Potential for increasing biological nitrogen fixation in soybean

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Abstract

The importance of soybean as a source of oil and protein, and its ability to grow symbiotically on low-N soils, point to its continued status as the most valuable grain legume in the world. With limited new land on which to expand, and emphasis on sustainable systems, increases in soybean production will come mostly from increased yield per unit area. Improvements in biological nitrogen fixation can help achieve increased soybean production, and this chapter discusses research and production strategies for such improvement.

The soybean-Bradyrhizobium symbiosis can fix about 300 kg N ha^{-1} under good conditions. The

factors which control the amount of N fixed include available soil N, genetic determinants of compatibility in both symbiotic partners and lack of other yield-limiting factors. Response to inoculation is controlled by the level of indigenous, competing bradyrhizobia, the N demand and yield potential of the host, and N availability in the soil.

Research efforts to improve BNF are being applied to both microbe and soybean. While selection continues for effective, naturally occurring bradyrhizobia for inoculants and the use of improved inoculation techniques, genetic research on bradyrhizobia to improve effectiveness and competitiveness is advancing. Selection, mutagenesis and breeding of the host have focused on supernodulation, restricted nodulation of indigenous *B. japonicum,* and promiscuous nodulation with strains of bradyrhizobia from the 'cowpea' cross-inoculation group. The research from the host side appears closer to being ready for practical use in the field.

Existing knowledge and technology still has much to offer in improving biological nitrogen fixation in soybean. The use of high-quality inoculants, and education about their benefits and use can still make a significant contribution in many countries. The importance of using the best adapted soybean genotype with a fully compatible inoculant cannot be overlooked, and we need to address other crop management factors which influence yield potential and N demand, indirectly influencing nitrogen fixation. The implementation of proven approaches for improving nitrogen fixation in existing soybean production demands equal attention as received by research endeavors to make future improvements.

Introduction

Soybean *(Clycine max L.* Merrill) is one of man's most important sources of food and feed. It is one of nature's most versatile plants, and produces an abundant supply of protein and oil in both temperate and tropical environments. Since its domestication around the 11th century B.C. in northeast China, soybean has been a staple food in eastern Asia (Hymowitz, 1970). Its significance as a world crop came after its introduction and highly successful adoption in the U.S.A. (Shanmugasundaram, 1989). From 1941 to present, soybeans ascended from an insignificant forage crop to become the second most valuable U.S. crop, surpassed only by maize (Anon., 1984). Brazil and Argentina have also seen large increases in the production of soybeans in the past two decades, leading the way in increased global production of soybean.

As a nodulating legume, soybean forms a nitrogen-fixing symbiosis with *Bradyrhizobium japonicum* and *Sinorhizobium* species. This attribute, in addition to its valuable oil and protein and wide environmental adaptation, ensures that it will continue to be an important world food crop in an era of increasing food demand and concerns for the sustainability of agricultural production systems. A great challenge will be meeting the food requirements of an increasing world population through comparable increases in productivity in sustainable systems (Cassman, 1990).

Several good reviews of biological nitrogen fixation (BNF) focusing on soybean have been written (Hardy and Havelka, 1976; Harper, 1987; Vest *et al*., 1973). The present paper is intended to serve as an update in this area, with emphasis on the established principles and new findings that have practical application, or a reasonable potential to be applied, toward increasing BNF in soybean.

Importance of soybean in world agriculture

Soybean is the world's premier oilseed crop. Of the eight major oilseeds traded in world markets (soybean, cottonseed, peanut, sunflower, rapeseed, flaxseed, copra and palm kernel), soybean's production has been twice that of any other oilseed since 1970 (Smith and Huyser, 1987).

The approximate composition of soybean is 40% protein, 21% oil, 34% carbohydrate and 5% ash (Scott and Aldrich, 1983). Soybean meal accounts for 60-70% of the value of the soybean, with the balance from oil. Soybean supplies one-fourth of the world's fats and oils, about two-thirds of the world's protein-concentrate animal feeds, and three-fourths of the

world trade in high-protein meals (Anon., 1984). Major importers of soybean include countries of the European Economic Community, Japan, and Eastern Europe. The demand for soybean is mainly for oil and meal products, rather than whole beans. In accounting for utilization of soybean, some 39 products have been identified ranging from livestock feed, salad oils and baby foods to industrial adhesives, putty and use in pharmaceuticals (Smith and Huyser, 1987).

By weight, the protein yield of soybean is about twice that of meat and of most beans and nuts, four times that of eggs and cereals, and twelve times that of milk (Anon., 1984). In countries with rapidly increasing populations, soybean is viewed as a crop that enhances nutritive value of the local diets and lessens national shortages of vegetable oil (Hume et al., 1985). With the diet of many people in the world deficient in protein and calories, soybean seems destined to remain an important commodity.

Recent soybean statistics

Over the past two decades, world soybean production has increased nearly two and one-half fold (Table 1). This can be attributed to nearly equal increases in area and yield.

