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Induction of sexual reproduction and zygospore patterns in the filamentous green alga *Spirogyra* Link (Conjugatophyceae: Zygnematales)

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ABSTRACT

Morphotaxonomy, ecological preferences and biological characterization of algal taxa of order Zygnematales (Conjugatophyceae, Chlorophyta), mainly the freshwater species *Spirogyra*, *Zygnema*, and *Mougeotia* are still poorly understood and need further in-depth investigations. In this study, different *Spirogyra* strains were examined to characterize their abilities in conjugation and formation of zygospores under different environmental conditions. We found that 16:8-h light:dark photoperiod is the best condition to induce the conjugation and zygospore formation. Moreover, the sexual reproduction was noticed to be triggered with increasing the light intensity to 85 µmol photons $m^{-2}s^{-1}$; no conjugation was observed at 35 µmol photons $m^{-2}s^{-1}$. The attribute of conjugation under red and blue irradiance, respectively, never equalized that in the white light even at elevated intensity. pH value (7.5) was the most suitable niche for induction of sexual reproduction. An increase of CO_2 in the atmosphere, provided by $NaHCO_3$ solution, did not enhance sexual reproduction. Cultivation of the investigated *Spirogyra* strains on 0.003% $CaCl_2$-containing agarized Pringhsheim´s medium (1/2 conc. and without nitrogen) induced the conjugation process as in case of $CaCl_2$ omitted. UV radiation completely inhibited the conjugation at all growth conditions.

Key words: environmental conditions, sexual reproduction, *Spirogyra*, zygospore formation

Introduction

The genus *Spirogyra* Link (order Zygnematales, Conjugatophyceae) is a common freshwater green alga with characteristic spirally coiled chloroplasts. In many cases, the unbranched filaments of *Spirogyra* form dense, slimy mats with a bright greenish appearance, floating near the surface of standing water bodies and areas of low current velocity in streams. The slimy texture due to its mucilaginous layers consisting of carbohydrates and serving to deter other organisms to attach as epiphytes (Marson, 2003; Weber & Schagerl, 2007). *Spirogyra* has a wide range of ecological preferences including small stagnant water bodies, ditches, as well as littoral habitats of lakes and streams. The accurate species delimitation mainly depends on the key morphotaxonomic features of the vegetative cells (mainly cell length and width, and number and shape of chloroplasts) and details of zygospores (Schagerl & Zwirn, 2015).

Several investigations have reported the importance of Zygnemataceae as a major source of food for snapping turtles, snails, mosquito larvae, large and small fishes, aquatic insects, and amphipods (Dineen, 1953; Delaney, 1954; Prescott, 1962). Species of *Spirogyra*, as one of the aforementioned group, is also a good source of bioethanol production (Sulfahri et al., 2016; Raju & Noufal, 2017). On the other hand, masses of *Spirogyra* often clog artificial waterways and foul filter systems of large reservoirs (Palmer, 1959). The genus *Spirogyra* is also used for bio-removal of uranium from mining wastewaters, biosorption of fluoride, copper, zinc, and acid blue dyes (Özer, et al., 2006; Lee and Chang, 2011). The concept of algal species complexes has recently been extended in algal studies due to the difficulty which one often encounters in species identification especially those depending on specific stages of life cycles (Simons et al., 1984). Therefore, cultivation of *Spirogyra* under laboratory conditions to induce and follow their complete life cycles are considered an important prerequisite, because in the natural environment this taxon is mainly observed in its vegetative state. Relations between various species of algal genera generally have only been inferred from similarity in observed morphology, rather than from any detailed cytological and genetic study. As a result, genera

comprising numerous species have been described often differing in only a single character. Simons et al., (1984) stated that nitrogen depletion and light intensity are key factors for induction of conjugation and zygospore formation in 31 *Spirogyra* sp., although eleven of those species showed no sexual reproduction. Simons et al., (1984) also reported that nitrogen depletion is a less successful driver in genera *Mougeotia* and *Zygynema* than in *Spirogyra*.

