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Distribution of blue green algae (BGA) in gardenland soils and their nature

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Abstract : Blue-Green algae (BGA) not only occur in low land rice fields which are flooded but also occur in gardenland soils rendered moist by irrigation. Although large mats do not appear small encrustations or growth occur along sides of irrigation channels, furrow slopes and certain cereals like maize fields. BGA occurred in these gradenland conditions exhibited a higher heterocyst frequency over wetland isolate under *in vitro* conditions. ***(Key words : Blue - Green algae, Irrigated gardenland fields, Heterocyst).***

Biological nitrogen fixation (BNF) mediated by microorganisms either through symbiotic association with plants or free living organisms play a vital role in contributing considerable quantum of nitrogen to the soil. Blue green algae have a greater potential as a biofertilizer in tropical rice soils as rice fields provide an ideal environment for the establishment of BGA and nitrogen fixation by them (Roger, 1982 and Venkataraman, 1989). Inoculation of BGA to rice has become a routine practice in the tropics to minimize nitrogenous fertilizer use and to boost yields. However, it is not always true that all cultivated lowland fields support abundant population of nitrogen fixing algal biotypes although recurrent combinations of algal species might occur in comparable habitats (Venkataraman, 1989).

Algal growth also occur in soils which are frequently made moist by intermittent irrigation leading to frequent wetting and gradual drying exhibiting moisture rich and moisture stress phases. This is particularly true for crops like sugarcane, banana, cotton and irrigated cereals like maize raised in gardenlands. In such soils although prominent BGA mats are never observed, some algal growth appear on soil surface particularly along the slopes of irrigation channels, on the sides of furrows and in the collar region of plants.

Although considerable investigations have been made on lowland, BGA studies on their distribution and nature in irrigated garden lands is lacking. With this idea of developing suitable BGA inoculants to gardenland crops, a study was made on algal flora of gardenlands and the results are reported hereunder.

Materials and Methods

A survey was made in irrigated gardenland crops like maize, cotton, sugarcane and collar regions of individual plants and of clumps. The visible algal encrustation observed in soil and algal growth on collar regions of plants were collected, brought to the laboratory, examined under microscope and identified.

BGA was isolated by spread plate method, the soil sample was serially diluted in sterile water and 1 ml aliquots of appropriate dilution (10^{-2}) were inoculated to 100 ml of N-free Bristol's medium. The flasks were incubated in light chamber at 28 $\pm$ 1°C for 4 weeks. The algal mat from the flask was picked up, homogenized in sterile water and inoculated to sterile solid medium containing antibiotics in plates (A mixture of 50 mg griseofulvin and 50 mg streptomycin was added to 10 ml of distilled water from the antibiotic mixture 0.2 ml was added to sterilised medium). The plates were incubated in light chamber and observed periodically. The colonies that appeared in the plate, were identified and compared with wet land isolate.

The two selected BGA cultures were examined under microscope and both vegetative cells and heterocysts were counted in ten fields at random. Heterocyst frequency was calculated by employing the formula and expressed in percentage.

$$\text{Heterocyst frequency(\%)} = \frac{\text{Total number of heterocysts}}{\text{Total number of cells}} \times 100$$

The ammonia excreted by the two algal cultures in the culture medium under *in vitro* was estimated by Nessler's method (Jackson, 1973). The algal cultures were inoculated to the N-free Bristol's medium and grown under the influence of light (2000 lux). The samples were drawn at periodical intervals and analysed. The culture medium was filtered to remove the algal biomass and 5 ml of the filtrate was taken in tubes to which 2 ml of 10 per cent sodium tartrate solution, 2 ml of 10 per cent acidified sodium chloride and 5 ml of Nessler's reagent were added. The tubes were allowed to stand for 30 min and then the absorbance was measured at 410 nm in a Bausch and lomb spectrionic 20 colorimeter. The quantity of ammonia was estimated using a standard curve prepared with ammonium chloride in ammonia free distilled water and expressed as µg/ml.

Results and Discussion

The survey made at different fields like sugarcane, cotton, banana which revealed a varying degree of algal growth as patches on soil surface and also in the collar region of the plants (Table 1). Microscopic examination showed a predominant *Nostoc* population in these gardenland.

