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Alterations in lipid and fatty acid composition of the cyanobacterium *Scytonema geitleri* bharadwaja under water stress

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Abstract

The composition of the glycerolipids [monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG)] and alterations in their saturation and unsaturation levels in response to osmotic and matric water potential have been investigated in the cyanobacterium *Scytonema geitleri* Bharadwaja. The level of MGDG in *S. geitleri* was high followed by PG, DGDG and SQDG. Whereas, the amount of fatty acids namely palmitic, stearic, oleic, linoleic and linolenic acid were high, arachidic and behenic acid were, however, present in traces in the four glycerolipids. A significant reduction in the level of total lipid as well as individual class lipid was observed in *S. geitleri* in response to matric water potential to that of its total lipid and individual class lipid in response to osmotic water potential. The levels of polyunsaturated and unsaturated fatty acids also increased in response to matric water potential to that of osmotic water potential.

Key words: Glycerolipids, Osmotic potential, Matric water potential, *Scytonema geitleri*, Saturated fatty acids, Unsaturated fatty acids.

Introduction

Cyanobacteria are able to cope with a wide range of environmental stresses such as heat, cold, desiccation, salinity, nitrogen starvation, photo-oxidation, anaerobiosis and osmotic stress (1). In natural habitats, they usually interact with frequent fluctuation of environmental conditions occurring in sharp contrast. In tropics, day temperature varies from 20 to 50°C, and the surface temperature of building tops can be as high as 70°C in the month of summer. Cyanobacteria growing on exposed buildings surfaces experience desiccation and high temperature stress (2), and show various structural and physiological adaptations.

Cytoplasmic and thylakoid membranes of cyanobacteria contain monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG) as major glycerolipids in addition to phospholipids (3). Cyanobacteria possess fatty acids belonging to four groups; the first group contains only saturated and monounsaturated fatty acids and the second group is characterized by the presence of α-18:3. The third and fourth group is characterized by the presence of γ-18:3 (3, 4) and 18:4 (5), respectively. Alteration in the membrane lipid often results in response to environmental stresses (6). Whereas, the role of membrane fluidity and lipid composition on survival of the desiccation - prone organisms at extreme temperature and salinity is well understood (4), a little is, however, known about the alterations (especially in lipid composition) associated with the drying of cells (7, 8). Since, water stress affects the cell membrane structure, and membrane function depends on the fluidity of the lipid components; it is desirable to see whether decrease in growth of the cyanobacterium *Scytonema geitleri* Bharadwaja (isolated from the roof-top of a building and physiologically adapted to water stress) under water stress is associated with alterations in the composition of glycerolipids (MGDG, DGDG, SQDG and PG). In the present study, an attempt has been made to find out the composition of glycerolipids (MGDG, DGDG, SQDG and PG) and alterations in their saturation and unsaturation levels in response to osmotic and matric water potential in *S. geitleri* Bharadwaja.

Materials and methods

Organism and culture conditions

Scytonema geitleri Bharadwaja was isolated from the roof-top of Botany Department, Banaras Hindu University, Varanasi, India. Cultures of *S. geitleri* were grown in 250 ml Erlenmeyer flasks containing 100 ml of Chu-10 medium (9) and maintained in a culture room at 28±1°C. The cultures were illuminated with combination of day and white light fluorescent lamps having the photon fluence rate of 95 µmol m^{-2} s^{-1} on the surface of the vessels under 14:10 light-dark rhythm. The pH of the medium was adjusted to 7.5 before autoclaving.

Maintenance of hydration level

In nature, rehydration (wetting) of naturally growing dry mats of *S. geitleri* takes place by direct availability of water whereas, dehydration (drying) results due to loss of water from wet mats to the atmosphere. The rehydration and dehydration of the cyanobacterium was controlled by regulating the osmotic status of the cells and matric availability of water. The stationary phase cultures of *S. geitleri* (28 day old) were harvested by centrifugation, dried by lyophilization, and placed onto

filter papers (henceforth mentioned as dry mat). The dried mats were rewetted at 0 MPa (double distilled water) and incubated for 72h (henceforth mentioned as growing mats). Dried mats were subjected to different matric potentials by putting them in NaCl solutions equivalent to different water potentials 0, -1.4, -2.8, -14.6 and -21.0 MPa, at 25°C as described before (10). Whereas osmotic water potential of *S. geitleri* mats was analyzed by incubating them onto filter papers soaked in different concentration of solutes, their matric water potential was calculated by equilibrating the mats in the atmosphere of a solution of defined water potential (isopiestic control) as described (11).

