Focal Theme (Botany) ISCA Symposium, 1987. 47-60

Legume-*Rhizobium* Interaction for Biological Dinitrogen Fixation: Its Use as Green Manure.*

S.K. Ghai and George V. Thomas
Central Plantation Crops Research Institute,
Kasaragod-670 124, India

ABSTRACT

Biological dinitrogen fixation by legume-*Rhizobium* combination is an excellent example of Plant-Microbe interaction. The beneficial effect of this interaction is derived mainly by two ways. One way is, using the dintitrogen fixing ability of the bacteria for direct benefit of the leguminous plants which gives higher yields of the grains (and also biomass). The other use of this plant-microbe interaction is indirect, which is the topic of this paper. Here the dinitrogen fixed by the legume-*Rhizobium* interaction is made available to the cereals and other nonlegumes, which otherwise are unable to enter into similar association with the rhizobia as the legumes. This process is called green manuring

*C.P.C.R.I. Contribution No. 620

where a leguminous plant, which has derived enough benefit from its association with appropriate species of *Rhizobium*, is ploughed into the soil and then a nonlegume is grown and allowed to take the benefit of the already fixed dinitrogen.

Research on green manuring in India and abroad is also reviewed here along with future scope of this practice in the agricultuire.

INTRODUCTION

A crop grown with the objective of burying in its green matter in the soil to decay and improve the soil fertility is called a green manure crop. Mainly legumes are grown as green manures because of their ability to fix significant amounts of dinitrogen biologically, though some nonlegumes are also grown as green manures. Documented literature suggests the prevalance of the practice of green manuring in India and China from early times. Varahamihara (circa 500 AD) reported that even before 500 BC sesamum was buried in the soil at flowering stage to improve the productivity of garden soils (Kakde, 1965). In China, the practice of green manuring has been practiced in the Hunan province atleast for 1000 years (Zehong & Xioliang, 1982). Similarly, in north Korea and Japan, green manure crops had long been grown extensively for improving the soil fertility (Watanabe, 1984) Introduction of intensive farming systems and availability of chemical fertilizers decreased the emphasis on green manuring. The first energy crisis due to 1973 West Asia war pushed the fertilizer prices from $30 to 140 per metric ton (Anonynous, 1979) and the second energy crisis of 1979 further pushed up the fertilizer prices. This has resulted in renewed interest in green manuring for providing biologically fixed nitrogen to the subsequent cereal crop.

NITROGEN FROM GREEN MANURES ONLY : A Strong Case Especially for Developing Countries

The industrial production of nitrogenous fertilizers is mainly by Haber-Bosch process where N_2 and H_2 are combined at high temperature (400-500°C) and pressure (200-1000 ATM). This process consumes large amounts of energy obtained mainly from the fossil fuels. Firstly, the fossil fuels are not going to last for ever (according to one estimate, the presently known oil reserves will not last until the middle of next century). Secondly, the prices of these fuels will keep rising in the world smitten by profit phobia. So under these circumstances we have to look for alternative sources of nitrogenous fertilizers. Biologically fixed nitrogen is one such source. In terms of energy economics also, biologically fixed nitrogen consumes 1/4-1/3 of the energy consumed by chemical fixation process (table 1). So for developing countries like India, which donot have enough reserves of fossil fuels and are unable to pay high prices for

Table 1. Energy input for biological fixation vs chemical fixation.

	Energy for i Kg N ($X10^2$ KWH)
Vigna unguiculata (L.) Walp	0.05
Cymopsis psoraliodes DC.	0.07
Sesbania aculeata (L.) Pers.	0.07
Chemical fixation (Haber-Bosch process)	0.20

Source: Singh, N.T. 1982. In Intt. Conf. organic matter & and rice". IRRI, Philippines.

their purchase, biological nitrogen fixation is very attractive. In addition to the above economy in energy, leguminous green manures (besides supplying biologically fixed nitrogen) also supply significant amount of organic matter which is essential for the health of the soil and hence for the following cereal crop. Ghai *et al.* (1965) have reported that *Sesbania aculeata*, the most popular green manure in north India, besides contributing about 70 kg of N also contributes 15-20 t ha^{-1} wet biomass to the soil. Because of their deep root system these crops can mobilise P and K from the deeper layers of the soil. Guevarra (1976) in a study on fertile, well drained soil from Hawaii reported that the foliage harvested from one hectare of *Leucaena leucocephala* plantation when used as green manure could contribute 44 Kg P and 187 Kg K besides 500-600 Kg of biologically fixed nitrogen. The green foliage is also rich in Calcium (0.8-2.0%) and magnesium (0.4-1.0%) and other micronutrients.

