ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 2(J), pp. 24498-24501, February, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

SALINITY STRESS INDUCED ALTERNATIVE PATHWAY OF PHOSPHATE DEPENDENT GLYCOLYTIC ENZYMES IN ANABAENA SP. BHUAR002

Aparna Rai*

Department of Botany, Banaras Hindu University (BHU), Varanasi 221005, India

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0902.1673

ARTICLE INFO

Article History:

Received 18th November, 2017
Received in revised form 6th December, 2017
Accepted 25th January, 2018
Published online 28th February, 2018

Key Words:

Adaptation; Cyanobacteria; Glycolytic Pathway; Salt Stress

ABSTRACT

Cyanobacterium *Anabaena* sp. BHUAR002 (Accession no. bankit1353506 HM235817) survive under energy depleted condition in salt stress because of different adaptations which facilitate organism survival. Inorganic phosphate which is the main source of energy in the form of ATP become diminished under stress condition, organism switch their dependency towards pyrophosphate which was unaffected under stress condition and all phosphate dependent glycolytic pathway enzymes alternate their pathway toward inorganic pyrophosphate dependent alternative glycolytic pathway enzymes., By observing enzyme activity under control and stressed condition, activity of main glycolytic pathway enzymes as inorganic phosphate or ATP dependent phosphofructokinase (PFP), Glyceraldehyde 3P-dehydrogenase, Phosphoglycerate Kinase and Pyruvate kinase found to be more in control. Whereas activity of alternative glycolytic pathway enzymes (inorganic pyrophosphate dependent enzymes) as PPi dependent phosphofructokinase (PFP), NADP- glyceraldehydes 3P-dehydrogenase, Phosphoenolpyruvate carboxylase (PEPC), Malate dehydrogenase and NAD – Malic enzyme was enhanced under stress condition.

INTRODUCTION

Excessive soil salinity is anincreasing agricultural problem in different regions of the World(Rai, 2015). One of the most important factors for the reduced growth and failure of plants and cyanobacteria to grow and survive under salinity is energy limitation to meet the adaptation. Pi starvation further deregulates the physiology of cells and cause ATP over intake(Plaxton, 2004). Plant metabolic flexibility allows the preferential utilization of inorganic pyrophosphate (PPi) as an energy donor, particularly when cellular ATP pools become limited during stresses and nutritional Pi starvation. PPi permits microbes to conserve ATP (Rai, 2011; Stitt, 1998)

Use of PPi (a byproduct of secondary metabolism and anabolism) as an energy donor helps to conserve inadequate ATP pools. Concentration PPi in the cytosol is up to about 0.5 mM which is remarkably unaffectedto abiotic stresses (Stitt, 1998). These large amounts may be engaged to enhance the energetic efficiency of cellular processes. During salt stress, there is aclear drop in cytoplasm Pi levels that follow large declines in intracellular levels of ATP. During stress conditions, PPi would continue to be generated (albeit at a lower rate) as a byproduct of the synthesis of essential macromolecules. PPi- motorized processes may become acrucial facet of the metabolic alterations of plants to environmental stress that cause low ATP (but not PPi) pools (Dobrota, 2006). However, this aspect has not been worked out especially in the case of salt stress.

Alternative glycolytic pathways are shown in Figure 1, in which PPi–dependent processes contribute a significant bioenergetic benefit that may extend the survival time of ATP-depleted cells during stresses. Glycolytic reactions can bypass Pi or ATP- demanding steps of glycolysis under environmental stress conditions (Duff *et al*., 1989; Theodorou *et al*., 1992).

One alternative glycolytic pathway is catalyzed by a PPi – dependent phosphofructokinase (PFP) which under Piscarcity, can bypass the ATP dependent phosphofructokinase (PFK) byproducing fructose -1,6-bisphosphate (Plaxton and Carswell, 1999).

Second alternative glycolytic pathway found in cyanobacteria is made by theaction of non-phosphorylating NADP – dependent glyceraldehyde -3P dehydrogenase (NADP-G3PDH) that alternate Pi dependent NAD – G3PDH and phosphoglyceratekinase (Duff *et al*., 1989; Theodorou *et al*., 1992).

