CHAPTER - 1

INTRODUCTION

1.1 Overview

The word Bryophyte is derived from the Greek word "*Bryon*" means moss and "*Phyton*" means plant. Bryophytes are the second largest group of land plants. (Buck and Goffinet, 2000). They are terrestrial but require water for completing their life cycle; hence they are called as "*Amphibians of Plant Kingdom*". They are about 400 million years or older. They are considered as the oldest living land plants (Shaw and Renzalia, 2004). They are found on soil, rocks and trees through out the world, from coastal Antarctica to the tundra of the Northern Hemisphere and from the Australian deserts to the Amazon rain forests. Although small in stature, they are an essential part of the earth's biodiversity and play a significant role in diverse terrestrial and aquatic ecosystems (Hallingback and Hodgetts, 2000). The gametophyte is the dominant phase in the life cycle of bryophytes while the sporophytic phase is short. They require water for germination and hence are mainly found in humid environment. They have sexual as well as asexual mode of reproduction.

Ecologically bryophytes assist in the stabilization of soil crust by colonising bare ground and soil. They protect the soil erosion due to the presence of net and web type of protonema and gametophytes especially mosses like *Atrichum*, *Pogonatum*, *Pohlia*, *Trematodon* etc (Bahuguna *et al*., 2013). They help in nutrient recycling, biomass production and carbon fixing (Turetsky, 2003). In general, they are very efficient at regulating water flow by means of an effective water-retention mechanism (Saxena and Harinder, 2004). They also have economic value, whether it is as peat for fuel, horticulture, oil absorption or as sources for a wide variety of chemical compounds. Bryophytes have long been used for medicinal purposes and their value as pollution indicators is also well known. They are also a food source for animals in cold environments (Goffinet and Shaw, 2008). In the temperate parts of the world they are used in construction of houses or for vertical gardening or for landscaping as well they are used in aquariums (Glime, 2019).

Bryophytes contains many novel compounds that have antibacterial, anti-tumor, antiseptic, anti-coagulant, cytotoxic properties (Asakawa, 1990; Asakawa, 1995). They are used as pollution indicator, they help in the ecosystem maintenance, are an important component of the food web, used as packing material, they also possess various phytochemical

compound that have medicinal value etc. (Mishra *et al.*, 2014). As per the doctrine of signature, liverworts (*Marchantia polymorpha*) have been used for liver disorders, *Plagiochasma appendiculatum* as well as *Targionia hypophylla* for skin infections and diseases *Polytrichum commune* for hair problems etc. They have anti-cancer property (*Polytrichum commune*) and can act as insecticides (*Conocephalum conicum, Dicranum scoparium*). Specifically, members of bryaceae are also very important medicinally viz, *Rhodobryum roseum* is used for nervous and cardiovascular disorders, *R giaganteum* is used as anti-pyretic, anti-hypertensive, *Bryum argenteum* is used as antipyretic and antifungal etc. (Glime, 2019).

In an evolutionary consideration, bryophytes occupy an intermediate position between algae and pteridophytes. Features in which bryophytes differ from algae and pteridophytes are shown in the table 1.1:

Table 1.1: Different features of algae, bryophytes and pteridophytes

Sr No.	**Features**	**Algae**	**Bryophytes**	**Pteridophytes**
1.	Habitat	Usually aquatic	Moist shady places & water	Usually land
2.	Plant body	Unicellular to multicellular	Thalloid or leafy	Differentiated into root, stem & leaf.
3.	Sex organs	Not covered by sterile jacket	Covered by sterile jacket	Not covered by sterile jacket
4.	Sporophytes	Independent / dependent	Dependent	Independent
5.	Vascular tissues	Absent	Not well developed, but hydroids & laptoids presence in some moss	Well developed tissues like xylem & phloem.

(Compiled from various sources)

Bryophytes have characteristic salient features which have been depicted in fig 1.1 below. They are broadly divided into three groups viz. liverworts, hornworts and mosses that occurs in two forms i.e. thalloid and foliose. As per Goffinet and Shaw (2008), they are classified into different divisions/phyllum viz. Marchantiophyta, Anthocerophyta and bryophyta, according to recent classification system of bryophytes .