Zygospores offer suitable features for such purposes and can be easily studied using SEM techniques (Lewis & Entwisle 2000; Novis 2004). Poulíková et al. (2007) provided key features based on light (LM) and scanning electron microscope (SEM) observations, which are essential for compiling a key for species identification based on details of cell wall structures of zygospores in some *Zygnema* morphospecies.

Vegetative filaments of the genus are common throughout the year, but reproductive filaments are rarely found in short peri¬ods (Transeau, 1951; Stancheva et al., 2013), which makes an identification down to the species level impossible. The effect of different environmental factors on growth and reproduction of *Spirogyra* species was reviewed by Singh & Singh (2015). They discussed the influence of light intensity, light quality, photoperiod, temperature, radiations, seasons, nutrients (inorganic and organic), biotic factors, osmotic stress, and pH gradients. They found that the conjugation process occurs through 2 days under laboratory conditions using Bold's basal medium at 20°C and a photoperiod of 16:8 h = light: dark (light = 20 $\mu m^{-2}s^{-1}$). Zwirn et al. (2013) also concluded that in the genus *Spirogyra* no general triggers could promote the sexual reproduction, but certain nutrient ratios are considered important. The depletion of nitrate from medium, in addition to red, green and white light sometimes promoted sexual reproduction stages, whereas ultraviolet radiation and blue light never resulted in zygospore formation. The taxonomy, biology, autecology and biogeographical distribution of the Zygnematalean taxa are still poorly understood (Kadlubowska, 2001). The main aim of the present study was to induce the sexual conjugation and formation of zygospores in some *Spirogyra* species isolated from different Austrian and Egyptian habitats. The influence of different environmental and growth conditions on such a process is included for possible identification.

Materials and Methods

Specimens collection and cultivation

Three different *Spirogyra* morphotypes were collected and isolated from Menoufia Governorate part El Gnabya drainage in Shibin El Koam in April and May 2007, Egypt (Figure 1), and two strains were isolated from Franzen pond in Zwettl, Austria, in October 2007 (Figure 2) and the other strains were collected by the members of phycology group. Water samples were collected for studying the fluctuations of phytoplankton and physicochemical characteristics of water. Samples were kept in clean stoppered polyethylene bottles.

