3

Methodology

3.1 Inventory of regional and Indian endemic plants in Gujarat state

In order to understand the status of endemic and threatened plants, various literatures were scrutinized. The present estimate is first of its kind that encompasses all endemic plant taxa of Indian boundary, occurring in Gujarat state and is based on an intensive survey of literature and extensive field studies.

3.1.1 Distribution database of endemic and threatened species in Gujarat

A distribution data of endemic and threatened plants was collected from the following different sources:

1) PhD theses were downloaded from Shodhganga: a reservoir of Indian theses, which can be accessed freely on the internet (shodhganga.inflibnet.ac.in). For each targeted species database was compiled from all the available theses.

2) Other scientific literature describing geographic distribution of endemic and threatened plants in Gujarat that includes published papers from 1832 till date. All source data describes the presence of species in political units excluding a few specimen records that describes them in point location. Villages, talukas and district levels are the basic geopolitical units in Gujarat.

3) Flora literature including the Flora of Gujarat (considered the most authoritative source, since 1978), the Flora of Bombay Presidency (Cooke 1901-1908), and Local Floras (Flora of Saurashtra)

4) Various countrywide and provincial monographs of endemic and threatened plants in India

5) Specimen records from the different herbaria in Gujarat, and virtual herbarium sites like waghaibotanicalgarden.in/searchHerbarium.aspx, a specimen information sharing platform that has digitized 3644 plant specimens, with majority of the collections by Dr. B.G. Vashi, Dr. J.R. Parmar and Mr. K.L. Dubey.

6) Field explorations carried out in different regions of Gujarat during the research duration (September, 2013 to December, 2017).

From the source data, all the distribution data was extracted and compiled after which a geographic distribution database of endemic and threatened plants in Gujarat was compiled. The database mainly consists of the scientific names of species, its endemic description, growth forms, present localities (in districts and villages units), and habitats. Perhaps, it is the most updated and precise information concerning the distribution of endemic and threatened plant species in Gujarat.

3.1.2 Field visits

The present work is the result of intensive and extensive field exploration to different corners and pockets of Gujarat during the period of September, 2013 to December, 2017. 18 long tours (5 to 9 days duration) and 34 short tours (2 to 4 days duration) were conducted.

Depending upon the need and convenience, different modes of travelling was adapted. Field explorations were carried out by motorbike at Pavagadh, Jambughoda, Rajpipla, Valsad and Dangs, while for distant areas private vehicle was hired. Irrespective of distant areas and different landscapes, maximum area for true survey to locate different species of plants and collecting samples for herbarium making was covered by foot. Wherever available, Gujarat Forest Department Rest houses were used as main camping centers in the forest villages.

During the field visits priority was given to those locations from where the plants have been previously reported and were explored for its present status. Most of the habitats in different forests were surveyed on foot. The plants were identified in the field and verified later with help of relevant floras and keys. Care was taken

during the field studies to take its GPS readings using a handheld Garmin Oregon 650 (Digital Global Positioning System). The number of individuals, threat faced by the species, were documented for each plant. Moreover, its seeds were brought for regeneration/conservation. A computerized database was generated incorporating details on their distribution, life-form type, habitat, phenology and local names.

3.1.3   Local Workshops

Figure 9: Taxon data sheets filled by local experts

Taxon data sheets were prepared and the information gathered during the workshop was compiled and used for analyzing the status of prioritized threatened plants. The stated sites were visited then to confirm the presence of the target species.

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3.1.4   Preparation of herbarium

Figure 10: Preparation of Herbarium sheets

3.1.5   Authentication of herbarium

Specimens were identified with flora and authentic literature. The specimens were also confirmed with different herbaraia *ie*. Department of Botany, Calicut University; Jawaharlal Nehru Tropical Garden and Research Institue, Thiruvanthapuram; Department of Botany, The Maharaja Sayajirao University Of Baroda, Vadodara

(BARO); Department of Botany, Shivaji University, Kolhapur; Botanical Survey of India, Jodhpur (BSJO) and Botanical Survey of India, Pune (BSI).

Several experts, Dr. Vinod Maina and Dr. S.L. Meena, BSI, Jodhpur; Prof. S.R. Yadav, Shivaji University; Dr. A.S. Reddy, S.P. University, also Prof. Minoo Parabia, Prof. S.D. Sabnis, Prof. Vinay Raole and Dr. Rinku Desai were consulted for the identity of threatened species.

