INDIA-JAPAN COOPERATIVE SCIENCE PROGRAMME (IJCSP)
DST, India – Japan Society for Promotion of Science (JSPS), Japan

Cell cultures and molecular investigation on *Polygonum tinctorium* and *Indigofera tinctoria* plants to understand indican biosynthesis

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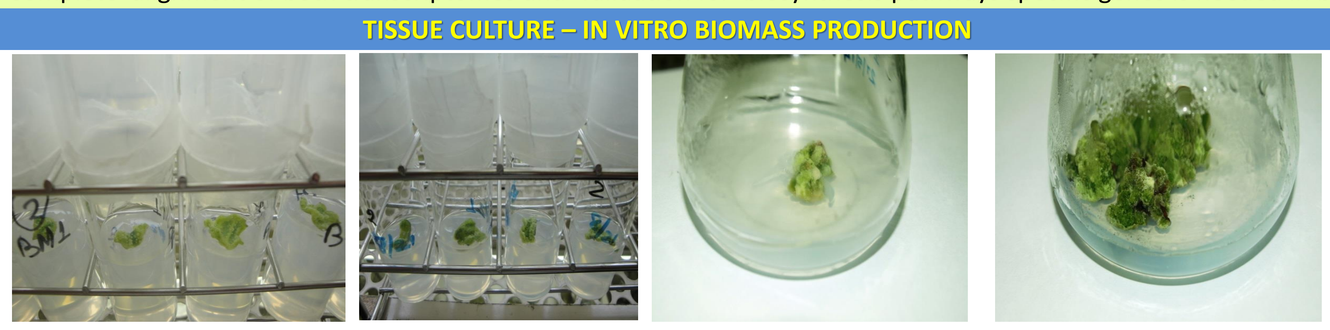
ABSTRACT

Indican biosynthesis in *Indigofera tinctoria* and *Polygonum tinctorium* 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. tinctorium* at Okayama University of Science, and *I. tinctoria* at CSIR-NEERI. Four different growth hormones i.e. BA, Kin, NAA, 2,4-D, at 0.01 – 2 mg L^{-1} 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. tinctorium* 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. tinctorium* presented higher indican synthesis ($p > 0.5$) in comparison to that of *P. tinctorium*. 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 transcriptome 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

GENESIS

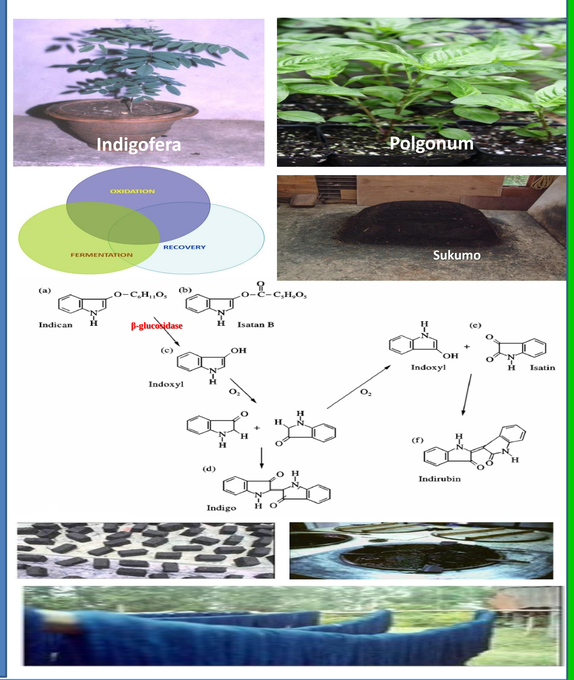
TISSUE CULTURE – IN VITRO BIOMASS PRODUCTION