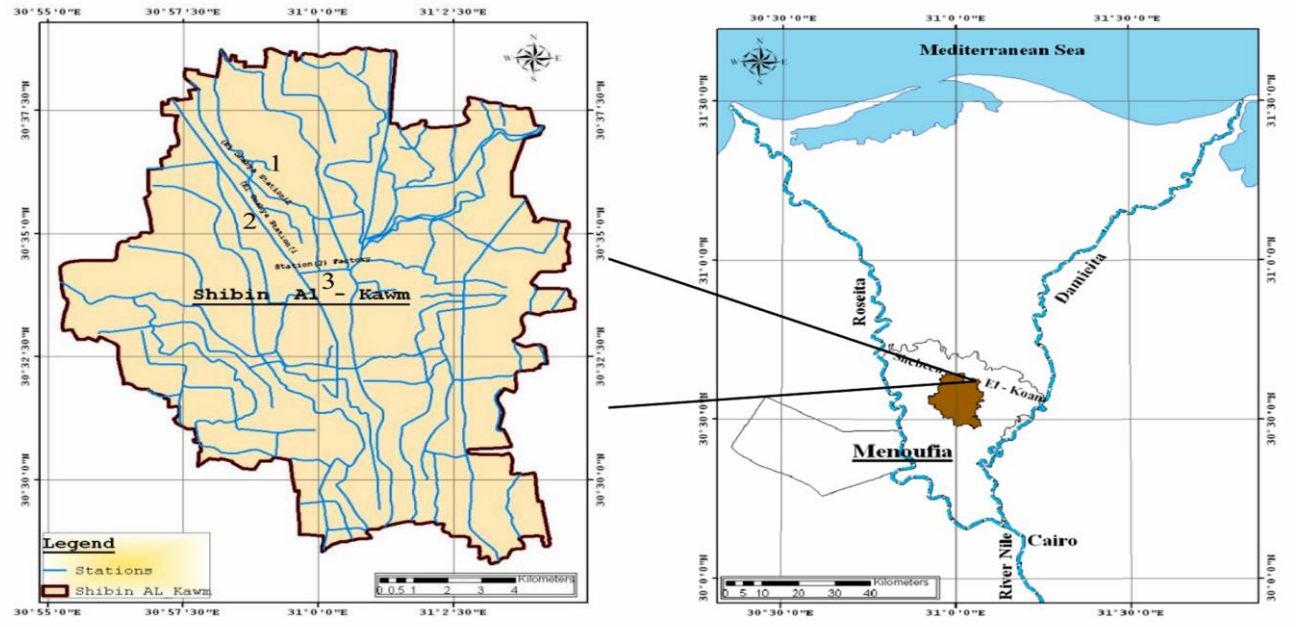

Figure 1. *Map of Egypt (El Gnabya in Shibin El Koam, sampling station). Sampling station in west of Shibin El Koam). Station 1: El Batanonya Station. Station 2: El Gnabya Station. Station 3: Factory Station*

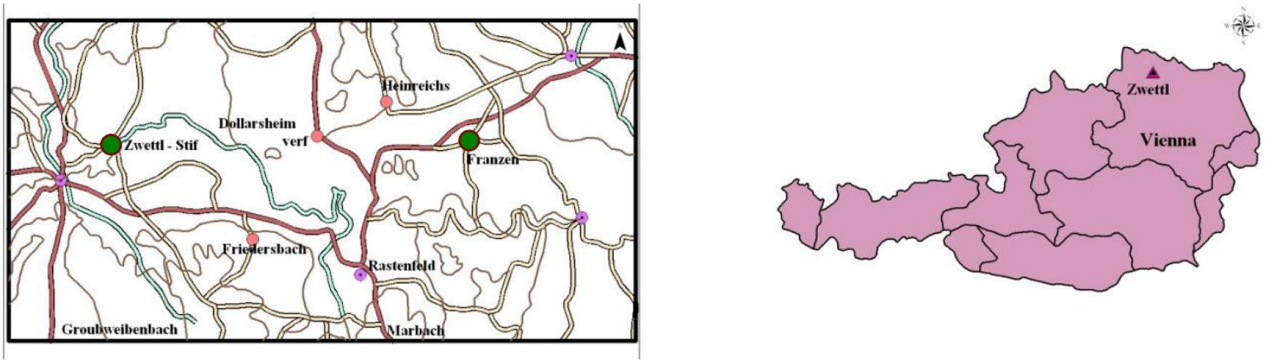

Figure 2. *The map of Zwettl area (Franzen, sampling station). Sampling Station located in Austria, north of Vienna city.*

The samples were stored refrigerated and analyzed within few hours after arrival to the laboratory.

Pringsheim´s culture medium (Ueno & Sasaki, 1978) was used for cultivation of vegetative filaments. Modified Pringsheim´s medium was used to induce sexual reproduction and zygospore formation: short filaments of *Spirogyra* were cultivated on Pringsheim´s medium (1/2conc.) without nitrogen. Vegetative material of *Spirogyra* was introduced into a small drop of old culture medium and gently spread over the agar surface following Allen (1958).

Effect of light intensity

Three different light intensities: 35 µmol photons $m^{-2}s^{-1}$, 72 µmol photons $m^{-2}s^{-1}$, 85 µmol photons $m^{-2}s^{-1}$ were applied to investigate the effect of light intensity on induction of the conjugation process and zygospores formation. The strains were grown under an illumination of a 16:8 L/D cycle at 20 to 23°C. Examination of the cultivated strain was carried out weekly and for four weeks.

Effect of the temperature niches

The *Spirogyra* specimens were cultivated at 15°C, 18°C, 22°C, 28°C, 30°C under white 16:8-h light intensity of 85 µmol photons $m^{-2}s^{-1}$. The strains were checked every week for four weeks.

