Summary and Conclusions

The present piece of investigation deals with “In vitro mutation and phytochemical studies in insulin plant Costus pictus D. Don.” Ayurveda is becoming a potential, global medicinal system as it facilitates good health and longevity instead of fighting with disease. Therefore people are again coming back towards ayurvedic medicines, herbal therapies and trying to avoid the allopathic medicines, due to side effects. Costus pictus plant is a native from Mexico and America and due to its medicinal value it is commonly known as the insulin plant. For India it is an exotic plant distributed in specific area only where it was cultivated. Its leaves and stem extracts have potential to overcome type 2 diabetes. Due to this potent activity, it is in great demand and to extract active principle compound, large plant material is getting exploited. Conventional propagation is hampered in this plant due to poor rooting ability of vegetative cutting; low seed germination and less viability of seeds. Due to this alternative methods have become necessary for large scale multiplication, conservation and to enhance concentrations of secondary metabolites carried by this plant material. Taking into considerations all such problems, present studies were carried out to standardize a protocol on tissue culture besides in vitro mutagenesis in C. pictus with phytochemical investigation. The outcome of the present research work is briefly summarized and concluded below.
Summary and Conclusions

➢ To achieve regeneration of plant, different explants were inoculated on MS medium incorporated with different concentrations of growth regulators. Minimum contamination of microbes and higher percentage of survival (80%) could be achieved at 0.1% of HgCl₂ treatment for 5 minutes to leaf explant, while for nodal segment and shoot tips, it was 0.3% of HgCl₂ for 3 minutes treatment. It was also noted that the lower concentration did not prove effective as it showed contamination while higher concentrations above 0.3% affected survival percentage of explants.

➢ Induction of callus was achieved on MS medium incorporating different auxins and cytokinins either alone or in combination from leaf, nodal segment and shoot tip explants. Friable white yellow callus was derived from 2, 4-D alone and in combination with 1.0 mg/L - 1.5 mg/L and 2.0 mg/L of 2, 4-D along with 1.0 mg/L of BAP effectively by using leaf and nodal segment explants.

➢ Higher concentration of 2, 4-D alone was found effective for induction of white granular callus and later development of somatic embryos after 3 weeks on nodal segment as well as leaf explants at more or less frequently.

➢ IAA in combination with BAP was not found effective for induction of callus. Poor callus was developed or swelling of explants could be recorded from these combinations from leaf and nodal segment explants.
Summary and Conclusions

- Regeneration of shoot percentage recorded was less from callus derived from different explants. The callus derived from nodal segment showed 50% of shoot regeneration with 1.40 ± 0.24 number of shoots on MS medium supplemented with 0.5 mg/L of IAA along with 1.5 mg/L of BAP. It was found the most suitable concentration to achieve shoot regeneration from callus.

- Leaf and shoot tip were also able to induce caulogenesis at lower ratio of shoot regeneration. Maximum shoot regeneration from callus derived from leaf was 30% with 1.00 ± 0.00 number of shoots in combination with 0.5 mg/L of IAA and 1.5 mg/L of BAP in MS medium. While 0.5 mg/L of IAA along with 2.0 mg/L of BAP were found suitable concentration to record regeneration of shoot from callus.

- Direct shoot regeneration could be recorded on MS medium supplemented with various concentrations of auxins and cytokinins in combination by using leaf and nodal segments as explants. Nodal segment as explants recorded best result for direct shoot regeneration on MS medium with IAA in combination with BAP and KIN with higher number of shoots.

- Direct shoot regeneration from nodal explants was noted at 0.5 mg/L IAA alongwith 0.5 mg/L - 3.5 mg/L of BAP. Most suitable concentration was found to be 3.0 mg/L of BAP, which recorded 93.33% of shoot regeneration with 2.06±0.300 number of shoots per explant. 4.0 mg/L of
KIN along with IAA was recorded as an effective combination for direct shoot regeneration from nodal segment.

- Shoot regeneration was also achieved from IAA in combination with BAP and KIN using leaf as explants. The effective concentration was found to be 0.5 mg/L of IAA with 2.5 mg/L of BAP and 4.0 mg/L of KIN supplemented in MS medium, respectively.

- Multiple shooting from leaf and nodal segment was achieved testing with 2.5mg/L and 3.0 mg/L of BAP with various concentrations of IAA. The highest mean number of shoots was recorded at 2.5 mg/L of BAP along with 0.5 mg/L of IAA using leaf as explant. Whereas for nodal segment explant, 3.0 mg/L of BAP with 0.5 mg/L of IAA could be found the most suitable combination.

