Ecological Relevance and Physical Morphology of Green Algae, *Spirogyra* sp. in Northern Thailand

Pheravut Wongsawad* and Yuwadee Peerapornpisal*

Abstract

This study aimed to investigate molecular profiling and ecological preference of *Spirogyra* spp. in Thailand, using the profiling of HAT-RAPD markers. Biological parameters; cell dimension (width and length), number of chloroplast spiral/granules and some ecological parameters; total dissolved solid (TDS), pH, dissolved oxygen (DO), salinity and conductivity were also performed to determine correlation between biological and ecological parameters. For the specimen collection; 11 sampling sites in Northern provinces were designed. By basing on cluster analysis of ecological parameter, significantly different was observed (p<0.05). Significantly different was also detected in biological parameters, by revealing 5 different character groups (p<0.05) among all 11 specimens. The major classified criteria seem to be number and arrangement of chloroplast spiral. Contrastingly, by basing on correlation coefficient analysis, conductivity and TDS showed significantly related with all biological parameters, whereas DO showed significantly related with TDS and number of chloroplast spiral. For molecular study, on the basis of 54 characters (fragment size) provided from 5 different HAT-RAPD primers, 3 groups of *Spirogyra* spp. were divided and represented according to their geographical distribution. This finding allowed the magnitude of HAT-RAPD markers for profiling and determining of geographical and ecological origins.

Keywords : Ecological relevance Physical morphology *Spirogyra* sp.

* Department of Biology Faculty of Science Chiang Mai University 239 Huaykaew road Suthep Mueang district Chiang Mai province 50202

ความสัมพันธ์เชิงนิเวศวิทยาและสัณฐานวิทยากายภาพ ของสาหร่ายเตา *Spirogyra* sp. ในภาคเหนือของประเทศไทย

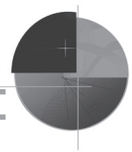
พีระวุฒิ วงศ์สวัสดิ์* และ ยุวดี พีระพรพิศาล*

บทคัดย่อ

การศึกษาคุณสมบัติเชิงโมเลกุล และนิเวศวิทยาเฉพาะของสาหร่ายเตา *Spirogyra* spp. ในประเทศไทย โดยใช้ เครื่องหมาย HAT-RAPD นอกจากนั้นปัจจัยทางชีววิทยา เช่น ขนาดของเซลล์ (ความกว้าง และความยาว) จำนวนของ chloroplast spiral และ granules รวมทั้ง ปัจจัยทางนิเวศวิทยา เช่น ปริมาณของแข็งที่ละลายน้ำ (TDS) ความเป็นกรด-ด่าง (pH) ปริมาณออกซิเจนที่ละลายน้ำ (DO) ความเค็มของน้ำ (salinity) และค่าการนำไฟฟ้าของน้ำ (conductivity) ยังถูกนำมาใช้เพื่อวิเคราะห์ความสัมพันธ์ระหว่างปัจจัยทางชีววิทยา และ นิเวศวิทยา จากจุดเก็บตัวอย่างทั้งหมด 11 จุด ในภาคเหนือ ผลการจัดกลุ่มปัจจัยทางนิเวศวิทยาพบว่ามีความแตกต่างอย่างมีนัยสำคัญ (p<0.05) นอกจากนั้นยังพบความแตกต่างอย่างมีนัยสำคัญระหว่างปัจจัยทางชีวภาพ โดยสามารถจัดกลุ่มลักษณะสัณฐานของตัวอย่างสาหร่ายได้ 5 กลุ่มจากทั้งหมด 11 ตัวอย่าง (p<0.05) พบว่าปัจจัยหลักที่ใช้ในการจัดกลุ่มคือ จำนวนและการจัดเรียงตัวของเกลียว chloroplast เป็นที่น่าสนใจว่า จากการวิเคราะห์สัมประสิทธิ์ความสัมพันธ์พบว่า ค่าการนำไฟฟ้า และปริมาณของแข็งที่ละลายน้ำทั้งหมด มีความสัมพันธ์อย่างมีนัยสำคัญกับปัจจัยทางชีวภาพทุกปัจจัย ขณะที่ ปริมาณออกซิเจนที่ละลายน้ำมีความสัมพันธ์อย่างมีนัยสำคัญกับปริมาณของแข็งที่ละลายน้ำทั้งหมดและ จำนวน chloroplast spiral ผลการศึกษาทางอณูชีววิทยาพบว่าลักษณะของแถบดีเอ็นเอทั้ง 54 แบบที่เกิดจาก HAT-RAPD primers จำนวน 5 ชนิด สามารถจัดกลุ่ม *Spirogyra* spp. ได้ 3 กลุ่ม ซึ่งพบว่ามีความสัมพันธ์กับภูมิศาสตร์และนิเวศวิทยาของจุดเก็บตัวอย่างแต่ละจุดด้วย ผลการศึกษาครั้งนี้สามารถยืนยันประสิทธิภาพของเครื่องหมาย HAT-RAPD ในการศึกษานิเวศวิทยาแบบเฉพาะของสาหร่ายเตาในภูมิศาสตร์ที่แตกต่างกันได้