The collected specimens were also authenticated and certificate was procured.
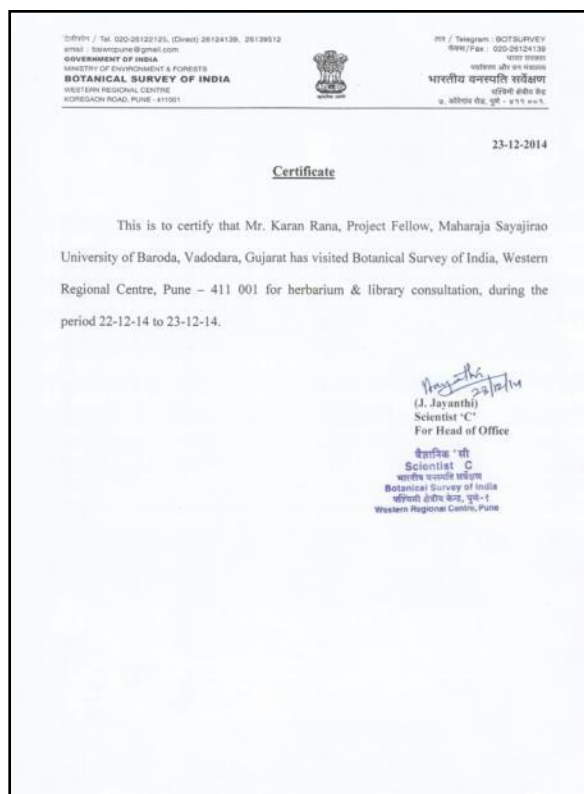
Herbarium verification and consultation with experts at BSI, Pune

Herbarium Authentication certificate issued by BSI, Jodhpur

3.2   Preparation of the inventory

This checklist is further processed through the following steps in order to construct taxonomically rich and accurate database.

- All the families in the present work have been arranged according to APG system of classification. The genera, species and infraspecific taxa are arranged

alphabetically which seems more convenient to follow. Nomenclature of each taxon has been checked properly and updated.

- Each and every scientific name is looked for the correct orthography from The International Plant Names Index (IPNI, 2012) database and corrected wherever required.

  Complete citation of the taxa, which includes correct name, basionym and synonyms wherever necessary, is given alongwith reference to original publications, Floras, *etc*. After citations, vernacular names (wherever available), flowering and fruiting period, habitat, distribution, status and notes are given.

- Distribution data is updated based on the information gathered from the published literature.

- Several insitutes were scrutinized in order to know the unpublished data in form of herbaria and relevant literature so as to update the distribution data of the enumerated species. The Botanical Survey of India, Arid Zone Circle, Jodhpur; the Botanical Survey of India, Western Regional Center, Pune; Sardar Patel University, Vallabh Vidyanagar; Veer Narmad South Gujarat University, Surat; Government Science College, Gandhinagar; GUIDE, Kachchh; Sahjeevan, Bhuj; Junagadh Agriculture University, Junagadh; Anand Agricultural Univserity, Anand; B.K.M. science college, Valsad, and Waghai Botanical Garden, Dangs.

3.3 Assessment strategy applying the IUCN Red List Categories and Criteria system

The IUCN Red List Categories and Criteria system is an international accepted assessment system for classifying species into different categories of extinction risk using simple quantitative rules (IUCN 2001). Among the listed endemic and threatened plant species in Gujarat, only a few have been cross-listed in IUCN global species assessments prior to 2014 (www.iucnredlist.org).

In order to assess the status of threatened plants at a regional scale, the IUCN Red List Categories and Criteria system was applied, following the guidelines for application at a regional level. The distribution database prepared for this study was

referred to when assessing the status of endemic and threatened plant species at a regional scale.

3.3.1 A general account on the categories and criteria of threatened plants and categories of endemic plants

Identification and conservation of species facing a high risk of extinction in the near future is one of the central objectives of modern conservation. And the categories and criteria for classifying threatened species seem to be the basic pre-requite of any conservation assessment, because it provides the mechanism for identification of the threatened species.

