Imaged Based Morphological Study of Bryophytes and Green Algae for Identifying Evolutionary Relationship between Them

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Abstract
It is well-known that all plants of the land evolved from a green algal ancestor. Bryophytes represent the first step in the evolution of plants from algae and also it is the first step in the transition of multicellular autotrophic eukaryotes from water onto the land. The latest phylogenetic studies prove that the bryophytes as a group share a common ancestor with green algae. Also, the DNA-study supports this close evolutionary relationship between bryophytes and green algae. A few other studies such as cell wall elongation, origin of cuticle etc. establish that both bryophytes and algae share a common ancestor. This paper presents the findings of our latest image based morphological studies on evolutionary relationship between bryophytes and green algae based on their morphologies. Features used in this study include compactness, area, perimeter and number of serrations of sample images of thallus of these two species.

Keywords
Processing, Bryophyte, Algae, C Value

I. Introduction
Relationships among the green algae and bryophytes remain one of the major unresolved questions in plant evolutionary biology. Green algae belong to the phylum Chlorophyta. They are unicellular, colonial, coenocytic, or multicellular photosynthetic in nature without leaf and stem like structure. Most species are aquatic, in both freshwater and marine habitats, although terrestrial forms are also known and include free-living species, as well as those symbiotic with lichens [1]. All land plants evolved from a green algal ancestor [2]. Bryophytes represent the first steps in the evolution of plants from algae, as well as the first steps in the transition of multicellular autotrophic eukaryotes from water onto the land. This transition and the pressures exerted on the evolving plants by life outside of the water, into the air, shaped every single aspect of the anatomy, morphology, development, and functioning of plants [3]. According to the latest phylogenetic studies, the bryophytes as a group share a common ancestor with green algae. The following figure shows the cladistic relationship between green algae with bryophytes.


The nature of the primitive bryophyte and the manner in which it originated is studied upon the comparative morphology and ontogeny of living bryophytes as there is no evidence of fossil bryophytes. Different evidences proved that bryophytes were originated from green algae i.e. Chlorophyceae because pigments chlorophylls and xanthophylls are present both in bryophytes and green algae Besides that, both green algae and primitive bryophytes i.e. liverworts shows the identical morphology i.e. both member contains filamentous thalli. It was generally thought that there was a migration of an alga from water to land and this was followed by an evolution of a bryophyte type of sexual plant [3]. The following evolutionary tree of bryophytes supports this view:

Fig. 2: Diagram Showing Interrelationship Between Liverworts and Green Algae

Cuticle is a protected layer which is common in vascular plants. The SEM and TEM studies on origin of cuticle of green algae and bryophytes revealed that both green algae and bryophytes diverged from a common ancestor. In angiosperms xyloglucan endotransglucosylase (XET)/hydrolase (XTH) is involved in reorganization of the cell wall during the growth and development. The location of oligo-xyloglucan transglycosylation activity and the
presence of XTH Expressed Sequence Tags (ESTs) in the earliest diverging extant plants, i.e. in bryophytes and algae were examined. The results provide information on the presence of an XET growth mechanism in bryophytes and algae and contribute to the understanding of the evolution of cell wall elongation in general [4]. In our present research work, we examined lot of images of Himalayan liverworts and green algal member Ulva sp. because Ulva shows similarity with liverworts. For example, multicellular thallus of Ulva has a sporic meiosis type of life cycle like bryophytes. The phylogenetic analysis has shown that the similar life cycles of Ulva and liverworts, these are the example of convergent evolution. Besides it, Mixed-linkage glucan (MLG) related polysaccharide is present in both Ulva and liverworts. We collected the images of Himalayan bryophytes from different region of Himalaya. The images of Ulva were collected from WWW. The rest of the paper is organized as follows: Part II describes the similarity of genome content among the members of algae and bryophytes, Part III depicts our image based study in details, Part IV describes the experimental result and Part V is the conclusion part of this paper.

II. C Value Paradox and Genome Study of Algae and Bryophytes

C value is used to refer to the haploid nuclear DNA content of a species. This genome size or C value is constant for a particular species but it varies from species to species. Accepting the genome size constancy within a species reflected the role of DNA as the hereditary substance. But comparative analysis of the genome size between different species shows some interesting facts. These are: some simple organisms have more DNA than complex ones, any given genome seems to contain much more DNA than would be needed to account for the predicted gene number, and some morphologically similar groups exhibit highly divergent DNA contents. These facts prove that DNA content does not correlate with the expected number of genes which is “paradoxical” in the sense that C values were presumed to be constant precisely because DNA is the stuff of genes. This is known as C value paradox [5]. For research and development purpose, plant C value database was developed in 1997. Till now, this database is growing and database version 2 contains C value of 3927 species including algae and bryophytes. Following are the histograms showing the distribution of DNA C values for 171 bryophytes and 85 green algae.

III. Image Based Study of Thallus of Bryophytes and Green Algae

Different stages of the proposed image analysis based method for finding the morphological similarities of liverworts and green algae are described below.

A. Collection of Sample Images

Bryophytes are abundant in hilly region. Himalayan regions are enriched with bryophytes. We have collected lot of images of liverworts from eastern as well as western Himalayan regions. The images of Ulva are collected from Internet.
B. Image Segmentation

General principle of image segmentation is to subdivide an input image based on sudden changes in gray values. Edges around the region of interest are computed to separate it from the remaining parts of the image. In the present work, to segment the region of thallus from the background in sample images, we have used watershed algorithm implemented in MatLab. In this process, an input image is subdivided into smaller parts until the required object i.e. thallus is isolated from its background. In Figure 5, a sample image of thallus and the result of its segmentation from the background are shown. Actually thalluses are densely distributed on the surface, some of which often may touch another thallus. So to facilitate the study of thallus, we used watershed algorithm, a special object partition algorithm, for separating single thallus from other neighboring thalluses.