Corresponding author:* **Aparna Rai
Department of Botany, Banaras Hindu University (BHU), Varanasi 221005, India

Third bypass of the glycolytic pathway is catalyzed by collective activities of PEPC, MDH andNAD-malic enzyme (Theodorou and Plaxton, 1996). When Pi stress under salt stress condition can severally bound pyruvate kinase (PK), an enzyme requiring Pi ADP, PEPC, MDH and NAD-malic enzymes bypass PK and by this continue the flow of carbon in glycolysis cycle by avoiding the use of ADP but producing free Pi (Plaxton and Carswell, 1999).

Cyanobacteria under salt stress condition use extra ATP for their metabolic activities. Due to this, cellular ATP pool of cyanobacteria becomes reduced or cellular Pi level falls to a very low level. For the better survival of the organism and for energy conservation during salt stress cyanobacteria may bypass the main glycolytic pathway enzymes by alternative glycolytic pathway enzymes which are ATP or Pi independent and PPi dependent pathway. Level of PPi has been found constant under stress condition.

MATERIALS AND METHODS

Estimation of intracellular Phosphate and Pyrophosphate

A known amount of *Anabaena* sp. BHUAR002 (Rai and Rai, 2011)culture was centrifuged and collected pellet was washed with sterilized DDW and inoculated which was then incubated for 24 h in control and stressed (500 mMNaCl) concentration of Allen Arnon medium(Allen and Arnon, 1955)

Intracellular Phosphate: 5 ml of homogeneous cyanobacterial cell suspension added with 5 ml combined reagent mixture containing 1.0 ml ammonium molybdate, 2.5 ml diluted H_2SO_4, 1.0 ml Ascorbic acid and 5.0 ml Antimony Potassium Tartrate (from prepared stock solutions of reagent). Absorbency was taken after 15 min at 885 nm by spectrophotometer in triplicates. Phosphate concentration was calculated by astandard curve of phosphate (K_2HPO_4).

Intracellular Pyrophosphate: 5 ml of *Anabaena* sp. BHUAR002 for control and stressed cells (500 mMNaCl, at 24 hr of incubation) was taken and added with liquid nitrogen and allowed to evaporate at room temperature then 1.5 ml of 1.41 M $HClO_4$ was added to the frozen cell which was broken up with mortar and pestle (Autoclaved). The resulting suspension was kept on ice for 90 min and then centrifuged for 2 min at 20,000 rpm at 2^0C. The sediment was rinsed with resuspension and centrifugation in two 0.5 ml portion of sterilized DDW. The initial supernatant and the washings were neutralized with 5 M K_2CO_3 and combined.

Further, centrifugation removed the resulting $KClO_4$ to give a final supernatant; the pH was adjusted to 7-8 by glacial acetic acid. The basis of enzyme assay was measurement of the fructose 1, 6 – bisphosphate formed when the extract was added to pure pyrophosphate: fructose 6 –phosphate 1 phosphotransferase. The assay mixture contained in 1 ml: 50 mM 2 amino – 2 (hydroxymethyl) – 1, 3 – propanediol (adjusted to pH 8.0 with glacial acetic acid), 2 mM Magnesium acetate, 1 mM fructose 6- Phosphate, 0.15 mM NADH, 20 µM Fructose 2, 6 bisphosphate, 0.45 unit fructose-bisphosphate aldolase, 1.7 unit glycerol – 3 – phosphate dehydrogenase, 5.0 unit triosephosphateisomerase and upto 0.87 ml extract (Edwards *et al*., 1984). The reaction was started with the addition of 0.1 unit pyrophosphate fructose 6 – phosphate 1-phosphotransferase and was allowed to go to completion. Oxidation of NADH was measured in triplicates by spectrophotometrically at 340 nm.

Measurement of rate of respiration

The rate of respiration (oxygen consumption in dark) was determined in *Anabaena* sp. BHUAR002 for control and stressed cells (500 mMNaCl, at 24 hr of incubation) by measuring total O_2 consumed in dark for a given period of time minus nonspecific O_2 uptake. To determine the non-specific O_2 uptake the consumption of O_2 was measured in presence of antimycin A (2 µM) (Grant, 1978).