Green manuring practice if adopted will result in restoration and maintenance of soil fertility which is basic and critical problem of developing countries. There are certain points like additional irrigation water requirement, and land, use of which could be put to grow other crops, go against green manuring practice but plus points out weigh against a few negative ones. So present situation still favours the adoption of leguminous green manures.

GREEN MANURING RESEARCH IN INDIA

(a) Green Manuring For Rice

India is one country where research and use of green manure have received the greatest attention mainly because sources of nutrients like compost and inorganic fertilizers are either lacking or short in supply or expensive. Research work on green manuring has been going on in India for more than 60 years as evident from reports published from 1922-1937 of Bal, Subasrabundhe, Mukerji etc. Many reports are also available from Agriculture Department of the Panjab State (Uppal, 1962) and Central Rice Research Institute, Cuttack (Vacchani & Murty, 1964) where lot of emphasis had been laid on beneficial effect of green manuring during fifties and sixties respectively. Exclusive booklets on green manuring were written by Uppal, (1962) and by Panse *et al* (1965) but these all dealt mainly with beneficial effects of green manures from agronomic point of view taking yields as the main criteria. No quantification of N contribution by green manures was attempted.

There was a lull in activities on green manuring front from mid sixties to early seventies because of availability of chemical fertilizers at reasonable prices. Spurt in prices of crude oil due to 1973 west Asia war resulted in escalation of prices of fertilizers and this resulted in renewed interest on green manuring. During this time, a very informative booklet but dealing mainly on agronomic aspects appeared (Chela & Gill, 1973). Later reports [Bhardwaj, 1982; Beri & Meelu, 1981; Singh, 1982; Meelu & Rekhi, 1983; ghai *et al.*, 1984, 1985] mainly dealt with N contribution by green manures.

(b) Green Manuring For Plantation Crops

The practice of green manuring in plantation crops is followed not strictly in the same sense as for cereals. Here the legume, besides supplying biologically fixed nitrogen also acts as a cover crop to conserve moisture, control weeds and prevent erosion by wind and rain water. As early as the year 1900, *Tephrosia candida* had been tried as green manure in tea plantation in Assam (child. 1974). In 1947, the Indian Central Coconut Committee recommended *Crotalaria striata* cultivation in coconut garden under west coast conditions (Gowda & Patil, 1976). The superiority of *C. striata* as green manure over that of other bushy legumes had been demonstrated later (John, 1952). Cultivation of shrubs such as

Gliricidia sepium, *Gliricidia maculata*, *Erythrina lithosperma* and *Indigofera teysmannii* on the borders of coconut gardens and harvesting of their foliage to be used as green manure has also been tried (Child, 1974; Krishna, 1956). *Calopogonium mucunoides* besides supplying nutrients, controlling weed growth, preventing erosion has also been used as green manure cum mulch (Albuquerque *et al*, 1956). Certain guidlines for green manuring of low fertility soils have also been suggested (Anonymous, 1966). Reviews on cover crops (child, 1974. Thampan, 1981) have described *Pueraria phasoeoloides calopogonium mucunoides* and *Centrosema pubesence* as the most popular cover crops for plantations in India. Recently conducted experiments at Kayangulam Regional Station of CPCRI (Thomas and shantaram, 1984) revealed the feasibility of growing leguminous green manure crops in the basins of coconut palms without any additional land requirement in the conventional cropping system. Of the nine green manures tried (table 2), *Mimosa invisa* contributed the maximum i.e. 150g N basin^{-1}. Green manure application also resulted in significant increase in the population of microorganisms and activities of soil enzymes. Table 3 gives a partial list of leguminous green manure as well as cover crops grown in different parts of the world.

