DEVELOPMENT OF ACID TOLERANT CYANOBACTERIAL MUTANTS WITH HIGHER N_2 FIXING POTENTIAL AND BIOFERTILIZER VALUE FOR RICE UNDER ACID SOIL CONDITIONS

Thesis submitted in part fulfilment of the requirements for the degree of Master of Science (Agriculture) in Agricultural Microbiology to the Tamil Nadu Agricultural University, Coimbatore - 641 003

By

Ms. K. MALATHY

(I.D.NO. 98-605-007)

CENTRE FOR ADVANCED STUDIES IN AGRICULTURAL MICROBIOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE - 641 003.

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CERTIFICATE

This is to certify that the thesis entitled "**DEVELOPMENT OF ACID TOLERANT CYANOBACTERIAL MUTANTS WITH HIGHER N_2 FIXING POTENTIAL AND BIOFERTILIZER VALUE FOR RICE UNDER ACID SOIL CONDITIONS**" submitted in part fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in AGRICULTURAL MICROBIOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a bonafide record of research work carried out by **Ms. K. MALATHY** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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(Dr. G. GOPALASWAMY)
Chairman

APPROVED BY

Chairman :

(Dr. G. GOPALASWAMY)

Members :

(Prof. Dr. S. KANNAIYAN)

(Dr. R. SAMIYAPPAN)

Date : 20.10.2k

(External Examiner)

ABSTRACT

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DEVELOPMENT OF ACID TOLERANT CYANOBACTERIAL MUTANTS WITH HIGHER N_2 FIXING POTENTIAL AND BIOFERTILIZER VALUE FOR RICE UNDER ACID SOIL CONDITIONS

By

K. MALATHY

Degree : Master of Science in Agriculture
(Agricultural Microbiology)

Chairman: **Dr. G. GOPALASWAMY,**
Associate Professor,
Centre for Plant Molecular Biology,
TNAU, Coimbatore - 3.

2000.

Development of acid tolerant cyanobacteria is cost effective and environmental friendly for improving the rice production in acid soils. Though several acid tolerant cyanobacterial cultures were isolated from acid soils of Tamil Nadu, development of efficient acid tolerant cyanobacterial mutants with higher biomass and N_2 fixing ability through induced mutagenesis is worth investigating.

The effect of the chemical mutagens *viz.*, Ethyl methane sulphonate, N-methyl N-nitro N-nitrosoguanidine and Colchinine at various concentrations and physical mutagenisis with UV rays on five acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$, *Oscillatoria*-AT-TGK-$5B_8$, *Westiellopsis*-AT-TGK-$4A_2$ and *Westiellopsis*-AT-TGK-$4A_7$ were studied. In general, mutation frequency varied with mutagen concentration as well as cyanobacterial cultures. Maximum mutation frequency was obtained at E_{250} and N_{150} for *Westiellopsis* cultures, while at C_{100}, *Oscillatoria* showed maximum

mutation frequency. For physical mutagenesis, lower the exposure time, higher was the mutation frequency. Maximum mutation frequency was at 10 min time exposure by all the acid tolerant cyanobacterial cultures. It is interesting to note that the lethal dose concentration of EMS is more followed by Colchinine and NTG. The reversion frequency of the acid tolerant cyanobacterial mutants developed by EMS was less and varies with mutagen concentration. It was less for *Westiellopsis* and *Nostoc* at E_{250} and for *Oscillatoria* and *Anabaena* at E_{200}. However, in the case of NTG, the reversion frequency was minimum at 150 ppm for all the acid tolerant cyanobacterial cultures.

The acid tolerant cyanobacterial mutants *viz.*, *Anabaena*-AT-MGK-$5A_6$ - E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria*-AT-MGK-$5B_8$-E_{200}, *Westiellopsis* - AT-MGK-$4A_2$-E_{250} and *Westiellopsis*-AT-MGK-$4A_7$-E_{250} were obtained through EMS mutagenesis and their growth, biomass production and other biochemical constituents *viz.*, ammonia excretion, chlorophyll a content, protein content, polysaccharide production, amino nitrogen content, nitrogenase activity, free amino acid liberation and methionine content were assessed and compared with their wild parents. All the mutants recorded higher growth, biomass production and the above biochemical constituents than their wild parents. Among the mutants, *Westiellopsis* cultures performed well. All the acid tolerant cyanobacterial mutants performed well under acidic pH *viz.*, 4,5 and 6 than the normal and the wild acid tolerant *Anabaena* culture.

The inoculation of acid tolerant cyanobacterial mutants significantly increased the seedling height, flood water ammonia content, total chlorophyll content and total nitrogen content of the ASD 16 rice seedlings compared to the uninoculated control at all the time intervals. Among the mutants, *Westiellopsis* cultures significantly increased the performance of the rice seedlings. The study clearly indicated the positive influence of the acid tolerant cyanobacterial mutants in augmenting the rice yield is acidic soils in a sustainable manner.

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(K. MALATHY)

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INTRODUCTION

1. INTRODUCTION

Asia accounts for 60 per cent of the global population, 92 per cent of world's rice production and 90 per cent of global rice consumption. India, the home of 16 per cent of world's population with 2.42 per cent of world's total area, has the largest area under rice cultivation (43 million ha) but stands next to China in production. World's annual rice production is 468 million tonnes y^{-1}, with China standing first (175 million tonnes y^{-1}) followed by India (100 million tonnes y^{-1}). This may be due to the low average productivity of 2.9 t ha^{-1} as compared with China's average of 5.8t ha^{-1} (IRRI, 1993). Tamil Nadu is one of the major rice producing states in India with an area of 21 lakh ha and ranks first with annual productivity of 3.5t ha^{-1}. The population in India may exceed 1.4 billion by the end of 2025 and the demand for rice is expected to increase by 46 per cent (IRRI, 1993).

Nitrogen is an important element essential for increasing the productivity of crops. The rice plant takes 1kg of N to produce 15-20 kg of grains. With the present day high yielding rice varieties, there is every chance for the depletion of nitrogen reserve in the soil. The nitrogen demand is partially met by chemical fertilizers and to some extent by BNF. There is a gap of 16 per cent between the supply and demand for nitrogenous fertilizers. This has resulted in the use of eco-friendly biofertilizers to increase rice production. Moreover indiscriminale use of inorganic nitrogenous fertilizer not only affects the productivity but also the soil health in the long run.

The vast resources of atmospheric nitrogen has been continuously tapped by microorganisms through BNF. Tropical lowland rice can use the nitrogen that is available either naturally through BNF or from mineralisation in soil to produce 2-3 t of grain ha^{-1}. Manufacturing the fertilizer for today's need requires 544×10^7 MJ of fossil fuel energy which is equivalent to 13 million t of oil. So the BNF derived N, assumes significance in the lowland system that provide about 80 per cent of the world's rice. Nitrogen fixing microorganisms convert about 139 to

170 million t of nitrogen every year into fertilizer nitrogen for which the energy bill is paid by nature (Kannaiyan, 2000). The fact that the total world's biological nitrogen fixation is three times that of industrially produced nitrogen, demonstrates the significance of BNF in agriculture and natural nitrogen cycles.

Biofertilizers constitute a highly potential, cheap and easily manageable input which not only augments the nutrient component of the soil but also increases its availability over a longer period of time. This helps the crop plants in utilizing more nutrients. Diazotrophic cyanobacteria form the most potential source of biologically fixed nitrogen for lowland rice. Cyanobacteria are unique group of photosynthetic prokaryotes, many of which are endowed with the capacity to fix nitrogen (Postgate, 1982; Stewart and Rowell, 1986) in an oxygen free atmosphere. The cyanobacteria perform the process of oxygen sensitive nitrogen fixation and oxygen evolving photosynthesis simultaneously (Hallenbeck, 1987). The oxygen free atmosphere is essential for nitrogen fixation by nitrogenase (Gallon, 1990), as its components Fe-protein and Mo-Fe protein are inactivated equally by oxygen (Donze *et al.*, 1974). Deposition of thick, three layers and complete elimination of phycobiliproteins associated with the loss of oxygen evolving photosystem II in heterocysts (Haselkorin, 1978; Wolk, 1982) afford conducive reducing environment for nitrogenase action in cyanobacteria.

The agronomic potential of cyanobacterial N_2 fixation in rice fields was first recognised in India during 1939 by De . Diazotrophic cyanobacteria have been found to play a vital role in sustaining the cultivation of wetland rice without added nitrogen fertilizer due to their inherent ability to add fixed nitrogen to such habitats under aerobic photosynthetic conditions (Singh, 1961). In recent years, the practice of utilizing cyanobacteria as an efficient source of biofertilizer for rice have been advocated and adopted in India (Venkataraman, 1981; Kannaiyan, 1990). The nitrogen fixing cyanobacteria, in addition to contributing upto 30 kg N ha^{-1} $season^{-1}$,

also add organic matter, excrete growth promoting substance (Venkataraman and Neelakantan, 1967), solubilize insoluble phosphates (Bose *et al.*, 1971), improve the fertilizer utilizing efficiency of the crop (Goyal, 1987), and mend the physical and chemical nature of the soil (Aiyer *et al.*, 1971). Algalization has been shown to have ameliorating effect on saline and saline alkali soils (Subhashini and Kaushik, 1981), increase the soil aggregate size (Kaushik and Krishnamurthy, 1981), reduce the oxidizable matter content of the soil (Aiyer *et al.*, 1972), remove soil compaction and narrow the C/N ratio.

The rice field ecosystem provides an environment favourable for the growth of cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability. However, cyanobacteria in rice fields are subjected to various abiotic stresses *viz.*, acidity, herbicide, salinity etc. which reduced the population and nitrogen fixation. To overcome the stresses, much emphasis was given for the development of location specific cultures adopted to the stress environment (Gopalaswamy, 2000). Several promising stress tolerant cyanobacterial cultures have been isolated. Their performance could still be improved by genetic manipulation of the desired organisms. Induced mutagenesis is one such way to manipulate the desired organisms. Induced mutagenesis which results in altered biochemical and genetical characteristics have been extensively used in uncovering the biochemical and genetical problems in microorganisms. Besides providing new material for evolution, mutagens also provide materials for recombinations and selection for breeding. The potential value of cyanobacteria as biofertilizer cannot be realized without the genetic improvement for important characters.

A number of studies have been made on the effects of chemical mutagens on various organisms. EMS has been recognised as a powerful mutagen of relatively low lethality to microorganisms (Strauss, 1961). NTG is an extremely potent mutagen for a wide spectrum of organisms (Neale, 1976). Diethyl sulphate and hy-

droxylamine have been shown to induce mutations in certain microorganisms and higher plants (Westergaard, 1957). Procedures for mutagenesis in acid conditions for selection by antibiotic enrichment in cyanobacteria have been developed. In view of the marked variations amongst cyanobacteria with respect to their biological activity and response to various ecological stresses, it is essential to screen and select promising stress compatible strains. With this aim, the following objectives have been set for this investigation.

i) To screen the cyanobacterial cultures maintained at *Azolla* laboratory for acid tolerance, for higher growth and biomass production.

ii) Induced mutagenesis of the selected acid tolerant cyanobacterial cultures using EMS, NTG, Colchicine and UV rays.

iii) Assessment of LD_{50} value, mutation frequency and reversion frequency of the mutants.

iv) Biochemical characterization of the mutants and wild type acid tolerant cyanobacterial cultures.

v) To study the effect of acidity on the growth, ammonia excretion, nitrogen fixation and other biochemical coustituents of the acid tolerant cyanobacterial mutants.

vi) Testing the performance of the mutants as biofertilizer for rice seedlings.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. CYANOBACTERIA - GENERAL

Cyanobacteria otherwise known as Blue Green Algae are the most thoroughly studied and understood group of prokaryotic microorganism, widely distributed in nature and use sunlight as the sole energy source for the fixation of carbon and nitrogen (Stewart, 1980). Cyanobacteria have very simple inorganic nutrient requirements and their mass production is much simpler than those of conventional chemical fertilizers or biofertilizers.

Many filamentous forms are capable of carrying out dinitrogen fixation in specialized cells termed `heterocysts', although a few nitrogen fixing forms in which no such differentiation occurs. Until the findings of Wyatt and Silvey (1969), it was believed that nitrogen fixing cyanobacteria are only heterocystous. Then many nonheterocystous forms like *Aphanothece, Oscillatoria, Gloeocapsa, Plectonema* and *Trichodesmium* have been found to fix nitrogen, which account for the major share of nitrogen fixed in the oceans of this planet (Stewart, 1970; Venkataraman, 1972 and Kannaiyan, 1979). Heterocysts are terminally differentiated cells, the interior of which is microanaerobic to carry out the oxygen sensitive process of nitrogen fixation. Heterocysts are surrounded by a thick coat and mature heterocysts differs from vegetative cells with respect to nitrogen metabolism in three ways by having nitrogenase, higher level of glutamine synthetase and a lower level of glutamate synthase (Haselkorn, 1978). There are strains of cyanobacteria that contain a single heterocyst at the end of a filament and others that form a patterned array within the filament. Heterocysts are usually spaced at regular intervals along the filament with upto 10 per cent of the cells differentiating in free living forms (Wolk *et al.*, 1994).

Biological nitrogen fixation (BNF) in rice fields and its use were reviewed by Roger and Watanabe (1986). Roger *et al.* (1988) have estimated *in situ* nitrogen

fixation rates in rice fields. It is generally assumed that the nitrogen fixed by cyanobacteria are made available to the rice plants through exudation, autolysis and microbial decomposition (Roger *et al.*, 1993). In India, cyanobacterial inoculants are grown in cement tanks and field plots and dried soil based algal biofertilizer comprising composite cultures were inoculated to rice soil where they multiply and fix nitrogen (Venkataraman, 1982 and Kannaiyan, 1984).

2.2. PHOTOSYNTHETIC PIGMENTS OF CYANOBACTERIA

One of the most important biological processes on earth is photosynthesis, the conversion of light energy to chemical energy. The central light harvesting pigment of photosynthesis is chlorophyll. Chlorophylls are located in membrane systems called photosynthetic membranes, where the light reactions of photosynthesis are carried out. Most chlorophyll molecules are antenna molecules and function only to harvest light energy and transfer it on to special molecules called reaction center chlorophylls (Brock, 1970).

Cyanobacteria are photosynthetic prokaryotes that carry out plant type oxygenic photosynthesis (Sprent and Sprent, 1990). They harbour light harvesting pigments such as chlorophyll a, phycocyanin, allophycocyanin, phycoerythrin and other phycobiliproteins (Ohki *et al.*, 1987 and Smith and Rogers, 1988). The chlorophyll a content ranged from 0.75 to 1 per cent of the dry weight of the cell (Chapman, 1973). Within the cell the light energy trapped by the phycobiliproteins is transferred to chlorophyll a with an efficiency approaching 100 per cent (Porter *et al.*, 1978 and MacColl and Guard Friar, 1987).

Pinevich (1986) found no change in the chlorophyll content of thylakoids in the mutant *A. variabilis* R-26, defective for nitrogen fixation. Rathore *et al.* (1993) reported that molybdenum deficiency slightly decreased the chlorophyll a content in N_2 fixing cyanobacterium, *Anabaena doliolum*.

The growth phase was slowed down and the chlorophyll synthesis in cyanobacteria was inhibited by acidic pH . At pH 5, the chlorophyll synthesis is very low in the initial stages after inoculation (Subramanian and Shanmugasundaram, 1987). However no inhibition in chlorophyll a was noticed at pH 5.0 (Dorling *et al.,* 1997). Even at pH 4 and 5, chlorophyll content was recorded by acid tolerant cyanobacterial cultures (Tamilselvam, 1998). Ruecker *et al.* (1995) studied the response of chlorophylls to variations of growth limiting factors such as nitrogen, phosphorus and light limitation in three filamentous blue green algae. They reported that the adaptive change due to nutrient limitation was independent of chlorophyll a content. Samal (1991) has recorded higher chlorophyll a synthesis in free living *Nostoc* and *Anabaena* than algal symbionts of *Azolla.* Mahesh (1992) has stated that the pigment synthesis was higher under immobilized state of both free-living and symbiotic cyanobacteria compared to free-living condition. Kannaiyan *et al.* (1992) recorded more chlorophyll content at 250 ppm sodium chloride concentration. Nam *et al.* (1995) reported that the phycocyanin and chlorophyll content and phycocyanin/chlorophyll ratio were increased in cells grown under blue green light compared with those grown in white light. Bisen and Shanthy (1993) have developed a DCMU resistant mutant of *A. doliolum* with high C-phycocyanin content when compared with the Chlorophyll a rich parent. The photosynthetic properties differed from the parent due to either alteration of the DCMU binding protein or removal of the protein. Bastia and Adhikary (1995) developed a phycoerythrin lacking mutant induced by DCMU in photoheterotrophically grown *Nostoc linckia* and the mutant strain grew well, synthesized high amount of chlorophyll and phycocyanin and possessed higher nitrogen fixing capacity in comparison to the parent strain. Chlorophyll content of herbicide tolerant cyanobacterial cultures was not much inhibited by butachlor(Selvakumar, 1999).

2.3. NITROGEN FIXATION

Many cyanobacteria are able to grow at the expense of atmospheric nitrogen under aerobic conditions and many more are able to perform N_2 fixation when anaerobic conditions are provided experimentally. Given the widespread distribution in nature of these microorganisms, it is believed that cyanobacteria contribute significantly to the process of biological nitrogen fixation and thus participate in restoring the soil fertility. Nitrogen can constitute as much as 11 per cent of the dry weight of a cyanobacterial cell (Wolk, 1973). The cyanobacteria mainly use inorganic compounds (nitrate, dinitrogen and ammonium) to fulfill their N requirements, but urea and other organic sources of N , such as some amino acids, can also be assimilated by some cyanobacteria.

Process of nitrogen fixation like in all other diazotrophs is facilitated by the enzyme nitrogenase (Stewart, 1980). The nitrogenase enzyme complex consists of two component proteins. One is Mo-Fe protein called dinitrogenase and the other is an Fe containing protein, dinitrogenase reductase. Algal nitrogenase resembles those of bacteria and legume in many respects such as oxygen sensitivity, cold lability, its requirement for ATP and reductant (Haystead *et al.*, 1970). Dinitrogenase is a tetramer composed of two pairs of different subunits which contains 4 (4Fe-4S) clusters and dinitrogenase reductase is a dimer composed of two identical subunits with a single (4Fe-4S) cluster. The Mo-Fe cofactor is thought to affect the binding and reduction of dinitrogen to ammonia (Burris *et al.*, 1980).

Electrons for the reduction of N_2 are supplied by dinitrogenase reductase. The reaction is highly endergonic requiring about 12 to 15 mol of ATP per mol of N_2 reduced. Nitrogenase can reduce a number of other substances such as acetylene, hydrogen azide, hydrogen cyanide or nitrous oxide. Molecules of these compounds contain a triple bond like N_2. Of these acetylene which is reduced to ethylene, is particularly important because both acetylene and ethylene can be

detected conveniently and with great accuracy by gas chromatography. The acetylene reduction assay is now used routinely to estimate nitrogenase activity (Bothe *et al.*, 1980).

The heterocyst is the actual site of N_2 fixation and the enzyme nitrogenase is present in heterocyst. Nitrogenase activity require a source of ATP and reductant (Shi and Hall, 1988) and photosynthesis is the ultimate source of all reductant and ATP (Losadda *et al.*, 1981). Absence of oxygen is one of the most crucial requirement for nitrogen fixation and cyanobacterial nitrogenase is irreversibly inhibited by oxygen (Stewart, 1980). The superoxide dismutase enzyme in *Plectonema boryanum, Anacystis nidulans and Anabaena variabilis* would function as a protection mechanism of nitrogenase (Okada *et al.*, 1979). Mackey and Smith (1983) have described superoxide dismutase activity in free living cyanobacterium, *Anabaena cylindrica.* Canini *et al.* (1991) have also investigated the occurrence of superoxide dismutase in the heterocysts of the symbiont *A. azollae.* Rodriquiz *et al.* (1991) have stated that Ca^{2+} is required for aerobic nitrogen fixation by heterocystous blue green algae. The role of Ca^{2+} seems to be related to protection of nitrogenase from inactivation by conferring heterocysts resistance to oxygen.

The nitrogenase of *Anabaena cylindrica* like its bacterial analogue is an acidic protein and it does not lack any of the activities associated with other nitrogenase (Haystead *et al.*, 1970). Reddy *et al* (1989) have reported that addition of nitrite to rapidly growing filaments of *Anabaena variabilis* caused an immediate drop in nitrogenase activity and elimination of combined nitrogen induced heterocyst differentiation.

Higher nitrogenase activity occurred in *Calothrix* strain D-764 for any particular light flux following a dark period than with continuous illumination at the same flux (Islam and Whitton, 1992). Kumar and Kumar (1994) reported that the exogenous supplementation of fructose at 0.1 per cent and 1.3 per cent to the cells

of *Anabaena* sp strain CA, stimulated light dependent growth and nitrogenase activity. Prosperi *et al.* (1992) reported that fructose was the best substrate for supporting the growth and dinitrogen fixation by cyanobacteria in mixotrophy, photoheterotrophy and heterotrophy. Samal (1991) reported higher nitrogenase activity in free-living cyanobacteria, *Anabaena* and *Nostoc*. Samal and Kannaiyan (1992) have reported that the incorporation of biotin and pantothenic acid stimulated the growth and heterocyst frequency of the cyanobacterial isolates and also exhibited higher nitrogenase activity and ammonia excretion. Amsaveni and Kannaiyan (1995) have reported that the phosphorus sources had positive influence on nitrogenase activity and ammonia excretion of salt tolerant cyanobacteria. Nitrogenase activity of the PUF immobilized cyanobacteria was significantly higher than that of cultures in the free living state (Uma and Kannaiyan, 1996).

Cells of the filamentous, non-heterocystous cyanobacterium *Microcoleus* sp, grown under a 10 h light / 14 h dark cycle with N_2 as inorganic nitrogen source showed highest nitrogenase activity at the end of the light phase (Sroger, 1997). Kapoor *et al.* (1997) recorded higher growth, nitrogen fixation, cell nitrogen as well as uptake of organic carbon sources under photoheterotrophic conditions in heterocystous, filamentous cyanobacterium *Camptylonema indicum*. Around 44 cyanobacterial isolates belonging to 8 genera which fixes nitrogen was recorded by Begum and Mandal (1997). Immobilization of the cells of *Anabaena variabilis* in calcium alginate gel increased heterocyst differentiation and nitrogenase activity in both wild type and its NaCl resistant strain (Chauhan *et al.*, 1999).

2.4. AMMONIA EXCRETION

Ammonia occupies an unique biochemical position, being the only inorganic form of nitrogen that is directly incorporated into organic linkage and thus an obligate intermediate in the utilization of other inorganic nitrogen sources. Cells growing in nitrate or dinitrogen will generate ammonia internally but this compound

may be totally absent extracellularly (Kleiner, 1981 and 1985). Though both the heterocystous and non-heterocystous forms are capable of N_2 fixation, it is rarely possible to detect extracellular ammonia in NO_3 or N_2 using cultures (Kerby *et al.*, 1986). A pH gradient between the inside and outside of the cell also influence the internal concentration of ammonia (Gibson, 1981). Cifferi and Tiboni (1985) have reported that the cyanobacteria can thrive in environments of higher pH range of 11 or above. However, they become sensitive to raised ammonia concentration at high pH (Boussiba, 1989). Low external pH would tend to drain the cells of ammonia through diffusion and are trapped in the protonated form in the outside compartment. *Anacystis nidulans* was characterized for the production of NH_4^+ from nitrate (Ramos *et al.*, 1982). Spiller *et al.* (1986) have reported that cyanobacterial strain *Anabaena variabilis* - SA_1 cultured with air/CO_2 produce 3.6 μM ammonia day^{-1} mg^{-1} dry weight.

Guerrero *et al.* (1982) reported that nitrate supplied to cyanobacterial cells pretreated with MSX is reduced to ammonia and released, and cells remain active for long periods. Sustained production of ammonia from dinitrogen and water was noticed in *Anabaena* sp strain 33047 and when MSX was added every 20 h. However, maximal rate of ammonia production was detected at 50 h after MSX treatment (Ramos *et al.*, 1984). Newton and Tyler (1987) have reported that herbicides that bind specifically to photosystem II greatly increased ammonia liberation by a heterocystous cyanobacterium incubated with MSX anaerobically in light. Boussiba and Gibson (1991) have shown that ammonia is lost from the cells as NH_3 in a process that is not energy dependent. Ammonia leaks from cells because of the inherent permeability of NH_3 and that no specific carrier is involved in its release. Cyanobacterial mutants capable of releasing ammonia continuously due to reduced glutamine synthetase activity together with increased nitrogenase have been developed (Thomas *et al.*, 1990 and Modi *et al.*, 1991).

Mahasneh *et al.* (1994) have shown that transposon induced mutants of *Anabaena* sp PCC 7120, are capable of extracelluar liberation in the absence or presence of glutamate analogue (MSX). Mahesh and Kannaiyan (1993a) have also shown higher ammonia excretion and nitrogen fixing activity by immobilized cyanobacteria in solid matrix. Suguna Rani (1997) showed that salt tolerant *Westiellopsis* sp recorded the highest levels of ammonia excretion in the growing medium. Tamilselvam (1998) recorded ammonia excretion by the acid tolerant *Westiellopsis* even at a pH of 4. Glutamine synthetase activity in NaCl resistant mutant strain was altered, which increases the liberation of ammonia in the external medium (Chauhan *et al.*, 1999).

2.5 FREE AMINO ACID LIBERATION BY CYANOBACTERIA

Aulosira and *Anacystis* liberate number of amino acids including glutamic acid, aspartic acid, proline and valine. In addition, a number of other amino acids are also excreted occasionally. The excretion of amino acids was maximum during the lag and stationary phases. It is indicative that some of the extracellular amino acids liberated in the medium are reutilized by the growing cyanobacteria (Singh and Trehan, 1973). Aspartic acid, glutamic acid and alanine were the amino acids present in the free form and they played an important role in the pathway of fixation mechanism (Taha *et al.*, 1962). The free amino acid content of the algal material increased with the solid waste of a chlor-alkali factory, which was also time dependent. (Mishra *et al.*, 1986).

Dimethoate, Karathane and Trifluralin decreased the synthesis of amino acid in *Anabaena oryzae* and *Nostoc muscorum*. Dursban treatment increased amino acid synthesis (Shabana *et al.*, 1991). Amino acid liberation by a mutant strain of *Anabaena variabilis*, ACA 15 was stimulated by increased irradiance, CO_2 limitation and by supplying nitrate to N_2 fixing cultures of the mutant strain (Yunes *et al.*, 1990). The production of abcissic acid (ABA) by cyanobacteria *Nostoc*

muscorum, *Trichormus variabilis* and *Synechococcus leopoliensis* into culture medium was investigated. More than 1000 ng/litre ABA was found 6 days after NaCl treatment in the culture medium (Marsalek *et al.*, 1992).

The cyanobacterium could be protected from Chromium and Lead toxicity with glutathione, ascorbic acid and sulfur containing amino acids, cysteine and methionine. Cysteine and methionine elicited little stimulatory effect and acted as a nitrogen source (Rai *et al.*, 1994). Amino acid transport systems are required for diazotrophic growth in the cyanobacterium *Anabaena* sp strain PCC 7120. Five amino acid transport systems were identified for strain PCC 7120. For basic amino acids, one high affinity active and one low affinity passive system, for neutral amino acids, two high affinity active systems and for two acidic amino acids, one putative system (Montesinos *et al.*, 1995). *Westiellopsis prolifica* Janet and *Chaetophora attenuata* Hazen cultures released sugars (glucose, fructose and sucrose), organic acids (oxalo acetic acid and oxalic acid), amino acids (glycine, serine, cystine, glutamic acid, aspartic acid, methionine, α-alanine) and protein. *Westiellopsis prolifica* and *Chaetophora attenuata* cultures of all ages released more extracellular protein than total free amino acids (Agarwal and Sharma, 1996).

2.6 CYANOBACTERIA IN ACID SOILS

The acid soils of India are located in Kerala, Assam, Manipur, Bihar, West Bengal, parts of Tamil Nadu and Karnataka. In Tamil Nadu, the acid soils are prevalent in Kanyakumari, parts of Pudukottai and Thanjavur districts. Out of the total 83,195 ha of rice cultivated area in Tamil Nadu, nearly 12,300 ha area is under acid soils representing approximately 14 per cent of the total area. Acidity has its own limit as it affect the availability of most of the essential elements and nutrients to the plants and microbes. Acidity also affect the pH balance of the cells and its activity (Das, 1996). Only acidophilic and acid tolerant microbes can survive in acidity (Sahai, 1990). In acid sulphate soils, the availability of iron and aluminium

become toxic to microorganisms. Among soil properties, pH is a very important factor in growth, establishment and diversity of cyanobacteria which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth (Brock, 1973). Acidic soils are therefore one of the stressed environments for these organisms.

Algalisation was found to significantly reduce the content of oxidizable matter, total sulphides and ferrous ions(Aiyer *et al.*, 1965). In acid soils, iron and sulphite toxicity can be reduced by algalisation and addition of lime. Aiyer (1965) reported that in acid soils, many cyanobacteria flourish well even in the acidity of pH 3.8. Jurgensen and Davey (1968) reported that cyanobacteria survive under acidic conditions also in field soils suggesting that they tolerate low pH levels. It is possible that under field conditions the soil components may act as a good buffer system, thus permitting algal growth even under unfavourable pH conditions and proved the growth maximization of *Anabaena spiroides* at pH 10.0 and tolerance down to a pH of 5.0 (Volk and Phinney, 1968). Sardespande and Goyal (1981) have reported many acid soil cyanobacterial isolates and they established their fair tolerance towards acidity of their native soil. They also established that, due to acidity, the nitrogen fixation was not affected to a great extent. Nair *et al.* (1993) reported that the usage of cyanobacteria in acid soils as biofertilizer was not fruitful. But native isolates have their own adaptability to their own regions. Algal cells are known to develop a certain electrical surface charge expressed as zeta potential, depending upon the pH of the surrounding medium, the size of which affects the permeability of the cell wall (Von Uexkull and Mutert, 1995).

Madhusoodanan and Dominic (1995) isolated many cyanobacterial strains which are tolerant to acidic pH of which, *Nostoc linckia, Calothrix marchina* and *C. fusca* were found below pH 4.0. Singh and Singh (1997) reported that they had isolated 83 cyanobacterial species from the acid soils (pH 3.64) of Arunachal

Pradesh. Singh *et al.* (1997) isolated *Westiellopsis, Nostoc, Anabaena, Hapalosiphon, Phormidium* and *Cylindrospermum* from the acid soils of Nagaland. Tamilselvam (1998) isolated acid tolerant cyanobacterial cultures which exhibited tolerance to acidity upto pH 5.0. Dominic and Madhusoodanan (1999) reported 42 species of acid tolerant cyanobacteria in Alapuzha district of Kerala, where the lowest pH at which cyanobacteria were found was 2.8. This is indicative of an efficient internal, pH regulating mechanism in these rare strains. The availability of certain nutrients, water holding capacities and other conditions are influenced by high H^+ concentrations.

2.7. INDUCED MUTAGENESIS

The concept of producing artificial mutants and utilizing them for breeding cultivated plants was first clearly suggested in 1901 by Hugo De Vries (Blackeslee, 1936.) In the first twenty five years of this century numerous research workers tried to induce mutations in a variety of organisms subjecting them to a number of physical and chemical agents. The discovery of chemical mutagens during second world war is another milestone in the history of induced mutations (Gaul, 1963).

