The last two decades have witnessed the conversion of a large amount of forest cover into agricultural land for human use. Moreover, the increasing interest in medicinal plant-based treatments has precipitated the over exploitation of these species from the wild. The populations of medicinal plants are drastically degraded due to anthropogenic interferences (Aguilar et al., 2008; Sarwat et al., 2008). The loss of one plant species means its depletion from the biological Gene Bank (Haas, 2008). Thus, biodiversity is an irreplaceable resource; once lost, it is lost forever.

Three species of genus Tylophora (family Asclepiadaceae), Tylophora indica Burm.f. Merrill, Tylophora rotundifolia Ham.ex Wight and Tylophora fasciculata Ham.ex Wight are important indigenous medicinal plants found in restricted localities and facing threats of different proportions. Though Tylophora indica has a spatially structured population and is found in the plains, hilly slopes and the outskirts of the forests of Gujarat, the other two species do not have a scattered population and are listed as endangered species. Tylophora rotundifolia and Tylophora fasciculata are over exploited to an extent that now it can be found in restricted regions in the inner recesses of Shoolpaneshwer sanctuary in South Gujarat (D’Cruz, 2003).

Tylophora indica (Fig. 1) previously called as Tylophora asthamatica, is a well-known medicinal plant used for the treatment of asthma (Shivpuri et al., 1972; Ali and Bhutani, 1989; Huntley and Ernst, 2000), bronchitis, whooping cough, inflammation, allergies (Shivpuri et al., 1968), dermatitis, dysentery, diarrhoea and rheumatic gouty
pains (Gopalakrishnan et al., 1979). It is also a folk remedy for psoriasis, seborrhoea, anaphylaxis, and leucopenia (Faisal et al., 2007). The roots have a fairly sweet and subsequently acrid taste, aromatic odour and a brittle fracture (Bentley and Trimen, 1880; Cooke, 1908). The powdered leaves, stem, and root contain several phenanthroindolizidine alkaloids including tylophorine (C_{24}H_{27}O_{4}N) (an anti-inflammatory agent), tylophorinine (C_{23}H_{25}O_{4}N) tylophorinidine (C_{22}H_{22}O_{4}N) and tyloindicine I (potential anticancer alkaloids) (Viswanathan and Pai, 1985; Abe et al., 1999; Chandrasekhar et al., 2006; Kimball et al., 2007; Nagarajan, 2008). The alkaloids are analgesic, smooth muscle relaxant (Dhananjayan et al., 1975) and have hepato-protective activity against induced hepatotoxicity (Gujrati et al., 2007; Malathi and Gomez, 2008; Mujeeb et al., 2009). It is used for its antitumor, anti-inflammatory, anti-anaphylactic properties (Ganguly, 2001) and is also used to treat jaundice in certain parts of India (Gujrati et al., 2007). The extract of *Tylophora indica* is marketed by several pharmaceutical companies (Modern Natural Products, Mumbai, India; Sabinsa Corporation, Piscataway, NJ, USA; Sanjivini Herbals, Salem, India) as antiasthmatic herbal drugs.

*Tylophora indica* is a perennial, slightly woody, not much branched, climbing shrub. It gives off numerous long fleshy roots. Its stems are slender, twining, tortuous, terete, reaching 10-12 feet in length. The leaves are broadly ovate, rounded or cordate. Flowering occurs from July to December and flowers are small and numerous, arranged in irregular order. The fruit is 3-4 inches long, producing numerous seeds and widely spreading (Bentley and Trimen, 1880; Cooke, 1908; Shah, 1978; Almeida, 2001).
Tylophora rotundifolia (Fig. 2) is a twining perennial, mostly unbranched, hairy herb. Its leaves are coriaceous, broadly ovate or subrotund, and petiolate. It produces numerous flowers from January to July and long, ovate seeds (Cooke, 1908; Shah, 1978; Almeida, 2001). The tribal medicinal men of Gujarat have been using Tylophora rotundifolia root extracts for chest pain, indigestion and as an emetic for insect bite.

Tylophora fasciculata (Fig. 3) is a tall, stout, slightly twining and a tuberous herb. Several branches arise from the roots. The leaves are ovate to ovate-lanceolate and are diminishing in size upwards. It produces chocolate brown small flowers from August to September. It produces long, ovate or oblong, flat seeds (Cooke, 1908; Shah, 1978; Almeida, 2001). Leprosy is treated with the root extracts of Tylophora fasciculata. It is also given in fever, abdominal pain, jaundice, diarrhoea, vomiting and duodenal ulcer. The root and leaf extracts are used as a nasal drop for snakebite (D’Cruz, 2003).

