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Azolla–Anabaena biological symbiotic system for rice production

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Abstract : **_Azolla_ is a small water fern that assimilates nitrogen in symbiotic association with _Anabaena azollae_. The algal symbiont resides in the leaf cavity of _Azolla_ and provides fixed nitrogen and other growth promoting substances to the host _Azolla_ in exchange for nutrients and carbon sources. The heterocyst frequency of algal symbiont _A. azollae_ under nitrogen limiting condition was 30 per cent. The algal symbiont showed polymorphism in different _Azolla_ cultures. The growth of _Azolla_ is influenced very much by season, phosphorus, growth regulators, herbicides and salinity. _Azolla_ mineralizes rapidly and its nitrogen is made available to the rice in a very short period. It is estimated that nitrogen contribution by _Azolla_ is 40-60 kg ha^{-1} besides improving the soil health. _Azolla_ inoculation at 500 kg ha^{-1} on 7 DAT along with 75 kg N ha^{-1} increased the grain and straw yield of rice significantly. Inoculation of _Azolla_ hybrid AH-C2 coupled with USG at 75 kg N ha^{-1} increased the grain and straw yield of CO 43 rice besides increasing the plant N content and N uptake in sodic soil.**

Key words : ***Ammonia assimilation, Azolla, A. azollae, Mineralization, N_2 fixation, N use efficiency, Phosphorus nutrition, Rice yield.***

Introduction

The water fern *Azolla* fixes atmospheric nitrogen in association with nitrogen fixing cyanobacterium – *Anabaena azollae* and thereby serves as an effective biofertilizer in agriculture (Neirzwicki-Bauer, 1990 and Peters, 1991). The seven extant species of *Azolla* are grouped under two sections, *viz. Azolla* and *Rhizosperma* (Sub genera) based on megaspore floats and glochidia (Moore, 1969). Section *Azolla*, exhibit three floats and the megaspore apparatus, whereas section *Rhizosperma* has nine floats. In *Azolla*, the surface of the massulae is covered by the long barbed glochidia (Perkins *et al.* 1985) whereas in *Rhizosperma*, the floats are arranged on the megaspore in two groups with three above and six below (Hills and Gopal, 1967).

The section *Azolla* comprises five species *viz. A. caroliniana* willdenow, *A. filiculoides* Lamarck (type species), *A. mexicana*, Persl, *A. microphylla* Kaulfuss and *A. rubra* R. Brown. The section *Rhizosperma*, comprises *A. nilotica* De Cailsne and *A. pinnata* R. Brown. Based on differences in leaf branching morphology, *A. pinnata* is further divided into the varieties *A. pinnata* var. *pinnata* and *A. pinnata* var *imbricata* (Sweet and Hills, 1971). Recently three distinct geographically related sub species of *A. pinnata* were identified and formally named and described as (1) Sub species *pinnata* (Northern and Eastern Australia) (2) Sub species *usiatica* (India, Southern China, South East Asia and Southern Japan) and (3) Sub species *africana* (Tropical Africa and Madagaskar) (Sanders and Fowler, 1992).

Azolla plants are triangular in shape as in *A.pinnata* or elongate, irregularly branched, often bipinnate as in *A. nilotica* or small, circular (or) fan shaped as in *A. microphylla, A. filiculoides* and *A. mexicana.* The size of the plants measures from 4-60 mm long, 5-12 mm wide as in *A. pinnata* and *A. nilotica* (Tan *et al.* 1986). New sporocarpic structures such as smooth type and punctured type of float morphology, glochidial septum, sporoderm structures and other characters associated with megaspores apparatus are explored to be useful in forming the recent advance of species concept and taxonomy of the genus *Azolla* (Dunham and Fowler, 1987). Recently enzyme electrophoresis was used to differentiate the sections of *Azolla* and demonstrate the value of this method in finger printing taxa (Zimmerman *et al.* 1989a and b).

The precise identification of the members of section *Azolla* is somewhat complex due to similarity in vegetative characters. But in *Rhizosperma* it is easy because members are distinct from each other. With the hope of resolving the complex taxonomical problems, attention has now been focussed on the cytology of the genus (Stergianou and Fowler, 1990). The basic chromosomal number in all species of the section *Azolla* as well as *A. pinnata* of the section *Rhizosperma* is n=22, whereas in *A. nilotica.* it is n=26. The chromosome number clearly

separated *A. nilotica* from all other species, and indicated that *A. pinnata* might have closer phylogenetic affinities with species of the section *Azolla*. This finding was supported further by DNA probe studies which indicated that the dominant symbionts in the section *Azolla* and *A. pinnata* generally belong to divergent evolutionary lines (Plazinski *et al.* 1990).