Enzyme Assay

It includes extraction of enzyme and estimation of nine different glycolytic enzymes (main glycolytic pathway and alternative glycolytic pathway enzymes) of *Anabaena* sp. BHUAR002 in control and stressed cells (500 mMNaCl) at 24 h of incubation.

Enzyme extraction

The experiment was carried out at 4^0C. Frozen cells (0.5 g) were ground (1:1) w/v in homogenization buffer for 10 min using sterilized autoclaved mortar and pestle and the homogenate was centrifuged for 10 min at 10000 rpm supernatant used for enzyme assay(Duff *et al*., 1989).

Estimation of Glycolytic Enzyme Activity

Different glycolytic enzymes and alternate glycolytic enzymes were assayed in triplicates at 25°C by reduction of NAD^+ or $NADP^+$ or the oxidation of NADH or NADPH at 340 nm using a Hitachi, model no. U-2900 Spectrophotometer.

Main Glycolytic Pathway Enzymes: Phosphate dependent Phosphofructokinase(PFK)(Bergmeyer, 1974), ATP dependent glyceraldehydes 3P dehydrogenase (Kelly and Gibbs, 1973),Phosphoglycerate kinase (Bücher, 1955)and Pyruvate kinase (Bergmeyer *et al*., 1974).

Bypass Glycolytic Pathway Enzymes: Inorganicpyrophosphate-dependent phosphofructokinase (PFP) (Schaftingen *et al*., 1982), NADP – dependent glyceraldehydes-3P dehydrogenase (NADP – G3PDH) (Kelly and Gibbs, 1973)Inorganic pyrophosphate-dependentphosphoenolpyruvate carboxylase (Wohl and Markus, 1972), Inorganic pyrophosphate-dependent Malate dehydrogenase (Bergmeyer, 1974), Inorganic pyrophosphate-dependent NAD – Malic enzyme(Geer *et al*., 1980).

RESULTS AND DISCUSSION

Acclimatization under salt stress by conserving ATP pool is mainly due to hyperactivity of ATP independent and PPi dependent glycolytic pathway enzymes. Energy-dependent plant stress acclimation explained by(Dobrota, 2006)mainly studied about the survival of the organism under salt stress condition when ATP was more required to cells for better survival. Under this condition, more ATP was consumed to separate different metabolic activity of cells, so thelevel of ATP or inorganic phosphate become diminished (Ashihara *et al*., 1988). Experimental results also show that rate of respiration and level of phosphate become diminished under stress conditions but pyrophosphate level was found more or less constant under control and stress condition (Table-1).

Table 1 Rate of respiration, intracellular phosphate and pyrophosphate in control and stressed cyanobacterial cells

Sl no	Parameters	Control	500 mMNaCl
1	Rate of Respiration	0.32 ±0.28 µM O_2 consumed ml^{-1}	0.52 ±0.2 µM O_2 consumed ml^{-1}
2	Intracellular Phosphate	20.1 ±0.18 µM ml^{-1}	9.2 ±0.26 µM ml^{-1}
3	Intracellular Pyrophosphate	8.02 ±0.2 µM ml^{-1}	7.14 ±0.11 µM ml^{-1}

Under stress condition when cellular ATP pool become diminished PPi can work as an energy donor (Plaxton and Carswell, 1999). Several studies i.e., MicroRNA399 signal for regulation of plant phosphate homeostasis by (Pant *et al*., 2008), Phosphoenolpyruvate Carboxylase activity measured by Meyer (Meyer *et al*., 1988) and malate dehydrogenase as an alternative pathway enzyme characterized in cyanobacterium Coccochlorispeniocystis (Norman and Colman, 1988), Contribution of Malic enzyme, Pyruvate kinase, and Phosphoenolpyruvate carboxylase (Edwards *et al*., 1998) indicate towards this fact.