Although algal presence is well known in all soils the present critical survey revealed their distribution in different soils that are frequently made moist by intermittent irrigation. Their presence in such soils also lends scope for exploiting them under garden land area in the country which is much larger than the lowland rice ecosystem. Such cultures can be utilized as biofertilizer with whatever minimum benefit they could bring to soil fertility.

It is generally accepted that tropical soils harbour larger populations of cyanobacteria than do temperature soils. Soils other than, under rice also exhibited the presence of BGA (Singh, 1961). The result of the present investigation also recorded that although a few members of *Tolypothrix, Scytonema* were observed, only *Nostoc* sp. was found to be more than other algal species.

Heterocyst frequency

The heterocyst frequency of the two BGA cultures are presented in Fig. 1. It was found that on an average gardenland isolate of BGA (GI) recorded more heterocyst frequency of 29.1 per cent and the wetland isolate of BGA (WI) recorded less heterocyst frequency of 10.9 per cent.

As herterocyst are the sites of N_2 fixation, it may be generally inferred that the GI might have a higher N_2 fixation potential. But since both the cultures were grown under identical conditions in N-free medium and heterocysts counted, it might also be construed that a single heterocyst in a WI might meet the N_2 demand of more number of vegetative cells than in the gardenland isolate of *Nostoc*. However, in gardenland due to alternating moisture stress and due to the prevailing acrobatic conditions unconducive for nitrogenase activity most number of heterocysts might meet the requirement of the vegetative cells. This variation in heterocyst frequency is a well established phenomenon and this is influenced by various factors. Sylvestor (1976) reported symbiont had an 8 fold higher heterocyst frequency than a free living *Nostoc,* perhaps to fix more nitrogen to meet the demand of non-algal partner. However in the present study a variation among the free living forms itself was observed due to changes in environment.

The higher heterocyst frequency in the GI of *Nostoc* should also be viewed from other angles. In a typical gardenland environment the moisture is not stagnant and no floating mass is seen and therefore a higher heterocyst may lead to breakage of shorter filaments aiding dispersal. As there is a chance for rapid desiccation of soil in between successive irrigation periods atleast in local sites to facilitate survival and maintenance of the BGA in gardenland might produce a large number of heterocysts as these also serve as propagules. Since heterocyst have a higher drought tolerance than vegetative cells, a higher number of them might also facilitate survival. Above all the role or heterocysts in serving as sites of salt accumulation should be taken note of. In flooded environments like rice fields, salt in soil and water is less and in a diluted fashion and cells also can excrete salts into water as they float. This facility is not there in garden land. If the vegetable cells absorbs that salts, it mustbe pushed to selective cells. Since in most garden land, the water drawn is from a well which might contain more salts besides salts in soil. Further there is no flooding as in rice fields to dilute or wash-off salts under such conditions, the higher heterocyst frequency might help in storing the salt absorbed and accumulated.

Ammonia excretion gradually increased upto 20 days, after which there was a reduction in the quantity of ammonia excreted by the algae.

Effect of BGA inoculation on soil available nitrogen levels

Pot culture experiments were conducted, on the effect of BGA inoculation with and without fertilizer application in Maize and Blackgram was investigated in sterile and unsterile soil and with different graded levels of fertilizers was also carried out in unsterile soil.

The results of the present study revealed that the unsterile soil recorded slightly more available nitrogen than sterile soil. This might be due to the presence of other soil microflora. The BGA isolates both WI and GI recorded almost same level of soil available nitrogen (Table 3). In the present study algalization with NPK showed a better effect than algalization alone (Table 4).

The survey of irrigated gardenland crops revealed that, *Nostoc* was the predominant alga although *Tolypothrix* and *Scytonema* were occasionally observed. The heterocyst frequency differed in both GI and WI with 29.1 and 10.9 per cent respectively. The soil available nitrogen both in Maize and Blackgram inoculated with BGA was enhanced more in unsterile soil, but the potential of GI and WI varied with applied fertilizers.

