CHAPTER - 2

REVIEW OF LITERATURES

2.1 Diversity and Distribution of Bryophytes

Bryophytes are the non-vascular green plants that evolved about 470-551 million years ago (Morris *et al.,* 2018). Across the globe, there about 9000 to 13, 000 species of mosses, 5000 to 7500 species of liverworts and 215 species of hornworts are present (Goffinet *et al*., 2008; Magill, 2010; Von Konrat *et al*., 2010; Soderstrom *et al*., 2016) They are the second largest group of land plants next to angiosperms (Rice, 2009). They are present in tropical to temperate and Arctic to Antarctic regions of world (Redfearn, 1990; Gradstein, 1992; Piippo, 1992; Blocher and Frahm, 2002; Staples *et al.*, 2004; Bramley-Alves *et al.*, 2014; Lewis *et al.*, 2017). The group of researchers working in the field of bryology is less in comparison to other group of plants especially angiosperm. The regional floras of higher plants i.e. angiosperms are available in contrast to regional authentic bryophytes literatures that are lacking. In India there are few scattered researchers that are working on this group of plants exploring the hot spot areas like Eastern Ghats, Western Ghats, Indo-Gangetic plains, Himalayan regions , Biosphere reserves etc (Gangulee, 1961; Gangulee, 1969-80; Asthana *et al*., 2005; Daniels and Kariyappa, 2007; Asthana and Sahu, 2013; Palani *et al*., 2017). But still there exists many areas that are potentially rich in bryophytes diversity but not yet explored fully. As a result of this, while the bryophyte diversity overall appears rich, but they contain little or no information for several regions that are not considered hotspots. In such areas regional documentation is lacking.

2.1.1 Pioneer studies in Bryophytes

Historically, several bryologist have studied the diversity of bryophytes from different geographical zones i.e. from temperate to tropical regions. Dillenius (1741) was one of the earliest researcher who worked on bryophytes and published book named *Historia muscorum.* Later, Linnaeus (1753) published book *Species plantarum.* In the past there was several database or indexes of bryophytes provided by various bryologist that included Jaeger and Sauerbeck's *Genera et Species Muscorum*(1879), the two editions of Paris's *Index Bryologicus*(1898), and Wijk, Margadant, and Florschütz's *Index Muscorum*(1959-67). Similarly, currently the Index of mosses provided by Tropicos in the name TROPICOS MOST database (MBG, 1995-2021). This database of names of mosses is prepared as a component of Index of Mosses at the Missouri Botanical Garden. In this chapter the diversity studies carried out in the field of bryophytes worldwide and in India has been discussed.

2.1.2 Global status

Globallydiversity of the bryophyte is studied in different part of the world by different researchers. The study of bryophytes is not just restricted to tropical regions but the temperate regions of the world have also explored its diversity. Among the tropical regions, countries like Bangladesh, Brazil, Cuba, Australia, Vietnam etc and among temperate regions countries like Japan, China, Canada, Finland, Sweden, etc. are well explored. The important studies on bryophyte diversity and distribution in tropical regions includes Costa (1999),Mota de Oliveira (2010),Gradstein and Costa (2016, 2018), Costa *et al*. 2020 from Brazil, Gradstein and Allen (1992), Fensham and Streimann (1997), Ramsay and Cairns (2004) Stevenso *et al*. (2012), Gradstein and Culmsee (2010) from Indonesia and Australia. The important studies on bryophyte diversity and distribution in temperate regions includes Nakanishi (2001), Higuchi (2011) from Japan, Wen-Zang *et al*. (2009), Wang *et al*. (2012), Sun *et al*. (2013) from China and Vitt *et al*. (1995) from Canada. Ignatov *et al*. (2006) had published the checklist of mosses of East Europe and North Asia. Similarly, Ros *et al*. (2013) had published the checklist of mosses of Mediterranean region. Hodgetts (2015) had brought out the list of eurpoean bryophytes and their IUCN status. Noguchi (1987-1994) had given the illustrated the moss diversity of Japan. Erdag & Kurschner (2017) had listed the mosses of Turkey. The studies carried out in India are described in details in the National status of Bryophyte, hence it is not discussed in this section.

2.1.3 National status

In the 19th century Hooker published the book- *Musci nepalensis* consisting of mosses from different parts of the Nepal (Hooker, 1808). Montagne (1842) had explored the Nilgiris of South India and recorded about 100 species of mosses. Griffith (1849) had discovered the monotypic liverwort - *Monoselenium tenerum* which was an important discovery. Mitten (1859) had extensively during that period and had recorded 95 genera and 800 species. He (1860, 1861) had also worked on the liverworts and published a book *Hepaticae Indiae* *orientalis* that included about 290 species of liverworts. The other important contribution was by Stephani (1906-1924), monograph of liverworts in the name *Species Hepaticarum* consisting of 6 volumes. It included collection from different parts of countries like India, Sri-Lanka, Myanmar and neighboring countries.

2.1.3.1 Liverworts and Hornworts

In India, Shiv Ram Kashyap was the pioneer in the field of bryology, hence he is called as "Father of Indian Bryology". He had published different papers and his major work included 'West Himalayan Hepatics' (1914) and Liverworts of the Western Himalayas and the Panjab Plains (1929-1932) (Kashyap 1914, 1929, Kashyap and Chopra, 1932). He had reported several species of hornworts and liverworts viz *Anthoceros erectus* and *A. himalyensis* (1915), *Aneura indica* (1917) from different parts of the country like Himalayas, Punjab plains and South India (Kashyap, 1915, 1917). *Aitchisoniella himalayensis* was collected and recorded by him from Uttarakhand for the first time. It is a monotypic genus and the absence of stalked carpocephala is the distinctive feature of this complex thalloid liverwort (Kashyap, 1914). He had intensively worked on some other species like *Anthoceros indica*, *Notothylas indica* and *N. levieri.* Mahabale (1941) had worked in Khandala region of Maharashtra and described *Aspirometus dixianthus.* Chopra (1938, 1943) had published *Census of Indian hepatics* which listed 628 species and 114 genera belonging to 18 families. Ahmad (1942) had described 3 new species of *Riccia* namely *Riccci amangalorica*, *R. gangetica* and *R.orientalis* from Mangalore, Uttar Pradesh and Kumaon Himalayas respectively. A detailed study on the morphology of *Anthoceros crispulus* was carried out by Bhardwaj (1950, 1960). Bapna had reported 24 species of liverworts from Rajasthan (Mount Abu). Udar and Srivastava (1975) had taxonomically described *Trichocolea tomentella* and *Notoscyphus lutescens* collected from the Eastern Himalayas. Udar and Singh (1979a and 1979b) had recorded *Notothylas dissecta* Steph as new record for India. Lal and Parihar (1979) recorded *Frullania muscicola* var *Inuera* as a new record for the country as well he also recorded 27 liverworts from Amarkantak (Madhya Pradesh). Nath and Asthana (1998) had given the taxonomic description and distribution of 12 species of *Frullania* collected from Southern India. Bapna and Kachroo (2000) had described liverworts and hornworts of India in the form of a two volume work