Table 2. Wet biomass and nitrogen contribution of green manure cover crops grown in basins of coconut palms.

Legume species	Wet biomass (Kg basin^{-1})	Nitrogen contributed (g basin^{-1})
Calopogonium mucunoides	14.71	102.61
Gylcine wightii	2.35	19.20
Leucaena leucocephala	2.95	16.55
Macroptilium atropurpureum	9.10	66.64
Macrotyloma axillaire	0.95	6.67
Mimosa invisa	17.00	153.19
Pueraria phaseoloides	19.43	121.29
Sesbania aegyptica	1.30	6.89
Stylosanthes guianensis	3.50	12.70

Source: Thomas, G.V. & Shantaram, M.V. 1984. Pl. Soil 80 373

GREEN MANURING– Adoption by other Countries

Green manuring practice is prevalent in many countries which include developed as well as under developed nations. Chinese have been growing leguminous green manures on a large scale. In the Hunan province of China, 60-70% of rice fields are planted with green manures (Zehong & Xioliang, 1982) and 30-40% of the N requirement is supplied by green manures. The green manure crops popular in China include *Rhaphanas grativus, Lolium perene, Astragalus sinicus, Vicia erassa, V. sativa, Sesbania cannabina* etc. Japanese, one of the highest consumers of chemical fertilizers per unit area, also practise green manure with *Astragalus sinicus*, soybean, *Medicago denticula, Vicia sativa* etc. In Columbia, *Desmodium trifolium* is used as green manure for rice crop. In south Korea, *Lespedeza bicolor* and *L. thunbergi* are used as green manure around pine seedlings, if cut and incorporated in soil at young age. However, if allowed to grow, these can also yield fuel wood besides foliage. In Papua New Guinea, *Leucaena leucocephala* is grown as a canopy above cacao plants and when cacao establishes properly, the foliage of *Leucaena* is clipped off regularly and used as green manure. In Hawaii, *Leucaena leucocephala* grown on the ridges of the farms is clipped off regularly at 3 months interval and the foliage is used as green manure which has been reported to contribute 500-600 Kg of biologically fixed nitrogen (Guevarra, 1976). In Ibadan, Nigeria and Cali, Colombia, *Leucaena Ieucocephala* is used as a support for yam and pole bean. The main stem provides support to these climbing crops and the foliage is harvested as the manure. In some central american countries, a slightly varied form of green manuring is practiced. Bean and maize are cultivated simultaneously in the same field. The bean plant not only fixes nitrogen for its own use but also supply it to the maize plant growing nearby. When the bean pods are harvested, the remaining part of the plant is cut and burried in the soil as green manure. Besides these there have been several reports on adoption of green manuring and research work from Burma, Indonesia, Pakistan, Phillippines etc.

Similarly, reports on use of leguminous green manures/cover crops in plantation crops are also available from many other countries. In Malaysia, *Centrosema pubescens, Calopogonium mucunoides, Pueraria phaseoloides, Tephrosia candida* are popular and the extent of N fixation can range from 226-353 Kg ha^{-1} in rubber plantation (Watson et al, 1963). The potential of N fixation by shade tolerent species like Calopogonium caeruleum is much more (Tan et. al, 1976) (694 Kg ha^{-1} from 3rd to 8th year). In Philippines, Pueraria phaseoloides and *Calopogonium caeruleum* have been reported to perform better (Cabato, 1970) whereas in Sri Lanka (Thampan, 1981) the most widely used cover crops are *Calopogonium mucunoides, Centrosema pubescens* and *Pueraria phaseoloides*. Recent work from Malaysia make conservate estimates of N fixation (150 Kg N

ha^{-1}) by cover crops in rubber (Broughton, 1977). In oil palm, the beneficial effect of leguminous crops has been quantified equal to 6% increase in fruit bunches (Tan & Ng, 1972).