There are many chemicals which cause mutations, hence called mutagens. In laboratory experiments mutations in DNA level have been induced by a number of chemicals, which either affect DNA directly or at the time of DNA synthesis (Drake, 1970). The alkylating agents such as Ethyl Methane Sulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) add ethyl or methyl groups to guanine, making it analogous to adenine (Lawley, 1968 and Cradock, 1969). The mutation which results in the substitution of one purine or pyrimidine with another is called transition. Auerbach (1967) observed that chemical mutagens are an important tool for plant improvement. Nitrogen fixing (Nif) mutants of *Klebsiella pneumoniae* are developed using EMS (Shanmugam and Valentine, 1980). Asato and Folsome (1969) tried nitrosoguanidine and UV irradiation in *Anacystis nidulans*.

Mutants of *Anabaena variabilis* and *Nostoc muscorum* resistant to the ammonium analogue ethylene - diamine and to the L-glutamate analogue L-methionine DL-sulfoximine (MSX) have been reported to release ammonium (Kerby *et al.*, 1988). A MSX mutant of *A. siamensis* was isolated and was found to be fast grower with high nitrogen fixing capacity (Thomas *et al.*, 1990). An ammonia execreting mutant of *Anabaena variabilis* strain SA was isolated and it was found to supply a major portion of inorganic N requirements of wheat grown in a hydroponic system (Spiller and Gunasekaran, 1990). Colchicine is another chemical mutagen which increases the ploidy level (Eigsti, 1940). A number of scientists have used Colchicine to increase the ploidy level and mutate the plants in their plant improvement studies (Dwivedi *et al.*, 1988 and Luckett, 1989).

Ribbi - Jaffe and Apitz - Castro (1979) have suggested that the primary effect of Colchicine on plantlet aggregation might be related to non-specific effect of the alkaloid on plasma membrane. Fisahn and Lucas (1990) have suggested that Colchicine disrupts the microtubule orientation. Tilak *et al.* (1994) tried Colchicine for inducing mutation in *A. pinnata* at five different concentrations *viz.*, 0.5, 1.0, 1.5, 2.0 and 2.5 per cent. It was found that 0.5 and 1.0 per cent induced mutations and other levels were found to be toxic.

Treatment with various concentrations of MNNG gave high frequencies of mutants. The pH of the incubation medium strongly influenced mutation. Neither chloramphenicol inhibited nor caffeine sensitive systems capable of repairing MNNG induced DNA damage (Chapman and Meeks, 1987). Mutant strains obtained by selection for resistance to either ethylenediamine or MSX have a derepressed nitrogenase and liberate NH_4^+ and aminoacids in significant quantities. They were immobilized for the continuous photo production of NH_4^+ and aminoacids (Kerby *et al.*, 1987). Mutations in only one of the three psb A genes of

Synechococcus R2 conferred herbicide resistance, suggesting that the molecular basis was similar to that in higher plants and algae (Ohad *et al.*, 1987).

The polyoxyethylene stearate detergents MYRJ45 and MYRJ 52 induced the liberation of amino acids by the parent strain and increased the liberation of aminoacids by the 6-Fluorotryptophan (FT-9) resistant mutant strain (Niven *et al.*, 1988). The toxic and mutagenic effects of the three pesticides alachlor, butachlor and carbaryl were similar to those of the mutagen MNNG (Vaishampayan, 1985). Two herbicide resistant strains of the cyanobacterium *Synechococcus* Sp PCC 7002 compared with the wild types with respect to the DNA changes which result in herbicide resistance. A point mutation at codon 211 in the psb A1 coding locus (TTC to TCC) resulted in an amino acid change from phenylalanine to serine in the D1 protein. This mutation conferred resistance to atrazine and diuron at 7 times and at 2 times, the minimal inhibitory concentration (MIC) for the wild type respectively (Gingrich *et al.*, 1988).

Two types of nitrosoguanidine induced rifampicin resistant mutants of *Nostoc muscorum* showing high growth rate, heterocyst frequency, nitrogenase activity, phycocyanin pigment, photosynthetic oxygen evolution and higher activities of phosphoribulokinase and Fd-NADP oxidoreductase compared to wild strain rif-1 were isolated (Dixit and Amla, 1989). Agrochemical resistant mutants of the nitrogen fixing cyanobacterium *Tolypothrix tenuis* were isolated after MNNG mutagenesis. The mutants exhibited greater nitrogenase activity and released greater quantities of extracellular nitrogenase substances such as ammonia, indole acetic acid like substances and amino acids when compared to the parent (Thomas *et al.*, 1992). Chlorate resistant mutants of the filamentous cyanobacterium, *Anabaena doliolum* were isolated by MNNG mutagenesis. Nitrite and ammonium metabolisms were not affected by this mutation. Both nitrate and chlorate acted like a pair of antagonists with nitrate protecting the growth against increased chlorate

concentration; similarly increasing chlorate concentration counteracted the growth protective action of nitrate (Bisen and Shanthy, 1992).

Kumar (1994) developed *Azolla* mutants using EMS, NTG and Colchicine. All the mutants registered higher protein content, chlorophyll content, higher activity of ammonia assimilating enzymes and their microporation as biofertilizer to rice resulted in higher N content, N uptake and higher nitrogen accumulation in soil than wild parents. The impact of UVB irradiation on five cyanobacterial strains revealed a considerable decrease in the Rubisco activity, decreased phycocyanin content and uptake of NaH $^{14}CO_3$ was affected. Cyanobacterial membrane disruption occurs during UVB irradiation leading to changes in membrane permeability (Sinha *et al.*, 1997). *Aulosira fertilissima* was sensitive to UVB and UVA and showed decreased pigment content particularly phycocyanin. Substantial decrease in the protein bands occurred after 3h of UVB exposure, particularly of these between 14.2 and 45 kDa (Banerjee *et al.*, 1996).

The effects of artificial UVB radiation on survival, growth, pigmentation and total protein profile have been studied in *Nostoc* sp, *Calothrix pulvenata* and *Plectonema boryanum. Nostoc* sp was comparatively more tolerant. Phycocyanin was severely affected by UVB irradiation in all strains. A linear decrease in the protein content with increasing UVB irradiation time was observed (Sinha and Hader, 1998). Application of UVB and heat shock stresses either separately or in combination showed a drastic photobleaching and alteration in the ratio of phycocyanin and chlorophyll a (PC/Chla) in the wild type strain as compared to its UV-HST mutant counterpart. Mutant strain showed 28 fold higher photosynthetic yield than that of its wild strain (Bisen *et al.*, 1999).

2.8. RICE SEEDLING RESPONSE TO CYANOBACTERIAL INOCULATION

Nitrogen fixation is especially important in flooded soils where cyanophyceae are recognised as important agents in the stabilization of soil surfaces

(Bailey *et al.*, 1973), primarily through the process of aggregate formation and stabilization (Burns and Davis, 1986). The inoculation of soil surfaces with the extracellular polysaccharide producing phototrophic microorganisms has been proposed as means of improving soil aggregate stability and soil structure (Lewin, 1977).

Since De (1939) attributed the internal fertility of the tropical rice field soils to the activity of nitrogen fixing cyanobacteria, considerable interest has been generated in exploiting the potential of these biological systems and thus recognized as nitrogenous biofertilizer (Santra, 1993) and are being used in rice fields (Venkataraman, 1981 and Kannaiyan, 1985). The beneficial effect of cyanobacteria on grain yield of many rice varieties has been demonstrated in a number of localities (Sankaran, 1971; Kannaiyan, 1978a; Venkataraman, 1982; Ahluwalia *et al.*, 1990 and 1993; Mandhare *et al.*, 1990). Cyanobacterial inoculation with composite cultures was reported to be more effective than single culture inoculation (Kannaiyan, 1978 and Nayak *et al.*, 1996). Many cyanobacteria contribute greatly to the nitrogen economy of aquatic and terrestrial habitats through their ability to fix atmospheric nitrogen (Fay, 1981).

Cyanobacteria are also important in reducing soil erosion and increasing the organic matter content of the soil and in producing certain substances which enhance the growth of higher plants (Venkataraman *et al.*, 1974). Cyanobacterial application resulted in a significant improvement in the aggregation status of the soil and also brought down appreciably the pH, electrical conductivity and exchangeable sodium status and increased considerably the hydraulic conductivity of the soil (Kaushik and Krishnamurthy, 1981). Increase in the growth parameters due to cyanobacterial inoculation, points towards the effect of biologically active growth factors like vitamin B_{12} and auxin like substances, which are reported to be secreted by the nitrogen fixing cyanobacteria, in addition to nitrogen contribution.

(Venkataraman and Neelakantan, 1967). Reasonable estimates place the nitrogen contribution by cyanobacteria in soil at 25 - 30 kg N ha^{-1} and to rice grain yield at 10 - 15 per cent (Kannaiyan, 1978).

Application of cyanobacterial biofertilizer facilitated the saving of around 25 - 30 per cent of fertilizer nitrogen (Venkataraman, 1972). It is generally assumed that the nitrogen fixed by cyanobacteria are made available to the rice plants through exudation, autolysis and microbial decomposition (Roger and Kulasooriya, 1980 and Venkataraman, 1986). Organic matter produced by cyanobacterial growth remain in soil and become available to the next crop (Sankaran, 1971). Venkataraman (1979) and Kannaiyan (1983) found in many sites where cyanobacterial inoculation was used for three to four consecutive cropping seasons, the inoculated culture established well and the effect persisted over subsequent years also.

Kannaiyan *et al.* (1980) have demonstrated different field trials under various seasons to assess the effect of cyanobacteria as biofertilizer for rice cultivation. Kannaiyan *et al.* (1982) demonstrated the effect of top dressing of fertilizer nitrogen for rice on the performance of cyanobacteria and noticed that split application of top dressing of fertilizer nitrogen did not affect the establishment and N_2 fixation by inoculated cyanobacteria in rice field. A combined application of urea and cyanobacterial inoculation was more effective than the application of urea alone (Panch pande, 1990). Kamuru *et al.* (1994) reported the combined response of both the rice plants and the cyanobacterium to high CO_2 levels, probably accounted for the higher dry matter and N at the elevated than at the ambient CO_2 concentration.

Besides increasing nitrogen fertility, cyanobacteria are known to produce vitamins, plant growth stimulating hormones and polysaccharides thereby improving soil aggregation (Roger and Kulasooriya, 1980). They also found that cyanobacterial inoculation has stimulated beneficial soil microorganisms, increased

soil organic matter which in turn might play a major role in increasing the productivity of rice crop as well as maintain long time soil fertility and sustainability. Chirriv *et al.* (1995) reported that BGA applied at the rate of 20 kg on the 7th day after transplanting could save N fertilizer upto 50 per cent.

A combination of fertilizer nitrogen and PUF immobilized cyanobacteria also significantly increased rice grain and straw yield (Kannaiyan *et al.*, 1997). Rice plants inoculated with the cyanobacterium *Anabaena variabilis*, a nitrogenase derepressed mutant produced more panicles, dry matter and total N contents of the root, shoot and grain were significantly higher than the plants inoculated with the parent strain (Kamuru *et al.*, 1998). Anitha and Kannaiyan (1999) reported that the rice seedlings inoculated with mixed culture of immobilized cyanobacteria recorded more root, shoot growth, biomass and total chlorophyll when compared to rice seedlings inoculated with individual inoculants.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. GENERAL

3.1.1. LOCATION

The experiments were conducted at *Azolla* laboratory of Tamil Nadu Agricultural University, Coimbatore, which is located at an altitude of 426.7 metres above mean sea level, 11°N latitude and 77°E longitude.

3.1.2. AGROCLIMATIC CONDITIONS

The mean maximum and minimum temperatures prevailing at Coimbatore is 31.5°C and 21.0°C respectively. The mean relative humidity is 61.1 per cent and the annual rainfall is 674.2 mm.

3.1.3. GLASSWARE

The glassware used for the biochemical studies were cleaned with chromic acid solution and finally washed with water. The glassware thus cleaned were rinsed with distilled water before use.

3.1.4. CHEMICALS

The analytical reagent (AR) grade chemicals of BDH, E.Merck, Himedia and Qualigens were used for biochemical studies. Protein markers for molecular biological works were obtained from M/s.Bangalore Genei, Bangalore.

3.1.4. CYANOBACTERIAL CULTURES

The Cyanobacterial cultures *viz.Anabaena* - AT-TGK-$5A_6$,*Nostoc* AT-TGK-$4C_4$,*Oscillatoria* -AT-TGK-$5B_8$,*Westiellopsis* -AT-TGK-$4A_2$ and*Westiellopsis* AT-TGK-$4A_7$ maintained at *Azolla* laboratory of Tamil Nadu Agricultural University were used for the mutation studies.

3.2. PURIFICATION OF THE CYANOBACTERIAL CULTURES

The cyanobacterial cultures were purified by repeated transfer to nitrogen free BG-11 medium atleast for 3 or 4 times. Further purification was done with treatment as detailed below.

3.2.1. PURIFICATION WITH TRIPLE ANTIBIOTIC SOLUTION

3.2.1.1. PREPARATION OF TRIPLE ANTIBIOTIC SOLUTION

One hundred mg of penicillin G (Na or K Salt) and 50 mg of Streptomycin were dissolved in 10 ml of distilled water. Ten mg of chloramphenicol was dissolved in 1 ml of 95 per cent ethanol and this solution was added into the penicillin streptomycin mixture.

3.2.1.2. PURIFICATION OF CYANOBACTERIA

A quantity of 1 g of freshly isolated cyanobacterial cultures were incubated with 1 ml of triple antibiotic solution for 6 - 10 h to kill other bacteria, fungi and green algae. After incubation the cultures were centrifuged at 3000 rpm for 5 min and the supernatant containing the triple antibiotic solution was discarded. The pellets were resuspended in nitrogen free BG-11 medium aseptically.

3.2.2. PURIFICATION OF CYANOBACTERIA BY AGAR PLATING

The algal suspension (1 ml) was homogenized using sonicator and the algal cultures were purified either by streaking or pour plating in nitrogen free BG-11 medium in clean sterile petriplates. The petri plates were incubated for 3- 4 weeks under 3000 lux light intensity at 28 ± 1°C.

3.2.3. IDENTIFICATION OF UNIALGAL CULTURES

Well developed unialgal cultures were examined under the binocular microscope (Euromax BM 1274) and identified using the cyanobacterial taxonomy hand books (Desikachary, 1959 and Anand, 1989).

3.2.4. MASS CULTURING THE CYANOBACTERIAL CULTURE

The cyanobacterial cultures were grown in nitrogen free BG-11 medium. One ml of homogenized cyanobacterial culture was transferred aseptically to 100 ml of sterilized nitrogen free BG-11 medium in 250 ml conical flasks. The flasks were incubated in a polynet house at 28±1°C with 3000 lux light intensity and in the culture room at 5°C with periodical shaking for 3 weeks. Sub culturing was done periodically in BG-11 medium to maintain the purity of the cultures and to replenish the nutrients needed for cyanobacterial growth.

COMPOSITION OF BG-11 MEDIUM (Rippka *et al.*, 1979)

Chemicals	**Stock solution** ($g100\ ml^{-1}$)	**Final solution** ($ml\ l^{-1}$)
$K_2HPO_4\ 3H_2O$	0.400	1.0
$MgSO_4\ 7H_2O$	0.750	1.0
$CaCl_2\ 2H_2O$	0.036	1.0
$C_6H_8O_7\ 7H_2O$	0.006	1.0
$Fe(NH_4)_2\ HC_6H_5O_7$	0.006	1.0
EDTA (Disodium Mg salt)	0.001	1.0
Na_2CO_3	0.020	1.0
* A_5 micronutrient solution		2.0
Distilled water		1000 ml
pH		7.0 - 7.5

***A_5 Micronutrient solution**	
H_3BO_3	2.86 g
$MnCl_2.4H_2O$	1.81 g
$ZnSO_4.7H_2O$	0.22 g
$Na_2MoO_4.2H_2O$	0.39 g
$CuSO_4.7H_2O$	0.08 g
$CoCl_2$	0.02 g
pH	5.6

3.3. INDUCED MUTAGENESIS OF THE ACID TOLERANT CYANOBACTERIAL CULTURES

The acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-5A_6, *Nostoc*-AT-TGK-4C_4, *Oscillatoria*-AT-TGK-5B_8, *Westiellopsis*-AT-TGK-4A_2 and *Westiellopsis*-AT-TGK-4A_7 were mutated with mutagens like Ethyl Methane Sulphonate (EMS), N-methyl - N' - nitro - N-nitrosoguanidine (NTG), Colchicine and Ultra Violet Radiation (UVR). Then the mutants were grown in BG-11 medium *viz.*, containing antibiotics Chloramphenicol and Kanamycin at 50 ppm each.

3.3.1. PREPARATION OF STOCK SOLUTIONS

3.3.1.1. PREPARATION OF ANTIBIOTIC STOCK SOLUTION

One gram of chloramphenicol and one gram of Kanamycin was dissolved in 100 ml of sterile distilled water separately to get 10,000 ppm each and stored under refrigerated condition.

3.3.1.2. PREPARATION OF STOCK SOLUTION OF MUTAGENS

3.3.1.2.1. EMS

One ml of EMS was dissolved in 100 ml of phosphate buffer to get 10,000 ppm stock solution and stored in a refrigerator.

3.3.1.2.2. NTG AND COLCHICINE

One gram of NTG and Colchicine was dissolved separately in 100 ml of sterile distilled water to get 10,000 ppm and stored in a refrigerated condition.

3.3.2. SAMPLE PROCESSING

Ten ml of the homogenised acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-5A_6, *Nostoc*-AT-TGK-4C_4, *Oscillatoria*-AT-TGK-5B_8, *Westiellopsis*-AT-TGK-4A_2 and *Westiellopsis*-AT-TGK-4A_7, were centrifuged at 3000 rpm for 15 min twice with physiological saline solution (0.85% NaCl) and then once with sterile distilled water. The pellets got were used for further treatment with EMS, NTG, Colchicine and UVR.

3.3.2.1. EMS TREATMENT (Bharathi and Giriyappanavar, 1988)

The pellets were suspended in 10 ml EMS solution at varied concentrations *viz.*, 100, 150, 200, 250 and 300 ppm for 15 min. Then 10 ml of sterile distilled water was added to stop the activity of EMS and then centrifuged again at 3000 rpm for 15 min thrice with sterile distilled water. Then the pellets were suspended in sterile BG-11 broth and incubated for 24 h. The cultures were then, spread plated using a sterile `L' glass rod on sterile BG-11 medium enriched with 50 ppm of chloramphenicol and 50 ppm of kanamycin. The petri plates were incubated for 3-4 weeks under 3000 lux light intensity at 28 ± 1°C. After the incubation period the plates were examined for the emergence of colony forming units.

3.3.2.2. NTG TREATMENT (Kumar and Kumar, 1984)

The pellets were suspended in 10 ml of NTG solution at varied concentration *viz.*, 100, 150 and 200 ppm for 30 min and centrifuged thrice at 3000 rpm for 15 min using 10 ml sterile distilled water for each cycle. Then the pellets were suspended in sterile BG-11 broth and incubated for 24 h. After the incubation period, the cultures were spread plated using a sterile `L' glass rod on sterile BG-11 medium enriched with 50 ppm each of kanamycin and chloramphenicol. Then the petri plates were incubated for 3-4 weeks under 3000 lux light intensity at 28 ± 1°C. After the incubation period, the plates were observed for the emergence of colony forming units.

3.3.2.3. COLCHICINE TREATMENT (Kumar and Kumar, 1984)

The pellets were suspended in 10 ml Colchicine solution at varied concentrations *viz.*, 100, 150 and 200 ppm for 30 min and centrifuged thrice with sterile distilled water as mentioned in previous section. Then the pellets were suspended in sterile BG-11 broth and incubated for 24 h, spread plated on sterile BG-11 medium enriched with 50 ppm of chloramphenicol and kanamycin each. The

petri plates were incubated for 3-4 weeks under 3000 lux light intensity at 28 ± 1°C. After the incubation period, the plates were observed for the colony emergence.

3.3.2.4. UV RADIATION TREATMENT (Srivastava, 1969)

The pellets were dispersed in 10 ml of sterile distilled water in separate petri plates and exposed to UV light in the laminar air flow chamber at a height of 15 cms from the base. At 10^{th}, 20^{th} and 30^{th} min after exposure, the cultures were withdrawn and suspended in sterile BG-11 broth and incubated for 24 h. After the incubation period, the cultures were spread plated using a sterile `L' glass rod on sterile BG-11 medium enriched with 50 ppm of chloramphenicol and kanamycin each. The petri plates were incubated for 3-4 weeks under 3000 lux light intensity at 28±1°C. After the incubation period, the plates were observed for the emergence of colony forming units.

3.3.3. ESTIMATION OF LD_{50} VALUE OF THE MUTAGENS FOR THE ACID TOLERANT CYANOBACTERIAL CULTURES

The acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$, *Oscillatoria*-AT-TGK-$5B_8$, *Westiellopsis*-AT-TGK-$4A_2$ and *Westiellopsis*-AT-TGK-$4A_7$ were exposed to the mutagens *viz.*, EMS, NTG, Colchicine and UVR as per the procedure detailed in sec 3.3.2.1, 3.3.2.2., 3.3.2.3 and 3.3.2.4 respectively. But for LD_{50} assessment, the cultures were spread plated after second centrifugation on sterile BG-11 medium without the antibiotics *viz.*, chloramphenicol and kanamycin. Then the petri plates were incubated under 3000 lux light intensity at 28±1°C for 3 - 4 weeks and examined for colony emergence.

3.3.4. ESTIMATION OF REVERSION FREQUENCY OF THE MUTATED CYANOBACTERIAL CULTURES

The acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$, *Oscillatoria*-AT-TGK-$5B_8$, *Westiellopsis*-AT-TGK-$4A_2$ and *Westiellopsis*-AT-TGK-$4A_7$ were exposed to the mutagens *viz.*, EMS, NTG,

Colchicine and UVR as per the procedure detailed in 3.3.2.1, 3.3.2.2, 3.3.2.3 and 3.3.2.4 respectively. But after the second centrifugation, the culture pellets were suspended in sterile BG-11 broth without antibiotics in it. Then the flasks were incubated unde 3000 lux light intensity at 28 ± 1°C for 5 generations. Then they were spread plated using a sterile `L' glass rod on sterile BG-11 medium enriched with 50 ppm of chloramphenicol and kanamycin each. Then the petri plates were incubated under 3000 lux light intensity at 28 ± 1°C for 3 - 4 weeks and observed for colony emergence. The reversion frequency was calculated using the formula given below :

$$\text{Reversion frequency} = \frac{\text{Number of revertants}}{\text{Number of mutants}} \times 100$$

3.4. GROWTH PERFORMANCE AND BIOCHEMICAL CONSTITUENTS OF THE ACID TOLERANT CYANOBACTERIAL CULTURES AND MUTANTS

The acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$, *Oscillatoria*-AT-TGK-$5B_8$, *Westiellopsis*-AT-TGK-$4A_2$ and *Westiellopsis*-AT-TGK-$4A_7$, EMS mutants *viz.*, *Anabaena*-AT-MGK-$5A_6$-E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria*-AT-MGK-$5B_8$-E_{200}, *Westiellopsis*-AT-MGK-$4A_2$-E_{250} and *Westiellopsis*-AT-MGK-$4A_7$- E_{250} were grown in sterile nitrogen free BG-11 medium. The growth, biomass production, ammonia execretion, chlorophyll a content, protein content, polysaccharide production, amino nitrogen content, nitrogenase activity, free amino acid content and methionine content were investigated under *in vitro* conditions for three times at regular intervals of 10 days.

3.4.1. GROWTH PERFORMANCE

The growth of the mutants and acid tolerant cyanobacterial cultures grown in nitrogen free BG-11 medium was determined at 10 days interval by measuring the

optical density at 660 nm in Beckman DU-64 spectrophotometer against uninoculated BG-11 medium as blank. The values were expressed as optical density at 660 nm.

3.4.2. BIOMASS PRODUCTION

One hundred ml of cyanobacterial culture suspension was filtered through a whatman No.1 filter paper of known weight. Then it was dried in an oven at 60°C for 3h till constant weights were recorded. The difference in weight was recorded and expressed as biomass of the cyanobacterial cultures. The results were expressed as μg of biomass produced per ml of the cyanobacterial suspension.

3.4.3. AMMONIA EXCRETION

Ammonia excretion by the cyanobacterial cultures were estimated at weekly intervals by following the method developed by Solorzano (1969).

Reagents

i. **Phenol reagent** : 10 g distilled phenol in 100 ml of 95 per cent ethanol.

ii. **Nitroprusside reagent** : 500 mg of sodium nitroprusside in 100 ml of double distilled water.

iii. **Alkaline stock** : 20 g of trisodium citrate and 5 g of sodium hydroxide in 100 ml of double distilled water.

iv. **Sodium hypochlorite stock** : Sodium hypochlorite solution with 4 per cent available chlorine.

v. **Oxidizing reagent** : Prepared freshly by mixing alkaline stock and sodium hypochlorite stock at 4:1 ratio.

Procedure

Three ml of culture filtrates were taken in cleaned test tubes. To this 0.2 ml phenol reagent was added and mixed thoroughly, then 0.2 ml of nitroprusside

reagent was added and mixed well. Then 0.5 ml freshly prepared oxidizing reagent was added, mixed well, completely covered with aluminium foil and incubated for 1 h at 28 ± 1°C in the dark with intermittent shaking. The intensity of blue colour developed was read at 620 nm in the Beckman DU-64 spectrophotometer. The standard graph was prepared at the time of estimation with pure ammonium chloride. The results were expressed as n moles of ammonia excreted per ml of culture filtrate.

3.4.4. ESTIMATION OF CHLOROPHYLL a CONTENT

Chlorophyll a content of the cyanobacterial cultures were determined by the method developed by Talling and Driver (1961). Ten ml of the cyanobacterial cultures were centrifuged at 5000 rpm for 5 min. The pellet was resuspended in a known volume of 95 per cent ethanol and the sample was ground in a pestle and mortar. The extract was allowed to settle in dark at room temperature (28 ± 1°C) for a period of 30 min and again centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was read at 665 nm in a Beckman DU-64 spectrophotometer against 95 per cent ethanol as blank. The chlorophyll a content of the cyanobacterial cultures was calculated using the following formula and expressed as mg ml^{-1}

$$\text{Chlorophyll a} = \frac{A_{665} \times \text{volume of ethanol}}{8.4}$$

3.4.5. ESTIMATION OF PROTEIN CONTENT

The amount of protein present in the cyanobacterial cultures was estimated by Bradford's method (Bradford, 1976).

Bradford's Reagent: 100 mg of coomassie brilliant blue G 250 was dissolved in 50 ml of 95 per cent ethanol. To this, 100 ml of conc. orthophosphoric acid was added and the volume was made upto 200 ml with distilled water. The solution was diluted by mixing one volume of it with 4 volumes of distilled water prior to use. The concentrated dye solution was stored in amber coloured bottles in a refrigerator.

To 0.1 ml of the culture extract prepared in 0.1 M phosphate buffer (pH 6.8), 5 ml of Bradford's reagent was added and kept for 15 min. The blue colour developed was read at 595 nm in a Beckman DU-64 spectrophotometer. From the standard graph prepared using bovine serum albumin, the protein content was calculated and the result was expressed as $\mu g\ ml^{-1}$ of the culture.

3.4.6. ESTIMATION OF POLYSACCHARIDE PRODUCTION

The polysaccharide production was estimated using anthrone reagent (Morris, 1948). To 0.2ml of the homogenized cyanobacterial culture, 1.8 ml of distilled water and 4.0 ml of anthrone reagent (0.2 per cent anthrone in 95 per cent sulphuric acid) were added and mixed gently by shaking. The tubes were kept in a boiling water bath for 10 min and cooled at room temperature. The absorbance was measured at 620 nm in a Beckman DU-64 spectrophotometer against a reagent blank. The standard graph with glucose at a concentration range of 10 - 100 μg was prepared at the time of estimation and polysaccharide content of the culture suspension was calculated from the standard curve and expressed as $\mu g\ ml^{-1}$ of culture.

3.4.7. ESTIMATION OF AMINO NITROGEN CONTENT

The amino nitrogen content was estimated following the method given by Spies (1955).

Ninhydrin reagent: Prepared by mixing 1 per cent ninhydrin in 0.5 M citrate buffer (pH 5.5), pure glycerol and 0.5 M citrate buffer(pH 5.5) in the ratio of 5:12:2.

The culture extract was prepared using 0.5 g of the cyanobacterial culture and 2 ml of 0.1M phosphate buffer (pH 6.8) and 0.1 ml of the extract was taken and the volume was made upto 1.0 ml by adding distilled water. To this, 5 ml of ninhydrin reagent was added and mixed vigorously. The mixture was heated in a boiling waterbath for 12 min and cooled. The absorbance of the purple colour

developed was measured at 570 nm against reagent blank. A standard graph was prepared using glycine as a standard amino acid and the amount of amino acid present in the sample was calculated from it and multiplied with the factor 14/75 and expressed as $\mu g\ ml^{-1}$ of the culture.

3.4.8. ASSAY OF NITROGENASE ACTIVITY

The nitrogenase activity was estimated by the acetylene reduction assay (Hardy *et al.*, 1968). One hundred ml of the culture grown in nitrogen free BG-11 broth was blot dried in a country filter paper and transferred to 10 ml glass serum vials and sealed. By using a sterile syringe, 10 per cent of the air inside the bottle ie., 3 ml was evacuated and replaced with 3.0 ml of pure acetylene. The bottles were incubated with 3000 lux light intensity for 20 h at 28 ± 1°C. One ml of gas mixture from the bottle was withdrawn and injected into the Nucon 2865 Gas Chromatograph. The gas chromatograph is fitted with oven, flame ionization detector and injector. The oven temperature and injector temperature was maintained at 100°C and detector temperature at 110°C. The oven is provided with a column of 2 m long stainless steel pipes of 2 mm inner diameter packed with poropack Q column with 80-100 mesh size. Peak height of ethylene was measured and recorded. Nitrogenase activity was calculated using the following formula :

$$\text{Nitrogenase activity} = \frac{\text{Peak height x Range x Attenuation x volume of gas occupied x } 0.001}{\text{Weight of sample material x volume of gas injected x Hours of incubation}}$$

The results were expressed as n mole of ethylene produced $h^{-1}g^{-1}$ fresh weight.

3.4.9. ESTIMATION OF TOTAL FREE AMINO ACID CONTENT

Total free amino acid content of the cyanobacterial cultures were estimated.

Reagents

1. **Ninhydrin** : 0.8 g stannous chloride was dissoved in 500 ml of 0.2 M citrate buffer (pH 5.0). To this solution 20 g of ninhydrin in 500 ml of methyl cellosolve (2 methoxyethanol) was added.
2. 0.2 M citrate buffer (pH 5.0).
3. **Diluent solvent**: Equal volumes of water and n-propanol was mixed and used.

Procedure

Pelleted ten ml of the culture by centrifugation at 5000 rpm for 5 min and resuspended in a known volume of 80 per cent ethanol and ground well in a pestle and mortar and again centrifuged. The extraction was done twice with the residue and all the supernatants were pooled and used for the estimation. To 0.1 ml of the extract, 1 ml of ninhydrin solution was added and made upto 2 ml with distilled water. Then the tubes were heated in a boiling water bath for 20 min. Five ml of the diluent was added and mixed well. After 15 min, the intensity of the purple colour developed was read at 570 nm in a colorimeter against a reagent blank which was prepared using 0.1 ml of 80 per cent ethanol following the above steps. Standard graph was prepared at the time of estimation with leucine and the results were expressed as $mgml^{-1}$ of the culture filtrate.