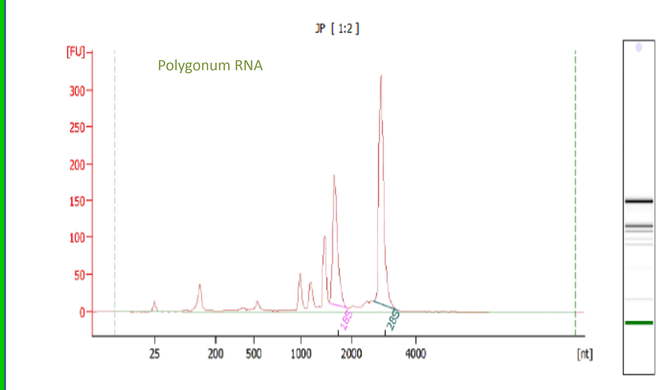
***Polygonum* tissue culture in Dept. of Biochemisry Okayama University**

***Indigofera* tissue cultures at CSIR-NEERI, Nagpur, India**
In vitro developed different types of cell biomass with diff. growth hormones

INDICAN – INDIGO DYE



Polygonum tinctorium

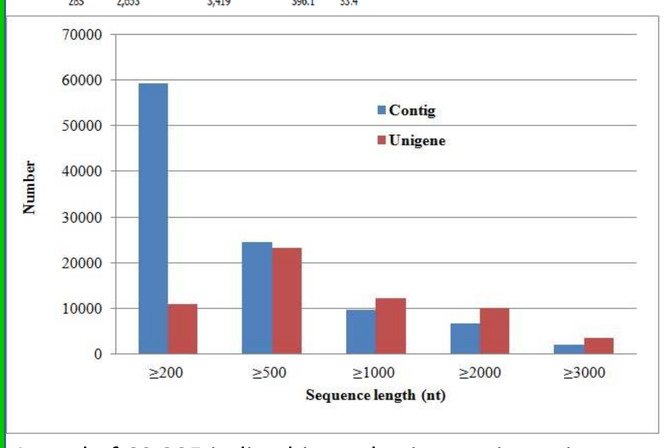


Overall Results for sample 10 : JP

RNA Area:	1,185.9	RNA Integrity Number (RIN):	8.3 (B.02.07, Anomaly Threshold(s) manually adapted)
RNA Concentration:	450 ng/µl		
rRNA Ratio [28s / 18s]:	1.6	Result Flagging Color:	
		Result Flagging Label:	RIN: 8.30

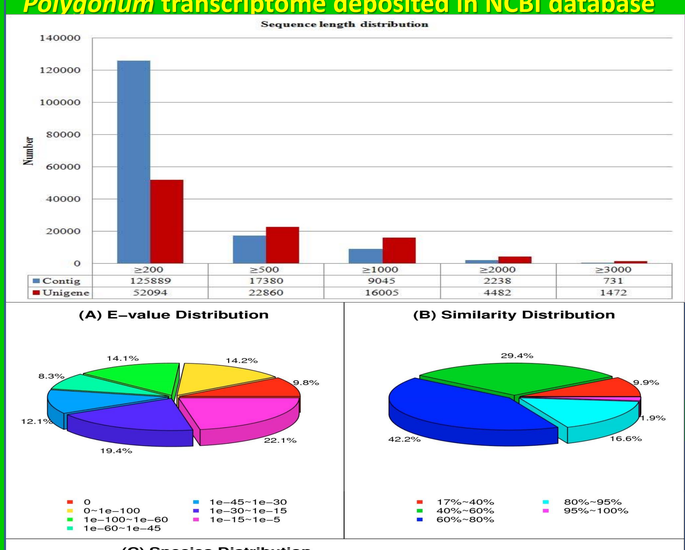
Fragment table for sample 10 : JP

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,565	1,920	243.4	20.5
28S	2,653	3,419	396.1	33.4



A total of 60,395 indigo biosynthesis putative unigenes were obtained from transcriptomes of *Indigofera tinctoria (*L.) through cDNA sequencing and assembly study.