NITROGEN CONTRIBUTION BY VARIOUS LEGUMINOUS GREEN MANURES

There are about 18000 species of legumes (Anonymous, 1979) but only a few hundred are cultivated and among these not many have been studied for nitrogen fixation and green manuring. Of the ones which have been studied include annual legumes, perennial legumes and wild annual legumes (table 3). The information about the exact N contribution by many of these is not known. *Sesbania aculeata* contributes 70-120 Kg N

Table 3. Crops which can be used as green manures

1. Cultivated annual legumes:

Astralagus sinicus, Crotalaria juncea, Crotalaria striata, Crotalaria anagyroides, Cassia mimosoides, Cyamopsis tetragonoloba, Desmodium canum, Desmodium hetrophylum, Desmodium ovalifolium, Desmodium virgatus, Glycine wightii, Indigofera anil, Indigofera linifolia, Indigofera teysmaii, Phaseolus lunatus, Psophocarpus ttetragonolobus, Sesbania aculeata, Sesbania rostrata, Sesbania speciosa, Vigna radiata, Vigna unquiculata etc.

II. Perinnial legumes:

Acacia nilotica, Cassia hirsuta, Desmodium gyroides, Gliricidia maculata, Sesbania aegyptica, Sesbania grandiflora, Tephrosia candida, Tephorisisa vogelle, Leucaena leucocephala, Prosopis juliflora etc.

III. Wild annual legume:

Aeschynomene americana, Calopogonium caerulum, Calopogonium muconoides, Cassia cobanensis, Cassa occodentalis, Cassia ttorraCentrosema pubescens, Erythrina lithosperma, Lathyrus sativus, Macrotyloma axillaire, Macroptilium atropurpureum, Macroptilium lathyroides, Mimosa invisa, Mucuna bracteata, Mucuna cohinensis, Pueraria phaseoloides, Stylosanthes guianensis, Styulosanthes hamata, Stylosanthes humilis, Tephrosia purpurea etc.

(Source: All referances)

ha^{-1} at the time of incorporation. (Ghai *et. al.*, 1965; Bhardwaj, 1982; Ghai et. al., 1984). The contribution from straw alone of summer mungbean has been reported to be about 70 Kg N ha^{-1} (Meelu & Rekhi, 1983). A report from IRRI has suggested that fast growing tropical lagumes when grown as green manures can accumulate more than 80 Kg N ha^{-1}. A study carried out in Florida, USA (Reddy et. al, 1986) on 7 tropical legumes has suggested that N contribution, alone from top grwoth ranges from 40-250 Kg N ha^{-1}. Research work at Senegal (child, 1974; Dreyfus *et. al.*, 1985) has suggested that *Sesbania rostrata* (which forms two types of nodules viz. *root* nodules and stem nodules in response to two different types of rhizobia)can contribute 200-280 Kg N ha^{-1} when used as green manure at 52 days growth stage. *Sesbania rostrata* has also been tried in alley cropping systems in Ibaden, Nigeria and its incorporation in soil has resulted in 65% increase (Mulongoy, 1986) in the yield of rice. *Leucaena leucocephala* has been reported to contribute 500-600 Kg N $year^{-1}$ from green foliage (Guevarra, 1976). Another study (Sanginga et. al., 1986) with this legume has estimated that the N fixed ranged from 448-598 Kg N ha^{-1} and the prunning from inoculated plots when used as green manure could contribute N to a maize crop equivalent to 80 Kg urea N ha^{-1}. Nitrogen contribution of other legumes range from 34-300 Kg N ha^{-1} when used as green manure (Ghai *et al.* , 1985) Table 4 gives a list of N contribution by some green manure legumes. For comparision purpose, amount of N fixed by other systems including nodulated non-legumes, blue green algae and asymbiotic nitrogen fixation have also been included.

HOW TO GET MAXIMUM BENEFIT OF BIOLOGICALLY FIXED NITROGEN FROM A GREEN MANURE CROP

Once the nitrogen has been fixed symbiotically by leguminous green manure—*Rhizobium* association, it has to be transferred to the subsequent cereal. In topical soils, upto 60% of the fixed N can be mineralised and hence available for use by other plants. So a green manure crop will be successful if it can contribute maximum N to the followng cereal crop. The sucess of a green manure crop depends upon the proper crop selection itself, depending upon the irrigation facilities available and soil type. *Sesbania aculeata* may be good for those north Indian soils which have assured irrigation supply (Ghai *et. al.*, 1984). This legume may also be grown in highly sodic soils (pH 9.5). Cowpea and cluster beans could be grown in soils with restricted soil water conditions. In coastal South

