Review Article

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Environmental stress: Response, mechanism and its regulation in *Cyanobacterium spirulina*

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Abstract: *Spirulina* is a photosynthesizing cyanophyte used as an important source of protein. It is multicellular, filamentous, unbranched and helically coiled blue-green microalgae which is mass cultured. Environmental stress is the major limiting factor for mass cultivation due to disturbances in this normal redox state caused by oxidative damage at the cellular level. Low temperature affects the biochemical composition of *Spirulina*. The balance between different pathways is disrupted and uncoupled resulting in transfer of electrons that have high energy state to molecular oxygen (O_2) to form reactive oxygen species (ROS). ROS damages DNA, PSII, carbon fixation process, enzymes activity, ATP production, causes lipid peroxidation and affects the protein flexibility & structural changes which results in initiation of apoptosis. To overcome the challenges of ROS the photosynthetic organisms developed robust antioxidant and redox buffering systems composed of enzymatic antioxidants and non-enzymatic antioxidants and also increases photorespiration.

Keywords: Oxidative Damage, Reactive Oxygen Species, Antioxidant, Lipid Membrane, DNA, Photosystem.

Introduction

Cyanobacteria are bluish green due to the presence of pigment phycocyanin and chlorophyll *a*, which they use to capture light for photosynthesis. Cyanobacteria are found in almost every conceivable environment, from oceans to fresh water to bare rock to soil (1). Cyanobacteria are the only organisms that actively evolve oxygen as a by-product of oxygenic photosynthesis within the same cell or colony of cells where nitrogen fixation occurs (1,2). Cyanobacteria are the oldest oxygenic photosynthetic organisms and they serve as rich source of novel bioactive metabolites, including many cytotoxic, antifungal, and antiviral compounds (3). Among these, *Spirulina* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates and alpha- linolenic acid (3; 4).

Spirulina:

Spirulina is a photosynthesizing cyanophyte (blue-green algae) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions (pH 8.5-11) (5). It is used as human food as an important source of protein and is collected from natural water, dried and eaten (6). It has gained considerable popularity in the human health food industry and it is used as protein supplement.

Morphology:

The cyanobacteria, *Spirulina* is multicellular, filamentous, unbranched and helically coiled (7) blue-green microalgae belonging to genus *Arthrospira* and consists of about 15 species. It belongs to family Oscillatoriaceae with a length of 200-300 µm and a breath of 5-10 µm (8). It has a cell wall similar to that of Gram-negative bacteria. It contains peptidoglycan, a lysozyme-sensitive heteropolymer that confers shape and osmotic protection to the cell, in addition to other material not sensitive to lysozyme. The body surface of *Spirulina* is smooth and without covering so it easily digestible by simple enzymatic systems. *Spirulina* reproduce by binary fission by forming transverse septa. The helical shape of the filaments (or trichomes) is characteristic of the genus and is maintained only in a liquid environment or culture medium. The presence of gas vacuoles in the cells, together with the helical shape of the filaments results in floating mats.

Physiology and Biochemistry:

Spirulina, like most cyanobacteria, is an obligate photoautotroph, i.e. it cannot grow in the dark on media containing organic carbon compounds. It reduces carbon dioxide in the presence of light and assimilates mainly nitrates. The main assimilation product of *Spirulina* photosynthesis is glycogen (9). *Spirulina* shows an optimum growth between 35°C and 37°C under laboratory conditions. The minimum temperature at which growth of *Spirulina* takes place is around 15°C during the day. The resistance of *Spirulina* to ultraviolet rays seems to be rather high (9). Beside phycocyanin *Spirulina* also contains pigments like chlorophyll *a* and carotenoids. Some contain the pigment phycoerythrin, giving the bacteria a red or pink colour. Other pigments

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include chlorophyll *a*, xanthophyll, beta-carotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin.