Effect of photoperiods on induction of the conjugation

The *Spirogyra* strains were cultivated under different photoperiods as the following: Continuous illumination, 16:8-h light/dark, and 13:11-h light:dark photocycle with an intensity of 85 µmol photons $m^{-2}s^{-1}$ and then the strains were examined every week until four weeks according to Allen (1958).

Effect of different light wavelengths

Control culture plates were illuminated using a typical white light of 85 µmol photons $m^{-2}s^{-1}$ at a 16:8-h L/D cycle. Test plates were grown under a 16-h white light illumination followed by 8 hours of different light types (red, blue, and green), and then the growth was examined.

Effect of UV radiation

The *Spirogyra*-inoculated plates were illuminated by white light (85 µ mol photons $m^{-2}s^{-1}$) plus UV (white bulb light at the wavelength 380nm) on a cycle of 16 hours light and 8 hours dark, and then *Spirogyra* was examined weekly for 4 weeks.

Effect of different pH values

Different strains of *Spirogyra* were cultivated for four weeks; the strains of *Spirogyra* were kept under light intensity 85 µmol photons $m^{-2}s^{-1}$ and a temperature of 20 to 23°C. The solution was prepared at different pH 7.2 and 7.5. Two controls of Czurda medium were included, (Allen, 1958), and then the stock solution measured with pH Meter 814 USB sample processor software.

Atmospheric CO_2

The plates of *Spirogyra* strains were surrounded by a solution which would influence the amount of CO_2 (5% $NaHCO_3$) and the other plates were surrounded by NaOH to increase the amount of atmospheric CO_2. The control plate was surrounded by an atmosphere influenced by 10 ml distilled water. The examination of cultivated *Spirogyra* strains was performed weakly and for 4 weeks.

Effect of different concentrations of sucrose and NaCl solutions

Inoculums of different strains of *Spirogyra* in different concentrations of sucrose at (0.2 – 4.0%) and NaCl at (0.2 - 5.0%) in distilled water as medium, were placed in reduced light (35 µ mol photons $m^{-2}s^{-1}$) to induce conjugation of *Spirogyra*, and then the strains were examined from one week to four weeks according to Allen (1958).

Identification of Spirogyra strains

The *Spirogyra* strains being investigated were identified by in-depth investigation of their key morphological taxonomic features: length of the cells, width of vegetative filaments, number of chloroplasts per cell, morphology of zygospore walls, and width and length of the male and the female conjugation canal. The monographs and classification systems of Transeau (1951), Randhawa (1959) and Kadlubowska (1984) have been used.

Results and Discussion

Collection of samples

The 1/2 conc. Pringhsheim´s without nitrogen on agar medium showed that some strains of *Spirogyra* (OSS_5A, SOT_1A, ZIP_2 LUA_1 and LHS_5) from culture collection of Vienna University and the strain S1 from Egypt were induced to sexual reproduction. Some strains, which were tested with NaCl and sucrose with distilled water medium, gave negative results. Twenty plates containing different strains gave negative results in 1/2 conc. nitrogen-free Pringhsheim´s agar medium. However, such a medium induced some strains of *Spirogyra* for sexual reproduction under the effect of different factors (temperature, light intensity, light quality, pH, inorganic salts, CO_2 and UV).

The *Spirogyra* strains which were induced for sexual reproduction include SOT_1A, OSS_5A, ZIP_2, LUA_1A and LHS_5A (obtained from culture collection of Vienna University and S1 (obtained from Egypt). Nitrogen-free Pringsheim agar medium supported these strains to conjugate, and this observation is in agreement with the hypothesis of Czurda (1933) who obtained conjugation in media with supported further growth, therefore, it could not be assumed that nitrogen deficiency was itself a causative factor for conjugation. A low nitrogen level in the medium would enhance the level of the enzyme glutamine synthetase which may play a role in sexual differentiation of vegetative cells. This enzyme is only activated in the presence of any nitrogen source except glutamine. O´kelly (1983) tested the effect of different N-sources on induction of sexual reproduction and confirmed the previous conclusion. Czurda (1933) indicated that if a complete mineral solution with nitrogen is provided, zygotes will be formed and matured after conjugation. If, however, any mineral constituent was lacking, the conjugation process and some fusion might also occur, but no ripened zygotes would be formed. This finding is in accordance with the results obtained for SOT_1A, which showed conjugation but no zygospores. Czurda (1933) concluded that neither dominance nor deficiency of any particular chemical component is essential for triggering conjugations, but a properly balanced composition for both conjugation and for properly developed zygospores.