Table 4. Contribution of N by some of the nodulated legumes when used as green manure/cover crops and their comparision with non legumes

N- Fixing system	Amount of N contributed (Kg ha^{-1})	Reference/s
I. Green manuring type legumes:		
Sesbania aculeata-Rhizobium	70-120	5,11,15,18
Sesbania sp. PLSe17-*Rhizobium*	70	5,18
Vigna radiata (straw)-Rhizobium	60	14,
Sesbania rostrata Rhizobium(root)		
Rhizobium(shoot)	280	35
Leucaena leucocephala -Rhizobium	500-600	6
Beans (broad bean/lupines/soybean/ lentil etc.)-*Rhizobium*	60-210	41
Fodders (*Trifolium/Medicago/ Melilotus etc.) -Rhizobium*	100-300	16
II. Cover crop type legumes:		
Pueraria phaseoloides/Calopogonium mucunoides/Centrosema pubescens Rhizobium	226-350	26
Lablab purpureus-Rhizobium	240	18
Glycine jawanica -Rhizobium	210	18
III. Non legumes:		
Casuarina equistifolia -Frankia	100.	42
Alnus -Frankia	30-300	43
Parasponia andersonil -Rhizobium	850	42
IV. Others:		
Azolla -Anabaena	25-150	41
Grasses - Azpospirillum	15-100	43
Azotobacter/Kleibsiella/Clostridia/ Beijernkia etc.	20-80	44

India, where the soil are either sandy or lateritic, *Calopogonium muconoids* and *Pueraria phaseoloids* are much better than sesbanias. The

N contribution from a green manure crop may also depend upon the stage of the crop when it is ready for incorporation in the soil. Incorporating the crop too early or too late gives less than full potential of the nitrogen contribution. The best stage of incorporation is at the flowering stage which is about 8 weeks after seeding in most of the legumes (annuals). Work done at Central Soil Salinity Research Institute, Karnal (Ghai *et. al.*, 1965, 1984'; Bhardwaj, 1982; Morris *et. al.*, 1986) has suggested that the incorporation age could be advanced to 45 days in case of *S. aculeata* and this can provide the much needed time to the farmer for rice transplantation at proper time. Another factor important for getting maximum benefit from a green manure in terms of N is the time allowed to decompose a green manure after incorporation. This mainly depends upon the climatic conditions. In Japan, for example 15-30 days decomposition period is allowed (Watanabe, 1984) whereas in India where the temperature is humid and hot during rice growing season, green manure does not need any decomposition period. So rice can be transplanted immediatly after incorporation of the green manure and flooding of the field. The N present in the green manure is first mineralised and then it becomes available to the next cereal. At 30°C, about 70% of the total N is mineralised in 4 weeks (Watanabe, 1984). Again to obtain maximum N benefit from a green manure crop it should be incorporated at proper depth in the soil. Significant N losses due to ammonia volatilisation occur if the green manure is incorporated less than 10 cm deep.

GREEN MANURING FOR RICE WITH SESBANIA ACULETA - An Ideal Nitrogen Fixing System

S. aculeata seems to be the best green manure crop under north Indian climatic and soil conditions. Ghai *et. al.* (1965,1983) have tested 38 sesbanias belonging to 15 identified and 8 unidentified species for their green manuring potential. This collection included annuals (Ghai *et. al.*, 1965) as well as tree types (Ghai *et.. al.* 1985 a, b.) Annual types have been found to be more suitable for green manuring (table 5) because of their fast growth and nitrogen accumulation rates. Among the annuals also, *S. aculeata* had highest N accumulation rate.

