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PN-ABI-689
72662 X C5-179

FINAL REPORT CDR-AID PROGRAM C5-179

"Optimization of Nitrogen Biofertilizer by Azolla for Rice"

reported by Prof. Elisha Tel-Or

Conducted by:

1. Prof. Elisha Tel-Or, Coordinator, Dept. of Agricultural Botany, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

2. Prof. Shmuel Malkin, Dept. of Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel.

3. Prof. S. A. Kulasoariya, Dept. of Botany, The University of Peradeniya, Peradeniya, Sri Lanka.

Summary:

The program aimed to introduce a novel analytical approach for assessment and improvement of N_2-fixation by Azolla in the rice fields. The photacoustic device, which monitors photosynthetic performance of Azolla, was brought to Peradeniya, and operated in the experimental field stations. This portable device proved useful to detect the effects of environmental conditions; high temperatures and phosphorus deficiency on the primary productivity of Azolla.

We established a monitoring system in the lab, measuring N_2-fixation by C_2H_2 reduction and photosynthesis by infra red gas analysis (IRGA) and O_2 electrode. This continuous gas flow system (CGFS) was tested to correlate photosynthetic activity with nitrogenase activity, demonstrating the regulation of nitrogenase activity by light. This mode of regulation was anlysed in detail and suggests unique feature of nitrogenase regulation in Anabaena azollae.

We also tested the interaction of Anabaena azollae, the N2-fixing cyamobiont, with photosynthates in the leaf cavity, sugars and starch, and suggest that starch breakdown by amylase is involved in photosynthate consumption by the cyanobiont.

I. Detailed description of the research.

1. Application of the photoaconstic device for Azolla.

Azolla fronds were tested by the photoacoustic device, in Prof. S. Malkin's lab in Rehovot, to correlate photosynthetic performance with nitrogenase activity, of single fronds and of leaves along the stem axis of Azolla. These studies, initiated during Prof. S.A. Kulasooriya's first visit to Rehovot, demonstrated that nitrogenase activity is located in the older leaves along the stem axis while highest photosynthetic activities were detected in the younger leaves. These studies have developed fast and direct measurements which were also applied to follow the influence of unfavourable growth conditions on productivity of Azolla.

We found that nitrogenase activity is sensitive to temperatures above 32°C, and is more sensitive in Azolla filiculoides than in Azolla pinnata, introduced from Sri Lanka. Phosphate-starved Azolla was also tested for the influence of Phosphate starvation on photosynthesis and N_2-fixation. These studies were reported in our joint contribution. Kulasooriya et. al. (1988) Symbiosis 6, 117-128.

The photoacoustic system was shipped to Peradeniya, and installed in Prof. Kulasooriya's laboratory by Prof. S. Malkin. After training, it was used by Prof. Kulasooriya and his students to detect the influence of local environmental stresses on photosynthesis of Azolla and these were compared to nitrogenase activities. Photoacoustics was proven in these measurements to become a useful, fast and easy detection device of photosynthesis by Azolla.

The Photacoustic device was adopted and transformed into a portable system, installed in Prof. Kulasooriya's portable lab, used for studies on rice production in two rice fields, in experimental stations. Prof. Malkin conducted the field analysis and confirmed with Prof. Kulasooriya the use and reliability of the field studies with the photoacoustic device to assess Azolla and rice productivity in the field.

This part of the project was not concluded, mainly due to the internal politcial problems in Sri Lanka, which forced Prof. Kulasooriya to stop the research in the experimental stations. The photoacoustics device was, however, left in Sri Lanka and will be used to further explore its use in assessment of outdoor Azolla and rice productivity.

2. The regulation of N_2-fixation by light in Azolla

Prof. S. A. Kulasooriya spent six months sabbatical in Rehovot, for studies on the correlations between photosynthesis and nitrogenase activity in Azolla. He joined Mr, Etan Bar, a Ph.D student in Prof. Tel-Or's lab, who designed and constructed a new monitoring device to follow synchroneously N_2-fixation by C_2H_2 reduction and photsynthesis by infrared gas analysis. This analytical apparatus, the contiuous gas flow system (GCFS) led us to identify the regulation of nitrogenase by light, in a direct mode which is not transformed by photosynthates. Hence, nitrogenase activity was switched on under extremely low light intensities of 3-io Einstein m^{-2}. sec $^{-1}$, which were affected directly. Mitrogenuse activation or initiated nitrogenase expression.