While the U.S. is still the largest producer of soybeans, over the past two decades Brazil and Argentina have made large gains. Most likely Argentina will surpass China in this decade. These four countries dominate the world soybean market and will continue to do so for some time. The world production reached in 1987 will have to almost double to meet the projected

production and demand of 190 million tonnes in the year 2002 (Smith and Huyser, 1987).

The need to increase soybean productivity within sustainable systems

A formidable challenge will be meeting future global food demands when faced with declining productivity in areas where soil and water resources are being depleted, combined with the diminishing supply of potentially arable land on which to expand agricultural production (Cassman, 1990; Larson, 1986; Russell *et al.,* 1989). This means that sustainable agricultural systems will have to intensify production (yield) per unit area.

Yield increases in soybean have typically been gradual. The U.S. national average yield has risen from 1579 kg ha^{-1} in 1960 to about 2150 kg ha^{-1} in 1982 (Anon., 1984). Studies on the rate of yield increase reveal that the average rate was 15.1 kg ha^{-1} yr^{-1}, a 0.6% average yield increase per year (Fehr, 1987). The world as a whole has also made steady yield improvement (Table 1). Experimental yields up to 8,500 kg ha^{-1} indicate that the genetic potential is far above current production levels (Lawn and Byth, 1989; Troedson, 1988). However, the rate of soybean yield increase has been well below that of cereals (Russell *et al.,* 1989). With advantages in yield, rate of yield increase and preference as a staple, the importance of cereal crops will remain or even increase, further limiting area for expansion of legume production (Cassman, 1990). It therefore appears likely that the bulk of future increased soybean production will

Table 1. World trend in soybean production and yield per ha during the past two decades

Region or country	Area (1000 ha)			Production (1000 t)			Yield (kg ha^{-1})		
	1967	1977	1987	1967	1977	1987	1967	1977	1987
World	33,556	49,243	51,567	40,638	79,206	98,288	1,210	1,608	1,906
Asia	1,595	15,986	12,002	1,186	14,647	15,437	740	916	1,286
Africa	48	250	398	34	145	414	710	580	1,039
Europe	56	306	1,023	50	399	2,454	890	1,305	1,299
Argentina	17	660	3,510	21	1,400	7,000	1,190	2,121	1,994
Brazil	612	7,070	9,161	716	12,513	16,876	1,170	1,770	1,842
China	14,050	14,236	8,404	11,100	12,955	11,816	790	910	1,406
USA	16,093	23,314	22,839	26,564	47,948	51,839	1,650	2,057	2,270

Source: FAO data summarized by Shanmugasundaram, 1989.

have to come from yield improvements rather than increased planting area. Improving the BNF component for soybean has been identified as part of the overall strategy for increasing productivity (Anon., 1984; Olsen, 1982; Russell *et al.*, 1989; Scott and Aldrich, 1983).

Biological nitrogen fixation in the soybean- bradyrhizobia symbiosis

The symbiosis involving soybean and bradyrhizobia (used in this chapter to refer to both Bradyrhizobium and Sinorhizobium, unless otherwise specified) is a well-organized system and goes through many steps, beginning at the root surface and resulting in a N_2-fixing nodule (Vincent, 1980). The host plant provides carbon substrate as a source of energy, and the bacteria reduce atmospheric N_2 to NH_3 which is exported to plant tissues for eventual protein synthesis. The efficiency of symbiotic BNF is markedly dependent on the mutual compatibility of both partners, and is influenced by a number of environmental factors (Sprent and Minchin, 1983; Vincent, 1980). Major factors that affect the symbiotic system are summarized in Table 2. The following discussion focuses on those significant principles of BNF in the soybean-brady-

rhizobia symbiosis which most influence our ability to manage and manipulate it at the field level.

Amounts of N, fixed

Soybean, like other nodulated legumes, utilizes two sources of N for its growth-mineral N in the soil (in the form of NO and NH_4) and atmospheric N_2 fixed in nodules. The soybean has been characterized as being rather non-responsive to the application of fertilizer N (Mengel *et al.*, 1987; Scott and Aldrich, 1983). If abundantly nodulated, soybean is capable of fixing substantial amounts of its required N from BNF. The N requirement of soybean is the highest among agronomic crops (Sinclair and de Wit, 1975). Each tonne of soybean seed requires the crop to assimilate approximately 100 kg N.

Different techniques have been used to evaluate N_2 fixation by soybean. They are reviewed elsewhere (Guffy *et al.*, 1989; Peoples *et al.*, 1989). The proportion of N derived from fixation varies substantially from zero to as high as 97%. As shown in Table 3, most estimates fall between 25% to 75%. LaRue and Patterson (1981) reported an average estimate of N_2 fixation in soybean to be 75 kg N ha⁻¹, using average commercial yields and assuming that 50% of the N was from fixation. Yet, field studies by Bez   `

