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BIOCHEMICAL PROFILE OF *IN VIVO* AND *IN VITRO* PRODUCED *BOUGAINVILLEA SPECTABILLIS* L.

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SUMMARY

***Bougainvillea spectabillis* rosea (Bougainvel) is an important medicinal plant used in the treatment of *Diabetes mellitus*. Plant tissue culture approach has been found to be advantageous as it provides a continuous and reliable source of natural product year round without the destruction of the entire plant. Biochemical profile of *in vivo* and *in vitro* produced metabolities of *B. spectabillis* was studied. The results revealed that the amount of sugar was notably less in *in vitro* while proteins, starch, amino acids, phenols, DNA and RNA did not show a significant variation. Enzymatic activities like peroxidase, invertase, IAA oxidase, polyphenol oxidase were higher in *in vitro* callus.**

***Key words*:** *Bougainvillea spectabillis,* metabolites

INTRODUCTION

Plants have been offering valuable and safe natural sources of medicines and agents of therapeutic, industrial and environmental utilities across the varied cultures and civilizations. *Bougainvillea spectabillis* (Bougainvel) is an important medicinal plant having anti-diabetic properties (Narayana *et al*. 1984). *B. spectabillis* extracts have been earlier studied for the presence of anti-diabetic compound pinitol and its effect on albino mice (Narayana *et al.* 1987, Purohit and Sharma 2006). On the basis of these studies present work was taken up to evaluate the biochemical profile of *in vivo* and *in vitro* produced materials of *Bougainvillea spectabillis*. Tissue culture technique could play an important role in the production of active phytochemical substances. Plant cells grown in culture have potential to produce and accumulate chemicals similar to the parent plant from which they were derived. There are numerous reports describing the production of diverse metabolites through cell line selection and/or addition of precursor into the production medium. (Khanna 1985, Mulabagal 2004, Haq 2005).

MATERIALS AND METHODS

Plants *B. spectabillis* required for tissue culture studies were grown in botanical garden of Gujarat University Campus and leaves were used as explants material. Explants were washed thoroughly under running tap water then the plants were washed twice to thrice in sterile double distilled water. Further the plant was sterilized with the series of various sterilizing reagents e.g. 10% Tween-20 solution, 5% Sodium hypochlorite, 0.1% $HgCl_2$, followed by washing with sterile double distilled water to remove the traces of $HgCl_2$ and sodium hypochlorite. Sterilized explants were excised into pieces of 0.5-1 cm^2 and carefully inoculated on basal media (Murashige and Skoog 1962) supplemented with different combinations of auxins and cytokinins. The cultures were incubated in culture room. They were observed regularly for any sign of contamination, swelling and initiation of

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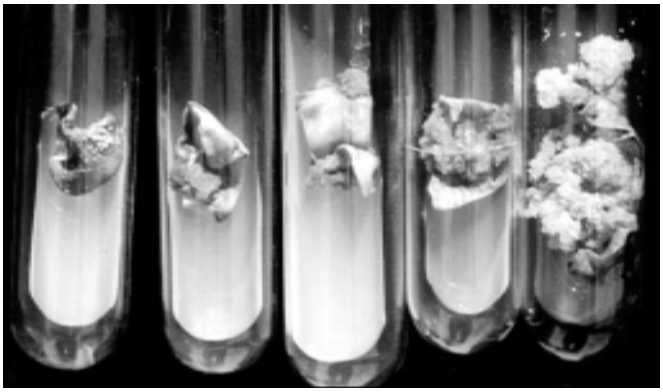
results. The callus obtained was harvested at the end of 2, 4, 6 and 8 weeks.

The evaluation of the biochemical profile of *B. spectabillis* was carried out from *in vivo* (leaf) and *in vitro* (callus of different age viz. 2, 4, 6 and 8 weeks) plant materials. Proteins, sugars, starch, phenols and amino acids (from fresh and dry materials) and enzymatic activities of peroxidase, IAA-oxidase, invertase, protease, α and β amylase, polyphenol oxidase (PPO), catalase and enzyme protein were estimated from cytoplasmic as well as wall-bound fractions of the fresh materials using the following standard methods:

