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Original Research Article

Biochemical analysis of *indigofera* L. species with special emphasis on protein content and phylogenetic analysis

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Abstract

Indigofera L. is a dicotyledonous plant and is a member of Leguminoceae-Papillionaeae family of largely herbs, shrubs and trees with a great variety of habitat. *Indigofera* has important medicinal uses. All the parts are useful. For the present study three species were selected *Viz. Indigofera linifolia* (Linn.f.) Retz, *I. cordifolia* Heyne ex Roth.. *I. trita* Linn. The sample proteins (seed proteins) were compared with a range of molecular weight marker. In *Indigofera trita* Linn. 15 bands were observed. The highest number of bands were observed in the range 75-50 i.e.. Lowest number of bands in the range 225-150, 100-75, 25-15 was 1 band in each range. Above 225 bands were absent likewise in the range 15-10 also bands absent. In *Indigofera cordifolia* Heyne ex Roth. 16 bands were observed. In *Indigofera linifolia* (Linn.f.) Retz 15 bands were observed. The highest number of bands in the range 25-15 is 4 bands and the lowest number of bands in the range above 225, 225-150, and 100-75 is 1 band in each range. It was found that the seeds have low molecular weight proteins. On the basis of banding pattern, the data was collected and analyzed for the phylogenetic analysis with the help of NTSYS software, showed close relation in *I. trita* and *I. linifolia* on the other hand *I. cordifolia* showed distantly related species.

Key Words: *Indigofera*, Protein, SDS PAGE.

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INTRODUCTION

Biochemical analysis techniques refer to a set of methods, assays and procedures that enable scientist to analyze the substances found in living organisms and the chemical reactions underlying life processes. The most sophisticated of these techniques are reserved for specially research and diagnostic laboratories, also simplified sets of these techniques are used in various fields. To perform a comprehensive biochemical analysis of a biomolecules in a biological processes or systems the biochemist typically needs to design a strategy to detect that biomolecule, isolate in pure form among thousands of molecules that are been found in an extracts from a biological sample, characterize it, and analyze its function. An assay, the biochemical test that characterizes a molecule, whether quantitative or semi-quantitative or qualitative, is important to determine the presence and the quality of biomolecule at each step of the study. In *Indigofera* there are many morphological variations within the species in different regions of the world. In India about 60 species and 10 varieties of *Indigofera* are found (Hajra *et al*., 1995). There are thirty-five species of *Indigofera* that are reported from Maharashtra (Almeida, 1998) at different places. They are *Indigofera angulosa* Edge worth from khandesh; *I. aspalathoides* Val ex DC. from Deccan; *I. astragalina* DC. from Ambali, Sawantwadi, Malvan, Marathwada and Vidarbha; *I. barberi* Gamble from (Osmanabad) Marathwada; *I. cassioides* Rottle. DC. from Koina nagar, Purandhar, Mahabaleshwar, Pune, Marathwada, Vidarbha and