Czurda (1933) studied the relationship between the conjugation process and nutrient supply. The results have demonstrated that the nature of the culture medium itself is important. Since in soil/water medium conjugation could eventually be obtained, it might be suspected that an internal "conjugation state" is triggered in such a medium and this supports well our results where the 1/2 conc.Pringsheim´s without nitrogen induced the conjugation in some Spirogyra strains, but not in others.

Effect of different environmental conditions on induction of conjugation process

The examination of *Spirogyra* strains showed that the best conditions for conjugation and zygospores formation were obtained under 16 hours daylight length and 8 hours in the dark as shown in Table 1. The amount of conjugation and zygote formation increased with increasing light intensity. The highest conjugation numbers were recorded at 85 µmol photons $m^{-2}s^{-1}$ and no conjugation obtained at low intensity 35 µmol photons $m^{-2}s^{-1}$. This observation is in a good agreement with the previous work of Allen (1958). The recent contribution of Singh and Singh (2015) also highlighted that the conjugation in *Spirogyra* species usually occurs throughout 2 days under laboratory conditions using Bold's basal medium at 20°C, photoperiod 16:8 h L:D and light intensity of 85 µmol photons $m^{-2}s^{-1}$.

Effect of light quality on conjugation of Spirogyra

Concerning different effects of light quality on induction of conjugation *Spirogyra spp*., the results in Table 2 show that the amount of conjugation and zygospore formation in red and blue lights not equal to the amount of conjugation and zygospore formation. This result is in an agreement with the previous study carried out by Allen (1958).Effect of temperature on induction of sexual reproduction of *Spirogyra*. In agreement with Allen (1958), and Singh & Singh (2015), the optimum temperature to induce of sexual reproduction of *Spirogyra* was at 22°C (Table 3).

Effect of UV radiation on conjugation of Spirogira

There is a remarkable negative effect of UV radiation on all *Spirogyra* strains investigated in this study (data not shown) with no conjugation observed.

Effect of pH gradients on conjugation of Spirogyra

The results in Table 4 show that pH 7.2 and 7.5 seemed to be the suitable for induction of sexual reproduction of *Spirogyra* strains, and this is in agreement with Allen (1958).

Atmospheric CO_2

The results showed that increased atmospheric CO_2 did not stimulate conjugation. An increase of CO_2 in the atmosphere, provided by $NaHCO_3$ solution, did not appear to enhance the sexual reproduction.

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Table 1. *Effect of different photoperiods on conjugation and zygospore formation in different Spirogyra strains*

Light-period	Filaments conjugation										Zygote formation									
SOT_1A																				
No. of days	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
16 h	0	0	0	0	3	3	3	4	4	4	0	0	0	0	15	15	15	24	24	24
13 h	0	0	0	0	0	3	3	3	3	3	0	0	0	0	0	15	15	15	15	15
continous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OSS_5A																				
16 h	0	0	0	3	3	3	3	4	4	5	0	0	0	18	18	18	18	24	24	32
13 h	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	15
continous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZIP_2																				
16 h	0	0	0	0	0	3	3	5	5	5	0	0	0	0	0	18	18	24	24	32
13 h	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
continous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1																				
16 h	0	0	0	0	0	0	3	3	5	5	0	0	0	0	0	0	18	18	35	35
13 h	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
continous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. *Effect of light quality on conjugation and zygospore formation in Spirogyra strains*