Polygonum transcriptome deposited in NCBI database

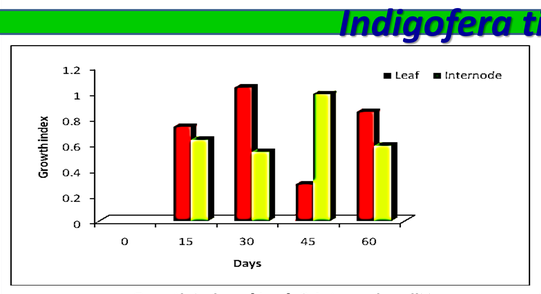


A total of 96,913 indigo biosynthesis putative unigenes were obtained from transcriptomes of *Polygonum tinctoriium* through cDNA sequencing and assembly study.

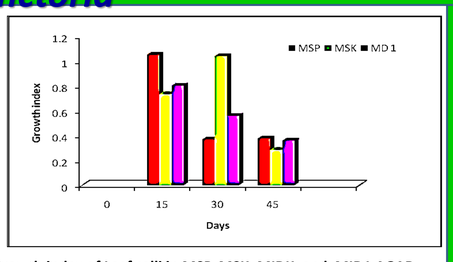
METHODS

MSP- Murashige and Skoog medium with 0.5mg/L BA + 2mg/L NAA
MSK- Murashige and Skoog medium with 0.5mg/L Kn + 2mg/L NAA
MID- Murashige and Skoog medium with 0.25mg/L 2,4-D
MIDK- Murashige and Skoog medium with 0.25mg/L 2,4-D + 0.1mg/L Kn
MIDI- Murashige and Skoog medium with 0.5mg/L 2,4-D