Khandesh; *I. colutea* (Burm. f.) Merrill. from Khandesh and Nagpur. *I. coerulea* Roxb. from Ahamednagar and Pune and Marathwada; *I. coerulea* Roxb., var. *occidentalis* Gillet and Ali from Ahamednagar and Marathwada; *I. constricta* (Thw) trimen from Kumbharli ghat and Ratnagiri; *I. cordifolia* Heyne ex Roth. from Thane, Nasik, Pune, Malvan, Marathwada, Vidarbha and Khandesh; *I. dalzelli* Cooke from Panchgani, Sawantwadi, Mahabaleshwar, Uran and Ramghat; *I. deccanensis* Sanjappa reported from Sautada (Beed); *I. trifoliata* Linn. from Ramling forest, (Osmanabad); *I. glabra* Linn. from Vidarbha and Melghat; *I. glandulosa* Wendl. from Thane, Khopli, Sion, Purandar, Matunga and Marathwada; *I. heteranta* Wall. ex Brandis from Vashi (New Bombay); *I. karuppiana* Pallithanum from dry hill slopes of Osmanabad, Marathwada, Vidarbha; *I. karnatakana* Sanjappa from Konkan; *I. linifolia* (Linn.f.)Retz from Juhu, Sawantwadi, Matheran, Marathwada, Vidarbha and Khandesh;. *I. linifolia* Retz. var. *campbellii* Wight ex Baker in Hook. from Malad, Pune, Ahamednagar, Marathwada Osmanabad and Vidarbha; *I. echinata* Willd. from Konkan, Malvan, Vidarbha, Wardha, Tadoba, Ballarpur; *I. oblongifolia* Forsk. from Khandesh Amalnar, Vidarbha, Wamanpalli; *I. parviflora* Heyne from Sawantwadi, Konkan, Junnar fort, Marathawada and Osmanabad; *I. santapau* Sanjappa from Purandar, Vazirgad fort; *I. semitrijuga* Forsk. from Bombay, Pune, Marathwada, khandesh and Vidarbha; *I. spicata* Forsk from Purandar, Sinhagad, Panchgani, Pune, Marathwada, Vidarbha and Melghat; *I. tinctoria* Linn. from Mumbai, Pune, Marathwada, Vidarbha and Melghat; *I. suffruticosa* Miller. from Powai and Victoria Garden-Bombay; *I. trifoliate* Linn. from Sawantwadi, Igatpuri, Purandar and Vidarbha; *I. trita* Linn. from Pune, Pashan, Solapur, Khandesh, Marathwada and Vidarbha. Three varieties of *Indigofera trita* i.e. *I. maffei* (Chiv) Gillet, *I. purandharensis* Sanjappa and *I. flccida* (Koen.ex Roxb) are reported by Almeida from the same regions and some from different regions of Maharashtra; *I. aspalathoides* Vahl ex DC. *I. wightii* Graham from Konkan and Marathwada; *I. hochststeri* Baker from Kolhapur, Pune and Chakan. These species are commonly found within Maharashtra State and they show great morphological variations. Out of these 35 species, 11 Species are present in Amravati district (Dhore, 2002). They are having edible and medicinal value. *Indigofera* has important medicinal uses. As there are large number of species each found its use in different manner depending upon its secondary constituents. *I. cordifolia* Heyne ex Roth.. used as a forage crops for animals due to the high crude protein and calcium contents and palability was good (Nath *et al.*, 1971). The seeds of *I. trita* Linn. are used as nutritive tonic, collected for goat fodder. Twigs are used as tooth brushes and preferred over more commonly used *Acacia nilotica* (Linn.) or *Azadirachta indica* Linn. twig. Siddhuraju and his colleagues (1995) analyzed the nutrients composition and antinutritional factors of *I. linifolia* (Linn.f.)Retz and their weight were determined. The mature seeds contained 296.6g Kg-1 crude proteins, 47.2g Kg-1 crude lipid, 56.7g Kg-1 Carbohydrates. The seeds of *I. linifolia* (Linn.f.) Retz were rich in K, Ca, Mn, and Cu, while albumins and globulins constituted the major portion of seed proteins.

MATERIALS AND METHODS

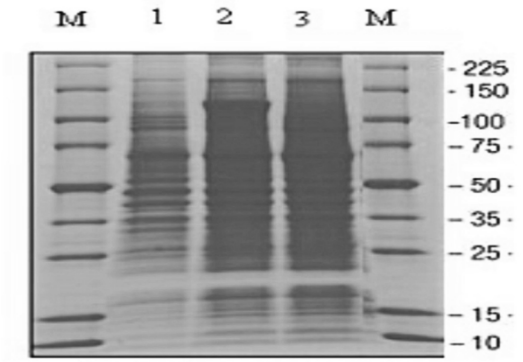
For the present study three different species of *Indigofera* viz. *I. linifolia* (Linn.f.)Retz, *I. cordifolia* Heyne ex Roth, *I. trita* Linn. were collected from Sant Gadge Baba Amravati University Campus. Variability of total seed storage proteins was investigated by using SDS-PAGE (Laemmli, 1970). Protein was extracted from dry seed by grinding it to a fine powder. An appropriate quantity (0.01 g) of this powder was taken in a 1.5 ml Eppendorf tube. Protein extraction buffer (400 ul) containing 0.05M Tris-HCl (pH 8.0), 0.4% SDS, 5M Urea and 1.5% 2-Mercaptoethanol then was added to the tube. The sample was vortexed for 10 min followed by centrifugation at 12000 rpm for 10 min at room temperature. The proteins extracted in the supernatant were size fractionated using SDS-PAGE (9.5% Acrylamide) and stained with Coomassie Brilliant Blue (CBB) dye. After destaining, gels were photographed by placing them on white light.