Nodulated *S. aculeata*, besides being a prolific N fixer (estimates range from 70-120 Kg N ha^{-1}) also helps in reclamation of problematic soils. In the Panjab, farmers have been able to reclaim alkali soils by growing *S. aculeata* for 2 consecutive seasons. During decomposition of the green manure, many different types of organic acids are released which

Table 5. Biomass and N accumulation rates of 6 sesbanias

Sesbania type	*Biomass* productivity [a] (Kg ha-1 day-1)	N accumulaation [a] (Kg ha-1 day-1)
S. aegyptica	54[b]	0.607[c]
S. grandiflora	56[b]	0.405[c]
S. glabra	81[c]	0.475[b]
Sesbania sp. Canning	95[c]	0.535[c]
S. aculeata	288[c]	1.973[b]
Sesbania sp. PLSe 17	373[d]	1.946[b]

a: calculated on the basis of data for 57 days
b,c,d: Significant at 0.1% , 1% and 5% level of significance respectively in individual column.
Source: Ghai, S.K. Rao, D.L.N. & Batra, L. 1965 Trop. Agri., 62 52

neutralise the high pH of alkali soils. Carbon dioxide produced as a result of decomposition of organic matter, also lowers the soil pH (and increase the availability of phosphorus). Work done at CSSRI, Karnal has suggested that alkali soils of even upto pH 9.5 could be successfully used to grow rice by first growing and incorporating *S. aculeata.*

In north India, *S. aculeata* can be seeded in a flooded field from end April to mid May, depending upon the time of availability of the field after wheat harvest. The cowpea group rhizobia, which nodulate *S. aculeata,* occur abundantly in these soils as profuse nodulation occurs without inoculation. Attempts to nodulate with standard *Rhizobium* strains generally fail (Ghai *et. al.*, 1983). Forty five days growth is ideal for incorporation for getting maximum benefit interms of nitrogen (Ghai *et. al.*, 1965; Bhardwaj, 1982; Bery & Meelu, 1981) and during this period it may need 3-5 irrigations (Ghai *et. al.*, 1984) depending upon the hot weather and rains. Immediately after incorporation, the field may be flooded and rice seedlings transplanted. There is no need to allow decomposition period after incorporation. From uptake of N by grains and straw, it has been estimated that the N contribution by *S. aculeata* equals to supply of 100-120 Kg of chemical nitrogenous fertilizers. (Ghai *et .al.*, un published data)

SYMBIOTIC SYSTEM WHICH FIX NITROGEN IN PRESENCE OF COMBINED NITROGEN

As a rule, symbiotic nitrogen fixation is inhibited by the presence of combined nitrogen but there are atleast two exceptions to this. The first one is *Azolla-Anabaena* systmes which can keep fixing nitrogen symbiotically even in the presence of 2.5 mM NH_4^+, though under this condition its N fixation ability is reduced by approximately 30%. The other is *Sesbania rostrata.* It has a remarkable property of being able to simultaneously absorb combined N with its root nodules and fix N_2 with its stem, nodules. Work done in Senegal (Renaudo, 1983), has shown that in young plant, roots of which were continuously bathed in a 3mM nitrate solution, had few root nodules (23 mg plantt^{-1}) exhibiting a very low acetylene reducing activity (ARA 95 nM roots system^{-1}) but had more stem nodules (133 mg plant^{-1}) which actively reduced acetylene (ARA 3944 nM C_2H_4 per stem). The stem ARA was higher (3944 nM) in plants growing in a N free nutrient solution with combined N than in plants growing in a N free nutrient solution (2639 nM). This system has been reported to fix 280 Kg N ha^{-1}under field conditions at 52 days growth stage, when used as green manure. Such systems can especially be suitable under soils with intermediate level of nitrogen.

SCOPE OF GREEN MANURING PRACTICE IN FUTURE

The adoption of the green manure practice is largely determined by the alternatives available to provide nitrogen and organic matter and the resources of the farmers. For a small and marginal farmer, green manuring may be important because chemical fertilizers are expensive. In some cases, land may be better used to grow green manure rather than left fallow. In case of problematic soils e.g. alkali soils, green manures may be adopted from reclamation point of view. In plantation crops, green manuring may aid in prevention of erosion (in addition to N contribution). Green manuring practice need to be encouraged because it not only contribute biologically fixed nitrogen but also contributes organic matter, P, K and many other nutrients which are essential for restoration and maintenance of the soil health. We have to look for efficient nitrogen fixing systems by selecting better *Rhizobium-legume* partners. We also have to find, improve or develop new symbiotic systems which can fix nitrogen in the presence of combined nitrogen.

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