–*Hepaticology of India*. Singh (1999) had given the status of diversity as well as the status of endemic and threatened liverworts of India. Negi (2001) had reported 19 liverworts dwelling on soil from Chopta-Tunganath region of Garhwal. Singh and Singh (2003) had recorded *Conocephalum japonicum* (Thumb) Grolle in India. Singh *et al* (2004) had recorded two liverworts belonging to family Porellceae *(Porella perrottetiana)* andPelliaceae *(Pellia necsiana).* Srivastava and Alam (2005) had reported a new family Scapaniaceae from Southern India. Asthana *et al.* (2005) had recorded *Phaeoceros kashyappi* Asthana Griv. as new record from the Eastern Himalayas. Nath and Singh (2006) had recorded *Frullania udarii* sp. as new record for Meghalaya. Daniels (2010) had listed 211 species of liverworts belonging to 56 (32 familes) genera and 8 species of hornworts belonging to 4 (2 families) genera from Tamil Nadu. Dey & Singh (2012) had worked on the Epiphyllous liverworts of Eastern Himalayas. Schwarz U. (2013) had documented the bryophyte diversity of Karnataka district comprising of a total of 338 bryophytes species that included by collecting secondary data from different sources.9 hornworts ,116 liverworts and 213 mosses. Singh and Kumar (2016) had recorded 101 taxa including 100 liverworts and 1 hornworts from North east region of India. They had also explored the liverwort and Hornwort flora of Jharkhand district recording 9 species of liverworts and single species of hornworts in the state for the first time. Asthana *et al*. (2015, 2016, 2019) has recorded many new species from India that included *Lejeunea minutiloba* A. Evans, *Archidium acanthophyllum* Snider and *Cololejeunea* (Spruce) Schiffn from various parts of India. Lavate (2020) had reported a total 42 taxa of bryophytes that included 32 species of liverworts and 10 species of hornwort. He also highlighted the threats to bryophyte diversity and their conservation measures.

2.1.3.2 Mosses

Major contribution in the early days was by Dixon (1914), who had recorded 40 genera and 50 species collected from South India and Ceylon. This also included three new species and 2 new varieties {*Stereophyllum ligulatum* C. Muell A. Jaeger var *sedgewickii* Broth & Dixon and *Lievierella fabroniaceae* var. *dilatinerve* Cardot. & Dixon}. Dixon (1921) recorded 43 species of mosses belonging 27 genera and 14 families collected from North Kanara. Dixon (1937) had reported 208 species of mosses from Naga hills of Assam.

Gangulee (1959, 1960, 1961) had described different families like Ditrichaceae (1959), Dicranaceae (1960) and Leucobryaceae (1961) in the form of papers on Mosses of Eastern India. Later on Gangulee (1969-80) had compiled his work in the form of a monumental three volume flora viz. *Mosses of Eastern India and adjacent regions - A monograph* where he had given the taxonomic description as well as distribution of different mosses. Foreau (1961) had recorded about 368 species of mosses that included 95 new species and 15 varieties from Palni hills. Raghavan and Wadhwa (1968,1970) had listed 21 genera and 28 species of bryophytes belonging to 16 families from Agumbe-Hulical ranges in the Shimoga district of Karnataka as well as 2 new records for India viz *Bryosedgwickia densa* and *Dendropogonella rufescens.* Dabhade (1969, 1970) had carried out detailed taxonomic study on the taxonomic on 4 species of *Bryum* as well recorded *Funaria nutans* from Khanadla for the first time in Western India. Chopra (1975) had described about 329 genera and 2000 species belonging to 56 families in the form of book viz. *Taxonomy of Indian Mosses*. It included the species collected from different parts of neighbouring countries like Pakistan, Nepal, Bhutan and Tibet.

Aziz & Vohra (2008) had provided monograph on Pottiaceae in India, wherein they had described 29 genera and 130 species. Daniels (2010) had listed 493 species of mosses belonging to 189 (44 families) genera from Tamil Nadu. Dandotiya *et al* (2011) compiled the available literature and listed 2489 taxa of bryophytes that included 675 species of liverworts belonging to 121 genera, 25 species of hornworts belonging to 6 genera and 1786 species of mosses belonging to 355 genera. Alam (2013) had compiled the work on the Western Himalayas reporting 745 species of mosses belonging to 230 genera. Asthana & Sahu (2013) had investigated the Mukteshwar region of Uttarakahand and had reported total 38 species. It comprised of 21 species of mosses from 20 genera, 15 species of liverworts from 10 genera and 2 species of hornwort from a single genus. Sahu & Asthana (2015) had worked on the diversity of bryophytes of Terai region of Uttar Pradesh. They had reported total 29 species of bryophytes consisted of 21 species of mosses, 6 species of liverworts and 2 species of hornworts. Bansal and Nath (2012) had recorded *Bryum coronatum* from Teele Tura hills of west Garo hills as a new record for the Meghalayas.

Magdum *et al*. (2017b) had listed the moss diversity of Western Ghats of Maharashtra constituted by 129 moss species belonging to 59 genera. Venugopal and Nair (2017) had documented the a total of 37 species of bryophytes from the Wayanad pass that included 7 species of liverworts, 2 species of hornworts and 28 species of mosses. Thamizharasi *et al*. (2018) had enumerated 55 species of mosses belonging to 36 genera and studied their phytosociological distribution of Kalrayan hills of Eastern Ghats located in Tamil Nadu. Benny & Mathew (2020) had recorded 28 species of bryophytes that included 17 species of mosses and 11 species of leafy liverworts. Sreenath *et al*. (2020) had documented new distributional record of three mosses viz. *Calymperes tenerumfrom*, *Hypopterygium tamarisci* and *Pterobryopsis acuminate* Andhra Pradesh.

2.1.4 Status of Bryophytes in Central India

In this chapter, Central India is deemed to be constituted by 6 states namely Gujarat, Rajasthan, Madhya pradesh, Chhattisgarh, Uttar Pradesh and some parts of Maharashtra (Northern region). Hence, the bryological investigation and documentation carried out in these regions are discussed below. Several bryologist who worked on bryoflora of the neighboring states like Rajasthan, Maharashtra and Madhya Pradesh viz. Chaudhary & Deora (1993) had studies the moss diversity of Rajasthan wherein they had reported 27 genera of mosses belonging to 7 families. Chaudhary *et al*. (2008) had recorded 6 species of liverworts belonging to 6 families, 4 species of hornworts belonging to 3 families and 34 species of liverworts belonging to 17 families from North Konkan region of Maharashtra. Nath *et al*. (2007) had listed 28 species of mosses falling under 21 genera and 12 families from Amarkantha, Madhya Pradesh.Alam *et al*. (2011) had explored the Ranthambore Tiger Reserve (Rajasthan-Central India) and had reported 13 species of liverworts falling under 7 genera belonging to 6 families comprised of single species of leafy liverwort and 12 species of thalloid liverworts. Nath *et al*. (2011a) had studies 22 members of Pottiaceae from Central India and provided illustrated account of 8 species from Panchmarhi Biosphere Reserve. Nath *et al*. (2011b) had worked on the genus *Fissidens* from Panchmarhi Biosphere Reserve of Madhya Pradesh State. They had reported 6 species of *Fissidens* consisting of one epiphytic species. Alam *et al*. (2014) had reported the 46 mosses species belonging to 30 genera and 12 families from the Mount Abu, Rajasthan. Bansal and Nath

