Chapter - 6

Elicitor and Precursor mediated L-dopa production
6. Elicitor and Precursor mediated L-dopa production

6.1. Introduction

In recent years, traditional system of medicine has become a topic of global importance. Although modern medicine may be available in developed countries, herbal medicines (phytopharmaceuticals) have often maintained popularity for historical and cultural reasons (Vanisree and Tsay 2004).

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world’s population (Balandrin and Klocke, 1988). These compounds belong to a group collectively known as secondary metabolites. These compounds may represent chemical adaptations to environmental stress or they may serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators. They are sometimes considered to be waste or secretary products of plant metabolism (Bhalsingh and Maheshwari 1998; Evans, 2001; Namdeo 2004). Many of the plant species that provide medicinal herbs has been scientifically evaluated for their possible medical applications. It has been mentioned that natural habitats for medicinal plants are disappearing fast and together with environmental and geopolitical instabilities. It is increasingly difficult to acquire plant-derived compounds (Vanisree and Tsay, 2004). Possible modes of obtaining these secondary metabolites are, extraction from natural sources, partial synthesis from structurally similar compounds, total synthesis and through plant biotechnology. Direct isolation from plants involves cultivation of plants, harvesting the desired parts, extraction and purification of desired product. This conventional method has a number of limitations such as plants are often not readily available because of geographical or governmental restrictions, requires huge area of land, which otherwise can be utilized for primary food crop. The process is labor intensive and time consuming, the quality may be affected by unforeseen environmental conditions and demands and supplies are difficult to manage. Total or partial synthesis of secondary metabolites proved to be extremely difficult and very expensive (Namdeo et al., 2007). This has prompted industries, as well as
industries, as well as scientists to consider the possibilities of investigation into cell cultures as an alternative supply for the production of plant pharmaceuticals (Vanisree and Tsay, 2004).

Advances in biotechnology particularly, methods for culturing plant cell cultures, have provided new means for the commercial processing of even rare plants and the chemicals they provide. These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals. There has been considerable interest in plant cell cultures as a potential alternative to traditional agriculture for the industrial production of secondary metabolites (Dicosmo and Misawa, 1995). Plant cell culture technologies were introduced at the end of 1960s as a possible tool for both studying and producing plant secondary metabolites. Different strategies using cell cultures systems have been extensively studied with the objective of improving the production of bioactive secondary metabolites. Cell culture systems could be used for the large scale culturing of plant cells from which secondary metabolites can be extracted. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. The major advantages of cell cultures includes, synthesis of bioactive secondary metabolites running in controlled environment, independently from climatic and soil conditions; negative biological influences that affect secondary metabolites production in the nature are eliminated (microorganisms and insects); it is possible to select cultivars with higher production of secondary metabolites; with automatization of cell growth control and metabolic processes regulation, cost price can decrease and production increase. The objectives of many industries are to develop plant cell culture techniques to the stage where they yield secondary products more cheaply than extracting either the whole plant grown under natural conditions or synthesizing the product. Although the production of pharmaceuticals using plant cell cultures have been highlighted, other uses have also been suggested as new route for synthesis, for products from plants difficult to grow, or in short supply, as a source of novel chemicals and as biotransformation systems. It is expected that
the use, production of market price and structure would bring some of the other compounds to a commercial scale more rapidly and *in vitro* culture products may see further commercialization.

For plant cell culture techniques to become economically viable, it is important to develop methods that would allow for consistent generation of high yields of products from cultured cells (Berlin and Sasse, 1985). However, the use of plant cell or organ cultures has had only limited commercial success. This is explained by the empirical nature of selecting high-yielding, stable cultures and the lack of understanding of how secondary metabolites are synthesized or how their synthesis is regulated (Sevon and Oksman-Caldentey, 2002; Verpoorte and Memelink, 2002). Careful selection of productive cells and cultural conditions resulted in accumulation of several products in higher levels in cultured cells. In order to obtain yields in high concentrations for commercial exploitation, efforts have focused on the stimulation of biosynthetic activities of cultured cells using various methods (Buite-laar and Tramper, 1992; Ravishankar and Venkataraman, 1993; Dixon, 1999; Ramachandra Rao, 2000). Culture productivity is critical to the practical application of plant cell culture technology to production of plant-specific bioactive metabolites. Until now, various strategies have been developed to improve the production of secondary metabolites using plant cell cultures. The tissue culture cells typically accumulate large amounts of secondary compounds only under specific conditions. That means maximization of the production and accumulation of secondary metabolites by plant tissue cultured cells requires, manipulating the parameters of the environment and medium, selecting high yielding cell clones, precursor feeding and elicitation (Vanisree and Tsay, 2004).

Number of chemical and physical factors like media components, phytohormones, pH, temperature, aeration, agitation, light affecting production of secondary metabolites has been extensively studied (Fett-Neto *et al.*, 1995; Goleniowski and Trippi, 1999; Wang *et al.*, 1999; Lee and Shuler, 2000). Several products were found to be accumulating in cultured cells at a higher level than
those in native plants through optimization of cultural conditions. Manipulation of physical aspects and nutritional elements in a culture is perhaps the most fundamental approach for optimization of culture productivity. For example, ginsenosides by *Panax ginseng* (Furuya *et al.*, 1984; Furuya, 1988; Choi *et al.*, 1994; Franklin and Dixon, 1994), rosmarinic acid by *Coleus blumei* (Ulbrich *et al.*, 1985), ubiquinone-10 by *Nicotiana tabacum* (Fontanel and Tabata, 1987), berberin by *Coptis japonica* (Matsubara *et al.*, 1989), shikonin by *Lithospermum erythrorhizon* (Takahashi and Fujita, 1991) were accumulated in much higher levels in cultured cells than in the intact plants.

6.2. Review of Literature

Studies on plant secondary metabolites have been increasing over the last 50 years. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of pharmaceuticals (Ramachandra Rao and Ravishankar, 2002). Many biotechnological strategies have been hypothesized and experimented for enhanced production of secondary metabolites from medicinal plants. Some of these include screening of high yielding cell line, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization, biotransformation, genetic manipulations and others (Dornenburg and Knorr, 1995; Bhalsingh and Maheshwari, 1998; Namdeo *et al.*, 2002; Rao and Ravishankar, 2002; Vanishree *et al.*, 2004).