![Sample Image of Liverwort and Its Segmentation](image)

Fig. 5: (a). Sample Image of Liverwort and (b). Its Segmentation

C. Preprocessing of Images

The boundary of the objects in the image is smoothed to eliminate possible noise. For this purpose, we considered morphological operations such as erosion and dilation. In fact, we first erode the segmented image and then dilated by using a 3 x 3 mask. Finally, we obtain the boundary of the thallus by the application of a non-linear convolutional filter, with two convolution kernels of the Sobel type.

D. Morphological Features of Thallus Considered in This Study

In this study, we considered images of thalluses of liverworts and Ulva. Thalluses (singular thallus) are multicellular organ where different types of growth such as cuticle, rhizoids etc. are present. Besides that, thallus also contains the sex organs. Normally, thalluses are green in color. In our study, following morphological features are computed from the thallus:

1. **Number of Serrations (N)**

   The thallus shows number of big and small ridges which are known as serrations. From our experimental result, we noticed that the number of serrations of a thallus shows distinct range. For calculation of number of serrations, a grid of specific size is imposed over the binary image of a thallus. For each small window, we calculate the pixel connectivity. If a small window contains a serration, then the pixel connectivity shows the value which is higher than a predefined threshold value. Otherwise it has a value which is smaller than the predefined threshold value.

   Area (A): Area is defined as the total set of pixels that constitute a Thallus. It can be calculated for the object region R as:

   \[ A = \int \int_R dx\, dy \]

   x and y in the above runs over horizontal and vertical axes respectively.

2. **Center of Gravity (m, n)**

   For a thallus surface described by the function \( f(m, n) \), consisting of N pixels, the coordinates of Center of Gravity \((\bar{m}, \bar{n})\) can be calculated as

   \[ \bar{m} = \frac{1}{N} \sum_{(m,n)\neq N} m \]

   \[ \bar{n} = \frac{1}{N} \sum_{(m,n)\neq N} n \]

3. **Compactness (CMP)**

   It is the following function involving the perimeter (thallus object) and Area.

   \[ CMP = 16.\frac{A}{(Perimeter)^2} \]

4. **Average Height/Average Width Ratio (HBWR) of Thallus**

   The algorithm for HBWR for an image is as follows:

   Divide image into several columns;
   While not End of Column
     Find height of the current column;
     Go to next column;
   End
   Calculate average height;

   Divide image into several rows;
   While not End of Row
     Find width of the current row;
     Go to next row;
   End
   Calculate average width;
   Calculate height/width;

   Tonal Distribution:

   Image Histogram gives the tonal distribution of an image. Here for a two-tone image, only logic 1 value (that represents the image) is considered.

   Acute Angle (AA) between lobs of Thallus : Those groove angles are <=90º.

   Obtuse angle (OA) between lobs of Thallus: Those groove angles are >90º.

   Groove Angles are calculated as follows:
Algorithms were developed in Windows environment (Windows XP) using MATLAB 7.1 programming language to extract the above mentioned morphological features of individual thallus from thallus pictures of liverworts and Ulva.

IV. Experimental Result

We performed our experiment on more than 150 images of Himalayan liverworts and more or less 80 images of Ulva. The number of serrations calculated for both liverworts and algae shows similarities. For example the following two figures depict this fact when 4x4 and 5x5 size of windows is used.

\[
\alpha = \frac{b}{\sin \alpha} = \frac{c}{\sin \beta} = \frac{d}{\sin \gamma},
\]

\[
\alpha = \arccos \left( \frac{h^2 + r^2 - a^2}{2bc} \right)
\]

\[
\beta = \arccos \left( \frac{a^2 + r^2 - h^2}{2ac} \right)
\]

\[
\gamma = \arccos \left( \frac{a^2 + h^2 - r^2}{2ab} \right)
\]

The compactness of thallus of liverworts shows that large number of thalluses is 40 to 90% compact in nature. It is more or less similar in case of Ulva. The following figure explains this fact.

![Chart showing compactness of thallus](chart.png)

Fig. 6: Number of Serrations Found in Ulva and Liverworts Using (a) 4x4 window and (b) 5x5 Window

We clustered both the images of Ulva and liverworts into several clusters based on there morphological features mentioned above. We consider the HBWR as primary element of clustering and take 3 clusters in the range of:

- Cluster 1: 0.90 to 0.98
- Cluster 2: 1.01 to 1.09
- Cluster 3: 1.40 to 1.45

![Cluster chart](cluster_chart.png)

Fig. 7: Compactness of the Thalluses of (a) Ulva and (b) Liverworts show the Similarities Between Them
HBWR Comparison between Bryophyte and Ulva

Now, we compare other parameters of the corresponding clusters and found the following results:

Tonal Distribution Comparison between Bryophyte and Ulva of same clusters

(Acute) Groove Angle Comparison between Bryophyte and Ulva of same clusters

(Obtuse) Groove Angle Comparison between Bryophyte and Ulva of same clusters

V. Conclusion

It was assumed that all the land plants were originated from green algae. Bryophytes are the next higher group from algae which are terrestrial in nature. Therefore it shows the similarities with green algae. The composition of pigments, growth pattern of cuticle and nature of cell wall elongation show the similarities between green algae and liverworts. Morphologically both of them show some similar features. The genome contents of green algae and bryophytes depict similarity. In our present image processing based research work, we studied several morphological features of Himalayan liverworts and green algal member Ulva. From this study we see that both these members show similarities in numerous cases. So from the above facts, we can conclude that the bryophytes as a group share a common ancestor with green algae.

References


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