คำสำคัญ : ความสัมพันธ์เชิงนิเวศวิทยา สัณฐานวิทยากายภาพ สาหร่ายเตา

* ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ 239 ถนนห้วยแก้ว ตำบลสุเทพ อำเภอเมือง จังหวัดเชียงใหม่ 50202



Introduction

Spirogyra is one of the most easily recognized green freshwater genera due to its spirally coiled chloroplasts. It is found in a wide range of habitats, including small stagnant water bodies, ditches as well as the littorals of lakes and streams. Distribution of *Spirogyra* sp. in northern Thailand is cosmopolitan with reaching abundance during hot dry and before entering rainy season. Because of the different of geographical distribution including habitat preferred, which may induce the emerging of variation at genetic level (1). *Spirogyra* can responses to the environmental condition through the expression of different filament type group (morphotypes), cell length/width and number of chloroplast spirals which related to physico-chemical parameters of water resource. By using morphological observation, light and SEM have been introduced to investigate the ultra morphology including morphotype characters as described before which can provides the useful information and significant taxonomic evidences. SEM has been introduced in many biological research viz., to study structure and properties of filamentous green algae (2), to investigate cell wall structure of the agarophytes *Gracillaria tikvahiae* and *G. cornea* (3) and to demonstrate productivity of marine diatom *Thalassiosira tumida* recorded in deglacial verves from the east Antarctica margin (4).

In recently year, a random amplified polymorphic DNAs (RAPD) approach have been employed to support taxonomic evidences and determine genetic variation related to various organism *viz*. green algae (5), Entomorpha fungus (6-7), gerbera plants (8) strawberry variety (9) and for detect fungal and algal symbionts of the lichen (10) and to investigate geneic variation of rice bean (*Vigna umbellata*) using ISSR and RAPD markers (11). This study aims to determine morphological variation of *Spirogyra* in northern Thailand using light microscpe. High Annealing Temperature Random Amplified Polymorphic DNA (HAT-RAPD) method was also performed to determine the variation at genetic level.

Methods

This study aimed to determine morphological by using topography of light microscope including to determine the genetic variation and ecological relevance of Spiogyra population in northern Thailand.

Specimen collection

Spirogyra specimens were collected from 11 different sampling sites along 9 upper-northern provinces of Thailand. Fresh specimens were kept in 15 ml plastic tube with few of habited water to maintain their normal structure and some were contained in plastic bag to preparing dry specimen. All collected specimen were placed on ice until used in subsequent processes.

Morphological study

Some biological parameters such as cell dimension (cell lengths/widths), number of chloroplast granule were also measured by using compound microscope. All measurements were taken with the aid of ocular and stage micrometer. The unit of measurement given was indicated by micrometer (μ).