Plants produce secondary metabolites in nature as a defense mechanism against attack by pathogens. Elicitors are signals triggering the formation of secondary metabolites. Use of elicitors for plant defense mechanisms, i.e. elicitation, has been one of the most effective strategies for improving the productivity of bioactive secondary metabolites (Roberts and Shuler, 1997). Biotic and abiotic elicitors which are classified on their origin are used to stimulate secondary metabolite formation in plant cell cultures, thereby reducing the process time to attain high product concentrations (DiCosmo and Tallevi,
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1985; Eilert, 1987; Barz et al., 1988). Production of many valuable secondary metabolites using various elicitors have been reported (Lee and Shuler, 2000; Dong and Zhong, 2001; Hu et al., 2001; Wang and Zhong, 2002a; 2002b). Elicitation is used to induce the expression of genes often associated with enzymes responsible for the synthesis of secondary metabolites. Gundlach et al., (1992) demonstrated that jasmonic acid and its methyl ester are signal transducers in a wide range of plant cell cultures. These compounds accumulated rapidly and transiently when plant suspension cultures of Rauvolfia canescens and Eschscholtzia californica were treated with a yeast elicitor. Exogenously applied methyl jasmonate was shown to induce the production of secondary metabolites in 36 different plant species.

In the past few years, jasmonic acid and methyljasmonate have been shown to be inexpensive effective elicitors of secondary metabolite production. Methyljasmonate is emerging as a useful chemical tool to increase the production of secondary metabolites. Elicitor dosage was found to be dependent on both the tissue density and the free elicitor concentration in the medium (Susan and Shuler, 1997). Several reports have documented on the impact of methyl jasmonate on the enhancement of secondary metabolite production like grapevines (Vitis spp.) (Omer et al., 2000), Norway spruce (Picea abies L.) (Martin et al., 2002; 2003; Nicole et al., 2006; Zeneli et al., 2006) and Poplar (Populus spp.) (Babst et al., 2005; Cheng et al., 2006).

The effect of yeast extract as a fungal elicitor on the accumulation of Rosmarinic acid (RA) has been described for cell cultures of two species of the Lamiaceae and Boraginaceae, namely Orthosiphon aristatus (Sumaryono et al., 1991; Sumaryono and Proksch, 1993) and Lithospermum erythrorhizon (Mizukami et al., 1992, 1993). Yeast extract enhanced RA accumulation up to ten-fold in cell cultures of O. aristatus independent of the growth stage. Highest RA levels were reached 72–96 h after addition of yeast extract to the cell cultures.
In cell cultures of *L. erythrorhizon*, RA accumulation was stimulated two to three fold by yeast extract with maximum levels 24 h. after addition.

Accumulation of various classes of flavonoid and triterpene molecules in cell cultures of the legume *Medicago truncatula* by yeast elicitor (YE) and methyl jasmonate have been described (Suzuki *et al*., 2002, 2005; Achnine *et al*., 2005; Broeckling *et al*., 2005; Farag *et al*., 2007; Naoumkina *et al*., 2007). The mechanisms by which the cells respond to two different elicitors, yeast elicitor (YE) and methyl jasmonate (MJ), differ not only in the final end products accumulating, but also in the nature of the underlying signal transduction pathways (Naoumkina *et al*., 2007). The differences are most likely orchestrated by rapid induction of different sets/combinations of transcription factors (Nandi *et al* 2003; Lorenzo *et al*., 2003, 2004; Zheng *et al*., 2007).

Chitin (N-acetylchitooligosaccharide) of a certain size could induce various cellular responses including phytoalexin production (Ren and West 1992; Yamada *et al*., 1993). In plants, chitin oligosaccharides have been known to induce various defense responses in a wide range of plant cells including both monocots and dicots. Chitin oligosaccharides were reported to induce defense responses also in mammalian and insect cells (Shibuya and Minami, 2001; Furukawa *et al*., 1999). Interestingly, specific modifications of chitin oligosaccharides by fatty acids, sulfate or some sugars generate “nod factors” that induce nodulation in legume roots in the symbiotic interaction with rhizobial bacteria (Truchet *et al*., 1991). Similarly chitin has been used as a stress molecule in the study of its effect on *Citrus aurantium* (Maria *et al*., 2007).

Pectin is one of the main structural components of the primary cell walls of dicots, induces numerous effects in plants including defense responses, control of organogenesis, inhibition of auxin-induced elongation and rapid responses at the cell surface (Darvill, *et al*., 1992). Amrita and Sharmila (2008) have reported a significant increase in menthol in *Mentha piperita* cell culture by pectin.
Exogenous supply of a biosynthetic precursor to culture medium may also increase the yield of the desired product. This approach is useful when the precursors are inexpensive. The concept is based on the idea that any compound, which is an intermediate, in or at the beginning of a secondary metabolite biosynthetic route, stands a good chance of increasing the yield of the final product. Attempts to induce or increase the production of plant secondary metabolites, by supplying precursor or intermediate compounds, have been effective in many cases (Moreno et al., 1993; Whitmer et al., 1998; Silvestrini et al., 2002). For example, amino acids have been added to cell suspension culture media for production of tropane alkaloids, indole alkaloids etc. Addition of phenylalanine to *Salvia officinalis* cell suspension cultures stimulated the production of rosmarinic acid (Ellis and Towers, 1970). Production of anthocyanin and phenylethanoid glycosides was enhanced by feeding phenylalanine and related precursors to the cell cultures of strawberry and *Cistanche deserticola* respectively (Edahiro et al., 2005; Quyang et al., 2005). Addition of the same precursor resulted stimulation of taxol production in *Taxus* cultures (Fett-Neto et al., 1993; 1994). Feeding ferulic acid to cultures of *Vanilla planifolia* resulted in increase in vanillin accumulation (Romagnoli and Knorr, 1988). Furthermore, addition of leucine led to enhancement of volatile monoterpenes in cultures of *Perilla frutiscens*, whereas addition of geraniol to rose cell cultures led to accumulation of nerol and citronellol (Mulder-Krieger et al., 1988). Attempt to increase the production of desired molecules by supplying precursor have been effectively studied in of *Solanum lyratum* and *Cistanche salsa* (Lee et al., 2007; Liu et al., 2007).

The perusal literature survey suggests that, there are limited tissue culture reports available on *Mucuna* species and all the reported studies till-date were carried out on *Mucuna pruriens* L. that produces black seeds. Brain (1976) found that the callus tissue of *Mucuna pruriens* accumulated 25 mg l\(^{-1}\) L-dopa in the medium containing relatively high concentrations of 2,4-D. Teramoto and Komamine (1988) induced callus tissues of *Mucuna hassjoo*, *M. Pruriense*, and
M. deeringiana and optimized the culture conditions. The highest concentration of L-dopa was obtained when M. hassjoo cells were cultivated in MS medium with 0.025 mg\textsuperscript{l}^{-1} 2.4-D and 10 mg\textsuperscript{l}^{-1} kinetin. The level of L-dopa in the cells was about 80 mmol g\textsuperscript{-1} fw. Wichers \textit{et al.}, (1993) have also reported the accumulation of L-dopa in callus cultures of \textit{Mucuna pruriense}. Till-date no reports are available on \textit{in vitro} studies in its synonymous member \textit{Mucuna prurita}.