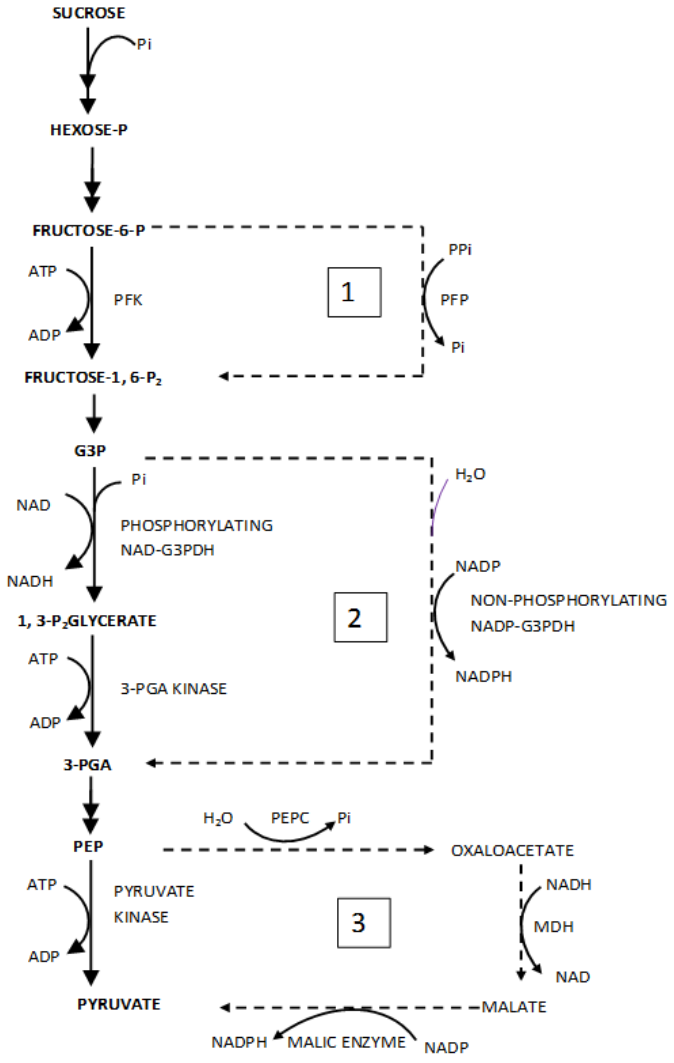

Figure 1 A model for plant(Dobrota, 2006; Duff *et al*., 1989)and cyanobacteria(Rai, 2011)suggesting the three salt stress inducible glycolytic bypass pathway in cyanobacteria, **1**- representing PPi dependent phosphofructokinase bypass of ATP dependent phosphofructokinase, **2**- represent Non-Phosphorylating NADP-G3PDH which bypasses two ATP dependent glycolytic enzymes i.e. Phosphorylating NAD-G3PDH and 3- PGA Kinase third bypass pathway **3**-represent combination of three ATP independent enzymes PEPC, MDH and NAD-Malic enzyme which alters ATP dependent Pyruvate Kinase of main glycolytic pathway.

It was found that under stressed condition when cellular ATP level become diminished and level of inorganic phosphate become lower, main glycolytic pathway enzymes as PFK, NAD-G3PDH, 3-PGA Kinase and Pyruvate Kinase bypasses by PFP, NADP-G3PDH, PEPC, Malate dehydrogenase and NAD- Malic enzyme inorganic pyrophosphate-dependent or ATP independent enzymes (Figure -2). A similar pattern of enzyme activities were observed in higher plants under stress condition(Stitt, 1998; Sung *et al*., 1988).