The lUCN Red List Categories and Criteria were first published in 1994 (lUCN 1994). In order to stabilize the assessment procedure these Categories and Criteria were extensively reviewed between 1997 and 1999. The revised Categories and Criteria (lUCN Red List Categories and Criteria *version 3.1*) were adopted by lUCN Council in February 2000 which is as follows:

3.3.2 IUCN Red List categories *(version 3.1)*

Assignments to the Red List take place only following a peer review by at least two qualified specialists and are reviewed at least once every 10 years. IUCN has recognized species in nine categories, within three principal groupings, whose definitions are presented in the following simplified form:

Extinct categories

- Extinct (EX): Taxa for which there is no reasonable doubt that the last individual has died. IUCN presumes that a taxon is extinct when exhaustive surveys in known or expected habitat, at appropriate times (diurnal, seasonal, annual), and throughout its historic range have failed to record an individual. Surveys should have been over a time frame appropriate to the taxon's life cycle and life form.

- Extinct in the wild (EW): Taxa known only to survive in cultivation, in captivity, or as a naturalized population (or populations) well outside the past range. IUCN presumes that a taxon is extinct in the wild when exhaustive

surveys in known or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual (as described for extinct). As for the extinct status, surveys should have been over a time frame appropriate to the taxon's life cycle and life form.

Threatened categories

- Critically endangered (CR): Taxa facing an extremely high risk of extinction in the wild.

- Endangered (EN): Taxa considered to be facing a very high risk of extinction in the wild.

- Vulnerable (VU): Taxa considered to be facing a high risk of extinction in the wild.

Lower risk categories

- Near threatened (NT): Taxa close to qualifying for, or likely to qualify for, a threatened category in the near future.

- Least concern (LC): A taxon is classified as least concern when it has been evaluated against the criteria and does not qualify for CR, EN, VU, or NT. Widespread and abundant taxa are included in this category.

- Data deficient (DD): A taxon is data deficient when there is inadequate information to make a direct or indirect, assessment of its risk of extinction based on its distribution and/or population status.

- Not Evaluated (NE): A taxon is not evaluated when it has been initially proposed but not discussed and not yet been evaluated against the criteria for any reason, including uncertainity about taxonomic or wild status.

Data Deficient is therefore not a category of threat. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that future research will show that threatened classification is appropriate. It is important to make positive use of whatever data are available. In many cases great care should be exercised in choosing between DD and a threatened status. If the range of a taxon is suspected to be relatively circumscribed, and a considerable period of time has elapsed since the last record of the taxon, threatened status may well be justified.

The word *threatened* in the NT conservation status can be especially misleading, as many NT species are actually quite common. Designation as NT rather than LC simply reflects some minimal evidence of possible future risk.

3.3.3   IUCN Red List Criteria *(version 3.1)*

A taxon is considered threatened if it meets any one of the criteria listed below. A taxon is assigned highest threat category as per the relevant criteria, if it meets two or more criteria or categories (IUCN, 2014).

The five criteria are:

a) Reduction in population size

b) Reduction in extent of occurrence

c) Reduction in area of occupancy

d) Reduction in mature individuals

e) Probability of extinction in the wild

3.3.4   Application of IUCN criteria at regional level

The word regional is means any subglobal geographically defined area, such as a continent, country, **state**, or province and regional assessment denotes process for determining the relative extinction risk of a regional population according to the guidelines. According to lUCN (2003) regional assessments should be carried out in a two-step process. In step one, the lUCN Red List Criteria are applied to the regional population of the taxon, resulting in a preliminary categorization. All data used in this initial assessment - such as number of individuals and parameters relating to area, reduction, decline, fluctuations, subpopulations, locations, and fragmentation- should be from the regional population.

In step two, the existence and status of any conspecific populations outside the region that may affect the risk of extinction within the region should be investigated. If the taxon is endemic to the region or the regional population is isolated, the Red List Category defined by the criteria should be adopted unaltered. If, on the other hand, conspecific populations outside the region are judged to affect the regional extinction risk, the regional Red List Category should be changed to a

more appropriate level that reflects the extinction risk as defined by criterion E (lUCN 2001). In most cases, this will mean downgrading the category obtained in step one, because populations within the region may experience a 'rescue effect' from populations outside the region. In other words, immigration from outside the region will tend to decrease extinction risk within the region. Normally, such a downgrading will involve a one-step change in category, such as changing the category from Endangered (EN) to Vulnerable (VU) or from VU to Near Threatened (NT). If the region is very small and not isolated by barriers from surrounding regions, downgrading by two or more steps may be necessary.