In the present chapter of the study, attempts were made to investigate the influence of some elicitor molecules viz., methyljasmonate, chitin, pectin and yeast extract, on L-dopa production. Apart from this, a precursor L-tyrosine molecule was also used to study its influence on L-dopa production, and on tyrosinase and polyphenol oxidase enzyme levels in callus cultures of \textit{Mucuna pruriens} L. and \textit{Mucuna prurita} H.

6.3. Materials and Methods

The surface sterilization and processing of plant tissue material or explants of both the cultivars of \textit{Mucuna} was described under general materials and methods (Chapter-3). Callus induction and proliferation was described under chapter-4 (Plantlet regeneration). Cell suspensions were established from 30 day old callus cultures that were induced on MS medium with IAA (2 mg\textsuperscript{l}^{-1}) and BAP (0.2 mg\textsuperscript{l}^{-1}) and 3% sucrose. Green calli (2 g) were inoculated in 250 ml Erlenmeyer flasks containing 100 ml liquid MS medium, supplemented with IAA (2 mg\textsuperscript{l}^{-1}) and BAP (0.2 mg\textsuperscript{l}^{-1}) and 3% sucrose. Cultures were maintained on a rotary shaker at 100 rpm at 22 °C, under a 16 h photoperiod (60 mmol m\textsuperscript{-2} s\textsuperscript{-1} PFD) (REMI, India). After two weeks, the cells released from calli were transferred into 4 volumes of fresh liquid medium and subcultures were done for every two weeks. Treatments of cell suspensions with L-tyrosine (50, 100, 150, 200 and 250 mM), methyljasmonate (50, 100, 150, 200 and 250 \textmu M) and chitin, pectin and yeast extract (50 mg, 100 mg, 150 mg, 200 mg and 250 mg) each, was performed 7 days after subculture when cells were in log phase of growth. At the same time, control cell suspensions in MS liquid medium, were supplemented with no
additive (elicit or precursor). Cell viability was determined by vital staining with methylene blue stain as described by Ravishankar (1998). The cells were harvested by vacuum filtration, weighted for growth analysis and analyzed on the same day.

6.3.1. Preparation of elicit and precursor solutions:

A 10 mM stock solution of methyljasmonate (MJ) (Sigma, USA) was prepared in 99% ethyl alcohol and then diluted with double distilled water upto 100 times to obtain the final concentration required. The 100 fold concentrated solution was added to autoclaved media after filter-sterilization (0.22 μm and 0.45 μm, Millipore, Intertech Inc., USA) to get the final concentrations (50 μM to 200 μM).

L-tyrosine was prepared by dissolving initially in 10% isopropanol with 1 or 2 drops of 1 N NaOH and later diluted with sterile double distilled water, pH was adjusted to 5.7. Solution was added to autoclaved media after filter-sterilization (0.22 μm and 0.45 μm, Millipore, Intertech Inc., USA) to get the final concentrations (50 mM to 200 mM).

Chitin was solubilized in 50% H₂SO₄ diluted with sterile double distilled water, pH was adjusted to 5.7. Solution was added to autoclaved media after filter-sterilization (0.22 μm and 0.45 μm, Millipore, Intertech Inc., USA) to get the final concentrations (50 mg to 200 mg).

Pectin was solubilized in sterile double distilled water, pH was adjusted to 5.7. Solution was added to autoclaved media after filter-sterilization (0.22 μm and 0.45 μm, Millipore, Intertech Inc., USA) to get the final concentrations (50 mg to 200 mg).

50g of yeast extract was dissolved in 250 ml of sterile double distilled water; to this 80% ethanol was added. The precipitate formed was allowed to settle for
four days at 6 °C. The supernatant was decanted and discarded. The gummy precipitate was dissolved in 250 ml of sterile double distilled water and once again precipitated with 80% ethanol. The second ethanol precipitate was dissolved in 200 ml of sterile double distilled water, pH was adjusted to 5.7. Solution was added to autoclaved media after filter-sterilization (0.22 μm and 0.45 μm, Millipore, Intertech Inc., USA) to get the final concentrations (50 mg to 200 mg).

6.3.2. Measurement of cell growth

Cell suspension culture material weighing 1 g FW was transferred into a 100 ml conical flask containing 20 ml of culture medium. The cell growth was monitored at every 3rd day (up to 30 days) by determining the fresh weight. Briefly the cells were harvested on every 3rd day to collect cells by vacuum filtration, weighted for growth analysis and analyzed on the same day. The fresh weight of the cells was recorded with the help of a physical balance. Alternatively cell growth was measured, by sedimented cell volume (SCV) measurement. Cell viability was determined by vital staining with methylene blue stain.

6.3.3. Extraction and quantification of L-dopa

The suspended cells were separated from the medium by vacuum filtration. The separated cells were washed with sterile distilled water to remove any adhering medium to the cell surface to record the fresh weight. Approximately 1 g of cells was taken and crushed using pestle and mortar by adding 10 ml of distilled water. The extract was boiled for 10 min and cooled and centrifuged for about 10 min at 5000 rpm. The supernatant was collected, boiled and cooled. Again the supernatant was centrifuged and the resulting supernatant was collected and was used for the estimation.

HPLC quantification L-dopa was carried out as described by Perumal Sidduraju and Klaus Becker (2001) with slight modifications as required. L-Dopa content in the extract was estimated by HPLC analysis using Waters, model 2487, pump 1515 with 2487 dual absorbance UV detection and
2414 RI detectors, manual sampler injector using C-18 column and a guard pre-column was packed with material, as in the main column. Isocratic elution was carried out using water:methanol:phsosphoric acid [975.5:19.5:1 (v/v)]. Separation was performed at room temperature (25 °C) and after the injection of 20 μl, the column was operated with the flow rate of 1.2 ml⁻¹. Absorbance was monitored at 282 nm and peak heights and areas were determined. Sample was eluted between 3rd and 4th min. The amount of L-dopa present in the tissue extracts was calculated with the help of standard curve for L- dopa standard (Sigma, St.Louis, MO., USA), amount of L-dopa present in tissue extract was expressed as mg g⁻¹ tissue dry weight. All the samples were passed through a glass-filter (0.20 μm pore size, Millipore, USA) and analyzed by HPLC for quantitative determination of L-dopa.