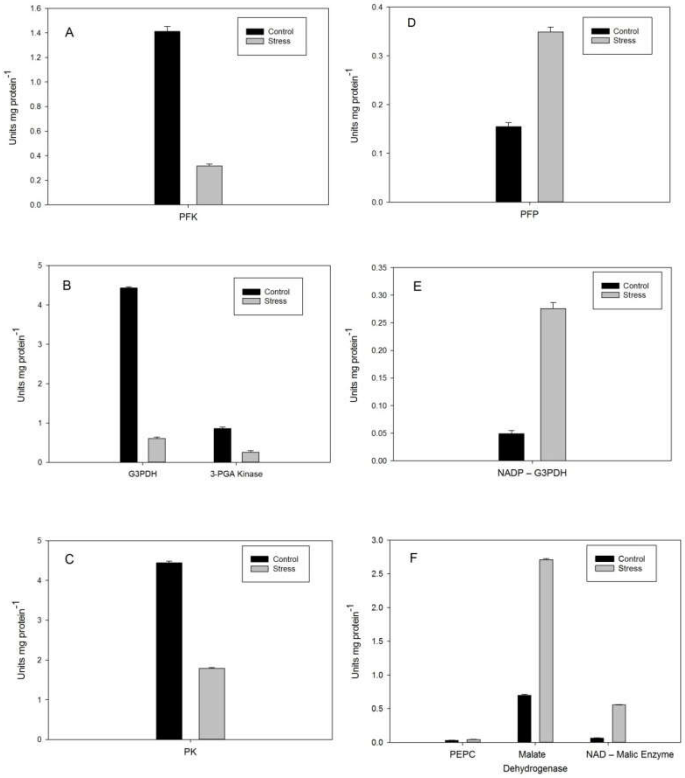

Figure 2 Activities of different glycolytic pathway enzymes (A, B, C) and alternative glycolytic pathway enzymes (D, E, F)

In *Anabaena* sp. BHUAR002 level of Pi dependent Phosphofructokinase (PFK) was found 77% less in stress (500 mMNaCl, at 24 h of incubation) compared to control and PPi dependent PFK was found 125.5% more in control compared to stress. NAD-G3PDH and Phosphoglycerate Kinase both were found more in control i.e., 86.4 and 70.7% respectively compared to stress and NADP-G3PDH was found 462% more in stress compared to control. 59.7% more pyruvate kinase was found in control compared to stress.

Table 2 Different Glycolytic enzymes activity in control and stressed cyanobacterial cells

Sl No	Name of the Enzyme	Enzyme activity (units/mg protein)		% age increase/ decrease
		Control	Stress	
1	PFK	1.412±0.039	0.317±0.016	-77.4936
2	PFP	0.154±0.008	0.349±0.010	125.5131
3	NAD-G3PDH	4.427±0.012	0.603±0.012	-86.3668
4	3-PGA Kinase	0.857±0.079	0.251±0.024	-70.665
5	NADP –G3PDH	0.048±0.005	0.275±0.010	462.9361
6	PK	4.440±0.045	1.789±0.021	-59.7064
7	PEPC	0.029±0.002	0.042±0.006	41.42083
8	Malate dehydrogenase	0.696±0.016	2.708±0.019	288.8753
9	NAD – Malic enzyme	0.062±0.002	0.555±0.006	787.3676

Phosphoenolpyruvate carboxylase, Malate dehydrogenase, a nd NAD – Malic enzymesare alternative glycolytic pathway enzymes were found to be 41.4, 288.8 and 787.4% respectively and was found more abundantin stress compared to control (Table-2).

Acknowledgments

This Work was supported by Council of Scientific and Industrial Research (CSIR), New Delhi, India and thanks to Prof. A. K. Rai, Department Of Botany, Banaras Hindu University, Varanasi, India, for suggestions.

References

Allen, M.B., Arnon, D.I., (1955). Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. *Plant Physiol.* 30, 366-372.

Ashihara, H., Li, X.-N., Ukaji, T., (1988). Effect of inorganic phosphate on the biosynthesis of purine and pyrimidine nucleotides in suspensioncultured cells of *Catharanthus roseus*. *Annals of Botany* 61, 225-232.

Bergmeyer, H.U., (1974). Methods of enzymatic analysis, 2nd ed ed.

Bergmeyer, H.U., Gawehn, K., Grassl, M., (1974). in: Bergmeyer, H.U. (Ed.) Methods of enzymatic analysis. Academic press, Inc, New York, NY, pp. 509-510.

Bücher, T., (1955). Methods in enzymology.

Dobrota, C., (2006). energy dependent plant stress acclimation. *Rev Environ Sci Biotechnol* 5, 243-251.

Duff, S.M.G., Moorhead, G.B.G., Lefebvre, D.D., Plaxton, W.C., (1989). Phosphate starvation inducible 'bypasses' of adenylate and phosphate dependent glycolytic enzymes in *Brassica nigra* suspension cell. *Plant Physiol* 90, 1275-1278.

Edwards, J., Tom ap, R., Wilson, P.M., Morrell, S., (1984). Measurement of the inorganic pyrophosphate in tissues of *Pisum sativum* L. *Planta* 162, 188-191.