Symbiotic association of Anabaena azollae with Azolla

Throughout its life cycle, the cyanobacterium *A. azollae* Strass is intimately associated with *Azolla* species. The algal symbiont has sinus trichomes (threads) composed of bead like or barrel shaped cells without a sheath. The symbiont resides in the leaf cavity of *Azolla*. The host *Azolla* provides nutrients and carbon source including sucrose and shelter to the algal symbiont in exchange for fixed nitrogen and other growth promoting substances (Kaplan and Peters, 1988).

Heterocysts are terminally differentiated cells which are the exclusive site of N_2 fixation (Bergman *et al.* 1986). In order to protect the O_2 sensitive enzyme nitrogenase, heterocyst differentiation is accompanied by substantial sub cellular and metabolic reorganization (Haselkorn *et al.* 1980). The number of active heterocysts increases from almost zero in leaves near the shoot apex to a maximum of 29.33 per cent in the 15th leaf. After the 20th leaf, however the heterocysts become senescent (Hill, 1975). In contrast, free-living *Anabaena* species exhibit uniformly spaced heterocysts that may reach frequencies of 6-7 per cent when grown in the absence of combined nitrogen source (Reynaud and Franche, 1986). Under nitrogen limiting conditions, the heterocyst frequency of algal symbiont *A. azollae* was 30 per cent in the intact association and it was only 10 per cent in free-living *A. azollae* Strass. (Braun-Howland and Neirzwicki-Bauer, 1990). The total nitrogen requirement of the *Azolla* frond is supplied by the endosymbiont. The ammonia from N_2 fixation by the endosymbiont is released into the leaf cavity and absorbed by the frond's ammonia assimilation enzymes.

During sporogenesis, the endosymbiont is packaged into sporocarps such that continuity of the symbiosis is maintained during sexual reproduction also. Lumpkin and Plucknett (1980) have reported the persistence of the endosymbiont in the mature megasporocarps. Plazinski *et al.* (1990) have shown the existence of numerous *A. azollae* genotypes in various *Azolla* species and ecotypes. The cyanobacterial partner in different *Azolla* sp. was not uniform throughout and substantial diversification had occurred. Subhashini *et al.* (2000) clearly demonstrated the polymorphism among all the algal symbionts of different *Azolla* cultures.

Growth of Azolla

Azolla has the potential of maintaining an exponential growth rate under optimum conditions. The growth of *Azolla* is initially slow in the growth medium as well as in the rice field followed by fast growth (Kannaiyan, 1993). The usual rate of multiplication of *Azolla* under field condition is about five fold over a period of five weeks (Kannaiyan, 1990a). Kumar and Kannaiyan (1999) reported higher growth rate in mutants of *A. microphyla* and *A. filiculoides*. Gopalaswamy and Kannaiyan (1998a) reported higher biomass production by *Azolla* hybrids. However the doubling time was maintained at 4-5 days upto 14 days and increased to 6-7 days on 21 days after inoculation. Similar trend was noticed for relative growth of *Azolla* hybrids. In *A. filiculoides*, Talley and Rains (1980) achieved a RGR of 0.245-0.277 g g^{-1} day^{-1}. In Brazil, Fiore and Gutbrod (1987) recorded a RGR of 0.25 – 0.30 g g^{-1} day^{-1} in *Azolla* cultures.

The growth potential of *Azolla* is subjected to various environmental factors such as temperature (Becking, 1979 and Kannaiyan, 1990a). light intensity (Roger and Reynaud, 1979) humidity (Becking, 1979); wind (Ashton, 1974), desiccation (Reynaud, 1985) season (Espinas *et al.* 1979); pests (Liu Chung Chu, 1979), planting methods (Liu Chung Chu, 1979), plant density and over crowding of fronds (Becking, 1979). The doubling time and relative growth rate of *Azolla* cultures were influenced by the salinity and temperature (Kannaiyan and Somporn, 1987). In China, the growth rate was maximum during May-June and Sep-Oct, while at Coimbatore, India it was between Oct-Feb (Kannaiyan, 1990a). Rajagopal *et al.* (1994) compared the biomass production and relative growth rate of seven *Azolla* strains and reported higher growth rate and biomass production of *Azolla* cultures during Nov-Dec and Mar-May. Maximum biomass was produced by *A. caroliniana* followed by *A. pinnata*. Gopalaswamy and Kannaiyan (2000a) observed

maximum biomass production by *Azolla* hybrids in winter months of Sep-Dec than in any other period. Their study clearly showed that apart from temperature, relative humidity and wind velocity, evaporation also would have a significant role on *Azolla* biomass production.