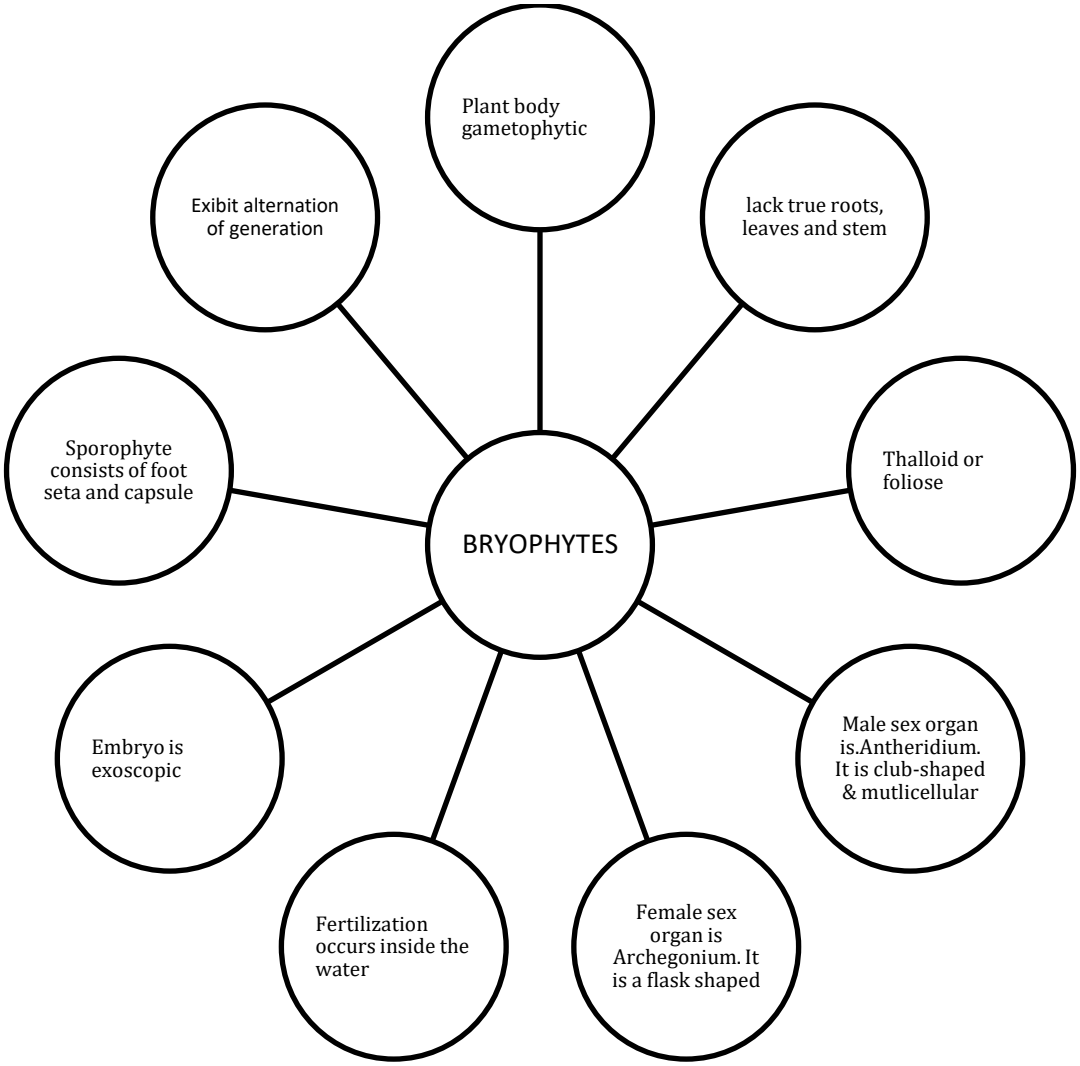

Fig. 1.1: Salient features of bryophytes

Liverworts: Liverworts are thalloid and occur in three different forms viz. simple thalloids, leafy thalloids and complex thalloids. Liverworts occurring in complex thalloid form belong to class Marchantiopsida and simple and leafy thalloid liverworts belong to class Jungermaniopsida. In the leafy liverworts the capsule is attached to the gametophyte by means of seta. In thalloid liverwort, the sporophyte is embedded in the thallus and

disperses the spores on maturity. The dispersal mechanism operates in two ways either with the help of elaters or splitting of capsule (Pandey, 2005; Sharma, 2014).