Alternatively L-dopa was also quantified by spectorophotometric analysis using nitrate-molybdate reagent as described by Earl Arnaw (1937). 1 g of callus was taken and crushed using pestle and mortar by adding 10 ml of distilled water. The extract was boiled for 10 min and cooled. Extract was centrifuged for about 10 min at 5000 rpm. The supernatant was collected, boiled and cooled. Again the supernatant was centrifuged and the resulting supernatant after centrifugation was collected and was used for the estimation. Reagents were added in the following order, 1 ml of 0.5N hydrochloric acid, 1 ml of nitrate molybdate reagent (a yellow colour product was formed at this point) 1 ml of 1N sodium hydroxide (a red colour product was formed), the absorbance was read the O.D at 520 nm.

The amount of L-dopa estimated by both HPLC and spectrophotometric methods in all the extracts were compared and the amount of L-dopa present in the callus cultures was determined with appropriate corrections required.
6.4.3. Estimation of Tyrosine hydroxylase and Polyphenol oxidase (PPO)

6.4.3.1. Preparation of enzyme extracts for assay

Enzyme extracts were prepared according to Jyotsnabaran Halder et al., (1998) with slight modifications as necessary. All preparatory operations were carried out at 4 °C. Cells or in vitro leaf material (approximately 200 mg) were homogenized into 2-3 ml cold 0.05 M potassium phosphate buffer (pH 7.0) containing 0.35M KCl and 0.5% TritonX100 and 50 μl of a cocktail mixture of protease inhibitors (mixture containing 70 mg phenyl methyl sulphonyl fluoride (PMSF), 1.4 mg leupeptin and 1 mg pepstatin per ml). Homogenates were filtered through two layers of cheese cloth, filtrates were centrifuged at 33,000 xg for 20 min. Supernatant was collected and used as enzyme source.

6.3.3.2. Tyrosine hydroxylase activity (Tyrosinase)

Tyrosine hydroxylase activity was measured by spectrophotometrically as described by Anderson and Craig (2001) using L-tyrosine as the substrate at 20 °C with modifications, in presence of 5 mM L-ascorbic acid to inhibit the polyphenol oxidase activity. The reaction mixture contained 2.95 ml of 10 mM L-tyrosine solution in 0.05 M Tris-HCl buffer (pH 8.5) and 0.05 ml of the enzyme extract. To distinguish between a phenoloxidase and the possibility of peroxidase activity responsible for hydroxylation of L-tyrosine, the tyrosine hydroxylase activity was performed in the presence of 6500 U catalase (Sigma, Sto.Louis, MO., USA). Absorbance was recorded at 475 nm and the enzyme activities were calculated using the standard curve for L-dopa (Sigma, St.Louis, MO., USA). One unit of tyrosine hydroxylase is that amount which catalyzes the formation of 1 μmol of product per min under the assay conditions. Specific activity is expressed as units mg^-1 protein.

6.3.3.3. Polyphenol oxidase activity

Polyphenol oxidase activity was determined by measuring the initial rate of quinine formation as indicated by an increase in absorbance at 420 nm (Coseteng and Lee, 1987). The reaction mixture contained 2.95 ml of 10 mM catechol
solution in 0.05 M phosphate buffer (pH 7.0) and 0.05 ml of the enzyme extract solution. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001 min$^{-1}$. Specific activity is expressed as units mg$^{-1}$ protein.

Protein concentration in the enzyme extracts was estimated by dye-binding method using bovine serum albumin as standard (Bradford, 1976).

6.4. Results and Discussion:

Standard L-dopa was quantified by HPLC, and the peak was emerged almost at 3$^{rd}$ minute of elution using the solvent system and conditions described under materials and methods of the current chapter (Fig. 6.4.1 a).

![Chromatogram showing peak of standard solution of L-dopa containing L-dopa in 1 mg ml$^{-1}$.](image-url)
In the present investigation the influence of the elicitor molecules, viz., methyljasmonate, chitin, pectin, yeast extract and a precursor L-tyrosine on L-dopa production and on tyrosinase and polyphenol oxidase enzyme levels in *in vitro* callus cultures of *Mucuna pruriens* L. and *Mucuna prurita* H. was investigated. The elicitors and precursor molecule were used in different concentrations as described in materials and method. The influence/effect of these elicitors and precursor molecule was studied at different time intervals from day 3 to day 15.

6.4.1. Effect of methyljasmonate on PPO, Tyrosinase activities and L-DOPA content in callus cultures of *M.pruriens* and *M.prurita*

Methyljasmonate was used at the concentrations of 50, 100, 150 and 200 μM l⁻¹ to study its effect on elicitation of L-dopa production, tyrosinase and PPO activities in the callus cultures of *M.pruriens* and *M.prurita*. The results of the studies were shown in Figures 6.4.1.1a, 6.4.1.1b and 6.4.1.1c for *M.pruriens* and Fig 6.4.1.2a, 6.4.1.2b and 6.4.1.2c *M.prurita*.

In both the cultivars of *Mucuna* there was gradual increase in the concentration of L-dopa from day ‘3’ to day ‘9’. In *M. pruriens* the concentration of L-dopa on day ‘9’ in 200 μM l⁻¹ was 45.93±0.32 mg g⁻¹ and in *M. prurita* it was 31.9±0.14 mg g⁻¹. From thereafter there was gradual decrease in the concentration of L-dopa till day ‘15’ (3.03±0.02 mg g⁻¹ in *M. pruriens* and 2.6±0.3 mg g⁻¹ in *M. prurita*). Nevertheless concentration of L-dopa did not fall below the control values (2.43±0.02 mg g⁻¹). The activity of PPO in *M.pruriens* was found to be increased from day ‘3’ (480.41±0.03 at 200 μM l⁻¹) and peaked on day ‘9’ (680.23±0.14 at 200 μM l⁻¹) and thereafter there was decrease in the activity. At 50 μM l⁻¹ MJ there was gradual increase in PPO from day ‘3’ (80.09±0.03) to day ‘9’ (240.63±0.06) whereas in 150 μM l⁻¹ and 200 μM l⁻¹, the activity remained same on day ‘3’ and day ‘6’ (120.10±0.02).
In *M. prurita* also there was increase in PPO activity from day ‘3’ (480.41±0.03 at 200 µM l⁻¹) and peaked in day ‘9’ (600.42±0.06 at 200 µM l⁻¹) and thereafter there was decrease in the activity. But the activity in 50 µM l⁻¹ MJ observed to be remained the same from day ‘3’ to day ‘15’ (120.10±0.02). At other concentrations the activity found to be remained same on day ‘3’ and ‘6’.