Table 1. Identified sources / places for the presence of algal growth in gardenland crops

S.No.	Location	Crop	Remarks
1.	Central farm Agricultural College, Madurai	Maize	Thin film along the furrow slopes
2.	Agricultural College, Madurai	Cotton	Algal growth in irrigation channels and in the collar regions of plants
3.	Agricultural College, Madurai	Banana	Algal growth around the pseudostem at collar region
4.	Agricultural College, Madurai	Sugarcane	Patches of algal growth and encrusations along irrigation channels
5.	Ambadurai, Dindugal	Sugarcane	Algal growth on the soil surface adjoining the clumps
6.	Ambadurai, Dindugal	Banana	Algal growth on pseudostems near the soil surface

Fig. 1. Heterocyst frequency of gardenland and wetland isolates of *Nostoc*

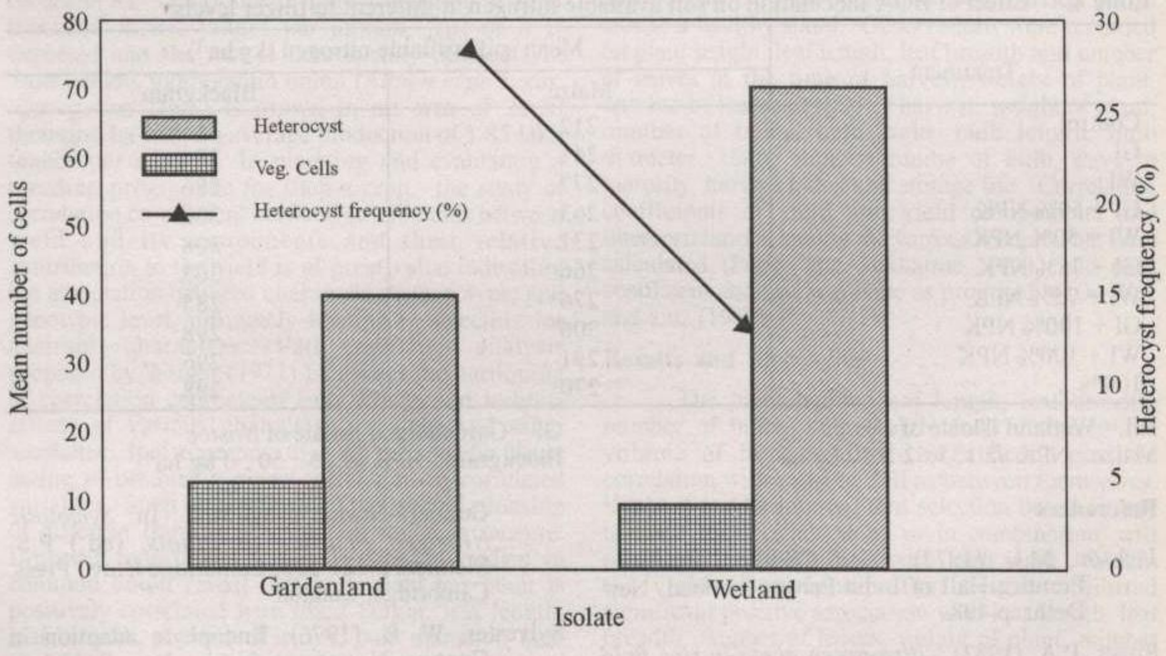

Table 2. Ammonia excretion by gardenland and wetland isolate of BGA in N-free Bristol's medium

Algal Isolates	Ammonia excretion (mg l^{-1})		
	5 DAI	15 DAI	40 DAI
Gardenland Isolate	0.84	4.72	0.60
Wetland Isolate	0.96	4.50	1.02

Table 3. Effect of BGA inoculation on soil available nitorgen with sterile and unsterile soils in maize and blackgram

Treatment	Mean soil available nitrogen (kg ha^{-1})	
	Maize	Blackgram
Sterile soil		
Control	245	250
GI	287	280
WI	301	277
GI + NPK	267	284
WI + NPK	267	281
NPK alone	294	266
Unsterile soil		
Control	230	207
GI	293	269
WI	329	299
GI + NPK	308	300
WI + NPK	291	291
NPK alone	328	295

Table 4. Effect of BGA inoculation on soil available nitrogen at different fertilizer levels

Treatment	Mean soil available nitrogen (kg ha^{-1})	
	Maize	Blackgram
Control	212	213
GI	241	284
WI	277	286
GI + 50% NPK	263	300
WI + 50% NPK	231	308
GI + 75% NPK	266	305
WI + 75% NPK	276	285
GI + 100% NPK	306	298
WI + 100% NPK	291	296
100%	270	298

WI = Wetland isloate of *Nostoc*
Maize : NPK @ 135:62.5:50 kg ha^{-1}

GI = Gardendland isolate of *Nostoc*
Blackgram : NPK @ 25 : 50 : 0 kg ha^{-1}

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