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SCREENING OF PGRs, L-DOPA PRECURSOR AND POLYPLOIDY INDUCER TREATMENTS ON PROTEIN CONTENT OF *MUCUNA PRURIENS* CALLUS.

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An investigation has been conducted to analyse the effect of different concentrations of phytohormones (2,4-D and kinetin) precursor (Tyrosine,1.0,1.5,and 2.0mM) and polyploidy inducer (colchicine 313 µM) treatments on protein content in *Mucuna pruriens* four weeks old calli. Highest amount of protein accumulated in callus raised on MS+ 3.0mg/l 2,4-D+0.1 mg/l Kn+1.5mM tyrosine as compared to all other treatments of colchicine and tyrosine individually. It was also noticed that the calli sub cultured after 12 hr treatment of colchicine in suspension media on MS+ 3.0mg/l 2,4-D showed higher concentrations of protein content as compared to all other treatments of colchicine. It indicates that treatment of tyrosine being a precursor of L-DOPA, led to increased level of protein while colchicine being an anti-mitotic, leads to accumulation of all those proteins, within the cell, which take part in the cell division. The study indicates supplementation of polyploidy inducer for shorter duration (313 µM for 12hr) and combination of phytohormone (3.0mg/l 2,4-D+0.1 mg/l Kn) with 1.5mM tyrosine may be preferred for increased accumulation of protein content in *Mucuna pruriens* calli.

Key words:-Tyr (Tyrosine),Col (Colchicine), L-DOPA (L-3,4-dihydroxyphenylalanine)

Mucuna pruriens is a widespread legume in tropical and sub-tropical regions of the world. It is considered a viable source of dietary proteins (Janardhanan *et al*. 2003 and Pugalenthi *et al.* 2005) due to its high protein concentration (23–35%) in addition to its digestibility, which is comparable to that of other pulses such as soybean, rice bean, and lima bean (Gurumoorthi *et al.* 2003). The beans have been traditionally used as food in a number of countries, viz., India, Philippines, Nigeria, Ghana, Brazil, and Malawi. It is therefore regarded a good source of food. It is one of the most popular Indian medicinal plant, which has long been used in traditional Ayurvedic Indian medicine, for diseases including parkinsonism (Sathiyanarayanan *et al.* 2007) and is a constituent of more than 200 indigenous drug formulations. According to Evans (2002) approximately 120 species of *Mucuna* have been reported from world wide. Out of 120, 15 species were identified in India (Anonymous, 2005). This plant is widely used in Ayurveda, which is an ancient traditional medical science that has been practiced in India since the Vedic times (1500–1000 BC). In many tropical countries, *Mucuna* beans are processed into flour as coffee substitute, or eaten as vegetable.

It contains novel alkaloids, saponins, and sterols (Manyam *et al*. 2004) and a high concentration of L-DOPA. L-DOPA, (L-3,4-dihydroxyphenylalanine), an-anti-nutritional compound is an important intermediate of secondary metabolism in higher plants and is known as a precursor of alkaloids, betalain, melanine, and others. According to Kumar *et al.* (2011) *in vitro* regenerated plant of *Mucuna pruriens* is a high accumulator of L-DOPA under salt stress. It is also a precursor of catecholamines in animals.

L-Dopa is produced via the oxidation of L-tyrosine by the copper-containing enzyme tyrosine hydroxylase in the presence of molecular O_2(Pattison *et al*. 2002).

In addition, serotonin and a number of indolic alkaloids structurally related to serotonin have been reported in various parts of the *Mucuna* plant, several of which have hallucinogenic properties of considerable strength (Szabo and Tebbett 2002). It would, however, be unlikely for these low-level alkaloids to have any effect on human and animal consumers because their absorption across the gastrointestinal tract is negligible (Szabo 2003).

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In the light of scarce literature regarding effect of PGRs, L-DOPA precursors and polyploidy inducers on protein content of *Mucuna pruriens,* the same was proposed to be undertaken during the present study.

MATERIALS AND METHODS

For the present study *Mucuna pruriens* has been used as experimental material. The explants were collected from Department of Botany, C.C.S.University campus Meerut.The cotyledeons of plant are selected as explant for the induction of callus. The explants were surface sterilized subsequently with the running tap water, Tween-20 (detergent) and finally with 0.1 % NaoCl for 4 to 5 minutes under laminar flow. The explants were then thoroughly rinsed with double distilled sterilized water 2-3 times and cut in an antioxidant (Ascorbic acid 5mg/ml) prepared in double distilled sterilized water, and the explants were directly inoculated on MS (Murashige and Skoog 1962) under aspectic conditions. Cultures were maintained at 25 ± 2 ºC in continuous light of 2000 lux intensity. Young cotyledons were excised in to 2-3 parts and inoculated after sterilization on MS (1962) nutrient medium supplemented with 2,4 D (1.0, II 2.0 and 3.0mg/l) and kinetin (0.05 and 0.1mg/l) their combinations for callus induction. In 14-18 days after inoculation a good amount of callus was formed on supplemented media. Callus, raised on differently supplemented MS medium, was transferred to suspension culture for treatment of Col (313 µM), Tyr (1.0, 1.5 and 2.0mM) and their combinations. The estimation of protein was carried out using (Lowry *et al.* 1951) protocol.