Ecological study

Ecological parameters including water temperature, pH, DO and conductivity were taken using multi-function meter (Consort^{TM}) to determine correlation between biological and ecological parameters in accordance with correlation coefficient in SPSS program.

DNA extraction

Spirogyra specimens were freezed by liquid nitrogen and grinded using mortar and pestle. Amount of 20 mg of each were loaded into 1.5 ml micro-centrifuge tubes and subjected for DNA extraction based on phenol-chloroform method. All extracted DNA were adjusted to obtain the throughout equivalent concentration and kept in – 20°C until used.

HAT-RAPD PCR

Five commercially available, arbitrary 10–mer primers (Operon technology, USA) were used individually for HAT–RAPD PCR and the reaction was carried out in a final volume of 20 µl, with common PCR compositions. The reactions were performed in a MyCyclerTM Thermocycler (Bio-RAD) and the PCR conditions were as follows: 1 cycle of 95°C for 5 min, 30 cycles of 95°C for 45 sec, 48°C for 45 sec, 72°C for 1 min and 1 cycle of final extension at 72°C for 7 min. HAT–RAPD PCR products were separated on 1.6% TBE agarose gel electrophoresis, stained with ethidium bromide, and photographed by a Kodak digital camera (Gel Logic 100). HAT-RAPD profiles were scored and analyzed using the solution of UPGMA in ClustalW2.

Results

The topological study of light microscope based on the biological parameters demonstrated that, among 11 *Spirogyra* specimens, 5 character groups were defined which the differences were mainly found in number of chloroplast granules including the arrangement of chloroplast spiral, that was condensed or scattered forms. The arrangement of chloroplast spiral and granules of group number 1 and 5 were highly condensed and compacted while groups 2, 3 and 4 were relatively scattered as indicated in figure 1. Morphological differences were mainly observed in cell dimension, cell length and width (cell border). For the ecological

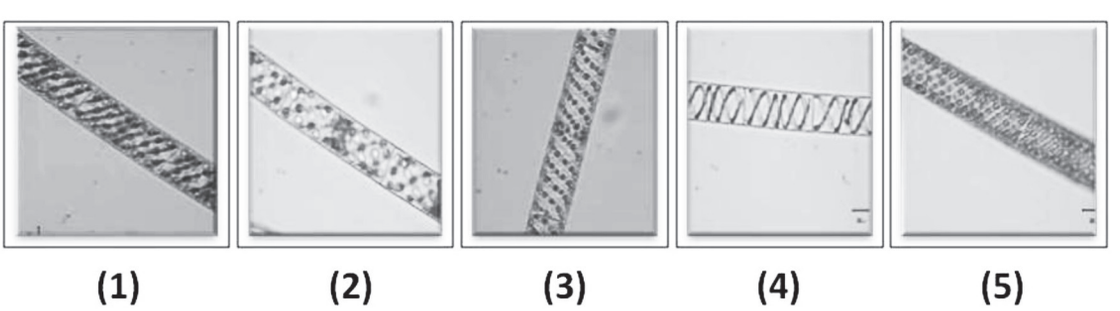


Figure 1 Five different morphological characters of *Spirogyra* population collected from northern part of Thailand (1): condensed and slightly compacted chloroplast spiral (2): short cell with scattered chloroplast spiral (3): long cell with less chloroplast spiral (4): short cell with less chloroplast spiral (5): long cell with condensed and compacted chloroplast spiral