The use of *Spirulina* as an experimental tool in biochemical studies has been very limited. *Spirulina* does not fix nitrogen and does not develop differentiated cells like heterocyst or akinetes as part of filament. *Spirulina* contains nitrite reductase which assimilates nitrate reduction via conversion of nitrite to ammonia. The absorption spectra of this enzyme have six major peaks relatively at 278, 402, 534, 572, 588 and 658 nm. Tiboni and Cifferi were the first to isolate *Spirulina* elongation factor EF-Tu in 1983. EF-Tu may be used in evolutionary studies for exploring phylogenetic relationship between *Spirulina* and other prokaryotic groups. The other important enzymes include Ferredoxin sulphite reductase (Fd-SiR) catalyzing reduction of sulphite to sulphide; ATP synthase, an enzyme associated with thylakoid membrane also known as ATPase coupling factor; Acetohydroxy acid synthase, the first common enzyme in biosynthesis of valine, leucine and isoleucine. A relatively high cytoplasmic pH (4.2 to 8.5) may account for the ability of this micro-organism to use ammonia as a source of nitrogen at high alkaline pH (5). *Spirulina* change their pigment composition under different stress conditions like high temperature, high light, oxidative stress, nitrogen deficient condition and salt stress conditions etc which provide protection to *Spirulina* in those conditions. The helicity of *Spirulina* has been used as a taxonomic criterion (10, 11) as well as a way to select for high quality strains (10, 12).

Economic importance:

Spirulina platensis is rich in easily digested protein and phytochemicals (13). The protein is considered as a complete protein since it contains all the essential amino acids. With around 65% protein (dry mass) *Spirulina platensis* is superior to all plant sources of proteins. *Spirulina platensis* has been recently gaining the attention of medical researchers. Studies are showing that *Spirulina platensis* exhibit antiviral, anticancer, antimicrobial and anti-inflammatory activity. It has beneficial effects on controlling cholesterol, diabetes, coronary artery disease, weight loss and wound healing. The β-carotene, B-group vitamin, vitamin E, iron, potassium and chlorophyll available in the *Spirulina* can promote the metabolism of carbohydrate, fats, protein, alcohol, and the reproduction of skin, muscle and mucosa.

Environmental stress:

Environmental factors such as light, temperature and salinity can affect the helical structure (10, 11, 14, 15, 16) and cellular homeostasis (17). Environmental stress is the major limiting factor for mass cultivation of *Spirulina* (18). Much of the injury caused by stress exposure is associated with oxidative damage at the cellular level. Oxidative stress is caused by an imbalance between the production of active oxygen and the ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell indulging proteins, lipids and DNA. Generally, the free radicals are more reactive than non-radicals and react with them to produce new free radicals in a chain reaction that can cause damage to molecules in the body (19).

Chilling stress:

In 1973, Layons (20) and Rasin (21) for the first time proposed that the thermotropic phase transition of membrane lipids might play role in chilling sensitivity. The phase separated biomembranes are not able to maintain ionic gradients and hence cellular metabolism becomes disrupted ultimately leading to the death of cell being (22). The occurrence of phase separation as the initial event has been demonstrated in unicellular cyanobacterium *Anacystis nidulans* (22). Unsaturation of lipids in thylakoid membranes protects the photosynthetic machinery from photoinhibition at low temperature (22). Changes in membrane fluidity are the initial events that lead to the expression of desaturases (22). The increase in degree of unsaturation of membrane lipids is correlated with the sustained activity of membrane bound enzymes at lower temperature. Cyanobacteria contains acyl lipid desaturases which are transmembrane protein and are specific to fatty acids esterifies to glycerolipids. The desaturase Josephano bacteria use ferredoxin as the electron donor and introduce double bonds in fatty acids