3.3.5 Adjustments to categories

The lUCN Red List Categories (lUCN 2001) should be used unaltered at regional levels, with three exceptions or adjustments.

1) Taxa extinct within the region but extant in other parts of the world should be classified as Regionally Extinct (RE).

2) The category of Extinct in the Wild (EW) should be assigned only to taxa that are extinct in the wild across their entire natural range, including the region, but they are extant in cultivation, in captivity, or as a naturalized population outside the past range.

3) Taxa not eligible for assessment at the regional level (mainly introduced taxa and vagrants) should be assigned the category Not Applicable (NA).

Table 3: Simplified overview of the IUCN Red List Criteria

| Criterion | Red List Categories | | | Additional sub-criteria |
	CR	EN	VU	
A1: Past reduction in population size	≥ 90%	≥ 70%	≥ 50%	Over 10 years/3 generations, where causes are reversible, understood and have ceased
A2: Past reduction in population size	≥ 80%	≥ 50%	≥ 30%	Over 10 years/3 generations
A3: Future reduction in population size	≥ 80%	≥ 50%	≥ 30%	Over 10 years/3 generations
A4: Reduction in population size	≥ 80%	≥ 50%	≥ 30%	Over a 10-year/3-generation window, which includes the present
B1: Geographic range (Extent of occurrence)	< 100 km^2	< 5000 km^2	< 20,000 km^2	AND at least two of below: (a) severely fragmented/no. of locations (= 1, ≤ 5, ≤ 10) (b) continuing decline (c) extreme fluctuations
B2: Geographic range (Area of occupancy)	< 10 km^2	< 500 km^2	< 2000 km^2	
C: Small and declining population	< 250	< 2500 mature individuals	< 10,000	Continuing decline either (1) over specified rate & period, or (2) with (a) specified sub-population structure, or (b) extreme fluctuations
D1: Very small population	< 50	< 250 mature individuals	< 1000	
D2: Very small range	not applicable		< 20 km^2 or ≤ 5 locations	Capable of becoming CR or EX within a short duration
E: Quantitative analysis (extinction risk)	≥ 50% in 10 years/3 generations	≥ 20% in 20 years/5 generations	≥ 51% in 100 years	Probability of extinction using quantitative models, *e.g.* Population Viability Analysis

3. 4 Red Listing Process

IUCN has partnered with ConservationTraining.org to offer a modular, self-timed course on the rules and standards used for IUCN Red List assessments. In this free curriculum, we get trained on Red List assessment process and how to compile a scientifically rigorous assessment.

After successful completion of the final exam for Global or Regional Assessors with a minimum grade of 75%, a Certificate of Achievement is provided.

CERTIFICATE OF ACHIEVEMENT

IUCN, International Union for Conservation of Nature, hereby recognizes

Karan Rana

As having successfully completed the Regional IUCN Red List Assessor exam for the online course

Assessing Species' Extinction Risk Using IUCN Red List Methodology

ConservationTraining
learn globally, conserve locally.

May 7, 2016

Figure 11: Certificate on completion of the IUCN Red List Assessor course

3.4.1 IUCN Species Information Service (SIS)

SIS is a centralized online database used for storing, managing and publishing data on the IUCN Red List.