Lipid extraction, identification and quantification

Total lipid from the treated as well as control mats was extracted as described before (12, 13). The cells of *S. geitleri* (1g fresh weight) were crushed with chloroform and methanol (2:1 v/v) and the cell debris was pelleted by centrifugation at 5000 rpm for 10-15 min. The process was repeated till the resulting pellet become whitish in color. The supernatant was evaporated to dryness in N_2 atmosphere at 37 ± 1°C to avoid autooxidation.

The dried extract was dissolved in chloroform: methanol: water (2:1:1, v/v/v) and water was added to this mixture to remove non-lipid substances. The resulting suspension was mixed well and transferred to a separating funnel. Bottom organic phase was collected and evaporated to dryness in N_2 atmosphere at 37 ± 1°C. Lipid thus obtained was stored in chloroform: methanol (2:1, v/v) at - 20 ± 1°C for further study.

Lipids were loaded on TLC plate about 2.5cm above the edge of silica gel plates and run in a solvent system consisting of acetone: benzene: water (91:30:8, v/v/v). Separated lipid spots were stained using iodine vapor (14). Total lipids in the extracts as well as in the spots separated on the TLC plates were quantified using K2Cr2O7 method (15), with palmitic acid as the standard. Glycerolipids, MGDG, DGDG, SQDG and PG were identified on the basis of their respective R_f values. R_f values were measured by calculating the ratio of the distance covered by the respective glycerolipid and the distance covered by the solvent from the origin of the spot.

Fatty acid extraction and transesterification

Lipid spots on the TLC plates were scrapped with razor blade and transferred to a centrifuge tube containing chloroform: methanol (2:1, v/v). After centrifugation, chloroform-methanol supernatant containing lipid was transferred to test tubes (15 X 15 mm) with a Teflon-lined screw-cap and evaporated to dryness in N_2 atmosphere. To the dried sample, 3 ml of 2.5% (w/w) HCl in anhydrous CH_3OH (HCl gas was bubbled into the CH_3OH until its weight increased by 2.5%) and 100µl of 1mM pentadecanoic acid (as internal standard in benzene) were added. The tubes were tightly capped and kept at 85°C in a water bath for 2.5 h. After cooling, 2.5 ml of n-hexane was added to each tube, and mixed by vortexing. The content was allowed to stand for 5 min and the upper (hexane) phase was carefully transferred to a clean test tube with a narrowly tapered bottom suitable for evaporation. The lower phase was extracted twice with additional hexane. Subsequently, 2 ml of distilled water was added to the remaining lower phase and the hexane extraction was repeated. The hexane extracts were pooled and the solvent was removed by evaporation under stream of N_2. The resulting methyl ester was dissolved in 20 µl of n-hexane. The tubes were wrapped with aluminum foil and placed on ice until analysis.

Table 1. R_f values of lipids of the cyanobacterium *S. geitleri.*

Lipid Class	Rf values
MGDG	4.0
DGDG	3.8
SGDG	2.8
PG	2.0

Analysis of fatty acids

Fatty acids recovered after TLC were analyzed in a Gas Chromatograph (Varian model GC-CP 3800) fitted with flame ionization detector (FID). Sample (2µl) was injected with the help of Hamilton syringe into a CP-Sil 8 CB column (5% diphenyl and 95% dimethyl polysiloxane, 30m by 0.32mm i.d, 1µm film thickness). The column temperature was maintained initially at 1700 C for 2 min followed by 5°C increase up to 230°C for 2 min, and at the rate of 15°C min-1 up to 250°C for 5min. The injector and detector temperature was kept at 280°C. N_2 was used as carrier gas with a flow rate of 30 ml min^{-1}. H_2 and O_2 were used as flame gas with a flow rate of 30 ml min-1and 300 ml min-1, respectively with split mode of 30:100. Fatty acids of methyl ester were identified using the standard of fatty acids of methyl ester (FAME) (Sigma-Aldrich).

Results

MGDG, DGDG, SQDG and PG were identified as the major glycerolipids in *S. geitleri* on the basis of their R_f values (Table 1). The data on gas chromatographic analysis of these glycerolipids further provided evidence for the presence of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0) and behenic (22:0) acid in *S. geitleri* (data not shown).