(2014) had pursued research on the status of *Bryum* in Rajasthan and had listed 15 species of *Bryum.* Alam *et al*. (2015) had listed the mosses recorded in different parts of Central that included Gujarat, Southern Rajasthan, Madhya Pradesh, Chhattisgarh and Jharkhand. They had listed 210 species of mosses falling in 94 genera (30 families). Rawat *et al*. (2015) had described 51 species of mosses that falling under 27 genera and 13 families from Rajasthan and Punjab plains. Gupta *et al*. (2017) had recorded a new species of *Fissidens* from Panchmarhi Biosphere Reserve (Madhya Pradesh). Apart from above mentioned literature, there were several other researchers that were carried out in central Gujarat viz. Bapna (1975), Bapna (1980), Bapna & Chaudhary (1989), Deora & Chaudhary (1996), Chaudhary & Sharma (2002), Nath *et al*. (2005), Nath & Bansal (2009), Nath & Gupta (2009), Kapoor *et al*. (2011), Nath *et al*. (2012), Nath *et al*. (2013), Gupta *et al*. (2013), Alam *et al*. (2014), Bansal & Nath (2014), Bansal *et al*. (2012), Alam *et al*. (2015). Gupta *et al*. (2016) had described 4 species of genus *Brachymenium*viz. *Brachymenium bryoides, Brachymenium sikkimense, Brachymenium acuminatum* and *Brachymenium ptychothecium*recorded from Pachmarhi Wildlife Sanctuary (Madhya Pradesh). Gupta *et al*. (2018) had documented the richness of family Hypnaceae at the Panchmarhi Sanctuary (India) reporting 12 species of mosses belonging to 7 genera. Magdum *et al*. (2017a) had explored the Panhalgad hill station in Western Ghats of Mahara reported and recorded 9 corticolous mosses. Rana (2020) had described three species of *Physcomitrium* genus viz. *Physcomitrium cyathicarpum* Mitt., *Physcomitrium eurystomum* Sendtn. and *Physcomitrium japonicum* (Hedw.) Mitt recorded from Banswara district of Rajasthan.

2.1.5 Status of Bryophytes in Gujarat

In Gujarat, the work on bryophytes was initiated by Chavan and his pioneering works included Chavan, 1937a; Chavan, 1937b; Chavan and Mahabale, 1945; Chavan *et al*. 1961. Chavan initiated research in the field of bryology in Gujarat and reported *Cyathodium barodae* as a new species (Chavan, 1937b). He worked for his Ph.D. on bryophytes at the University of Nebraska, USA. Chavan and Mahable (1945) reported 19 species of Bryophytes that included 9 liverworts, 2 hornworts and 8 mosses. This work included the area of the Bombay Presidency of which Gujarat was a part. Patel (1977a) had reported *Riella affinis* which is the only aquatic bryophyte from Central Gujarat till date and apart from these Patel (1977b) also recorded *Riella cossoniana* as new record for the state. All these work constituted about 22 species of bryophytes recorded till the end of the twentieth century. The other important work included a book on the bryophytes of Gujarat by Chaudhary *et al*. (2006) wherein about 18 locations of the state were visited (Fig. 2.1& 2.2). In this book, total 67 species of bryophytes have been recorded that included 13 liverworts, 10 hornworts and 44 mosses. Only 11 species of mosses described were collected in the fertile state. Gujar and Shah (2019) had studied the diversity of bryophytes of Wilson hill and the surrounding area of Valsad district of Gujarat recording 15 species of bryophytes that included 5 liverworts, 2 hornworts and 8 mosses.

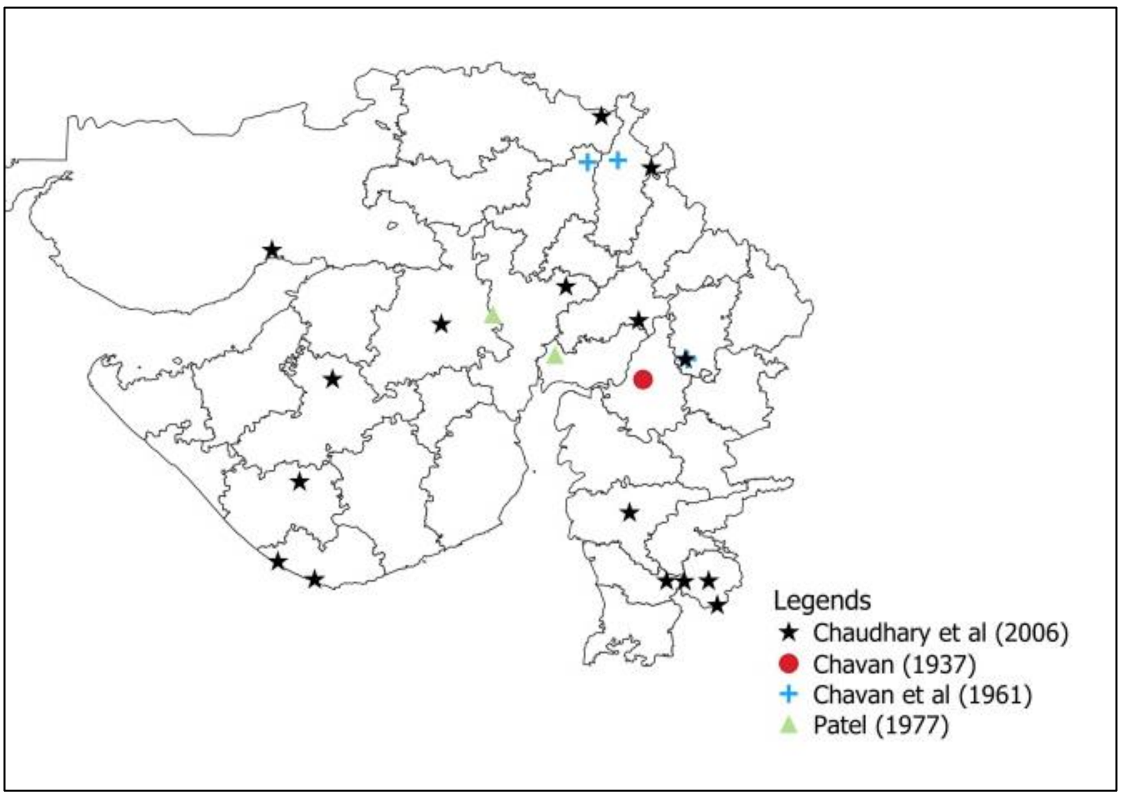

Fig 2.1: Figure showing the locations of Gujarat state visited by the Researchers in past study

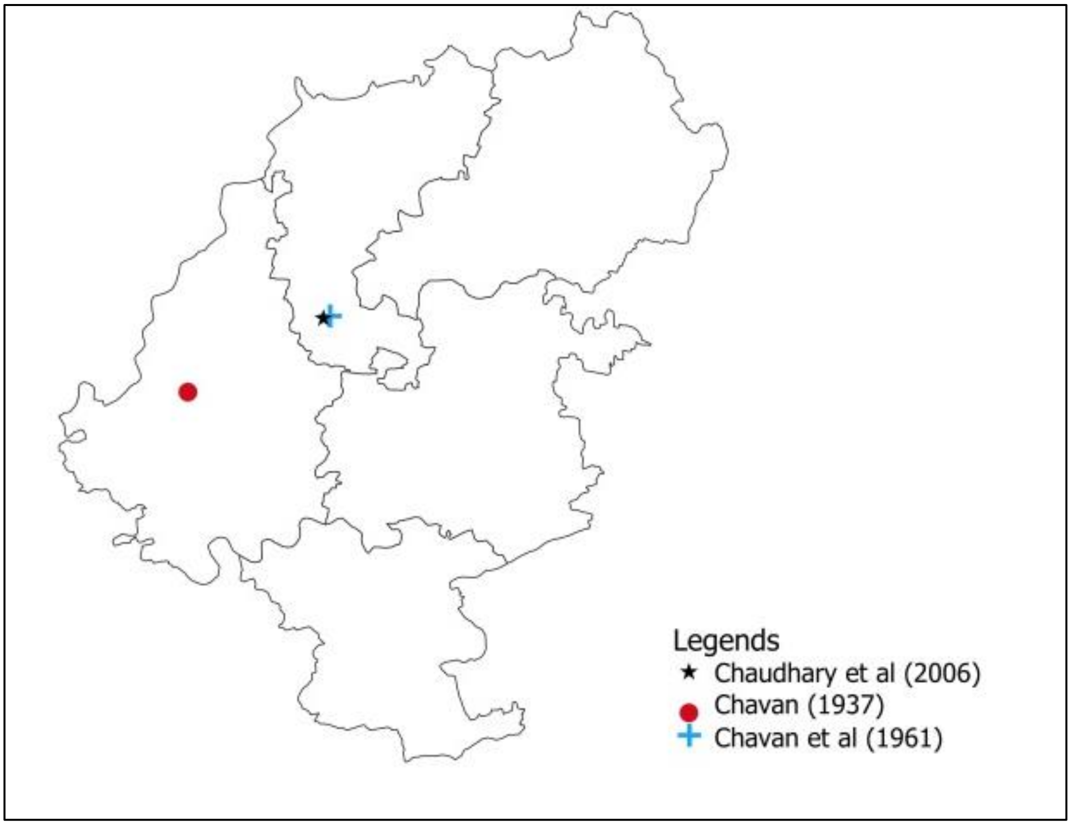

Fig 2.2: Figure showing the locations of study area state visited by the Researchers in past study