Light-quality	Filaments conjugation										Zygote formation									
SOT_1A																				
No. of days	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
white 35µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
red 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	3	3	4	4	4	0	0	0	0	0	15	15	24	24	24
blue 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
white 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	3	3	3	4	4	4	0	0	0	0	15	15	15	24	24	24
OSS_5A																				
white 35µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
red 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blue 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	3	3	3	0	0	0	0	0	0	0	18	18	18
white 85µmol photons $m^{-2}s^{-1}$	0	0	0	3	3	3	3	4	4	5	0	0	0	18	18	18	18	24	24	32
ZIP_2																				
white 35µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
red 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blue 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
white 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	3	3	5	5	5	0	0	0	0	0	18	18	24	24	32
S1																				
white 35µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
red 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blue 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
white 85µmol photons $m^{-2}s^{-1}$	0	0	0	0	0	0	3	3	5	5	0	0	0	0	0	0	18	18	35	35

Table3. *Effect of different temperature on induction of sexual reproduction of Spirogyra*

Temperature	Filaments conjugation	Zygote formation
15 °C	0	0
22 °C	+	+
28 °C	0	0
30 °C	0	0

Effect of different concentrations of sucrose and NaCl on Conjugation

The studied *Spirogyra* strains survived 0.2% NaCl (data not shown), but growth was decreased in 1% conc. NaCl after one week. The strains died shortly after exposure to 2- 5% NaCl. Fifteen strains of *Spirogyra* were tested for conjugation under different concentrations of NaCl and neither conjugation nor zygospores have been formed. The strains grew in 0.2 – 4% sucrose, but died in 5% within the first

week. No conjugation or zygospores were observed among the fifteen strains tested for conjugation under different concentrations of sucrose.

Identification of Spirogyra strains

Spirogyra strain OSS_5A (*Spirogyra fluviatilis* Hilse: Figure 3

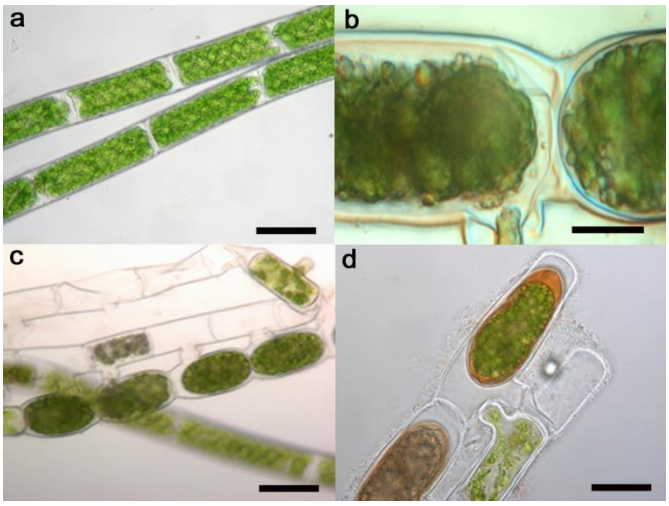

Figure 3. *Vegetative filament of Spirogyra fluviatilis (a); the stages of sexual reproduction of Spirogyra fluviatilis (b, c, d). Scale bar 50 µm.*

Description: Vegetative cells have an average length of 147 µm (minimum 142 µm -maximum 152 µm) and width of 31 µm (31 to 32 µm) and are characterized by plane cross walls. Chloroplasts 3–5 making 1.5 to 3.5 turns (Figure 3a).Cytoplasm contents of the male gametes pass through the scalariform conjugation tubes and fuse to form a zygote (Figures 3b-d). Length of the male cell 96-99 µm, and width 27-29 µm. Length of the female cell 115-125 µm, width 40-45µm; fertile cells shortened and inflated. Mature zygospore length 84-91 µm and width 38-42 µm (Figure 3c), ovoid, with brown median spore walls, corrugated or finely wrinkled. Branched rhizoids develop from some of the cells.This species was identified as *Spirogyra fluviatilis*.