The statistical analysis of the data obtained from all studies was expressed as mean ± standard deviation (SD) of three separates experiments using the Microsoft excel 2010. In order to detect the significance of differences ($p \leq 0.05$)of variables two-way ANOVA was used.

RESULT AND DISCUSSION

In the present study the higher concentration of protein content (22.64±1.45 mg/gdw) was observed on MS+ 3.0mg/l 2, 4-D after 12 hr treatment of Col as compared to all other treatments of Col. However, 18 hr treatment of Col. decreased the concentration of protein content of the callus on all the combinations of PGRs except on MS+3mg/l 2, 4-D.(Fig-1).

Maximum protein content (22.87 ±2.76 mg/gdw) was observed in callus raised on MS+ 3.0mg/l 2, 4-D+0.1 mg/l Kn+1.5mM Tyr as compared to all other treatments of Col and Tyr individually. It was also noticed that increased concentrations of Tyr led to enhanced protein concentrations. However, 2.0mM Tyr led to decrease in protein concentration. Minimum protein content among all the treatments of Tyr was observed in the calli raised on MS medium supplemented with 1.0mg/l 2,4-D+0.05mg/l Kn +1.0mM Tyr .(Fig-2)

Treatment of Col for 6 hr followed by 1.0 mM Tyr led to higher protein content (7.59 ±2.31 mg/gdw) of callus than individual Col (6 hr) (7.34 ±0.57 mg/gdw) or Tyr (1.0mM) (5.93 ±0.91 mg/gdw) treatment in MS+1.0mg/l 2,4-D and in 1.0mg/l 2,4-D+0.1mg/l Kn supplemented medium. Longer treatment with

Figure 1: % rise or fall in protein content in 4 week old callus of *Mucuna pruriens* raised on MS+PGRs along with different time period of Col. treatment as compared to control callus.

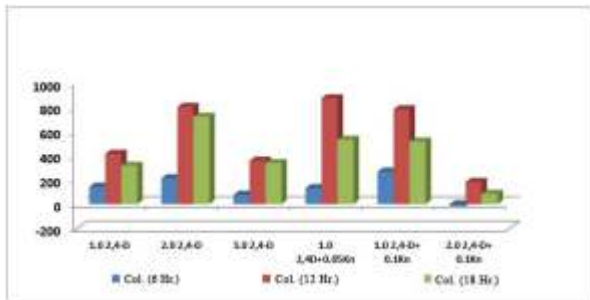


Figure 2: % rise or fall in protein content in 4 week old callus of *Mucuna pruriens* raised on MS+PGRs along with different concentration of Tyr supplementation as compared to control callus.

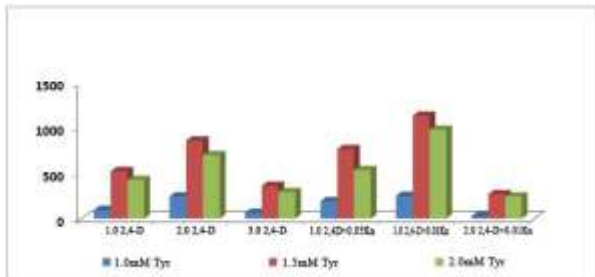


Figure 3: % rise or fall in protein content in 4 week old callus of *Mucuna pruriens* raised on MS+PGRs along with different combination of Tyr supplement and Col. treatment as compared to respective control callus.

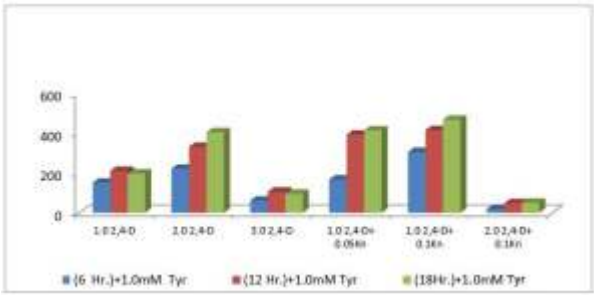


Figure 4 : % rise or fall in protein content in 4 week old callus of *Mucuna pruriens* raised on MS+PGRs along with different combination of Tyr supplement and Col as compared to respective control callus.

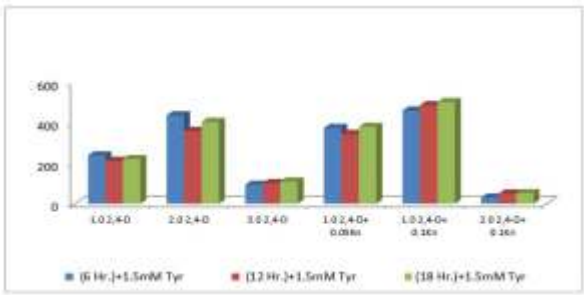
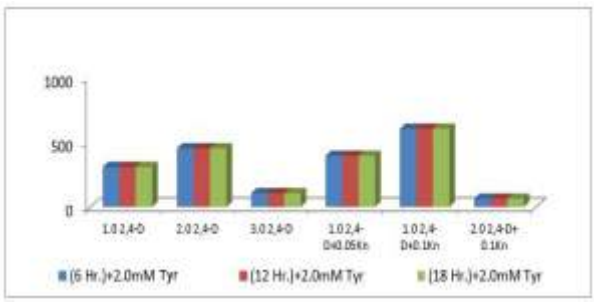


Figure 5 : % rise or fall in protein content in 4 week old callus of *Mucuna pruriens* raised on MS+PGRs along with different combination of Tyr supplement and Col. as compared to respective control callus.