2.2 Culturing of Moss

2.2.1 Studies of Culturing Bryophytes and their important Parameters

The *in-vitro* culturing of plants was successfully employed first in mosses (Servettaz, 1913) though this techniques has been subsequently used extensively for vascular plants. *In-vitro* studies on bryophytes have been carried out due to their applications in physiological, genetic, morphogenetic, ecological and evolutionary studies (Sabovljevic *et al*., 2003). Apart from its importance in above studies it is also an important means of conservation of rare and endangered bryophytes species (Bijelovic *et al*., 2004; Sabovljevic *et al*., 2005). Bryophytes possess many bioactive compounds and this had gained attention of many phytochemists (Sabovljevic and Sabovljevic, 2008). Review of the past studies reveals that the mosses have been used as experimental models while the liverworts are the most preferred group for phytochemical studies (Cove *et al*., 1997, Asakawa, 1999, Thornton *et al*., 2005; Klavina *et al*., 2015**,** Deora and Suhalka, 2016, Muller *et al*., 2016; Williams *et al*., 2016, Bhadauriya *et al*., 2018). The first in-vitro studies on bryophytes was carried out in mosses (Servettaz, 1913). Many researchers (Sabovljevic *et al*., 2005; Asthana and Sahu, 2010; Zavala *et al*., 2012; Srivastava *et al*., 2018) had worked on the different combination of media, hormones, sugars etc. The methods employed differed with the type of species and the duration of culture and storage. Varying treatment of media, hormones, sugars etc. are provided to the explant depending upon the species as well as the objective of the study. The graphic below depicts the important components of a moss tissue culture experiment.

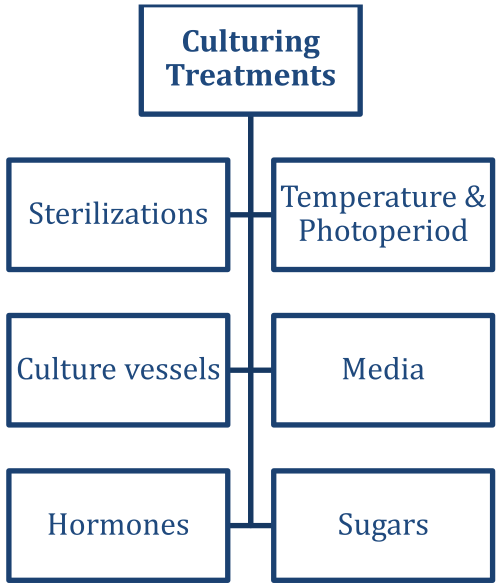

Fig 2.3: Important components of Moss tissue culture experiments.

2.2.1.1 Sterilization

The most crucial step in the *in-vitro* culture of mosses is sterilization. The success of *in-vitro* culture depends on the removal of the exogenous as well as endogenous contamination (Buckley and Reed, 1994). The surface of mosses is naturally contaminated as they are attached to the muddy substratum, which hinders the process of culturing by causing contamination i.e. by growth of fungi or bacteria. Thus, contamination by algae, fungi or bacteria is a serious problem in culturing of bryophytes (Shaw, 1986). Bryophytes especially mosses are a very delicate plant group, as the leaves of most of the species are single layered. Hence, the sterilizing agents should be used carefully as some sterilizing agents like combination of Tween reagent and sodium dichloroisocyanurate (NADCC) damages the tissue. Generally, the process of sterilization depends mainly on the plant species and the plant part used in culturing. But in mosses, there are a limited number of chemicals that have been used for sterilization. The most commonly used chemical are sodium hypocholorite ($NaOCl$) and mercuric chloride ($HgCl_2$). The various concentration of different sterilization agents including the most commonly used agent sodium hypocholoride are given below in table 2.1.

Table 2.1: Sterilizing agents and their concentrations

Sr. No	Sterilizing agent	Concentration (%)	References
1.	Sodium hypocholorite	0.01	Asthana *et al*., 2018
2.		0.5	Pandey *et al.,* 2014
3.		1	Sahu and Asthana, 2013; Sabovljevic *et al*., 2012 Pandey *et al*., 2014
4.		1.5	Silva-e-Costa *et al*.,2016; Silva *et al*., 2010
5.		2	Pandey *et al.,* 2014
6.		3	Sabovljevic *et al.*, 2012
7.		4	Pandey *et al.,* 2014, Sahu & Asthana, 2013
8.		5	Liang *et al.,* 2010, Sabovljevic *et al.*, 2012
9.		7	Sabovljevic *et al.*, 2012
10.		8	Pandey *et al.,* 2014
11.		10	Post *et al.*, 2016; Sabovljevic *et al.*, 2012
12.		13	Bijeleovic *et al*., 2004; Sabovljevic, 2002, 2005; 2010, Sabovljevic *et al.*, 2012
13.		15	Sabovljevic, 2010; Bijeleovic *et al.*, 2004; Sabovljevic, 2005
14.	Mercuric chloride	0.1	Chen *et al*., 2009; Liang *et al*., 2010
15.	Ethanol	10, 30, 50, 70	Sabovljevic *et al.*, 2012
16.	Sodium dichloroisocyanurate	1,3,5	Sabovljevic *et al.*, 2012

2.2.1.2 Temperature

Temperature control is an important factor for the growth of plants and for bryophytes evaporative cooling is important (Proctor, 2014). Bryophytes being C3 plant, their net photosynthetic rate is high at low temperature (Kallio and Heinonen, 1973). Even the tropical bryophytes grow best at comparatively low temperature than the tracheophytes. It is because beyond the optimum temperature they become dormant due to reversible depression of photosynthesis (Weis *et al.*, 1986). In case of bryophytes, the temperature refers to the temperature of the microclimatic condition in which it grows and not the external climatic temperature (Gabriel, 2000). The temperature for the initiation of the growth is generally between 21-27 °C (Chopra and Rawat, 1977; Pressel *et al.*, 2007; Silva *et al.*, 2010, Bijeleovic *et al.*, 2004; Sabovljevic, 2005, Sabovljevic *et al.*, 2002, 2010; Pandey *et al.*, 2014, Awasthi *et al.*, 2012a, Awasthi *et al.*, 2012b; Post *et al.*, 2016, Sahu and Asthana, 2018). Chopra R. N and Rawat M.S. (1977) had studied the effect of temperature on the behaviour of different species belonging to family Bryaceae viz. *Bryum klinggraeffi* Schimp., *Bryum coronatum* Schwaegr. and *Leptobryum pyriforme* (L.) Schimp. Post *et al.* (2016) had studied growth of silvery moss (*Bryum argenteum*) at different temperatures (8.4-35.7°C). The optimum growth was recorded at a temperature 29.5°C. Gametophytes initiation was recorded at a 10 °C while it was maximum at 22.5 °C. Awasthi *et al* (2010) had carried out *in-vitro* studies on twelve bryophytes species wherein optimum gametangia production in *Funaria hygrometrica* Hedw. and *Bryum coronatum* was observed between 18-20°C while the rest of the species produced asexual bodies like gemma optimally at temperature ± 25°C.