Cultivations of these important medicinal species have not been attempted due to nonavailability of adequate quality planting materials. At present only the wild population is used for medicinal purpose. Due to overexploitation and lack of organised cultivation, these wild populations have fast declined (D’Cruz, 2003; Chandrasekhar et al., 2006). There is an urgent need to conserve these species in view of their widespread
medicinal importance (Faisal et al., 2007). However, no significant efforts have been made to characterise the genetic diversity and the genetic structure of these plant populations. It is very important to understand the complex processes involved in the long term evolutionary history of species such as genetic drift, mutation, gene flow within the populations, and selection pressure. Understanding and assessing genetic diversity within and among populations can contribute valuable guidelines for conservation strategies (Jayanti and Mandal, 2001; Faisal et al., 2007).

The main goal of biodiversity studies is the preservation of existing genetic diversity. There could possibly be a genetic basis for the endangered nature if it has very low allele frequency. Survival of a species depends on the maintenance of genetic variability within and among populations to accommodate new selection pressures brought about by environmental changes. Restricted populations have lower genetic diversity than widespread species. The plant is threatened by increasing agriculture, cattle grazing and ethnomedicinal usage. Information on how genetic variation is distributed among the remaining population of an endangered species can be used in designing recovery programmes. Moreover, opting for conservation measures depends on their economic and medicinal importance to individuals, a particular community or society at large. Hence protecting an endangered species becomes an individual conservation priority.

The distinguishing mark of every organism is its unique set of DNA. Mutations occurring in the DNA sequences of an organism are expressed as variations, which create new alleles in a population. This phenomenon is termed as polymorphism (meaning, having many forms). Characterizing such alterations in genetic structure is
very informative in understanding genetic polymorphism in a population. A population is in a state of balanced polymorphism when non-identical alleles for a trait are being maintained at frequencies greater than 1%. The extent of polymorphism within the gene pool of a species determines not only the survival of a species but also its evolutionary potential. In plants, about 15 to 30% of genes coding for enzymes are polymorphic. It means that genetic variation is very common in natural populations of most plants. Such variations that distinguish within species and among species can be called Molecular Markers.

DNA polymorphisms are important genetic markers in natural populations. Knowledge about molecular markers based on polymorphism have been exploited by plant biologists in the last two decades for breeding, selection of high yielding varieties, identifying a species, solving taxonomic issues, estimating phylogenetic relationships, formulating conservation strategies, etc. Polymorphic traits are influenced by environmental factors.

Until two decades ago, morphological and biochemical traits like allozymes have been used to analyse genetic diversity within the gene pool of a species. However, allozymes have proved insufficient to assess genetic diversity within populations (Heun et al., 1994). Therefore, the development of molecular based technologies such as RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat), RFLP (Restricted Fragment Length Polymorphism) have broadened the scope of such studies at the population level. Among molecular markers, RAPDs have been extensively used in genetic research owing to their speed and simplicity (Penner, 1996). RAPD is a DNA polymorphism assay based on random amplification of a DNA segment using
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arbitrary sequence primers. This method is used in conservation, population and evolutionary biological studies because of its technical swiftness, cost effectiveness and less labour intensiveness. The technique does not require prior knowledge of DNA sequences and generates DNA markers from very little tissue by screening the entire genome. Visualisation of the result does not require the use of hazardous radioisotopes and can be directly observed from the gel by ethidium bromide staining.

In the case of endangered species, information beyond morphological variation is not available. Plant species with finite populations usually have low levels of genetic variation. Moreover, small populations are often subject to the loss of alleles through genetic drift or through random fluctuations in allele frequency. Gene flow assures genetic differentiation, which is essential for the survival of any species. For greater variation and greater survival rate the species should be spread over a wide geographical area. This may suggest a species-specific reason for reduced diversity in small population size. At present, no study has been carried out to assess the genetic diversity and to correlate it with the existing endangered nature of the species. Keeping this in mind, the present study was undertaken with the following specific objectives:

1. To locate, identify and collect the three species of *Tylophora* namely, *Tylophora indica* (from Gujarat and Maharashtra), *Tylophora rotundifolia* and *Tylophora fasciculata* from various regions of Gujarat

2. To generate species-specific fingerprints for *Tylophora indica*, *Tylophora rotundifolia* and *Tylophora fasciculata*

3. To assess genetic diversity expressed as percentage polymorphism and evaluate significant variations within and among different subpopulations using RAPD and HPTLC methods
4. To generate *trnL-Leu* (UAA) intron based molecular barcode and compares the patterns of chloroplast DNA diversity within *Tylophora* species. Using this information, assess the competence of chloroplast DNA to quantitatively differentiate species and populations.

5. To examine genetic relationship (identity and distance) within and among populations and correlate this data with the threatened status of the species under study.

6. To suggest management choices for efficient conservation that could help in maintaining genetic variation within the species of *Tylophora indica*, *Tylophora rotundifolia*, and *Tylophora fasciculata* using the baseline information generated.