Ecology: This strain was collected from Ossiacher See (Austria) by the phycology group in Vienna University. The habitat is alkaline (pH = 8.82). Nitrate conc.: 0.105 $mg.l^{-1}$, Nitrite conc.: 0.0055 $mg.l^{-1}$, Ammonium conc.: 0.0187 $mg.l^{-1}$

Spirogyra strain SOT_1A

This strain was collected from Stierofenteich (Austria) by the phycology group in Vienna University. The water was slightly alkaline with pH=8.03. The vegetative cell dimensions are 163 – 273 x 33 – 34 µm, and the number of chloroplasts 2-4 (Figure 4a). The average width of zygotes was 68 µm, the average of the length of zygote was 122 µm. Conjugation scalariform, tubes are formed by both gametangia, fertile cells cylindrical, zygospores ellipsoid to cylindrical – ellipsoid, median spore wall yellowish – brown, smooth. The identification is *Spirogyra irregularis* Nägeli (Figure 4b).

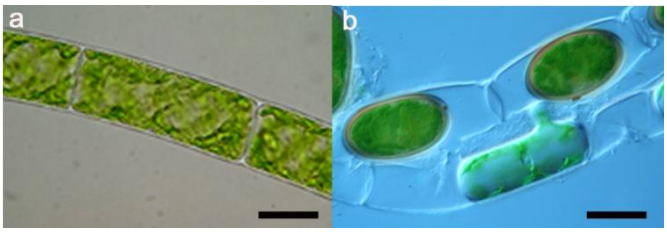

Figure 4. *Vegetative filament of Spirogyra irregularis (a) ; Spirogyra strain SOT_1A (Spirogyra irregularis): the outer layer is thin and hyaline and the mesospore is thick and brownish, scalariform conjugation canals (b). Scale bar 50 µm..*

Spirogyra strain (S1) isolated from Egypt

Spirogyra porticalis (O.F.Müller) Dumortier

This strain was collected from El Ganabya (Menouifa, Egypt). The water sample was slightly alkaline pH = 7.5, the nitrate and phosphate content of water sample was high with a nitrate concentration of 0.97 $mg.L^{-1}$ and a phosphate amount of 0.32 $mg.L^{-1}$, so this station is highly eutrophicated. Vegetative cells are 40 - 41 µm x 143 - 150 µm, with plane cross walls and contain a single chloroplast (Figure 5a). Conjugation scalariform with tubes formed by both gametangia. Fertile cells enlarged, zygospores ovoid with 45 - 50 x 73 - 83 µm in size; median spore wall finely wrinkled, or corrugated and yellow colour, smooth (Figure 5b).

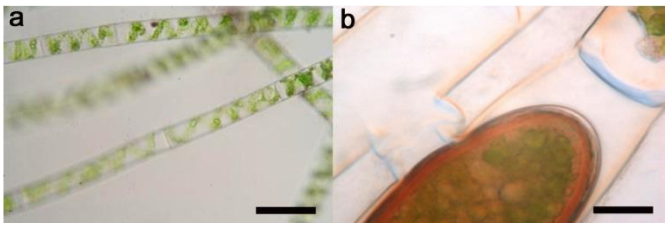

Figure 5*. Vegetative filament of Spirogyra porticalis (a); the zygospore of Spirogyra porticalis (b). Scale bar 50 µm.*

Spirogyra strain LUA_1

This strain was collected from Lunzer – Untersee - Ausrinn (Austria) by the phycology group, the water sample is slightly alkaline pH 8.26. The aplanospore average width is 41µm; length is 64 - 75 µm, vegetative cell have a size of 23 – 25 x 42 – 80 µm, and contain one chloroplast (Figure 6a) with plane end walls. The zygote is ovoid, less frequently ellipsoid Exospore, smooth, without colour, mesopore wall wider, yellowish brown and smooth. Cells bearing aplanospores are long and bent in the center, one chloroplast

with the nucleus moved into the central swelling (Figure 6b). The taxon was identified as *Spirogyra mirabilis* (Hassll) Kützing.