Phosphorus nutrition of Azolla

The response of *Azolla* to phosphorus is marked and phosphorus fertilization is necessary for its growth and N_2 fixation (Kannaiyan, 1990b). *Azolla* grown in the phosphorus deficient solution had a reddish brown discolouration that spread from the centre of the frond to the tip of the body with reduction in size of the frond. The fronds become fragile and the roots of *Azolla* turn reddish brown, grow longer and tend to detach easily from the *Azolla* body (Watanabe *et al.* 1977).

The phosphorus concentrations at or below 0.03 ppm level decreased the growth, chlorophyll content, phosphorus and nitrogen contents and acetylene reduction activity of *Azolla* (Subudhi and Watanabe, 1981). Ali and Watanabe (1987) reported that the phosphorus content in flood water limited the growth of *Azolla* and markedly affected the growth and N_2 fixation when the phosphorus content of the *Azolla* frond was below 0.1 per cent.

Addition of phosphorus is most effective in stimulating the growth of *Azolla*. Kannaiyan (1985) reported higher biomass of *Azolla filiculoides* at 20 ppm of phosphorus but found that 5-10 ppm level was adequate for the growth and multiplication. Kannaiyan (1993) compared the response of *Azolla filiculoides* and *Azolla microphylla* to different levels of phosphorus and found that *Azolla filiculoides* produced significantly higher biomass at different phosphorus levels than *Azolla microphylla*.

Tilo *et al.* (1989) compared different phosphorus sources *viz.* potassium dihydrogen phosphate, phosphoric acid and potassium sulphate and rock phosphate in increasing the growth and biomass production of *Azolla*. They concluded that potassium dihydrogen phosphate as well as phosphoric acid were equally effective in increasing the biomass production of *Azolla*, but not rock phosphate which was effective only in acid soils.

Phosphorus is the major nutrient limiting *Azolla* growth (Liu Chung Chu, 1979). Under field condition, *Azolla* growth improved markedly with split application of phosphorus fertilizer (Kannaiyan and Oblisami, 1981). Singh and Singh (1990) reported that split application of phosphorus at 10 kg P_2O_5 ha^{-1} in two splits at 0 and 20 DAI showed better growth and N-accumulation in *Azolla*. Gopalaswamy and Kannaiyan (2000b) suggested 30 ppm as the optimum level of phosphorus nutrition for *Azolla* hybrids.

Symbiotic nitrogen fixation in Azolla

Symbiotic association between the *Azolla* and *Anabaena azollae* resulted in the fixation of atmospheric nitrogen through a process involving reduction of atmospheric dinitrogen mediated by an enzyme nitrogenase. Since most of the energy is supplied by photosynthates in host *Azolla* and the presence of a characteristically low level of ammonia assimilating enzymes in the endosymbiont *A. azollae*, the nitrogen fixation process is more efficient only in the cells of *A. azollae* which are in active symbiotic state (Ladha and Watanabe, 1987).

In the presence of a combined nitrogen source, the *Azolla-Anabaena* symbiosis can assimilate the exogenous nitrogen available and fix atmospheric nitrogen simultaneously but the fixation rate was low (Ito and Watanabe, 1983). The N_2 fixing activity was stimulated in *Azolla* by the systemic fungicides benlate (Kannaiyan, 1987b) and in algal symbiont by bavistin (Uma and Kannaiyan, 1996) and PUF immobilisation (Kannaiyan *et al.* 1993). However, sodium chloride at 80-100 ppm was inhibitory to N_2 fixation (Sukumar and Kannaiyan, 1987).