Table 2. Factors that affect symbiotic nitrogen fixation

Factor	Effect
Macrosymbiont	
Variety	Nodulation and nitrogen fixation
Nodulin (e.g. Leghemoglobin)	Nodule function
Photosynthate availability	Nitrogen-fixing efficiency
Tolerance of stress	Establishment of symbiosis
Microsymbiont	
Infectiveness	Nodule formation
Effectiveness	Nitrogen fixation
Competitive ability	Nodule occupancy
Saprophytic competence	Persistence of rhizobia in soil
Environment	
Combined nitrogen	Nodulation and nitrogen fixation
Light	Nitrogen-fixing efficiency
Temperature	Soybean growth and nitrogen fixation
Water and aeration	Nitrogenase activity
Salinity	Reduce nitrogenase activity
Biotic agent	Rhizobium viability and infection

Table 3. Estimates of symbiotic nitrogen fixation by soybean

Location	N_2 fixed			Reference
	kg N ha^{-1} per year	Ndfa[a]	Method	
Delaware, USA	80–120	25–30	C_2H_2 reduction	Hardy et al. (1973)
Nigeria	125–188	84–87	N difference	Eaglesham et al. (1982)
Alberta, Canada	33–151	14–62	N^{15} isotope dilution	Rennie (1984)
Jilin, China	57–151	43–85	C_2H_2 reduction	Zhang et al. (1986)
Jilin, China	45–100	50–70	N^{15} isotope dilution	Gao et al. (1987)
Australia	0–236	0–71	Ureide	Herridge and Holland (1987)
Hawaii, USA	166–237	66–97	N difference	George et al. (1988)
USA	41–237	36–48	N^{15} isotope dilution	Guffy et al. (1989)
Washington, USA	0–311	0–80	N difference	Bezdicek et al. (1978)

[a] Nitrogen derived from the atmosphere, percent of total N.

dicek *et al.* (1978) show that soybeans are capable of fixing over 300 kg N ha -' when the soil is low in available N and effective strains of bradyrhizobia are supplied in high number.

Available soil N has a large influence on BNF. George et al. (1988) found that soil-N availability at different sites determined the relative contribution of symbiotic N, fixation, regardless of crop duration and total N accumulation by different varieties. The amounts and proportion of N derived from the atmosphere is also influenced by several other factors, including temperature, soybean cultivar, bradyrhizobial strain, root nodule position, quantity and form of fertilizer N and management practices (Buttery and Dirks, 1987; Danso *et al.*, 1987; Eaglesham *et al.*, 1983; George *et al.*, 1988; Hardarson *et al.*, 1989; Herridge and Holland, 1987).

It has been suggested that increasing the amounts of N, fixed in soybean, and the portion of total plant N derived from fixation, may only be achieved with concomitant yield increases (Herridge and Bergersen, 1988). Experimentally, it is not easy to separate N, fixation from yield. This is apparent from studies comparing the two parameters in soybeans of differing maturities; the late maturing cultivars fix more N, and yield more than earlier types due to a longer reproductive phase, when rates of N_2 fixation and seed biomass accumulation are high (Patterson and LaRue, 1983; George *et al.*, 1988). However, it appears that the portion of total N derived from fixation remains fairly constant for cultivars of different maturity at a given

site (George *et al.*, 1988), as summarized below in Table 4.

Assimilation of nitrogen

The N requirement of soybean can be met by both mineral N assimilation and symbiotic N, fixation. Although each N input system has independent pathways and control points, the soybean plant under almost all field conditions will use both systems, and these systems are interdependent (Harper, 1987).

Nitrogen fixation generally reaches a peak at early podfill and declines during late reproductive phases (Imsande, 1989; Latimore et al., 1977; Lawn and Brun, 1974; Thibodeau and Jaworski, 1975). The plant mobilizes a large quantity of N from vegetative tissue to meet the demand for seed N, whereas the net rate of NO_3 uptake gradually declines throughout pod fill. On the other hand, nodules produced with the first infections on the primary root of soybean only have an average duration of 65 days, and undergo rapid aging just after flowering (Bergersen, 1958). Because of the time lag (approximately 4 weeks) between infection and rapid N_2 fixation, infection must occur at approximately the R2 stage (see Fehr *et al.*, 1971), if rapid N, fixation is to occur during R5 when pod fill proceeds at an appreciable rate (Imsande, 1989). Therefore nodulation on lateral and deep roots may be essential for maximum N2 fixation, in order to match the high N demand during pod fill (Imsande, 1989; Zapata *et al.*, 1987).

Table 4. Total N accumulation and percent of total N derived from symbiotic fixation by five soybean varieties grown at three different elevations

Site (elevation, m)	Varieties					
	Clay 00[a]	Clark IV	D68-0099 VI	N77-4262 VII	Hardee VIII	Mean
	Total N accumulation (kg ha^{-1})					
Kuiaha (320)	236	317	316	260	349	295A[b]
Haleakala (660)	199	246	271	258	276	250B
Olinda (1050)	44	99	128	186	144	120C
	Percent N from fixation					
Kuiaha (320)	85	82	85	70	80	80B
Haleakala (660)	62	71	65	66	68	66C
Olinda (1050)	95	98	98	98	96	97A

[a] Indicates maturity group designation of the variety.
[b] Data followed by the same letter within the column are not significantly different ($p = 0.05$) by Duncan's Multiple Range test.
Source: George et al., 1988.