specifically at the specific Δ or ω positions of glycerides (23). The cyanobacterium *Synechocystis* encodes four *Des* proteins, *DesA, DesB, DesC* and *DesD* which introduces double bond at the Δ^{12}, Δ^{15} (ω^3), Δ^9 and Δ^6 (23- 25) positions of C-18 fatty acids at the sn-1 position (23-31). In *Spirulina platensis*, three genes for acyl lipid desaturases named *desA, desB* and *desD* has been cloned (23, 32) from cyanobacterial strains *Anabaena variabilis* (23, 29) and *Synechococcus* (23). Temperature can also affect the biochemical composition of *Spirulina*. Increasing temperature increases photosynthetic activity (33, 34). Growth and lipid content of *Spirulina platensis* are affected in temperature change from 25°C to 38°C (33, 35). Cyanobacteria respond to detrimental effect of cold stress by increasing membrane lipids fluidity of fatty acids with the help of desaturases (36) and increasing the transcription and translation by synthesizing enzymes that help in enhancing of this process (37-39) and therefore protect themselves.

Stress Response, Mechanism and its Regulation:

The balance between different pathways in different organelles is disrupted during temperature stress as different pathways have different temperature optima (17). When different pathways are uncoupled, electrons that have high energy state are transferred to molecular oxygen (O_2) to form reactive oxygen species (ROS) (17, 40, 41). The reactive oxygen species include singlet oxygen, superoxide radicals, hydrogen peroxide and hydroxyl radicals. Singlet oxygen is often produced.

The sites for production of ROS in photosynthetic cell are chloroplast and microbodies. The chloroplast contains PSI and PSII. The ROS ($^1O_2$, O_2^- and $HO^\bullet$) are produced in PSII reaction center (42). The PSII is susceptible to oxidative stress as these ROS can damage the PSII through degradation of D1 protein and inhibition of repair of damage to PSII (43). The reduction site of PSI is also the site of O_2^- production through Mehler reaction (44, 45). Under stress condition the production is enhanced at such an extent that it overwhelms antioxidant defenses to cause damage to both PSII and carbon fixation process. The H_2O_2 is synthesized by peroxisomes as it contains the enzymes involved in β- oxidation of fatty acids and in photorespiration. The H_2O_2 is produced by glycolate oxidase by transferring two electrons from glycolate to oxygen and O_2^- by xanthine oxidase reaction with purines (46,47).

Lipids are the major constituents of cell membranes. ROS especially $HO^\bullet$ react with lipids and cause cellular injury and is dependent on the degree of membrane fluidity, which in turn is a function of the saturation state of lipid bilayer. Aldehydes like malondialdehyde and hydrocarbons like ethane and ethylene are the degradation products of lipid peroxidation (48, 49). Enzyme activity and ATP production are affected in mitochondria by lipid peroxidation and result in initiation of apoptosis (50). The results are particularly cytotoxic.

Protein flexibility plays an important role in functional interactions and enzyme catalysis. These processes often involve structural changes at various levels of organization ranging from side-chain movements to domain rotations and quaternary structural changes. Each of these structural changes is affected by thermal fluctuations and hence is acutely temperature dependent. At low temperature when kinetic energy lowers down these kinds of structural changes take place rarely. Gerday *et al.,* (51) explain enthalpy driven interaction between the proteins. More flexible structure is required for the improvement of functionality of proteins at low temperature.

Feller *et al.,* (52) proposed that structural adaptations for cold-active proteins from psychrophilic over mesophilic and thermophilic protein characteristics. These include- (1) reduction in the number of salt bridges (2) reduction of aromatic interactions (3) reduced hydrophobic clustering (4) reduced proline content (5) reduced arginine content (6) additional loop-structures (7) more solvent interaction.

It is important to note that not all these types of changes would be expected to occur in a single protein because the molecular context of the changes is important in altering the stability and activity of the protein. It may generally be expected, however, that cold-active enzymes would have reduced thermo stability and a lower apparent temperature optimum for their activity in comparison to enzymes active at high temperatures. The consequences of oxidative attack on proteins are site specific amino acid modifications,

fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge, and increased susceptibility to removal and degradation. The primary, secondary and tertiary structure of a protein determine the susceptibility of each of amino acids to attack by ROS (49, 53) and the different forms of ROS diffusion their reactivity with different amino acids. Some enzymes become inactive upon the oxidation of O^{2-} of iron- sulphur centers while others like histidine, lysine, proline, arginine, and serine inactivate when oxidized and form carboxyl group (54). The accumulation of degraded proteins by ROS has been hypothesized to be involved in the process of ageing (55).