Becking (1985) reported that under favourable field conditions, *Azolla* can fix 5.0-9.0 mg N g^{-1} dry wt. plant tissue day^{-1}. Several workers have reported that the estimates of total input of N by *Azolla* in rice soil is variable. The average N_2 fixing rates by various *Azolla* spp were in the range of 0.4-3.4 kg N ha^{-1} day^{-1} under field conditions (Kikuchi *et al.* 1985). The maximum estimated nitrogen inputs during exponential growth of different *Azolla* species under unlimited growth condition range from 103-840 kg N $ha^{-1}y^{-1}$ (Watanabe, 1981). However, Roger and Ladha (1990) have estimated the nitrogen input by *Azolla* as 20-150 kg N ha^{-1}.

Ammonia assimilating enzymes

The ammonia assimilation mediated by the enzyme glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) is carried out both under symbiotic and free-living conditions. Yates and Eady (1989) have reported that GDH appears to be involved in NH_4^+ assimilation when N_2 fixing organisms are grown at high NH_4^+ concentrations but under N-limited conditions the GS-GOGAT pathway operates. However Meeks *et al.* (1985) have reported that ammonia assimilation is mainly mediated by GS-GOGAT pathway and GDH has very little role. Under normal physiological conditions, the nitrogen fixed in the heterocysts of the endosymbiont *A. azollae* is transported to the adjacent vegetative cells and incorporated in the amide position of glutamine and transferred to α position of α-keto glutarate in the host *Azolla* and metabolized via the most important GS-GOGAT pathway and enters the cellular metabolic pool both for exogenous excretion and internal generation of ammonia (Boussiba and Gibson, 1991).

In the *Azolla-Anabaena* symbiosis, although both partners had GS-GOGAT and GDH activity, the host was estimated to account for more than 75 per cent of GDH and GS activities (Ray *et al.* 1978). Peters *et al.* (1979) stated that the host contributes about 90 per cent of the association's GS activity and 80 per cent of the total GDH activity. Uheda (1986) reported higher GS and GDH activity in cavity hairs than in *Azolla* leaves. Ladha and Watanabe (1987) have attributed the GDH activity of the endosymbiont to incomplete removal of cavity hairs.

GS activity was inhibited by combined nitrogen. At higher nitrogen and phosphorus levels the GS and GDH activities were inhibited. Foliar application of the herbicide butachlor drastically affected the ammonia assimilating enzymes in *A. pinnata* (Venkataramanan and Kannaiyan, 1986).

Kannaiyan *et al.* (1994) have immobilized the algal symbiont *A. azollae* (AS-DS) and free-living *A. variabilis* (DOH) in different foams and found increased ammonia production in immobilized foams or hollow fibres or maize husk. Uma and Kannaiyan (1994) reported that the insecticide carbofuran could induce significant ammonia production by immobilized algal symbiont of *Azolla*. Gopalaswamy and Kannaiyan (1998a) reported that the *Azolla* hybrids exhibited higher activity of nitrogenase and ammonia assimilating enzymes. Kumar and Kannaiyan (2000) developed mutants of *A. microphylla* and *A. filiculoides* and found that the mutant *A. microphylla*-KK-SK-E_{20} exhibited maximum activity of ammonium assimilating enzymes.

Salt tolerance in Azolla

Nandabalan (1984) noticed poor tolerance by *Azolla* cultures to salinity. At 0.2 per cent NaCl concentration, the growth of *Azolla* was reported to be inhibited by 31.9 per cent over control. Nandabalan and Kannaiyan (1985) found an ameliorating effect of neem cake at 500 ppm when amended with NaCl in soil extract solution and recorded low salinity effect on *A. pinnata*. Sukumar and Kannaiyan (1987) reported that *A. filiculoides* was less susceptible to salinity than *A. pinnata*. Kannaiyan (1985) found an inhibitory effect of NaCl on the growth of *Azolla*. San Valentin (1989) reported that *Azolla* is sensitive to salinity and growth of *Azolla* decreased with increased salt concentration.

Shang Deng hui *et al.* (1987) found 0.7 per cent (salinity) and 0.3 per cent (alkalinity) respectively as salt and alkali resisting limit of *A. filiculoides* and suggested the possibility of using *A. filiculoides* for saline and alkali soil reclamation. They observed that in the presence of sea water containing 0.1-0.5 per cent salt, only *A. filiculoides* exhibited nitrogenase activity and not *A. imbricata*.