3.4.10. ESTIMATION OF METHIONINE

Methionine content was estimated by the method developed by Horn *et al.* (1946).

Reagents

1. 10 per cent Sodium hydroxide
2. 10 per cent sodium nitroprusside
3. 3 per cent glycine
4. Orthophosphoric acid (S.G - 1.75).

5. 2N hydrochloric acid
6. 10 N sodium hydroxide

Procedure

In a 50 ml conical flask, 0.5 g of the culture was taken, 6 ml of 2 N HCl was added and autoclaved at 15 lb pressure for 15 min. A pinch of activated charcoal was added, boiled for 10 min and filtered. The filtrate was neutralised with 10 N NaOH. The volume was made upto 50 ml with distilled water. Then 25 ml of the culture was transferred to a 100 ml conical flask and 3 ml of 10 per cent NaOH and 0.15 ml of sodium nitroprusside was added. After 10 min, one ml of 3 per cent glycine solution was added. After 10 min, 2 ml of orthophosphoric acid was added and shaken vigorously. The intensity of red colour developed was read after 1 h incubation at 520 nm. Standard graph was prepared using methionine and the result was calculated using the following formula and expressed as mg g^{-1} of the culture.

Methionine content in the sample = Methionine content from the graph x 4

3.6. EFFECT OF ACIDITY ON THE GROWTH AND BIOCHEMICAL CONSTITUENT OF THE MUTATED ACID TOLERANT CYANOBACTERIAL CULTURE

The mutants *viz.*, *Anabaena*-AT-MGK-$5A_6$-E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria*-AT-MGK-$5B_8$-E_{200}, *Westiellopsis*-AT-MGK-$4A_2$-E_{250} and *Westiellopsis*- AT- TGK-$4A_7$-E_{250}, were selected and their performance was compared with the normal soil isolate *Anabaena* - NS-$5G_2$ and acid tolerant culture *Anabaena*-AT-TGK-$5A_6$ by growing them in BG-11 broth at pH 4, 5 and 6 separately. The flasks were incubated under light (3000 lux) at 28±1°C for 4 weeks period and investigated for growth, biomass production, ammonia excretion, Chlorophyll-a content, protein content, polysaccharide production, amino nitrogen content, nitrogenase activity, free amino acids and methionine content under *in vitro* conditions by following the methods described earlier in sec. 3.4.

3.7. EFFECT OF INOCULATION OF THE MUTATED ACID TOLERANT CYANOBACTERIAL CULTURES ON RICE VARIETY ASD 16 SEEDLINGS

The experiment was conducted in plastic tubs (15 cm dia and 6 cm height) with three replications in completely randomized design. The tubs were filled with 2 kg of unsterilized acid soil collected from Rice Research Station, Ambasamudram. The pregerminated seeds of rice variety ASD-16 were sown at the rate of 15 seeds tub^{-1}. Acid tolerant mutant cultures *viz.*, *Anabaena*-AT-MGK-$5A_6$-E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria*-AT-MGK-$5B_8$-E_{200}, *Westiellopsis*-AT-MGK-$4A_2$-E_{250} and *Westiellopsis*-AT-MGK- $4A_7$-E_{250}, were inoculated as fresh weight individually and the normal soil cyanobacterial culture *Anabaena* - NS-$5G_2$ and acid tolerant cyanobacterial culture were also inoculated for comparision at the rate of 1 g of fresh weight of the culture tub^{-1} after sowing. Sterilized water were used to maintain the level for a depth of 5 cm throughout the study period in unsterile soil. The plant height, ammonia excretion, total leaf chlorophyll and the total plant nitrogen content were determined as follows :

3.7.1. PLANT HEIGHT DETERMINATION

The shoot growth (in cm) of the rice seedlings was determined at 10 days interval from 10th day after sowing upto 40 days.

3.7.2. ESTIMATION OF AMMONIA EXCRETION

Ammonia excretion by the acid tolerant mutants and cyanobacterial cultures inoculated to the rice seedlings was estimated by collecting the flood water samples at 10 days interval from 20^{th} day of sowing upto 40 days by following the method of Solorzano (1969) as described earlier in sec. 3.4.3.

3.7.3. ESTIMATION OF TOTAL CHLOROPHYLL CONTENT

Total chlorophyll content of the rice seedlings was estimated by following the standard procedure given by Wintermans and Demotes (1965). One g of leaf

samples was homogenized with acetone and the extract was allowed to settle in dark at room temperature for 15 min. Again the extract was centrifuged at 5000 rpm for 5 min. The supernatant was collected and the absorbance was read in Beckman DU-64 spectrophotometer at 665 nm and 649 nm against acetone.

Total chlorophyll content was determined by using the formula :

$$\text{Total chlorophyll} = \frac{6.10(A_{665}) + 20.04\ (A_{649})\ \text{X Volume of acetone (ml)}}{1000}$$

The results were expressed in terms of mgg^{-1} fresh weight.

3.7.4. ESTIMATION OF TOTAL NITROGEN IN ASD-16 RICE SEEDLINGS

Total nitrogen content in plant samples was estimated by following the Kjeldahl method (Humphries, 1956 and Jackson, 1973).

Reagents

1. 1 g of anhydrous Na_2SO_4 and 1 g dry $CuSO_4$
2. 40 per cent NaOH
3. Conc. H_2SO_4
4. Salicyclic acid
5. Sodium thiosulphate
6. Bromocresol green - methyl red indicator
7. Boric acid - indicator mixture - 40 g of H_3BO_4 was dissolved in 1 litre.

Dried and powdered plant samples 0.5 g was transferred to a dry digestion tube of VELP SCIENTIFICA UDK 126 digester. Twenty five ml of conc. sulphuric acid was added and the contents were thoroughly mixed and allowed to stand for 30 min. Then 0.5 g of catalyst mixture was added to the tubes and heated slowly till frothing continues and then digested for 1 h. The tubes were then cooled and 100 ml of distilled water was added slowly with shaking and this mixture was used for the

determination of total nitrogen. The nitrogen present was estimated in a VELP SCIENTIFICA UDK 126 semiautoanalyser, after adding approximately 100 ml of 40 per cent sodium hydroxide. In the semiautoanalyser, the ammonia liberated was collected in the titrating vessel containing boric acid indicator solution and titrated against 0.5N sulphuric acid. From the titre value, the nitrogen content was calculated using the formula,

$$\text{Nitrogen content (\%)} = \frac{a \times 0.00028 \times V \times 100}{10 \times W}$$

a - ml of the N/50 H_2SO_4 used

0.00028 - nitrogen equivalent of 1 m l of N/50 H_2SO_4 used

V - Volume of diacid extract prepared (ml)

10 - Volume of diacid extract pipetted out for analysis (ml)

W - Weight of the sample (g)

The results were expressed as per centage of nitrogen present.

3.8. Statistical analysis

The data were subjected to statistical scrutiny as per the methods detailed by Panse and Sukhatme (1985).

4. EXPERIMENTAL RESULTS

4.1. EFFECT OF VARIOUS CHEMICAL MUTAGENS (EMS/NTG/COLCHICINE) ON ACID TOLERANT CYANOBACTERIAL CULTURES

The effect of the chemical mutagens on the population and mutation frequency of different acid tolerant cyanobacterial cultures were tested separately by exposing them to varied concentration of EMS/NTG/Colchicine and the results are given in Tables 1-3 , Figs.1-3 and Plates1-5. In general, mutation frequency varied with mutagen concentration as well as cyanobacterial cultures. Among the cultures, *Westiellopsis*-AT-TGK-4A_7, *Westiellopsis*-AT-TGK-4A_2 followed by *Nostoc*-AT-TGK-4C_4 showed maximum mutation frequency, while *Anabaena*-AT-TGK-5A_6 and *Oscillatoria*-AT-TGK-5B_8 showed minimum mutation frequency. Maximum mutation frequency was obtained at E_{250} and N_{150} by *Westiellopsis* cultures, while at C_{100} *Oscillatoria* showed maximum mutation frequency. In general there was a reduction in mutation frequency at the higher concentration tested for any of the mutagen.

4.2. EFFECT OF PHYSICAL MUTAGENESITY OF UV RAYS ON THE ACID TOLERANT CYANOBACTERIAL CULTURES

The acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-5A_6, *Nostoc*-AT-TGK-4C_4, *Oscillatoria*-AT-TGK-5B_8, *Westiellopsis*-AG-TGK-4A_2 and *Westiellopsis*-AT-TGK-4A_7 were exposed to UV rays for different exposure time and the results are shown in Table 4 , Fig. 4 and Plate 6. Lower the exposure time higher will be the mutation frequency. Maximum mutation frequency was at 10 min time exposure by all the acid tolerant cyanobacterial cultures. Though *Anabaena*-AT-TGK-5A_6 and *Oscillatoria*-AT-TGK-5B_8 have shown higher mutation frequency, *Nostoc*-AT-TGK-4C_4 had shown the lower mutation frequency. *Westiellopsis* cultures showed the minimum mutation frequency. However, there was not much variation beween the *Westiellopsis* cultures.

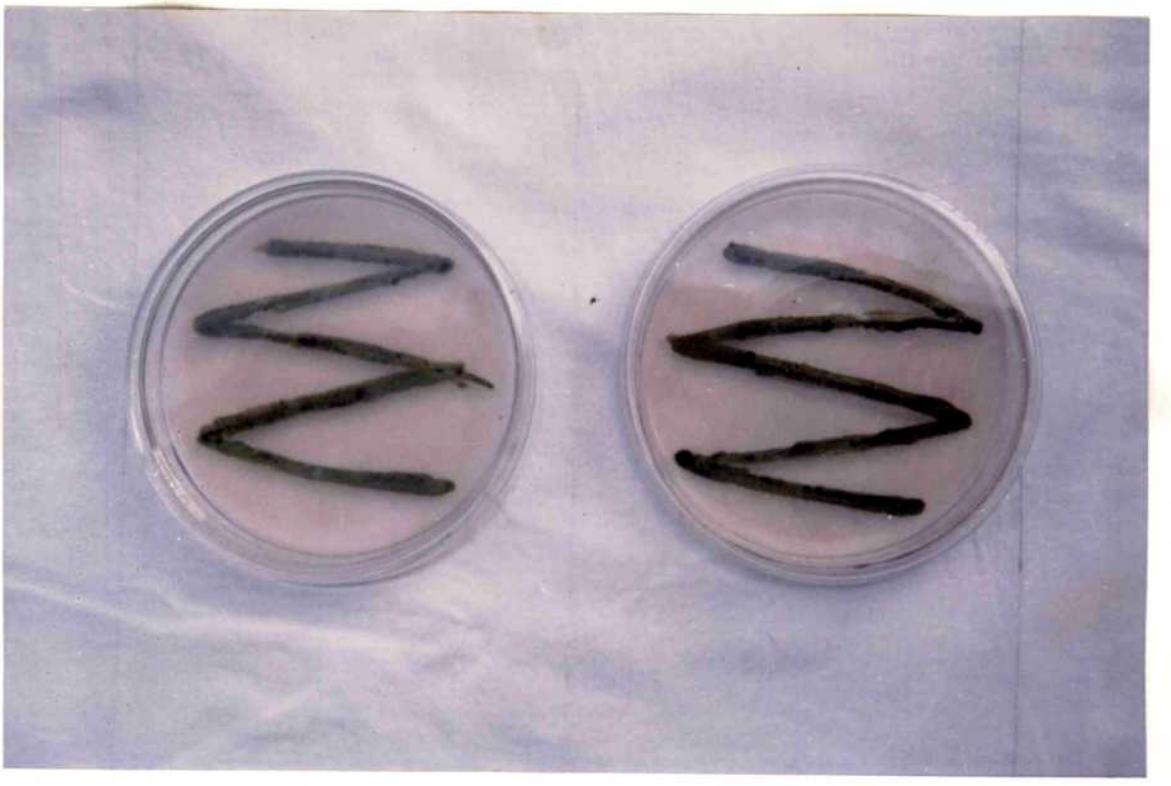


Plate 1. **Purification of acid tolerant cyanobacteria by agar streaking**

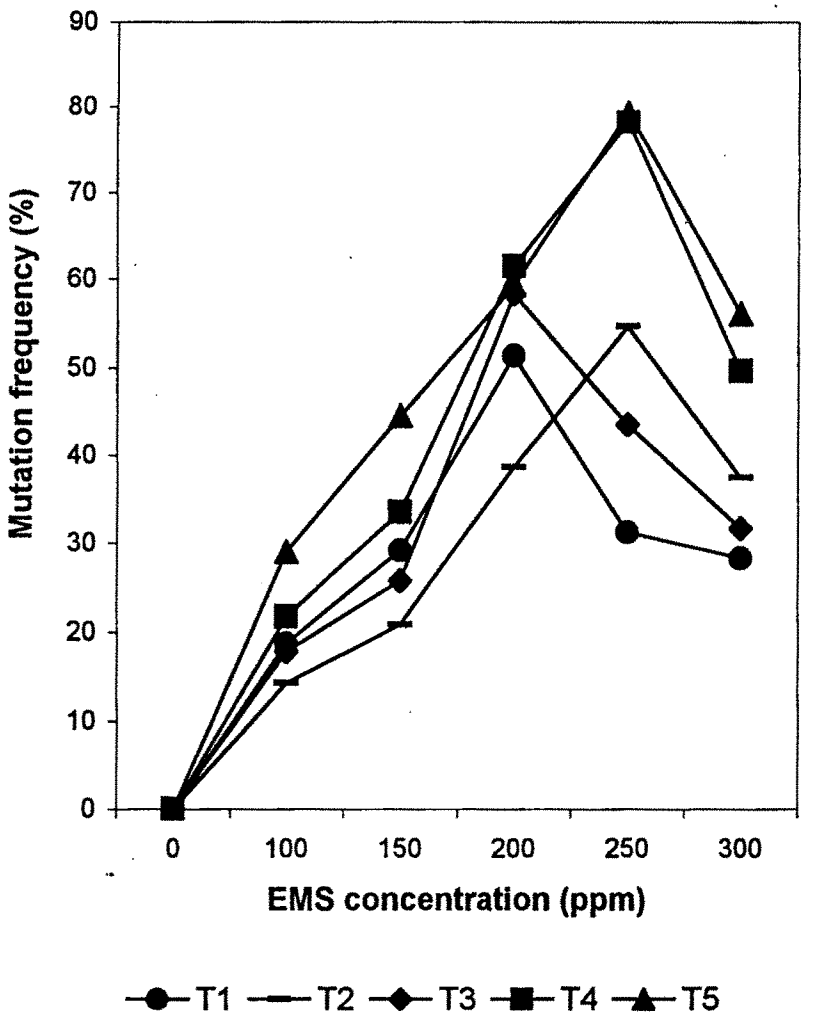
Plate 2. **Acid tolerant cyanobacterial cultures.**

Table 1. Effect of EMS on acid tolerant cyanobacterial cultures.

Cultures	EMS concentration (ppm)	Population (x 10^2 cfu)	Mutation frequency (%)
Anabaena-AT-TGK-$5A_6$	0	48.00	0.00
	100	8.66	18.75
	150	13.60	29.16
	200	24.66	51.38
	250	15.00	31.25
	300	13.60	28.30
Nostoc-AT-TGK-$4C_4$	0	56.00	0.00
	100	8.00	14.28
	150	11.66	20.82
	200	21.66	38.67
	250	30.66	54.75
	300	21.00	37.50
Oscillatoria-AT-TGK-$5B_8$	0	33.66	0.00
	100	6.00	17.82
	150	8.66	25.74
	200	19.66	58.42
	250	14.66	43.57
	300	10.66	31.68
Westiellopsis-AT-TGK-$4A_2$	0	53.66	0.00
	100	11.66	21.74
	150	18.00	33.54
	200	33.00	61.49
	250	42.00	78.27
	300	26.66	49.69
Westiellopsis-AT-TGK- $4A_7$	0	51.66	0.00
	100	15.00	29.03
	150	23.00	44.52
	200	30.66	59.36
	250	41.00	79.36
	300	29.00	56.13

	SEd	CD
Cultures	0.57	1.14
Concentration	0.62	1.25
Cultures x Concentration	1.40	2.79

Fig 1 . Effect of ethyl methane sulphonate on acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Nostoc*- AT-TGK-$4C_4$
T3-*Oscillatoria*- AT-TGK-$5B_8$
T4-*Westiellopsis* -AT-TGK-$4A_2$
T5-*Westiellopsis* -AT-TGK-$4A_7$

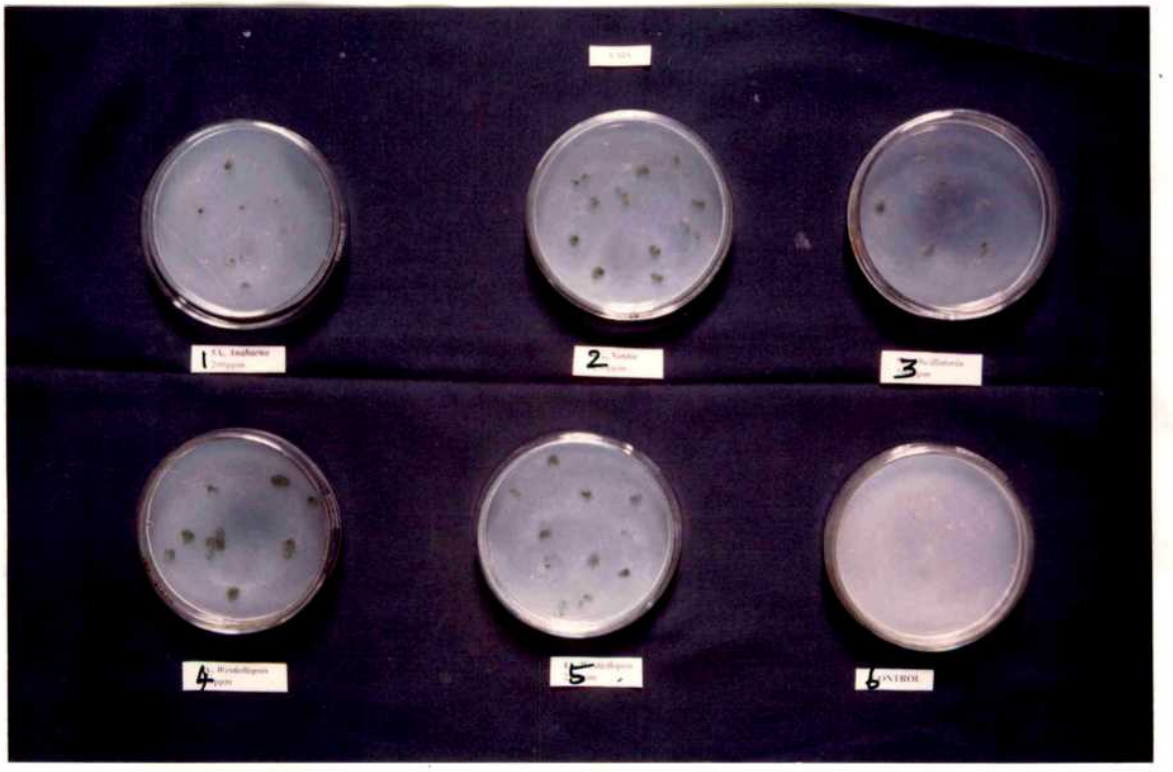


Plate 3. **Colony forming units of EMS induced acid tolerant cyanobacterial mutants**

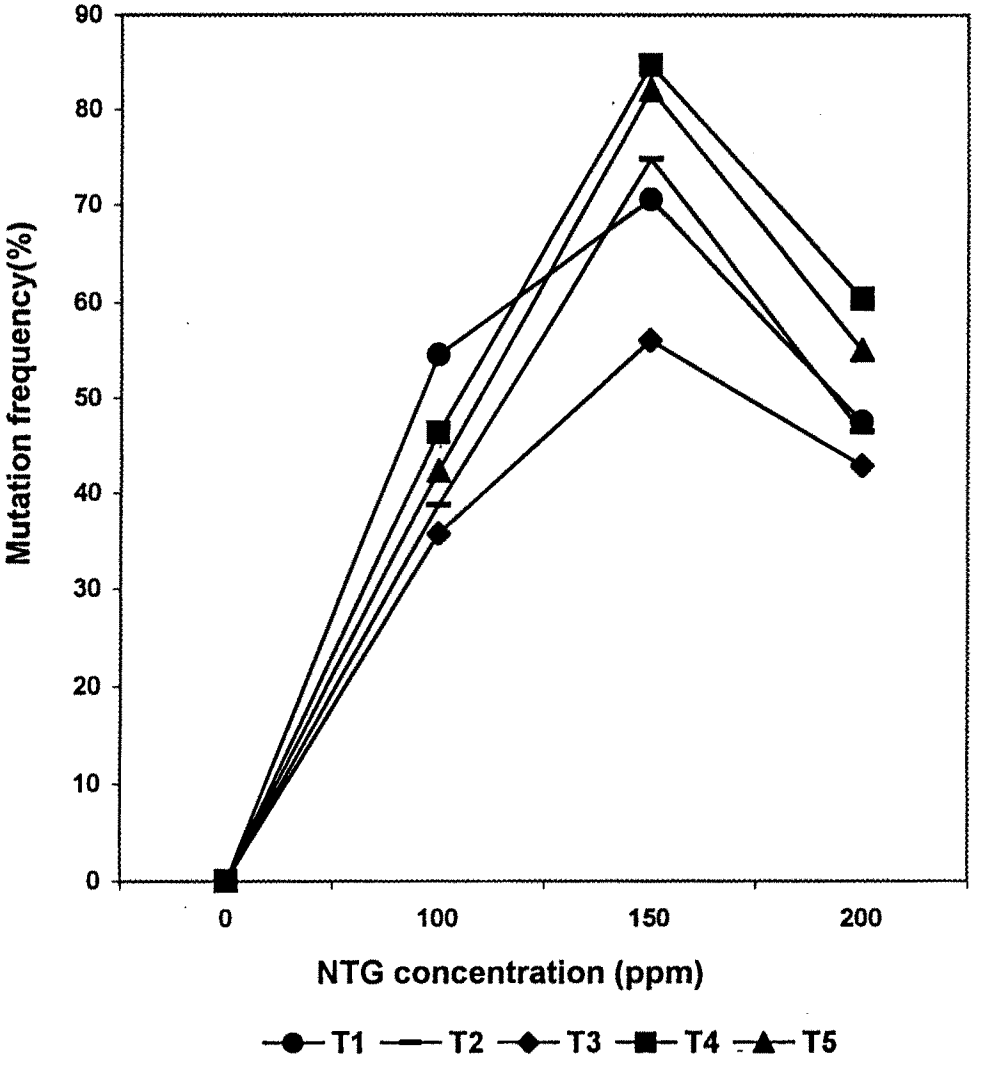
1. *Anabaena* – AT –MGK-5A_6-E_{200}
2. *Nostoc* – AT –MGK-4C_4-E_{250}
3. *Oscillatoria* – AT –MGK-5B_8-E_{200}
4. *Westiellopsis* – AT –MGK-4A_2-E_{250}
5. *Westiellopsis* – AT –MGK-4A_7-E_{250}
6. Control

Table 2. Effect of NTG on acid tolerant cyanobacterial cultures.

Cultures	NTG concentration (ppm)	Population (x 10^2 cfu)	Mutation frequency (%)
Anabaena-AT-TGK-5A_6	0	47.66	0.00
	100	26.00	54.55
	150	33.66	70.63
	200	22.66	47.55
Nostoc-AT-TGK-4C_4	0	51.66	0.00
	100	20.00	38.71
	150	38.66	74.84
	200	24.00	46.45
Oscillatoria-AT-TGK-5B_8	0	32.66	0.00
	100	11.66	35.72
	150	18.33	56.13
	200	14.00	42.86
Westiellopsis-AT-TGK-4A_2	0	49.66	0.00
	100	23.00	46.31
	150	42.00	84.57
	200	30.00	60.41
Westiellopsis-AT-TGK- 4A_7	0	52.00	0.00
	100	22.00	42.30
	150	42.66	82.03
	200	28.66	55.11

	SEd	CD
Cultures	0.79	1.60
Concentration	0.71	1.43
Cultures x Concentration	1.58	3.20

Fig 2 . Effect of NTG on acid tolerant cyanobacterial cultures



T1-*Anabaena*-AT-TGK-$5A_6$ T4-*Westiellopsis*-AT-TGK-$4A_2$

T2-*Nostoc*-AT-TGK-$4C_4$ T5-*Westiellopsis*-AT-TGK-$4A_7$

T3-*Oscillatoria*-AT-TGK-$5B_8$

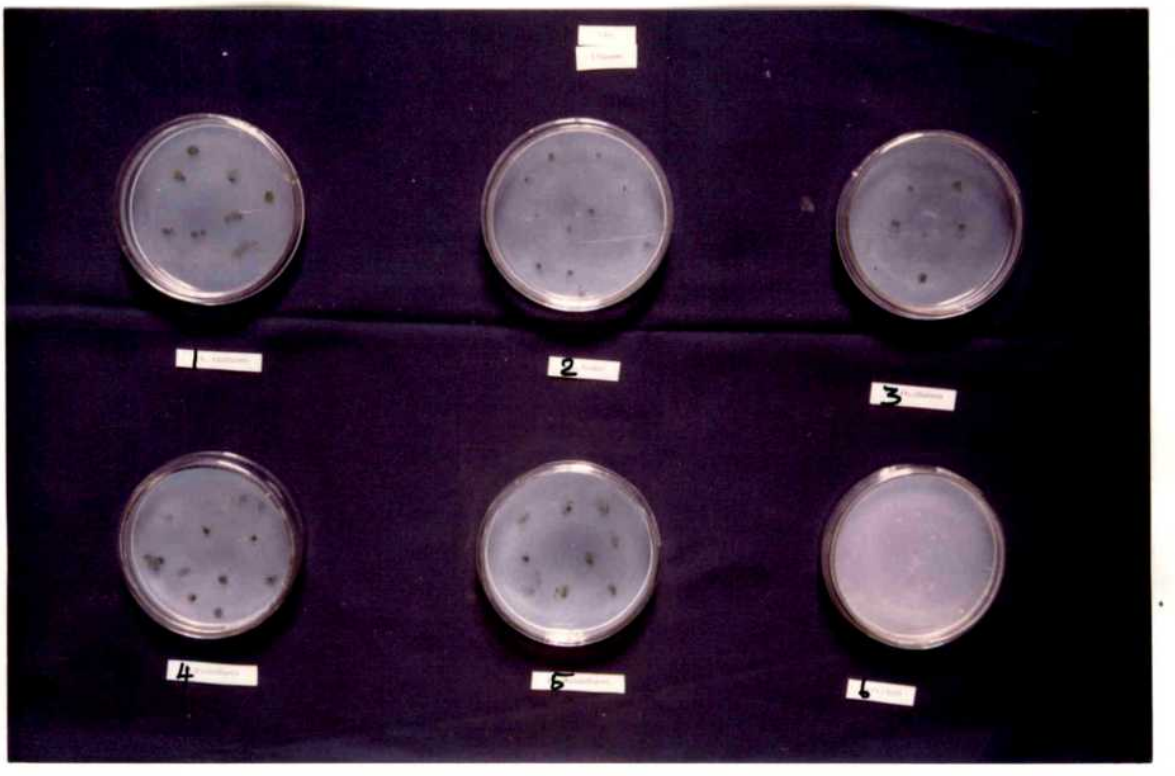


Plate 4. **Colony forming units of NTG induced acid tolerant cyanobacterial mutants at 150 ppm**

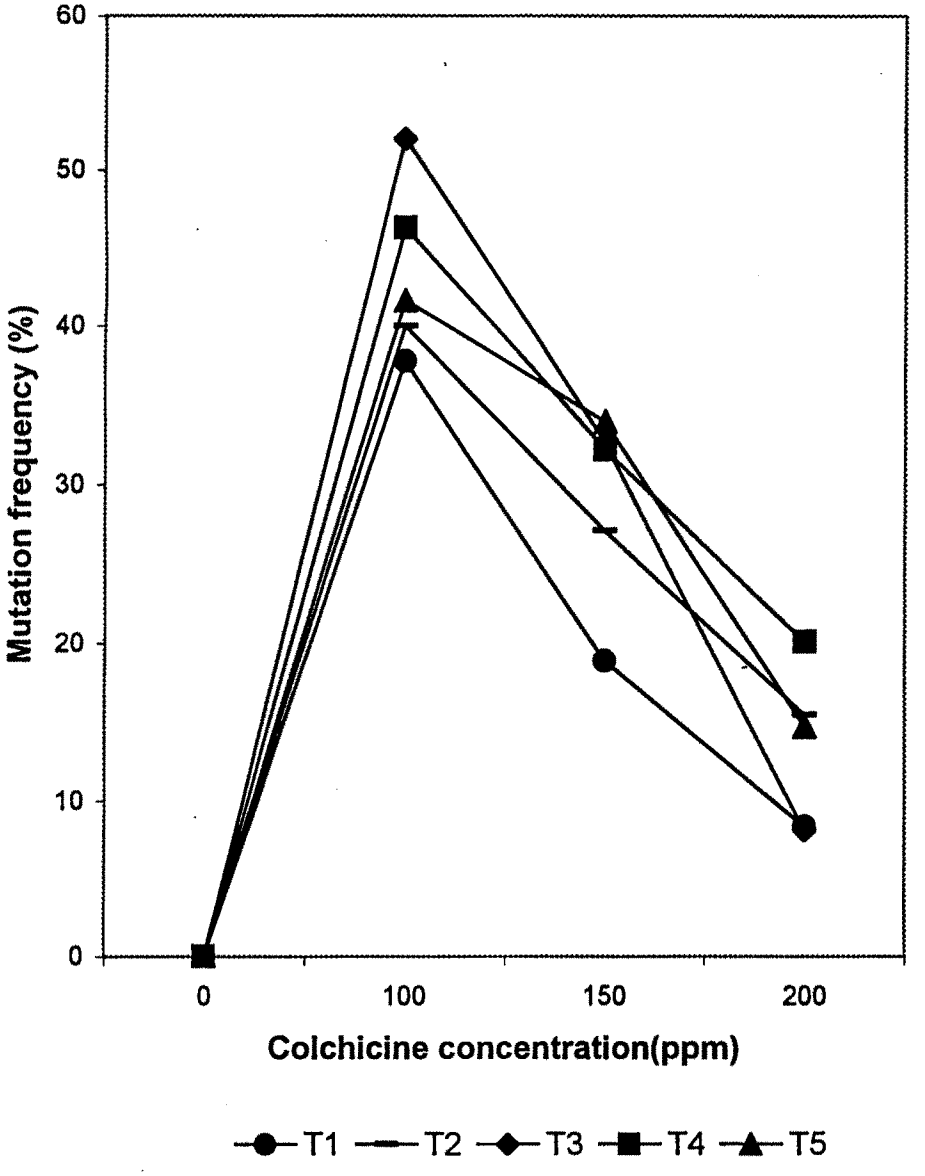
1. *Anabaena* – AT –TGK-5A_6
2. *Nostoc* – AT –TGK-4C_4
3. *Oscillatoria* – AT –TGK-5B_8
4. *Westiellopsis* – AT –TGK-4A_2
5. *Westiellopsis* – AT –TGK-4A_7
6. Control