Effect of osmotic water potential on lipid, lipid class and fatty acid content

The level of total as well as individual lipid class was significantly affected in *S. geitleri* in response to osmotic water potential (Fig.1, 2). The levels of MGDG, DGDG, SQDG and PG in *S. geitleri* were 5.60, 2.03, 2.12, and 2.93 mg g^{-1}dry weight, respectively at 0h. However, the level of MGDG increased maximally with increase in incubation period and attained its maximum level (35.37%) at 72 h; followed by PG (27.64%), DGDG (14.28%) and SQDG (9.90%) over the level of their respective lipid class at 0h.

The data on fatty acid composition of different lipid classes in *S. geitleri* in response to osmotic water potential are presented in (Fig.3). In MGDG; the level of palmitic acid (16:0) was maximum followed by stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0) and behenic (22:0) acid. The level of palmitic (16:0) and stearic (18:0) acid also increased

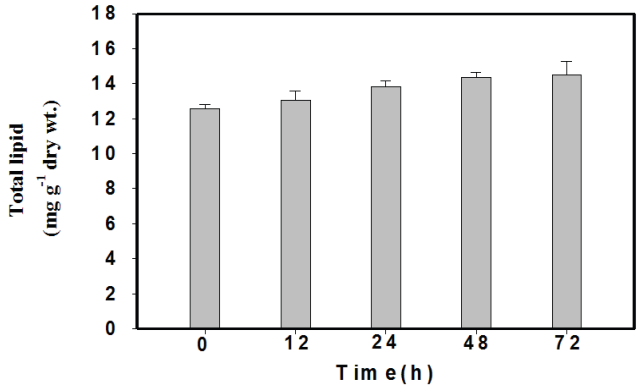

Figure 1. Effect of osmotic water potential (0MPa) on total lipid of *S. geitleri* incubated for different time period. Values are the mean of n=3 ± SD.

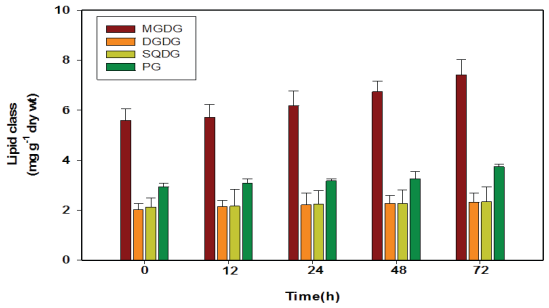

Figure 2. Effect of osmotic water potential (0MPa) on content of lipid classes in *S. geitleri* incubated for different time period. Values are the mean of n=3 ± SD.

maximally with increase in incubation period upto 72h. However, the increase in oleic (18:1), linoleic (18:2) and linolenic (18:3) acid was minimal with increase in incubation period. Similar increase in fatty acids was also recorded in glycerolipids PG, DGDG and SQDG, however, the level of fatty acids was low in these glycerolipids to that of MGDG.

Effect of matric water potential on lipid, lipid class and fatty acid content

The total lipid content in *S. geitleri* was also significantly affected in response to matric water potential (Fig.4). The cells of *S. geitleri* grown for 72h at 0MPa and treated with different matric water potential (-1.4 to -21.0 MPa) exhibited an alteration in their MGDG, DGDG, SQDG and PG levels. A significant decrease in MGDG, DGDG, SQDG and PG level was observed with increase in matric water potential and maximal decrease (45.4%) was recorded in MGDG at -2.8 MPa (Fig.5). The level of palmitic (16:0) and stearic (18:0) acid also decreased in response to matric water potential in MGDG, maximum decrease being recorded at -2.8MPa. In contrast, the level of oleic (18:1), linoleic (18:2) and linolenic (18:3) acid increased in response to different matric water potential over their respective controls. Quantitatively, a minor variation was recorded in arachidic (20:0) and behenic (22:0) acid. Similar pattern of variation in fatty acid contents was also recorded for PG, DGDG and SQDG; however, the level of fatty acids was relatively low in these lipid classes when compared with MGDG (Fig.6).