Herbicide tolerance in Azolla

The recommended herbicides for transplanted rice include the phenoxys (2,4-D, MCPA etc.) amides (Propanil and butachlor), thiocarbamates (thiobencarb), dinitroaniline (trifluraline), pyrimide (bentazon) and a heterocyclic organic compound oxadiacinon (Lales *et al.* 1989). and a dithiophosphate (anilofos) (Pandey and Shukla, 1990). Among the herbicides, triazine, phenylureas and bipyridyls are inhibitors of photosynthesis by affecting electron transport as well as morphology, while phenoxy compounds, phenyl carbamates and acylanilides act as inhibitors not primarily involving photosynthesis (Tripathi, 1988).

Jania and Moody (1981) found that herbicides are not suitable for weed control in rice fields inoculated with *Azolla*. They observed that the

ibicides dicamba and simazine at 1 ppm gnificantly affected the biological nitrogen xation of *Azolla* by causing 99 per cent reduction, hereas thiobencarb was less toxic to *Azolla* showing only 29 per cent reduction in *Azolla* omass and complete mortality was seen in olinate-simetryn-MCPB combination. Mabbayad id Lapitan (1985) reported that the biomass oduction of *A. microphylla* was adversely affected hen inoculated either immediately after the pplication of butachlor or 2-3 weeks after ,4-D application. Kannaiyan (1987a) reported iat *Azolla* growth decreased gradually with ıcreasing concentrations of butachlor and that mmonia assimilating enzymes are inhibited at gher concentrations.

Differential persistence of different herbicides has been reported (Kearney and Kaufman, 988). According to Kumar and Prakash (1993), he persistence decreased with time and the effect ersisted even after 90 days. Most of the herbicides re deleterious to *Azolla*. Delaying the inoculation of *Azolla* after herbicides application reduces or eliminates the problem (Moody and Janiya, 1992). *Azolla* and 2,4-D can be applied together but only after 3-4 weeks in the case of butachlor and thiobencarb (Singh *et al.* 1982). Lales *et al.* (1989) reported that thiobencarb, 2,4-D and piperophos + 2,4-D were safe to use, provided field inoculation was done 4 days after herbicide application. Janiya and Moody (1988) recommended that butachlor be applied 3 to 7 days before *Azolla* inoculation. Srinivasan *et al.* (1990) found that there was a significant reduction in fresh biomass and relative growth rate of *Azolla* when it was inoculated on the same day of application of thiobencarb + 2,4-D and anilofos. Delaying inoculation until 3 and 6 days after herbicide application resulted in significant increases in both biomass production and relative growth rate of *Azolla*. Moody and Janiya (1992) observed herbicide toxicity to *Azolla* only if *Azolla* was inoculated within 10 days after the application of butachlor and oxadiazinon and not afterwards (i.e., 17 days after herbicides application).

Mineralization of Azolla in rice soil

Watanabe (1977) has shown that 75 per cent of total N of *Azolla* was mineralized in 6-8 weeks after soil incorporation. The rate of mineralization of organic-N from *Azolla* proceeded at a much faster rate during the first two weeks after which mineralization was more gradual (Subramani and Kannaiyan, 1987). In the rice soil ecosystem, the availability of nitrogen in ammoniacal form is very important. Ali and Watanabe (1987) reported peak NH_4^+-N formation during 1st week after *Azolla* incorporation but decreased in subsequent weeks in low land rice soil.

The rate of mineralization of *Azolla* is determined by C:N ratio. A low C to N ratio of 10:1 favoured very rapid mineralization and release of nitrogen within a few weeks after soil incorporation (Lumpkin, 1982). Wang (1982) observed that *A. imbricata* with low C:N ratio mineralized in 2 days, while *A. filiculoides* with high C:N ratio mineralized in 5 days. However, Wang De Xian *et al.* (1987) have reported that *A. filiculoides* released more nitrogen at a faster rate than *A. imbricata*. Watanabe *et al.* (1991) have correlated the tissue N and P contents with *Azolla* mineralization. They have reported that *Azolla* plants deficient in P and low in N show low mineralization ratios.

The fate of mineralization of *Azolla* in fallow flooded soil has been studied. The release of nitrogen from *Azolla* goes through a succession of ammonification, nitrification and denitrification steps. Initially NH_4^+-nitrogen was released which was then oxidised to NO_3 nitrogen in the aerobic surface layer. About 45 per cent of *Azolla*-N was released in 60 days, 55 per cent remained in soil as undecomposed material. Out of 45 per cent of *Azolla*-N, 34 per cent was recovered by rice crop and the remaining 11 per cent was lost as gas through denitrification process (Mian and Stewart, 1985). About 50 per cent of the *Azolla*-N incorporated into the soil was recovered by rice between 42 and 123 days after transplanting (Ito and Watanabe, 1985).