Total proteins - material was extracted for total proteins using Trichloro acetic acid (TCA) (Lowry *et al.* 1951), reducing and non-reducing sugars (Nelson 1944), starch (Chinoy 1939), total phenols (Bray and Thorpe 1954), free amino acids (Lee and Takahashi 1966), RNA (Bonner and Zeevaart 1962), DNA (Bonner and Zeevaart 1962). Enzyme activities of peroxidase (George 1952), IAA-oxidase (Mahadevan 1964), invertase (Hatch and Glasziou 1963), protease (Penner and Ashton 1967, modified by Cruz *et al*. 1970), α and β amylase (Sumner and Howell 1935), polyphenol oxidase (PPO) (Kar and Mishra 1976), catalase (Chance and Maehly 1955), enzyme protein (Lowry *et al.* 1951)

RESULTS AND DISCUSSION

The concentrations of different combinations of different hormones showed varied results. However, medium containing 6mg/l kinetin and 2,4-D each was most effective for callus culture (Plate 1). Biochemical changes observed in the leaf and callus during the growth at different age of callus (2, 4, 6 and 8 weeks) of *Bougainvillea spectabillis* revealed certain interesting features. Amount of protein was highest in both *in vivo* and *in vitro* materials at the age of two weeks old callus. It decreased with the increase in age of callus in dry materials (Fig 2A). Reducing and non-reducing sugars were higher in dry materials of the leaf (Fig 1, ab). Amount of reducing sugar increased with the increase in age of callus (Fig 1a). Starch content was maximum at 2 week old fresh callus (Fig1d). Amino acid content increased linearly in order from leaf to 2, 4, 6 and 8 week callus in both dry and fresh materials (Fig 1e). Fresh and dry callus showed highest amount of phenol at 6 week stage. During the analysis of enzymatic pattern polyphenol oxidase in callus was higher than *in vivo* and was maximum in 8 week old callus (Fig 2b). Peroxidase increased with increasing age of callus and was



Initiation, 2, 4, 6, 8 weeks old callus

Plate 1. Callus Culture of *Bougainvillea spectabillis*

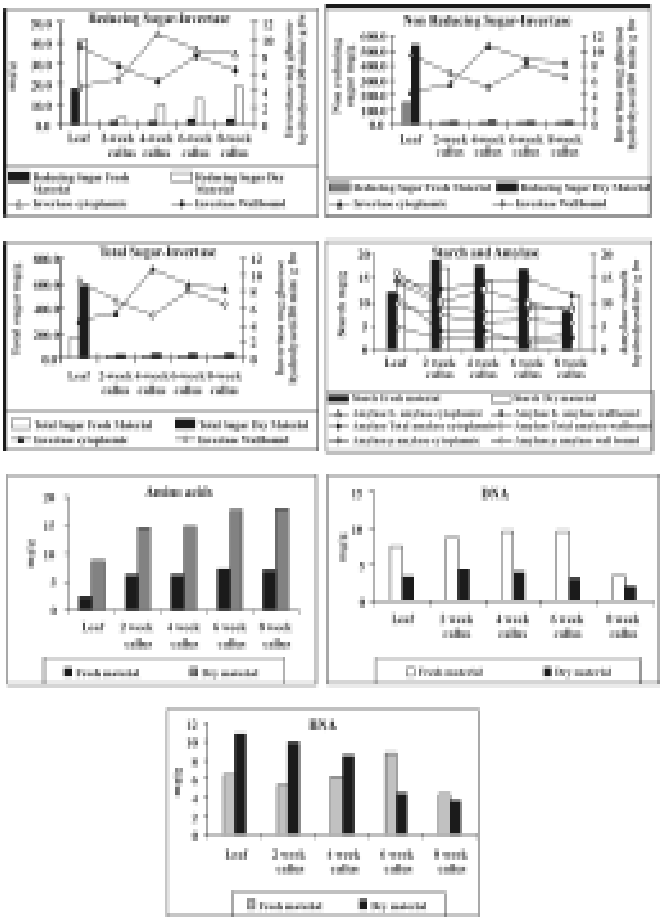


Fig. 1. Sugar, invertase, starch, amylase, amino acids, DNA and RNA contents of *in vivo* and *in vitro* produced *B. spectabillis*