It is well established that increasing levels of mineral N in the rhizosphere inhibit soybean nodule formation and functioning. Concentration of available N above 2 mM generally decreases N2 fixation in symbioses (Phillips and DeJong, 1984). Under controlled conditions, 15 mM N has been found to decrease nodule numbers 2.5-fold (Malik *et al.*, 1987). Imsande (1986) reported that short-term (3-6 days) exposure to 4 mM N only temporarily delayed nodule formation, and the late steps of nodule development were reversibly inhibited. Extended growth in the presence of 4 mM N blocked both early and late steps of nodule development. Split-root experiments have shown that N inhibited soybean nodule formation through localized effects on the root system rather than as a function of whole plant nutrition (Eaglesham, 1989b; Hinson, 1975).

Symbiotic N$_2$ fixation may not meet the soybean N requirement during the early and late phases of growth. Small amounts of available N (0-2 mM), supplied early, often promote growth and N$_2$ fixation in legumes, indicating N-limited early growth (Phillips and DeJong, 1984). Increasing the N supply to soybean during flowering and pod fill have increased total N and seed yield in the field (Brevedan *et al.*, 1978; George et al., 1988; Thies et al., 1991). Field response of soybean to fertilizer N may be related to the amount of NO$_3$ in the root zone. When this amount was low, the use of N fertilizer significantly increased soybean seed yield at several soil moisture levels (Al-Ithawi *et al.*, 1980). In the absence of indigenous rhizobia, Thies et al. (1991) found that inoculation response was directly proportional to the availability of mineral N in the soil.

Nitrogen fixation requires about 10 kg of carbohydrates/kg of N, fixed, and the equivalent of 25-28 molecules of ATP for each molecule of N, fixed (Havelka *et al.*, 1982). Soybean has the relatively inefficient C$_3$ photosynthetic system. Soybean growth rate is thought to be photosynthate source-limited rather than sink-limited. Due to large energy requirements, BNF is thought to be closely coupled to photosynthate production, particularly during the reproductive stages (Hardy and Havelka, 1976). A recent proposal based on experimental and theoretical evidence indicates that carbon and nitrogen are simultaneously limiting soybean yield increases (Sinclair, 1989). Related proposals by others (Imsande, 1989; Millhollon and Williams, 1986) indicate that this area of research is quite complicated, but future findings should be valuable in guiding strategy to increase soybean yields.

Host-strain compatibility

The establishment and functioning of an effective symbiosis is dependent on genetic determin-

ants in both plant and bacteria. The fully compatible symbiosis proceeds from recognition, penetration, stimulation of host-cell division, differentiation of rhizobia into bacteroids, leghemoglobin synthesis, nitrogenase synthesis and its activity. Host-strain compatibilities for nodulation, effectiveness and efficiency of N, fixation, and competitiveness for nodule occupancy have been studied intensively in the soybean-bradyrhizobia system, and some serve as classic models for other symbioses (Cregan *et al.*, 1989a; Evans et al., 1980; Vest *et al.*, 1973).

To date, some 45 genes across eight legume species have been identified as affecting modulation and N_2 fixation, including at least eight genes in soybean (Vance *et al.*, 1988). Five of these genes control restricted nodulation and are summarized in Table 5. These host genes control nodulation at the species, serogroup and strain level within *B. japonicum* and *Sinorhizobium* spp. The ril gene that conditions non-modulation has been transferred through conventional breeding to several soybean cultivars for use in estimating response to inoculation and the amount of N, fixed.

A unique incompatibility expressed in many soybean genotypes when nodulated by some strains of certain serogroups is the production of rhizobitoxine (Johnson and Clark, 1958; La Favre and Eaglesham, 1986). The toxic compound is produced in the roots and translocated to the newly developing leaves, where it produces a chlorosis. The effect can be severe enough in contained systems (such as small plant growth assemblies) to kill the plant. In the field, expressions of the chlorotic symptoms are tran-

sient and have been associated with high rates of nodule occupancy by these same serogroups (D.F. Weber, unpublished data). Many of these same strains are als o capable of nodulating the non-modulating (rjlrj1) soybean under controlled conditions (Clark, 1957; Devine and Weber, 1977).

Reports have shown that the number of nodules and their distribution patterns on soybean roots are largely dependent on host influence (Carroll *et al.*, 1985; Kosslak and Bohlool, 1984). Singleton and Stockinger (1983) demonstrated that the soybean will compensate for ineffective nodulation by producing more mass in those nodules containing effective strains. Soybean mutants have been developed which lack normal regulation of amount of modulation, allowing `supernodulation' to occur (Carroll *et al.*, 1985).

Competition for nodule occupancy

One of the major problems in inoculation technology with soybean is the establishment of an introduced inoculant strain of *B. japonicum* in the nodules of soybean grown in soils which contain indigenous populations of bradyrhizobia (Ham, 1976, 1980; Tang, 1979; Vest *et al.*, 1973). Previous inoculation and continued cropping of soybean confer a formidable advantage in numbers and environmental adaptation to the indigenous population in competition with the introduced strains. Thus, when the indigenous strains dominate the nodules, response to inoculation is not observed (Ge and Xu, 1982; Kapusta and Rouwenhorst, 1973; Kvien *et al.,* 1981).