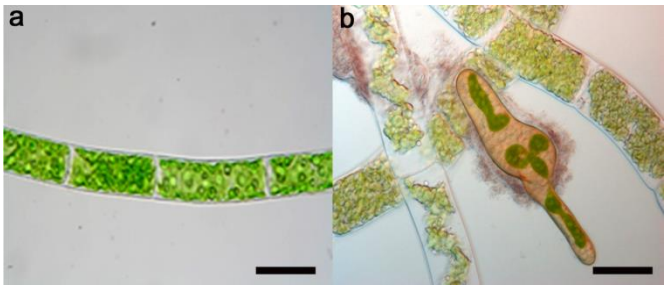

Figure 6. *Vegetative filament of Spirogyra mirabilis (a); Spirogyra mirabilis: Aplanspore is germinating up (b). Scale bar 50 µm.*

Spirogyra strain LHS$_5$

Spirogyra articulate Transeau

This strain was collected from Langhagensee (Germany) with slightly alkaline conditions (pH 6.72). Vegetative cells: width 24 - 28 µm, length 300 – 600 µm (Figure 7a). Number of chloroplasts one to two making 3 to 8 turns in the cell. The wall is folded with replicate cross walls. The width of aplanospores is 36 - 40 µm and the length is 60 - 88 µm; median spore wall yellow, smooth. Sporangia cylindrical, enlarged or slightly inflated, sometimes straight, often bowed or bent towards the middle. Cells with aplanospore swollen, mesospore, smooth, yellow (Figure 7b).

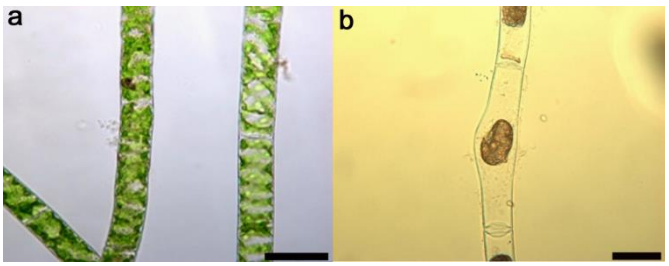

Figure 7. *Vegetative filament of Spirogyra articulate (a); Spirogyra articulate with aplanospore (b). Scale bar 50 µm.*

In the present investigation, the success of induction of sexual reproduction in *Spirogyra* was very low. This may be due to the male gamete develop a papilla first before the female cell in scalariform conjugation (Czurda, 1925; Lloyd, 1926). Sauners (1931) concluded that cell might first initiate a papilla, before the female cell enlarges over a less restricted area. According to Smith (1950), the factors inducing conjugation are not wholly connected with changes in the external environment, and fruiting cannot be induced by altering the conditions of illumination, temperature, and mineral content of the surrounding water. This statement expresses the present state of knowledge on the conjugation process. Smith was probably referring to the statement by Czurda (1930) on the internal conditioning of cells which Czurda referred to as an inner physiological state. Czurda (1925), on the other hand, concluded that the cell volume of the two gametes is important in successful of zygote formation. Czurda suggested that the size and the shape of the female gametangium determine the successful hybrids. Thus, according to Transeau (1951), one might expect to obtain successful hybrids in any combination since the form and the size of the zygote are determined by the female gamete. According to Czurda (1925), however, the relationships of the zygote volume and female gametangium would determine the probable success of a cross. Between forms of different ploidy, however, there is a partial barrier to successful zygote formation, the size of the female gametangium restricting the success of zygote formation when the maternal cell is the smaller of the two gametes.

Conclusion

In this work, the taxonomy of the *Spirogyra* was studied using the morphology of hypnozygotes. In order to examine zygospores, the sexual reproduction of *Spirogyra* was induced in the laboratory. Nitrogen depletion and light supply appeared to be the key factors for the induction of hypnozygote development. Also, variations in pH, inorganic salts, and CO_2 supply were effective parameters. Characters of the zygote wall (colour, shape, and ornamentation), conjugation tubes, and female gametangia were studied, which are of considerable importance for species identification in *Spirogyra*. Much work still needed in order to further understanding the induction of sexual reproduction of *Spirogyra* and classification.

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