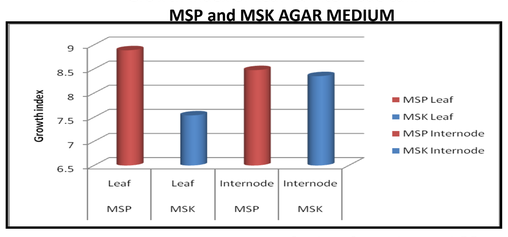
Indigofera tinctoria

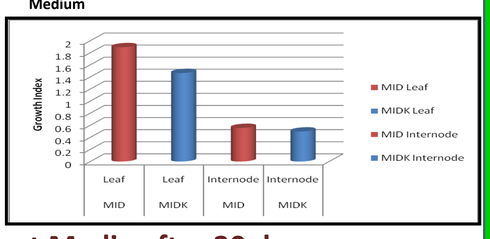


Growth index of Leaf & Internode calli in MSP and MSK AGAR MEDIUM

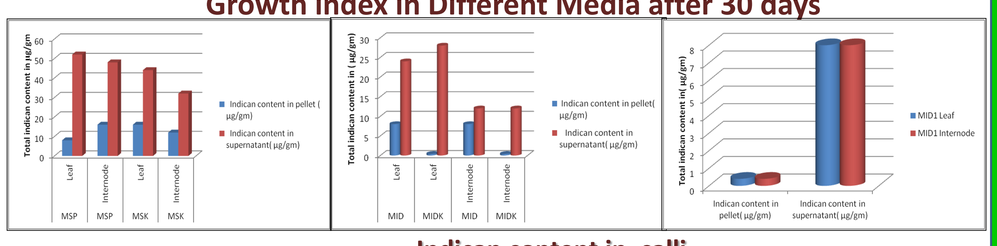


Growth index of Leaf calli in MSP, MSK, MIDK, and MID1 AGAR Medium



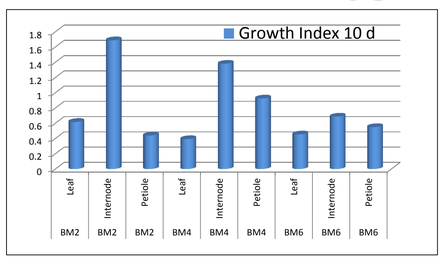


Growth index in Different Media after 30 days

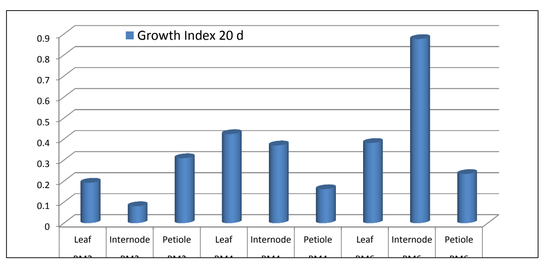


Indican content in calli

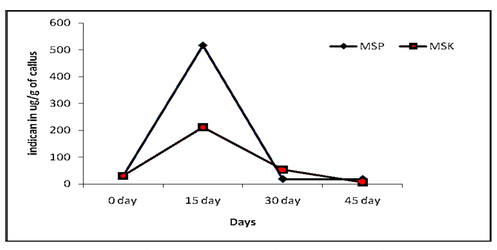
Polygonum tinctorium



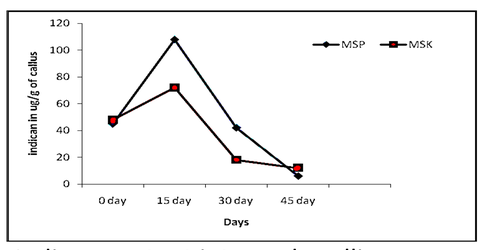
GI *Polygonum* calli



GI *Polygonum* calli after 20 days



Indican content in leaf calli



Indican content internode calli

Indigofera and *Polygonum* transcriptome deposited in NCBI GenBank database

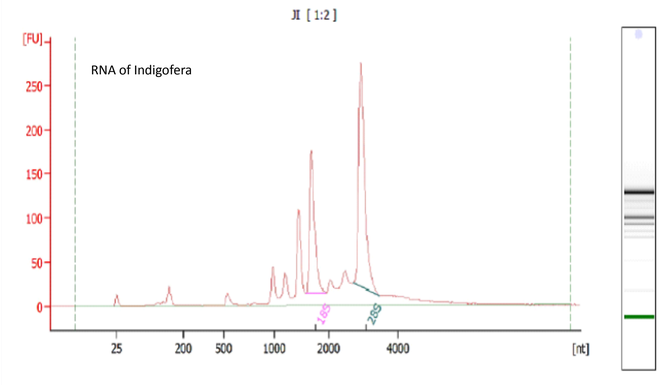
Sample: SAMN03015981 - Transcriptomic analysis of Indigo dye plant, Indigofera tinctoria L. using Illumina HiSeq2000 sequencing platform (less...)
Organism: Indigofera tinctoria
Attributes:
cultivar: tinctoria
biomaterial_provider: CSIR-NEERI, Nehru Marg, Nagpur - 440020, India
tissue: Leaf
age: 2 month
Library: IT_Lib (less...)
Strategy: RNA-Seq
Source: TRANSCRIPTOMIC
Selection: cDNA
Layout: SINGLE
Platform: Illumina (less...)
Instrument model: Illumina HiSeq 2000
Spot descriptor:
1 forward
Experiment attributes:
Authors: Bijaya K. Sarangi, Sanjog T. Thul and Yoshiko Mina (hide...)
Bijaya K. Sarangi, Sanjog T. Thul and Yoshiko Minami.
Total: 1 run, 26.7M spots, 4.8G bases, 3Gb

#	Run	# of Spots	# of Bases	Size
1.	SRR1565381	26,668,889	4.8G	3Gb

ID: 975250

The cDNA transcriptome sequence of indigo dye plant, *Indigofera* & *Polygonum* has been inventoried in GenBank (Acc. No. SAMN03015981 & SRX692542).

