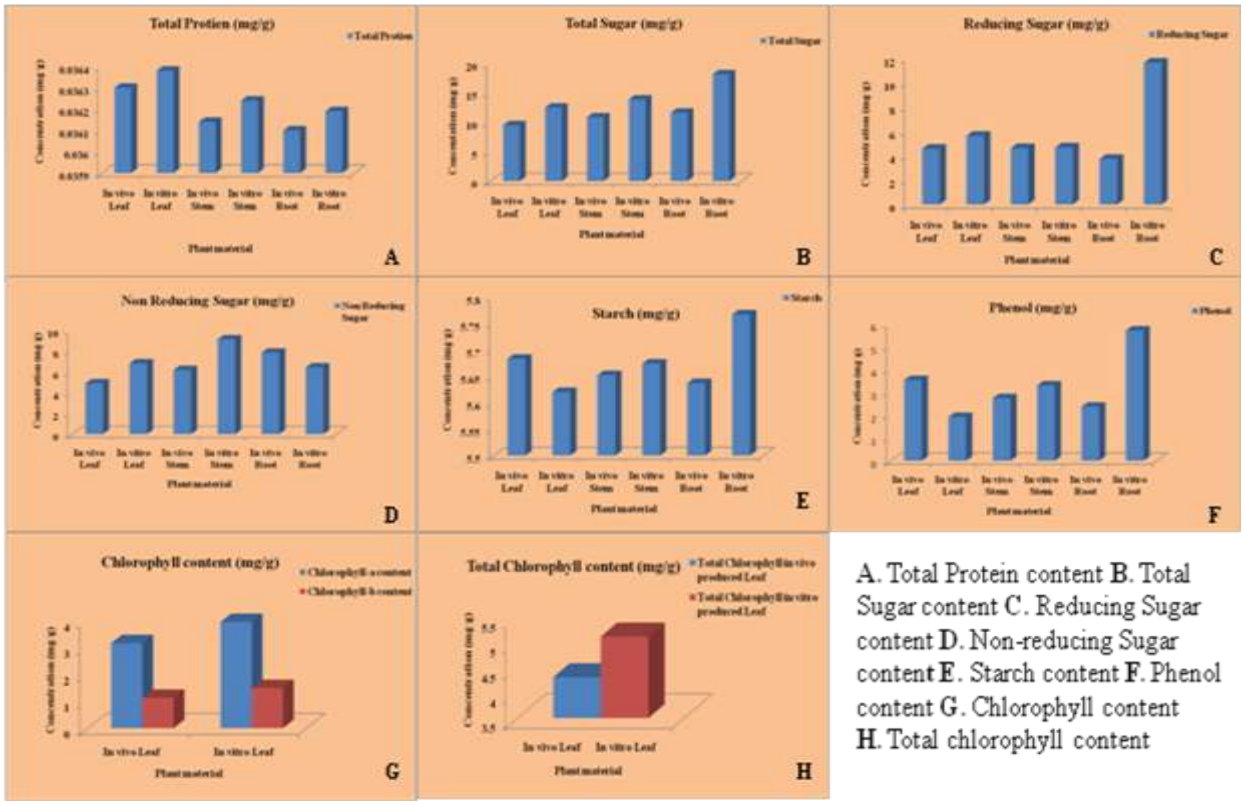


Figure 1: *In vitro* mass multiplication of *Mentha arvensis* L. A). *Nodal explants: MS medium supplemented with 2.0 mg/l BAP and 0.4 mg/l NAA.* ***B)*** *Initiation of shoots within 10 days of inoculation.* ***C).*** *Shoot formation within 16 days on MS medium supplemented with 2.0 mg/l BAP and 0.4 mg/l NAA* ***D).*** *Shoot proliferation: within 28 days on MS medium supplemented with above same medium.* ***E).*** *well developed root after one week on MS medium with IAA (1.0 mg/l)* ***F).*** *Acclimatized plant*

Table 1: Biochemical parameters of *in vitro* and *in vivo* produced plant parts

Biochemical Parameters	Plant material (mg/g)					
	Leaf		Stem		Root	
	In vivo	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*
Protein	0.03630	0.03638	0.03614	0.03624	0.03610	0.03619
Reducing sugar	4.586	5.651	4.627	4.688	3.746	11.690
Non-reducing sugar	4.915	6.839	6.205	9.174	7.884	6.458
Starch	5.683	5.626	5.652	5.674	5.637	5.767
Phenol	3.542	1.923	2.751	3.308	2.373	5.719
Chlorophyll a	3.1802	3.9763				
Chlorophyll b	1.1334	1.5001				



A. Total Protein content B. Total Sugar content C. Reducing Sugar content D. Non-reducing Sugar content E. Starch content F. Phenol content G. Chlorophyll content H. Total chlorophyll content

Conclusion

In vitro plants contain high amount of various primary metabolites and roots were most moneyed parts for carbohydrates and phenol. To explore the standardized protocol for root production through different plant tissue cultured methods. The menthol and other metabolites requirement in the industry is increasing and a large quantity have to import from outside countries. If a fresh approach for intensive cultivation through *in vitro* condition of mint is taken up, the country can produce sufficient quantity of crop to yield the required demand of various metabolites.

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