During the decomposition of *Azolla* in soil, bacterial and fungal populations were low whereas actinomycetes, urea hydrolysing, cellulolytic and heterotrophic nitrogen fixing bacterial populations were increased (Kannaiyan and Kalidurai, 1995).

Activity of soil enzymes such as urease and dehydrogenase was positively correlated with increasing organic carbon, total nitrogen and total phosphorus content (Barush and Mishra, 1984). The incorporation of *Azolla* has resulted in the increased soil urease activity (Thangaraju and Kannaiyan, 1989). Significant increase in the activity of soil enzymes *viz.* dehydrogenase,

phosphatase, cellulase and amylase due to incorporation of N_2 fixing green manures such as *S. rostrata, S. speciosa* and *A. microphylla* has been noticed (Kumar and Kannaiyan, 1992). Gopalaswamy and Kannaiyan (2000c) reported that incorporation of *Azolla* hybrids stimulated the microbial population, total bacterial, cellulolytic, phosphate solubilizing and urea hydrolyzing bacteria; N_2 fixing *Azospirillum, Azotobacter,* fungi, actinomycetes and soil enzymes L-asparaginase, urease, cellulase, dehydrogenase and phosphatase activity in soil significantly over prilled urea (PU) application.

Use of Azolla biofertilizer as dual crop in rice

Nitrogen can be effectively supplied to rice by the use of *Azolla* biofertilizer. The most effective way to accomplish transfer of biologically fixed nitrogen from *Azolla* to rice under tropical situation in India is to grow *Azolla* as dual crop with rice after transplanting and then incorporating the fronds into the soil *in situ* when the *Azolla* grows and covers the field as a thick mat (Kannaiyan, 1985). *Azolla* inoculated at 0.2 kg m^{-2} at 7-10 days after transplanting was found to establish and cover the area within 15-30 days (Kannaiyan, 1984). In recent years the inoculation level has been reduced to 0.05 kg m^{-2} in dual cropping and efforts are on to reduce the inoculum further using *Azolla* cultures exhibiting relatively higher growth rate to make the technology a viable one. It has been well established that application of *A. microphylla* could contribute 40-60 kg N ha^{-1} when inoculated at 500 kg ha^{-1} as dual crop (Kannaiyan, 1995).

Venkataramanan and Kannaiyan (1984) studied the inoculation of *A. pinnata* as dual crop with rice in double row planting system and found significant increase in grain yield of rice. Wang Pu and Wang Zai de (1987) compared the influence of different spacing *viz.* wide narrow, wide row and traditional on *Azolla* dual cropping and found higher rice yield and *Azolla* biomass with wide row planting. However, spacing apparently did not affect the growth of *Azolla* and rice yield (Watanabe, 1981).

Talley *et al.* (1977) have reported that dual cropping of *A. filiculoides* and *A. microphylla* with rice increased the rice yield by 23 and 67 per cent respectively. Inoculation of *Azolla* not only increased the grain and straw yield but also improved the grain quality by increasing its protein content (Liu Chung Chu, 1979). A positive rice crop response with *Azolla* inoculation was reported in 4 different sites of Tamil Nadu *viz.* Coimbatore, Aliyar Nagar, Ambasamudram and Tirurkkuppam (Kannaiyan *et al.* 1983).

Kannaiyan (1984) reported that *Azolla* application along with 60 kg and 90 kg N ha^{-1} increased the grain yield of rice significantly over control. Nazeer and Prasad (1984) observed that *Azolla* application coupled with 75 kg N ha^{-1} level recorded 24.2 per cent increase in grain yield over the uninoculated control. Significant increase in the productive tillers of rice was also noticed due to inoculation of *Azolla* as dual crop (Kannaiyan *et al.* 1983; Gopalaswamy *et al.* 1994).

Singh *et al.* (1988) have reported that *Azolla* application showed positive responses to rice crop by increasing the panicle number and panicle weight, grain and straw yields, nitrogen uptake in rice significantly at all nitrogen levels. Intercropping of *Azolla* in rice with 30, 60 and 90 kg N ha^{-1} as urea showed yield increase at par with 60, 90 and 120 kg N ha^{-1} alone besides improving soil organic carbon, available nitrogen and phosphorus contents (Singh and Singh, 1990). Gopalaswamy and Kannaiyan (1999) have investigated the potential of *Azolla* hybrids and reported that inoculation of *Azolla* hybrids significantly improved the soil organic carbon status besides increasing the rice yield.