Table 1 Correlation coefcient among ecological and biological parameters in SPSS program showed the signicantly relationships which indicated by (*). (* =0.05, **=0.01 condence level)

		pH	Conduc	TDS	Salinit	DO	Width	Length	Spiral	Granule
pH	Pearson Corre.	1	.138	.131	.152	-.143	.268	.236	.177	-.011
	Sig. (2-tailed)		.394	.421	.350	.378	.094	.143	.274	.948
	N	11	11	11	11	11	11	11	11	11
Conduc	Pearson Corre	.138	1	.686(**)	-.038	-.242	.525(**)	.709(**)	.764(**)	.510(**)
	Sig. (2-tailed)	.394		.000	.817	.133	.001	.000	.000	.001
	N	11	11	11	11	11	11	11	11	11
TDS	Pearson Corre	.131	.686(**)	1	.180	-.364(*)	.449(**)	.436(**)	.534(**)	.326(*)
	Sig. (2-tailed)	.421	.000		.267	.021	.004	.005	.000	.040
	N	11	11	11	11	11	11	11	11	11
Salinity	Pearson Corre	.152	-.038	.180	1	-.282	-.153	-.156	-.062	-.001
	Sig. (2-tailed)	.350	.817	.267		.078	.346	.336	.705	.993
	N	11	11	11	11	11	11	11	11	11
DO	Pearson Corre	-.143	-.242	-.364(*)	-.282	1	-.183	-.105	-.457(**)	-.291
	Sig. (2-tailed)	.378	.133	.021	.078		.257	.519	.003	.068
	N	11	11	11	11	11	11	11	11	11
Width	Pearson Corre	.268	.525(**)	.449(**)	-.153	-.183	1	.592(**)	.492(**)	.365(*)
	Sig. (2-tailed)	.094	.001	.004	.346	.257		.000	.001	.021
	N	11	11	11	11	11	11	11	11	11
Length	Pearson Corre	.236	.709(**)	.436(**)	-.156	-.105	.592(**)	1	.553(**)	.592(**)
	Sig. (2-tailed)	.143	.000	.005	.336	.519	.000		.000	.000
	N	11	11	11	11	11	11	11	11	11
Spiral	Pearson Corre	.177	.764(**)	.534(**)	-.062	-.457(**)	.492(**)	.553(**)	1	.641(**)
	Sig. (2-tailed)	.274	.000	.000	.705	.003	.001	.000		.000
	N	11	11	11	11	11	11	11	11	11
Granule	Pearson Corre	-.011	.510(**)	.326(*)	-.001	-.291	.365(*)	.592(**)	.641(**)	1
	Sig. (2-tailed)	.948	.001	.040	.993	.068	.021	.000	.000	
	N	11	11	11	11	11	11	11	11	11

relevance, based on biological characters, significantly different was also detected in biological parameters by revealing 5 different character groups (p<0.05) among all 11 specimens. The major criteria classified seem to be number and arrangement of chloroplast spiral. Contrastingly, by basing on correlation coefficient analysis, conductivity and TDS showed significantly related with all biological parameters, and the relative values (r) were described in table 1. Moreover, significantly relation between the same ecological parameters including in the same biological parameters were also observed (see table 1). For the molecular study, 5 HAT-RAPD profiles were generated and scored for determine the variation. It was found that DNA profiles of each character showed relatively similar (Figure 2). UPGMA analysis based on DNA profiles of all *Spirogyra* specimens (figure not shown) revealed that 2 separated groups were defined; first was comprised with 4 different clades while the remaining groups was consisted with 1 clade (figure 3). Interestingly, all *Spirogyra* specimens consisted in this clade were morphological characterized as group number