maximum in wallbound fractions of 6 week old callus (Fig 2c). Enzyme protein was maximum in cytoplasmic fraction of callus at the age of 6 week, wall bound fraction of 2 week old and was higher than its *in vivo* fraction (Fig 2f). IAA oxidase activity was quite close among 2, 6 and 8 week old callus. Maximum amount was observed in cytoplasmic fraction of the leaf and the wall bound fraction of 8 week old callus (Fig 2d). Catalase was maximum in cytoplasmic fraction of leaf and wall bound fraction of 6 week old callus (Fig 2e). α and β amylase activity reduced with the increase in age of callus (Fig 1d). Increase in polyphenol activity was observed in the wall bound fractions while cytoplasmic fraction showed the contradictory results (Fig 2b). Protease activity in cytoplasmic fraction was maximum in the leaf as well as in wall bound fraction in 8 week callus (Fig 2a). Invertase activity of the wall bound fraction showed and declining pattern while cytoplasmic fractions showed maximum activity at 4 week age of callus (Fig 1c).

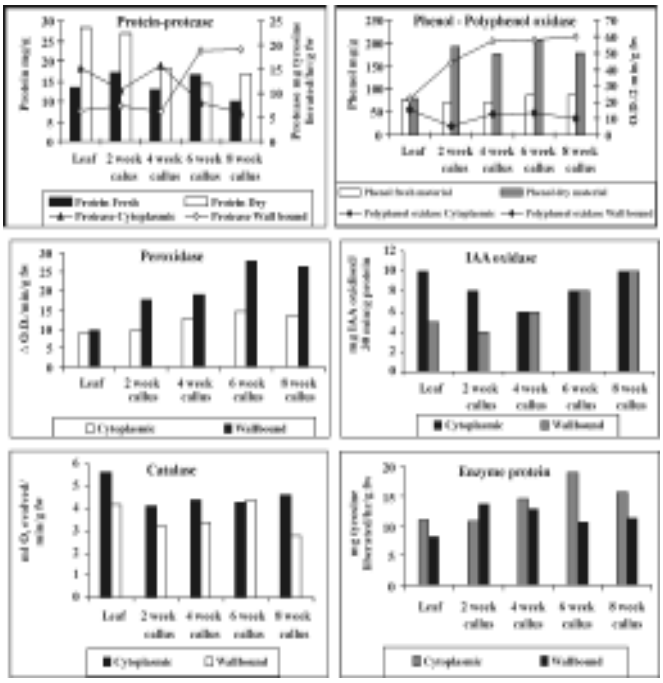


Fig. 2. Protein protease, phenol-polyphenol oxidase, peroxidase, IAA oxidase, catalase, enzyme protein of *in vivo* and *in vitro* produced *B. spectabillis*

The interaction between phenolic compound and protein with reference to the inhibition of enzyme activity has been reviewed by Loomis and Bottaile (1966). According to them phenolic compounds can bind to protein by hydrogen bonding or may be oxidised to quinones which co-polymerise with protein through covalent bonding. Quinones may also condense to form tannins or brown pigments which inactivate enzyme like polyphenol oxidase and may cause precipitation of soluble proteins (Anderson and Rowan 1967). Numerous endogenous phenolic compounds are recognized as protein precipitants (Pirie 1959) and enzyme inhibitors (Hartdegen and Rupley 1964, King 1971).

Kavikishor *et al.* (1992) studied the activity of wall-bound enzymes in callus cultures of *Gossypium hirsutum* L. during growth. Activities of ß-glucosidase, ß-galactosidase, -mannosidase, ß-1,3-glucanase, acid and neutral invertase were detected in the cytoplasmic fraction as well as in cell wall isolated from callus cultures of cotton. Bhardwaj *et al.* (1995) observed changes in the composition of membrane lipids in relation to differentiation in *Aegle marmelos* callus cultures. Gutmann *et al.* (1996) observed an increase in the protein levels in the cells of *Larix leptoeuropaea* during the first 2 weeks of culture. Jeyaseelan and Rao (2005) measured physiological and biochemical changes between embryogenic and non-embryogenic callus obtained from *Cardiospermum halicacabum*. As a result of these metabolic reactions various products are formed, out of which some products are further needed in growth (e.g. amino acids, proteins, carbohydrates, lipids, vitamins, nucleotides etc.). Their large diversity in nature, permit the identification of lead molecules of great interest for the development of new therapeutic agents, as well as to understand the biochemical and molecular mechanism of action involved in most physiological and pathological processes. The biological functions of plants are also due to their diverse chemical properties.

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