Research Article

www.ijrap.net

TAXONOMICAL, ANATOMICAL, CYTOLOGICAL AND PALYNOLOGICAL ASSESSMENT OF A GERMPLASM OF *INDIGOFERA TINCTORIA* L. (FABACEAE): AN AYURVEDIC PLANT

Bapi Ghosh¹, Tanmoy Mallick², Asok Ghosh², Animesh Kumar Datta^{1*} and Ankita Pramanik¹

¹Department of Botany, Cytogenetics, Genetics and Plant Breeding Section, University of Kalyani, Kalyani, West Bengal, India

²Department of Botany, Taxonomy of Angiosperms and Biosystematics, University of Burdwan, Burdwan, West Bengal, India

Received on: 02/09/16 Revised on: 29/09/16 Accepted on: 05/10/16

*Corresponding author

E-mail: dattaanimesh@gmail.com

DOI: 10.7897/2277-4343.075227

ABSTRACT

A germplasm of *Indigofera tinctoria* L. (Fabaceae, dye yielding plant with immense therapeutic importance and also with potential phyto-constituents) is grown in West Bengal plains (Nadia) and characterized taxonomically, anatomically, cytologically and palynologically. The objective of the work is to keep the germplasm under *ex-situ* conservation and sustainable cultivation for further genetic exploration in enhancing bioactive compounds and crop improvement.

Keywords: *Indigofera tinctoria*, Cytotaxonomical characterization, Anatomical features, SEM, Acetolysis

INTRODUCTION

Indigofera tinctoria L. (Family: Fabaceae; common name Indigo, Nili) is an annual, biennial or perennial shrub and found to grow wildly throughout India and rarely cultivated for commercial purposes. The whole parts of the plant species are useful¹. The leaves and twigs produce colorless precursor that is extracted to produce blue dye - indigo, useful for textile industries². Apart from its dye yielding and therapeutic significances, the plant species is extremely important as it possesses essential phytochemical constituents^{1,3,4}. The juice extracts from leaves contain indirubin and indigotone, essential for treatment of hydrophobia⁵. It also possesses anti-hyperglycemic⁶, antibacterial⁷, antioxidant and cytotoxic³, anti-inflammatory^{8,9}, anti-hepatoprotective^{4,10}, anti-diabetic¹¹, anti-epileptic¹², antinociceptive¹³, anthelmintic¹⁴, anti-proliferative¹⁵, anti-dyslipidemic¹⁶ properties among others.

The ayurvedic importance of *I. tinctoria* highlights the essentiality of its sustainable cultivation and further genetic exploration for increasing raw as well as value added products as suggested in *Andrographis* plant species¹⁷. For the purpose, the present investigation describes the taxonomical, anatomical, palynological and cytological (mitotic as well as meiotic) attributes of the germplasm of *I. tinctoria* under study for proper cataloguing.

MATERIALS AND METHODS

Germplasm

Seeds samples (moisture content: 12.50%; seeds size: length-2.40 mm $\pm$ 0.06, breadth-1.71 mm $\pm$ 0.04; 100 seed weight: 0.52 g $\pm$ 0.06; seeds color: RAL 8008-111-079-040—olive brown) of *Indigofera tinctoria* L. (Family: Fabaceae) were obtained from Medicinal Plant garden, Narendrapur, Ramkrishna Mission, Govt. of West Bengal, India.

Raising of plant population

Seeds of mother stock were sown in the experimental field plots of Kalyani University (West Bengal Plains, Nadia: 22°99'N, 88°45'E, elevation 48 ft above sea level, sandy loamy soil, soil pH 6.85) during the months of February to December, 2014 and subsequently characterized.

Taxonomic characterization

Morphological traits (each part of the plant species) including seeds (surface ornamentation was studied following Scanning Electron Microscopy-SEM) were studied from plants of identical maturity. The study includes detailed description of every part of the specimen. The specimens as well as seed size and morphology were examined under Stereomicroscope (*Leica* stereo, photographs taken by EC3 Digital camera).

SEM analysis of seed-coat surfaces

Seeds were mounted on specimen stubs with double sided adhesive tape. The stubs were placed on a revolving disc and coated with 200-300 Å thick layer of gold in a vacuum evaporator (Polaron, East Sussex, UK) sputter coating system. The specimen stubs were then observed under SEM (EVO LS 10, Zeiss) in instrumentation center, DST-PURSE, Kalyani University. Ten seeds were observed and photomicrograph was taken from suitable preparations.