The species has been assessed using the information and data recorded in this study, and follow the IUCN Red List Categories and Criteria *version 3.1* and current version of the Guidelines for Using the IUCN Red List Categories and Criteria for guidance on applying the IUCN criteria. The available data for population sizes, trends, decline rates, ranges, etc. was then entered in the SIS tool to compare against the IUCN Red List Criteria thresholds.
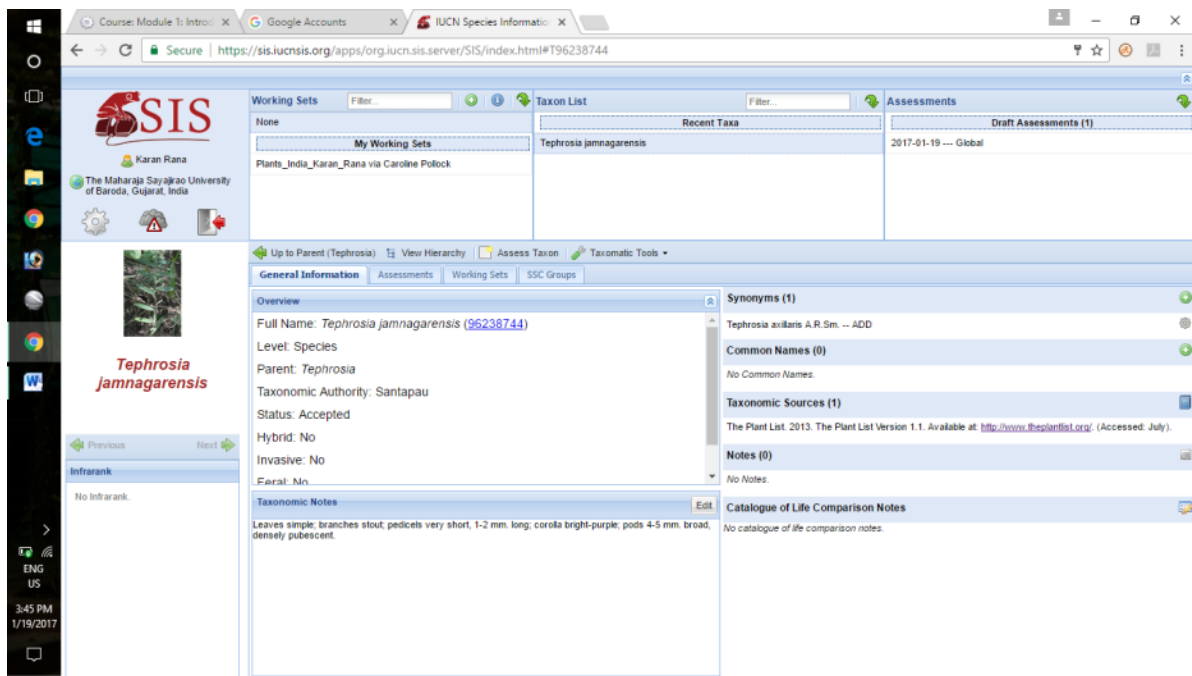
Figure 12: IUCN Species Information Service

First of all in the assessments section, all the assessments that are currently attached to the species, including all published and current draft assessments (including global and regional assessments) are seen. Then, a new draft assessment can be created. SIS saves data automatically. It also includes a function to attach additional documents to an assessment (e.g. figure or table, etc. that does not display well in the text field).

Next, the distribution section allows entering all the information available for the taxon's geographical distribution. AOO and EOO can be recorded in the fields provided in their respective pages. The number of locations can be recorded in the Locations page, along with whether there is any evidence of continuing decline or extreme fluctuations in number of locations. The 'very restricted' page is used to record taxa that have a very restricted range and with a plausible threat (either a current threat or a realistically potential threat) that could cause the taxon to rapidly move into the Critically Endangered or Extinct categories. Also, Elevation/Depth ranges can be recorded in this page. The Biogeographic Realms page is used to record which realms the taxon occurs within.

The occurrence section is to record the taxon's occurrence in specific countries. Then after typing the country name (India) into the text box, within India, this species (*T. jamnagarensis*) actually occurs in Gujarat. To add that sub-country

unit to the assessment, click on the arrow next to India to expand the list of Indian sub-country units, and then click in the check box next to Gujarat. After saving the selection, the entries selected will now appear in the country occurrence list, lastly click on done.

The population section holds all of the data fields for recording all available information for population size, trends, etc. In the documentation page, a summary should be entered of the population information available for the taxon. Next, the population page includes many data fields to record the current population trend, estimated population size, whether there is evidence of continuing decline or extreme fragmentation, estimated rate of decline over different time frames, etc. These data fields are used by the automatic Red List Criteria Calculator to determine the appropriate Red List status for the taxon. The pages for Past Reduction, Future Reduction and Ongoing Reduction should be used to record rate of population size reduction over 10 years or three generations.

Then after in the habitats and ecology section, all available and relevant information on habitats and ecology should be recorded here. The coded habitats page allows selecting habitat types from the Habitats Classification Scheme. In the same section, it also allows data fields to record continuing decline in area, extent or quality of habitat. The Life History page holds many fields related to life history parameters that may be used to estimate generation length. In the systems page, tick on terrestrial. The last page (Plant Specific), the fields are used to record whether the species is a crop wild relative, and what general growth form it is (*e.g.*, tree, shrub, hydrophyte, geophyte, epiphyte).