ROS can induce deletions, mutations and other lethal genetic defects in DNA. The damaging effects of ROS may be due to base degradation, single strand breakage and cross linking to proteins because both sugar and base moieties are susceptible to oxidation (56). *In vivo* toxicity of H_2O_2 & O^{2-} is due to the result of fenton reaction in the presence of transition metal as these can't by themselves cause strand breaks under normal physiological conditions in vitro. The in situ produced hydroxyl radical in the proximity of DNA molecules damages the DNA and form alkaline labile sites as well as DNA strand breaks (57).

The induction of antioxidant defense system such as superoxide dismutase, catalase and peroxidases (57-61), increase de novo synthesis of molecular antioxidants such as ascorbic acid and carotenoids and increased repair efficiency (57, 58) decreases oxidative DNA strand breaks which is one of the key aspects in process of adaptation of cyanobacteria (57). The highly efficient DNA repair systems Josephano bacteria allow them to survive under several environmental stresses during evolution and adapt to these stresses (57, 58). In addition to DNA repair, the recovery of the photosynthetic apparatus and its function also determine adaptation and recovery of the cyanobacteria exposed to stress.

The ATP and NADPH consumption decreases under stress as there is limited CO_2 fixation which results in excess NADPH content and hence there is decline in level of $NADP^+$ which is a major electron acceptor in photosystem I, this depletion of $NADP^+$ accelerates the transport of electrons from photosystem I to molecular oxygen resulting in generation of H_2O_2 via O_2. (62). The elevated level of ROS inhibits the repair of damaged photosystem II and leads to photo-inhibition (43, 62-65).

The enhancement of photoinhibition of photosynthesis in intact cells at low temperature is due to disruption of recovery process. Acidification of the lumen due to transfer of protons, inactivates the oxygen evolving system and allow the formation of P_{680}^{+}, the oxidized reaction centre damages the D1 protein in the lumen due to the transfer of protons (66,67). The damage to D1 protein of PSII is major cause of Photo inactivation (22). Its recovery involves several steps. First is proteolysis of damaged D1 protein, secondly pre- D1 protein is synthesized de novo, the pre D1 protein is sorted into PSII complex which in turn is matured by processing and reconstruction of oxygen evolving complex, hence recovery of active PSII complex. From all these processes transcription of *psbA* gene (the structural gene for pre- D1 protein) was not affected by unsaturation of membrane lipids. So, Nishida and Murata (1996) proposed that either the reassembly of the pre- D1 protein with PSII complex or the processing of pre- D1 protein that yield mature D1 protein is accelerated by the unsaturation of membrane lipids. Nishiyama *et al.,* (43, 66, 68) identified the process that is suppressed by ROS (formed during stress) by analyzing levels of *psbA* mRNA and pre- D1 protein with northern and western analysis. They found that translation of *psbA* mRNA is suppressed by $^1O_2$ and H_2O_2. The subcellular localization of polysomes with bound *psbA* mRNA suggested that the primary target of $^1O_2$ and H_2O_2 might be a step of elongation (43, 66, 68). In green alga *Chlamydomonas reinhardtii* under excess light, change in Rubisco and D1 protein was found to be closely related to ROS increase and changes redox status regulated by glutathione pool (57, 69). UV-B induced expression of the *psbA2* and *psbA3* genes has been suggested to be a defence response against stress that allow cyanobacteria to repair UV-B induced damage of PSII via de novo synthesis of D1 and D2 proteins (57,70,71).

