BRIEF COMMUNICATION

OPTIMIZATION OF CALLUS INDUCTION IN GLYCYRRIZHA GLABRA USING GROWTH REGULATORS

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In vitro multiplication of Glycyrrhiza glabra was achieved using thin sections of nodes and mature leaves as explant source. Thin sections of nodes (1.0 -0.15 mm), were cultured on MS medium containing varying concentrations of different growth regulators IAA, Kinetin, NAA and 2,4-D (1.0, 1.5, 2.0, 2.5 mg/l), respectively. The callus initiation by IAA / Kinetin 124 D, Kinetin, NAA, IAA at MS+2.5 mg/I concentration showed varied callus ratings of 40, 86.7 and 93.3% respectively. IAA / Kinetin combination showed very high callus rating, while IAA/NAA combination failed to initiate callus induction in G. glabra.

Discriminate use, demographic pressure, ecological imbalance of excessive utilization of plants in various medical and other purposes have made the available supplies of the medical plants exhausted to a considerable extent (George et al 1984). To compete this, plant tissue culture is one of the most promising and potentially valuable technique (Mantell et al 1985). The current and potential importance of medicinal plants has been internationally recognized. The World Health Organization (WHO) initiated programs world wide to increase the medical self-sufficiency of developing countries (Julia Novy 1997). The use of plants as medicine is as old as human civilization itself (Agarwal 1997).

Glycyrrhiza glabra is one of the important medicinal plant and it belongs to the family of leguminosae (Iran Ross 1984). It is a perennial plant grows only in specific pH (5.5 - 8.2), rainfall and temperature (5.7 - 25°C). The plant has a deep tap root system which produces horizontal rhizomes that spread out from the main plant just under the soil surface (Kirtikan & Basu 1991). The main constituent of G. glabra, a glycoside allied to saponin called glycyrrhizic acid. It is 50 times sweeter than sugar (Toe Graedon 1999).

Mass multiplication by tissue culture is also the best way of propagation of slow growing, exotic plant with a variety of medicinal uses like G. glabra (Kumar 1999). Extensive reports are available on modern pharmacological research on this rhizome (Andrew 1996). Licorice has long been valued for its medicinal uses in ancient Greece for asthma, mouth ulcers and anti-inflammatory effect (Arockiasamy 2000).
The explant *G. glabra* was collected from herbal garden of Kolli Hills, maintained by state government farm Namakkal district, Tamil Nadu. The explant was surface sterilised with sterile distilled water and then with 0.1% (w/v) HgCl for 1 min followed by five washings with double distilled sterile water. Leaf segments (1-2 cm) excised aseptically are cultured on MS medium supplemented with growth regulators with varying concentrations of IAA, Kinetin, NAA and 2.4 D (1.0 mg/l to 2.5 mg/l) in varying combinations.

The medium was adjusted to pH 5.8 and maintained 38% (w/v) agar (Qualigens, India). The sterilised medium was poured to sterile culture tubes (25 x 150 mm) inoculated with explant and incubated at (25° ± 2°C) for 12 hours. Continuous light provided by cool white fluorescent tubes. Observations were made at regular intervals for 7 days. All treatments had 5 replicates and repeated thrice.

The results obtained from the present study clearly indicate the efficiency of the developed protocol. It was observed that the growth regulators (IAA, NAA, Kinetin & 2, 4, D) elicit response (Table 1).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Callus (%)</th>
<th>Callus rating</th>
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<tbody>
<tr>
<td>MS+2.5 mg/l IAA+2.5 mg/l Kinetin</td>
<td>93.3±0.08</td>
<td>+++</td>
</tr>
<tr>
<td>MS+2.5 mg/l Kinetin + 2.5 mg/l, 2,4,D</td>
<td>86.7±0.05</td>
<td>++</td>
</tr>
<tr>
<td>MS+2.5 mg/l, IAA + 2.5 mg/l, 2,4,D</td>
<td>86.7±0.05</td>
<td>++</td>
</tr>
<tr>
<td>MS+2.5 mg/l, IAA +2.5 mg/l, NAA</td>
<td>40.0±0.08</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ - Good callus, ++ - Moderate callus, + - Poor callus

Many combinations of growth regulators of varying concentrations were supplemented to observe their effect on callus induction and survival percentage. Callus was initiated in the leaves of *G. glabra* on MS medium supplemented with varying concentrations of different growth hormones namely IAA, NAA, Kinetin and 2,4, D. The effect of IAA of callus induction from *G. glabra* showed very high callus rating of 93.3% of MS+2.5 mg/l IAA + 2.5 mg/l Concentration followed by IAA / 2,4, D and kinetin / 2,4,D combination for growth regulator showed 86.7% callus rating at MS +2.5 mg/l, 2,4,D and MS+2.5 mg/l, 2,4,D concentration, respectively. The IAA/NAA combination showed very low callus rating (40%) at the MS+2.5mg/l IAA+2.5 mg/l NAA concentration.