Table 3. Effect of Colchicine on acid tolerant cyanobacterial cultures

Cultures	Colchicine concentration (ppm)	Population (x 10^2 cfu)	Mutation frequency (%)
Anabaena-AT-TGK-5A_6	0	14.66	0.00
	100	18.00	37.76
	150	9.00	18.88
	200	4.00	8.39
Nostoc-AT-TGK-4C_4	0	51.66	0.00
	100	20.66	40.00
	150	14.00	27.10
	200	8.00	15.48
Oscillatoria-AT-TGK-5B_8	0	32.66	0.00
	100	17.00	52.05
	150	10.66	32.65
	200	2.66	8.16
Westiellopsis-AT-TGK-4A_2	0	49.66	0.00
	100	23.00	46.31
	150	16.00	32.21
	200	10.00	20.13
Westiellopsis-AT-TGK-4A_7	0	52.00	0.00
	100	21.66	41.66
	150	17.66	33.97
	200	7.66	14.74

	SEd	CD
Cultures	0.52	1.06
Concentration	0.47	0.95
Cultures x Concentration	1.05	2.13

Fig 3 . Effect of colchicine on acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Nostoc* -AT-TGK-$4C_4$
T3-*Oscillatoria* -AT-TGK-$5B_8$
T4-*Westiellopsis* -AT-TGK-$4A_2$
T5-*Westiellopsis* -AT-TGK-$4A_7$

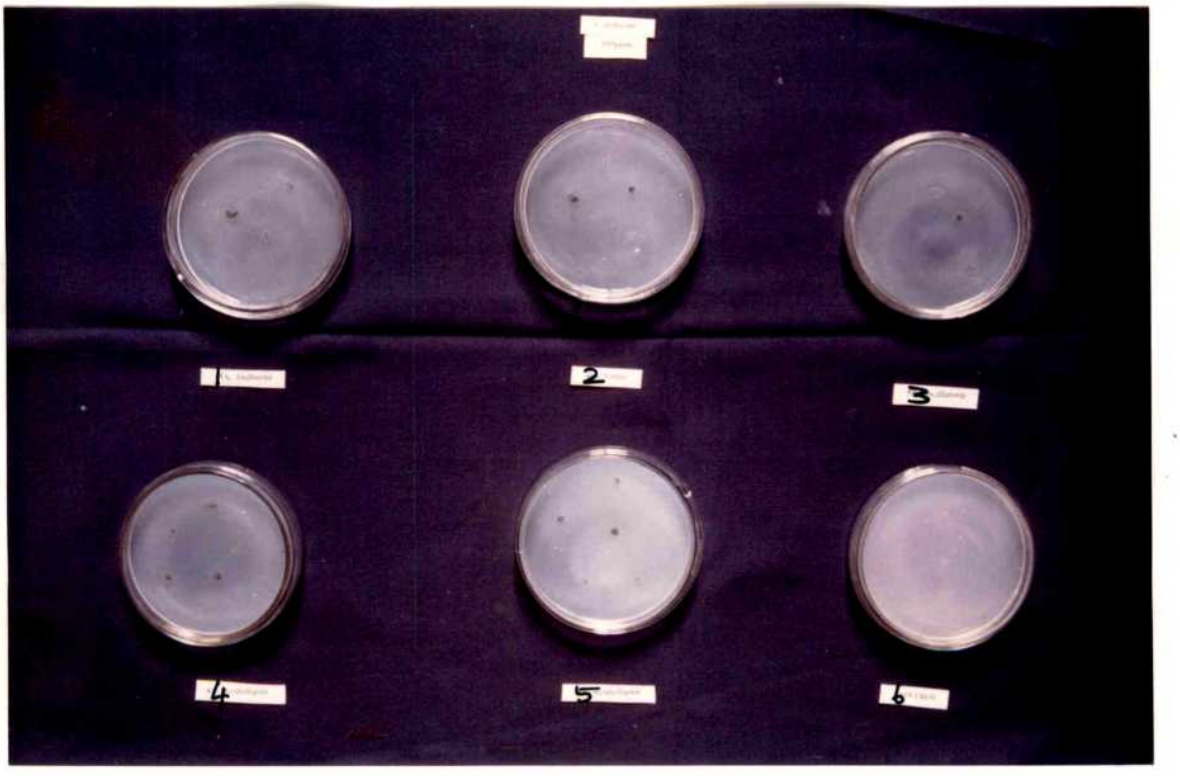


Plate 5. **Colony forming units of Colchicine induced acid tolerant cyanobacterial mutants at 100 ppm.**

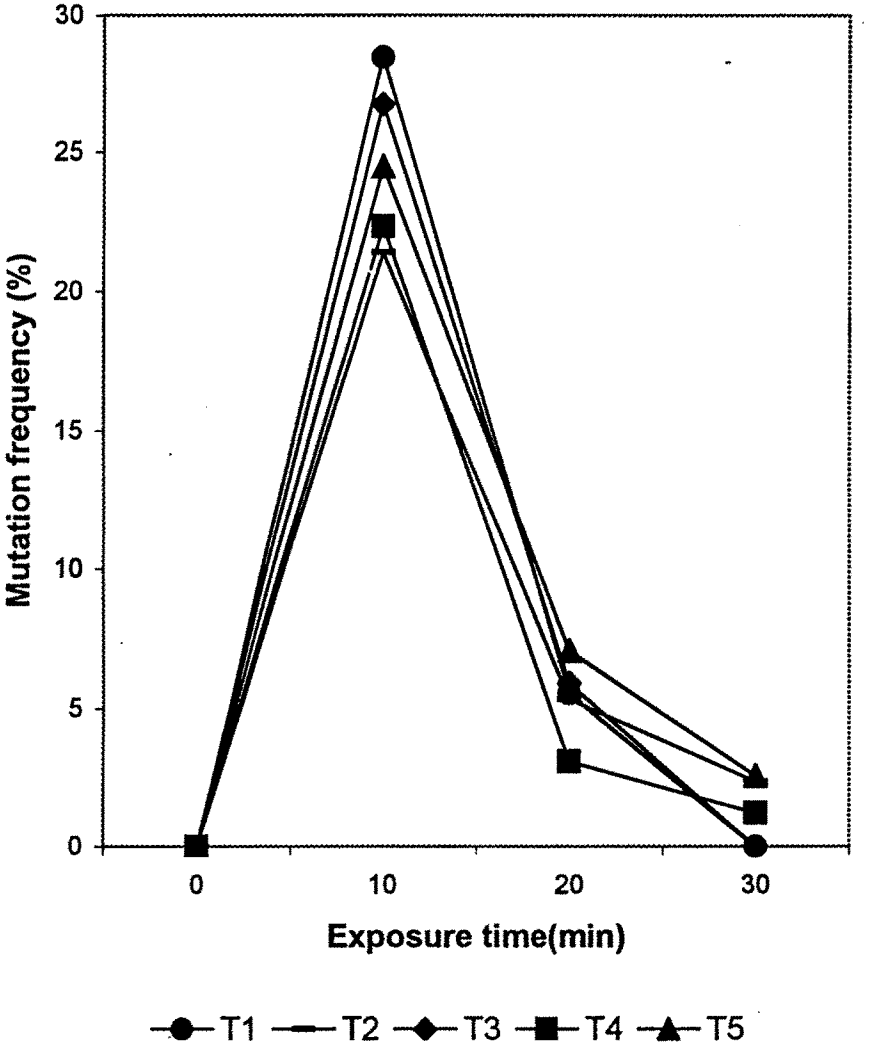
1. ***Anabaena*** **– AT –TGK-5A_6**
2. ***Nostoc*** **– AT –TGK-4C_4**
3. ***Oscillatoria*** **– AT –TGK-5B_8**
4. ***Westiellopsis*** **– AT –TGK-4A_2**
5. ***Westiellopsis*** **– AT –TGK-4A_7**
6. **Control**

Table 4. Effect of UV rays on acid tolerant cyanobacterial cultures

Cultures	Exposure time (min)	Population (x 10^2 cfu)	Mutation frequency (%)
Anabaena-AT-TGK-$5A_6$	0	48.00	0.00
	10	13.60	28.47
	20	2.66	5.55
	30	0.00	0.00
Nostoc-AT-TGK-$4C_4$	0	56.00	0.00
	10	12.00	21.42
	20	3.00	5.35
	30	1.33	2.38
Oscillatoria-AT-TGK-$5B_8$	0	33.66	0.00
	10	9.00	26.73
	20	2.00	5.94
	30	0.00	0.00
Westiellopsis AT-TGK-$4A_2$	0	53.66	0.00
	10	12.00	22.36
	20	5.00	3.10
	30	0.66	1.24
Westiellopsis AT-TGK-$4A_7$	0	51.66	0.00
	10	12.66	24.50
	20	3.66	7.09
	30	1.33	2.58

	SEd	CD
Cultures	0.55	1.11
Duration	0.49	0.99
Cultures x Duration	1.10	2.22

Fig 4 . Effect of UV rays on acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Nostoc* -AT-TGK-$4C_4$
T3-*Oscillatoria* -AT-TGK-$5B_8$
T4-*Westiellopsis*- AT-TGK-$4A_2$
T5-*Westiellopsis* -AT-TGK-$4A_7$

Plate 6. **Colony forming units of UV induced mutants of acid tolerant cyanobacterial mutants after 10 min exposure**

1. *Anabaena* – AT –TGK-5A_6
2. *Nostoc* – AT –TGK-4C_4
3. *Oscillatoria* – AT –TGK-5B_8
4. *Westiellopsis* – AT –TGK-4A_2
5. *Westiellopsis* – AT –TGK-4A_7
6. Control

4.3. ASSESSMENT OF LD_{50} TO VARIOUS MUTAGENS FOR THE ACID TOLERANT CYANOBACTERIAL CULTURES

The acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$, *Oscillatoria*-AT-TGK-$5B_8$, *Westiellopsis*-AT-TGK-$4A_2$ and *Westiellopsis*-AT-TGK-$4A_7$ were exposed to different concentration of mutagens *viz.*, EMS, NTG and Colchicine and also different exposure time of UV rays and the results are given in Tables 5-8 and Figs. 5-8. The survival percentage of the acid tolerant cyanobacterial cultures decreased as the concentration of the chemical mutagens and the exposure time of the physical mutagen increased. The LD_{50} value of the tested mutagens on the acid tolerant cyanobacterial cultures was compared and given in Table 9. It is interesting to note that the lethal dose concentration of EMS is more followed by Colchicine and NTG. Among the acid tolerant cyanobacterial cultures, the LD_{50} value of EMS was more for *Westiellopsis*-AT-TGK-$4A_7$ followed by *Westiellopsis*-AT-TGK-$4A_2$, *Oscillatoria*-AT-TGK-$5B_8$, *Nostoc*-AT-TGK-$4C_4$ and *Anabaena*-AT-TGK-$5A_6$. The LD_{50} value of NTG was more for *Westiellopsis*-AT-TGK-$4A_2$ followed by *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$, *Westiellopsis*-AT-TGK-$4A_7$ and *Oscillatoria*-AT-TGK-$5B_8$. The LD_{50} value of Colchicine was more for *Westiellopsis*-AT-TGK-$4A_2$, followed by *Westiellopsis*-AT-TGK-$4A_7$, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AT-TGK-$4C_4$ and *Oscillatoria*-AT-TGK-$5B_8$. The LD_{50} value of UV rays was more for *Westiellopsis*-AT-TGK-$4A_2$ followed by *Nostoc*-AT-TGK-$4C_4$, *Westiellopsis*-AT-TGK-$4A_7$, *Oscillatoria*-AT-TGK-$5B_8$ and *Anabaena*-AT-TGK-$5A_6$.

4.4. REVERSION FREQUENCY OF THE ACID TOLERANT CYANOBACTERIAL MUTANTS DEVELOPED BY EMS AND NTG

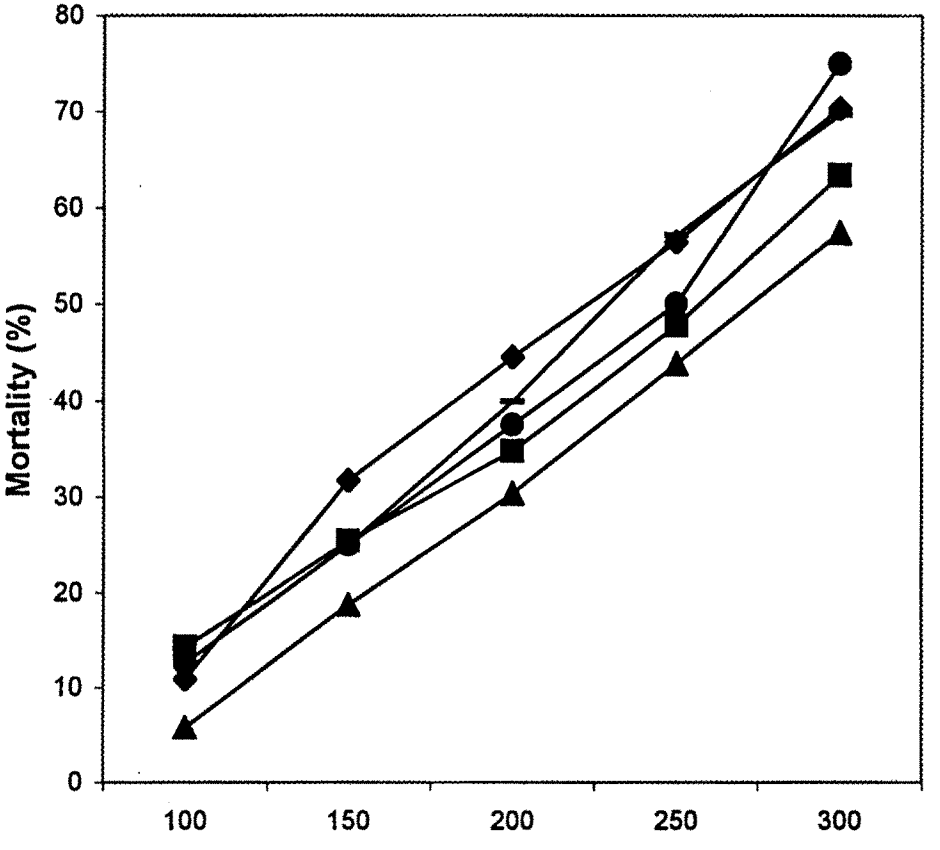
Based on the *in vitro* studies on the mutation frequency of the acid tolerant cyanobacterial cultures, mutants developed by EMS and NTG which had more mutation frequency was selected and their reversion frequency were studied and the results are given in Tables 10 and 11 respectively. The reversion frequency of the

Table 5. Assessment of LD_{50} to EMS for the acid tolerant cyanobacterial cultures

Cultures	EMS concentration (ppm)	Population (x 10^2 cfu)	Survival (%)	Mortality (%)
Anabaena-AT-TGK-5A_6	0	48.00	100.00	0.00
	100	42.00	87.50	12.50
	150	36.00	75.00	25.00
	200	30.00	62.50	37.50
	250	24.00	50.00	50.00
	300	12.00	25.00	75.00
Nostoc-AT-TGK-4C_4	0	56.00	100.00	0.00
	100	49.00	87.50	12.50
	150	42.00	75.00	25.00
	200	33.66	60.10	39.90
	250	24.00	42.85	57.15
	300	17.00	30.35	69.65
Oscillatoria-AT-TGK-5B_8	0	33.66	100.00	0.00
	100	30.00	89.12	10.88
	150	23.00	68.33	31.67
	200	18.66	55.45	44.55
	250	14.66	43.57	56.43
	300	10.00	29.70	70.30
Westiellopsis AT-TGK-4A_2	0	53.60	100.00	0.00
	100	46.00	85.82	14.18
	150	40.00	74.62	25.38
	200	35.00	65.29	34.71
	250	28.00	52.23	47.77
	300	19.60	36.69	63.31
Westiellopsis AT-TGK-4A_7	0	51.66	100.00	0.00
	100	48.66	94.19	5.81
	150	42.00	81.30	18.70
	200	36.00	69.68	30.32
	250	29.00	56.13	43.87
	300	22.00	42.58	57.42

	SEd	CD
Cultures	0.46	0.92
Concentration	0.50	1.00
Cultures x Concentration	1.12	2.24

Fig 5. Assesment of LD $_{50}$ to EMS for the acid tolerant cyanobacterial cultures



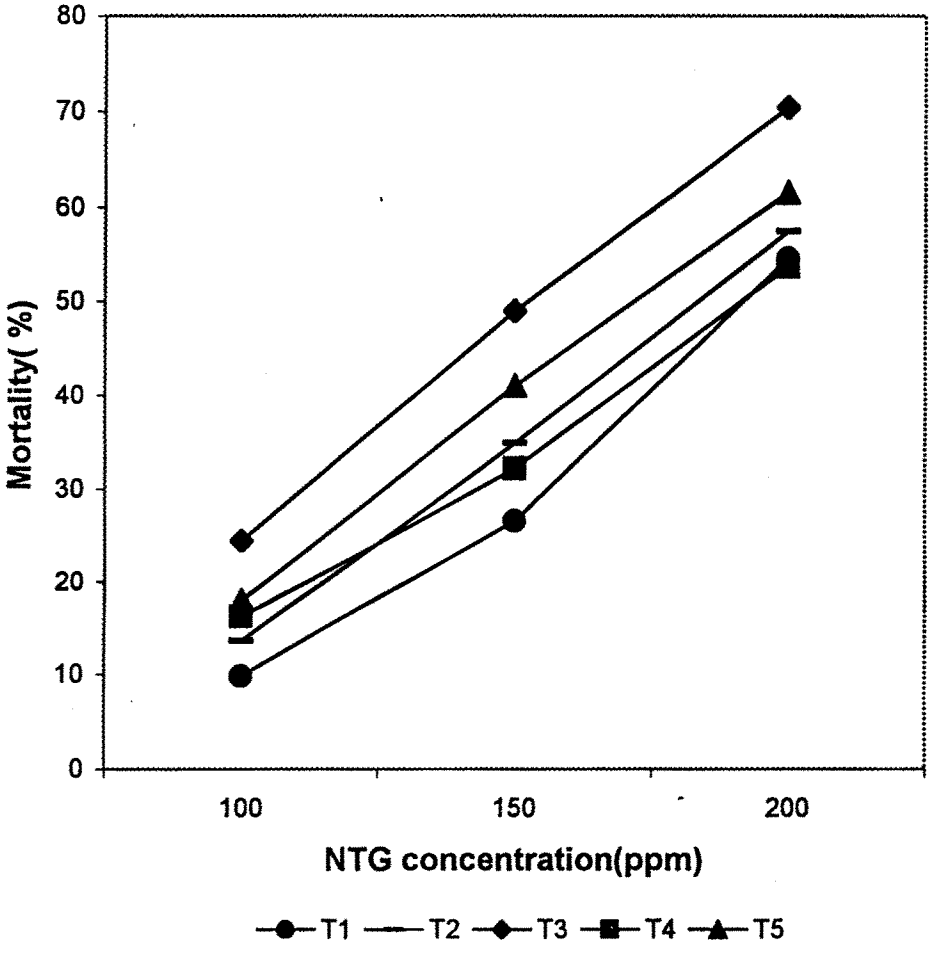
T1-*Anabaena*-AT-TGK-5A$_6$
T2-*Nostoc*-AT-TGK-4C$_4$
T3-*Oscillatoria*-AT-TGK-5B$_8$
T4-*Westiellopsis*-AT-TGK-4A$_2$
T5-*Westiellopsis*-AT-TGK-4A$_7$

Table 6. Assessment of LD_{50} to NTG for the acid tolerant cyanobacterial cultures.

Cultures	NTG concentration (ppm)	Population (x 10^2 cfu)	Survival (%)	Mortality (%)
Anabaena-AT-TGK-$5A_6$	0	47.66	100.00	0.00
	100	43.00	90.22	9.78
	150	35.00	73.43	26.57
	200	21.66	45.46	54.54
Nostoc-AT-TKG-$4C_4$	0	51.66	100.00	0.00
	100	44.66	86.46	13.54
	150	33.66	65.16	34.84
	200	22.00	42.58	57.42
Oscillatoria-AT-TGK-$5B_8$	0	32.66	100.00	0.00
	100	24.66	75.52	24.48
	150	16.66	51.03	48.97
	200	9.66	29.59	70.41
Westiellopsis-AT-TGK-$4A_2$	0	49.66	100.00	0.00
	100	41.66	83.90	16.10
	150	33.66	67.79	32.21
	200	23.00	46.31	53.69
Westiellopsis-AT-TGK-$4A_7$	0	52.00	100.00	0.00
	100	42.66	82.03	17.97
	150	30.66	58.96	41.04
	200	20.00	38.46	61.54

	SEd	CD
Cultures	0.62	1.26
Concentration	0.55	1.12
Cultures x Concentration	1.24	2.52

Fig 6. Assessment of LD_{50} to NTG for the acid tolerant cyanobacterial cultures



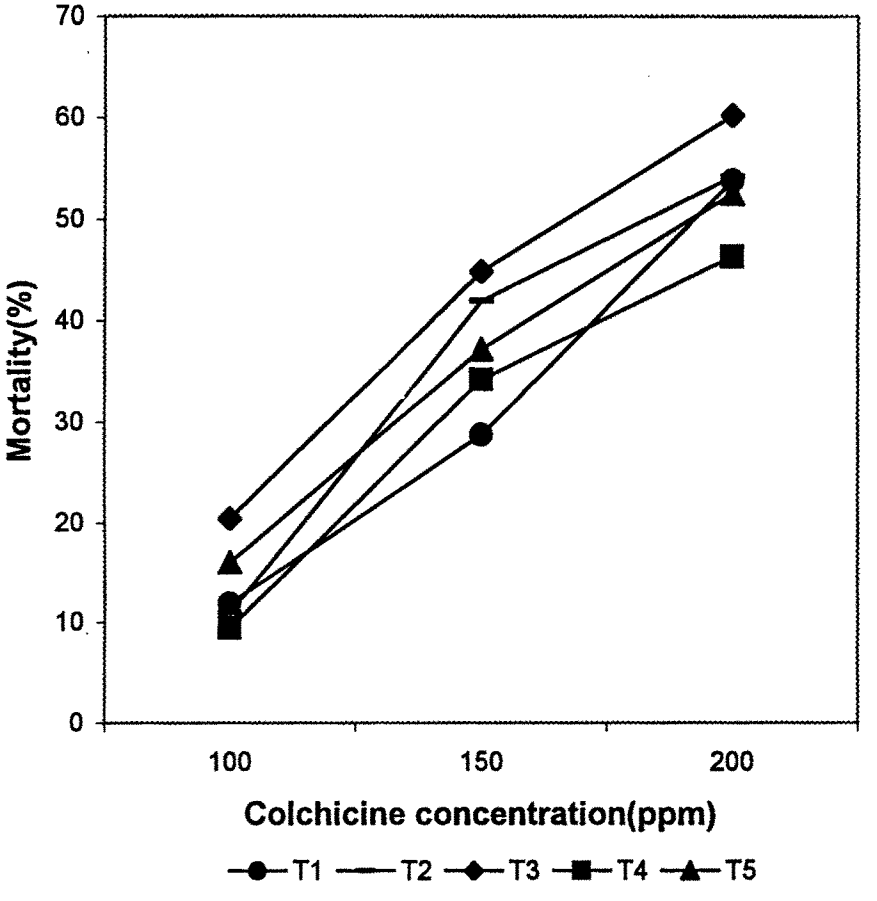
T1-*Anabaena*-AT-TGK-$5A_6$
T2-*Nostoc*-AT-TGK-$4C_4$
T3-*Oscillatoria*-AT-TGK-$5B_8$
T4-*Westiellopsis*-AT-TGK-$4A_2$
T5-*Westiellopsis*-AT-TGK-$4A_7$

Table 7. Assessment of LD_{50} to Colchicine for the acid tolerant cyanobacterial cultures

Cultures	Colchicine concentration (ppm)	Population (x 10^2 cfu)	Survival (%)	Mortality (%)
Anabaena-AT-TGK-5A_6	0	47.66	100.00	0.00
	100	42.00	88.12	11.88
	150	34.00	71.33	28.67
	200	22.00	46.16	53.84
Nostoc-AT-TGK-4C_4	0	51.66	100.00	0.00
	100	46.00	89.04	10.96
	150	30.00	58.07	41.93
	200	23.66	45.81	54.19
Oscillatoria-AT-TGK-5B_8	0	32.66	100.00	0.00
	100	26.00	79.60	20.40
	150	18.00	55.11	44.89
	200	13.00	39.80	60.20
Westiellopsis-AT-TGK-4A_2	0	49.66	100.00	0.00
	100	45.00	90.61	9.39
	150	32.66	65.78	34.22
	200	26.66	53.69	46.31
Westiellopsis-AT-TGK-4A_7	0	52.00	100.00	0.00
	100	43.66	83.97	16.03
	150	32.66	62.80	37.20
	200	24.66	47.43	52.57

	SEd	CD
Cultures	0.71	1.44
Concentration	0.63	1.29
Cultures x Concentration	1.42	2.88

Fig 7. Assessment of LD $_{50}$ to colchicine for the acid tolerant cyanobacterial cultures



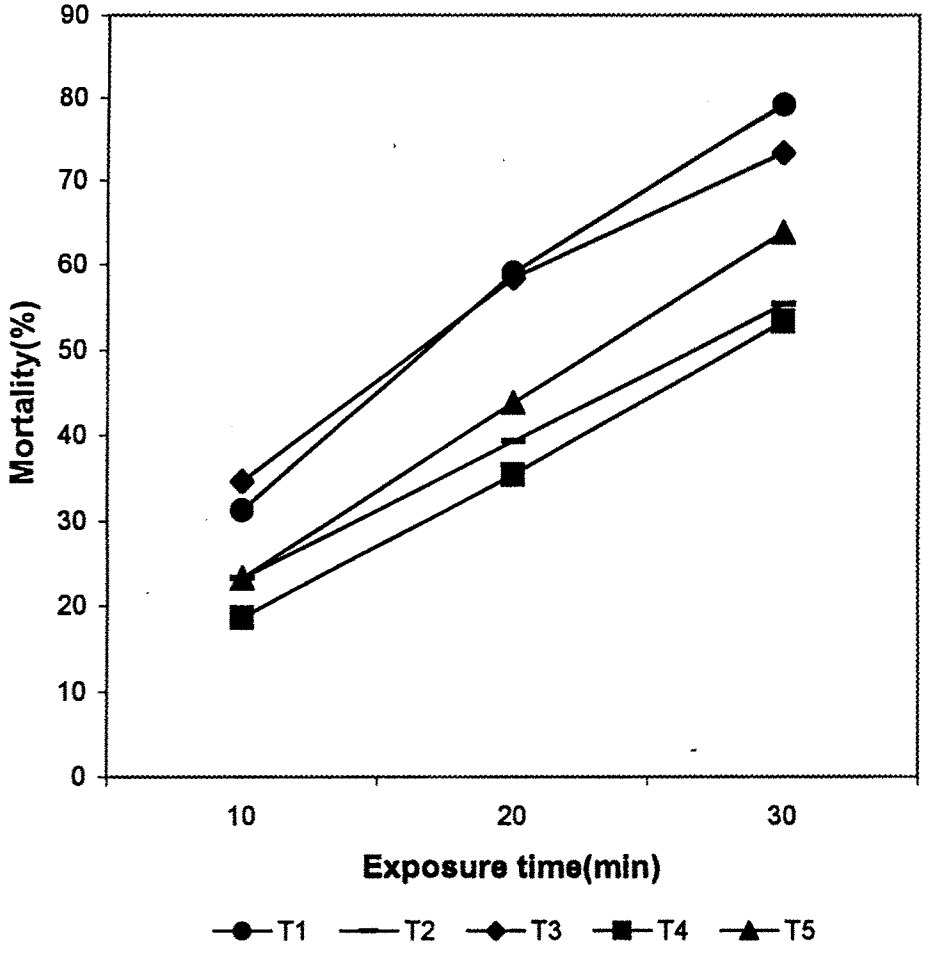
T1-*Anabaena*-AT-TGK-5A$_6$
T2-*Nostoc*-AT-TGK-4C$_4$
T3-*Oscillatoria*-AT-TGK-5B$_8$
T4-*Westiellopsis*-AT-TGK-4A$_2$
T5-*Westiellopsis*-AT-TGK-4A$_7$

Table 8. Assessment of LD_{50} to UV rays for the acid tolerant cyanobacterial cultures.

Cultures	Exposure time (min)	Population ($X10^2$cfu)	Survival (%)	Mortality (%)
Anabaena-AT-TGK-$5A_6$	0	48.00	100.00	0.00
	10	33.00	68.75	31.25
	20	19.66	40.95	59.05
	30	10.00	20.83	79.17
Nostoc-AT-TGK-$4C_4$	0	56.00	100.00	0.00
	10	43.00	76.78	23.22
	20	34.00	60.71	39.29
	30	25.00	44.64	55.36
Oscillatoria-AT-TGK-$5B_8$	0	33.66	100.00	0.00
	10	22.00	65.35	34.65
	20	14.00	41.59	58.41
	30	9.00	26.73	73.27
Westiellopsis-AT-TGK-$4A_2$	0	53.66	100.00	0.00
	10	43.66	81.37	18.63
	20	34.66	64.60	35.40
	30	25.00	46.58	53.42
Westiellopsis-AT-TGK-$4A_7$	0	51.66	100.00	0.00
	10	39.66	76.78	23.22
	20	29.00	56.13	43.87
	30	18.66	36.13	63.87

	SEd	CD
Cultures	0.69	1.41
Duration	0.62	1.26
Cultures x Duration	1.39	2.82

Fig 8. Assessment of LD_{50} to UV rays for the acid tolerant cyanobacterial cultures



T1-*Anabaena*-AT-TGK-$5A_6$
T2-*Nostoc*-AT-TGK-$4C_4$
T3-*Oscillatoria*-AT-TGK-$5B_8$
T4-*Westiellopsis*-AT-TGK-$4A_2$
T5-Westiellopsis-AT-TGK-$4A_7$

Table 9. LD_{50} value for acid tolerant cyanobacterial cultures exposed to various mutagens

Cultures	LD_{50}			
	EMS (ppm)	NTG (ppm)	Colchicine (ppm)	UVR (min)
Anabaena-AT-TGK-$5A_6$	226.05	195.71	190.00	15.58
Nostoc-AT-TGK-$4C_4$	225.67	183.84	181.65	26.68
Oscillatoria-AT-TGK-$5B_8$	218.45	150.69	168.45	15.73
Westiellopsis AT-TGK-$4A_2$	254.28	195.21	202.44	28.80
Westiellopsis -AT-TGK-$4A_7$	272.85	170.83	190.95	22.27

Table 10. Reversion frequency of acid tolerant cyanobacterial mutants developed by EMS

Cultures	EMS concentration (ppm)	Mutants population (x 10^2 cfu)	Revertants population (x 10^2 cfu)	Reversion frequency (%)
Anabaena-AT-TGK-5A_6	100	8.33	2.33	27.97
	150	13.33	9.00	67.51
	200	24.33	2.00	8.22
	250	15.00	5.00	33.33
	300	13.33	5.33	39.98
Nostoc-AT-TGK-4C_4	100	8.00	3.00	37.50
	150	11.33	4.00	35.30
	200	21.33	5.00	23.44
	250	30.33	1.33	4.38
	300	21.00	5.33	25.38
Oscillatoria-AT-TGK-5B_8	100	6.00	3.00	50.00
	150	8.33	3.33	39.97
	200	19.33	1.33	6.88
	250	14.33	5.33	37.19
	300	10.33	4.00	38.72
Westiellopsis-AT-TGK-4A_2	100	11.33	3.33	29.39
	150	18.00	5.00	27.77
	200	33.00	7.33	22.21
	250	42.00	2.33	5.54
	300	26.33	5.00	18.98
Westiellopsis-AT-TGK-4A_7	100	15.00	3.33	22.20
	150	23.00	5.33	23.17
	200	30.33	4.33	14.27
	250	41.00	1.33	3.24
	300	29.00	3.33	11.48

	SEd	CD	SEd	CD
Cultures	0.62	1.25	0.33	0.67
Concentration	0.62	1.25	0.33	0.67
Cultures x Concentration	1.40	2.81	0.74	1.50

Table 11. Reversion frequency of acid tolerant cyanobacterial mutants developed by NTG

Cultures	NTG concentration (ppm)	Mutants population ($x10^2$ cfu)	Revertants population ($x 10^2$ cfu)	Reversion frequency (%)
Anabaena-AT-TGK-$5A_6$	100	26.00	6.00	23.07
	150	33.33	2.00	6.00
	200	22.33	4.33	19.39
Nostoc-AT-TGK-$4C_4$	100	20.00	5.00	25.00
	150	38.33	2.00	5.21
	200	24.00	5.00	20.83
Oscillatoria-AT-TGK-$5B_8$	100	11.33	2.33	20.56
	150	18.00	1.33	7.38
	200	14.00	4.00	28.57
Westiellopsis-AT-TGK-$4A_2$	100	23.00	5.33	23.17
	150	42.00	2.00	4.76
	200	30.00	5.00	16.66
Westiellopsis-AT-TGK-$4A_7$	100	22.00	5.00	22.72
	150	42.33	2.33	5.50
	200	28.33	5.00	17.64

	SEd	CD	SEd	CD
Cultures	0.95	1.95	0.39	0.81
Concentration	0.74	1.51	0.30	0.62
Cultures x Concentration	1.65	3.39	0.68	1.40

acid tolerant cyanobacterial mutants developed by EMS was less and varies with mutagen concentration. The reversion frequency for *Westiellopsis* - AT-TGK-$4A_7$, *Westiellopsis* -AT-TGK-$4A_2$ and *Nostoc* - AT-TGK-$4C_4$ was less at E_{250} and for *Oscillatoria* -AT-TGK-$5B_8$ and *Anabaena* -AT-TGK-$5A_6$, it was less at E_{200}. However in the case of NTG, reversion frequency of the acid tolerant cyanobacterial mutants was minimum at 150 ppm for all the acid tolerant cyanobacterial cultures.