- Multiple shooting was also achieved from combination of BAP and NAA incorporated in MS medium. The highest number of shoot from leaf explants was recorded at 2.5 mg/L of BAP along with 0.5 mg/L of NAA. While 3.0 mg/L of BAP with 0.5 mg/L of NAA showed the best results for nodal segment explant.

- MS media incorporated with KIN in combination with IAA, NAA and 2, 4-D for induction of multiple shoot as compared to BAP proved less effective but was able to induce multiple shoots.
Summary and Conclusions

- Leaf and nodal explants showing multiple shooting on KIN in combination with IAA, and leaf explants showed 70.00% of shoot induction with 1.50±0.372 mean number of shoots per explant at 4.0 mg/L of KIN with 0.5 mg/L of IAA while the nodal segment showed 80.00% of shoot induction with 2.40±0.305 number of mean shoots per explant.

- NAA in combination with KIN recorded multiple shoot induction. Higher number of shoots from leaf explants was achieved at 4.0 mg/L of KIN in combination with 0.5 mg/L of NAA. It revealed 93.33% of shoot induction with 2.53±0.236 number of shoots per explant by using nodal segment as an explant.

- Leaf and nodal segment induced multiple shoots on MS medium supplemented with KIN and 2, 4-D. The most suitable concentration was found to be 0.5 mg/L of 2, 4-D along with 4.0 mg/L of KIN. It showed maximum shoot induction percentage of 80.00% with 1.20±0.200 and 2.00±0.292 number of shoots per explant for leaf and nodal segment, respectively. Higher concentration of 2, 4-D, 2.5 mg/L showed shoot formation along with moderate callus induction.

- After six to seven weeks, well-developed shoots were transferred to another medium for rhizogenesis. Various concentrations of IAA and NAA were found potential for induction of roots of two types namely fibrous roots and adventitious roots.
Summary and Conclusions

➢ Well rooted microshoot cultures were exposed to external conditions of interval and then transferred to coco peat, soil: FYM (1:1) and finally in soil put in pot after particular time of period. All above steps were carried out in aseptic and controlled polyhouse conditions and 60.00% of hardening of *C. pictus* could be achieved.

➢ *In vitro* mutagenesis experiment was carried out to induce variability and reduce the concentration of palmitic acid which was a main obstacle in utilization of plants for treatment of diabetes, by using SA and EMS as chemical mutagens.

➢ Observations of *in vitro* mutagenesis comprised sprouting of bud percentage, besides studies of morphological and chlorophyll variants. It revealed that, 0.10% EMS and 0.01% of SA showed maximum percentage of sprouting of bud from nodal segment explant.

➢ It was found that higher concentration of mutagen showed effect on sprouting of bud, where it decreased with an increase in concentration of mutagen. The lower concentration of mutagen was found most suitable to induce mutation *in vitro*.

➢ Observation on *in vitro* mutagenesis revealed morphological changes in M₁ generation through SA and EMS treatments. Morphological variation included long shoot, dwarf shoot, broad stem, thin stem and change in leaf lamina. They were observed at lower concentrations of SA and EMS.
Mutagen EMS was found better for induction of morphological variations under \textit{in vitro} mutagenesis compared to SA. The lower concentration of SA induced poor variation in M\textsubscript{1} generation.

\textit{In vitro} mutagenesis experiment also revealed chlorophyll variants in the form of \textit{albino}, \textit{xantha}, \textit{chlorina} and \textit{virdis}. Leaf having \textit{albino} sector did not survive for more than 20 days while other types showed stunted growth as compared to control.

Chlorophyll variants were induced at higher frequency using nodal segment explant at various concentrations of EMS and SA. 0.10\% of EMS was found the best concentration in this regard.

The frequency of chlorophyll variants could be recorded in the order \textit{xantha} followed by \textit{albino} and \textit{virdis}.

Phytochemical analysis revealed that, the secondary metabolites got altered due to change in condition. More number of metabolites were recorded from mutagen treated leaf extract compared to tissue culture and \textit{in vivo} leaf extract.

Variability in number of secondary metabolites was reported at lower degree from stem extract of \textit{in vivo}, \textit{in vitro} and mutagen treated grown plantlets.

\textit{C. pictus} plant referred to as an insulin plant is having potent herbal drugs against type-2 diabetes. It is necessary to screen the plant in detail in
regard to its contents and to assess their activities, another phytochemical and antidiabetic assay deserved to be formulated and carried out systematically in succession.