An increase in the grain yield of rice by the application of *Azolla* as green manure, dual crop or both with rice was equivalent to 30-40 kg N ha^{-1} and this has been well documented by several investigators in various countries such as China (Liu Chung Chu, 1987), the Philippines (Mabbayad, 1987; Watanabe, 1987), India (Singh, 1979; Kannaiyan, 1981), Thailand (Loudhapasitiporn and Kanareugsa, 1987), Sri Lanka (Kulasooriya *et al.* 1987), USA (Talley *et al.* 1977) and West Africa (Diara *et al.* 1987). The use of *Azolla* in rice field has considerably increased the soil microbial as well as enzyme activity besides playing a vital role in improving soil quality management in sustainable rice farming (Kannaiyan,1995) as well as in the bio-reclamation of sodic soil (Gopalaswamy and Kannaiyan, 1998b)

Nutrient availability to rice crop

Azolla contains 3-6 per cent nitrogen, 0.5-0.9 per cent phosphorus, 2-4.5 per cent potassium

on dry weight basis besides other major and minor nutrients (Watanabe *et al.* 1977). The availability of *Azolla* N may be a function of species, strain, N content and chemical composition (Watanabe *et al.* 1989). However, Ventura and Watanabe (1993) observed a direct relationship between the nitrogen content and the amount of *Azolla* nitrogen available to rice. Talley and Rains (1980) concluded that availability of *Azolla* nitrogen is roughly equivalent to chemical fertilizer nitrogen on a per kg N basis.

The availability of N from the incorporation of dried *A. caroliniana* was 19 per cent compared to 61 per cent in ammonium sulphate (Mian and Stewart, 1985). However, Watanabe and Roger (1984) have reported that fresh *Azolla* incorporation released 60 per cent N compared to 47 per cent N released by dried *Azolla* in 4 weeks time. The availability of *Azolla*-N to rice could be enhanced by the method and time of *Azolla* application to rice. Watanabe *et al.* (1991) have shown that *Azolla*-N became available to rice after its mineralization and found that when *Azolla* was incorporated, the recovery of nitrogen was higher than when it was placed on the surface of flood water. Ito and Watanabe (1985) reported that rice plants absorbed more than 50 per cent of ^{15}N labelled *Azolla*-N incorporated at the time of transplanting and when *Azolla* was kept on the surface of water, less than 10 per cent of its N was available to rice plants.

Watanabe *et al.* (1987) obtained higher recovery of *Azolla*-N in early incorporation compared to lower recovery of *Azolla*-N in later incorporation and concluded that the best time to incorporate *Azolla* is 30 days after transplanting (Watanabe *et al.* 1989). They have reported that rice crop recovers 39 and 63 per cent of *Azolla*-N as against 27 and 48 per cent of urea N during basal and top dressings respectively.

It has been well established that *Azolla* inoculation as dual crop could supplement 30-50 kg N ha^{-1} $crop^{-1}$ (Watanabe *et al.* 1977; Kannaiyan *et al.* 1983). Singh and Singh (1987) reported that 2 crops of *Azolla* grown as dual crop registered 44-61 and 43-59 kg N ha^{-1} respectively. Roger and Ladha (1992) pointed out that estimates of per cent Ndfa and *Azolla* by ^{15}N dilution and delta ^{15}N methods ranges from 51-49 per cent. Thangaraju and Kannaiyan (1993) have reported that *Azolla* inoculation individually or in combination with different forms of urea, recorded more total-N accumulation in soil during early and wet seasons.

Phosphorus availability was found to increase significantly in *Azolla* amended soil compared to control (Kannaiyan, 1990b). Besides nitrogen and phosphorus, *Azolla* is an excellent and effective potassium source and may be considered as a potential biological-K in rice producing areas. When the soil water-K concentration is maintained at 2 ppm or above, *Azolla* absorbs 'K' only from water and has little interference on soil 'K'. During decomposition it releases 'K' for utilization by rice crop (Liu Chung Chu, 1987). Singh *et al.* (1988) have reported that *Azolla* incorporation increased the total nitrogen, organic carbon contents and available 'P' content in soil. Kannaiyan (1990b) reported that besides nitrogen, *Azolla* incorporation increased the availability of phosphorus, potassium, zinc and iron to rice crop. Gopalaswamy and Kannaiyan (1998b) observed that incorporation of *Azolla* hybrid AH-C2 reduced the pH and EC of sodic soil from the initial level. Significant reduction in exchangeable cations and increase in CEC from the initial soil level was noticed when the *Azolla* hybrid AH-C2 was incorporated with USG.