Molecular oxygen is produced as a result of the oxidation of water by the photosynthetic electron transport chain. The latter, however, can also use oxygen as an electron acceptor In addition; molecular oxygen is assimilated

during photorespiration producing phosphoglycolate. Both of these reactions have positive and negative effects. Superoxide, produced by the transport of electrons to oxygen, is not compatible with metabolism and must be eliminated by the antioxidative defense system while recycling of phosphoglycolate to phosphoglycerate (in order to re-enter the Benson – Calvin cycle) results in a considerable loss of assimilated carbon. In addition, large amounts of H_2O_2 are produced during the oxidation of the glycolate in the peroxisomes. Although much of this H_2O_2 is destroyed by catalase, some chemical decarboxylation of keto acids by H_2O_2 still occurs (72). Nevertheless, photosynthesis benefits since photorespiration protects the photosynthetic membrane against light-induced damage at times when carbon assimilation is limited (73). This may indeed be regarded as the principal function of photorespiration, which is far more effective than electron transport to oxygen (termed pseudocyclic electron flow or the Mehler reaction) in protecting against photo inhibition (74). Due to presence of PUFA in thylakoid membrane (75) photosynthetic apparatus are more prone to photodynamic damage (76). Due to presence of antioxidant compounds and mechanisms (which causes removal of ROS), the photosynthetic apparatus of cyanobacteria which produce O_2 are not damaged (76). Photo inhibition occurs as a result of the reduction in photosynthetic electron transport combined with the continued high absorption of excitation energy and the production of ROS. ROS have many cellular targets, including photosystem II and the primary carboxylating enzyme, Rubisco, in zooxanthella (77, 78). Elevated temperatures functionally lower the set point for light-induced photo inhibition. Enzymic defenses in the cnidarian host occur in proportion to the potential for photo-oxidative damage in symbiotic cnidarians (79, 80). However, high fluxes of ROS in the host (79,81) or zooxanthellae (78, 82, 83) can overwhelm the protective enzymatic response and result in hydroxyl radical production via the Fenton reaction (45,53,84). Both the cnidarians host and zooxanthellae expressCu/Zn and Mn SODs (82,85,86), whereas zooxanthellae also express an Fe SOD (87). To overcome the challenges of ROS the photosynthetic organisms developed robust antioxidant and redox buffering systems composed of enzymatic antioxidants and non-enzymatic antioxidants (88, 89). The enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX), Monodehydroxy ascorbate reductase, dehydroxy ascorbate reductase, glutathione reductase, glutathione peroxidase and catalase.

Superoxide dismutase, is the family of metallo enzymes that catalyzes the destruction of O^{2-} free radical (90). It protects oxygen metabolizing cells against harmful effect of superoxide free radicals (91).

$$2O^{2-} + 2H^{+} \longrightarrow O_2 + H_2O_2$$

SOD is found as multiple isozymes but in Oscillatoriaceae species they are found in only one form. Hydrogen peroxide is detoxified by ascorbate peroxidase and catalase. APX detoxifies hydrogen peroxide by utilizing two molecules of ascorbic acid to reduce H_2O_2 to water with generation of Monodehydro ascorbate (MDHA) (Fig 1) (92). MDHA is converted to ascorbate and dehydro ascorbate (DHA) either directly or by MDHAR borrowing electrons from b type cytochrome (92), reduced ferredoxin (92, 93) or NAD (P)H (92,93). APX isozymes of chloroplasts in higher plants are encoded by only one gene, and their mRNAs are generated by alternative splicing of the genes to 3'- terminal exons (95). Chloroplastic APX isozymes are sensitive under oxidative stress conditions and hence high level of endogenous ascorbate is essentially effective to maintain APX activity under stress conditions (95). The two copies of chloroplastic APX located in thylakoid membrane and stroma are key regulators of intracellular level of H_2O_2 and has significant role in response to photooxidative stress (96). The enzymes MDHAR, DHAR and GR are involved in recovery of ascorbic acid that are oxidized by ascorbate peroxidase (Fig 2) (97). The enzymes APX, MDHAR, DHAR, GPX and GR are found in multiple isozymes that exist in chloroplast, cytoplasm and microbodies. Catalase is localized in microbodies and in Oscillatoriaceae specie they are found in only one form. It converts hydrogen peroxide to water and molecular oxygen. Catalase has high catalytic rates but low substrate affinity (92). As catalase is present in microbodies, the cytosolic protection from H_2O_2 is done by peroxidases which are found throughout the cell. Peroxidases have higher affinities for hydrogen peroxide.