Effect of osmotic water potential (0MPa) on relative

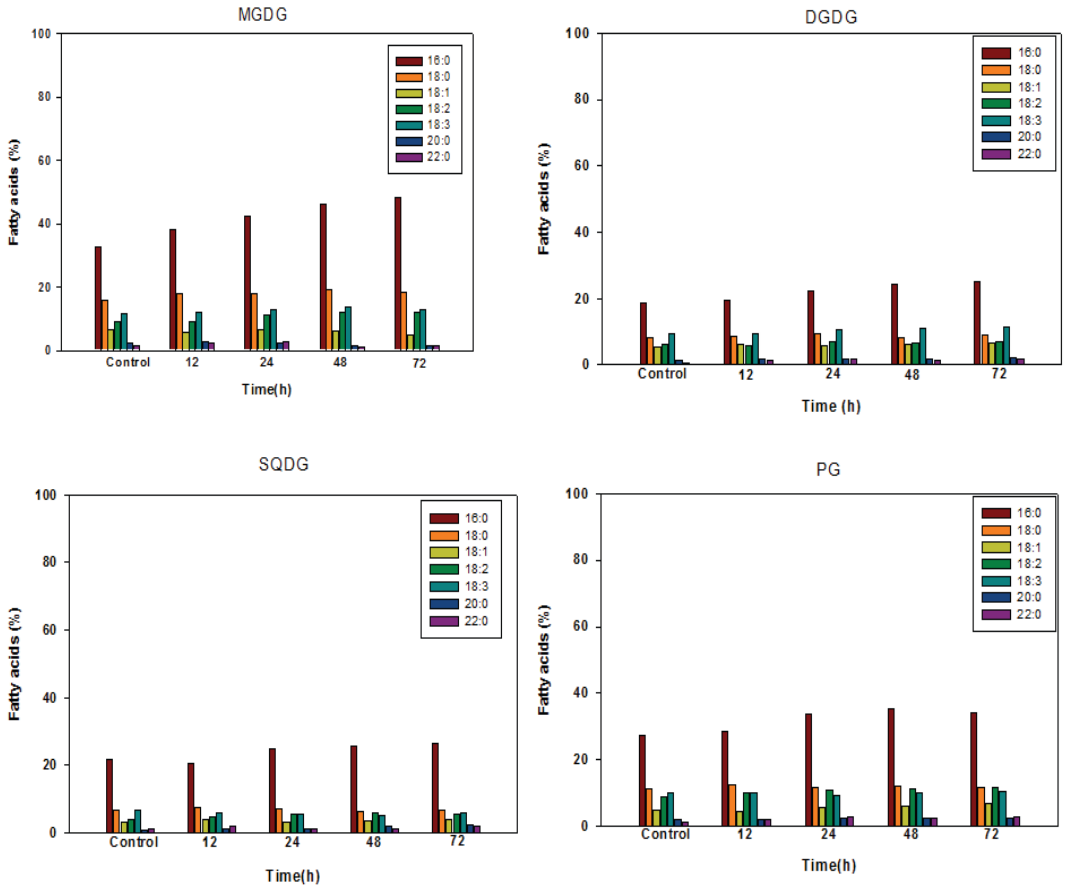

Figure 3. Effect of osmotic water potential (0MPa) on fatty acid composition of lipid classes in *S. geitleri* incubated for different time period.

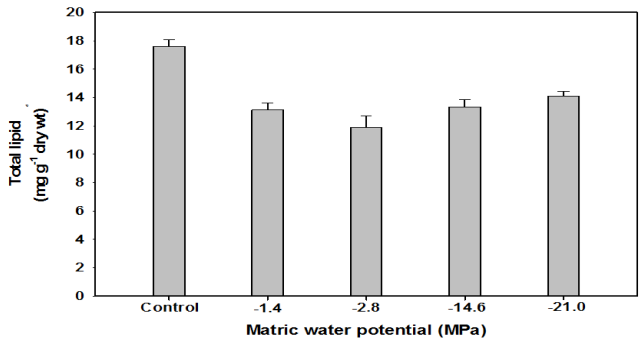

Figure 4. Effect of matric water potential (MPa) on total lipid content of 72h grown culture of *S. geitleri*. Values are the mean of n=3 ± SD.

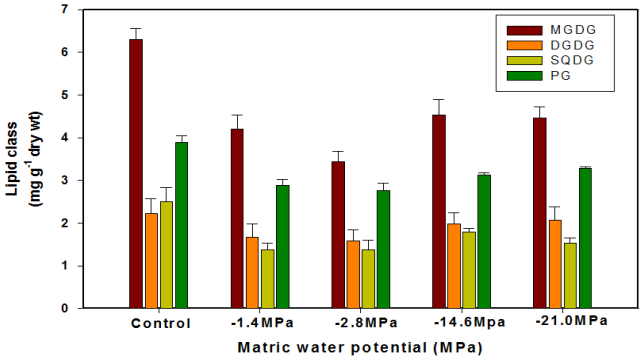

Figure 5. Effect of matric water potential (MPa) on content of lipid classes in 72h grown cells of *S. geitleri*. Values are the mean of n=3 ± SD.

level of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of MGDG, DGDG, SQDG and PG

The amount of SAFA in *S. geitleri* increased with increase in incubation period and osmotic water potential (Fig.7). However, a relatively minor increase in MUFA and PUFA content was recorded in MGDG. Similar increase in SAFA, MUFA and PUFA content was also recorded for DGDG, SQDG, and PG. However, the level of SAFA in SQDG whereas declined at 12h, the level of PUFA in PG increased at 24h of incubation.