Mr. Bar continued the investigation of the mode of nitrogenuse regulation by light throughout 1988-1990, and this objective became the major chapter of his Ph.D dissertation, under the supervision of Prof. Tel-Or. The molecular analysis of nitrogenase polypeptides demonstrated a different profile in the light and in the dark; the Fe-protein of nitrogenase showed an additional polypeptide in the dark of 31 KD, which could be the inactive form of the 30 KD. Modification of nitrogenase Fe-protein was earlier in the photosynthetic bacterium Phodospirillum rubrum, where the Fe-protein was bound to ADP-ribose in its inactive form. With nitrogenase of Anabaena azolla we have not clarified the nature of the transition from inactive form in the dark to active form in the light, but the regulation of nitrogenase in the light may be beneficial to photsynthetic symbiosis. We have confirmed light reulation of nitrogenase in Azolla filiculoides and in Azolla pinnata, intorduced from Sri Lanka.

This part of the work was reported by Bar et. al (1988) and Bar et. al (1991).

3. Azolla outdoor cultivation

Four years ago, we were restricted to Azolla outdoor cultivation in the botanical gardens of the society for nature conservation in Israel. Azolla suffered from chilling stress in the winter, at temperatures below 14°C and suffered also from high dozes of irradiance in the summer.

We designed a very simple and cheap solution for the chilling and irradiance problems, setting the Azolla in shallow tunnels, of 8 x 2 meters, 20 cm deep covered with 0.1 mm plolyethylene. the tunnels were fertilized according to nutrient composition of IRRI

(International Rice Research Institute) and Azolla was covered by polyethylene in the winter, to provide protection from cold winds, rain and night frost. The temperature inside the protected tunnels never dropped throughtout the winter (between December till March) to harmful temperatures for Azolla. In April, under increasing irradiation and higher temperature, the Azolla tunnels were protected by plastic nets cutting 50 percent of incident irradiation. This protection was sufficient to protect Azolla against photobleaching and photoinhibition.

Azolla growth was monitored, throughout the year, and the biomass was doubled within 4-10 days. These results yield Azolla crop of 5 tons dry biomass/1000 m^2, annually, or 65 tons fresh weight Azolla/1000m^2. annum. This crop may be equivalent to 300 Kg Nitrogen per hectqr per year.

The single, cheap and easy to handle Azolla tunnels could be employed to operate Azolla nurseries, in rice growing sites, all year round.

II. The Project's technological Progress

Three technological developments were achieved throughout our studies:

1. The photoacoustic system was introduced to Sri Lanka and is being used by Prof. S.A. Kulasooriya in the lab and in field conditions in the University of Peradeniya experimental farms.

2. A novel device for synchronous analysis of nitrogenase and of photosynthesis was designed and consturcted - the continuous gas flow system, where Azolla fronds (1.5 g) are set in their growth medium, illuminated from above and the gas phase containing C_2H_2 is monitored for C_2H_2 reduction (nitrogenase activity) and the CO_2 content in the gas phase is monitored for CO_2 produciton or consumption by infrared gas analysis.

3. The cultivation of Azolla in polyethylene tunnels, protected under environmental stress, is a device useful for Azolla nurseries under nonfavourable growth conditons. In most rice farming countries Azolla must be stored and maintained in nurseries out of season.

III. Mode of Cooperation

We have established a very constructive collaboration with Prof. Kulasorriya during his first visit to Rehovot in Dec. 1986, and throughout his 6 months sabbatical in Rehovot, 1988. These joint studies also led to Prof. Malkin's visit to Peradeniya to introduce the photoacoustic system in Sri Lanka for lab and field studies.

Prof. Kulasooriya brought Azolla pinnata from Sri Lanka to Rehovot and this species was compared with Azolla filiculoides.

Unfortunately, the insecure situation in Sri Lanka since 1988, caused severe difficulties in the application of the photoacoustic system in the field stuides. The University was also suffering from restriction, lack of supplies, and hence Prof. Tel-Or's exchange visit was not possible.

We trust that many of the ideas and developments achieved throughout this research program will be further developed in the future by Prof. Kulasooriya and his coworkers in Peradeniya.

IV Publications:

1. S.A. Kulasooriya, h. Arad, O. Canaani, E. Tel-Or, S. malkin (1988) Distribution of the N_2-fixation and Photosynthetic activities in the Azolla-Anabaena symbiosis. Symbiosis 6, 117-128.

2. E. Bar, S.A. Kulasooriya, E. Tel-Or (1988) Nitrogen fixation in Azolla is controlled by the availability of photosynthates to the cyanobiont. In: Nitrogen Fixation Hundered Years After, Rothe/de Bruijn/Newton (eds), Gustav Fisher Stuttgart p. 229.

3. E. Bar, S.A. Kulasooriya, E. Tel-Or (1991). Regulation of nitrogenase activity by light in the Azolla-Anabaena symbiosis. Bioresources Technology (in press).