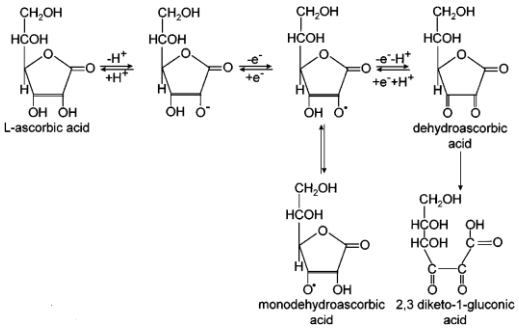

Fig.1: Schematic diagram showing oxidation of ascorbic acid. (source; Noctorr and Foyer, 1998).

Peroxidases, like catalase, catalyze the reduction of H_2O_2 to H_2O, but they require a source of electrons that subsequently becomes oxidized. Ascorbate peroxidase (EC 1.11.1.11) is a heme-containing monomeric enzyme with a molecular mass of 30 kD (98). It has a significantly lower *Km* for H_2O_2 than does catalase and uses a large pool (10–20 mM) of ascorbate as its specific electron donor to reduce H_2O_2 to H_2O in the stroma and on the thylakoids of chloroplasts (98).

Glutathione peroxidase (EC 1.11.1.9) is a tetrameric enzyme with a molecular weight of 84 kD. The enzyme is found in both selenium-containing and seleniumin dependent forms in the cytosol and mitochondria of animal tissues, but not in plants (53). This enzyme catalyzes the oxidation of glutathione, a low-molecular weight tripeptide thiol compound, with H_2O_2 (53). Glutathione is very abundant in animal tissues through the action of glutathione reductase, which regenerates reduced glutathione (53).

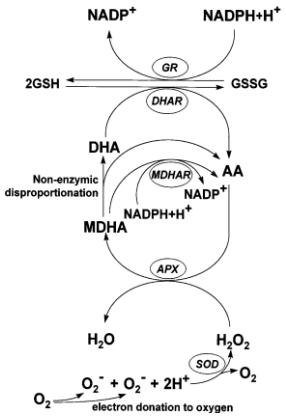

Fig.2: The Ascorbate- glutathione cycle (Source; Noctor and Foyer, 1998).

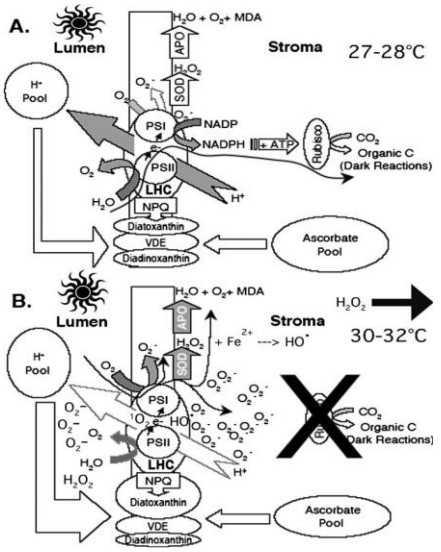

Fig.3: Schematic diagram showing oxidative stress. Normal temperature (A) and elevated temperature (B). APO, ascorbate peroxidase; LHC, light-harvesting complex; VDE, violaxanthin de-epoxidase. *(Source; Micheal, 2006)*