4.5. GROWTH PERFORMANCE AND BIOCHEMICAL CONSTITUENTS OF THE ACID TOLERANT CYANOBACTERIAL CULTURES AND MUTANTS

4.5.1. GROWTH AND BIOMASS PRODUCTION OF THE ACID TOLERANT CYANOBACTERIAL CULTURE AND MUTANTS.

Based on the earlier *in vitro* studies, the mutagen EMS was selected for further study. The growth and biomass production of the EMS mutants of acid tolerant cyanobacterial cultures *viz.*, *Anabaena* -AT-MGK-$5A_6$-E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria* -AT-MGK-$5B_8$-E_{200}, *Westiellopsis* -AT-MGK-$4A_2$-E_{250} and *Westiellopsis* -AT-MGK-$4A_7$-E_{250} were compared with the wild acid tolerant cyanobacterial cultures. The results are presented in Table 12 and Figs.9 and 10. In general, linear increase in growth was observed with increased incubation time. The growth and biomass production were higher in the case of mutants than wild cultures. Among the cyanobacterial cultures, the growth and biomass production was significantly higher in *Westiellopsis* mutants than any of the mutant and wild acid tolerant cyanobacterial cultures.

4.5.2. AMMONIA EXCRETION

The ammonia excretion by the mutants of acid tolerant cyanobacterial cultures was compared with wild type and the data are given in Table 13 and Fig.11. All the mutants showed increase in ammonia excretion into the surrounding medium than the wild type. The acid tolerant cyanobacterial mutant *Westiellopsis* - AT-MGK-$4A_7$-E_{250} registered higher ammonia excretion among the experimented

Table 12. Growth performance and biomass production by the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Growth (OD at 660 nm)			Biomass in dry wt. ($\mu g\ ml^{-1}$)
	10^{th}day	20^{th}day	30^{th}day	
Anabaena-AT-TGK-$5A_6$	0.21	0.36	0.70	77.38
Anabaena-AT-MGK-$5A_6$-E_{200}	0.28	0.42	0.73	83.28
Nostoc-AT-TGK-$4C_4$	0.27	0.40	0.72	71.79
Nostoc-AT-MGK-$4C_4$-E_{250}	0.21	0.42	0.77	78.73
Oscillatoria-AT-TGK-$5B_8$	0.17	0.27	0.58	63.66
Oscillatoria-AT-MGK-$5B_8$-E_{200}	0.15	0.31	0.65	69.23
Westiellopsis-AT-TGK-$4A_2$	0.24	0.42	0.75	87.34
Westiellopsis-AT-MGK-$4A_2$-E_{250}	0.22	0.51	0.85	93.81
Westiellopsis-AT-TGK-$4A_7$	0.29	0.47	0.76	89.50
Westiellopsis-AT-MGK-$4A_7$-E_{250}	0.23	0.52	0.85	95.54

	SEd	CD
Cultures	0.01	0.01
Days	0.01	0.01
Cultures x Days	0.01	0.02
Biomass	0.01	0.02

Fig 9 . Growth performance of the wild and mutant acid tolerant cyanobacterial cultures

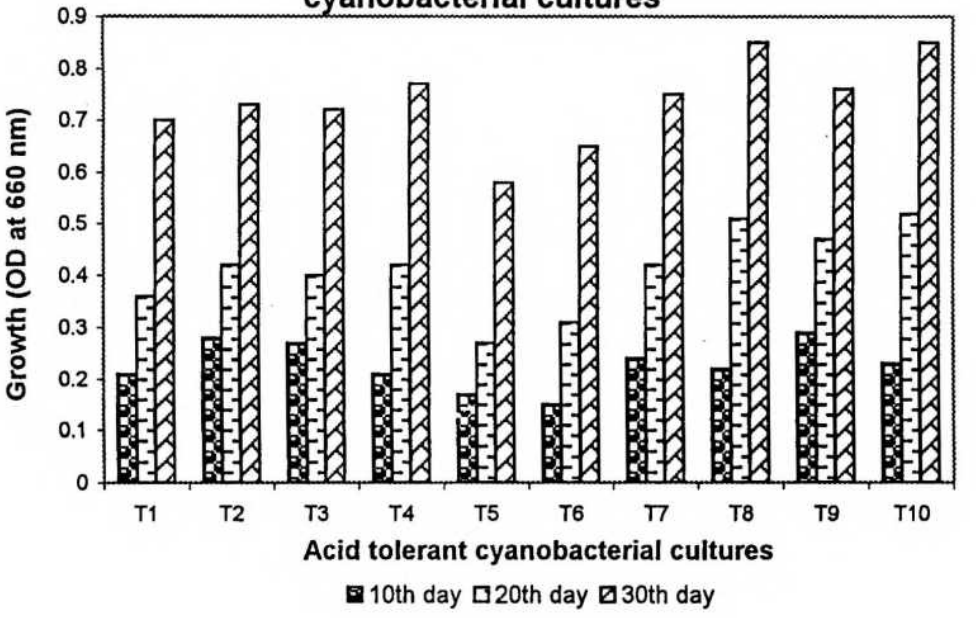
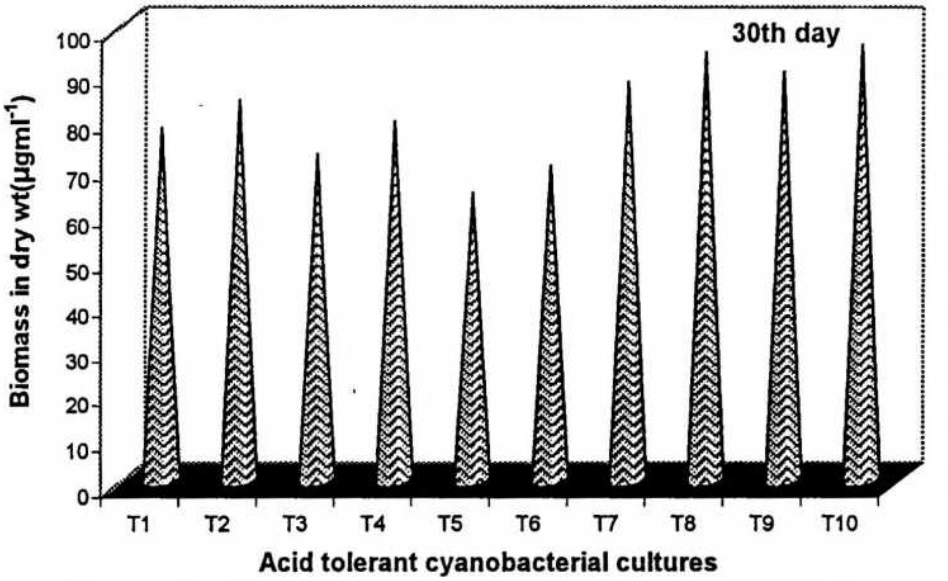


Fig 10 . Biomass production by the wild and mutant acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Anabaena*-AT-MGK-$5A_6$-E_{200}
T3-*Nostoc*-AT-TGK-$4C_4$
T4-*Nostoc*-AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*-AT-TGK-$5B_8$
T6-*Oscillatoria*-AT-MGK-$5B_8$-E_{200}
T7-*Westiellopsis*-AT-TGK-$4A_2$
T8-*Westiellopsis*-AT-MGK-$4A_2$-E_{250}
T9-*Westiellopsis*-AT-TGK-$4A_7$
T10-*Westiellopsis*-AT-MGK-$4A_7$-E_{250}

Table13. Ammonia excretion by the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Ammonia excretion (n moles ml^{-1})		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	57.94	157.36	126.19
Anabaena-AT-MGK-$5A_6$-E_{200}	68.51	194.67	165.41
Nostoc-AT-TGK-$4C_4$	63.26	204.32	145.75
Nostoc-AT-MGK-$4C_4$-E_{250}	68.26	214.56	185.44
Oscillatoria-AT-TGK-$5B_8$	48.25	146.86	108.31
Oscillatoria-AT-MGK-$5B_8$-E_{200}	57.47	175.99	136.20
Westiellopsis-AT-TGK-$4A_2$	68.14	204.48	165.46
Westiellopsis-AT-MGK-$4A_2$-E_{250}	78.34	234.38	186.78
Westiellopsis-AT-TGK-$4A_7$	72.52	205.17	176.59
Westiellopsis-AT-MGK-$4A_7$-E_{250}	88.37	251.82	194.66

	SEd	CD
Cultures	5.07	10.14
Days	2.78	5.55
Cultures x Days	8.78	17.56

cultures. The ammonia excretion by the cyanobacterial cultures were maximum at 20th day after inoculation and it declined at 30th day after inoculation. Both wild and mutant of *Oscillatoria* culture showed minimum ammonia excretion.

4.5.3. CHLOROPHYLL a CONTENT

The acid tolerant cyanobacterial cultures and the mutants were tested for their chlorophyll a content and the results are given in Table 14 and Fig.12. In general, the mutants recorded higher chlorophyll a content than wild parents. Among the cyanobacterial cultures, *Westiellopsis* - AT-MGK-$4A_7$-E_{250} registered maximum chlorophyll a content. There was a linear increase in chlorophyll a content with respect to aging of cultures. However the increase was gradual with respect to both wild and mutant of *Oscillatoria.*

4.5.4. PROTEIN CONTENT

The protein content of the acid tolerant cyanobacterial cultures and the mutants are shown in Table 15 and Fig.13. Among the mutants, *Westiellopsis* -AT-MGK-$4A_2$-E_{250} recorded more protein content and *Oscillatoria* - AT-MGK-$5B_8$-E_{200} registered the least. All the mutants recorded higher protein content than their wild parent.

4.5.5. POLYSACCHARIDE PRODUCTION

The polysaccharide production by the acid tolerant cyanobacterial culture and the mutants are represented in Table 16 and Fig.14. Enhanced polysaccharide production was observed in mutants than their wild parents. *Westiellopsis*-AT-MGK-$4A_2$-E_{250} and *Westiellopsis* -AT-MGK-$4A_7$-E_{250} produced significantly higher amount of polysaccharide than other mutants and *Anabaena* cultures produced the least amount of polysaccharide.

4.5.6. AMINO NITROGEN CONTENT

The acid tolerant cyanobacterial cultures and their mutants were analysed for their amino nitrogen content and the results are given in Table 17 and Fig.15. All

Table 14. Chlorophyll a content of the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Chlorophyll a content (mg ml^{-1})		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	1.09	2.97	4.06
Anabaena-AT-MGK-$5A_6$-E_{200}	1.44	3.67	4.63
Nostoc-AT-TGK-$4C_4$	1.20	3.11	4.19
Nostoc-AT-MGK-$4C_4$-E_{250}	1.57	3.82	4.76
Oscillatoria-AT-TGK-$5B_8$	0.96	2.53	2.99
Oscillatoria-AT-MGK-$5B_8$-E_{200}	1.20	2.74	3.33
Westiellopsis-AT-TGK-$4A_2$	1.57	3.35	4.29
Westiellopsis-AT-MGK-$4A_2$-E_{250}	2.04	4.07	5.05
Westiellopsis-AT-TGK-$4A_7$	1.70	3.47	4.42
Westiellopsis-AT-MGK-$4A_7$-E_{250}	2.15	4.17	5.14

	SEd	CD
Cultures	0.01	0.02
Days	0.01	0.01
Cultures x Days	0.02	0.03

Fig11 . Ammonia excretion by the wild and mutant acid tolerant cyanobacterial cultures

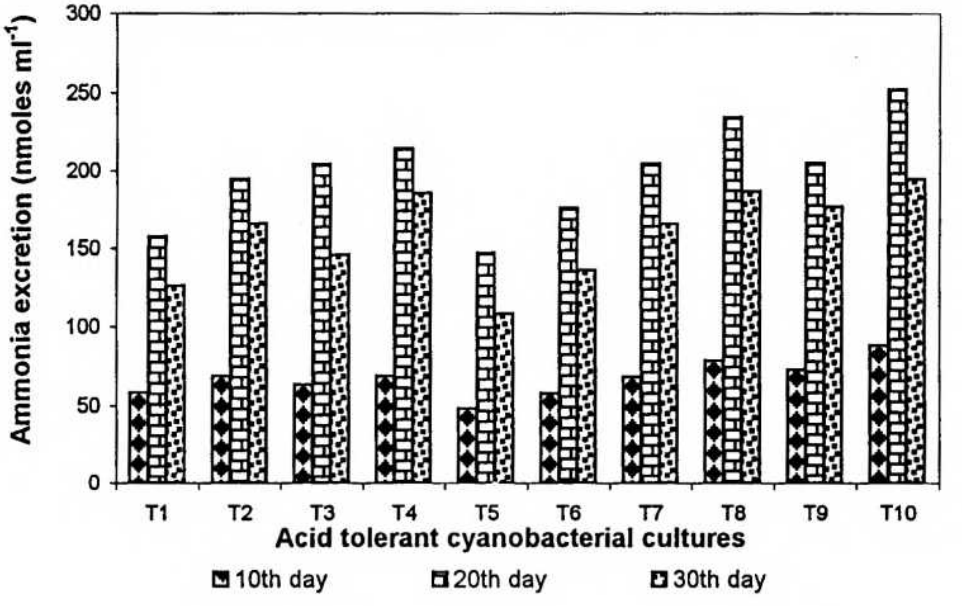
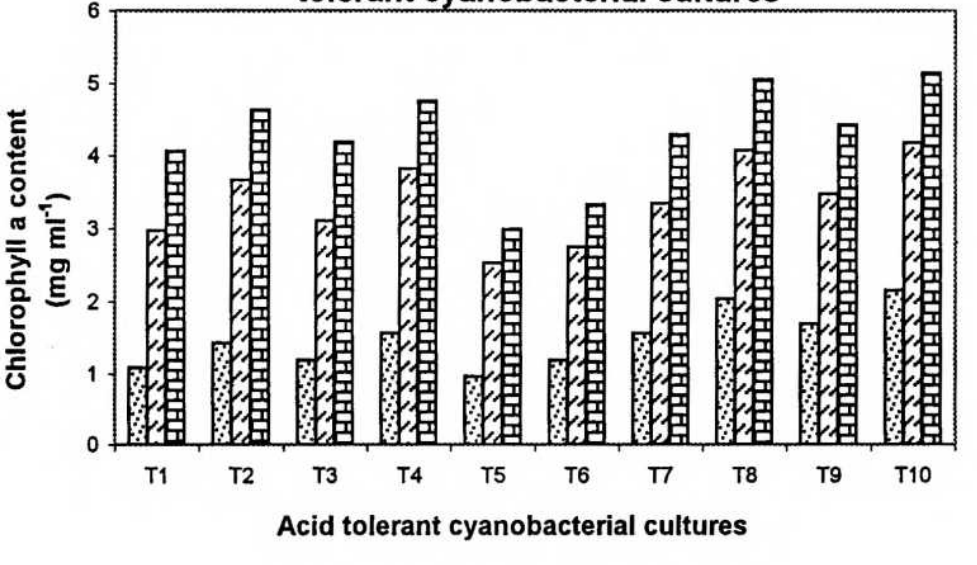


Fig 12 . Chlorophyll a content of the wild and mutant acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Anabaena* -AT-MGK-$5A_6$-E_{200}
T3-*Nostoc*- AT-TGK-$4C_4$
T4-*Nostoc* -AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*- AT-TGK-$5B_8$
T6-*Oscillatoria* -AT-MGK-$5B_8$-E_{200}
T7-*Westiellopsis*- AT-TGK-$4A_2$
T8-*Westiellopsis* -AT-MGK-$4A_2$-E_{250}
T9-*Westiellopsis* -AT-TGK-$4A_7$
T10-*Westiellopsis* -AT-MGK-$4A_7$-E_{250}

Table 15. Protein content of the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Protein content (µg ml^{-1})		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	42.23	57.48	77.35
Anabaena-AT-MGK-$5A_6$-E_{200}	42.72	62.37	82.80
Nostoc-AT-TGK-$4C_4$	45.39	60.45	75.15
Nostoc-AT-MGK-$4C_4$-E_{250}	50.28	62.86	85.09
Oscillatoria-AT-TGK-$5B_8$	37.48	55.55	70.45
Oscillatoria-AT-MGK-$5B_8$-E_{200}	44.70	57.31	75.52
Westiellopsis-AT-TGK-$4A_2$	47.52	62.83	86.76
Westiellopsis-AT-MGK-$4A_2$-E_{250}	52.63	65.78	97.48
Westiellopsis-AT-TGK-$4A_7$	45.78	62.90	82.61
Westiellopsis-AT-MGK-$4A_7$-E_{250}	55.36	66.31	93.85

	SEd	CD
Cultures	0.10	0.19
Days	0.05	0.10
Cultures x Days	0.17	0.33

Table 16. Polysaccharide production by the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Polysaccharide content (μg ml^{-1})		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	2.43	5.74	7.32
Anabaena-AT-MGK-$5A_6$-E_{200}	3.04	7.11	8.52
Nostoc-AT-TGK-$4C_4$	2.62	7.52	8.79
Nostoc-AT-MGK-$4C_4$-E_{250}	3.22	8.06	9.83
Oscillatoria-AT-TGK-$5B_8$	2.23	7.05	8.22
Oscillatoria-AT-MGK-$5B_8$-E_{200}	2.62	7.55	8.57
Westiellopsis-AT-TGK-$4A_2$	2.85	8.47	11.05
Westiellopsis-AT-MGK-$4A_2$-E_{250}	3.11	8.78	11.83
Westiellopsis-AT-TGK-$4A_7$	3.05	9.03	11.27
Westiellopsis-AT-MGK-$4A_7$-E_{250}	3.82	10.23	11.63

	SEd	CD
Cultures	0.05	0.10
Days	0.03	0.06
Cultures x Days	0.09	0.18

Fig 13. Protein content of the wild and mutant acid tolerant cyanobacterial cultures

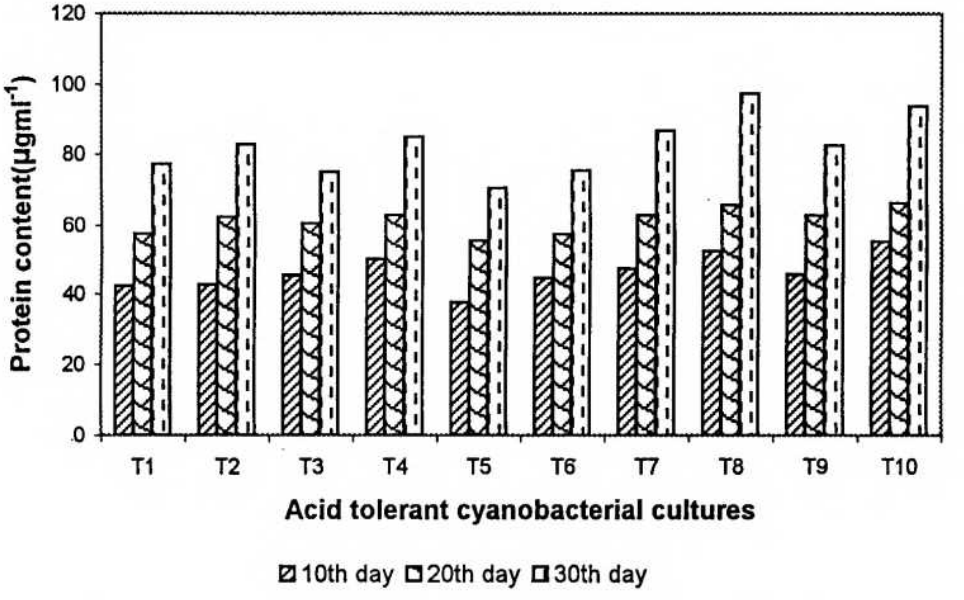
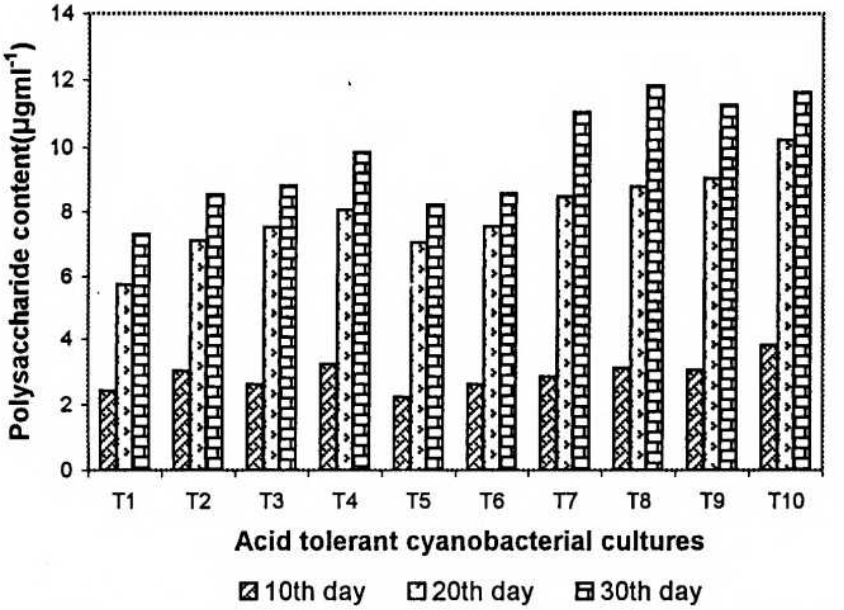


Fig 14 . Polysaccharide production by the wild and mutant acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Anabaena*- AT-MGK-$5A_6$-E_{200}
T3-*Nostoc* -AT-TGK-$4C_4$
T4-*Nostoc* -AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria* -AT-TGK-$5B_8$
T6-*Oscillatoria* -AT-MGK-$5B_8$-E_{200}
T7-*Westiellopsis* -AT-TGK-$4A_2$
T8-*Westiellopsis* -AT-MGK-$4A_2$-E_{250}
T9-*Westiellopsis* -AT-TGK-$4A_7$
T10-*Westiellopsis* -AT-MGK-$4A_7$-E_{250}

Table 17. Amino nitrogen of the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Amino Nitrogen content (µg ml^{-1})		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	48.51	61.59	84.04
Anabaena-AT-MGK-$5A_6$-E_{200}	52.25	70.93	87.76
Nostoc-AT-TGK-$4C_4$	35.57	70.95	87.73
Nostoc-AT-MGK-$4C_4$-E_{250}	56.06	74.65	93.36
Oscillatoria-AT-TGK-$5B_8$	44.78	56.06	78.44
Oscillatoria-AT-MGK-$5B_8$-E_{200}	50.41	65.33	84.08
Westiellopsis-AT-TGK-$4A_2$	46.66	63.44	112.04
Westiellopsis-AT-MGK-$4A_2$-E_{250}	57.83	70.93	130.70
Westiellopsis-AT-TGK-$4A_7$	50.47	63.46	102.64
Westiellopsis-AT-MGK-$4A_7$-E_{250}	59.74	67.23	121.30

	SEd	CD
Cultures	0.02	0.05
Days	0.01	0.02
Cultures x Days	0.04	0.08

mutants showed increased amino nitrogen content. Both *Westiellopsis* mutants have shown higher amino nitrogen content. However, least amino nitrogen content was registered by *Oscillatoria* culture.

4.5.7. NITROGENASE ACTIVITY

The nitrogenase activity of the acid tolerant cyanobacterial cultures and the mutants are given in Table 18 and Fig.16. All the mutants registered significantly increased nitrogenase activity than their wild parents. Among the mutants *Westiellopsis* cultures expressed higher nitrogenase activity and *Oscillatoria* -AT-MGK-$5B_8$-E_{200}, recorded the lowest activity.

4.5.8. FREE AMINO ACID CONTENT

The acid tolerant cyanobacterial cultures and the mutants were tested for the liberation of the free amino acid and the results are given in Table 19 and Fig.17. All the mutants registered higher amount of free amino acids than their parents. Among the mutants *Westiellopsis* -AT-MGK-$4A_2$-E_{250} and *Westiellopsis* -AT-MGK-$4A_7$-E_{250} registered more amount of free amino acids than other mutants. These were followed by *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Anabaena* -AT-MGK-$5A_6$-E_{200} and *Oscillatoria* -AT-MGK-$5B_8$-E_{200}.

4.5.9. METHIONINE CONTENT

The sulphur containing amino acid *viz.*, methionine content of both wild and mutant acid tolerant cyanobacterial cultures were estimated at three intervals and are given in Table 20 and Fig 18. Not much variation was seen in the methionine content due to time intervals. However, all the mutants showed increased methionine content than that of their parents. Both wild and mutant *Westiellopsis* cultures registered significantly higher methionine content, while *Oscillatoria* -AT-MGK-$5B_8$-E_{200} showed minimum methionine content.