Col (12 hr and 18) followed by 1.0mM Tyr treatment led to protein values of the callus to be less than individual Col (12/18 hr) and higher than Tyr (1.0mM) treatments. (Fig-3)

It was noticed that as the duration of Col was increased from 6-18 hr, protein content increased in the callus raised on MS+3.0mg/l 2,4-D, MS+1.0mg/l 2,4-D+0.1mg/l Kn and MS+2.0mg/l 2,4-D+0.1mg/l Kn compared to control, in case of 1.5mM Tyr supplemented media. However, remaining combination of PGRs with same concentrations of Tyr did not show enhanced level of protein. (Fig-4)

Maximum protein content (12.36 ± 2.01mg/gdw) was observed in the callus subcultured, after 6 hr treatment of Col on MS media supplemented with 1.0mg/l 2,4-D +2.0mM Tyr as compared to all combinations of treatments as well as control. It was found that 18 hr treatment of Col, followed by 2.0mM Tyr led to decrease in protein content on all the combination of PGRs except on MS+1.0mg/l 2,4-D+0.05mg/l Kn.(10.01±2.12 mg/gdw) (Fig-5).

The study indicates the treatment of col being an antimitotic, leads to accumulation of all those proteins, within cell, which take part in the cell division. Longer treatments of Col also led to decrease in protein content as already reported by Mansouri and Bagheri (2014) that Col with higher concentration and longer duration was more destructive than lower concentration and shorter treatment. The treatment of tyrosine, being a precursor of L-DOPA, leads to increased level of protein. However, higher amount of tyrosine could lead to substrate inhibition because it decreased protein content as nutritional in lower concentration of tyrosine.

CONCLUSION

The study indicates supplementation of polyploidy inducer for medium duration (313 µM for 12hr) and combination of phytohormone (3.0mg/l 2,4-D+0.1 mg/l Kn) with 1.5mM tyrosine may be preferred for increased accumulation of protein content in *Mucuna pruriens* calli.

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REFERENCES

Anonymous 2005 *"The wealth of India- Raw materials*" NISCAIR Publishing New Delhi L-M, **6** 439-444

Evans W C, 2002 In:(Trease and Evans) *Pharmacognosy*,15th edn, W.B. SAUNDERS Edinburgh London:26

Gurumoorthi P, Pugalenthi M and Janardhanan K 2003 Nutritional potential of five accessions of a south Indian tribal pulse *Mucuna pruriens* var. utilis ; II. Investigation on total free phenolics, tannins, trypsin and chymotripsin inhibitor, phytohaemagglutinins, and *in vitro* protein digestibility. *Trop. Subtrop. Agroecosys.* **1** 153-158

Janardhanan K, Gurumoorthi P and Pugalenthi M 2003 Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens* var. utilis . Part I. The effect of processing methods on the contents of L-DOPA, phytic acid, and oligosaccharides. *Journal of Tropical and Subtropical Agro-ecosystems* **1** 141-152.

Kumar U, Singh I, Priyanka and Vimala Y 2011 *In vitro* production of L-DOPA in tissue culture of *Mucuna Pruriens L.Vegetos –An International journal of plant research* **24(1)** 111-113.

Lowry O N, Rosenbrough N J, Farr A L and Randell R J 1951 Protein measurement with folin-phenol reagent. *J.Biol.Chem*. **193** 265-275

Murashige T, Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. plantarum*. **15** 473-497.

Pattison D I, Dean RT, Davis M J 2002 Oxidation of DNA, proteins and lipids by DOPA, protein-bound DOPA, and related catechol (amine) s. *Toxicology* **177** 23-37

Pugalenthi M, Vadivel V and Siddhuraju P 2005 Alternative food/feed perspectives of an under-utilized legume *Mucuna pruriens*. Utilis-A Review/ Linn. *J. Plant Foods Human Nutr*. **60** 201-218.

Sathiyanarayanan L and Arulmozhi S (2007) *Mucuna pruriens*. A comprehensive review. *Pharmacognosy Review* **1**157-162.

Szabo N J 2003 Indolealkylamines in *Mucuna* species. *Tropical and Subtropical Agroecosystems* **1** 295-307.

Szabo N J and Tebbett I R 2002 The chemistry and toxicity of *Mucuna* species. In: B. M. Flores, M. Eilitta, R. Myhrman, L.B. Carew and R. J. Carsky (Eds), Food and Feed from *Mucuna*: Current Uses and the Way Forward. Proceedings of the *Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO) Workshop*, Tegucigalpa, Honduras, 120-141.