Tyrosinase activity was observed to be peaked at 200µM l⁻¹ on day ‘9’ in both the cultivars (1280.23±5.26 in *M.pruriens* 800.54±1.86 in *M.prurita*). There was gradual increase in the activity between day ‘3’ to day ‘9’ and thereafter there was decline in the activity. In both the enzymes even though there was decrease in the activity the activity did not fall below the control treatments.
Figure-6.4.1b: Chromatogram showing peak of L-dopa in suspension culture of (a₁) methyl jasmonate (M. pruriens), (a₂) methyl jasmonate (M. prurita).
Fig 6.4.1a-Effect of methyljasmonate on L-dopa content in callus cultures of *M. pruriens*

Fig 6.4.1b-Effect of methyljasmonate on PPO activity in callus cultures of *M. pruriens*
Fig 6.4.5.1c-Effect of methyljasmonate on tyrosinase activity in callus cultures of *M. pruriens*

Fig 6.4.1.2a-Effect of methyljasmonate on L-dopa content in callus cultures of *M. prurita*
Fig 6.4.1.2b-Effect of methyljasmonate on PPO activity in callus cultures of *M. prurita*

![Graph showing the effect of methyljasmonate on PPO activity.](image)

Fig 6.4.1.2c-Effect of methyljasmonate on tyrosinase activity in callus cultures of *M. prurita*

![Graph showing the effect of methyljasmonate on tyrosinase activity.](image)
6.4.2. Effect of chitin on PPO, Tyrosinase activities and L-dopa content in callus cultures of *M. pruriens* and *M. prurita*

Chitin was used in the concentration 50, 100, 150 and 200 mg l\(^{-1}\). Figures 6.4.2.1a, 6.4.2.1b and 6.4.2.1c shows the concentration of L-dopa, PPO activity and tyrosinase activity in callus cultures of different chitin concentration in *M. pruriens* and Figures 6.4.2.2a, 6.4.2.2b and 6.4.2.2c shows the concentration of L-dopa, PPO activity and tyrosinase activity in callus cultures of different chitin concentration in *M. prurita*.

There was increase in the concentration of L-dopa on day ‘3’ and day ‘6’ in both the cultivars and thereafter there was decrease in the concentration. Here the concentration of L-dopa was found to be more in 200 mg l\(^{-1}\). The PPO activity and tyrosinase activity was highest in 200mg l\(^{-1}\), peaked on day ‘6’ and thereafter there was gradual decrease in the activity of PPO and tyrosinase.

The concentration of L-dopa on day ‘3’ in *M. pruriens* was 5.9±0.02 mg g\(^{-1}\) in 50 mg l\(^{-1}\) and 12.02±0.01 mg g\(^{-1}\) in 200 mg l\(^{-1}\). On day ‘6’ it was 9.23±09 mg g\(^{-1}\) in 50 mg l\(^{-1}\) and in 200 mg l\(^{-1}\) it was 14.09±0.12 mg g\(^{-1}\). The PPO activity was 360.41±0.32 in 200 mg l\(^{-1}\) on day ‘3’ and ‘6’. Thereafter there was decrease in the activity and was 80.09±0.03 in all the concentrations of chitin on day ‘15’. The tyrosinase activity gradually decreased from day ‘3’ (840.92±3.02 in 200 mg l\(^{-1}\)) to day ‘15’ (420.52±0.31).

In *M. prurita* the concentration of L-dopa was peaked on day ‘6’ (12.62±0.01 mg g\(^{-1}\) in 200 mg l\(^{-1}\)) and thereafter there was decrease in its concentration. On day ‘15’ in 50 mg l\(^{-1}\) was 2.54±0.6 mg g\(^{-1}\) and in 200 mg l\(^{-1}\) it was 2.45±0.32 mg g\(^{-1}\). The PPO activity reached a maximum of 360.41±0.32 on day ‘6’ in 200 mg l\(^{-1}\) and it was 80.09±0.03 in all the concentrations of chitin on day ‘15’. The tyrosinase activity gradually decreased from day ‘3’ (840.92±3.02 in 200 mg l\(^{-1}\)) to day ‘15’ (420.52±0.31) as was in *M. pruriens*. 
Figure-6.4.1c: Chromatogram showing peak of L-dopa in suspension culture of (b₁) Chitin (M. pruriens), (b₂) Chitin (M. prurita)
Fig 6.4.2.1a-Effect of chitin on L-dopa content in callus cultures of *M. pruriens*

![Graph showing L-dopa content in callus cultures of *M. pruriens*. The graph displays the amount of L-dopa in mg g\(^{-1}\) F.W. over days of incubation. The x-axis represents the days of incubation (3, 6, 9, 12, 15), and the y-axis represents the amount of L-dopa. Different lines represent different chitin concentrations (C, 50, 100, 150, 200).

Fig 6.4.2.1b-Effect of chitin on PPO activity in callus cultures of *M. pruriens*

![Graph showing PPO activity in callus cultures of *M. pruriens*. The graph displays the activity of PPO in mg g\(^{-1}\) protein over days of incubation. The x-axis represents the days of incubation (3, 6, 9, 12, 15), and the y-axis represents the activity of PPO. Different lines represent different chitin concentrations (C, 50, 100, 150, 200).
**Fig 6.4.2.2c**-Effect of chitin on tyrosinase activity in callus cultures of *M. pruriens*

![Graph showing the effect of chitin on tyrosinase activity in callus cultures of *M. pruriens*.](image)

**Fig 6.4.2.2a**-Effect of chitin on L-dopa content in callus cultures of *M. prurita*

![Graph showing the effect of chitin on L-dopa content in callus cultures of *M. prurita*.](image)
Fig 6.4.2.2b-Effect of chitin on PPO activity in callus cultures of *M. prurita*

![Graph showing the effect of chitin on PPO activity in callus cultures of *M. prurita*]

Fig 6.4.2.2c-Effect of chitin on tyrosinase activity in callus cultures of *M. prurita*

![Graph showing the effect of chitin on tyrosinase activity in callus cultures of *M. prurita*]
6.4.3. Effect of pectin on PPO, Tyrosinase activities and L-dopa content in callus cultures of *M.pruriens* and *M.prurita*

Pectin was the third elicitor used in the present study. It was used in the concentration 50, 100, 150 and 200 mg l⁻¹. Concentration of L-dopa and the PPO activity of *M. pruriens* are shown in 6.4.3.1a, 6.4.3.1b and 6.4.3.1c and for for *M. prurita* it is Figures 6.4.3.2a, 6.4.3.2b and 6.4.3.2c.