Anatomical studies

Transverse hand sections of the stem (from base about 1.5 cm) and root (2.0 cm below) were made from 45 days old plant. The sections were doubled stained using 1.0% safranin dissolved in 50% alcohol and 1.0 % light green dissolved in 90% alcohol as per Puri et al.¹⁸.

Cytological studies

Mitosis: Seeds were presoaked (overnight in ddH₂O) and allowed to germinate (23°C $\pm$ 1°C) in Petri plates lined with moist filter paper. Healthy roots (2 mm in sizes) were pretreated

(0.05% colchicine for 4h at room temperature-23°C $\pm$ 1°C), fixed (acetic-alcohol 1:3 v/v) overnight, stained (2% orcein-1N HCl mixture 9:1 following gentle warming in a spirit lamp for 10 min), and the root tips were squashed in 45% acetic acid in a glass slide before observing under the microscope. Well scattered and properly condensed metaphase chromosomes (3 plates) were assessed. Data for karyotype analysis were computed using computer programming software (LAS EZ). A suitable metaphase plate was photomicrographed.

Metaphase chromosomes in the species were classified as metacentric (F%: 40.0 to 50.0%) and submetacentric (F%: 30.0 to 39.99%) types as per Hirahara and Tatuno¹⁹. Karyomorphological attributes studied were mean length of individual chromosomes measured in micrometer (μ m), relative length of each chromosome represented as per cent length of longest chromosomes, total form per cent (TF%) represented the total length of short arm as per cent of total chromosomes complement²⁰, S per cent representing relative length of smallest chromosome $\times$ 100 and total haploid chromatin length measured in μ m. Karyotype formula was also determined.

On the basis of chromosomes (long: > 3.0 μ m and medium: < 3.0 μ m) and F%, the chromosomes in the species were classified into following types: type A - long metacentrics, type B - long submetacentrics, type C - medium chromosomes with submetacentric constrictions and type D - medium metacentrics.

Meiosis: Young inflorescences of 2.0 to 3.0 cm (from randomly selected 5 plants) were fixed in Carnoy's fixative during 9.00 am to 9.30 am in the month of July. One change is given in the fixative after 24 h. The inflorescences were preserved in 70% alcohol under refrigeration.

Pollen mother cells (PMCs) and pollen grains obtained from anther squash preparations were stained in 2% propionocarmine solution. Fully stained pollen grains were considered fertile²¹. Meiotic observations were made from diplotene-diakinesis, metaphase I (MI) and anaphase I (AI) preparations. Photomicrographs were taken from temporary squash preparations and suitably magnified.

Acetolysis of Pollen grains

Acetolysis technique was adopted as per Erdtman²² to study the shape, size, ornamentation of the pollen grains.

RESULT AND DISCUSSION

Taxonomical studies

Annual and perennial shrubs and shrublets, erect, 1 to 2.5 m tall (Figure 1:A and 2:A). Stem brownish on maturity, branching generally started from certain height above ground, sometimes also started immediately forming a bushy appearance. Young stem with dichotomously branched trichomes, trichomes (Figure 2:E-F), medifixed more or less parallel to the stem surface, unicellular (rarely multicellular), trichome tips slightly raised. Leaves pinnately compound, unipinnate, imperipinnate, petiole with pulvinus base, alternate 8 to 15 cm, 9-13 foliate (Figure 2:B), stipulate, stipule minute (Figure 2:G-H), petiole and rachis adaxially grooved with trichomes, petiole pulvinate, 1.32 to 2.60 cm; stipels minute; leaflets typically green, variable in shapes, obovate to nearly elliptical, tips rounded (Figure 1:B and 2:C-D), emarginate, sometimes truncated and even emarginate to mucronate: base of leaflets sometimes unequal; length of petiolules also variable; blades opposite to subopposite, obovate-oblong to obovate, 1.4-3.2 $\times$ 0.7-1.3 cm²; surface with trichomes, adaxially glabrous (Figure 2:C); midvein adaxially impressed, secondary veins inconspicuous, green, base cuneate; stipels present, triangular, caducous. Inflorescence a simple