Table 18. Nitrogenase activity of the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Nitrogenase activity (n moles of ethylene produced h^{-1} g^{-1} dry wt.)
	30^{th}day
Anabaena-AT-TGK-$5A_6$	905.62
Anabaena-AT-MGK-$5A_6$-E_{200}	921.11
Nostoc-AT-TGK-$4C_4$	916.34
Nostoc-AT-MGK-$4C_4$-E_{250}	930.22
Oscillatoria-AT-TGK-$5B_8$	843.80
Oscillatoria-AT-MGK-$5B_8$-E_{200}	890.50
Westiellopsis-AT-TGK-$4A_2$	1002.44
Westiellopsis-AT-MGK-$4A_2$-E_{250}	1013.82
Westiellopsis-AT-TGK-$4A_7$	1005.53
Westiellopsis-AT-MGK-$4A_7$-E_{250}	1016.20
SEd	0.71
CD	1.48

Fig15 . Amino nitrogen content of the wild and mutant acid tolerant cyanobacterial cultures

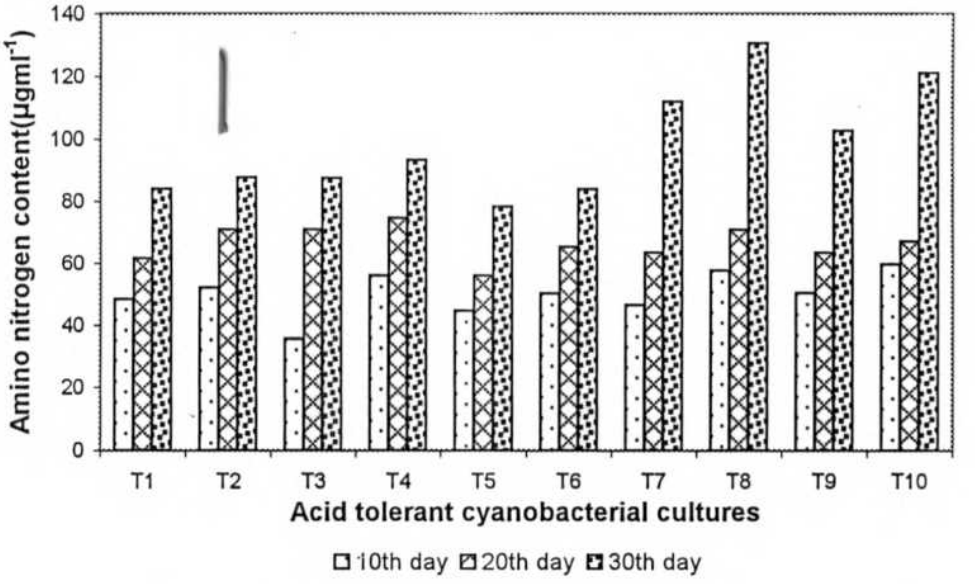
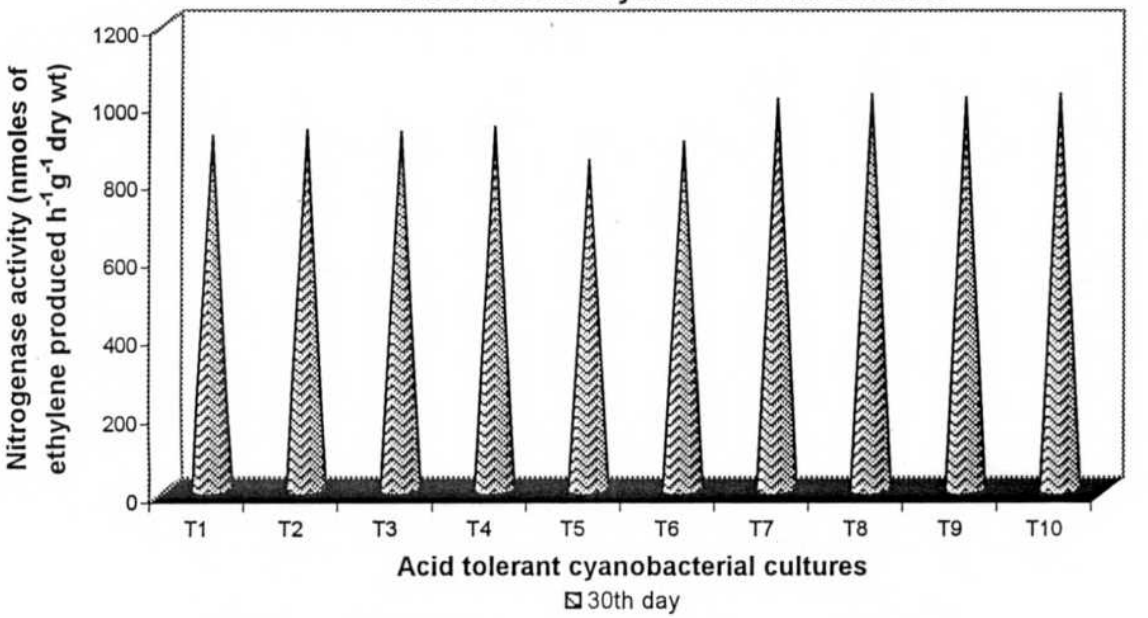


Fig 16 . Nitrogenase activity of the wild and mutant acid tolerant cyanobacterial cultures



T1-*Anabaena* - AT-TGK-$5A_6$

T2-*Anabaena* -AT-MGK-$5A_6$-E_{200}

T3-*Nostoc*- AT-TGK-$4C_4$

T4-*Nostoc* -AT-MGK-$4C_4$-E_{250}

T5-*Oscillatoria* -AT-TGK-$5B_8$

T6-*Oscillatoria*- AT-MGK-$5B_8$-E_{200}

T7-*Westiellopsis* -AT-TGK-$4A_2$

T8-*Westiellopsis* -AT-MGK-$4A_2$-E_{250}

T9-*Westiellopsis* -AT-TGK-$4A_7$

T10-*Westiellopsis* -AT-MGK-$4A_7$-E_{250}

Table 19. Free amino acid content of the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Free amino acid content (mg ml^{-1})		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	0.19	0.27	0.39
Anabaena-AT-MGK-$5A_6$-E_{200}	0.21	0.29	0.41
Nostoc-AT-TGK-$4C_4$	0.15	0.29	0.38
Nostoc-AT-MGK-$4C_4$-E_{250}	0.18	0.35	0.43
Oscillatoria-AT-TGK-$5B_8$	0.14	0.25	0.35
Oscillatoria-AT-MGK-$5B_8$-E_{200}	0.15	0.26	0.36
Westiellopsis-AT-TGK-$4A_2$	0.20	0.30	0.43
Westiellopsis-AT-MGK-$4A_2$-E_{250}	0.24	0.35	0.49
Westiellopsis-AT-TGK-$4A_7$	0.21	0.30	0.44
Westiellopsis-AT-MGK-$4A_7$-E_{250}	0.25	0.34	0.48

	SEd	CD
Cultures	0.001	0.001
Days	0.001	0.001
Cultures x Days	0.001	0.002

Table 20. Methionine content of the wild and mutant acid tolerant cyanobacterial cultures

Cultures	Methionine content (mg g^{-1} fresh wt.)		
	10^{th}day	20^{th}day	30^{th}day
Anabaena-AT-TGK-$5A_6$	0.06	0.09	0.11
Anabaena-AT-MGK-$5A_6$-E_{200}	0.08	0.11	0.14
Nostoc-AT-TGK-$4C_4$	0.09	0.11	0.13
Nostoc-AT-MGK-$4C_4$-E_{250}	0.10	0.12	0.15
Oscillatoria-AT-TGK-$5B_8$	0.02	0.04	0.05
Oscillatoria-AT-MGK-$5B_8$-E_{200}	0.04	0.06	0.07
Westiellopsis-AT-TGK-$4A_2$	0.12	0.15	0.18
Westiellopsis-AT-MGK-$4A_2$-E_{250}	0.15	0.18	0.21
Westiellopsis-AT-TGK-$4A_7$	0.13	0.16	0.19
Westiellopsis-AT-MGK-$4A_7$-E_{250}	0.16	0.18	0.22

	SEd	CD
Cultures	0.002	0.003
Days	0.001	0.002
Cultures x Days	0.003	0.005

Fig 17 . Free amino acids content of the wild and mutant acid tolerant cyanobacterial cultures

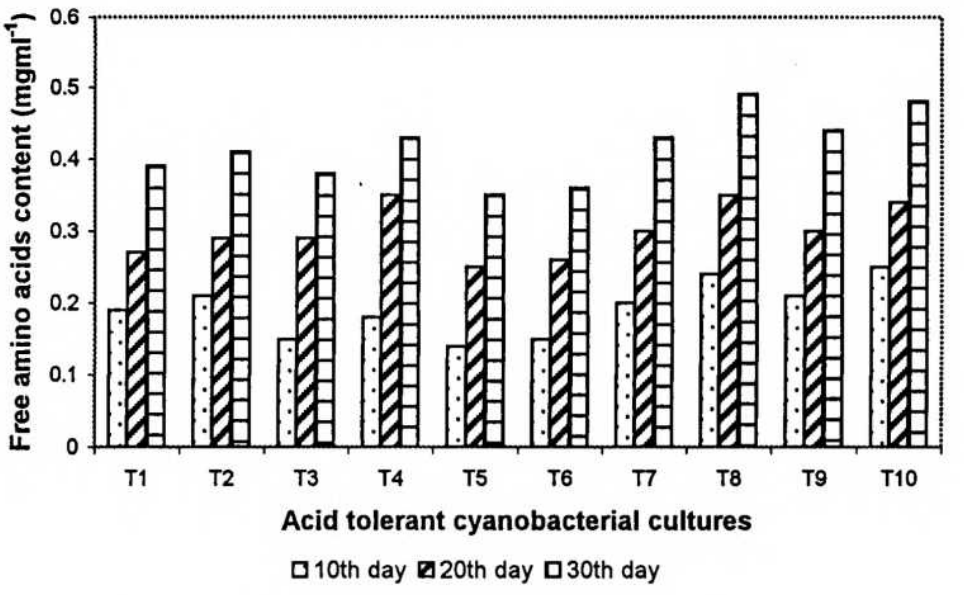
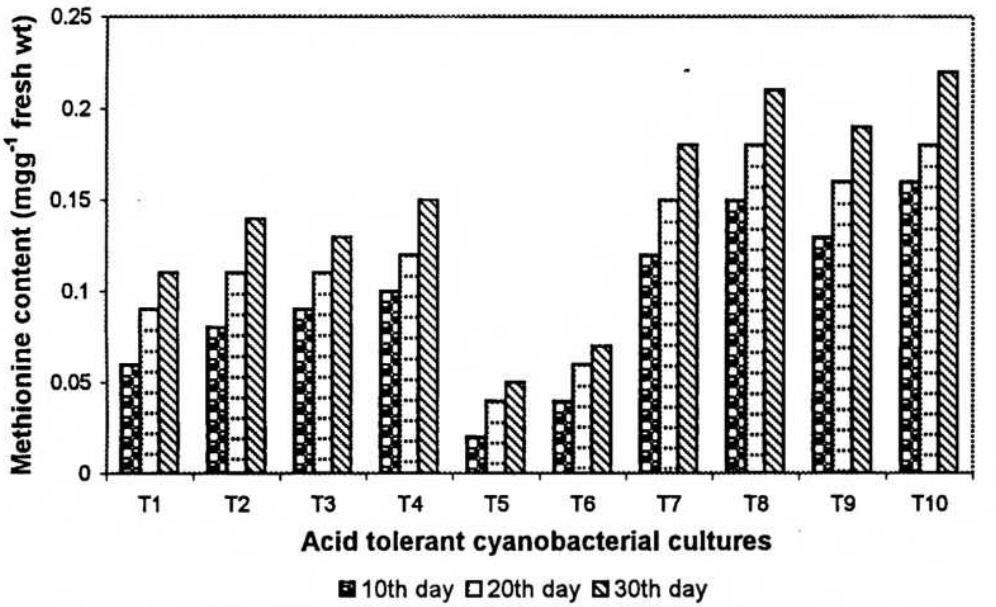


Fig 18 . Methionine content of the wild and mutant acid tolerant cyanobacterial cultures



T1-*Anabaena* -AT-TGK-$5A_6$
T2-*Anabaena* -AT-MGK-$5A_6$-E_{200}
T3-*Nostoc* -AT-TGK-$4C_4$
T4-*Nostoc* -AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria* -AT-TGK-$5B_8$
T6-*Oscillatoria* -AT-MGK-$5B_8$-E_{200}
T7-*Westiellops* is-AT-TGK-$4A_2$
T8-*Westiellopsis*- AT-MGK-$4A_2$-E_{250}
T9-*Westiellopsis* -AT-TGK-$4A_7$
T10-*Westiellopsis* -AT-MGK-$4A_7$-E_{250}

4.6. EFFECT OF ACIDITY ON THE GROWTH AND THE BIOCHEMICAL CONSTITUENTS OF THE MUTANTS

The effect of acidity on the growth performance and the biochemical constituents of the EMS mutants *viz.*, *Anabaena* -AT-MGK-$5A_6$-E_{200},*Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria* -AT-MGK-$5B_8$-E_{200}, *Westiellopsis* -AT- MGK-$4A_2$-E_{250} and *Westiellopsis* -AT-MGK-$4A_7$-E_{250} were analysed and compared with the normal soil isolate *Anabaena* -NS-$5G_2$ and wild acid tolerant cyanobacterial culture *Anabaena* -AT-TGK-$5A_6$ at varied pH levels *viz.*, 4,5 and 6.

4.6.1. GROWTH AND BIOMASS PRODUCTION

The data on the growth and biomass production by the mutants of acid tolerant cyanobacterial cultures in Table 21 , Figs.19 and 20 and Plate 7, revealed that the cyanobacterial cultures tolerated acid pH by registering higher growth and biomass production. On the other hand, the normal soil culture registered very low growth and biomass production at low pH level. Linear increase in growth and biomass production due to increase in incubation time was seen. All the cyanobacterial mutants tolerated well the acid pH compared to wild acid tolerant *Anabaena* -AT-TGK-$5A_6$ by recording higher growth and biomass production. Maximum growth and biomass production was noticed in *Westiellopsis* mutants followed by *Anabaena* -AT-MGK-$5A_6$-E_{200} mutant.

4.6.2. AMMONIA EXCRETION

The effect of acid pH levels on the ammonia excretion by the cyanobacterial mutants was studied and the data are given in Table 22 and Fig 21. In general, ammonia excretion is directly proportional to increased pH of the medium. However maximum excretion was detected at 20^{th} day after inoculation which declined thereafter. Significant increase in ammonia excretion was seen in cyanobacterial mutants compared to wild and normal soil *Anabaena* culture. Not much variation with respect to ammonia excretion was noticed between

Table 21. Effect of acidity on the growth and biomass production by the acid tolerant cyanobacterial mutants

Cultures	pH	Growth (OD at 660 nm)			Biomass in dry weight ($\mu g\ ml^{-1}$)
		10^{th}day	20^{th}day	30^{th}day	30^{th} day
Anabaena-NS-$5G_2$	4	0.01	0.08	0.11	11.21
	5	0.09	0.11	0.22	32.89
	6	0.22	0.34	0.54	69.97
Anabaena-AT-TGK-$5A_6$	4	0.11	0.19	0.38	53.67
	5	0.19	0.27	0.48	63.18
	6	0.23	0.35	0.69	77.38
Anabaena-AT-MGK-$5A_6$-E_{200}	4	0.19	0.31	0.62	60.56
	5	0.23	0.37	0.67	71.40
	6	0.29	0.42	0.76	83.28
Nostoc-AT-MGK-$4C_4$-E_{250}	4	0.18	0.29	0.61	55.74
	5	0.20	0.36	0.66	66.90
	6	0.22	0.41	0.74	78.73
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	0.09	0.21	0.49	52.85
	5	0.12	0.27	0.57	61.94
	6	0.14	0.32	0.63	69.23
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	0.16	0.36	0.69	77.95
	5	0.19	0.42	0.76	84.52
	6	0.22	0.49	0.83	93.81
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	0.15	0.39	0.71	80.92
	5	0.20	0.44	0.78	87.90
	6	0.23	0.50	0.85	95.53

Growth	SEd	CD
Cultures	0.003	0.005
Days	0.002	0.003
pH	0.002	0.003
Cultures x Days x pH	0.008	0.016
Biomass		
Cultures	0.35	0.70
pH	0.23	0.46
Cultures x pH	0.60	1.22

Fig 19 . Effect of acidity on the growth of the acid tolerant cyanobacterial mutants

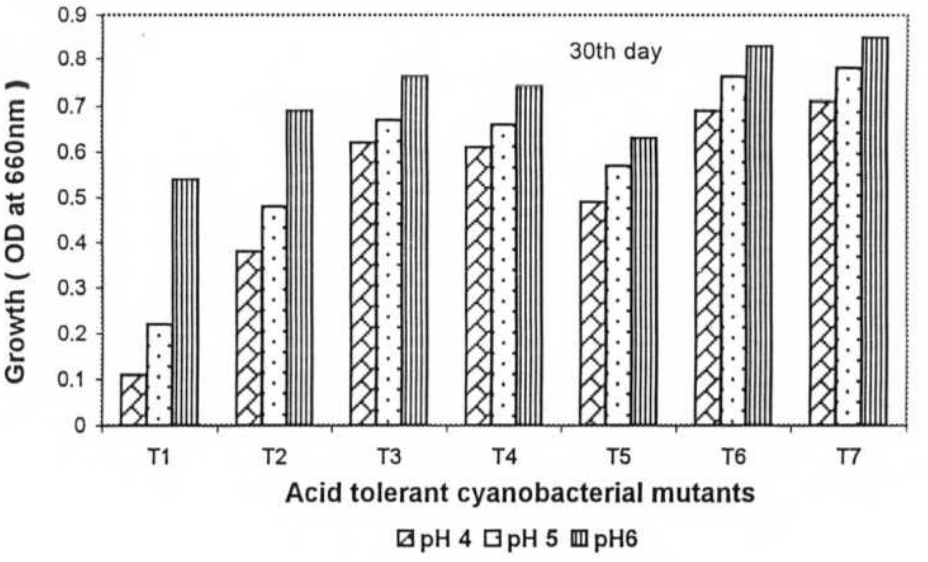
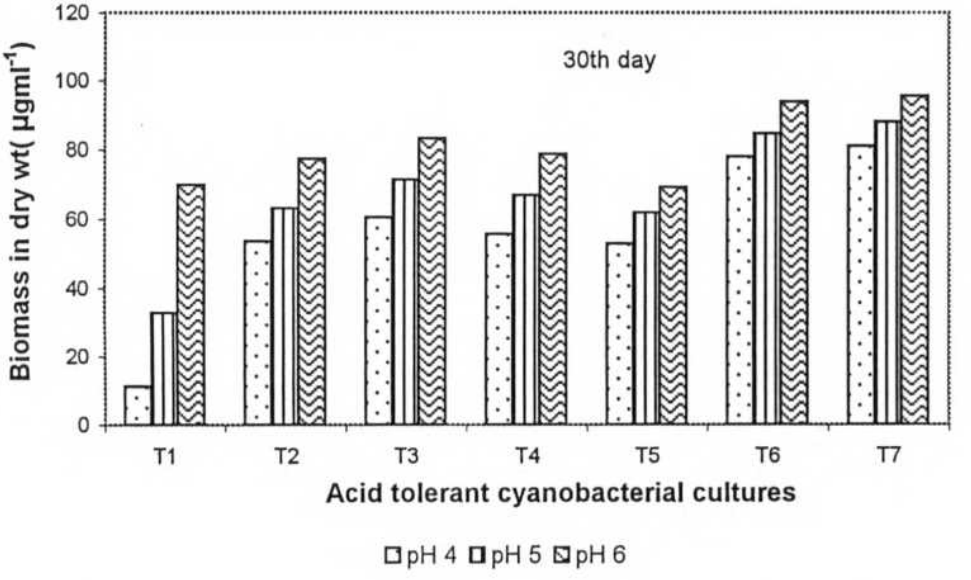


Fig 20 . Effect of acidity on the biomass production by the acid tolerant cyanobacterial mutants



T1-*Anabaena*-NS-$5G_2$
T2-*Anabaena*-AT-TGK-$5A_6$
T3-*Anabaena*-AT-MGK-$5A_6$-E_{200}
T4-*Nostoc*-AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*-AT-MGK-$5B_8$-E_{200}
T6-*Westiellopsis*-AT-MGK-$4A_2$-E_{250}
T7-*Westiellopsis*-AT-MGK-$4A_7$-E_{250}

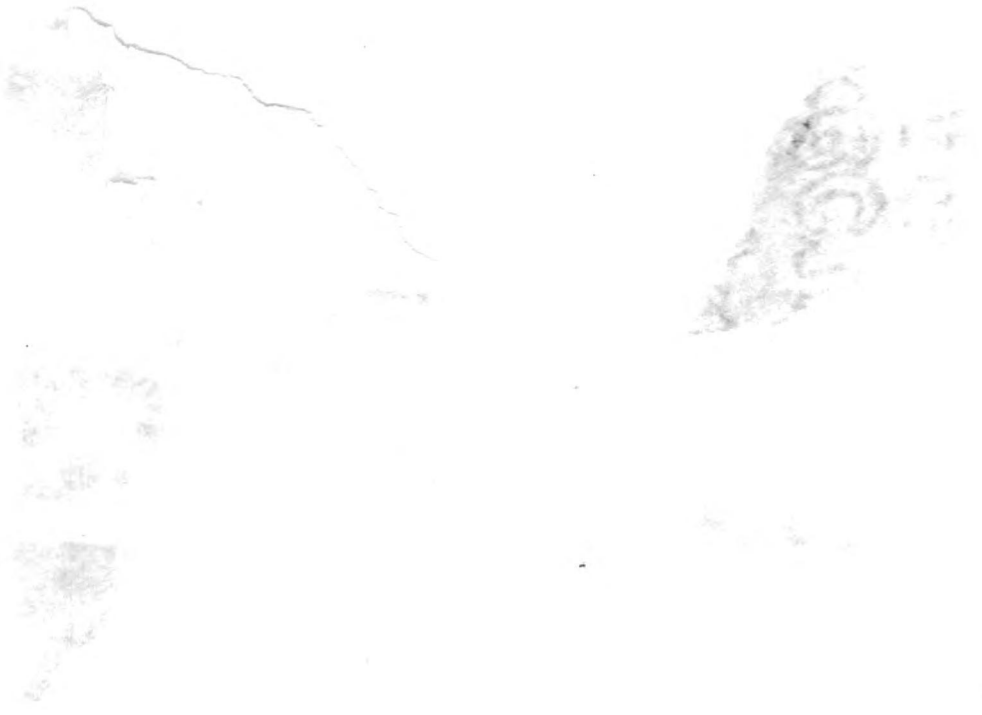


Plate 7. **Growth of *Westiellopsis* mutants at different acidic conditions**

Table 22. Effect of acidity on ammonia excretion by the acid tolerant cyanobacterial mutants

Cultures	pH	Ammonia excretion (n moles ml^{-1})		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	3.50	9.44	7.64
	5	11.76	30.17	22.10
	6	48.63	116.83	86.71
Anabaena-AT-TGK-$5A_6$	4	7.81	108.39	86.40
	5	11.10	147.39	102.07
	6	49.48	166.40	116.53
Anabaena-AT-MGK-$5A_6$-E_{200}	4	20.34	127.61	102.37
	5	48.97	176.65	148.33
	6	68.56	195.58	162.79
Nostoc-AT-MGK-$4C_4$-E_{250}	4	19.93	116.73	85.11
	5	39.39	155.50	102.21
	6	69.26	214.34	186.54
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	9.25	107.44	84.37
	5	30.33	116.65	95.05
	6	59.43	175.48	123.11
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	30.13	165.63	132.57
	5	49.41	204.74	177.37
	6	78.87	222.68	191.99
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	30.41	185.36	132.17
	5	49.24	214.28	161.21
	6	88.40	226.91	196.36

	SEd	CD
Cultures	0.27	0.54
Days	0.18	0.35
pH	0.18	0.35
Cultures x Days x pH	0.82	1.62

Westiellopsis mutants. The ammonia excretion was very minimum in the case of normal soil *Anabaena* -NS-$5G_2$ culture.

4.6.3. CHLOROPHYLL a CONTENT

The chlorophyll a content of the acid tolerant cyanobacterial mutants at pH 4,5 and 6 were analysed and given in Table 23 and Fig. 22. All the mutants performed significantly well than the acid tolerant and the normal cyanobacterial culture at acidic pH conditions. *Westiellopsis* -AT-MGK-$4A_7$-E_{250} registered higher amount of chlorophyll a content than all other mutants at all low pH conditions. It was followed by *Westiellopsis* -AT-MGK-$4A_2$-E_{250}, *Nostoc* -AT-MGK-$4C_4$-E_{250}, *Anabaena* -AT-MGK-$5A_6$-E_{200} and *Oscillatoria* -AT-MGK-$5B_8$-E_{200} in the descending order.

4.6.4. PROTEIN CONTENT

The data on the influence of acidic pH on the protein content of the acid tolerant cyanobacterial mutants are shown in Table 24 and Fig.23. Among the mutants, *Westiellopsis* -AT-MGK-$4A_2$-E_{250} recorded more protein content than other mutants at pH 6. At pH 4, *Westiellopsis* -AT-MGK-$4A_7$-E_{250} registered more protein content. At pH 5, both the *Westiellopsis* cultures were on par with each other. *Oscillatoria* -AT-MGK-$5B_8$-E_{200} recorded lowest protein content among the mutants at acidic conditions.

4.6.5. POLYSACCHARIDE PRODUCTION

The polysaccharide production of the cyanobacterial cultures at various acidic conditions are given in Table 25 and Fig.24. All the mutants produced significantly increased amount of polysaccharide at low pH conditions than the normal and acid tolerant cyanobacterial cultures. Among the mutants, *Westiellopsis* cultures produced significantly higher amount of polysaccharide at pH 4 and 5 followed by *Nostoc* -AT-MGK-$4C_4$-E_{250}. *Oscillatoria*-AT-MGK-$5B_8$-E_{200} produced more polysaccharide than *Anabaena* -AT-MGK-$5A_6$-E_{200} at pH 5.

Table 23. Effect of acidity on chlorophyll a content of the acid tolerant cyanobacterial mutants

Cultures	pH	Chlorophyll a content (mg ml^{-1})		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	0.15	0.64	0.95
	5	0.36	0.85	1.33
	6	1.15	3.25	4.22
Anabaena-AT-TGK-$5A_6$	4	0.63	1.93	2.54
	5	0.85	2.94	3.34
	6	1.23	3.35	4.44
Anabaena-AT-MGK-$5A_6$-E_{200}	4	0.86	2.34	3.15
	5	1.14	3.24	3.73
	6	1.42	3.74	4.64
Nostoc-AT-MGK-$4C_4$-E_{250}	4	0.95	2.54	3.24
	5	1.25	3.34	3.93
	6	1.53	3.83	4.84
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	0.57	2.14	2.62
	5	0.86	2.43	3.04
	6	1.23	2.52	3.34
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	0.97	2.74	3.64
	5	1.43	3.55	4.44
	6	2.05	4.05	5.03
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	1.15	2.93	3.74
	5	1.53	3.64	4.53
	6	2.15	4.22	5.14

	SEd	CD
Cultures	0.01	0.02
pH	0.01	0.01
Days	0.01	0.01
Cultures x Days x pH	0.03	0.07

Fig 21 . Effect of acidity on the ammonia excretion by the acid tolerant cyanobacterial mutants

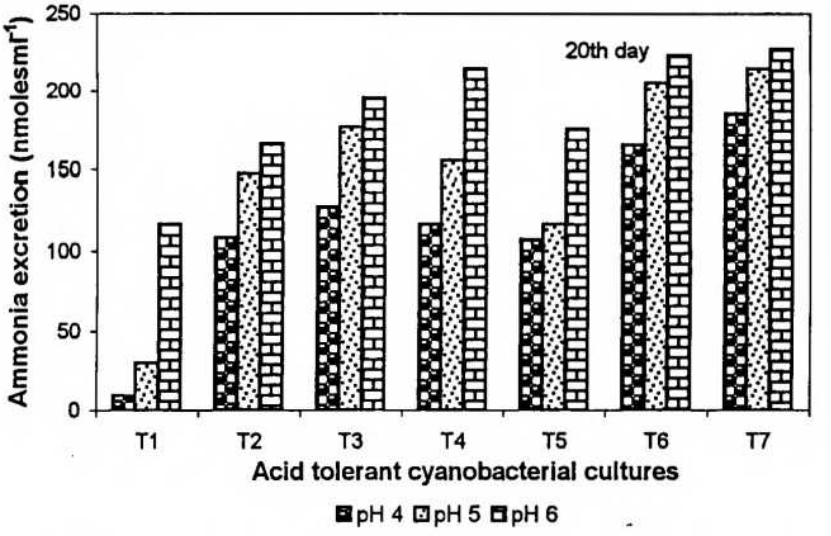
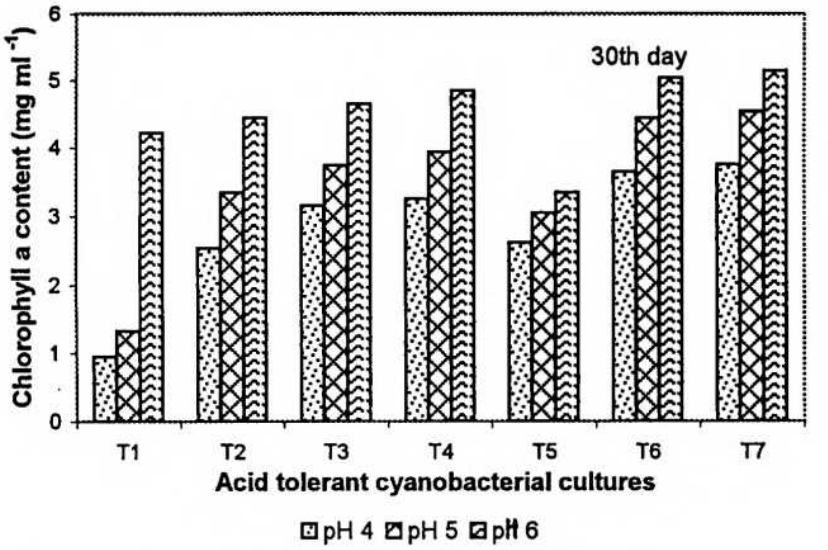


Fig 22 . Effect of acidity on chlorophyll a content of the acid tolerant cyanobacterial mutants



T1-*Anabaena*-NS-$5G_2$
T2-*Anabaena*-AT-TGK-$5A_6$
T3-*Anabaena*-AT-MGK-$5A_6$-E_{200}
T4-*Nostoc*-AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*-AT-MGK-$5B_8$-E_{200}
T6-*Westiellopsis*-AT-MGK-$4A_2$-E_{250}
T7-*Westiellopsis*-AT-MGK-$4A_7$-E_{250}

Table 24. Effect of acidity on protein content of the acid tolerant cyanobacterial mutants

Cultures	pH	Protein content (μg ml^{-1})		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	3.24	20.15	27.37
	5	20.93	30.52	42.74
	6	35.57	55.59	72.41
Anabaena-AT-TGK-$5A_6$	4	20.58	27.34	40.52
	5	27.34	40.60	57.32
	6	18.18	57.52	77.53
Anabaena-AT-MGK-$5A_6$-E_{200}	4	27.41	37.52	55.20
	5	35.05	52.20	67.51
	6	42.30	62.47	82.52
Nostoc-AT-MGK-$4C_4$-E_{250}	4	27.32	42.33	52.38
	5	37.39	57.59	70.41
	6	52.94	62.13	85.12
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	20.36	27.42	42.15
	5	27.32	42.48	55.51
	6	45.34	57.36	75.49
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	30.56	42.49	57.48
	5	45.31	57.14	80.10
	6	52.17	65.55	97.10
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	27.27	40.51	60.44
	5	45.33	57.15	77.35
	6	55.54	65.59	92.38

	SEd	CD
Cultures	0.19	0.37
pH	0.12	0.24
Days	0.12	0.24
Cultures x Days x pH	0.56	1.11

Table 25. Effect of acidity on the polysaccharide production by the acid tolerant cyanobacterial mutants

Cultures	pH	Polysaccharide content (μg ml^{-1})		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	0.16	1.06	2.23
	5	1.26	2.10	3.22
	6	2.24	4.22	6.75
Anabaena-AT-TGK-$5A_6$	4	1.43	3.65	4.43
	5	2.26	4.74	6.07
	6	2.64	5.53	7.74
Anabaena-AT-MGK-$5A_6$-E_{200}	4	1.65	4.23	5.73
	5	2.43	6.14	7.54
	6	3.10	7.13	8.54
Nostoc-AT-MGK-$4C_4$-E_{250}	4	1.83	3.84	5.75
	5	2.64	6.08	7.73
	6	3.23	8.06	9.75
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	1.23	2.63	4.45
	5	1.85	4.03	6.16
	6	2.63	7.54	8.54
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	2.10	6.32	7.05
	5	2.83	8.04	8.74
	6	2.96	8.74	11.76
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	2.22	6.14	7.54
	5	3.10	8.24	9.73
	6	3.84	10.07	11.51

	SEd	CD
Cultures	0.02	0.05
pH	0.02	0.03
Days	0.01	0.03
Cultures x pH x Days	0.07	0.13

Fig 23 . Effect of acidity on protein content of the acid tolerant cyanobacterial mutants

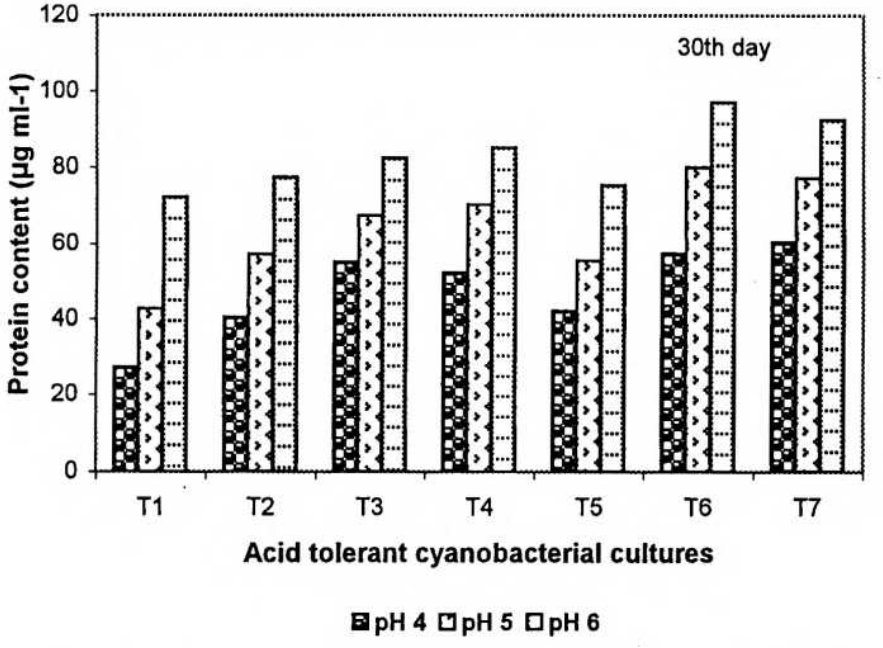
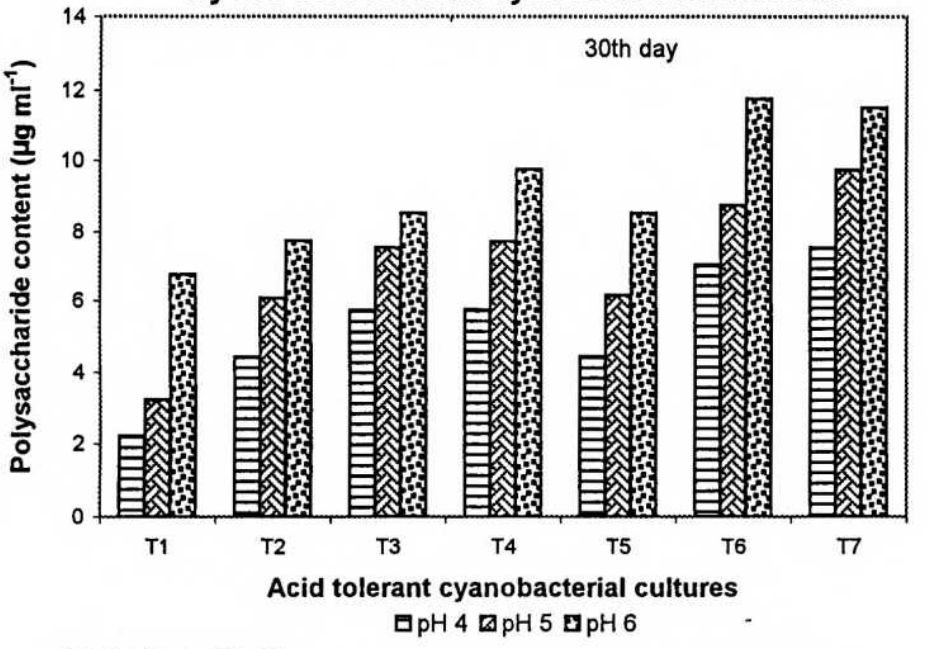


Fig 24 . Effect of acidity on the polysaccharide production by the acid tolerant cyanobacterial mutants



T1-*Anabaena*-NS-$5G_2$
T2-*Anabaena*-AT-TGK-$5A_6$
T3-*Anabaena*-AT-MGK-$5A_6$-E_{200}
T4-*Nostoc*-AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*-AT-MGK-$5B_8$-E_{200}
T6-*Westiellopsis*-AT-MGK-$4A_2$-E_{250}
T7-*Westiellopsis*-AT-MGK-$4A_7$-E_{250}

4.6.6. AMINO NITROGEN CONTENT

The amino nitrogen content analysed for the cyanobacterial cultures at pH 4,5 and 6 are given in Table 26 and Fig.25. In general, all the mutants performed well than the normal soil and the acid tolerant cyanobacterial culture. *Westiellopsis* -AT-MGK-$4A_2$-E_{250} produced higher amount of amino nitrogen at pH 6. At pH 4 and 5, *Westiellopsis* -AT-MGK-$4A_7$-E_{250} produced more amount of amino nitrogen. *Oscillatoria* -AT-MGK-$5B_8$-E_{200} produced lowest amount of amino nitrogen among the mutants.