Table 5. Host genetic control of nodulation in soybean

Allele	Phenotype	Reference[a]
rj1	Non-nodulating	1
Rj2	Cortical proliferations or ineffective nodules formed by strains in serogroup 6(c1) and 122 with soybean cv. Hardee	2
Rj3	Small, nodule-like structures with white interior by strain USDA 33 with cv. Hardee	3
Rj4	Very few cortical proliferations by strain USDA 61 with cv. Hill	4
Dominant undesignated	Swellings or rudimentary nodules by *Sinorhizobium fredii* strain USDA 205 with cv. Kent.	5

[a] 1) Williams and Lynch, 1954; 2) Caldwell, 1966; 3) Vest, 1970; 4) Vest and Caldwell, 1972; 5) Devine, 1984.

The soil population density of indigenous bradyrhizobia is a major factor determining competition for nodule occupancy and response to inoculation. Using the estimate of a hectare of plow-layer soil to weigh 2.24×10^6 kg, and assuming an indigenous bradyrhizobial population of 10^4 cells g^{-1} soil and an inoculation rate of 10 g peat kg^{-1} seed (assuming an inoculum density of 10^8 cells g^{-1} peat), calculation shows that at normal seeding rates the indigenous population has about a three hundred-fold advantage in population density over the inoculum. Field trials have demonstrated that to achieve nodule occupancy of greater than 50%, inoculant bradyrhizobia must be applied at a rate at least 1,000 times greater than the estimated number of indigenous bradyrhizobia (Weaver and Frederick, 1974). Use of massive inoculation rates can overcome competition from indigenous strains (Kapusta and Rouwenhorst, 1973), but such a delivery system is not yet practical or economical. Recent experiments from multi-site, standardized field inoculation trials with several legumes revealed that 59% of the variation in inoculation responses could be accounted for by the relationship of inoculation responses to numbers of indigenous rhizobia (Thies et al., 1991).

Despite the strong influence indigenous population density has on competition, it is a complex problem with interactions involving the bacterial genomes, host genomes and the environment (Dowling and Broughton, 1986; Triplett, 1990a). As yet, mechanisms responsible for competition per se have not been identified. For instance, it has been demonstrated that some factor(s) other than comparative numbers in the rhizosphere is (are) determining the outcome of competition (Ellis *et al*., 1984; Moawad *et al*., 1984). Indeed, the mechanism(s) may be so multifaceted that their identification will be slow and piecemeal. Some factors determined by genomes of both partners have been identified, such as host range (Cregan *et al*., 1989a; Sadowsky *et al*., 1987), mobility of rhizobia (Catlow *et al*., 1990; Mellor *et al*., 1987), and bacteriocin production (Hodgson et al., 1985; Schwinghamer and Brockwell, 1978; Triplett, 1990a). In the absence of indigenous bradyrhizobia, the pattern of competition between inoculum strains was found to be a stable (and therefore selectable) characteristic,

independent of rhizosphere population size, nitrogen application, elevation (temperature) or soil type (Abaidoo *et al*., 1990; George *et al*., 1987).

Symbiotic effectiveness seems to have no direct relationship with competitiveness (Triplett, 1990b). Cregan and Keyser (1988) showed that host genotypes of *Glycine max* and *G. soja* have great influence on competition and effectiveness, both with *B. japonicum* and strains of *Sinorhizobium* (formerly R. *fredii)*. This host control was exploited to select genotypes that would restrict nodulation of the commonly occurring, very competitive and yet relatively ineffective *B. japonicum* serogroup 123, found in the midwestern U. S. soybean growing region. Such genotypes were found which drastically altered competition (compared to commercial cultivars) in favor of inoculant strains over strains in the 123 serogroup (Cregan and Keyser, 1986; Keyser and Cregan, 1987).

Research strategies to increase BNF in soybean

The need to devote research efforts towards improvement of BNF in legumes in general and soybean in particular has been recognized frequently (Anon., 1984; Olsen, 1982; Russell *et al*., 1989; Scott and Aldrich, 1983). Areas of research with good prospects for contributing to this objective are discussed below.

Selection and engineering of bradyrhizobia

Soil microbiologists have extensive experience in the selection of rhizobia and bradyrhizobia for symbiotic effectiveness with legumes (Brockwell *et al.,* 1982; Burton, 1980; Date, 1976). To date, the provision of superior strains for use in inoculants remains the primary applied contribution from the field of BNF. In addition to selecting strains which are highly effective and efficient at nitrogen fixation with a given legume genotype, Brockwell *et al*. (1982) list 10 other important attributes; 1) competitive ability, 2) N_2-fixing ability over a range of environmental conditions, 3) nodulation and N_2 fixation in the presence of soil nitrogen, 4) ability to multiply in broth and survive in inoculant carriers, 5) ability to survive

when incorporated in seed pellets, 6) persistence in soil, 7) ability to migrate from initial site of inoculation, 8) ability to colonize soil away from influence of host roots, 9) ability to survive adverse physical conditions such as desiccation, heat, or freezing, and 10) strain-stability during storage and growth. Selection of naturally occurring strains for these attributes continues to be an important research activity in soybean programs worldwide, especially as production expands on to less desirable soils.