upright raceme of variable length 2.3 to 8.1 cm, 24-41 flowers per raceme, laxly flowered, peduncle absent (Figure 1:C and 2:I); flowers complete zygomorphic, bisexual, hypogynous, pedicel 0.7 to 2.3 cm long (Figure 1:D and 2:J). Sepals 5, gamosepalous, persistent, unequal, 1-1.6 mm (Figure 1:E and 2:L-M), outer side of the bract with trichomes (Figure 2:K), inside glabrous, standard greenish, outside heavily adorned with brown trichomes, pinkish streak at the base, wing pink colored, veined; vein 2.3 mm to 3.1 mm long; keel as long as wings, valvately connate along lower margin from about middle to apex, greenish, transparent; lateral spur absent (Figure 1:F and 2:N-Q). Stamens diadelphous (9+1) (Figure 1:G and 2:S), filament persistent, free, variable in length, length varies from 4.2 to 5.7 mm; anthers, basifixed, cordate, uniform; connective apiculate (Figure 1:H and 2:R).

Carpel 1; Ovary superior, glabrous, sessile, linear-flattened, many ovuled; style curved upward; stigma capitate; placentation marginal, 3.2-4.1 mm (Figure 1:I). Legume linear to narrowly cylindrical, brown colored straight sometimes curved at the tip, 1.8 to 4.1 cm, glabrous; dehiscent; seeds 7 to 13 per legumes (Figure 1:L and 2:T); seeds rectangular/quadrangular to cylindrical; hilum central to sub central. Seed length ranges from 2.10 to 2.80 mm, width 1.48 to 2.00 mm, length and width ratio 1.40 to 1.51; seed color light brown to deep brown (sometimes olivaceous when immature), deep brown to chocolate colored and wrinkled on maturity; testa texture smooth-creased, blackish; hilum shape circular to elliptical; aril absent (Figure 1:M).

SEM analysis of seed surface

Testa under SEM shows simple perforated to reticulate-perforated ornamentation pattern, pores/ pits are irregularly shaped and irregularly arranged; boundaries of anticlinal wall not clear; testa glossy on maturity (Figure 1:N).

Anatomy

TS of stem consists of intact epidermis, thin cortex, narrow secondary phloem and very wide, strong secondary xylem and conspicuous pith. Corners of the stem contain thick patch of collenchymas. Secondary xylem thick; vessels occur in thin uniseriate rows of 2-8; each row of vessel separated by wide gap between them; in immature/young stem vessels are singly arranged. Vessels narrow to medium sized, thin walled, circular, oval and elliptical in outline (Figure 1:J).

TS of root shows that secondary xylem is dense, very compact comprising of circular thick walled vessels (mostly arranged tightly) within conjunctive tissue (Figure 1:K).

It is observed that both stem and root show secondary growth at the very early stage of plant growth.

Cytological studies

Mitosis: Karyomorphological data for different attributes are presented in Table 1. Karyotype analysis reveals four ($2n=16=6A_m^L+6B_{sm}^L+2C_{sm}^M+2D_{sm}^M$) morphologically distinct chromosome types (Figure 3:A). The length of the chromosome in the complement ranges from 2.56 μ m to 3.96 μ m. Total haploid chromatin length; TF% and S% are noted to be 27.13 μ m $\pm$ 1.22, 40.10 and 64.60 respectively. The somatic chromosome complement in *I. tinctoria* is symmetric in nature (Figure 3:B). The chromosome number is in conformity with earlier report of Gupta and Agarwal²³, however, the authors suggested a different karyotype formula ($1M+5m+2sm$) for the species.

Table 1: Karyomorphological data of *I. tinctoria*

Chromosome types	Somatic Chromosome Pair	Chromosome length in μm			Arm Ratio LA/SA	Relative Length (%)	F (%)	Nature of Primary constriction
		Long Arm (LA)	Short Arm (SA)	Total				
A	A ₁ A ₁	2.22	1.76	3.96	1.26:1.00	100.0	44.2	metacentric
A	A ₂ A ₂	2.20	1.62	3.82	1.35:1.00	96.5	42.4	metacentric
A	A ₃ A ₃	1.95	1.54	3.49	1.26:1.00	88.1	44.1	metacentric
B	B ₁ B ₁	2.41	1.31	3.72	1.83:1.00	93.9	35.2	Sub-metacentric
B	B ₂ B ₂	2.17	1.27	3.44	1.70:1.00	86.9	36.9	Sub-metacentric
C	C ₁ C ₁	1.89	1.03	2.92	1.83:1.00	73.7	35.3	Sub-metacentric
D	D ₁ D ₁	1.42	1.14	2.56	1.24:1.00	64.6	44.5	metacentric