Effect of matric water potential on relative level of SAFA, MUFA and PUFA of MGDG, DGDG, SQDG and PG

The cells of *S. geitleri* subjected to different matric water potential and grown for 72 h exhibited decreased level of SAFA. The level of SAFA in MGDG and PG decreased with increase in matric water potential and attained its minimal level at -2.8 MPa. DGDG and SQDG, however, exhibited the minimum level of SAFA at -1.4 and -14.6 MPa, respectively. In contrast, an increase in MUFA and PUFA level in MGDG, DGDG, SQDG and PG was recorded in *S. geitleri* in response to different matric water potential (Fig.8).

Effect of osmotic water potential (0MPa) on unsaturation of lipid class

The cells of *S. geitleri* subjected to 0MPa osmotic water potential for 12 to 72h exhibited a significant va-

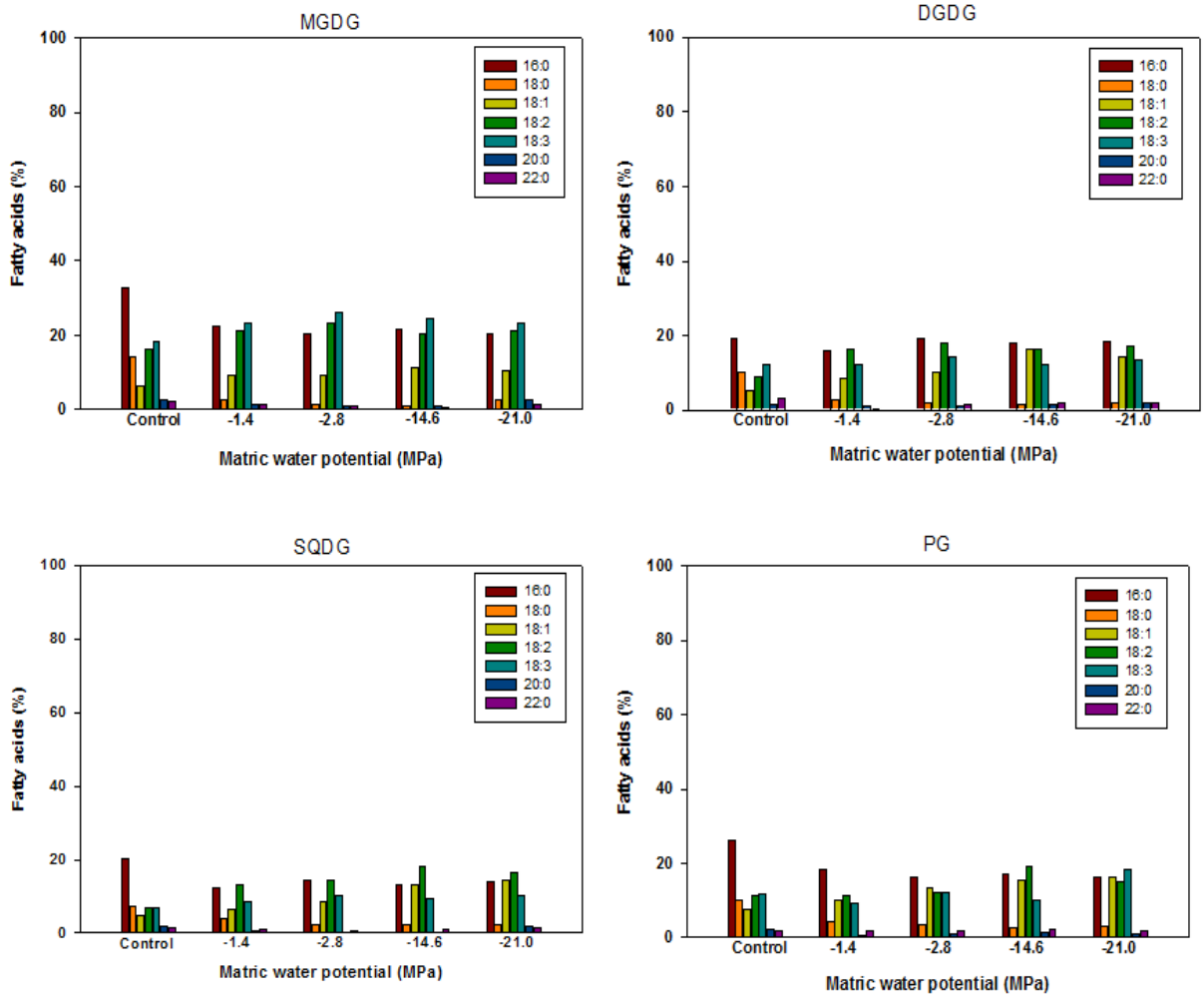

Figure 6. Effect of matric water potential (MPa) on fatty acid composition of lipid classes in 72h grown cells of *S. geitleri.*

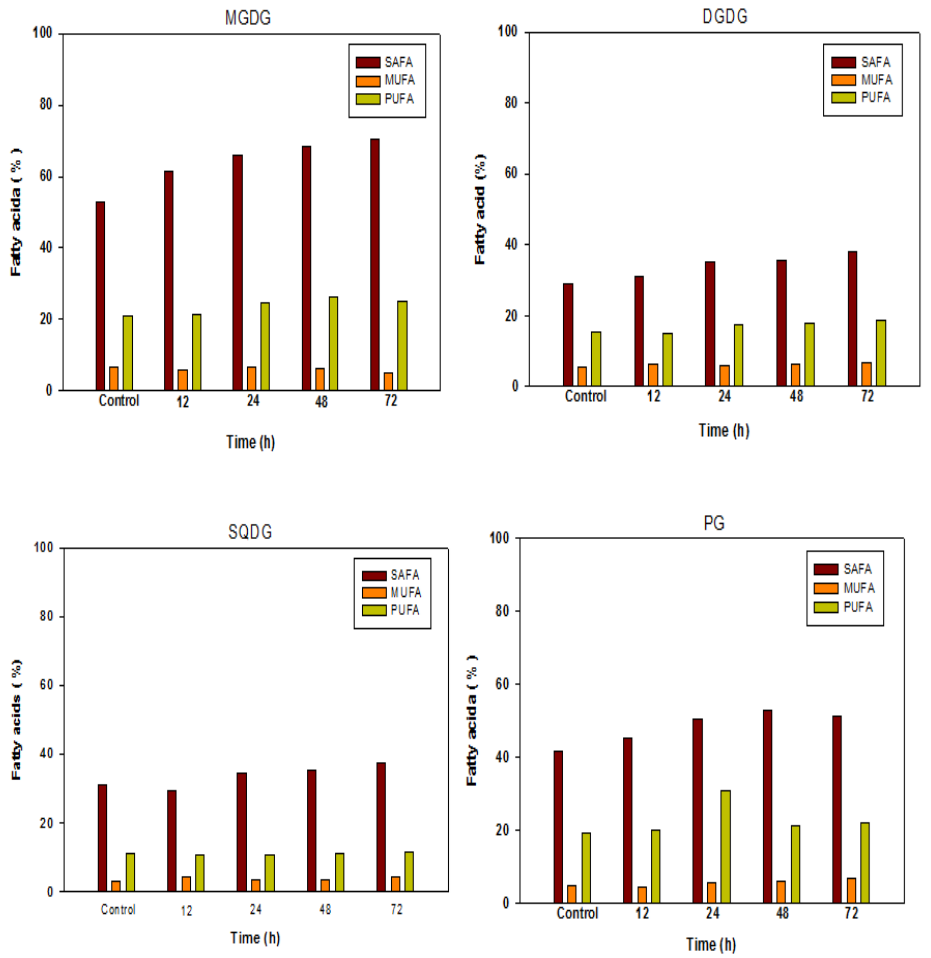

Figure 7. Effect of osmotic water potential (0MPa) on relative level of saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of different lipid classes in *S. geitleri.*