Competition for nodule occupancy is a complex problem which is of practical concern. In response to this there have been methods developed to screen inoculant strains for their competitive ability. The most straightforward method is to assess the response of the legume to an effective inoculant in the presence of ineffective (unable to fix N_2) competitors (Amarger, 1981; Jones et al., 1978). The value of this method was demonstrated in field trials of inoculant strains competitive against ineffective, indigenous rhizobia nodulating subclover *(Trifolium subterraneum L.)*. The inoculants produced large responses by subterranean clover (Jones *et al.*, 1978). A more complicated method is that proposed by Paau (1989) that involves isolation of indigenous strains, which are assumed to be well adapted and competitive in their specific environment, subjecting them to mutagenesis, and selecting mutants for increased N_2-fixing ability for use as inoculant. Multiple-site field tests of these mutants in soils with indigenous *B. japonicum* over a four-year period showed an average soybean yield increase of 169 kg ha^{-1}, or 6.7% (Paau, 1989).

Significant progress has been achieved in the studies of genetics of rhizobia and bradyrhizobia since the mid 1970s (Johnston *et al.*, 1987). *Bradyrhizobium japonicum is* becoming a genetically well-studied bacterium as far as nodulation and symbiotic nitrogen fixation genes are concerned (Hennecke *et al.*, 1988). Some of the first attempts at genetic manipulation for practical purposes focused on increasing the nitrogen fixation rate of the bacteria through mutagenesis (Maier and Brill, 1978; Williams and Phillips, 1983). The mutant used by Williams and Phillips produced a significant soybean yield increase above that of the parent strain USDA 110 in

field trials. Introducing the hydrogen uptake (hup) genes into bradyrhizobial strains has been proposed as a manipulation to increase their energy efficiency (Lim *et al.*, 1980). The Eisbrenner and Evans (1983) review of this topic showed that the majority of reports on the comparison of Hup+ and Hup- strains found a significant response in total N with Hup+ strains. Evans *et al.* (1985) reported that soybean yields were higher when plants were nodulated by a strain of *B. japonicum* that was Hup+ as compared to plants nodulated by an isogenic Hup- strain. However, there are Hup- strains of *B. japonicum* that are equal or superior to Hup+ strains in symbiotic performance in the field (Hume and Shelp, 1990).

Genetic manipulation to alter competitive ability in the soybean bacteria has not been successful as yet. However, there is still a need to pursue this approach. In a review of this subject, Triplett (1990b) identifies bacterial phenotypes found to play a role in competition, including motility, cell-surface characteristics, speed of infection, and bacteriocin production. Given the rapid rate of advances made in the field of molecular genetics of rhizobia and bradyrhizobia, it seems quite likely that it will contribute to our understanding, and ability to manage, competition for nodule occupancy.

Selection and breeding of soybean genotypes

On the host side, improving BNF has been approached by selection for improved nitrogen fixation per se (if indeed this can be separated from yield), selection for ability to nodulate and fix nitrogen in the presence of high soil-N levels, the development of soybeans with the ability to restrict nodulation by selected indigenous populations and still nodulate with effective inoculant strains, and the development of soybeans that nodulate promiscuously with indigenous *Bradyrhizobium spp*. Several methods are available to undertake these objectives, including selection of existing germplasm, conventional plant breeding, mutagenesis, and gene transfer (Dreyfus *et al.*, 1988).

Traditional legume breeding programs have not included enhancement of BNF as a direct objective (Phillips and DeJong, 1984). A review

of the reports on soybean, examining the relationship between total N fixed and yield, shows them to be positively and highly correlated (Leffel, 1989). It appears that direct selection for yield improvement in soybean has indirectly included improved capacity to fix N_2. Further, the potential for yield increase due directly to increased BNF exists (Burias and Planchon, 1990; Imsande 1989). The one preliminary report that shows differences between BNF in soybean genotypes of equal yield and maturity is that of Cregan *et al*. (1989b). They found that selected high seed-protein genotypes attained higher total N from higher N, fixation compared to normal genotypes.

Soybean genotypes have recently been identified which have greater numbers of nodules in the presence of high mineral N (Betts and Herridge, 1987; Herridge *et al*., 1988). Similar studies have been conducted on a smaller scale (Danso *et al.,* 1987; Gibson and Harper, 1985). The benefit of this approach is that soybean would fix more at a given level of mineral N, thus saving or 'sparing' that soil N for subsequent crops. Mutagenesis of soybean has produced supernodulating mutants which are also more nitrate-tolerant (Carroll *et al.,* 1985; Gremaud and Harper, 1989). A mutant characterized as a moderate supernodulator has produced increased yields in the field under some conditions (Boerma and Ashley, 1988; Carroll *et al*., 1988), indicating the potential of this research to increase yields through manipulation of a trait related to BNF.