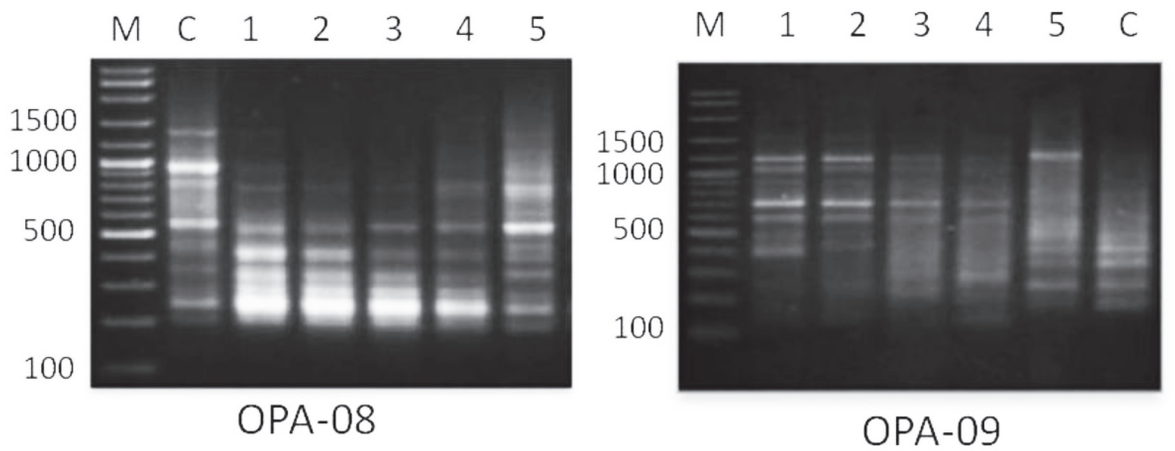


Figure 2 RAPD prole (OPA-08 and OPA-09) of *Spirogyra* sp. in each character group M: 100 bp molecular markers, lane 1-5: *Spirogyra* Character group 1-5, C: Cladophora sp. (out group)

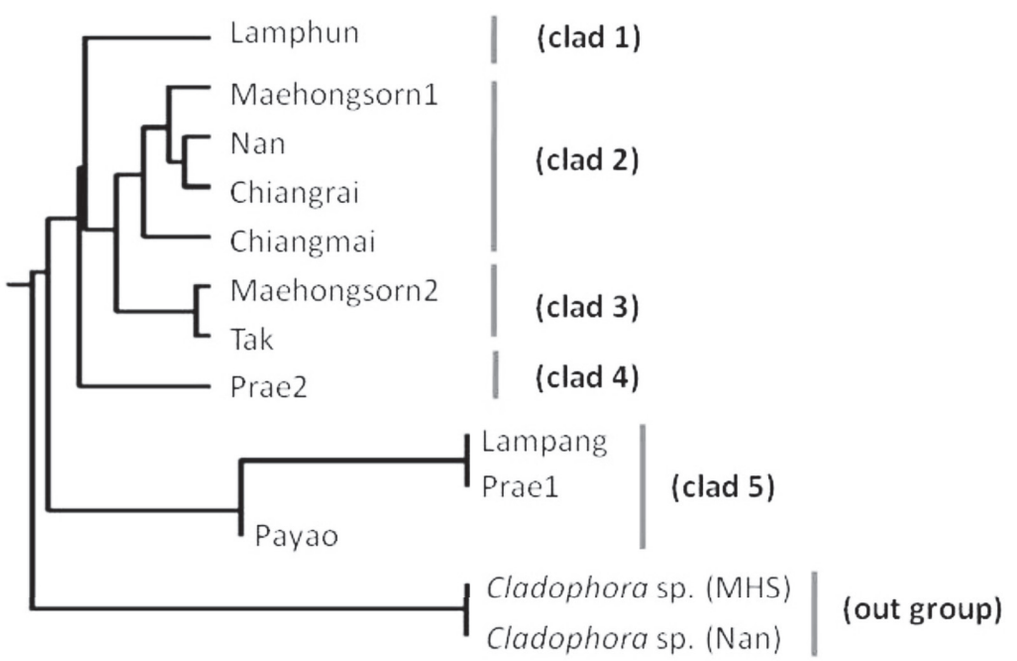


Figure 3 UPGMA dendrogram demonstrated the variation based on HAT-RAPD proles of 11 localities *Spirogyra* sp. collected from 9 upper Northern provinces, Cladophora samples were used as out group control (MHS: Maehongsorn)

5 as detailed in figure 1 while character of *Spirogyra* specimens consisted in clades 1-4 were mixed.