General use and trade information is recorded in the Use and trade section within an assessment. The Non-consumptive page is used to record uses of the taxon that do not involve it being removed from the wild (to attract tourists to a particular area). The livelihoods section allows adding available data indicating the commercial value of the taxon.

In the threats section, there is a documentation page to record a summary of available information on threats that may be affecting the taxon. The conservation section includes a text field to record summary of all conservation actions currently

in place for the taxon, and those actions that are required to help improve its status or to improve our knowledge of the taxon to be able to better understand its extinction risk.

The Conservation-Needed and Research-Needed pages give access to the Conservation and Research Classification Schemes to record actions that are required to help improve the taxon's status.

In the All Fields view, the 'Red List Assessment' section is where the actual assessment for the taxon recorded based on all the other information entered in other pages. An automatic Red List Criteria Calculator has been built into SIS. The calculator uses the data entered into the various data fields throughout the assessment and compares these against the IUCN Red List Criteria thresholds to calculate an appropriate Red List status for the taxon.

After selecting the appropriate reference type (books, edited books, conference proceedings, manuscripts, theses, reports, etc.), enter the information for the reference into the appropriate fields. Once completed, click on 'Generate' to see how the citation will appear on the Red List website when the assessment is published.
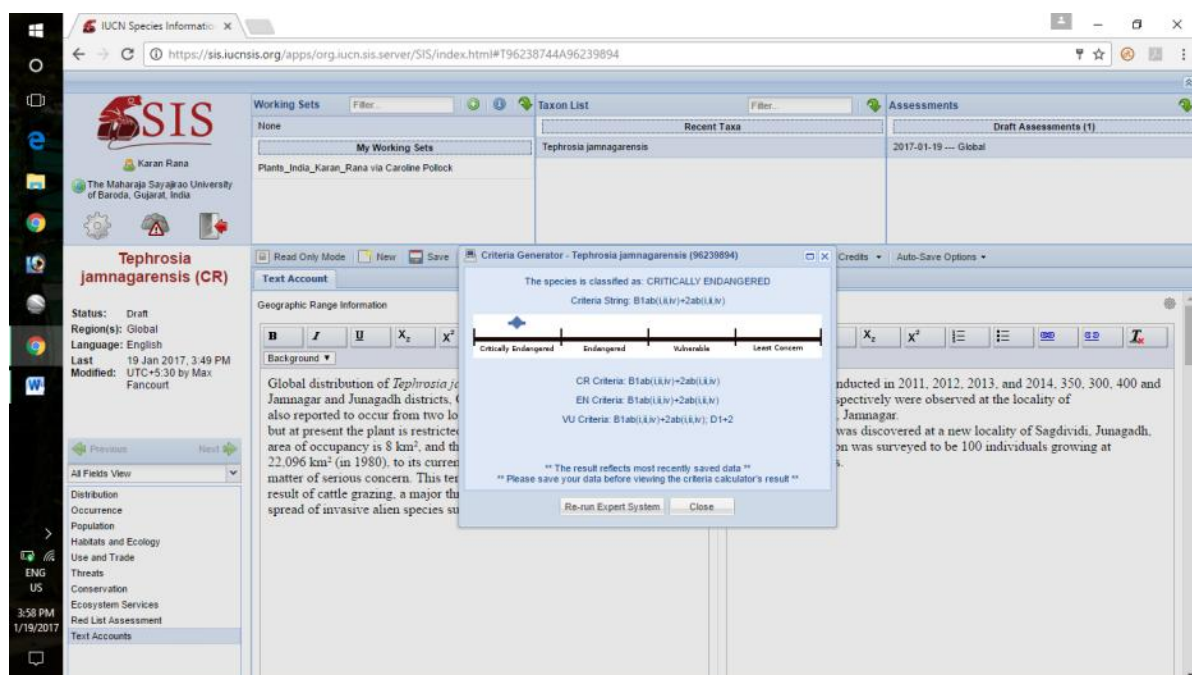

Figure 13: IUCN (SIS) Red List Criteria Generator

From anywhere in the Assessment Page, we can check the Red List status of the taxon based on the data entered so far by selecting the Summary function in the

toolbar and opening the Quick Criteria Generator. In the example shown here, the EOO has been recorded as 86 km², and AOO just 8 km² restricted to only one location. The taxon (*Tephrosia jamnagarensis*) clearly falls within the Endagared (EN) category because the data recorded in SIS are within the EN thresholds for criteria B1ab(i,ii,iv)+2ab(i,ii,iv).