As mentioned above in the section on competition, soybean genotypes have been selected which restrict nodulation with serogroup 123, an indigenous, heterogeneous group of *B. japonicum* present in much of the northern midwest U.S.A. (Cregan and Keyser, 1986). The objective is to permit a higher portion of the nodules to be occupied by highly effective inoculant strains. This could lead to higher levels of N_2 being fixed, or a greater portion of total N from BNF, thereby sparing soil N. Generations of backcrossing to productive, commercial cultivars will be necessary to adequately evaluate the success of this approach in production fields. However, preliminary data, summarized below in Table 6, indicate that the restriction of the

Table 6. Nodule occupancy in selected soybean genotypes[a]

Soybean genotype	Percent of nodules occupied by		
	USDA 123	USDA 122 or USDA 138	Other
Williams	75.7	20.5	3.8
PI 371607[b]	3.0	88.7	8.3
PI 377578[b]	5.0	91.5	3.5

[a] Average of 2 years, field experiments.
[b] Genotypes identified as restricting USDA 123 in greenhouse trials.
Source: Keyser, Cregan and El-Maksoud, unpublished data.

serogroup-type strain, USDA 123, is expressed under field conditions where 123 was mixed into the soil and inoculant was applied on the planted seed.

Soybean genotypes have also been selected for ability to nodulate with indigenous strains of *B. japonicum* and for the ability to nodulate preferentially with the effective inoculant strain USDA 110 (Kvien *et al*., 1981). Further development of this approach indicates that while selection for preferential recovery of USDA 110 will be difficult, a positive relationship of nodule mass to seed yield was found indicating that further selection for increased nodulation with native *B. japonicum* may be warranted (Greder *et al.,* 1986).

Selection and breeding of soybeans for a lack of dependence on *B. japonicum* has been carried out by workers at the International Institute of Tropical Agriculture (IITA) in Nigeria. Because the production and distribution of inoculant in parts of Africa is difficult, the researchers at IITA have sought soybean lines which are promiscuous in their nodulation. These lines nodulate with indigenous bacteria, presumably strains of *Bradyrhizobium spp.,* which nodulate legumes in the 'cowpea' cross-inoculation group, thus obviating the need to inoculate with *B. japonicum* (Bromfield and Roughley, 1980; Nangju, 1980; Pulver *et al*., 1982; 1985). Selected promiscuous lines bred to lines with superior agronomic traits and compared for yield and nodulation with non-promiscuous (B. *japonicum* requiring) genotypes show that progress has been made (Dashiell *et al*., 1985). However, there is some concern over the potential of this system. Eaglesham (1985) indicated that many of the brady-

rhizobia which do nodulate these lines are relatively ineffective, and may be a subset of the general population of *Bradyrhizobium spp.* which could be quite variable in its representation throughout African soils. It is clear that further yield increase through better BNF is possible in some promiscuous lines (Pal, 1989). Also, it needs to be clearly demonstrated that there are not indigenous, true *B. japonicum,* which have persisted from earlier studies, at sites where these lines are evaluated. Such characterization of the microsymbiont population structure in the soils where these lines are evaluated would seem a necessary component to assist the evaluation and progress of this approach.

Improved inoculation techniques

Application of peat-based inoculant to the seed just prior to planting is the most common form of inoculation. This technique does not always give abundant nodulation of soybean (Li *et al*., 1986; Wadisirisuk *et al*., 1989). Evidence is accumulating that other techniques of inoculation can provide better nodulation and plant growth (Danso and Bowen, 1989; Hardarson *et al*., 1989; Kamicker and Brill, 1987; McDermott and Graham, 1989). Under conditions where soybean is a new crop, or where stress such as high temperature is encountered, soybean may respond to the higher numbers of bacteria provided in granular or spray inoculation as compared to seed applied inocula (Bezdicek *et al*., 1978; Scudder, 1974).

Hardarson *et al*. (1989) found that when inoculant was distributed throughout the soil it gave profuse nodulation throughout the root system, and nodules formed in the bottom part where roots were younger and contributed large amounts of fixed N to the soybean during seed formation. Inoculation through irrigation water at the third-node stage (V3) produced nodules that were very active in N_2 fixation during the reproductive stage, resulting in an increase in seed yield and seed protein (Ciafardini and Barbieri, 1987).

Burton (1980) reviewed the need for improved inoculant-delivery systems. The importance of delivering large numbers of bradyrhizobia is a challenge, and the best systems identified to date

are the soil-applied granular and seed bed sprayed inoculants. Higher numbers of inoculant rhizobia will be required if soybean production moves onto marginal soil that is acidic, has high temperatures at the time of planting, or has other stresses which affect inoculant viability. Also, as the levels of agricultural inputs increase in developing-country agroecosystems, inoculant compatibility with pesticides and insecticides will need to be addressed.

Production strategies to increase BNF in soybean

A century of research on BNF has brought tremendous progress in both our basic understanding of the process and in its application for improving legume growth (Nutman, 1987; Quispel, 1988). In recent years the majority of research on BNF has focused on bacterial genetics and biochemistry, with elaborate and sophisticated knowledge accumulating at an impressive rate. Gene transfer systems for plants are also being developed. Developments in these areas may be applicable in the future for enhancing BNF in soybean. Until then, such improvement will probably come from those strategies that were established many years ago, and while they are proven approaches, their use is far from ubiquitous and they probably provide the best potential for improvements at the field level.