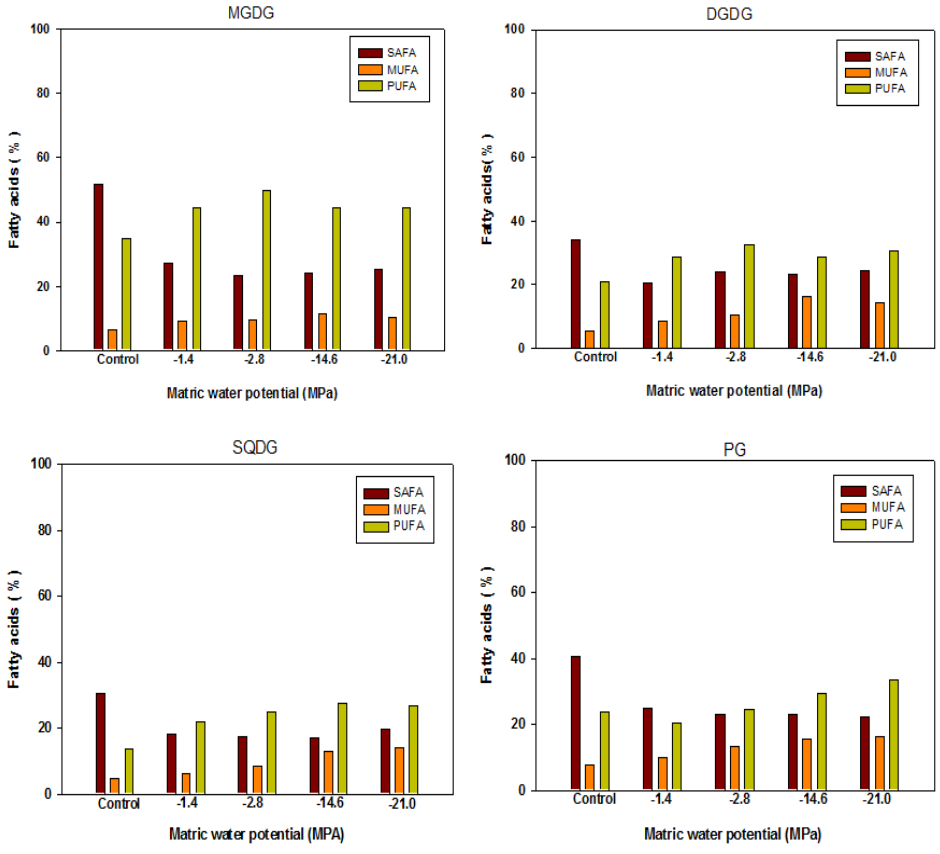

Figure 8. Effect of matric water potential (MPa) on relative level of SAFA, MUFA and PUFA of different lipid classes in 72 h grown cells of *S. geitleri.*

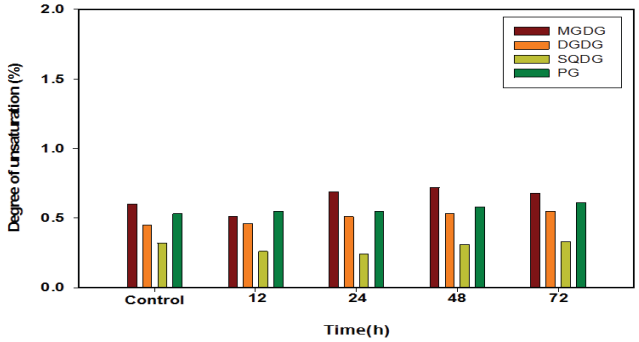

Figure 9. Effect of osmotic water potential (0MPa) on unsaturation of lipid classes in *S. geitleri* incubated for different time period.

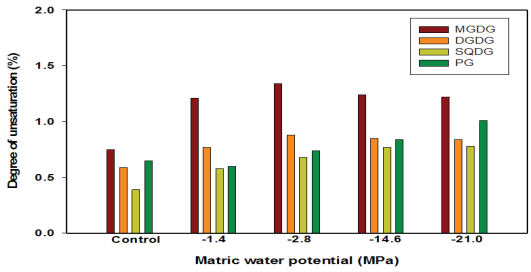

Figure 10. Effect of matric water potential (MPa) on unsaturation of lipid classes in 72h grown cells of *S. geitleri.*

riation in the degree of unsaturation in MGDG, DGDG, SQDG and PG. However, the degree of unsaturation varied from 0.60-0.68%, 0.45-0.55%, 0.32-0.33% and 0.53-0.61% in MGDG, DGDG, SQDG and PG, respectively in response to osmotic water potential (Fig.9).

