Chapter 8

BIOLOGICAL NITROGEN FIXATION IN PEANUTS

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BIOLOGICAL NITROGEN FIXATION

The ability of procaryotic organisms to reduce atmospheric nitrogen to usable forms has been known for over 100 years. It is generally estimated that biological nitrogen fixation accounts for about 175 million metric t of fixed nitrogen per year, compared with about 80 million t of ammonia produced annually through the Haber-Bosch process, 75% of which is available for fertilizer. Thus, the most important yield of fixed nitrogen results from biological nitrogen fixation. The symbiotic nitrogen fixation system involving rhizobia and legumes is estimated to produce about 35 million metric t/year of fixed nitrogen. The range of nitrogen fixation by different legume/*Rhizobium* combinations is from 24 to 584 kg/ha/year (Gibson *et al.*, 1982).

The energy cost of biological nitrogen fixation is significant. Currently, 1-2% of the world's fossil energy is used for fertilizer, while about 1-2 billion t of plant carbohydrate from photosynthesis is consumed in biological nitrogen fixation (Hardy, 1980). Nitrogen is a unique element in that almost no nitrogen is present in mineral form in nature and, yet, above every hectare of soil at sea level there are 78 million kg of inert nitrogen gas. Plants, as eucaryotic autotrophs, need an oxidized or reduced form of nitrogen for anabolism. Only a few procaryotic organisms are able to "fix" nitrogen directly, a technology scientists have learned to simulate using the energy-expensive Haber-Bosch process. The procaryotes in the Eubacteria and Archaea kingdoms that are able to fix nitrogen are metabolically diverse, representing autotrophs, heterotrophs, photosynthetics, aerobes and anaerobes, single-celled and filamentous, free-living and symbiotic. The ability to fix nitrogen is present in a wide range of procaryotes but is restricted to a very small percentage of the total number of procaryotic species. Dixon and Wheeler (1986) identified 38 genera of bacteria (out of a total of 1500), 20 genera of cyanobacteria and about 87 species (in two genera) of Archaea as nitrogen fixers.

It appears that biological nitrogen fixation depends upon a highly conserved enzyme complex common to all bacteria with this property, although organization and genomal location varies. It is interesting to note that the nitrogen-fixing process is similar in different procaryotic systems and depends upon the following factors: nitrogenase enzyme complex, a high energy requirement as ATP, anaerobic conditions (for nitrogenase activity), and source of strong reductant (Haaker and Klugkist, 1987). Nitrogenase consists of two components: dinitrogenase (an iron-molybdenum protein), and

dinitrogenase reductase (an iron protein) (Haaker and Klugkist, 1987). Both enzymes are needed for activity. Nitrogenase reduces atmospheric nitrogen and H^+ simultaneously, using $\pm$75% of the ATP in nitrogen reduction and $\pm$25% in H^+ reduction. A key characteristic of this enzyme complex is that both components are quickly and irreversibly lost by interacting with free oxygen, regardless of the oxygen requirements of the microbe. Evans *et al.* (1980) estimated the energy requirement for biological nitrogen fixation as 28 mol of ATP theoretically consumed in the reduction of 1 mol of nitrogen. Other estimates vary from 12 to 29 mol of ATP per mol of nitrogen. Energy is required not only for the reduction but also to maintain the anaerobic environment required for the reaction. The best known protective mechanism occurs in the legume-*Rhizobium* association where the nitrogenase system of the aerobic rhizobia is protected from excess oxygen by nodule leghemoglobin.

THE LEGUME-RHIZOBIAL SYMBIOSIS

The Leguminosae are one of the largest plant families, with worldwide distribution of about 750 genera and an estimated 16,000-19,000 species. The Leguminosae have traditionally been divided into three distinct subfamilies based on floral differences: Mimosoideae, Caesalpinoideae, and Papilionoideae. Although only about 15% of the total species have been examined for nodulation, these species are representative of all three subfamilies of legumes. Virtually all species within the Mimosoideae and Papilionoideae are nodulated, but only about 70% of the species in the subfamily Caesalpinoideae are nodulated (Allen and Allen, 1981). There is only one verified nitrogen-fixing association between a *Rhizobium* and a nonlegume. This involves a *Bradyrhizobium* of the cowpea and peanut type which has been shown to form an effective symbiosis with a nonlegume, *Parasponia*, a member of the *Ulmaceae* (elm family).