Inoculant production, quality control and training

The greatest impacts on world agriculture from inoculation have been made with soybean. While there may not be large new areas planted to soybean on a global scale, there are still going to be regional and local needs for inoculation that will produce economic returns and improve BNF. In a global survey of inoculant use and availability, Eaglesham (1989a) found that inoculant is still perceived as being needed in many cases, especially where it is not available. Concerted efforts are needed to deliver and adapt existing inoculant technology to local conditions, especially in developing countries.

Many countries have no mandatory quality-

control standards for legume inoculant. The result can be products that are highly variable in quality as determined by symbiotic effectiveness, population density and shelf-life. The production of high-quality inoculant and proper quality control cannot be addressed here (see Roughley, 1976; Roughley and Pulsford, 1982; Thompson, 1984), but their importance is critical to the improvement of BNF. An essential part of the adoption of this technology is its adaptation to the needs of individual or highly localized situations (Hubbell, 1988). In many developing countries this means improvising with the non-traditional materials and equipment available to achieve economic production of a satisfactory product.

Education about the benefits and use of legume inoculants can still play a significant role in improving BNF in many countries. Many farmers may not know of the existence of inoculant, especially if the crop is new, as soybean often is, and the agricultural extension agents may only have a limited understanding of the technology. Having perceived the need for such practical training, NifTAL (University of Hawaii - U.S.A.I.D.) developed a BNF training course for extension specialists. This course was presented to Indonesian and Ugandan government and private-agency extension personnel, and was met with tremendous enthusiasm. The course was designed to give the extension specialists sufficient applied experience and theoretical knowledge to set up mini-courses on BNF and inoculation for their district farmers. Such training should be viewed as part of the overall inoculant technology transfer to other countries
.

Matching soybean genotypes to the environment

There is tremendous germplasm diversity in soybean, and using a genotype well adapted to a given site is probably one of the best and simplest strategies for improving BNF, through improving yield. This of course assumes that the soybean is well nodulated with effective bradyrhizobia. Some excellent data are found in INTSOY's International Soybean Variety Experiment (ISVEX) verifying that the varieties may differ in their yield performance in a given environment, and even at different sites in the

same environmental zone (Jackobs *et al*., 1985; Judy and Whigham, 1978). This world-wide variety evaluation program provides a wealth of information concerning environmental influences on several agronomic traits of soybean, and identifies important relationships which can assist plant breeders. A standard granular inoculant was provided to network cooperators for use in ISVEX. Any breeding program should include cooperation with a microbiologist to ensure that strains of bradyrhizobia are identified which are compatible with a given soybean genotype in a particular environment.

Management of other inputs

As with selecting the best genotype for yield, other management variables that increase yield should also increase the amount of N_2 fixed. As emphasized by Eaglesham (1989a) N is not always the primary limiting factor, and when it is not there will not be a response to inoculation. Other factors which limit soybean yield will then by definition also limit inoculation and N response.

Phosphorus is also a common limiting nutrient in many soils, and its management is important for attaining high yields of soybean. Cassman *et al*. (1981) found that field-grown soybean has a higher P requirement when it is dependent on BNF for its N supply as compared to mineral N dependency. In this study, soybean dependent on BNF but not supplied with P attained only 28% of the maximum yield obtained at optimum P levels.

Further evidence of the importance of other management factors such as P is supplied below in Table 7. These are results from 140 on-farm demonstration plots established in four districts of Uganda during 1989. The addition of P and inoculation improved yields. Averaged over all trials, each input gave approximately 300 kg yield increase, and applied together a 600 kg increase. The return on investment from the combined inputs was about 12-fold.

Supplying an adequate symbiosis to the soybean provides it only with a biological source of N. It is not a panacea against poor growth resulting from limitations of water, lime, nutrients, disease or any other factors. This per-

Table 7. Response of soybeans in Uganda, 1989 on-farm demonstration trials

District	Average yield of soybeans (kg ha^{-1})				LSD 5%
	Local variety in rows	Improved variety			
		in hills	in hills + P^a	in hills + P^a + inoculum	
Masindi	920	750	1,080	1,290	107
Mubende	738	588	899	1,260	102
Luwero	1,070	1,050	1,450	1,790	253
Kasese	843	772	1,021	1,309	357

^{a} 45 kg P$_2$O$_5$ ha^{-1}.
Source: Simkins, Kalule and Baguma, unpublished data.

spective is necessary for evaluating the potential for improving BNF in soybean in a given situation.

Concluding remarks

The world soybean production is continuing to increase, and the prospects for future increases in sustainable systems will likely come mostly from improvements in yield per area. Research on BNF in the soybean-bradyrhizobia symbiosis will have a role in contributing to such improvements. Manipulations of both the bacteria and the host plant for attributes related to BNF continue to be impressive. Still, there is much of the proven technology of BNF that has not yet been applied in cases where it could make immediate improvements in soybean yields. Research agencies, national planning bodies and international development agencies need to place proper emphasis on both research and application.

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