Here also in both the cultivars of *Mucuna* there was gradual increase in the concentration of L-dopa from day ‘3’ to day ‘9’. From thereafter there was gradual decrease in the concentration of L-dopa till day ‘15’ in all the concentrations of pectin used. In *M. pruriens* it was 42.82±0.3 mg g⁻¹ in 200 mg l⁻¹ on day ‘9’ and 2.45±0.32 mg g⁻¹ on day ‘15’, whereas in *M. prurita* it was 40.43±0.12 mg g⁻¹ in 200 mg l⁻¹ on day ‘9’ and 2.36±0.02 mg g⁻¹ on day ‘15’.

The PPO activity in *M. pruriens* increased from day ‘3’ and peaked in day ‘9’ and thereafter there was decrease in the activity. The activities remain the same in 50 mg l⁻¹ in day ‘3’ and day ‘6’ (360.41±0.32) and there was gradual decrease in the activity. Whereas in 100 mg l⁻¹ there was increase in the activity on day ‘3’ (360.41±0.32) and ‘6’ and remained the same on day ‘9’ (480.41±0.03) and further there was decrease in the activity. In 150 mg l⁻¹ the activity remained the same on ‘3’ and ‘6’ (480.41±0.03) and increased on day ‘9’ (600.42±0.06) and further there was decrease in the activity. In 200 mg l⁻¹ there was gradual increase in the activity of PPO from day ‘3’ (480.41±0.03 ¹) to day ‘9’ (680.74±1.36) and further there was decrease in the activity.

In tyrosinase activity there was gradual increase in the activity from ‘3’ to day ‘9’ in all the concentrations used and the activity peaked in day ‘9’. It was 840.92±3.02 in 50 mg l⁻¹ and 680.56±0.83 in 200 mg l⁻¹ on day ‘3’ and on day ‘9’ it was 880.72±3.12 in 50 mg l⁻¹ and 1280.23±5.26 in 200 mg l⁻¹. After day ‘9’ there was gradual decrease in the activity till day ‘15’ (420.52±0.31) in all the concentrations.
In *M. prurita* the activity of PPO remained the same in day ‘3’ to day ‘9’ in 50 mg l\(^{-1}\) (360.41±0.32) and thereafter there was decrease in the activity. The activity was peaked on day ‘9’ in 200 mg l\(^{-1}\), reached 680.74±1.36.

The tyrosinase activity in *M. pruriens* increased from ‘3’ to day ‘9’ in all the concentrations used and the activity was maximum on day ‘9’ (1120.74±5.36 in 50 mg l\(^{-1}\) and 1520.79±6.14 in 200 mg l\(^{-1}\)). After day ‘9’ there was gradual decrease in the activity till day ‘15’ (420.52±0.31 in 50 mg l\(^{-1}\) and 800.54±1.86 in 200 mg l\(^{-1}\)). The results were the same in *M. prurita* also. But in 50 mg l\(^{-1}\) the activity remain the same in day ‘3’ and day ‘6’ (840.92±3.02) and there was increase in the activity on day ‘9’ (880.72±3.12) and further there was decrease in the activity. In 200 mg l\(^{-1}\) the activity increases from ‘3’ (680.56±0.83) to day ‘6’ remained the same on day ‘9’ (1120.74±5.36) and thereafter there was decrease in the activity.
Figure-6.4.1d: Chromatogram showing peak of L-dopa in suspension culture of (C₁) Pectin (*M. pruriens*), (C₂) Pectin (*M. prurita*)
Fig 6.4.3.1a. Effect of pectin on L-dopa content in callus cultures of *M. pruriens*

![Graph showing effect of pectin on L-dopa content](image)

Fig 6.4.3.1b. Effect of pectin on PPO activity in callus cultures of *M. pruriens*

![Graph showing effect of pectin on PPO activity](image)
Fig 6.4.3.1c-Effect of pectin on tyrosinase activity in callus cultures of *M. pruriens*

![Graph of tyrosinase activity over days of incubation with different pectin concentrations.]

Fig 6.4.3.2a-Effect of pectin on L-dopa content in callus cultures of *M. prurita*

![Graph of L-dopa content over days of incubation with different pectin concentrations.]
Fig 6.4.3.2b-Effect of pectin on PPO activity in callus cultures of *M. prurita*

![Graph showing the effect of pectin on PPO activity](image)

Days of Incubation: 3, 6, 9, 12, 15

Activity mg⁻¹ protein

Fig 6.4.3.2c-Effect of pectin on tyrosinase activity in callus cultures of *M. prurita*

![Graph showing the effect of pectin on tyrosinase activity](image)

Days of Incubation: 3, 6, 9, 12, 15

Activity mg⁻¹ protein
6.4.4. Effect of yeast extract on PPO, Tyrosinase activities and L-dopa content in callus cultures of *M. pruriens* and *M. prurita*

The fourth elicitor used in the present study was yeast extract (YE). It was used at the concentration of 50, 100, 150 and 200 mg l\(^{-1}\). Concentration of L-dopa PPO activity and the tyrosinase activity of both the cultivars of *Mucuna* were shown in 6.4.4.1a, 6.4.4.1b and 6.4.4.1c for *M. pruriens* and 6.4.4.2a, 6.4.3.2b and 6.4.4.2c for *M. prurita*.

In both the cultivars of *Mucuna* there was gradual increase in the concentration of L-dopa from day ‘3’ to day ‘9’. It was 16.53±0.35 mg g\(^{-1}\) in 50 mg l\(^{-1}\) and 42.78±0.14 mg g\(^{-1}\) in 200 mg l\(^{-1}\) on day ‘3’ and 31.52±0.12 mg g\(^{-1}\) in 50 mg l\(^{-1}\) and 62.35±5.43 mg g\(^{-1}\) in 200 mg l\(^{-1}\) on day ‘9’ in *M. pruriens* and it was 14.73±0.12 mg g\(^{-1}\) in 50 mg l\(^{-1}\) and 40.69±1.32 mg g\(^{-1}\) in 200 mg l\(^{-1}\) on day ‘3’ and 29.65±0.18 mg g\(^{-1}\) in 50 mg l\(^{-1}\) and 60.45±5.13 mg g\(^{-1}\) in 200 mg l\(^{-1}\) on day ‘9’ in *M. prurita*. Thereafter there was gradual decrease in the concentration of L-dopa till day ‘15’ in all the concentrations used in both the cultivars. However, the extent decrease was observed to be not as that of with the other elicitor molecules.

