Indigenous biosynthesis in *Polygonum tinctorum* and *Indigofera tinctoria* plants is of topical interest due to its academic and industrial relevance for indigo dye production. Dye yield depends on indican content in the plant biomass. Cell culture and molecular biological investigations were carried out to assess indican biosynthesis in these plants. Tissue culture protocols were optimized for explant identification, decontamination, in vitro culture medium & suitable growth regulators and culture conditions for *P. tinctorum* at Okayama University of Science, and *I. tinctoria* at CSR-NEERI. Four different growth hormones (i.e. BA, Kn, NAA, 2,4-D) at 0.01 – 2 mg/L culture medium, in random combination, and two different explants i.e. leaf and internode of both plants were experimented. In both plants, callus proliferation was better from leaf tissue with growth index (GI) up to 10 on MS agar gelled medium fortified with BA+NAA in comparison to BA+2,4-D. Suspension cell cultures of *I. tinctorum* were induced in MS liquid medium with only 2,4-D through 3 stages with GI up to 30. In vitro raised cell biomass of *I. tinctorum* presented higher indican synthesis (p<0.5) in comparison to that of *P. tinctorum*. Both of these plants synthesize indican, but the differential response under in vitro is interesting. Total transcriptomes of both plants were worked out and annotated. Comparative analysis of transcription profile indicated >80% genes are similar for the indican biosynthetic pathways. Complete alignment of both transcriptomes and validation for biosynthesis pathways specific genes is needed in both the plants to ascertain their differential expression. Indican biosynthesis in these plants is interesting.

**METHODS**

A total of 60,395 Indigo biomass synthesis putative unigene were obtained from transcriptomes of *Polygonum tinctorum* through cDNA sequencing and assembly study. Four different growth hormones (BA, Kn, NAA, 2,4-D) at 0.01 – 2 mg/L culture medium in random combination and two different explants i.e. leaf and internode of both plants were experimented. In both plants, callus proliferation was better from leaf tissue with growth index (GI) up to 10 on MS agar gelled medium fortified with BA+NAA in comparison to BA+2,4-D. Suspension cell cultures of *I. tinctorum* were induced in MS liquid medium with only 2,4-D through 3 stages with GI up to 30. In vitro raised cell biomass of *I. tinctorum* presented higher indican synthesis (p<0.5) in comparison to that of *P. tinctorum*. Both of these plants synthesize indican, but the differential response under in vitro is interesting. Total transcriptomes of both plants were worked out and annotated. Comparative analysis of transcription profile indicated >80% genes are similar for the indican biosynthetic pathways. Complete alignment of both transcriptomes and validation for biosynthesis pathways specific genes is needed in both the plants to ascertain their differential expression.

**CONCLUSION – FUTURE SCOPE**

- *Biotechnological*:
  - Complete extraction of indigo from biomass
  - Indigo fermentation process
  - Reducing fermentation duration
  - Reduce fermentation duration
- **INDICAN BIO-SYNTHESIS STRATEGIES**
  - Store glucoraphanase gene (*Polygonum* microbial) to expression vector under strong promoter
  - Identify genes and enhance tryptophan synthesis
  - Metabolomics
- **INDICAN DYE PRODUCTION STRATEGIES**
  - Complete extraction of indigo from biomass
  - Indigo fermentation process
  - Reduce fermentation duration
  - Reduce fermentation duration
- **FUTURE SCOPE**
  - Indigo dye production through indigo fermentation system
  - Develop different types of cell biomass with different growth hormones
  - Enhance quantitative traits through rapid cell growth and differentiation with wild genes through indirect gene transfer
  - Induced mutation to Mass culture of tissue with high inherent indican content
  - Future scope:
    - Mass culture of tissue with high inherent indican content
    - Enhance quantitative traits through rapid cell growth and differentiation with wild genes through indirect gene transfer
    - Induced mutation to Mass culture of tissue with high inherent indican content
  - FUTURE SCOPE