Hornworts: The plant body is thalloid without internal tissue differentiation and lack of midrib. Several members also have pyrenoids. Scales and tuberculated rhizoids are absent, only smooth walled rhizoids are present. Sporophyte bears capsule and foot, seta is absent. However, capsule have sterile columella with overarching archesporium (Pandey, 2005; Sharma, 2014).

Mosses: Many times the word bryophyta and bryophytes are synonymously used for one another, though they are different. Bryophyta is one of the division of bryophytes. Bryophyta division/phylum comprises of all the mosses. Mosses are of two type, 1) growing erect called acrocarpic and 2) growing prostrate called pleurocarpic. Mosses have two stages in its life cycle – Protonema- prostrate stage and mature gametophore- erect stage. Mature gametophyte is leafy, bearing multicellular rhizoids with oblique septa. The sporophyte possess capsule, seta and foot (Pandey, 2005; Sharma, 2014).

This diversity had been depicted in the fig 1.2 given below.

Liverwort (Thalloid)

Liverwort (Foliose)

Hornworts

Moss (Acrocarpic)

Moss (Pleurocarpic)

Fig. 1.2 Morphological diversity of different groups of bryophytes

1.2 Diversity of Bryophytes

The number of bryophyte species in the World are about 15000 to 16000 while in India it accounts for about 2562 species whereas in Gujarat about 67 species of bryophytes are recorded (Goffinet and Shaw, 2008; Chaudhary *et al*., 2006; BSI ENVIS, 2021). Table 1.2 below shows the global, national and regional distribution of different groups of bryophytes.

Table 1.2: Distribution of bryophytes

Sr. No.	Regions	Liverworts (spp)	Hornworts (spp)	Mosses (spp)	References
1	World	5000	150	13000	Goffinet and Shaw,2008
2	India	675	25	1786	BSI ENVIS, 2021
3	Gujarat	13	10	44	Chaudhary et al, 2006

1.3 History of Classification

The term *Bryophyta* was given by Braun (1864) and it included alage, lichen and mosses. Eicher (1883) was first to classify bryophytes into Hepaticae and Musci. He divided the class hepaticae into 3 orders namely; Marchantiales, Jungermanniales and Anthocerotales. Later on several researchers (like Smith, 1938, 1955; Takhtajan, 1953; Wardlaw, 1955 and Schuster, 1958) worked and understood the basic differences between Anthocerotales and other members of hepaticae, hence divided bryophya into Hepaticae, Anthocerotacea and Musci. However, the status of class 'Anthocerotes" was given by Howe (1899). Rothmaler (1951) added suffix "opsida" to the classes due to rule of International Code of Botanical Nomenclauture to use suffix "opsida" for class. Thus, Hepaticae was converted into Hepaticopsida, Anthocetes converted into Anthoceropsida and Musci converted into Bryopsida. Anthoceropsida was further changed into Anthocerotopsida by Proskauer (1957). Holmes (1983) had given an outline classification in her book "Outline of plant classification". The most recent classification for liverworts was proposed by Crandall-

Stotler *et al*. (2008), hornworts was proposed byDuff *et al*.(2007) (and mosses was proposed byGoffinet *et al*., (2008).

This group of plants isnot as familiar as the flowering plants. There are several reasons for this:

- **Less amount of bryophyte research:** Majorly the biodiversity hotspots of India like Western ghats, Eastern ghats, Himalayan region, Gangetic plains etc are explored (Nair *et al*., 2005; Manju *et al*., 2009; Palani et al., 2017; Rao and Dora, 2019, Tewari *et al*., 2020) extensively. In India, the bryodiversity of only few other areas have been explored like in the Central India few parts of Rajasthan and Panchmarhi Biosphere reserve of Madhya pradesh are explored (Chaudhary *et al*., 2006; Kapoor *et al*., 2011;Alam *et al*., 2014;Alam *et al*., 2011; Bansal and Nath, 2014; Ashthana and Gupta, 2015; Shah and Gujar, 2014; Mehta *et al*., 2016; Gujar *et al*., 2016). Comparatively, very few researchers had expored the diversity of bryophytes and several districts of Gujarat like Vadodara, Narmada, Dahod etc were not explored (Chaudhary *et al*., 2006).
- **Lack of Expert:** Identification of plants need basic education along with experience, proficiency and botanical expertise in the concerned field (Ramasubbu *et al*., 2015; Waldchen and Mader, 2018). Moreover, bryophytes are very small plants and its identification requires more expertise. Experts in this field are very few and are decreasing with time (Mishra *et al*., 2014).
- **Lack of reporoductive structures and sporophytes:** Bryophytes are seasonal plants and their growth and development depend on the availability of moisture. Hence, in case of areas of erratic and scanty rainfall, the sexual reproductive structures do not develop and hence the the sporophytes are absent or poorly developed. The identification of bryophytes in absence of them is difficult.
- **Neglected in School and College curriculum**: In many of the schools and colleges, proportion of the bryophytes and its specimen are much less studied in comparison to other groups of plants especially the flowering plants. This creates less interest in the young researchers towards taking this group in research in future.
- **Less Literature available:** The authentic literature for these groups of plants is verty few in comparison to the angiosperms. Regional floras are available for the