In *M. pruriens* PPO activity was found to be increased between day ‘3’ (360.41±0.32 in 50 mg l\(^{-1}\) and 680.74±1.36 in 200 mg l\(^{-1}\)) to day ‘9’ (600.42±0.06 in 50 mg l\(^{-1}\) and 840.92±3.02 in 200 mg l\(^{-1}\)) and thereafter a gradual decrease in the activity was recorded till day ‘15’ at all the concentrations of yeast extract supplemented in the medium.

In *M. prurita* also there was a gradual increase in the PPO activity from day ‘3’ (360.41±0.32 in 50 mg l\(^{-1}\) and 680.74±1.36 in 200 mg l\(^{-1}\)) to day ‘9’ (480.41±0.03 in 50 mg l\(^{-1}\) and 840.92±3.02 in 200 mg l\(^{-1}\)) and thereafter followed a declining trend till day ‘15’. But at 50, and 100 mg l\(^{-1}\) concentrations the enzyme activity was observed to be remained constant between day ‘12’ to day ‘15’ (360.41±0.32), while at the other two (higher) concentrations enzyme activity was recorded to be followed a declining trend during the same period.
The tyrosinase activity was found to be similar to PPO in both the cultivars of *Mucuna*. In *M. pruriens* the activity on day ‘3’ was found to be 800.54±1.86 in 50 mg l\(^{-1}\) and 1280.23±5.26 in 200 mg l\(^{-1}\) and no day ‘9’ it was 1120.74±5.36 in 50 mg l\(^{-1}\) and 1520.79±6.14 in 200 mg l\(^{-1}\), and thereafter a gradual decrease in the activity was recorded till day ‘15’ at all the concentrations of yeast extract supplemented in the medium. The activity of tyrosinase in *M. prurita* on day ‘3’ was found to be 840.92±3.02 in 50 mg l\(^{-1}\) and 1280.23±5.26 in 200 mg l\(^{-1}\) and no day ‘9’ it was 720.74±2.56 in 50 mg l\(^{-1}\) and 1520.79±6.14 in 200 mg l\(^{-1}\), and thereafter a gradual decrease in the activity was recorded till day ‘15’ at all the concentrations of yeast extract supplemented in the medium.
Figure-6.4.1e: Chromatogram showing peak of L-dopa in suspension culture of (d₁) Yeast extract (M. pruriens), (d₂) Yeast extract (M. prurita)
Fig 6.4.4.1a-Effect of yeast extract on L-dopa content in callus cultures of *M. pruriens*

![Graph showing L-dopa content over days of incubation with different yeast extract concentrations.]

Days of Incubation

Fig 6.4.4.1b-Effect of yeast extract on PPO activity in callus cultures of *M. pruriens*

![Graph showing PPO activity over days of incubation with different yeast extract concentrations.]

Days of Incubation
Fig 6.4.4.1c-Effect of yeast extract on tyrosinase activity in callus cultures of *M. pruriens*

Fig 6.4.4.2a-Effect of yeast extract on L-dopa content in callus cultures of *M. prurita*
Fig 6.4.4.2b-Effect of yeast extract on PPO activity in callus cultures of *M. prurita*

![Graph showing the effect of yeast extract on PPO activity in callus cultures of *M. prurita*.](image)

Days of Incubation

Fig 6.4.4.2c-Effect of yeast extract on tyrosinase activity in callus cultures of *M. prurita*

![Graph showing the effect of yeast extract on tyrosinase activity in callus cultures of *M. prurita*.](image)

Days of Incubation
6.3.5. Effect of tyrosine on PPO, Tyrosinase activities and L-dopa content in callus cultures of *M. pruriens* and *M. prurita*

Tyrosine, the precursor molecule (Pattison *et al.*, 2002) for the production of L-dopa was used in the concentration 50, 100, 150 and 200 mM l⁻¹. Fig 6.4.5.1a-c, and Fig 6.4.5.2a-c shows the concentration of L-dopa, PPO activity and the tyrosinase activity in both the cultivars of *Mucuna*.

There was gradual increase in the concentration of L-dopa from day ‘3’ to day ‘15’ in all the concentrations of tyrosine used in both the cultivars of *Mucuna*. In *M. pruriens* the concentration of L-dopa on day ‘3’ was 10.52±0.23 mg g⁻¹ in 50 mM l⁻¹ and 39.42±1.32 mg g⁻¹ in 200 mM l⁻¹. On day ‘15’ the concentration was 30.47±0.72 mg g⁻¹ in 50 mM l⁻¹ and 94.72±7.06 mg g⁻¹ in 200 mM l⁻¹, whereas in *M. prurita* the concentration of L-dopa was 10.52±0.23 mg g⁻¹ in 50 mM l⁻¹ and 35.46±0.42 mg g⁻¹ in 200 mM l⁻¹ on day ‘3’. On day ‘15’ it was 30.47±0.72 mg g⁻¹ in 50 mM l⁻¹ and 90.54±7.05 mg g⁻¹ in 200 mM l⁻¹.

The activity PPO and tyrosinase was also increased from day ‘3’ to day ‘15’. In *M. pruriens* the PPO activity in 50 mM l⁻¹ remained the same from day ‘3’ to day ‘9’ (320.32±0.21) and further there was increase in the activity till day ‘15’ (600.42±0.06). In 100 mM l⁻¹ the activity remained the same in day ‘3’ and ‘6’ (320.32±0.21) and thereafter there was increase in the activity till day ‘15’ (800.54±1.86). In 150 and 200 mM l⁻¹ there was increase in the PPO activity from day ‘3’ (480.41±0.03 in 150 mM l⁻¹ and 600.42±0.06 in 200 mM l⁻¹) to day ‘15’ (1400.49±3.16 in 150 mM l⁻¹ and 1400.49±3.16 in 200 mM l⁻¹). In *M. prurita* the PPO activity in both 50 mM l⁻¹ and 100 mM l⁻¹ remained the same from day ‘3’ to day ‘9’ (320.32±0.21) and further there was increase in the activity till day ‘15’. In 150 and 200 mM l⁻¹ there was increase in the PPO activity from day ‘3’ (480.41±0.03 in 150 mM l⁻¹ and 680.74±1.36 in 200 mM l⁻¹) to day ‘15’ (840.92±3.02 in 150 mM l⁻¹ and 1400.49±3.16 in 200 mM l⁻¹).
There was gradual increase in the tyrosinase activity in both the cultivars of *Mucuna*. In 50mM l\(^{-1}\) the activity remained the same from day ‘3’ to day ‘9’ (840.92±3.02) and further there was increase in the activity till day ‘15’ in both the cultivars of *Mucuna*. And further there was increase in the activity from day ‘3’ (840.92±3.02 in 100 mM l\(^{-1}\) and 1280.23±5.26 in 200 mM l\(^{-1}\) in *M. pruriens* and 840.92±3.02 in 100 mM l\(^{-1}\) and 1120.74±5.36 in 200 mM l\(^{-1}\) in *M. prurita*) to day ‘15’ (1420.43±5.35 in 100 mM l\(^{-1}\) and 2080.26±7.34 in 200 mM l\(^{-1}\) in *M. pruriens* and 1280.23±5.26 in 100 mM l\(^{-1}\) and 1120.74±5.36 in 200 mM l\(^{-1}\) in *M. prurita*).
Figure-6.4.1f: Chromatogram showing peak of L-dopa in suspension culture of (e₁) Tyrosine (M. pruriens), (e₂) Tyrosine (M. prurita)
Fig 6.4.5.1a-Effect of tyrosine on L-dopa content in callus cultures of *M. pruriens*