Discussion

In this study, it is difficult to determine the variation of *Spirogyra* due to HAT-RAPD profiles of each groups are relatively similar

which may depend on a less number of primers used as described in (11). The morphological differences found in this study ought to be the result of different developmental stages or maturity which may relates to trophic status of water as shown in (1), which does not be the different at genetic level. More specific molecular approaches such as nucleotide sequencing and ISSR marker are required to verify the variation in such morphological differences as described in (5, 8-11). Interestingly, HAT-RAPD profiles and ecological relevance show the similar result by group number 5 was clustered separately. This group gives positive relation with conductivity and pH which its water resource is running down from limestone rock where possesses the extremely high carbonate and cation. However, this study provides the advantages of combination methods used that valuable for investigate the genetic variation due to morphological different of *Spirogyra* specimens.

Morphological differences were observed among *Spirogyra* population collected in northern part of Thailand but it should be the result of different developmental stages or maturity and trophic status of water resource than the variation at genetic level. Water habitat with high conductivity seems to be suitable for growth and development of *Spirogyra*. It is difficult to determine the variation of *Spirogyra* in this study by using HAT-RAPD profiles alone. Other specific molecular approaches are required to verify the genetic variation in such morphological differences.

Acknowledgements

This work was facilitated by the Applied Technology in Biodiversity Research Unit, Institute for Science and Technology Research and the Economic Plant Genomes Research and Service Center, Faculty of Science, Chiang Mai University.

References

1. Hainz, R., Wobber, C., Schagerl, M. : The relationship between *Spirogyra* (Zygnematophyceae, Streptophyta) filament type groups and environmental conditions in Central Europe. Aquatic Botany 91: 173–180, 2009.
2. Johnson, M., Shivkumar, S., Berlowiz-Tarrant, L. : Structure and properties of filamentous green algae. Materials Science and Engineering 38:103-108, 1996.
3. Dawes, C. J., Teasdale, B.W., Frielander, M. : Cell wall structure of the agarophytes Gracillaria tikvahiae and G. cornea (Rhodophyta) and penetration by the epiphyte Ulva lactuca (Chlorophyta). Journal of Applied Phycology 12:567-575, 2000.
4. Stickley, C.E., Pike, J., Leventer, A. : Productivity event of marine diatom Thalassiosira tumida (Janisch) Hasle recorded in deglacial verves from the east Antarctica margin. Marine Micropaleontology 59:184–196, 2006.
5. Shen, S.: Genetic diversity analysis with ISSR PCR on green algae Chlorella vulgaris and Chlorella pyrenoidosa. Chinese Journal of Oceanology and Limnology 26(4), 380-384, 2008.

6. Lihme, M., Jensen, A.B., Rosendahl, S. : Local scale population genetic structure of Entomophthora muscae epidemics. Fungal Ecology 2:81-86, 2009.
7. Alaniz, S., Armengol, J., Leo'n, M., A-Jime' Nez, G.J., Abad-Campos, P. : Analysis of genetic and virulence diversity of Cylindrocarpon liriodendri and C. macrodidymum associated with black foot disease of grapevine. Mycological Research 113:16-23, 2009.
8. Bhatia, R., Singh, K.P., Jhang, T., Sharma, T.R. : Assessment of clonal fidelity of micropropagated gerbera plants by ISSR markers. Scientia Horticulturae 119:208–211, 2009.
9. Hussein, T.S., Tawfik, A.A., Khalifa, M.A. : Molecular Identification and genetic relationships of six strawberry varieties using ISSR markers. International Journal of Agriculture & Biology 10(6):677–680, 2008.
10. Widmer, I., Grande, F.D., Cornejo, C., Scheidegger, C. : Highly variable microsatellite markers for the fungal and algal symbionts of the lichen Lobaria pulmonaria and challenges in developing biont-specific molecular markers for fungal associations. Fungal Biology. 114: 538-544, 2010.
11. Muthusamy, S., Kanagarajan, S., Ponnusamy, S. : Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (Vigna umbellata) landraces. Electronic Journal of Biotechnology 11(3):1-10, 2008.