Edwards, S., Bich-Ty, N., Do, B., Roberts, J.K.M., (1998). Contribution of Malic Enzyme, Pyruvate Kinase, Phosphoenolpyruvate Carboxylase, and the Krebs Cycle to Respiration and Biosynthesis and to Intracellular pH Regulation during Hypoxia in Maize Root Tips Observed by Nuclear Magnetic Resonance Imaging and Gas Chromatography-Mass Spectrometry. *Plant Physiol.* 116, 1073-1081.

Geer, B.W., Krochko, D., Oliver, M.J., Walker, V.K., Williamson, J.H., (1980). A comparative study of the NADP-malic enzymes from *Drosophila* and chick liver. *Comp. Biochem. Physiol.* 65B, 25-34.

Grant, N.G., (1978). Respiratory Process in mitochondria, in: Hellebust, J.A., Craigie, J.S. (Eds.), Handbook of phycological methods: physiological and biochemical methods. Cambridge University Press, Cambridge, pp. 329-336.

Kelly, G.J., Gibbs, M., (1973). Nonreversible D-glyceraldehyde 3- phosphate dehydrogenase of plant tissues. *Plant Physiol.* 52, 111-118.

Meyer, C.R., Rustin, P., Wedding, R.T., (1988). A Simple and Accurate Spectrophotometric Assay for Phosphoenolpyruvate Carboxylase Activity. *Plant Physiol* 86(2), 325-328.

Norman, E.G., Colman, B., (1988). Evidence for an incomplete glycolate pathway in cyanobacteria. *J Plant Physiol* 132, 766-768.

Pant, B.D., Buhtz, A., Kehr, J., Scheible, W.R., (2008). MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *The Plant J.* 53(5), 731-738.

Plaxton, W., (2004). Plant responce to stress: Biochemical adaptations to phosphate deficiency in: Goodman, R. (Ed.) Encyclopedia of palnt and crop science Marcel Dekker, nc, NY.

Plaxton, W.C., Carswell, M.C., (1999). Metabolic aspects of the phosphate starvation response in plants., in: Lerner, H.R. (Ed.) Plant responses to environmental stress: from phytohormones to genome reorganization. Marcel-Dekker, New York, USA, pp. 350-375.

Rai, A., (2011). Cyanobacterial stress acclimation under energy depleted condition. *Journal of Plant Development Sciences* 3(1&2), 113-116.

Rai, A., (2015). Salt tolerance by cyanobacteria and reclamation of usar soil *Indian Journal of Plant Sciences* 4(2), 59-62.

Rai, A., Rai, A.K., (2011). Morphological and molecular characterization of two usar soil cyanobacterial isolates. *Journal of Scientific and Applied Research* 2(2), 136 - 141.

Schaftingen, E.V., Lederer, B., Bartrons, R., Hers, H.G., (1982). A kinetic study of pyrophosphate: fructose-6-phosphate phosphotransferase from potato tubers.Application to a microassay of fructose 2,6-bisphosphate. *Eur. J. Biochem*. 129, 191-195.

Stitt, M., (1998). Pyrophosphate as an energy donar in the cytosol of plant cells: an enigmatic alternative to ATP *Bot Acta* 111, 167-175.

Sung, S., Dian-Peng, X., Galloway, C., Black, C., (1988). A reassessment of glycolysis and gluconeogenesis in higher plants. *Physiol. Plant* 72, 650-654.

Theodorou, M.E., Cornel, F.A., Duff, S.M., Plaxton, W.C., (1992). Phosphorus starvation-inducible synthesis of the alpha-subunit of the pyrophosphate-dependent phosphofructokinase in black mustard suspension cells. *J Biol Chem* 267, 21901-21905.

Theodorou, M.E., Plaxton, W.C., (1996). Purification and Characterization of pyrophosphate-dependent phosphofructokinase from phosphate-starved *Brassica nigra* suspension cells. *Plant Physiol* 112, 343-351.

Wohl, R.C., Markus, G., (1972). Phosphoenolpyruvate Carboxylase of *Escherichia-Coli* - Purification and Some Properties. *J. Biol. Chem*. 247(18), 5785-5792.

****