Indigofera tinctoria



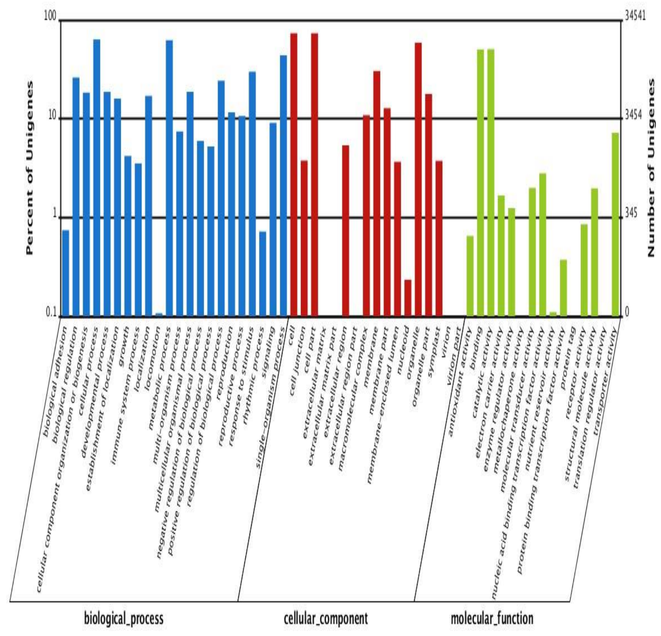
Overall Results for sample 9 : JI

RNA Area:	1,331.7	RNA Integrity Number (RIN):	8.4 (B.02.07, Anomaly Threshold(s) manually adapted)
RNA Concentration:	505 ng/µl		
rRNA Ratio [28s / 18s]:	1.8	Result Flagging Color:	
		Result Flagging Label:	RIN: 8.40

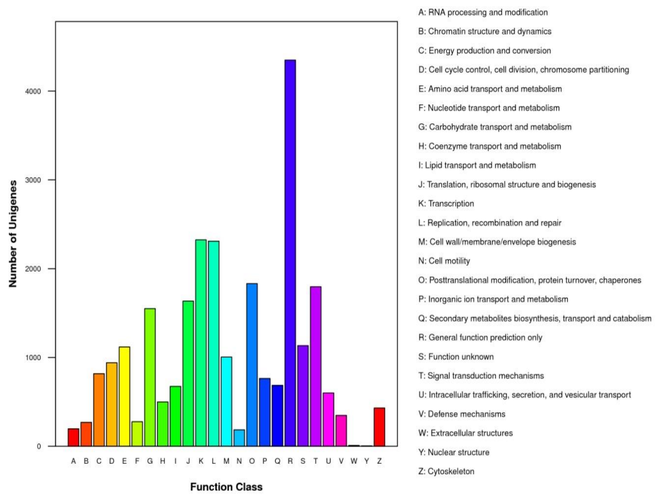
Fragment table for sample 9 : JI

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,581	1,946	210.4	15.8
28S	2,682	3,463	374.8	28.1

Total RNA



At least 23,721 unigenes mapped onto 128 pathways involved in secondary metabolite biosynthesis using the KEGG pathway database.



Unigenes mapped onto 128 pathways using the KEGG pathway database involved in the biosynthesis of secondary metabolites

CONCLUSION - FUTURE SCOPE

INDICAN BIO-SYNTHESIS STRATEGIES

- Clone β-glucosidse gene (*Polygonum* / microbial) to expression vector under strong promoter
- Identify genes and enhance tryptophan synthesis
- Metabolomics

Biotechnological

- Mass culture of tissue with high inherent indican content
- Selection of clonal variants
- Induced mutation to select mutant cell lines with enhanced indican content
- Rapid cell growth and differentiation with wild genes through indirect gene transfer
- Enhance quantitative traits through para sexual hybridization
- Genetic transformation

Contact: minami@dbc.ous.ac.jp ; bk_sarangi@neeri.res.in

INDIGO DYE PRODUCTION STRATEGIES

Biomass Fermentation:
- Complete extraction of indican from biomass
- Reduce fermentation duration
- Reduce biomass : water ratio

Ferment Oxidation:
- Mechanical aeration arrangement
- Optimize DO concentration to prevent rate limiting
- Reduce oxidation time