![Graph of L-Dopa content in mg g\(^{-1}\) F.W. vs Days of Incubation](image)

Fig 6.4.5.1b-Effect of tyrosine on PPO activity in callus cultures of *M. pruriens*

![Graph of Activity mg\(^{-1}\) protein vs Days of Incubation](image)
Fig 6.4.5.1c-Effect of tyrosine on tyrosinase activity in callus cultures of *M. pruriens*

![Graph showing the effect of tyrosine on tyrosinase activity in callus cultures of *M. pruriens*.](image)

Fig 6.4.5.2a-Effect of tyrosine on L-dopa content in callus cultures of *M. prurita*

![Graph showing the effect of tyrosine on L-dopa content in callus cultures of *M. prurita*.](image)
Fig 6.4.5.2b-Effect of tyrosine on PPO activity in callus cultures of *M. prurita*

![Graph showing the effect of tyrosine on PPO activity](image)

Fig 6.4.5.2c-Effect of tyrosine on tyrosinase activity in callus cultures of *M. prurita*

![Graph showing the effect of tyrosine on tyrosinase activity](image)
Elicitation has been one of the most effective strategies for improving the productivity of bioactive secondary metabolites (Roberts and Shuler, 1997). Production of many valuable secondary metabolites using various elicitors have been reported (Lee and Shuler, 2000; Dong and Zhong, 2001; Hu et al., 2001; Wang and Zhong, 2002a; 2002b). Methyljasmonate, chitin, pectin, yeast extract were used in the present study for the study of their effect on the production of L-dopa. The significant effects of these molecules to enhance the production of L-dopa were shown (Fig 6.4.1.1a to 6.4.5.1a and Fig 6.4.1.2a to 6.4.5.2a). Of the four elicitors used yeast extract, proved to be very efficient compared to methyljasmonate, chitin and pectin to increase the L-dopa content. Eventhough methyljasmonate has also significantly increased the L-dopa content, but it was not as much as yeast extract. Chitin and pectin have also increased the L-dopa content, but comparatively lesser efficient than the other two elicitors tried. Of the four elicitors used for the L-dopa elicitation, chitin was found to be least efficient. Several reports have documented on impact of the MJ on enhancement of secondary metabolite production in other plant cell cultures like grapevines (Vitis spp.) (Omer et al., 2000), norway spruce (Picea abies L.) (Martin et al., 2002, 2003; Nicole et al., 2006; Zeneli et al., 2006) and poplar (Populus spp.) (Babst et al., 2005; Cheng et al., 2006). Accumulation of various classes of secondary metabolite molecules in cell cultures of legumes by yeast elicitor (YE) and methyljasmonate have been described (Suzuki et al., 2002, 2005; Achnine et al., 2005; Broeckling et al., 2005; Farag et al., 2007; Naoumkina et al., 2007). Pectin has been reported to be as an effective elicitor that has increase significantly menthol in Mentha piperita cell cultures (Amrita and Sharmila, 2008), similarly chitin has also been used as a stress molecule in the study of its effect on Citrus aurantium (Maria et al., 2007). But during the present it was observed that both pectin and chitin fall behind the yeast extract and methyjasmonate in eliciting L-Dopa content in the cell cultures of both M. pruriens and M. prurita. There was decrease in the L-dopa content in all the elicitors used after nine days of treatment. This may be because of loss of cell viability in culture after nine days.
of exposure as was confirmed in by staining of the cells for viability. (Stefania et al., 2002; Walker et al., 2002; Sonja et al., 2007)

Attempt to increase the production of desired molecules by supplementing precursors in the medium have been effectively studied in case of Solanum lyratum and Cistanche salsa (Lee et al., 2007; Liu et al., 2007). Production of anthocyanin and phenylethanoid glycosides was enhanced by feeding L-phenyl alanine and related precursors to the cell cultures of strawberry and Cistanche deserticola respectively (Edahiro et al., 2005; Quyang et al., 2005).

L-dopa is produced via the oxidation of tyrosine by the copper containing enzyme tyrosine hydroxylase in the presence of molecular O₂ (Pattison et al., 2002). Hence in the present study L-tyrosine was used as precursor to study its influence (supplementation in the medium) on the production of L-dopa. Of the precursor tyrosine and all the four elicitors tested, tyrosine has increased the concentration of L-dopa significantly to a much higher extent compared to the elicitor molecules. Amongst the elicitors tested yeast extract was found to be superior that elicited higher concentration of L-dopa, while chitin was found to be inferior that showed the poor elicitation of L-dopa production in the cell suspension culture of two Mucuna species. The order elicitor enhanced production L-dopa was evaluated for 4 elicitors as yeast extract > pectin > methyljasmonate > chitin. The efficacy of L-dopa production was found to be higher for suspension cultures of M. pruriens L. compared to M. prurita H. in all the treatments (Fig. 6.4.1.1a-c to 6.4.5.1a-c and 6.4.1.2a-c to 6.4.5.2a-c).

From the results of the present investigations, it could be concluded that precursor molecule L-tyrosine has been evaluated to be the best choice for L-dopa production, that yielded the highest amount of L-dopa by increasing the L-tyrosine hydroxylase activity at all the concentrations tested, followed by yeast extract among the elicitors tested. All the elicitors could able to elicit L-dopa production upto either 6th or 9th day of incubation, and failed to show the consistency
throughout the period of study. The amount of L-dopa yield was found to be
dependent on concentration of elicitor used. At higher concentrations, especially
in case of chitin and methyljasmonate the yield of L-dopa was very poor, though
it was estimated to be higher than respective control values. The low yield might
be attributed to the loss of cell viability in presence of higher concentrations of
elicitor molecule and also even at low concentrations because of exposure for the
longer durations (15 days).