flowering plants. Similarly in Gujarat, sufficient authentic literature for the identification of bryophytes is scarce except few researcher in the late 1970's and the stateflora of bryophyte (Chavan, 1937a; Chavan *et al*.,1961; Patel, 1977a; Chaudhary *et al*., 2006). Even in the State Flora there is a major lacunae as the flora had covered only 18 location across the state and few districts viz. Anand, Amreli, Dahod, Jamnagar, Narmada, Patan, Porbandar, Bhavnagar, Surat, Vadodara etc. were not covered in the study.

1.4 New Techniques in plant Identification

From the above points it is evident that the identification of bryophytes is demanding task and especially in absence of reproductive structures. This problem can be resolved to an extent by modern techniqued/tools like Computer aided Identification (CAI) and by the use of molecular method like DNA barcoding.

1.4.1 Computer aided Identification (CAI)

Computer aided identification provides a broad scope for its use in the field of taxonomy and provides the user friendly platform for the beginner also (Saucede *et al*., 2020). CAI are mainly of 4 types viz. multi access keys, hypertext, expert system and neural network (Edwards and Morse, 1995). Multi access key is one of the most widely used CAI type. Multi access key has advantage over the traditional biological dichotomous key (also called as single access key) identification key. In dichotomous, it is mandatory to follow the fixed sequence of the key whereas in multi access key it is not requisite (Hagedom *et al*., 2010). There are several identification modules available but the INTKEY is one of the most widely used CAI tool for plants (Edwards and Morse, 1995). Such keys can be developed for any groups of organism and it can used for identification by selecting any character desired available with the user and thereby by narrowing the search. CAI tools are used in species identification of different groups of plants like gymnosperms (Watson and Dallwitz, 2008), mangroves (Prosperi *et al.*, 2005), grasses (Watson *et al.*, 1992) and other flowering plants (Watson and Dallwitz, 1992). There are several sofwares like Dkey (Tofilski, 2018), Delta (Dallwitz, 1997), Xper2 (Ung *et al*., 2010), Xper3 (Vignes-Lebbe *et al*. 2017), Lucid (Bittrich, *et al*., 2012) and IPEZ (Fischer, 2013) that are available for preparing computer aided

identification tools for the identification of plants and animals. CAI tool have been used for identification of different plant groups i.e. from fern to angiosperms but havenot been explored for the bryophyte group. Bryophytes are very small group of plants and as mentioned earlier many times the sporophytes fail to grow due to unfavourable environment. In such cases the development of a CAI module will help in the identification.

1.4.2 DNA Based identification

The method of identification of the plants and animals using specific regions of the DNA strand is called DNA barcoding and is considered to be the most powerful method of identification (Suesatpanit *et al.*, 2017). The use of DNA barcode for the identification of the species requires a reference DNA library/database of known taxa within which the unknown sample can be matched. Globally as well locally the DNA database for the higher plant is more available in comparison to lower plants like algae, bryophytes, pteridophytes. There are several researchers that have used this technique in genera as well species level identification. This technique is employed for the identification of several members of angiosperms in and around the world (Cuenoud *et al*., 2002). They were used for the identification of member of Lemnaceae, medicinal plants, flowering plants of Sumatra, members of lamiaceae (Indonesia) (Wang *et al*., 2010, De Mattia *et al*., 2011; Techen *et al*., 2014, Amandita *et al*., 2018).