4.6.7 NITROGENASE ACTIVITY

Nitrogenase activity of the cyanobacterial cultures at various acidic conditions are given in Table 27 and Fig.26. At pH 6, the nitrogenase activity of all the mutants were more. *Westiellopsis*-AT-MGK-$4A_7$-E_{250} recorded more nitrogenase activity at all low pH conditions which was followed by *Westiellopsis* -AT-MGK-$4A_2$-E_{250}, *Nostoc* -AT-MGK-$4C_4$-E_{250} ,*Anabaena*-AT-MGK-$5A_6$-E_{200} and *oscillatoria* -AT-MGK-$5B_8$-E_{200}.

4.6.8. FREE AMINO ACID CONTENT

The data on the free amino acid content of the cyanobacterial cultures at varied acidic conditions are presented in Table 28 and Fig.27. All the mutants performed well except *Oscillatoria* -AT-MGK-$5B_8$-E_{200} at all acidic conditions. *Westiellopsis* -AT-MGK-$4A_2$-E_{250} registered higher liberation of free amino acids than all other mutants at pH 6. At pH 4 and 5, *Westiellopsis* -AT-MGK-$4A_7$-E_{250} registered higher liberation of free amino acids. *Nostoc* -AT-MGK-$4C_4$-E_{250} recorded more liberation of amino acids than *Anabaena* -AT-MGK-$5A_6$-E_{200} at all low acidic conditions.

4.6.9. METHIONINE CONTENT

The sulphur containing amino acid, methionine content of the cyanobacterial cultures at pH 4,5 and 6 were estimated at three intervals and given in Table 29 and

Table 26. Effect of acidity on the amino nitrogen content of the acid tolerant cyanobacterial mutants

Cultures	pH	Amino nitrogen content (μg ml^{-1})		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	22.53	28.23	28.83
	5	30.60	38.63	43.55
	6	46.57	58.77	74.72
Anabaena-AT-TGK-$5A_6$	4	28.67	40.38	48.57
	5	39.33	48.50	61.57
	6	48.67	63.69	81.57
Anabaena-AT-MGK-$5A_6$-E_{200}	4	35.62	47.73	56.23
	5	45.63	57.62	71.66
	6	51.40	69.39	86.77
Nostoc-AT-MGK-$4C_4$-E_{250}	4	38.53	48.70	60.63
	5	49.50	59.63	75.70
	6	57.83	74.60	93.57
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	31.80	45.63	49.67
	5	40.37	56.80	66.50
	6	49.63	65.37	84.33
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	39.40	47.70	56.50
	5	47.77	59.63	70.63
	6	58.50	70.80	130.63
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	41.57	49.63	57.70
	5	48.67	60.63	72.63
	6	59.60	67.40	121.50

	SEd	CD
Cultures	0.07	0.13
pH	0.04	0.09
Days	0.04	0.09
Cultures x pH x Days	0.20	0.39

Table 27. Effect of acidity on the nitrogenase activity of the acid tolerant cyanobacterial mutants

Cultures	pH	Nitrogenase activity (n moles of ethylene produced h^{-1} g^{-1} dry wt.)
		30^{th}day
Anabaena-NS-$5G_2$	4	355.31
	5	551.32
	6	823.38
Anabaena-AT-TGK-$5A_6$	4	571.24
	5	733.10
	6	905.28
Anabaena-AT-MGK-$5A_6$-E_{200}	4	651.53
	5	821.61
	6	920.66
Nostoc-AT-MGK-$4C_4$-E_{250}	4	686.19
	5	856.22
	6	931.07
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	612.24
	5	756.36
	6	891.21
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	776.99
	5	902.35
	6	1013.28
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	756.34
	5	903.35
	6	1015.76

	SEd	CD
Cultures	0.27	0.54
pH	0.17	0.35
Cultures x pH	0.46	0.93

Fig 25 . Effect of acidity on the amino nitrogen content of the acid tolerant cyanobacterial mutants

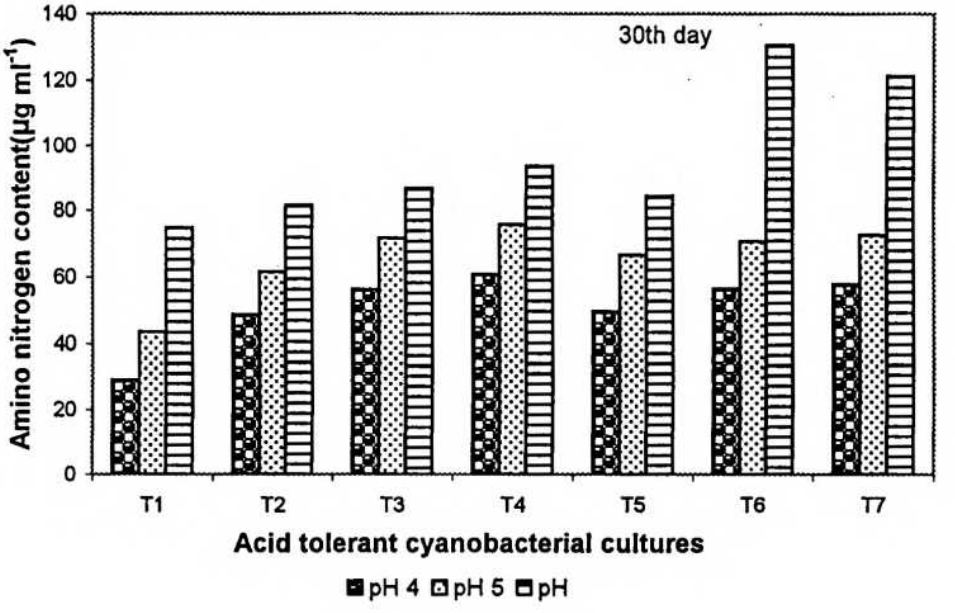
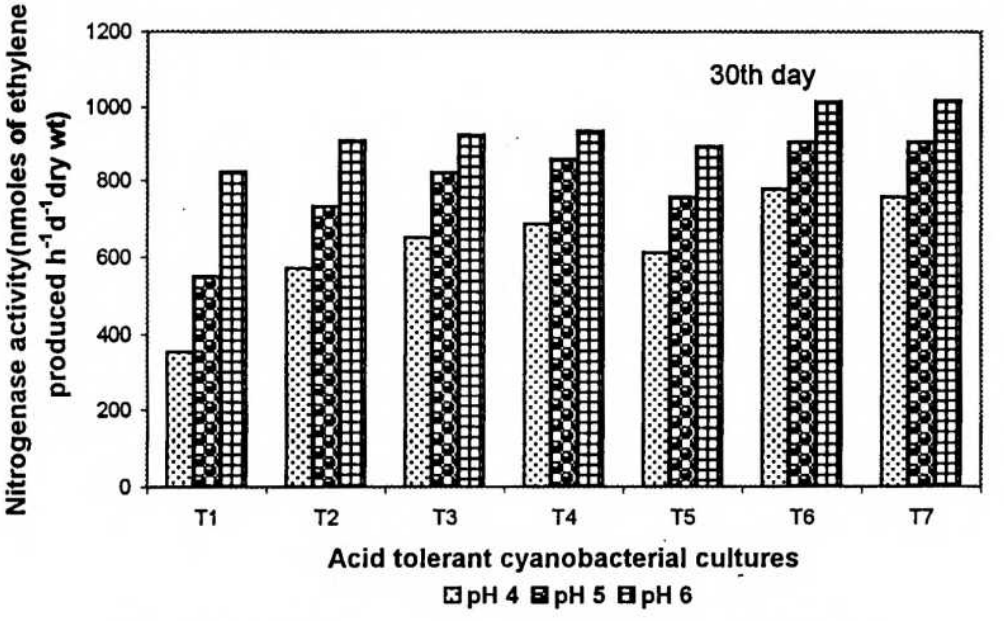


Fig 26 . Effect of acidity on the nitrogenase activity of the acid tolerant cyanobacterial mutants



T1-*Anabaena*-NS-$5G_2$
T2-*Anabaena*-AT-TGK-$5A_6$
T3-*Anabaena*-AT-MGK-$5A_6$-E_{200}
T4-*Nostoc*-AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*-AT-MGK-$5B_8$-E_{200}
T6-*Westiellopsis*-AT-MGK-$4A_2$-E_{250}
T7-*Westiellopsis*-AT-MGK-$4A_7$-E_{250}

Table 28. Effect of acidity on free amino acid content of the acid tolerant cyanobacterial mutants

Cultures	pH	Free amino acid content (mg ml^{-1})		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	0.01	0.08	0.12
	5	0.06	0.12	0.16
	6	0.17	0.24	0.34
Anabaena-AT-TGK-$5A_6$	4	0.09	0.13	0.20
	5	0.14	0.16	0.28
	6	0.19	0.25	0.36
Anabaena-AT-MGK-$5A_6$-E_{200}	4	0.13	0.20	0.27
	5	0.18	0.24	0.34
	6	0.22	0.29	0.42
Nostoc-AT-MGK-$4C_4$-E_{250}	4	0.09	0.21	0.26
	5	0.13	0.28	0.35
	6	0.18	0.35	0.43
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	0.05	0.14	0.27
	5	0.13	0.20	0.32
	6	0.17	0.27	0.37
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	0.14	0.20	0.32
	5	0.19	0.27	0.38
	6	0.24	0.35	0.49
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	0.15	0.22	0.33
	5	0.19	0.28	0.38
	6	0.25	0.37	0.47

	SEd	CD
Cultures	0.002	0.004
pH	0.001	0.002
Days	0.001	0.003
Cultures x pH x Days	0.006	0.012

Table 29. Effect of acidity on the methionine content of the acid tolerant cyanobacterial mutants

Cultures	pH	Methionine content (mg g^{-1} fresh wt.)		
		10^{th}day	20^{th}day	30^{th}day
Anabaena-NS-$5G_2$	4	0.004	0.01	0.02
	5	0.01	0.02	0.03
	6	0.06	0.08	0.12
Anabaena-AT-TGK-$5A_6$	4	0.04	0.05	0.09
	5	0.04	0.06	0.10
	6	0.06	0.08	0.11
Anabaena-AT-MGK-$5A_6$-E_{200}	4	0.05	0.09	0.13
	5	0.06	0.09	0.13
	6	0.09	0.12	0.15
Nostoc-AT-MGK-$4C_4$-E_{250}	4	0.05	0.08	0.12
	5	0.07	0.10	0.14
	6	0.10	0.12	0.15
Oscillatoria-AT-MGK-$5B_8$-E_{200}	4	0.004	0.02	0.04
	5	0.01	0.04	0.05
	6	0.04	0.05	0.07
Westiellopsis-AT-MGK-$4A_2$-E_{250}	4	0.09	0.13	0.17
	5	0.11	0.16	0.19
	6	0.15	0.18	0.21
Westiellopsis-AT-MGK-$4A_7$-E_{250}	4	0.10	0.13	0.18
	5	0.12	0.15	0.20
	6	0.15	0.18	0.22

	SEd	CD
Cultures	0.001	0.001
pH	0.001	0.001
Days	0.001	0.001
Cultures x pH x Days	0.002	0.004

Fig 27 . Effect of acidity on free amino acids content of the acid tolerant cyanobacterial mutants

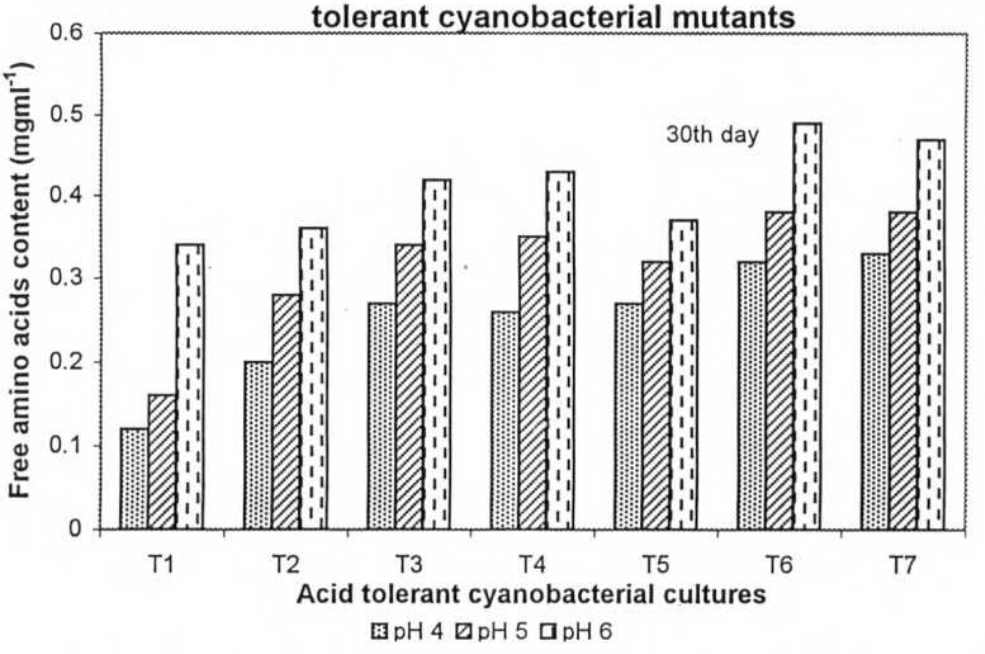
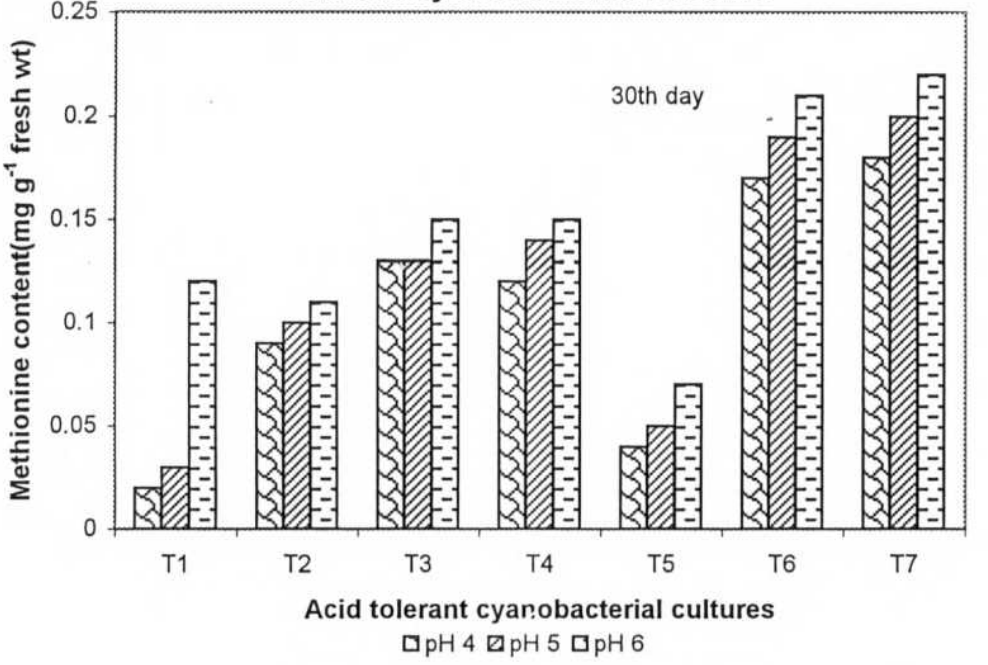


Fig 28 . Effect of acidity on the methionine content of the acid tolerant cyanobacterial mutants



T1-*Anabaena*-NS-$5G_2$
T2-*Anabaena*-AT-TGK-$5A_6$
T3-*Anabaena*-AT-MGK-$5A_6$-E_{200}
T4-*Nostoc*-AT-MGK-$4C_4$-E_{250}
T5-*Oscillatoria*-AT-MGK-$5B_8$-E_{200}
T6-*Westiellopsis*-AT-MGK-$4A_2$-E_{250}
T7-*Westiellopsis*-AT-MGK-$4A_7$-E_{250}

Fig.28. All the mutants except *Oscillatoria* -AT-MGK-$5B_8$-E_{200} registered higher methionine content than the normal and acid tolerant cyanobacterial culture. *Westiellopsis* mutants recorded significantly higher methionine content than other mutants at pH 4,5 and 6. At pH 4, *Anabaena* -AT-MGK-$5A_6$-E_{200} produced more methionine than *Nostoc* -AT-MGK-$4C_4$-E_{250}.At pH_5, *Nostoc*- AT-MGK-$4C_4$-E_{250} produced higher methionine content than *Anabaena* -AT-MGK-$5A_6$-E_{200}.

4.7. EFFECT OF INOCULATION OF THE ACID TOLERANT CYANOBACTERIAL MUTANTS ON SEEDLING GROWTH OF RICE VARIETY ASD 16

4.7.1. EFFECT ON SEEDLING HEIGHT

The data are presented in Table 30 , Fig.29 and Plates 8-10. The inoculation of acid tolerant cyanobacterial mutants significantly increased the height of the ASD 16 rice seedlings than the uninoculated control and other normal and the acid tolerant cyanobacterial culture. Among the individual applications, *Westiellopsis* mutants increased the plant height significantly followed by *Nostoc* -AT-MGK-$4C_4$-E_{250}, *Anabaena* -AT-MGK-$5A_6$-E_{200} and *Oscillatoria* -AT-MGK-$5B_8$-E_{200}.

4.7.2. FLOOD WATER AMMONIA

The results are given in Table 31 and Fig.30. In general, the treatments inoculated with acid tolerant cyanobacterial mutants recorded highly significant production of flood water ammonia. Significant level of ammonia excretion was noted in the flood water at 20^{th} day after inoculation and it reduced gradually thereafter. Among the mutants, *Westiellopsis* mutants showed higher ammonia excretion followed by *Nostoc* -AT-MGK-$4C_4$-E_{250}, *Anabaena* -AT-MGK-$5A_6$-E_{200} and *oscillatoria* -AT-MGK-$5B_8$-E_{200}.

4.7.3. TOTAL CHLOROPHYLL CONTENT

The total chlorophyll content of the plants with various treatments inoculated with the acid tolerant cyanobacterial mutants are given in Table 32 and Fig.31. The

Table 30. Effect of inoculation of the acid tolerant cyanobacterial mutants on the plant height of ASD 16 rice seedlings

Cultures	Plant height (cm)			
	10^{th} day	20^{th} day	30^{th} day	40^{th} day
Uninoculated	5.54	12.60	21.66	32.62
Anabaena-NS-$5G_2$	5.65	13.46	23.53	34.49
Anabaena-AT-TGK-$5A_6$	6.06	15.48	24.58	35.40
Anabaena-AT-MGK-$5A_6$-E_{200}	6.26	16.74	25.58	37.66
Nostoc-AT-MGK-$4C_4$-E_{250}	6.54	17.54	27.53	39.39
Oscillatoria-AT-MGK-$5B_8$-E_{200}	5.96	13.51	23.57	35.66
Westiellopsis-AT-MGK-$4A_2$-E_{250}	6.85	18.48	29.29	43.70
Westiellopsis-AT-MGK-$4A_7$-E_{250}	6.96	19.45	29.47	45.57

	SEd	CD
Cultures	0.10	0.20
Days	0.07	0.14
Cultures x Days	0.19	0.39

Table 31. Effect of inoculation of the acid tolerant cyanobacterial mutants on the flood water ammonia content

Cultures	Flood water ammonia (n moles ml^{-1})		
	20^{th} day	30^{th} day	40^{th} day
Uninoculated	20.56	18.56	15.58
Anabaena-NS-$5G_2$	53.15	27.41	21.49
Anabaena-AT-TGK-$5A_6$	68.61	48.42	31.62
Anabaena-AT-MGK-$5A_6$-E_{200}	73.36	54.49	43.44
Nostoc-AT-MGK-$4C_4$-E_{250}	77.64	58.64	45.57
Oscillatoria-AT-MGK-$5B_8$-E_{200}	65.57	43.68	32.69
Westiellopsis-AT-MGK-$4A_2$-E_{250}	84.49	64.64	49.68
Westiellopsis-AT-MGK-$4A_7$-E_{250}	87.68	67.68	50.54

	SEd	CD
Cultures	0.17	0.33
Days	0.10	0.21
Cultures x Days	0.29	0.58

Fig 29 . Effect of inoculation of the acid tolerant cyanobacterial mutants on the plant height of ASD16 rice seedlings

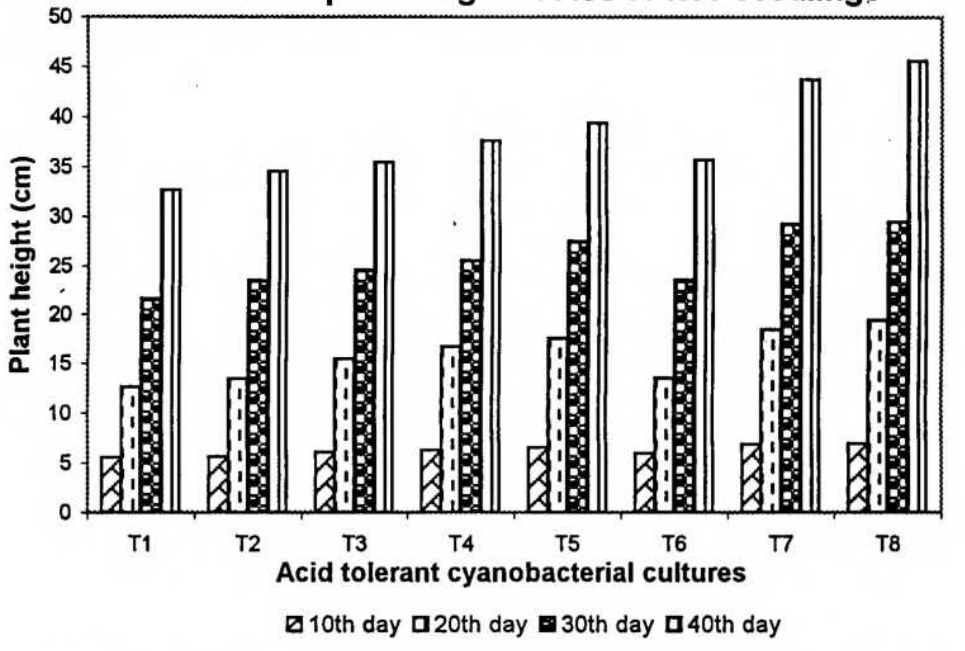
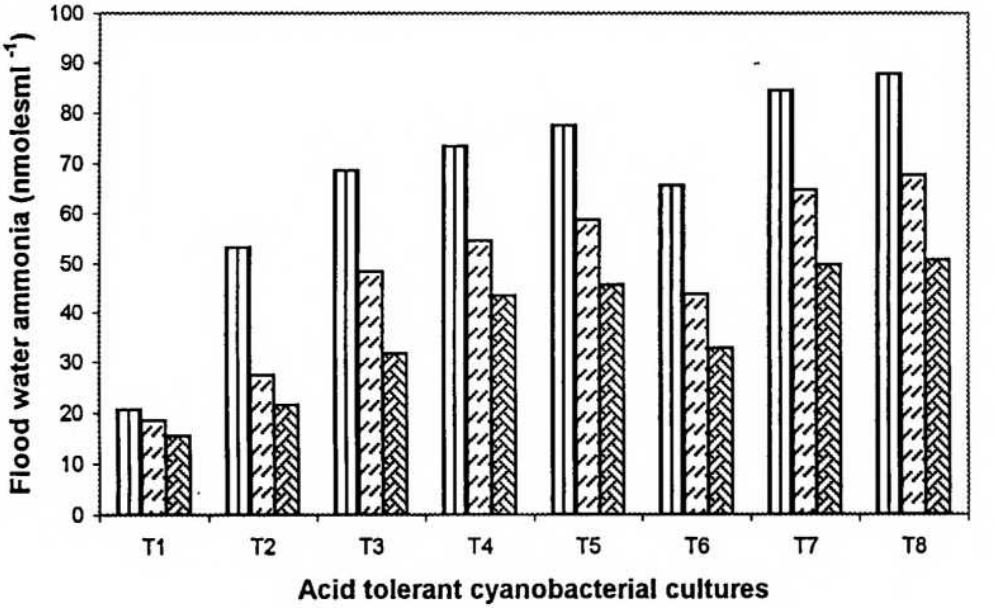


Fig 30 . Effect of inoculation of the acid tolerant cyanobacterial mutants on the flood water ammonia content



T1-Uninoculated
T2-*Anabaena* -NS-5G_{72}
T3-*Anabaena* -AT-TGK-5A_6
T4-*Anabaena* -AT-MGK-5A_6-E_{200}
T5-*Nostoc* -AT-MGK-4C_4-E_{250}
T6-*Oscillatoria* -AT-MGK-5B_8-E_{200}
T7-*Westiellopsis* -AT-MGK-4A_2-E_{250}
T8-*Westiellopsis* -AT-MGK-4A_7-E_{250}

Plate 8. **Pot cultures experiment with ASD 16 seedlings in acid soils.**

Plate 9. Establishment of *Westiellopsis* mutants in plastic tubs.

Plate 10. **Inoculation effect of *Westiellopsis* mutants on ASD 16 rice seedlings.**

1. *Westiellopsis* – AT –TGK-4A_7

2. *Westiellopsis* – AT –MGK-4A_7-E_{250}

Table 32. Effect of inoculation of the acid tolerant cyanobacterial mutants on the total chlorophyll content of ASD 16 rice seedlings

Cultures	Total chlorophyll content (mg g^{-1} fresh wt.)		
	20^{th} day	30^{th} day	40^{th} day
Uninoculated	0.79	1.08	1.26
Anabaena-NS-$5G_2$	0.86	1.18	1.45
Anabaena-AT-TGK-$5A_6$	0.95	1.23	1.65
Anabaena-AT-MGK-$5A_6$-E_{200}	1.05	1.24	1.75
Nostoc-AT-MGK-$4C_4$-E_{250}	1.15	1.31	1.85
Oscillatoria-AT-MGK-$5B_8$-E_{200}	0.86	1.17	1.54
Westiellopsis-AT-MGK-$4A_2$-E_{250}	1.25	1.42	2.06
Westiellopsis-AT-MGK-$4A_7$-E_{250}	1.27	1.51	2.16

	SEd	CD
Cultures	0.01	0.01
Days	0.01	0.02
Cultures x Days	0.02	0.04

mutants increased the total chlorophyll content of the ASD 16 rice seedlings compared to the control at all time intervals. Among the mutants, *Westiellopsis* -AT-MGK-$4A_7$-E_{250} significantly increased the total chlorophyll content of the ASD 16 rice seedlings followed by *Westiellopsis* -AT-MGK-$4A_2$-E_{250}, *Nostoc* -AT-MGK-$4C_4$-E_{250}, *Anabaena* -AT-MGK-$5A_6$-E_{200} and *Oscillatoria* -AT-MGK-$5B_8$-E_{200}.

4.7.4. TOTAL NITROGEN CONTENT

The total nitrogen content of the ASD 16 rice seedlings are presented in Table 33 and Fig.32. In general, the total nitrogen content of the seedlings inoculated with mutants was more than the uninoculated control and other normal and acid tolerant cyanobacterial culture. Among the inoculants, *Westiellopsis* -AT-MGK-$4A_7$-E_{250} significantly increased the total nitrogen content of the seedlings followed by *Westiellopsis* -AT-MGK-$4A_2$-E_{250}, *Nostoc* -AT-MGK-$4C_4$-E_{250}, *Anabaena* -AT- MGK- $5A_6$-E_{200} and *Oscillatoria* -AT-MGK-$5B_8$-E_{200}.