The soil-improving properties of legumes were recognized by ancient agriculturalists. For example, Theophrastus (370-285 B.C.) in his "Enquiry into Plants" wrote as follows: "Of the other leguminous plants the bean best reinvigorates the ground;" and in another section, "Beans...are not a burdensome crop to the ground; they even seem to manure it." However, it was only in 1888 that Hellriegel and Wilfarth established positively that atmospheric nitrogen was assimilated by root nodules. This was quickly followed by the experiments of Beijerinck (1888), who used pure culture techniques to isolate the root nodule bacteria and proved that they were the causative agents of dinitrogen assimilation. He proposed the name *Bacillus radicicola* for these organisms. In 1889, the root nodule bacteria were renamed *Rhizobium* by Frank.

Early researchers considered all rhizobia to be a single species capable of nodulating all legumes. Extensive cross-testing on various legume hosts led to a taxonomic characterization of rhizobia based on bacteria—plant cross-inoculation groups, which were defined as "groups of plants within which the root nodule organisms are mutually interchangeable." The concept of cross-inoculation groupings as taxonomic designators has gradually fallen into

disrepute, although some of this philosophy is retained in the current taxonomic scheme. There is a wide range in the efficiency of the symbiosis. Estimates for the amounts of nitrogen fixed are summarized in Table 1.

The bacteria of the family Rhizobiaceae as cells without endospores are normally rod-shaped, motile, with one polar or subpolar flagellum or two to six peritrichous flagella, aerobic, and gram-negative, with many carbohydrates utilized. Considerable extracellular slime is usually produced during growth on carbohydrate-containing media. Some strains of rhizobia and *Agrobacterium* show a close relationship in DNA base composition.

Traditionally, rhizobia have been divided into two groups according to growth rate. The term "fast growers" commonly refers to rhizobia associated with alfalfa, clover, bean, and pea because, in culture, these grow much faster (less than one-half the doubling time of "slow growers," or <6 hours) than the "slow growers" exemplified by soybean and cowpea rhizobia (generation time >6 hours). Although there is phenotypic and genotypic diversity within these major groupings, and some overlap, numerous studies have demonstrated the validity of this approach.

The relative fastidious nutrition of the slow growers has been substantiated by more recent studies. While the major biochemical pathways seem to be similar, evidence suggests that the preferred pathway may be different. 16S RNA analysis of the fast and slow growing rhizobia confirmed that these groupings indeed represent different genetic phyla since the similarity coefficient (S_{AB}) of the RNA is 0.53. Thus, with gene analysis, the fast and slow growers, again, fall into widely separate groups. Recent findings using numerical taxonomy, carbohydrate metabolism, antibiotic susceptibilities, serology, DNA hybridization, RNA analysis, and DNA base ratio all

Table 1. Nitrogen fixed by pulses (after Elkan, 1992).

Plant	Avg	Range
	------kg N/ha------	
Vicia faba (faba beans)	210	45-552
Pisum sativum (peas)	65	52-77
Lupinus spp. (lupines)	176	145-208
Phaseolus aureus (green gram)	202	63-342
Phaseolus aureus (mung)	61	—
Cajanus cajan (pigeon pea)	224	168-280
Vigna sinensis (cowpea)	198	73-354
Canavalia ensiformis (jack bean)	49	—
Cicer arietinum (chickpea)	103	—
Lens culinaris (lentil)	101	88-114
Arachis hypogaea (peanut)	124	100-152
Cyamopsis tetragonolobus (guar)	130	41-220
Glycine max (soybeans)	95	85-155

Table 2. Differences between fast and slow growing rhizobia (after Elkan, 1992).