2.2.1.3 Light

Bryophytes require light for the photosynthesis and in its absence growth is limited (Tixier, 1979). The period of light and darkness is also important factor in the growth of bryophytes. In almost all the *in-vitro* culturing experiments referred to the photoperiod was 16/8 day/light (Bijeleovic *et al.*, 2004; Sabovljevic *et al.*, 2005; Sabovljevic *et al.*, 2002, 2010; Pandey *et al.*, 2014) while only one used a 12/12 day/light (Silva *et al.*, 2010). The optimum intensity of light used is mostly in the range of 2000-8000 lux (Sahu and Asthana, 2013, 2018; Srivastava *et al.*, 2018). In *Funaria hygrometrica*, total darkness caused the morphological abnormalities in capsule while relatively low light intensity decreased the abnormalities (French and Paollillo, 1976). Very few studies have carried out work on the role of light intensity, color, duration of exposure, quality and quantity and they are given below in table 2.2.

Table 2.2: Light intensities studies carried out by various researchers.

Sr.No	Light	Species	Reference
1.	4000-5000 lux	*Philonotis thwaitesii* Mitt. and *Brachythecium plumosum* (Hedw.) B.S.G.	Awasthi *et al.* (2012b)
2.	White, Red, Blue light or diffused day light	*Anthoceros miyabeanus* spores	Wada *et al.*(1984)
3.	1400-1500 lux 2500-2800 lux 3000-3500 lux	*Pohlia ludwigii* (Spreng. Ex Schwagr. Broth	Sahu & Asthana (2018)

2.2.1.4 Culture vessels

In most cases, petri plate is widely used for the culturing of the moss as it is easy to calculate its colony diameter or its growth in it and it also provides large surface area for the proliferation of the protonema. Most of the researchers have carried out the experiments in petri plates *viz* Cove *et al* (2009), Chen *et al* (2009), Sahu and Asthana (2013), Post *et al.* (2016), Bijelovic *et al.* (2004), Pandey *et al.* (2014) etc. However, Silva e costa *et al* (2016) had used test tube while Silva *et al* (2010) have used conical flask. For experimenting with petri plate, 90 mm diameter petri plate is considered the most ideal one for culturing of bryophytes (Duckett *et al.*, 2004). Transparent petri plate makes the monitoring of the moss growth easy. This petri plates can be covered with paraffin tape to avoiding contamination.

2.2.1.5 Media

Since early 1900's *in-vitro* culturing of bryophytes have been attempted using defined media (Beneke, 1903; Marchal and Marchal, 1906). Commonly used media are Knop media (Cano *et al.*,1996; Chen *et al.*, 2009; Liang *et al.*, 2010, Awasthi *et al.*, 2012a, Sahu and Asthana, 2013, Silva-e-Costa *et al.*, 2016, Sahu and Asthana, 2018; Srivastava *et al.*, 2018), MS media (Post *et al.*, 2016, Sabovljevic *et al.*, 2002, 2010, Bijelovic *et al.*, 2004, Sabovljevics(2005), Pandey *et al.*, 2014) and Hoagland's media (Asthana *et al.*, 2018). Other less known media like White media Chen *et al.* (2009), Parkers nutrient (Pressel *et al.*, 2007), BCD (Liang *et al.*, 2010), Beneke (Liang *et al.*, 2010) have also been used. Change in the concentrations of the media or the concentration of any single elements in the medium can bring about changes in the growth pattern of species (Bopp, 1983). Full strength of nutrient medium generally favours the formation of protonemal gemma while the in its absence tubers are more readily formed (Goode *et al.*, 1993; Duckett *et al.*, 2001). Mosses can be easily experimented using the solidified media, hence in most studies the solidified media has been preferred. The most commonly used solidifying agent is agar, at least in India. However, some other agents like Gelrite or Phytagel (Pressel *et al.*, 2007) are also available which forms a gel at a temperature lower than agar or agarose. They are also almost transparent and it is easy to detect any contaminants or impurities. It can hence be used as a substitute to agar. They however, get liquefied over a long period of time due to changes in pH or loss in salts that are necessary for crosslinking and so are not much preferred gelling agent. Phytagel and Gelrite are mostly preferred for calcifuge bryophytes as well for the study of the endophytic fungus.(Duckett *et al.*, 2004). Wide use of solid media does not neglect the fact the mosses cannot grow in liquid media. In case of aquatic species liquid media is more preferred (Machlis and Doyle, 1962; Glime and Knop, 1986), also for the continuous culture (Simola, 1975; Rudolph, 1978, Kajita *et al.*, 1987) and for protoplast culture (Bopp, 1983).

The results of major experiments conducted on various bryophyte species by researchers using different media is summarized in table 2.3.

Table 2.3: Results of In-vitro studies on different species of bryophytes

Sr. No	**Media**	**Result**	**References**
1.	Parkers nutrient medium	Asexual reproduction by protonemal gemmae (32 species) was more common than the tubers (19 species) and bulbils (5 species). Morphologically all the gemmae are almost same except some of the endangered species *viz Bryum schleicheri* and	Pressel *et al.*, 2007

Sr. No	Media	Result	References
		B. radiculosum.	
2.	Murashige and Skoog and Knop medium	Authors had used modified knop medium, modified white medium and basic MS medium for the invitro culture of *Rhodobryum giganteum* as it one of the medicinally important plant of china. The study of the colony diameter revealed that the modified knop medium is best for protonemal culture, the modified white is best for gametophore formation and MS medium was good for branching of protonemata.	Chen *et al.,* 2009
3.	Murashige and Skoog Hoagland medium	Highest percentage and fast growth of *Brachymenium capitulatum* was obtained in Murashige and Skoog and Hoagland respectively	Asthana and Sahu, 2010
4.	Knop, BCD and Beneke	The protonemal multiplication and gametophore production in case of Knop liquid medium was better than Beneke and BCD	Liang *et al.*, 2010
5.	Murashige and Skoog	Optimum growth of *Marchantia polymorpha* L. ssp. *ruderalis* Bischl. & Boisselier was obtained in half strength of MS medium	Vujicic *et al.*, 2010
6.	Knop medium + Nitsch's trace elements	Optimum growth of two mosses *viz Philonotis thwaitesii* Mitt. and *Brachythecium plumosum* (Hedw.) B.S.G was recorded in in Knop medium containing Nitsch's trace elements with 10 ppm ferric citrate	Awasthi *et al.,* 2012b
7.	Murashige and Skoog	Optimum growth of *Bryum coronatum* of protonema and rhizoids was recorded in 1/4 MS medium containing 1.5% sucrose.	Pandey *et al.,* 2014
8.	Knop medium	Half strength knop macronutrients showed best for all the species of *Bryum* studied .ie *Bryum argenteum, B. billardieri, B. pallescens*	Asthana *et al.*, 2015
9.	Murashige and Skoog	The study revealed that the ability of the turf protection products to suppress the growth of the Silvery moss (*Bryum argenteum*) differs in spores and bulbils.	Post *et al.*, 2016

Sr. No	Media	Result	References
10.	Knop's nutrient solution	Spore germination and gametopyte development of the Brazilian hornworts species – *Notothylas vitalii* was studies in Knop's nutrient solution	Oliveira *et al.*, 2017
11.	Knops macronutrients, BAP, Hogland medium	The best results were obtained in Hoagland no 2 basal salt mixture forming dichotomously branched thalloid liverwort.	Asthana *et al.*, 2018

2.2.1.6 Hormones

In case of *in-vitro* studies on higher plants as well as bryophytes, hormones are produced internally as well as artificially supplemented (Beutelmann and Bauer, 1977). In case of *in-vitro*, the externally supplemented hormones play a key role in the development process. In bryophytes, the simple body organization as well the lack of major morphological adaptation limits the selection of the hormones to a few like auxins, gibberellins, cytokinin etc only (Christianson, 2000a). A review of their use in bryophyte culture experiments which is detailed in Table 2.4 shows that very few hormones have been in *in-vitro* cultures.