Nitrogen use efficiency of Azolla with urea super granules

In wetland ecosystem, the productivity of rice could be increased by raising the efficiency of applied nitrogen supplied either by natural means or by chemical fertilization (Murayama, 1979). The supply of N from different organic sources must always synchronise with plant nitrogen demand. Improperly matched nitrogen supply and demand would result in lower yields and increased nitrogen losses (Upendra Singh, 1995). When nitrogen is applied in flooded soil, it undergoes a series of losses thereby minimizing the N use efficiency. Reddy and Patrick (1977) found that only 49 per cent of applied N was recovered by rice even under the best cultural conditions. However, N recovery of 61 per cent by rice with good and best agronomic practices have been reported (Mikkelsen and De Datta, 1979).

Nitrogen use efficiency in rice ecosystem is governed by several factors such as soil, variety, season, planting time, water management, weed control, insect and disease control, cropping sequence, source, rate, time and method of N

application (Upendra Singh, 1995). Among the different methods to reduce the nitrogen losses and to increase the N use efficiency, use of urea super granules (USG) is considered to be one of the promising techniques. Mahajan and Tripathi (1992) reported that USG application significantly increased the grain yield, nitrogen concentration and recovery in rice compared to split application of PU.

Under flooded soil situations, combined application of organic and inorganic nitrogen sources was found to be better for effective utilization of nitrogen. Latha *et al.* (1988) have recorded significantly higher grain yield of rice when *Azolla* inoculation was coupled with USG application. Thangaraju and Kannaiyan (1993) reported that *A. microphylla* and *A. filiculoides* coupled with USG application significantly increased the grain yield of rice. Kannaiyan (1990a) compared the application of 60 kg N ha^{-1} as PU and USG with 2 incorporation of *A. microphylla* (equivalent to 60 kg N ha^{-1}) and found that USG was superior by recording a higher grain yield in IR 20 and CO 43 rice varieties. Manjappa *et al.* (1991) have reported that *Azolla* application coupled with USG at 30 kg N ha^{-1} level gave rice yield equivalent to 60 kg N ha^{-1} level. Shanmugasundaram and Kannaiyan (1994) have reported that inoculation of *Azolla* hybrid RS-SK-TNAU 1, with PU as well as USG recorded higher grain and N uptake in rice. The nitrogen fixing green manure plants *viz. Azolla, S. rostrata* could be effectively utilized by incorporation with USG to increase the rice production under lowland rice ecosystem (Kumar *et al.* 1995). They concluded that by adopting the Integrated Nutrient Management system using nitrogen fixing green manures or *Azolla*, the N use efficiency could be improved considerably. Gopalaswamy and Kannaiyan (1998b) reported that inoculation of *Azolla* hybrid AH-C2 coupled with USG at 75 kg N/ha increased the grain and straw yields of CO 43 rice besides increasing the plant N content and uptake in sodic soil.

Conclusions

Biological nitrogen fixation is an important process in rice farming systems because it is an inexpensive source of nitrogen for increasing the productivity of crops. The nitrogen fixing biosystems such as *Azolla* are able to adapt well under wetland rice field ecological conditions and fix considerable amounts of nitrogen. The biomass of *Azolla* decomposes rapidly in rice soil and supplies the nitrogen to rice crop. Besides nitrogen addition, *Azolla* contributes significant amounts of phosphorous, potassium, sulphur, zinc, iron, molybdenum and other micronutrients. The organic acids released during the mineralization process would accelerate the phosphorus availability in rice soil. In TNAU, significant contribution have been made on *Azolla* biofertilizer particularly on the selection of new strains of *Azolla microphylla* which is highly tolerant to high temperature and salinity with higher biomass producing capacity (25 t ha^{-1}). An *Azolla* hybrid was also developed by sexual hybridization and the hybrid is also highly adaptive and has higher N_2 fixing potential. A suitable *Azolla* hybrid AH-C2 was identified for the bioreclamation of sodic soil. Though *Azolla* is ecofriendly, its efficient use depends upon the agro-ecological environment and local availability.

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