Figure 1

Figure 1: *Indigofera tinctoria* (A) A mature plant; (B) Leaflet; (C) Inflorescence; (D) Flower; (E) Calyx; (F) Corolla; (G) Reproductive organs; (H) Stamen; (I) Gynoecium; (J) TS of stem; (K) TS of root; (L) Fruit; (M) Seeds; (N) Seed surfaces following SEM; (O-P) Pollen grains following acetolysis.

Figure 2

Figure 2: Diagrammatic representation of *L. tinctoria* (A) A flowering twig; (B) Compound leaf; (C) Leaflets; (D) Leaf surface; (E-F) Stem; (G-H) Stipules; (I) Inflorescence; (J) Flower; (K) Bract; (L-M) Calyx; (N) Vexillum-actual; (O) Vexillum-pressed; (P) Wings; (Q) Keel; (R) Stamen; (S) Reproductive organ; (T) Fruit.

Figure 3

Figure 3: Chromosomes of *I. tinctoria* (A) Somatic cell showing $2n=16$ chromosomes; (B) Idiogram; (C-F) Meiotic chromosome configurations showing 8II formation and 8/8 separation (C. Diplotene, D-E. Metaphase I, F. Anaphase I).

Meiosis: Meiocytes at diplotene-diakinesis (Figure 3:C) and MI (Figure 3:D-E) always show $2n=16$ chromosomes with preponderance of rod bivalents as reported earlier by Ghosh et al.²⁴ (2016). The chromosomes mostly form bivalents (predominant association: 8II formation-87.86%) with nearly (94.12%) regular (8/8) segregation of chromosomes at AI (Figure 3:F). Pollen grain fertility in the species is high (93.60%). Ghosh et al.²⁴ reported secondary association of chromosomes as persistent feature (89.39%) in MI cells, and statistical analyses of cytological data reveal that the probable basic chromosome number in the species is $X=5$ suggesting polyploid lineage.

Acetolysis of Pollen grains

Pollen monad, size ranges from 26.7 to 45.2 µm, prolate to spheroidal; triangular (broadly) in polar view, tectate; exine sculpture granulate to slightly perforated; polar length $34.69 \mu\text{m} \pm 3.82$; equatorial length $31.83 \mu\text{m} \pm 2.37$ (Figure 1:O-P).

CONCLUSION

Characterization of the germplasm under study has been significant as it offers scope for proper indexing for further exploration in enhancing bioactive compounds and crop improvement.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Sudha Gupta, Assistant Professor in Botany, Kalyani University for providing necessary facility for photomicrography.

REFERENCES

1. Saraswathi MN, Karthikeyan M, Rajasekar S, Gopal V. *Indigofera tinctoria* Linn—A Phytopharmacological Review. Int J Res Pharm Biomed Sci 2012; 3:164–169.
2. <https://www.prota4u.org/> [Homepage on the Internet]. Kenya: Plant Resources of Tropical Africa; [update July 1, 2016]. Available from <https://www.prota4u.org/>.
3. Verma SM, Suresh KB. Phytochemical investigations of *Indigofera tinctoria* Linn. leaves. Anci Sci Life 2002; 21:235–239.
4. Renukadevi KP, Sultana SS. Determination of Anti-bacterial, Antioxidant and Cytotoxicity effect of *Indigofera tinctoria* on Lung cancer cell line NCI-h69. Int J Pharmacol 2011; 7:356–362.
5. Muthulingam M, Mohandoss P, Indra N, Sethupathy S. Antihepatotoxic efficacy of *Indigofera tinctoria* (Linn.) on paracetamol induced liver damage in rats. Indian J Pharm Biomed Res 2010; 1:13–18.