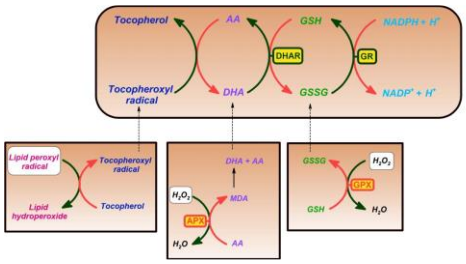

Fig.4: Schematic diagram showing protection mechanism. (Source Andras *et al.,* 2012)

Micheal (99) explained the detail of events leading to oxidative stress on the thylakoid membrane of the chloroplast of zooxanthellae is shown in Fig 3. During normal temperatures and irradiances, light is absorbed by the light-harvesting complex (LHC) and photochemistry in PSI and PSII produce ATP and NADPH for the dark reactions, in which CO_2 is fixed by the enzyme Rubisco (Fig3 A). The efficiency of photochemistry is regulated by the inter conversion of the two pigments diatoxanthin and diadinoxanthin, which is part of the xanthophylls cycle used to protect the photosystems from overexcitation (99). Superoxide dismutase (SOD) and ascorbate peroxidase enzymes in the chloroplast degrade reactive oxygen species (ROS). During heat stress, membrane fluidity changes (Fig 3 B) result in the production of ROS. Subsequently, the simultaneous over reduction of photosynthetic electron transport and the decreased fixation of CO_2 (i.e., sink

limitation) result in the over excitation of the photosystems and the flow of excitation energy primarily through PSI. The excess absorbed energy cannot be dissipated by the xanthophyll cycle (NPQ, nonphotochemical quenching) (99). More ROS are formed than can be quenched by the available enzymatic and nonenzymatic antioxidants, and some species (e.g., H_2O_2) can be exported from the chloroplast (bold horizontal arrow).

Chien and Vonshak (100) studied the enzymatic antioxidant response to low temperature acclimatization in cyanobacterium *Arthrospira platensis* (Kenya and M2) and found that when transferred to low temperature of 15°C from 33°C there was immediate cessation of growth which was followed by new acclimated growth rates. They found that during acclimatization process both strains showed changes in their antioxidant activities and found that faster growth rate of Kenya at lower temperature as compared to M2, is due to higher antioxidant enzymatic activities and hence efficient regulatory strategy of enzymatic antioxidant response to low temperature induced photo inhibition. Under salt stress also, there is a great role of antioxidant enzymes. Diego *et al.,* (101) showed that salt tolerant varieties have better protection against reactive oxygen species by increasing the activity of antioxidant enzymes under salt stress. Choudhary *et al.,* (102) investigated that under toxic effect of lead, copper and zinc in *Spirulina platensis* showed reduction in growth and increased level of MDA, SOD and proline contents. Breanne *et al.,* (103) purified 21-KDa Fe-SOD polypeptide and sequenced the N terminus and found that *sodF* encodes an open reading frame of 200 codons and is expressed as monocistronic transcript from a region of genome which includes genes involved in nucleic acid synthesis and repair. *sodF* mRNA was abundant and stable in cells after long term desiccation.

Kumar *et al.,* (104) found reduction in growth, photosynthetic pigments and carbohydrate of *Aulosira fertilissima, Anabaena variabilis* and *Nostoc muscorum* were accompanied with increase in their total protein, proline MDA, superoxide dismutase, ascorbate peroxidase and catalase under higher doses of endosulfan.

The non-enzymatic antioxidants include low-molecular-weight antioxidants such as ascorbate, glutathione (GSH) and tocopherols that mitigate the harmful effects of elevated ROS production (62). Non-enzymatic antioxidants can affect gene expression associated with abiotic stresses, altering acclimation responses (62). These antioxidants function as redox buffers that interact with ROS and act as a metabolic interface that modulates the appropriate induction of acclimation responses or programmed cell death (62,105-107). Ascorbate is the most abundant antioxidant and serves as an electron donor to many important reactions (62,108-110). It generally reaches a concentration of over 20 mM in chloroplasts and occurs in all cell compartments including the cell wall. It is the best-known molecule for detoxifying H_2O_2, especially as a substrate of ascorbate peroxidase (APX), an essential enzyme of the ascorbate-glutathione cycle, present in most compartments of cell (62).