Table 2.4: List of hormones experimented by different researchers.

Sr. No	Hormone	Derivative	References
1.	Auxin	Indole-3-acetic acid	Bhatla and Chopra, 1981, Srivastava *et al.,* 2018
2.		Indole-3-butric acid	Bijelovic *et al.,* 2004, Decker *et al.,* 2006, Asthana *et al.,* 2018
3.		α- Naphthalene acetic acid	Goode *et al.*, 1993, Bijelovic *et al.,* 2004
4.	Cytokinin	6-benzylaminopurine or Kinetin	Chopra and Sood, 1973; Vashistha, 1987, Srivastava *et al.,* 2018, Asthana *et al.,*2018

Sr. No	Hormone	Derivative	References
5.		6-furfurylaminopurine	Bhatla and Chopra, 1981, Bijelovic *et al*, 2004
6.		6-Benzylaminopurine (BAP)	Bijelovic *et al.,* 2004, Asthana *et al.,*2018
7.	Gibberelins	GA3 & GA7	Bhatla & Chopra, 1981, Chopra and Kumra, 1984
8.	Abscisic acid (ABA)	Abscisic acid (ABA)	Bhatla and Chopra, 1981

2.2.1.7 Sugars

Sugars like glucose, fructose and sucrose are important for regulation various metabolic processes of plants including bryophytes (Hassid and Putman, 1950, Sabovljevic *et al.,*2005). , Research suggest that they are essential signaling molecules of plant development and especially during the abiotic stress (Smeekens, 2000, Loreti *et al.,*2001). They are essential for the physiological development as well for other metabolic processes (Gibson, 2004; Smeekens, 2000). The change in the solubility level and the type of sugars affect different developmental as well the signaling processes. Two members of bryaceae *vizBryum argenteum* and *Atricum undulatum*have been tested for the effect of three sugars namely glucose (hexose), fructose (hexose) and sucrose (dissachharide). Results showed that all the sugars i.e. sucrose, fructose and glucose had positive effect on the growth and development of protonema of *Bryum argenteum*, but in case of *Atricum undulatum* it affected negatively (Sabovljevic*s et al.,* 2005). Optimum growth of *Marchantia polymorpha* L. ssp. *ruderalis* Bischl. & Boisselier was found in 1.5% concentration of sucrose (Vujicic *et al*, 2010). Sucrose had increased the germination rate of the *Entosthodon hungaricus* (Funariaceae) (Sabovljevic*s et al.,* 2012).The addition of sugars as a carbon sources is usually not necessary in agar cultures except to accelerate growth and development or for use in experiments without light (Bopp and Knoop, 1984).

2.3 DNA Barcoding of Bryophytes

2.3.1 Classical taxonomy vs Molecular studies

Naming of organisms and their method of classification is an ancient science (Kranz *et al*, 1994). The main goals of taxonomy are to identify, describe and classify the species based on their natural relationship. In the 350 BC to 287 BC, Theophratus had described the morphological characters of the herbs, shrub and trees in his book "*Historia plantarum* (Negbi, 1989; Ogilvie, 2003). Earlier the system of classification was descriptive and was difficult to follow. To solve this difficulty, Carl Linnaeus proposed the hierarchical system of classification that formed the base of the modern taxonomy. In the 18th century, Linnaeus proposed the binomial system of nomenclature (Reid, 2009). Classical taxonomy uses the morphological and anatomical methods for identification and classification of organisms (Narendran, 2008). On the other hand, molecular taxonomy uses the genetic sequence for the identification and classification of species (Alexander *et al*, 2015). Some of the morphological or pheonotypic characters used in classical taxonomy are variable as they change with the change in the climatic condition, hence cannot be totally relied upon it for the identification of the species. Contrary to it, genetic sequence used in the molecular taxonomy is highly stable, therefore can be used as tool for identification.

2.3.2 Methods of molecular taxonomy

There are various methods used in molecular taxonomy and all uses the genetic sequence as the basis of identification (Alexander *et al*, 2015) (Table 2.5). Molecular studies can be carried out using molecular markers either for DNA or RNA. Two type of methods are included in DNA based study that involved, one is multi locus system or second is single or oligo-locus system of DNA fingerprinting.

Apart from DNA, r RNA (ribosomal RNA) is also sued in the molecular identification (Fox *et al*, 1980; Woese and Fox, 1977). There are several other approaches for achieving the above target like using different restriction endonuclease sites genetic loci, repetitive DNA method and gene sequence methods (viz. MLSA, SNP typing). Most of these methods are used in identification of species at strain-level However, for species level identification multilocus sequence analysis (MLSA) has been widely used technique (Maiden, 2006).

Table 2.5: Methods in DNA based studies.

Sr. No.	Single or Oligo Locus	Multi-locus
1.	Simple sequence repeat PCR (ssr PCR)	Restriction fragment length polymorphism (RFLP)
2.	Polymorphic sequence (CAPS)	Randomly amplified polymorphic DNA (RAPD)
3.	Single nucleotide polymorphism (SNP's)	Amplified fragment length polymorphism (AFLP)

2.3.3 Molecular studies in bryophytes

The molecular studies in bryophytes are carried out to determine the evolution of land plants and second for the identification of species. The study of origin of evolutionary sequence of land plants have been prime interest of evolutionary biologists. The arrival of land plants about 480 million year ago was an important phenomenon in the history of earth. Bryophytes are considered to be the first plants to colonise the land. With the passage of the time they gained several adaptations that helped them to survive in terrestrial environment that included alternation of generation, special reproductive structures, desiccation tolerance capacity etc (Renzaglia *et al.*, 2000). Phylogentic studies on the different groups of bryophytes had proposed that the liverworts are sister to all land plants while the mosses or hornworts are sisters to the vascular plants (Mishler and Churchill, 1984; Lewis *et al.*, 1997). It is also supported by the paleonotological studies wherein spores of bryophytes (especially liverworts and hornworts) were recorded in Sirulian, upper Devonian and upper Cretaceous periods (Taylor, 1995; Richardson, 1985; Hueber, 1961; Nemejc and Pacltova, 1974).

Identification of bryophytes using molecular biology has been proposed as an easy and accurate method of identificationas its identification is difficult due to its small size, undistinguishable morphological characters, as well as sometimes absence of sporophytes adds to the difficulty of identification of bryophytes (Bergamini and Peintinger, 2002; Hassel *et al*., 2005). Moreover, traditional method of identification is sometimes misleading as they based on morphological characters that show variation due to fluctuations in the environmental conditions. Hence, such methods were considered less reliable. In such situation, DNA barcoding method of identification of species has been proposed as a useful tool.