Table 33. Effect of inoculation of the acid tolerant cyanobacterial mutants on the total nitrogen content of ASD 16 rice seedlings

Cultures	Total nitrogen content (%)
Uninoculated	0.49
Anabaena-NS-$5G_2$	0.53
Anabaena-AT-TGK-$5A_6$	0.56
Anabaena-AT-MGK-$5A_6$-E_{200}	0.66
Nostoc-AT-MGK-$4C_4$-E_{250}	0.67
Oscillatoria-AT-MGK-$5B_8$-E_{200}	0.57
Westiellopsis-AT-MGK-$4A_2$-E_{250}	0.69
Westiellopsis-AT-MGK-$4A_7$-E_{250}	0.73
SEd	0.004
CD	0.009

Fig 31 . Effect of inoculation of the acid tolerant cyanobacterial mutants on the total chlorophyll content of ASD16 rice seedlings

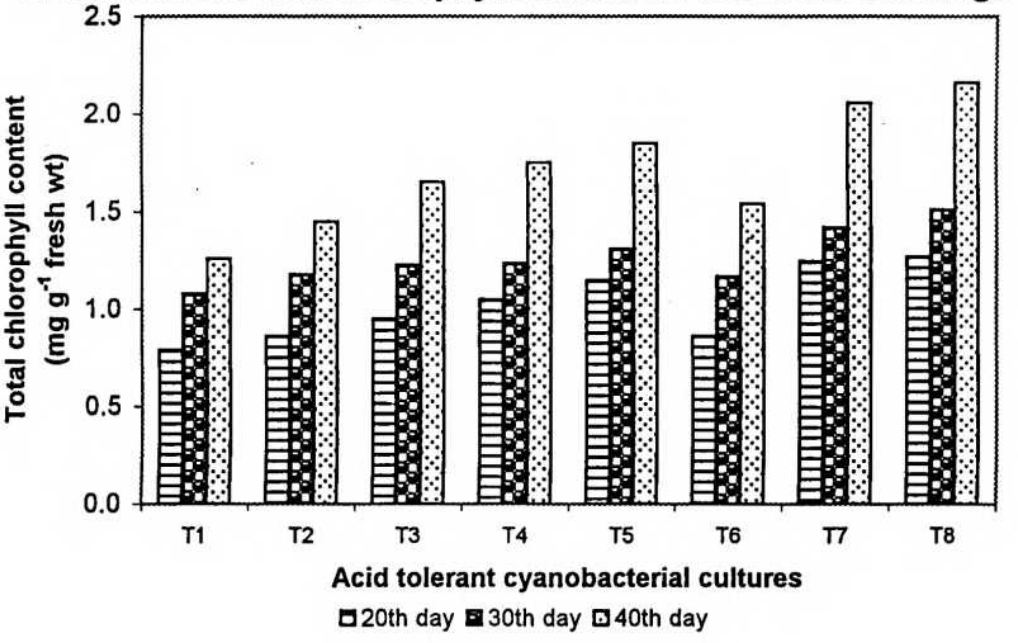
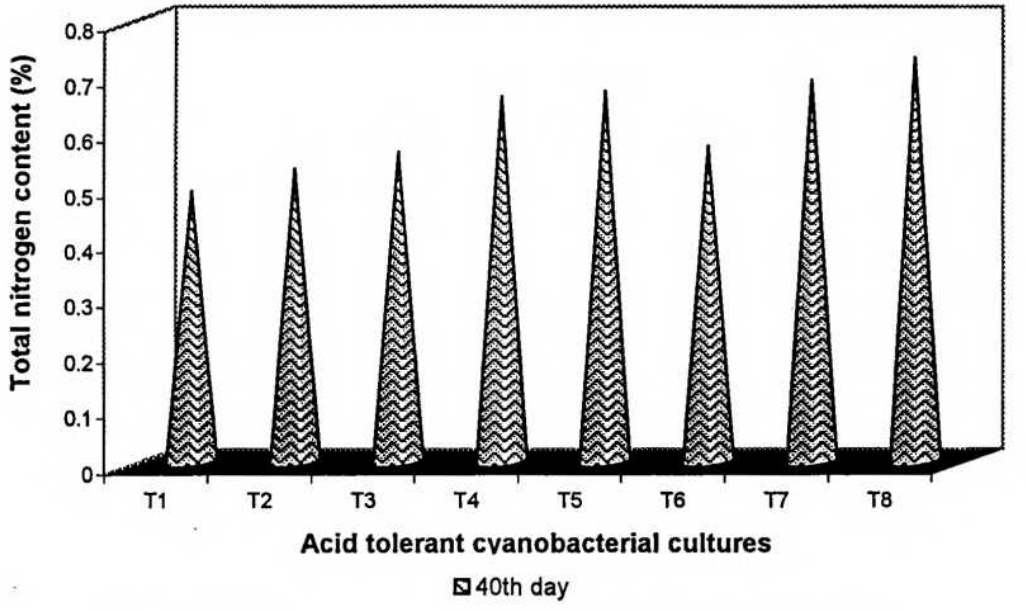


Fig32 . Effect of inoculation of the acid tolerant cyanobacterial mutants on the total nitrogen content of ASD 16 rice seedlings



T1-Uninoculated
T2-*Anabaena* -NS-$5G_2$
T3-*Anabaena* -AT-TGK-$5A_6$
T4-*Anabaena* -AT-MGK-$5A_6$-E_{200}
T5-*Nostoc* -AT-MGK-$4C_4$-E_{250}
T6-*Oscillatoria* -AT-MGK-$5B_8$.E_{200}
T7-*Westiellopsis* -AT-MGK-$4A_2$.E_{250}
T8-*Westiellopsis* -AT-MGK-$4A_7$.E_{250}

DISCUSSION

5. DISCUSSION

The burgeoning population increased the demand for food to feed them from shrinking land and limited water supply, which resulted in desperate problems of malnutrition and starvation. In view of world's food crisis, much attention has been turned to increase the productivity of agricultural crops, through sustainable farming methods, which are ecofriendly, economically rewarding and intellectually stimulating.

Although consumption of all three primary nutrients *viz.*, N, P and K increased in tropical soils, consumption of N use increased more rapidly. Nitrogen is the most critical nutrient for rice productivity. In India the total supply of nitrogenous fertilizer in the recent past was 9 million tonnes against the demand of 11 million tonnes resulting in a gap of 16 per cent. This gap is likely to widen further and could be narrowed down through biological nitrogen fixation (BNF). The main agents of BNF in rice field are free - living cyanobacteria (30 - 40 kg N ha^{-1} y^{-1}), *Azolla* - *Anabaena* symbiosis (40 - 60 kg N ha^{-1} y^{-1} and free living bacteria (15kg N ha^{-1} y^{-1}) (Kannaiyan, 2000).

Under flooded rice ecosystem, BNF by cyanobacteria is considered the best biological system due to their capacity of fixing atmospheric nitrogen efficiently by utilizing the solar energy. The nitrogen fixed by cyanobacteria made available to rice plants either through excretion of nitrogenous compounds or by decay and subsequent mineralization (Roger and Kulasooriya, 1980). Cyanobacteria are ubiquitous in nature and their occurrence and succession is regulated by many interacting factors such as soil properties, temperature, light, nutrient concentrations, growth promoting and growth inhibiting substances and selective grazing. Amongst the various soil factors affecting the distribution of edaphic cyanobacteria, hydrogen ion concentration is an important factor which directly influences their distribution,

abundance and nitrogen fixation in soil (Sharma and Kumar, 1975). Though the cyanobacteria performed well in normal and saline soil, very little is known about the performance in acid soils. These soils are poor in nutrient status, which ultimately reduce the rice yield. Recently several acid tolerant cyanobacterial cultures were isolated from acid soils of Tamil Nadu (Tamilselvam *et al.*, 1999).

One of the recent suggestions for effective use of cyanobacteria is to select good, effective and promising cultures through several approaches (Goyal and Goyal, 1998). Induced mutagenesis is one such approach. In the present study attempts were made to select efficient cultures of cyanobacteria for better performance and to increase the rice production in acid soil ecosystem through induced mutagenesis.

5.1. INDUCED MUTAGENESIS OF THE ACID TOLERANT CYANOBACTERIAL CULTURES

Selection of mutant strains of cyanobacteria is very important so as to increase their potentialities, since the study of mutations and isolations of wide spectrum mutants in different cyanobacteria has subtle role in terms of their biochemical constituents. An array of both physical and chemical mutagens are used for selection of mutants for desirable traits, particularly higher growth and biomass production and higher nitrogen fixation. The chemical mutagens such as EMS and NTG have been widely used for inducing various types of mutations in nitrogen fixing cyanobacteria (Mishra and Tiwari, 1986).

A number of studies have been made on the effects of chemical mutagens on various organisms. EMS has been recognised as a powerful mutagen of relatively low lethality to microorganism (Strauss, 1961). NTG is an extremely potent mutagen for a wide spectrum of organisms (Neale, 1976). Diethyl sulphate and hydroxylamine have been shown to induce mutations in certain microorganisms and higher plants (Westergaard, 1957). Procedures for mutagenesis in acid condition for

selection by antibiotic enrichment in cyanobacteria have been developed. In view of the marked variations amongst cyanobacteria with respect to their biological activity and response to various ecological stresses, it is essential to screen and select promising stress compatible strains.

It was found that chemical mutagenesis with EMS at different time exposure on *Anabaena oryzae* and *Nostoc entophytum* revealed that minimum exposure of 15 min had less lethal effect by showing increased growth and chlorophyll a content after a lag phase (Bharthi and Giriyappanavar, 1988). Non-heterocystous and non-nitrogen fixing stable mutants of *Nostoc linckia* were isolated by NTG mutagenesis after penicillin enrichment (Kumar and Kumar, 1984). Several stable mutants of cyanobacteria were isolated by UV mutagenesis (Singh and Singh, 1964a and Srivastava, 1969).

In the present study, the effect of various mutagens *viz.*, EMS, NTG, Colchicine and UV rays on 5 acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-5A_6,*Nostoc*-AT-TGK-4C_4, *Oscillatoria*-AT-TGK-5B_8,*Westiellopsis*-AT-TGK-4A_2 and *Westiellopsis*-AT-TGK-4A_7 were studied. In case of chemical mutagenesis using antibiotic enrichment it was observed that mutation frequency varied with mutagen concentration as well as cyanobacterial culture. Among the acid tolerant cyanobacterial cultures, *Westiellopsis* followed by *Nostoc* showed maximum mutation frequency at E_{250}, N_{150} and C_{100}, while *Anabaena* and *Oscillatoria* showed maximum frequency at E_{200}, N_{150} and C_{100}. Antibiotic enrichment increased mutation frequency and decreased reversion frequency (Kumar and Kumar, 1984). The results are in accordance with the above findings.

Physical mutagenesis with UV rays,showed increased mutation frequency with lesser exposure time. Maximum mutation frequency was obtained at 10 min time exposure. *Anabaena* and *Oscillatoria* had shown higher mutation frequency followed by *Westiellopsis* and *Nostoc* cultures. However not much variation was

seen between *Westiellopsis* cultures. The growth of UV induced mutants was comparatively less to chemically induced mutants. The results are in accordance with the findings of Singh and Singh (1964b) who demonstrated the slow growth of the UV induced mutants of *Anabaena doliolum.*

The LD_{50} value of various mutagens on the acid tolerant cyanobacterial cultures was compared and it is interesting to note that the lethal dose concentration of EMS is more followed by Colchicine and NTG. Among the cultures, *Westiellopsis*-AT-TGK-$4A_7$ was found to possess more LD_{50} value for EMS, while *Westiellopsis*-AT-TGK-$4A_2$ registered higher LD_{50} value for Colchicine, NTG and UV rays. *Oscillatoria*-AT-TGK-$5B_8$ registered minimum LD_{50} value for EMS, NTG and Colchicine and *Anabaena*-AT-TGK-$5A_6$ registered minimum LD_{50} value for UV rays.

Based on the mutation frequency and LD_{50} value studies, EMS and NTG mutants were chosen for the assessment of the reversion frequency. Among them, EMS mutants possessed less reversion frequency than the NTG mutants. All the acid tolerant cyanobacterial mutants of EMS had less reversion frequency at E_{250} except *Anabaena* -AT-TGK-$5A_6$ and *Oscillatoria*-AT-TGK-$5B_8$.

The alkylating agent, EMS add ethyl or methyl groups at carbon 7 of guanidine. This weakens the linkage of guanine to deoxyribose and the guanine is lost from DNA, leaving a gap. Depending on which of the four bases fills the gap, transitions or transversions may eventually arise (Evers, 1980). The mutagenic effects of EMS was first demonstrated by Loveless and Howart (1959) and it has been recognised as a powerful mutagen of relatively low lethality to microorganism (Strauss, 1961). EMS has been observed to be a potent mutagen in *Neurospora* (Kolmark, 1956) and in *Drosophila* (Ehrenberg *et al.*, 1961). It was also used to mutate wheat (Swaminathan *et al.*, 1962) and sorghum (Sharma and Chatterji, 1962). The present results are in accordance with the above findings.

5.2. GROWTH,BIOMASS PRODUCTION AND CHLOROPHYLL CONTENTS OF ACID TOLERANT CYANOBACTERIAL MUTANTS AT ACIDIC pH

Cyanobacteria are able to grow in a wide range of pH, but prefers alkaline pH. Biswal *et al.* (1975) tested the pH effect on the growth of the cyanobacterium *Westiellopsis prolifera* and observed that reduction in pH to the acid range led to sudden reduction in growth and chlorophyll a content with alteration in branching habit. Tamilselvam (1998) isolated cyanobacterial cultures which tolerated the acidity range upto pH 4.0. They showed better growth and biomass production under acidic conditions. With a view to improve the genetic potential of efficient acid tolerant cyanobacterial cultures, induced mutagenesis with EMS was performed and mutants were obtained. The growth and biomass production as well as biochemical characterization was attempted.

In the present study, with 5 EMS mutants *viz.*, *Anabaena* - AT-MGK-$5A_6$-E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria*-AT-MGK-$5B_8$-E_{200}, *Westiellopsis*-AT-MGK-$4A_2$-E_{250} and *Westiellopsis*-AT-MGK-$4A_7$-E_{250}, there was a linear increase in growth with increased incubation time and pH. The growth and biomass production were higher in the mutants than wild cultures. Among the mutants *Westiellopsis* cultures showed maximum growth and biomass production at all acidic conditions. Kumar (1994) noticed increased biomass production, doubling time, relative growth rate in *A.microphylla* mutated with EMS. Bharathi and Giriyappanavar (1988) have shown increased growth after a lag phase in the EMS induced mutants of *Anabaena oryzae* and *Nostoc entophytum*. This is in agreement with our results.

EMS induced mutants of *A. microphylla* have registered maximum chlorophyll content which is attributed to the faster growth rate and better nutrient uptake than their wild parent (Kumar, 1994). The mutant strains of *A. microphylla*-C50-SK-JV and *A.microphylla*-C25-SK-JV have revealed higher chlorophyll

contents in them than their wild parents (Vasanthan Paul, 1994).The results of the present study revealed that there was linear increase in chlorophyll a content with respect to aging of cultures and increase in pH. In general, the mutants possessed higher chlorophyll a content than their wild parents at all acidic conditions. Among the mutants *Westiellopsis* - AT-MGK-$4A_2$-E_{200}-showed higher chlorophyll a content than others. The results are in accordance with the findings of Subramanian and Shanmugasundaram (1987) who observed maximum chlorophyll a content in *Anabaena* ARM-220 at acidic pH of 5.0.

5.3 NITROGEN FIXATION AND AMMONIA EXCRETION BY ACID TOLERANT CYANOBACTERIAL MUTANTS AT ACIDIC pH

Kumar (1994) isolated EMS induced mutants of *A. microphylla* which exhibited higher nitrogenase activity than their wild parents. Acid tolerant cyanobacterial cultures with higher nitrogenase activity has been isolated (Tamilselvam, 1998). Forty four cyanobacterial cultures with high nitrogen fixing ability has been isolated by Begum and Mandal (1997).In the present study, mutants possessed higher nitrogenase activity than their wild parents even at high acidic conditions. The strong interaction between photosynthesis and nitrogen fixation has also been demonstrated by determining the action spectra for nitrogenase catalysed acetylene reduction (Tyagi *et al.*, 1980). The present study has also indicated a positive correlation between chlorophyll contents and nitrogen fixation since the *Westiellopsis* mutants with higher chlorophyll content have exhibited better nitrogenase activity.

Stewart (1969) observed that although pH of the growth medium may not affect specifically the nitrogen fixation, it may affect the general metabolism. Raghava Reddy *et al.* (1980) demonstrated that the pH of the growth medium exerted a marked influence on the nitrogen content of the cells as well as the total nitrogen fixed. The amino nitrogen content of the acid tolerant cyanobacterial mutants was low at high acidic conditions and it increased with decrease in acidic

conditions. All the mutants possessed more amino nitrogen content than the wild acid tolerant cyanobacterial cultures. The accumulation of amino N and ammonium N in the symbiotic association of *A. azollae* has been demonstrated by Tung and Watanabe (1983). Associative cyanobacteria with high amino nitrogen content has been isolated by Subhashini (1999). The present results are in conformity with the above findings.

Nitrogen fixing cyanobacteria are known to liberate ammonia under natural ecological condition. It has been found that certain strains such as *Anabaena variabilis* are capable of excreting the ammonia into the environment (Spiller *et al.*, 1986). In the present study, ammonia excretion is directly proportional to increased pH of the medium. However maximum excretion was detected at 20^{th} day after inoculation which declined thereafter. Increased ammonia excretion was seen in cyanobacterial mutants compared to their wild parents. Among the mutants, *Westiellopsis* cultures showed maximum ammonia excretion which has direct correlation with ARA activity. Mutants of symbiotic *Anabaena azollae* and free living *A. variabilis* were developed by mutagenesis with NTG which were found to excrete higher amounts of ammonia (Mahesh and Kannaiyan, 1993b). The results are also in conformity with the findings of Singh *et al.* (1984) who have isolated MNNG mutants of *Cylindrospermum* sp with increased ammonia production. Maximum liberation of ammonium ions during nitrogen fixation into the growth medium was formed by mutant cyanobacterium *A. variabilis* ED81 and ED92 was demonstrated (Orozohoeva *et al.*, 1990). Mutants of *Anabaena* 7120 had the capacity to excrete N_2 fixed ammonia continuously into the culture medium (Singh and Tiwari, 1998). The results of the present study are in accordance with the above findings.

5.4 PROTEIN , AMINO ACID AND POLYSACCHARIDE CONTENT OF THE ACID TOLERANT CYANOBACTERIAL MUTANTS AT ACIDIC pH

Proteins form a major component of cyanobacteria, contributing about 1.7 per cent of the dry weight and contains 90 per cent of the total nitrogen (Show,

1981), protein is the major nitrogen component in the C/N ratio, varies with nitrogen availability in nitrogen fixation. When supplemented nitrogen is provided to the cyanobacterium, the C/N ratio could be reduced to 4-8/1 range (Ryther and Hanisak, 1982). In the present study, all the mutants showed more protein content than their wild parents at all acidic conditions. Protein content was directly proportional to the increase in pH. *Westiellopsis* mutants produced more protein content at acidic conditions than other mutants. The results are in accordance with the findings of Angadi and Shyamala Datta (1990) who have shown that the mutagenic agent N-methyl-N-nitro-N-nitrosoguanidine induced the protein synthesis in the mutants obtained from *Hapalosiphon stuhlmanii*.

Many microorganisms in addition to minerals, carbon and energy source need some accessory nutrients known as growth factors *viz*, amino acids, purine and pyrimidine bases and the vitamins which are the components of proteins, nucleic acids and co-enzymes respectively (Schlegel, 1996). Amino acids are also known to carry out osmoregulatory role in cyanobacteria in order to adapt to the change in environmental conditions (Reddy *et al.*, 1989 and Thomas and Shanmugasundaram, 1991). Reddy and Sarada (1982) demonstrated that cysteins, protected *A. doliolum* from the UV irradiation damage, since it also absorbed UV light. When *Anabaena azollae* grown on media containing NH_4^+, the level of total free amino acids plus ammonia increased markedly, whereas it decreased when the plants were grown on media containing NO_3^-(Kitoh *et al.*, 1992). Aspartic acid, glutamic acid and alanine were the amino acids present in the free form and they played an important role in the pathway of fixation mechanism (Taha *et al.*, 1962).

In the present study, the free amino acid content of the mutants decreased with decrease in pH of the growth medium. The sulphur containing amino acid (methionine) in the acid tolerant cyanobacterial mutants was directly proportional to the aging of the cultures and increase in pH of the growth medium. All the mutants

liberated higher amount of free amino acids and methionine than the normal and acid tolerant cyanobacterial culture at high acidic conditions. The results are in conformity with the findings of Agarwal and Sharma (1996) who witnessed the release of amino acids (glycine, serine, cystine, glutamic acid, aspartic acid, α-alanine) by *Westeillopsis prolifica* Janet and *Chaetophora attenuata* Hazen cultures.

The halotolerant, filamentous, heterocystous cyanobacterium *Anabaena* sp ATCC 33047 released large amounts of exopolysaccharide to the culture medium (Moreno *et al.*, 1998). The enormous exopolysaccharide secretion from *Nostoc muscorum* increased the soluble C content, microbial activity and the amount of water soluble aggregates in a saline sodic soil (Zulpa de caire *et al.*, 1997). Cells of the cyanobacterium *Nostoc commune* secrete a complex, high molecular weight extracellular polysaccharide which accumulates to more than 60 per cent of the dry weight (Hill *et al.*, 1997).

The present study revealed that the polysaccharide production of the acid tolerant mutants increased with increase in pH and inoculation time. All the mutants produced increased polysaccharide content at acidic conditions than the wild acid tolerant cyanobacterial cultures. Among the mutants *Westiellopsis* mutants produced higher polysaccharide content. Sugunarani (1997) observed that saline tolerant cyanobacteria cultures produce higher amount of polysaccharides at high pH than at neutral pH range. Holeberg and Margalith (1981) and Karube *et al.* (1980) suggested that the presence of polysaccharides even at a low level has substantial effects on biochemical reactions which are water dependent. The development of the polysaccharide mucilage has an effect on water activity and as a consequence on the metabolites of the cell. Increased quantities of metabolites are produced with the increased polysaccharides (Yamamotc *et al.*, 1974). The results are in accordance with the above findings

5.5. PERFORMANCE OF ACID TOLERANT CYANOBACTERIAL MUTANTS AS BIOFERTILIZER FOR RICE SEEDLINGS

Nitrogen fixation by both symbiotic and non symbiotic associations have been demonstrated as potential nitrogen source for crop production in agriculture (Kannaiyan, 1991). The availability of fertilizer nitrogen from inorganic sources or biological fixation or mineralization of organic matter is subjected to a series of physical, chemical and biological changes in soil. Integrated use of organic and inorganic nitrogen sources apparently increases the nitrogen utilization by rice crop. In low land rice ecosystem, the nitrogen could be efficiently supplied by *Azolla* and cyanobacteria (Kannaiyan, 1990).

Nitrogen fixing cyanobacteria, both symbiotic and free living species are known to provide ammonia to rice plants under natural ecological conditions (Kannaiyan *et al.*, 1992). The importance of cyanobacteria as a source of nitrogen has been well documented by Roger and Kulasooriya (1980) and Kannaiyan (1990). Cyanobacterial biofertilizers are a promising alternative to avoid soil pollution caused by agrochemicals and recover the nutrient content and structure lost after harvest as they bring to soil combined N (some are N_2 fixers), exopolysaccharide that improve soil structure and bioactive substances that enhance seedling growth. Singh (1961) estimated the cellular composition of different cyanobacterial biomass and recorded 6 to 9 per cent nitrogen, 1 to 3 per cent phosphorus and 6 to 8 per cent ash with C:N ratio as narrow as 5 to 8:1. They provide fixed nitrogen to rice plants through both secretion of nitrogenous substances and on their decay and subsequent mineralisation of organic substances (Santra, 1993). Nitrogen contributions of 14 to 40 kg ha^{-1} has been attributed to the activities of the N_2 fixing cyanobacteria in different rice growing areas (De and Mandal, 1956; Sankaran, 1971 and Yoshida and Ancaja, 1973). Relwani and Subramanyan (1953) inoculated composite culture of *Nostoc* sp., *Microcoleus* sp, *Phormidium* sp and *Aphanothece* sp to experimental

pots and fields with rice crop and suggested that cyanobacteria could be an effective susbstitute for ammoniacal nitrogen.

The results of the present study revealed that the inoculation of acid tolerant cyanobacterial mutants increased the plant height, chlorophyll content, total nitrogen content and flood water ammonia when compared to uninoculated control. *Westiellopsis* cultures increased plant height, total chlorophyll content, total nitrogen content and flood water ammonia production. The cultures grew well in the tubs and covered the soil surface in 30 days of inoculation. It might be due to the adaptive nature of the mutants to acidic conditions. The higher accumulation of the chlorophyll and nitrogen content in rice seedlings might possibly be attributed to the vigorous growth of cyanobacteria, which facilitated the uptake of nutrients by rice seedlings. The results has been confirmed by the findings of Mule (1999) who showed that *Nostoc muscorum* and *Tolypothrix tenius* increased the shoot length, total N content and soluble C content in the rice seedlings. Spiller and Gunasekaran (1990) have shown that the growth of wheat plants was significantly higher by the inoculation of ammonia excreting mutant strain of cyanobacterium, *Anabanea variabils*. The present results are in accordance with the above findings. Moreover increase in plant height and total chlorophyll content by *Westiellopsis* mutants has been confirmed by the findings of Tamilselvam (1998) who showed increased plant height and total chlorophyll content by the rice seedlings due to inoculation of the acid tolerant cyanobacterial cultures. The results clearly suggested that the inoculation of acid tolerant cyanobacterial mutants would increase the growth of rice seedlings by virtue of their continuous photo production of ammonia in flood water ecosystem.

SUMMARY

6. SUMMARY

Cyanobacteria are probably the most diverse group of prokaryotes, capable of performing simultaneously both carbon assimilation and N_2 fixation, thereby enhancing the productivity in a variety of environments. Among soil properties, pH is a very important factor influencing the growth, establishment and diversity of cyanobacteria which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth. So induced mutagenesis has been tried to develop suitable strains of cyanobacteria which may possess higher nitrogen fixing ability, higher growth and biomass production coupled with better adaptation to acidic conditions. Though number of studies have been made on the effect of chemical mutagens on various organisms, very little work was carried out on cyanobacteria.

In the present investigation, five acid tolerant cyanobacterial cultures *viz.*, *Anabaena*-AT-TGK-$5A_6$, *Nostoc*-AK-TGK-$4C_4$, *Oscillatoria* AT-TGK-$5B_8$, *Westiellopsis*-AK-TGK-$4A_2$ and *Westiellopsis*-AK-TGK-$4A_7$ were mutated with chemical mutagens viz., EMS, NTG and Colchicine at various concentrations and physical mutagenesis with UV rays at different exposure time. Based on the *in vitro* studies on mutation frequency and reversion frequency, 5 EMS mutants *viz.*, *Anabaena*-AT-MGK-$5A_6$-E_{200}, *Nostoc*-AT-MGK-$4C_4$-E_{250}, *Oscillatoria*-AT-MGK-$5B_8$-E_{200}, *Westiellopsis*-AT-MGK-$4A_2$-E_{250} and *Westiellopsis*-AT-MGK-$4A_7$-E_{250} were selected and used for further characterization. The effect of acidity on these mutants were studied and the inoculation effect on ASD16 rice seedlings was tested and the salient features are summarised below

1. The effect of the chemical mutagens on the population and mutation frequency of different acid tolerant cyanobacterial cultures were tested separately by exposing them to varied concentration of EMS/NTG/ Colchicine. In general, mutation frequency varied with mutagen concentration as well as cyanobacterial cultures. Maximum mutation

frequency was obtained at E_{250} and N_{150} by *Westiellopsis* cultures, while at C_{100} *Oscillatoria* showed maximum mutation frequency.

2. The acid tolerant cyanobacterial cultures were exposed to UV rays for different exposure time. Lower the exposure time, higher will be the mutation frequency. Maximum mutation frequency was at 10 min time exposure by all the acid tolerant cyanobacterial cultures. *Anabaena*-AT-TGK-$5A_6$ and *Oscillatoria*-AT-TGK-$5B_8$ have shown higher mutation frequency followed by *Nostoc*-AT-TGK-$4C_4$ and *Westiellopsis* cultures.

3. The LD_{50} value of the tested mutagens on the acid tolerant cyanobacterial cultures was compared. It is interesting to note that the lethal dose concentration of EMS is more followed by Colchicine and NTG. The survival capacity of *Westiellopsis* cultures was more for the mutagens when compared to other acid tolerant cyanobacterial cultures.

4. The reversion frequency of the acid tolerant cyanobacterial mutants developed by EMS was less and varies with mutagen concentration. The reversion frequency for *Westiellopsis* cultures and *Nostoc* was less at E_{250}, for *Oscillatoria* and *Anabaena*, it was less at E_{200}. However, in the case of NTG, reversion frequency of the acid tolerant cyanobacterial mutants was minimum at 150 ppm for all the acid tolerant cyanobacterial cultures.

5. The acid tolerant cyanobacterial mutants were compared with their wild parents for their performance. The growth and biomass production were significantly higher than their wild acid tolerant cyanobacterial parents. Among the mutants, *Westiellopsis* mutants showed higher growth and biomass production. In general, linear increase in growth was observed with increased incubation time.

6. All the mutants showed increase in ammonia excretion into the surrounding medium than the wild type. The ammonia excretion by the cyanobacterial

cultures was maximum at 20th day after inoculation and it declined at 30th day after inoculation. *Westiellopsis* -AT-MGK-$4A_7$-E_{250} registered higher ammonia excretion among the experimented cultures.

7. All the mutants recorded higher chlorophyll a content, protein and polysaccharide content than wild parents. Among the mutants, *Westiellopsis* cultures produced higher chlorophyll a content, protein and polysaccharide production. In general, there was a linear increase in biochemical constituents as the incubation time increases.

8. All the acid tolerant cyanobacterial mutants recorded higher amino nitrogen content and nitrogenase activity, than their wild parents. Among the mutants, *Westiellopsis* cultures expressed higher amino nitrogen content and nitrogenase activity and *Oscillatoria* recorded the lowest activity.

9. The liberation of free amino acids and sulphur containing amino acid (methionine) was more in the mutants than their wild parents. Not much variation was seen in the methionine content due to time intervals. Among the mutants, *Westiellopsis* liberated more amount of free amino acids than other mutants. Both wild and mutant *Westiellopsis* cultures registered significantly higher methionine content, while *Oscillatoria*-AT-MGK-$5B_8$-E_{200} showed minimum methionine content.

10. pH had a direct influence on the acid tolerant cyanobacterial mutants. All the mutants performed better at pH 4.0 than the normal soil isolate *Anabaena*-NS-$5G_2$and wild acid tolerant cyanobacterial culture *Anabaena*-AT-TGK-$5A_6$. Linear increase in growth and biomass production due to increase in incubation time was seen. Maximum growth and biomass production was noticed in *Westiellopsis* mutants followed by *Anabaena*-AT-MGK-$5A_6$-E_{200}.

11. Ammonia excretion is directly proportional to increased pH of the medium. However maximum excretion was detected at 20th day after inoculation which

declined thereafter. Significant increase in ammonia excretion was seen in cyanobacterial mutants compared to wild and normal *Anabaena* culture.

12. The chlorophyll a content, protein content and polysaccharide production of the acid tolerant cyanobacterial mutants decreased with decrease in acid pH of the surrounding medium. All the mutants performed well at acidic condition than the wild and normal soil isolate. *Westiellopsis* cultures showed higher chlorophyll a content, protein content and polysaccharide production at acidic conditions than other mutants.

13. *Westiellopsis* mutants recorded higher amino nitrogen content, nitrogenase activity, free amino acids liberation and methionine content than other mutants at pH 5.0. *Oscillatoria*-AT-MGK-$5B_8$-E_{200} was the least producer of the biochemical constituents among the mutants.

14. The inoculation of acid tolerant cyanobacterial mutants significantly increased the height of the ASD 16 rice seedlings and excretion of ammonia in the flood water than the uninoculated control. Among the cultures, *Westiellopsis* mutants increased the plant height significantly and showed higher ammonia excretion in the flood water. Significant level of ammonia excretion was noted in the flood water at 20^{th} day after inoculation and it reduced gradually thereafter.

15. Inoculation of cyanobacterial mutants increased the total chlorophyll content and total nitorgen content of the ASD 16 rice seedlings compared to the control at all the time intervals. Among the mutants, *Westiellopsis*-AT-MGK-$4A_7$-E_{250} significantly increased the total chlorophyll content and total nitrogen content of the rice seedlings.

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