Glutathione is a small, ubiquitous tripeptide molecule that is involved in plethora of cellular process in addition to its role as an antioxidant and in maintenance of cellular homeostasis (88, 111). GSH is present in high concentration than other thiols in cell (62). In stress cell maintain GSH: GSSG ratio of 20:1 (62, 112, 113). The ratio of GSH/GSSG is often used as an indicator of oxidative stress in cells, and glutathione functions as an antioxidant in many ways by reacting with $^1O_2$, O_2^-, and $HO^\bullet$. Glutathione can also act as a chain-breaker of free radical reactions and is an essential substrate for glutathione peroxidase (53). The maintenance of GSH levels, and therefore the reducing environment of cells, is crucial in preventing damage to cells exposed to conditions that promote oxidative stress. Less is known regarding the roles of glutathione in photosynthetic organisms, despite an array of studies (88, 114- 116). Cyanobacteria gene families involve glutathione metabolism compared to that of plants (88, 116). GshA from the cyanobacteria *Anabaena* sp. has been biochemically characterized but there is no evidence that its activity is redox modulated (117).

Genes required for tocopherol (vitamin E) synthesis in plants and cyanobacteria have been identified (118, 119). Tocopherols are essential for controlling non-enzymatic lipid peroxidation (118). Tocopherols are formed of

chromanol head group and prenyl side chain and are amphipatic molecule, in which the hydrophobic prenyl tail associates with membrane lipids and the polar chromanol head groups are exposed to the membrane surface (62). The antioxidant activity of tocopherols as free-radical scavenger is associated with the ability to donate its phenolic hydrogen to lipid free radicals, and with specific requirements of the molecule (Fig 4) (62). These are the degree of methylation in the aromatic ring (α > β = γ > δ), the size of the heterocyclic ring, the stereochemistry at position 2, and finally the length of the prenyl chain (optimum between 11 and 13 carbons) (62). The tocopherol levels in cells increase under higher light conditions and low carbon dioxide supply (120).

Cyanobacteria enhance their carotenoid pool in response to high light conditions, as increased production of other carotenoids with photo- protective abilities has also been observed under high irradiance levels (121). The total carotenoid content of *Lyngbya-Plectonema-Phormidium* sp. nearly doubled with a selective increase in the concentrations of β-carotene and zeaxanthin when the cells were grown in red light compared with white light. There is apparently a direct relationship between total carotenoid content and phycobilisome size for *Lyngbya-Plectonema-Phormidium*. sp. and *F. diplosiphon*, which is associated with the protective effect of carotenoids against photosensitized oxidation (122). *β*-carotene can quench both excited triplet-state chlorophyll and $^1O_2$ because they have highly conjugated double bonds.

Carotenoids can also dissipate excess excitation energy through the xanthophylls cycle (45, 53, 84, 98), a process, also known as dynamic photoinhibition, that prevents the overexcitation of the photosynthetic apparatus. Many carotenoids also serve as effective quenchers of ROS and can prevent lipid peroxidation in marine animals (123). The first cold-inducible genes to be characterized in cyanobacteria were genes for fatty acid desaturases (24, 124). The fatty acid desaturases maintain the appropriate physical state of the cell membranes (36). Subsequently, genes for RNA-binding proteins (*Rbps*) were identified as cold inducible genes (38). The *Rbps* appear to act similarly to the Csp RNA chaperones of *Escherichia coli* and *Bacillus subtilis.* The S21 protein in the small subunit of ribosomes was also shown to be induced by cold and to accumulate transiently in ribosomes at low temperatures (39). Next, *Clp* proteins were discovered as a novel family of cold-shock chaperones and proteases (125). Most recently, genes for RNA helicases (126), which appear to establish the appropriate secondary structure of mRNAs, were identified as being inducible by cold.

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