2.3.4 DNA barcoding as identification tool

DNA barcoding is a method of identification of any species by extracting a sequence of DNA from any tissue of an organism (Kress and Erickson, 2012). The use of DNA for the identification of species was first proposed by Paul Hebert and its colleagues (Hebert *et al*., 2003, 2004). It was proposed as a quick and reliable method of species level identification for animals, plants and microorganisms. The most common gene region for plants were three plastids (rbcL, mat k, trnH-psbA) and one nuclear (ITS) were discovered. (CBOL, 2009; Kress *et al.*, 2009; Schoch *et al.*, 2012).

The technique of DNA barcoding is broadly used in the following cases:

- For systemic identification of unknown species
- To measure the genetic variability in the species
- For identification and purification of the biological products like herbal medicine, seafood etc.
- Identification of cryptic species
- In ecological and evolutionary studied to understand community succession and assemblage.

The DNA barcoding broadly involves two steps

1) Building the barcode library of identified species

2) Matching the unknown barcode sequence with the sequence available in the library. The sequence if matched, the species is identified and if not than the sample is identified by other sources and then added to the library of sequence with new identity. In the first step the sample is either obtained from the voucher specimen or from fresh from field.

Gene region of the DNA barcode should meet the following criteria to give the best and accurate results:

- Should have good length of conserved region
- Should possess species level genetic variability
- Suitable length of sequence for extraction and sequencing.

The use of specific sequences used in the identification and barcoding of different bryophyte species by different authors was analysed. The table 2.6 below summarises this information.

Table 2.6: List of different primers used in the molecular studies of Bryophytes.

Sr. No	Species (Family)	Plant part used	Extraction protocol (Ref) /Kit	Primers/Locus	Reference
Liverworts and Hornworts					
1.	19 species (including algae, bryophyte, pteridophyte, gymnosperms and angiosperms)	Not specified	CTAB (Doyle & Doyle, 1990)	trnT , trnL ,	Taberlet, 1991
2.	*Jensenia*spp. (5 species), *Pallavicinia* spp. (4 species), *Podomitrium phyllanthus, Symphyogyna marginata*.	Not specified.	Manual method (Forrest & Crandall –Stotler, 2004,2005)	rbcL, rps4, psbA, trnT-trnF, atpB-rbcL, LSU, nad5	Forrest *et al.*, 2005
3.	*Herbertus* sp (Herbertaceae)	Vegetative	Invisorb spin plant minikit (Invitek,	ITS1, ITS2, 5-8S, trnL-trnF	Feldberg *et al.*, 2007

Sr. No	Species (Family)	Plant part used	Extraction protocol (Ref) /Kit	Primers/Locus	Reference
		part	Berlin, Germany)		
4.	*Porella* sp., *Ascidiota* sp, *Macvicaria* sp	Shoot	Invisorb spin plant minikit (Invitek, Berlin, Germany)	rbcL, trnT-trnF, nrITS	Hentschel *et al*, 2007
5.	*Plagiochasma appendiculatum*	Thalli	CTAB (Doyle & Doyle, 1990)	RAPD (60 markers used)	Soni *et al.,* 2009
6.	*Plagiochasma appendiculatum*	Thalli	CTAB (Doyle & Doyle, 1990)	RAPD (OPA, OPB & OPD)	Soni & Kumar, 2009
7.	9 species of liverworts	Not specified	Plant DNA genomic kit (TiaGen, Beijing, China)	atpF-atpH, ITS2, matK, psbK-psbI,rbcL,rpoB, rpoC1,trnH-psbA,rps4, trnL-trnF	Liu *et al.,* 2010
8.	*Herbertus* sp (Herbertaceae)	Stem tips fried in Silica gel	Qiagen DNeasy minikits (Qiagen Ltd)	matK, rbcL, trnH-psbA, ITS	Bell *et al*,2012
Mosses					
9.	4 orders (48 taxa) Bryales, Leucodontales, Hypnales and Hookeriales	Not specified	Manual CTAB (Rogers & Bendich) Wizard DNA clean up kit (Promega) was also used	rbcL, rps4, trnL-trnF, 18S	Cox *et al*, 2000
10.	*Pulchrinodus inflatus* (Family-Pterobryaceae)	Not specified	Doyle & Doyle (1990)	trnL-F, psbT-H, ITS-2	Stech *et al*, 2003
11.	*Totula subulata* (Pottiaceae)	Not specified	NaoH Method ((Werner, Ros & Guerra, 2002b)	18S and ITS4	Cano *et al.*, 2005
12.	*Tylimanthus* sp	Not specified	Mixer mill MM200 (Retsch) and NucleoSpin Plant Extraction Kit (Macherey-Nagel)	trnL , -trnF.	Stech *et al.*, 2006
13.	9 Mosses (*Amphidium mougeoti, Breutelia chrysocoma, Bryum*	Shoot and Leaf	Small fragments of shoot and leaf (<10mg) were taken	trnL-F, trnG, ITS	Pedersen *et al.*, 2006

Sr. No	Species (Family)	Plant part used	Extraction protocol (Ref) /Kit	Primers/Locus	Reference
	bicolor, Ctenidium molluscum, Dicranum scoparium, Garovaglia elegans, Garovaglia powellii, Homalothecium sericeum, Rhytidiadelphus squarrosus) 1 Liverwort (*Herbertus aduncus*).		and Liquid nitrogen was added and then crushed in micropestle. Then, diluted in Tris buffer (10M) and incubated for 15 min at 60°C.The sample was centrifugated and then the supernatant was used for further analysis.		
14.	85 moss species belonging to Bryidae, Dicranidae, Timmidae, Funariidae, Encalyptidae, Diphysciidae classes.	Not specified.	Mixer mill MM200 (Retsch) followed by Doyle & Doyle (1990)	trnLatpB-rbcL spacer, psbA-trnH	Stech and Frey, 2008
15.	50 mosses belonging to Sphagnales, Takakiales, Andreales, Polytrichales, Tetraphidales etc	Not specified	Plant DNeasy kit (Qiagen, Hilden, Germany)	nad5-nad4,nad5,nad2, rbcL,rps4	Wahrmund *et al*., 2009
16.	49 species of mosses & 9 species of liverworts	Not specified	Plant DNA genomic kit (TianGen, Beijing, China)	atpF-atpH, ITS2,matK, psbK-psbI,rbcL,rpoB, rpoC1,trnH-psbA,rps4, trnL-trnF	Liu *et al*., 2010
17.	Racomitrium spp.(13 species), Coscinodon sp. (1 species),Grimmia spp. (5 species), Schistidium spp. (1 species).(Family-Grimmiaceae)	Not specified	Plant DNA genomic kit (TianGen, Beijing, China)	trnH-psbA, rps4, rbcL-a, trnL	Liu *et al*., 2011
18.	*Racomitrium canescens*		DNeasyH Plant Kit (Qiagen) or the NucleoSpin H Plant II kit (Macherey	rps4-trnT, trnT-trnL, ITS1, ITS2	Stech *et al*., 2014

Sr. No	Species (Family)	Plant part used	Extraction protocol (Ref) /Kit	Primers/Locus	Reference
			Nagel)s		
19.	*Dicranum* spp(16 species) *(*Dicranaceae*)*		Nucleospin plant II kit	rpoB, trnHGuGpsbA, rps19-rp12, trnS-F, nrITS1, 5-8 S, ITS2	Lang *et al*., 2014
20.	*Dicranum* (27 species) *(*Dicranaceae*)*	Shoot	Nucleospin plant II kit (Macherey-Nagel, Duren, Germany)	Rps4-trnT, trnl-trnF,trnH-psbA, rps19-rpl2.	Lang *et al*., 2015
21.	*Schistidium* sp		Qiagen DNeasy minikits (Qiagen Ltd)	matK, rbcL, trnH-psbA, ITS2	Hofbauer *et al.,* 2016

An analysis of the above literature shows that *rbcL*, *mat*k, *trn*H-*psb*A and ITS2 were the four most widely used makers in bryology.