3.4.2   Mapping the Extent of Occurrence (EOO) and Area of Occupancy (AOO)

The categorization of different taxa was done mainly by own observations in the field after getting the preliminary information from literature and herbaria. The information of some newly described species was gathered by personal communication either with the original authors or with other experts in this field. During categorization mainly the criterion 'B' (Extent Of Occurrence - EOO or Area Of Occupancy - AOO) of IUCN's guidelines with their sub-criteria B1 and B2 have been followed. The categories were determined by the main criteria 'B' supported by either single or double subcriteria B1 and B2, instead of at least two subcriteria compulsorily among the three as recommended by IUCN. This is due to lack of any detailed prior information in this field. In some cases the criterion 'D' (number of mature individuals) and criterion 'E' (probability of extinction in the wild) have also been followed.

GeoCAT tool was usedto analyse the EOOand AOO. These two measures are the foundation of the 'B' criterion of the IUCN Red List system (IUCN, 2001). The most appropriate scale will depend on the taxon in question, and the origin and comprehensiveness of the distribution data (IUCN Standards and Petitions Subcommittee, 2010). Within GeoCAT the cell size can be selected, while in the present study the cell size was 2km² as recommended in the IUCN guidelines (IUCN, 2010).

3.4.3   Mapping the number of locations

By the grid adjacency method, all contiguous grid cells from the AOO calculations are considered to be one subpopulation and thus, the count of subpopulations is obtained.

3.4.4 AOO Density

The AOO density is used to study the density and distribution of occurrence of localities for the species (Lakadong, 2009; Barik *et al*. 2018). The value is calculated as the number of AOO subpopulations divided by the number of AOO cells. A value between 0 and 1 is assigned where **0 is sparsely occupied**, i.e. all AOO cells are isolated and **1 is densely occupied**. The AOO density is calculated as follows:

$$\textbf{AOO density = 1 -} \left(\frac{\textbf{AOO subpopulations}}{\textbf{AOO number of cells}}\right)$$

In the present study, a standard grid size of 4 sq. km is used as per the IUCN criteria.

3.5 Conservation

Palmer (1987) reviewed rare plant monitoring, and emphasized the need to develop monitoring efforts that are most consistent with project goals, and suggested demographic and experimental studies for threatened species. Criteria for determining the intensity of monitoring efforts should be based on the degree of endangerment.

During the present study *in-situ* conservation will be carried out by creating the awareness prgrammes among the local people. To raise the awareness among the local peoples and tribal's the workshops will be held in the different areas of Gujarat. People will be trained to distinguish the threatened species. Booklets and Posters were made in a local language to make the people aware about the endangered species and their importance for *in situ* conservation.

While the *ex-situ* conservation of threatened plants like *Sterculia guttata, Entada rheedei, Sterculia villosa, Butea monosperma* var. *lutea, Firmiana colorata, etc.* was carried out in the arboretum of The Maharaja Sayajirao University of Baroda. The below stated methodology was followed:

- During field visits, those localities of previously reported occurrence were searched for recording the targetted species. Additionally entire habitat was also searched. After a species was located, its seeds/bulbs/cuttings were

brought for regeneration/multiplication. Different botanical parameters and initial germination tests were carred out. Thereafter, regeneration protocols of each species were studied. These saplings were re-planted in the wild at selected localities of their natural habitat, to monitor damage due to abiotic and biotic factors as well as growth, so as to understand threats faced by these species in nature.

- Seeds were collected from intact pods before split. Surface sterilization was done with sodium hypochlorite. Seeds were treated with concentrated sulphuric acid for 5 minutes, and then washed thoroughly with water. Seeds were then sown in a mixture of cocopeat and soil (1:1). But they were sown in two different ways: (1) Directly in polythene bags and (2) Seeds presoaked overnight were sown. Germination was higher in presoaked seeds than in direct sown seeds. On emergence of plumule, seedlings were exposed to sunlight. Later they were transferred to the arboretum and conserved.

For stem cuttings, they were treated with IBA and planted in beds with organic manure.