Effect of matric water potential on unsaturation of lipid class

The cells of *S. geitleri* subjected to different matric water potential also exhibited a significant alteration in the degree of unsaturation in MGDG, DGDG, SQDG and PG. Whereas MGDG exhibited higher degree of unsaturation (1.34%) at -2.8MPa, DGDG, however, exhibited a low degree of unsaturation (0.88%) at the same matric water potential. Unlike MGDG and DGDG; SQDG and PG showed higher degree of unsaturation at -21.0MPa matric water potential (Fig.10).

Discussion

Since, water deficit affects cell membrane structure resulting in its breakdown, a full reconstitution of the membrane integrity during rehydration is a prerequisite for the cells to survive. *S. geitleri* contained mostly acyl lipids (glycerolipid as the major component) and MGDG, DGDG, SQDG and PG as major glycerolipids. These results are in agreement with the lipid composition of cyanobacteria (3, 16, 17). Amongst the glycerolipids, MGDG has been reported to be the main glycerolipid in cyanobacteria (18). In the present study, whereas MGDG constituted 50-60% of the total glycolipid content, DGDG, SQDG and PG, however, contributed to 10-25% each. Similar variation in the lipid composition has also been reported in green algae (19).

Membrane lipids play an important role in stabilizing the structural arrangement via lipid-protein interactions, in integrating the protein complexes and in maintaining their spatial distribution (20). Under different abiotic stresses such as drought, many plants make alterations in their membrane composition and phase behavior to optimize the fluidity (21).

Under rehydration, the dry mat of *S. geitleri* whereas exhibited an increase in the amount of lipids with increasing incubation period, dehydration, however, resulted in reduction of the lipid content (Fig. 1, 4). The increase in lipid content in rehydrated dry mat of *S. geitleri* may presumably be due to its requirements by the organism to increase the rate of metabolic activities, as metabolic activities recovered on rehydration (22). Since, optimization of fluidity is regulated by the content and composition of lipids in the membrane, the least variation in the content of lipids in *S. geitleri* in response to drying and wetting suggested that the fluidity of membrane in *S. geitleri* is least affected by both drying and wetting.

PG has been suggested to hold the light harvesting chlorophyll-protein complex of photosynthesis (23, 20). The variation in the level of DGDG, SQDG and PG on changing the level of hydration during wetting and drying of *S. geitleri* suggests that structures associated with photosynthetic apparatus such as ATP synthase and light harvesting complexes are least affected during rehydration and dehydration. A non-specific association of MGDG to light-harvesting complex of PS II and its reaction center has been suggested (24). MGDG form a cylindrical inverted hexagonal configuration instead of the lipid bilayer configuration under isolated conditions. It is believed that the excess of MGDG in lipid mixtures facilitates the formation of non-lamellar phases (25). A reduction in the formation of non-bilayer structure by MGDG on increasing its level of saturation has also been reported (26).

S. geitleri contained higher amount of SAFA in MGDG, DGDG, SQDG and PG as compared to MUFA and PUFAs in response to osmotic water potential. Among these fatty acids, palmitic acid (16:0) was highest in all the four lipid class. In contrast, the amount of oleic (18:1), linoleic (18:2) and linolenic (18:3) acid was high to that of palmitic acid (16:0) in response to matric water potential. Membrane containing high level of PUFAs tends to decrease the phase-transition temperature and increase the fluidity of membrane. In addition, PUFAs are important for growth, and also assist in preventing photoinhibition of photosynthesis at low temperature (27, 28). PUFAs in membrane lipids are also essential for the growth of cells at low temperatures (29).

Functional change in the fatty acids 18:1, 18:2 and 18:3 has been suggested to be a major adaptation mechanism (17). In the present study *S. geitleri* exhibited maximum fatty acid desaturation in MGDG followed by PG, SQDG and DGDG. Change in the fatty acids unsaturation is related to the change in bilayer thickness and fluidity; and increase or decrease in fatty acid unsaturation causes increase or decrease in membrane fluidity (30).

Nostoc flagilliforme grown under nutrient depleted condition and temperature stress accumulates carbon photosynthate in the form of neutral lipids (31), with a

high proportion of MUFA and PUFA (32). In the present study *S. geitleri* had a much higher content of PUFAs and lower content of MUFAs in response to matric water potential, leading to a higher degree of fatty acid unsaturation. Growth conditions especially, the shift in growth temperature and water stress could induce the processes of desaturation and acyl chain elongation to a greater extent through the feedback of an array of desaturases and elongases.

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Other articles in this theme issue include references (33-48).

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