2.3.5 BOLD system- a molecular database

After the identification of the sample with the help of DNA barcoding, the identified sample can be submitted to the Barcode of life data system (BOLD) for future reference. Barcode of life data system is an online platform with DNA bardcode database freely available for the scientific community (Ratnasingham and Hebert, 2007). It allows free database search of about more than 1.7 m records via Public data portal system across the globe (http://v4.boldsystems.org/). Some part of the database is made freely available to all the individual. After the submission of data in bold, a barcode is generated for the submitted species which is unique to each submission. The submission of data in bold is free however acquisition of final barcode requires some preliminary data, only after which the barcode status is gained. Following data are needed for the submission in bold system (Rantnasingham and Hebert, 2007):

- Name of species
- Catalogue number and Name of Institution sample deposited

- Name of collector, Date of collection and GPS locations
- Name of Identifier
- COI sequence
- Name of Primers used
- Trace files

Bold database of bryophytes is very weak in India and in Gujarat as very few species of bryophytes are submitted in the BOLD system. The following table 2.7 shows the status of bryophytes records and species across World, India and Gujarat.

Table 2.7: Status of bryophytes records and species across World, India and Gujarat.

Groups	World		India		Gujarat	
	Records	**Species**	**Records**	**Species**	**Records**	**Species**
Marchantiopsida	8113	2128	25	15	4	0
Anthecerotopsida	257	101	0	0	0	0
Bryopsida	16380	3288	53	38	3	0

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

2.4 Computer Aided Identification.

The Identification of species is an important task in the any biodiversity study which is not only a difficult but also needs expertise. Also plant identification is not exclusively the job of botanists and plant ecologists. It is required or useful for large parts of society, from professionals to the general public. But the identification of plants by conventional means is difficult, time consuming, and frustrating for beginners (Waldchen *et al*., 2018). To aid this process of identification of species, scientists started working with computer technology to develop tools or softwares for species identification (Waldchen *et al*., 2018; Kaur and Kaur, 2019). The development of computer aided identification tool is one of the most important break through for scientific studies of the life sciences. Contrary to the conventional dichotomous key, computer aided identification involves use of computer and its programs for the species identification (Ayob and Kadir, 2016). The characters in a dichotomous key appear in a definite order and so comparison of more than one characters at time is not possible, making the identification process difficult and strenuous (Cerretti *et* *al*., 2012). Due to the development of science and technology several softwares have been designed and are used for the identification of organism in various disciplines. There are many researchers across the world as well as in India that have developed Computer aided identification tool for the identifying different taxa. These include various softwares like Delta (Dallwitz, 1993), Dkey (Tofilski, 2018) etc. that are used for the generation of such identification modules. Many of them are free like Dkey (Tofilski, 2018), Delta (Dallwitz, 1997), Xper2 (Ung *et al*., 2010), Xper3 (Vignes Lebbe *et al*., 2015) etc while some like Lucid (Bittirich *et al*., 2012) and IPEZ (Fischer, 2013) are paid ones.

The use of computers for the identification in the field of taxonomy began in early sixties. Boughey *et al*. (1968) and Morse (1974) were pioneers in preparing interactive keys as an identification tool. In 1984, a program named XPER was developed by Lebbe (1984) for the identification of mushroom and toadstools. Zeltyn and Pchelkin (1993) had prepared a tool for the identification of lichens of North-Western and Central Asia. A similar tool for fungus and selected genera of lichens of North western United states was developed (Pasarell, 1999). Delta key has been used as interactive identification key for various groups of plants like flowering plants (Watson and Dallwitz, 1992b), gymosperms (Watson and Dallwitz, 2008), grass genera of world (Watson *et al*., 1992) etc. Prosperi *et al*. (2005) had developed a software for the identification of mangroves in the form of DVD. Du *et al*. (2006) had documented a new computer aided method of identification of plant species by matching the leaf shape of plants. Sasidharan (2006) developed tool called TreeID for the identification of trees of Kerala. Similarly, Ramesh *et al*. (2010) developed the same for the identification trees of Western Ghats.

Bittrich *et al*. (2012) had constructed an interactive key using Lucid 3.5 software for the identification of the angiosperms of the Amazon region. Herdiyeni *et al*. (2013) had developed a method for the identification of the medicinal plant which is computer based and uses Probabilistic Neural Network for identification using leaf architecture. Fischer (2013) had developed software named IPOFIS (Integrated photo-based online Fish identification System) and IPEZ for the identification of marine and fresh water fishes based on the morphological characters. Such interactive tools are not only used for the species identification but is also used for developing tools to study the anatomical variations in the plant part. Thomas and Franceschi (2013) had developed tool to identify the palm stem based on the anatomical differences. This had helped the non-taxonomic field researchers like archeologist and paleontologist in their research.

Valliammal (2014) had developed a software for the identification of plants that used the leaf recognition technique for the identification. Ramasubbu *et al*. (2015) had used GRIBIOTAX software for the identication of families of angiosperms of South India with the help of characteristic feature of the particular plant/family. Dellinger-Johnston (2015) had developed a visual interactive key for the identification of *Quercus* species constructed using the standardized photographs. Vorontsova *et al*. (2015) had developed a database of about 11, 290 grasses of the world using Delta software. Zuquim *et al*. (2017) had developed a free online database for the identification of the ferns of Amazon using the Delta software.

Ayob and Khadir (2016) had developed computer aided software for the identification of the invasive lady birds. Attigala *et al*., (2016) had web based interactive identification software called WEBikey for the identifying the members of the grasses family. Tofilski (2018) had introduced a new open source software 'DKey' that was used to develop computer aided identification tool in the taxonomic studies. A group of Antarctic biologists had recently developed a tool for the identification of the marine biodiversity based on computer technology (Saucede *et al*., 2020).

This technique was not just limited to plants but wasalso employed for the identification of insect taxa which had a very large diversity. Holzenthal *et al*. (2011) had constructed a key for the identification of different species of genera *Amphoropsyche* recorded from Eucador using the Delta software. Similarly, Sharkey *et al*. 2011 prepared description, dichotomous key and interactive key of the species falling under genera *Lytopylus* recorded from Costa Rica using the same software. Cerretti *et al*. (2012) had developed MOSCH-web based interactive identification tool for the genera of family tachinidae belonging to order Diptera of Palaearctic region. Baena-Bajarano and Heads (2015) had used delta software for describing the new species belonging to genera *Ripipteryx* recorded from Columbia. Yang *et* *al*. (2015) developed a tool called DAIIS (Developing an Automatic Insect Identification System) for identification of insects with the help of wing outline.

Waldchen *et al.* (2018) recently reviewed the trends and future trajectories of automated plant identification. They discussed the use of several techniques like artificial neural networts (ANN) and machine learning for plant identification. They concluded that the identification of species with minimal input from users and in which the extensive use of image data and its comparison with image databases would be the trend in the future. In another review Waldchen and Mader (2018) focused on the use of computer imaging and analysis of different plant parts in the identification of species. Their emphasis again was on the identification of plants by non-botanists or non-taxonomists.

The Delta system which is an integrated set of programs is a very powerful and flexible system for recording taxonomic descriptions and it's processing by computer programmes (Dallwitz, 2018).The system has been used to generate descriptions of large groups of plants like the Grass Genera of the world which incorporates about 800 genera using upto 500 characters (Watson and Dallwitz, 1992). It is also been accepted as a standard of data exchange by the 'Biodiversity Information Standards' (Dallwitz, 2018). The extensive use of the Delta system by different researchers as mentioned earlier and its universal acceptability had prompted the use of the Delta system in the current study