Data Analysis: A matrix (for SDS-PAGE) was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the lines. Genetic similarity coefficients were computed following Nei and Li (1979). The data were subsequently used to construct a dendrogram using the unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYSpc (Numerical Taxonomy and Multivariate Analysis System), version 2.1 (Rohlf, 2000). Correlations coefficients were calculated using similarity coefficients obtained from SDS-PAGE analyses.

RESULT AND DISCUSSION

The genetic information in a cell which is present in DNA, converts into proteins. These proteins are remarkable molecules and largely responsible for the structure and function of the cells of plants. The protein content can also determine some medicinal properties of the plants. Electrophorograms showing the protein banding patterns of *Indigofera* lines. A total of forty two bands were scored among the 3 species studied. Of these

46 bands, 24 were polymorphic giving 50.1% polymorphism. Size of the protein bands generated by SDS-PAGE (measured by protein standard marker ranging in molecular weight from 10-225 KDa) ranged from 10-150 KDa. Maximum polymorphism was observed with high molecular weight glutenin subunits (HMW-GS) proteins in all species. In *Indigofera trita* Linn. 15 bands are observed. The highest number of bands are observed in the range 75-50 i.e. 4 bands. Lowest number of bands in the range 225-150, 100-75, 25-15 is 1 band in each range. Above 225 bands are absent likewise in the range 15-10 also bands are absent. In *Indigofera cordifolia* Heyne ex Roth. 16 bands are observed. The highest number of bands are observed in the range 25-15 i.e. 5 bands and lowest number of bands in the range above 225, 150-100 is 1 band in each range. In the range 100-75 bands are absent and also in 35-25 absence of band. In *Indigofera linifolia* (Linn.f.) Retz 15 bands are observed. The highest number of bands in the range 25-15 is 4bands and the lowest number of bands in the range above 225, 225-150, and 100-75 is 1 band in each range. It is found that the seeds have low molecular weight proteins (Fig. 1). Genetic similarity coefficients grouped the 3 genotypes into two clusters at 0.67 coefficient level (Figure 2). Cluster A comprised of 2 species, viz *I. linifolia* and *I. trita* Cluster B comprised of single species i.e. *I. cordifolia*. Similarity coefficients ranged from 0.33 to 1.0 Two species which showed close relation showed 66.0% similarity with each other (Table1).



Protein Gel Electrophoresis in seeds of *Indigofera sp.*

Figure 1:

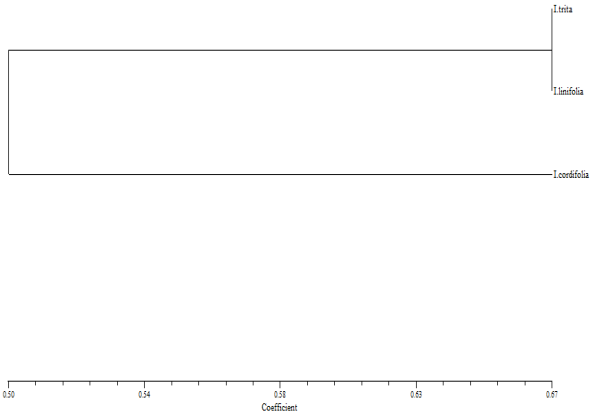


Figure 2: Dendrogram of three species

Table 1: Similarity and dissimilarity index of three *Indigofera species*

	I. trita	I. cordifolia	I. linifolia
I.trita	1	0.66	0.34
I.cordifolia	0.33	1	0.34
I.linifolia	0.66	0.66	1

Below diagonal similarity index, above diagonal dissimilarity index

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