Molecular database of fewer species of bryophytes are available in comparison to angiosperms (Table 1.3 & 1.4). DNA Barcoding of bryophytes are largely carried out in the temperate regions like Holland, Germany etc (Valente *et al*., 2019). Regions like brazil have a high (1567 species) bryophyte diversity, yet in such regions also the DNA barcoding technique is mostly restricted to the flowering plants (Palhares *et al*., 2015; Rivera-Jimenez *et al*., 2017). DNA barcoding in bryophytes is difficult as the successful barcoding of bryophytes need designing of specific barcodes for each group (Slipiko *et al*., 2020). Hence, they are not largely explored unlike the flowering plants.

Table 1.3: Global, National and Regional published barcodes records of different groups of plants **

Group	World record	India Record	Gujarat Record
Magnoliophyta	272288	4532	1799
Rhodophyta	29885	44	-
Chlorophyta	7122	155	-
Lycopodiophyta	952	12	2
Pteridophyta	8373	77	34
Marchantiophyta	8113	25	4
Anthocerotophyta	257	0	0
Bryophyta	16380	53	3

Table 1.4: Global, National and Regional identified barcode species records of different groups of plants *

Group	World species	India Species	Gujarat Species
Magnoliophyta	78436	1972	1222
Rhodophyta	3003	20	-
Chlorophyta	2048	80	-
Lycopodiophyta	282	11	2
Pteridophyta	3604	53	24
Marchantiophyta	2128	15	0
Anthocerotophyta	101	0	0
Bryophyta	3288	38	0

Note: **Source: BOLD System V4 http://v4.boldsystems.org/ accessed on 26th February 2021

1.5 Evaluating the role of tissue culture in micropropagation of select bryophyte specimens

Bryophytes are the second largest group of terrestrial plants, yet they are overlooked in terms of conservation and protection in comparison to vascular plants. (Vujicic *et al*., 2009; Sabovljevic *et al*., 2012). Bryophytes are seasonal plant and hence their availability all-round the year is difficult. Tissue culture can be used for the *in vitro* culturing of bryophytes and storing them *in situ*, thereby making them available in non-season period. Tissue culturing was first successfully and effectively used in bryophytes especially mosses, but it not been widely used in comparison to the vascular plants (Servettaz, 1913;Vujucic *et al*., 2011). Numerous studies have been carried out for the long-term storage and preservation several important vascular plants. However, there are few successful studies have been documented for the culturing and long term preservation of bryophytes from the different parts of the world (Gonzalez *et al*., 2006; Awasthi *et al*., 2012a, 2012b). Encapsulation technique can also be applied for the conservation of the buds of the mosses (Mallon *et al*., 2006) using calcium alginate matrix. The culturing of bryophytes encounters few problems like availability of material, its genetic variability, the endophytes bryophytes and lack of expertise in identification of species (Vujicic *et al*., 2009) The culturing of bryophytes from the sporophyte is easy and successful than from leafy gametophores, hence is mostly preferred from the spores (Sabovljevic *et al*., 2003; Rowntree, 2006). This is because the contamination of the specimenscan be easily removed by surface sterilizing the sporophytes. On the contrary, the gametophytes are more prone to contamination by endophytes and also they are fragile for sterilization (Schussler, 2000; Costa *et al*., 2001; Davey and Currah, 2006). Using these, techniques bryophytes can be made available for the study in non-season period also.

1.6 Objectives

From the above points, it is clear there exist several lacunae in information on the diversity and distribution of bryophytes in Gujarat. Hence, the present study was conceived to fill the regional information gaps. Objectives were also included to facilitate the identification of this difficult group of plants by workers with limited taxonomic expertise as well as in scenarios in which plants with all life stages are not available. In addition to this, efforts were made to design a general protocol for conserving the important bryophyte specimens. To achieve this mentioned task, following objectives were proposed.

1. Assessment of diversity of bryophytes in the study area
2. Delineating the distribution of different species of bryophytes in the study area.
3. Standardization of protocol for tissue culture of bryophytes of importance.
4. To evaluate barcoding of bryophytes and their role in molecular identification.
5. Preparation of a Computer Aided identification (CAI) Module in Delta INTKEY for morpho-anatomical identification of bryophytes of the study area.