6. Bangar AV, Saralaya MG. Anti-hyperglycemic activity of ethanol extract and chloroform extract of *Indigofera tinctoria* leaves in streptozotocin induced diabetic mice. Res J Pharm Biol Chem Sci 2011; 2:445–455.
7. Vijayan M, Jacob K, Govindaraj Y. Antibacterial activity and mutagenicity of leaves of *Indigofera tinctoria* Linn. J Exp Integr Med 2012; 2(3):263–269.
8. Tyagi PK, Rai VK, Pahlia AK and Kumar SS, Singh Y, Sharma M, Goyal M. Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Ethanolic Extract of Leaves of *Indigofera tinctoria* Linn. J Curr Pharmaceut Res 2010; 3(1):47–50.
9. Mohan EM, Mohan CK, Amudha P. Effect of *Indigofera tinctoria* on Neurotransmitters concentrations in Rat Brain After Induction of Seizure. Int J Phytopharmacol 2010; 1:23–27.
10. Felicia FA, Muthulingam M. Phytochemical and HPTLC studies of methanolic extract of *Indigofera tinctoria* (Fabaceae). Int J Pharm Life Sci 2012; 3:1670–1674.
11. Verma SM, Suresh KB, Verma A. Antidiabetic Activity of Leaves of *Indigofera tinctoria* Linn (Fabaceae). Int J Toxicol Pharm Res 2010; 1:42–43.
12. Asuntha G, Prasannaraju Y, Prasad KVSRR. Effect of Ethanol Extract of *Indigofera tinctoria* Linn (Fabaceae) on lithium Pilocarpine-Induced Status Epilepticus and Oxidative Stress in Wistar Rats. Trop J Pharm Res 2010; 9:149–156.
13. Saravana Kumar A., Gandhimathi R, Mohana Lakshmi S, Nair R, Ashok.Kumar.C.K. Evalution of the antinociceptive properties from *Indigofera tinctoria* leaves extracts. J Pharmaceut Sci Res 2009; 1(2):31-37.
14. Balamurugan G, Selvarajan S. Preliminary Phytochemical Screening and Anthelmintic Activity of *Indigofera tinctoria* Linn. Int J Drug dev Res 2009; 1:157–160.
15. Kameswaran TR, Ramanibai R. The Anti proliferate activity of Flavonoidal Fraction of *Indigofera tinctoria* is through Cell Cycle Arrest and Apoptotic Pathway in A-549 Cell. J Bio Sci 2008; 1–7.
16. Puri A, Khaliq T, Rajendran SM, Bhatia G, Chadra R, Narender T. Antidyslipidemic activity of *Indigofera tinctoria*. J Herb Phamacother 2007; 7:59–64.
17. Ghosh Bk, Datta AK, Mandal A, Dubey PK, Halder S. An overview on *Andrographis paniculata* (Burm. F.) Nees. Int J Res Ayurveda Pharm 2012;3(6):752–760. DOI: 10.7897/2277-4343.03610
18. Johansen DA. Plant Microtechnique 1st ed, McGraw Hill Book Co Inc., New York & London, 1940, Pp 104–106.
19. Hirahara S, Tatuno S. Cytological studies in *Narcissus*. I. Karyotype and nucleolus of *Narcissus jonquilla*. Cytologia 1967; 32:553–559.
20. Huziwara Y. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosome of *Aster*. Am J Bot 1962; 49:116–119.
21. Marks, GE. An acetocarmine glycerol jelly for use in pollen fertility counts. Stain Technol 1954; 29:277.
22. Erdtman G. Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist & Wiksell, Stockholm, 1952, Pp 539.
23. Gupta PK, Agarwal K. Cytological Studies in the Genus *Indigofera* L. Cytologia 1982; 47:665–681.
24. Ghosh B, Datta AK, Mandal A, Das D, Kumbhakar DV. Meiotic Configurations and Secondary Chromosome Associations in *Indigofera tinctoria* L. (Family: Fabaceae). Cytologia 2016; 81:1–6.

Cite this article as:

Bapi Ghosh, Tanmoy Mallick, Asok Ghosh, Animesh Kumar Datta and Ankita Pramanik. Taxonomical, anatomical, cytological and palynological assessment of a germplasm of *Indigofera tinctoria* L. (Fabaceae): An Ayurvedic plant. Int. J. Res. Ayurveda Pharm. Sep - Oct 2016;7(Suppl 4):90-95
<http://dx.doi.org/10.7897/2277-4343.075227>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IJRAP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJRAP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IJRAP editor or editorial board members.