Since the plants of medicinal values do not survive in all agro climatic conditions, an appropriate media and standardised conditions for successful tissue culture is desired. The breeding of *G. glabra* is a slow and difficult process due to long generation time and difficult in cultivating in other climatic zones by conventional breeding techniques. Hence, this approach was undertaken to optimize callus induction in *G. glabra* using various growth regulators.
National Symposium on Medicinal and Aromatic Plants (maps): Bioprospecting and Conservation Concerns on October 10 – 11, 2003

THE EFFECT OF *Glycyrrhiza glabra* ON THE LEVEL OF MARKER ENZYMES IN SERUM/LIVER IN ADJUVANT INDUCED ARTHRITIC RATS

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Rheumatoid arthritis is chronic inflammatory disease that affects the joints. The Comparative results pertained from the present study is categorized into lysosomal, cytoplasmic enzymes with the effect induced by the selected plant species “*Glycyrrhiza glabra*” the lysosomal marker (serum) enzymes in namely, ACP, Cat-D, decreased significantly, in treated group. The cytoplasmic marker enzymes in serum i.e. AST, ACT, ACP, & LDH showed significant reduction treated groups when compared with that of arthritic rats.

National Seminar on Biotechnology Update for Teachers / Researchers (Sponsored by the University Grants Commission) July 7-9, 2004

ISOLATION, PURIFICATION AND CHARACTERISATION OF SECONDARY METABOLITES FROM *Glycyrrhiza glabra*

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Plants produce large diverse array of Organic compounds that appear to have no direct function in growth and development. These substance are known as Secondary metabolites. Secondary metabolites are synthesized in specialized cells during distinct developmental stages. *Glycyrrhiza glabra* L. is a perennial herb, which are mostly used for flavoring and medicinal purposes. The phytochemical analysis revealed the presence of secondary metabolites (Alkaloids, Flavonoids, Phenols, Tannins, Steroids). Efficient extraction of secondary metabolites was performed by Accelerated Solvent Extraction (ASE) using three different solvents namely Hexane, Benzene & Ethyl acetate. TLC analysis of *Glycyrrhiza glabra* showed the presence of Flavonoids.
PHYTO HORMONE INDUCED INVITROORGANOGENESIS
IN Glycyrrhiza glabra

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Plant derived medicines have been part of traditional health care in most parts of the
world for thousands of years. More than 80 % of the population in developing
countries depend on plants for their medicinal needs. In India medicinal plants are
widely used by all sections of people. Glycyrrhiza glabra (Licorice) is a perennial
plant belonging to leguminosae family. It is grown in Spain, Sicily, USSR, Iran, Iraq,
Creece and Asia Minor. It is exotic innature.

Licorice root has been used therapeutically for several thousand years in both Western
and Eastern systems of Medicine. It was used in ancient Arabia to treat coughs and to
relieve the unwanted effects of laxatives. It is also used for dry cough, asthma and all
pectoral diseases.

INTERNATIONAL CONFERENCE
Indo Australian Conference on Biotechnology in Medicine – IISC – Bangalore
on February 9 – 11, 2004

THE ANTIOXIDANT EFFECT OF Glycyrrhiza glabra IN FCA
INDUCEDARTHRITIC RAT

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Phytonutriens have been demonstrated functionally to be antioxidant in enzyme
modulation, cellular proliferation and apoptosis. All the biological membranes are
characterized by the presence of PUFA associated with lipids and a variety of
membrane protein. ROS has been implicated in pathogenesis and induction of arthritis.
Immuno modulation using herbal drugs (Rasayana) could provide an alternative to
conventional chemotherapy. (Glycyrrhiza glabra) is a perennial and belongs to
leguminaceae family. The rhizome (roots) are used for the study and posses antiviral
and hepatoprotective activity. The lipid peroxidation level as measured by
Concentration of MDA was elevated in /FCA induced arthritic rats, on treatment with
root extract (Glycyrrhiza glabra) was reduced and resorted to near normal. The levels
of antioxidant (Enzymatic / bonenzymatic) Gpx, peroxidase, catalase, Sod, GSH and
Vitamin C reduced in arthritic condition, on treatment with herbal root extract showed
an positive modulation. The reduction in LPO levels and increase in antioxidant levels
increase in antioxidant levels and increase in antioxidant levels exemplifies the positive
effectiveness of Glycyrrhiza glabra against arthritis.