Characteristic	Rhizobial type[a]	
	Fast growing	Slow growing
Generation time	<6 hours	>6 hours
Carbohydrate substrate	Uses pentoses, hexoses, & mono-, di-, & trisaccharides	Uses pentoses and hexoses solely
Metabolic pathways	EMP—Low activity Strain-specific ED—Main pathway TCA—Fully active PP pathway present	EMP—Low activity ED—Main pathway TCA—Fully active Hexose cycle present
Flagellation type	Peritrichous	Subpolar
Symbiotic gene location	Plasmid & chromosome	Chromosome only
Nitrogen-fixing gene location	*nif* H, D, & K on same operon	*nif* H, D, & K on separate operons
Intrinsic antibiotic resistance	Low	High

[a]EMP, Embden-Meyerhoff-Parnas pathway; ED, Entner-Doudoroff pathway; TCA, tricarboxylic acid cycle; PP, pentose phosphate pathway.

demonstrated the validity of the fast and slow growing groupings. A summary of some of the differences between these groups is found in Table 2.

On the basis of the differences between the fast and slow growing rhizobia, the traditional rhizobia were divided into two genera. The slow growing strains were placed in the genus *Bradyrhizobium* comprising two species—*B. japonicum* and *B. elkanii*, which nodulate soybeans. Other bradyrhizobia occur (e.g., the peanut bradyrhizobia) but have not been classified to species or biovar levels. Researchers suggested that until further taxa within the genus are proposed, these should be described with the appropriate host plant given in parentheses [i.e., the peanut rhizobia *Bradyrhizobium* sp. (*Arachis*)].

The fast growing rhizobia have been placed in the genus *Rhizobium* comprising nine species—*R. leguminosarum, R. meliloti, R. loti, R. galegae, R. fredii*, and *R. tropici, R. huakuii, R. etli*, and *R. ciceri*. Three former species—*R. phaseoli, R. trifolii*, and *R. leguminosarum*—have been combined into the species *R. leguminosarum*. *Rhizobium fredii* is a new species consisting of fast growing rhizobia that effectively nodulate Chinese soybean cultivars ordinarily nodulated by *B. japonicum*. This species of *Rhizobium* has recently been assigned to a new genus, *Sinorhizobium*, with the type species being *Sinorhizobium fredii*. A new genus, *Azorhizobium* with one species, *A. caulinodans* containing the stem nodule-forming rhizobia, was

Table 3. Current taxonomic classification of the rhizobia.

Recognized genus	Recognized species
Bradyrhizobium (Jordan, 1982)	*B. japonicum* (Jordan, 1982)
	B. elkanii (Kuykendall *et al.*, 1992)
Rhizobium (Jordan, 1984)	*R. leguminosarum* (Jordan, 1984)
	R. leguminosarum bv. *phaeseoli* (Jordan, 1984)
	R. leguminosarum bv. *trifolii* (Jordan, 1984)
	R. leguminosarum bv. *viciae* (Jordan, 1984)
	R. meliloti (Jordan, 1984)
	R. loti (Jordan, 1984)
	R. fredii (Scholla *et al.*, 1984)
	R. galegae (Lindstrom, 1989)
	R. tropici (Martinez-Romero *et al.*, 1991)
	R. huakuii (Chen *et al.*, 1991)
	R. etli (Segovia *et al.*, 1993)
	R. ciceri (Nour *et al.*, 1994)
Azorhizobium (Dreyfus *et al.*, 1988)	*A. caulinodans* (Dreyfus *et al.*, 1988)

recently recognized. The current taxonomic scheme is summarized in Table 3.

The taxonomy of the rhizobia is in a state of transition. As more molecular information accumulates, such genetic data will, no doubt, further displace cross-inoculation approaches to classification. The present scheme, however, does function to allow generalized identification of isolates.

NODULE FORMATION

Nodule initiation and subsequent maturation is an interactive process involving the eukaryotic host legume and the prokaryotic *Rhizobium*. The process is complex, resulting in biochemical and morphological changes in both symbionts and leading to the capacity of reducing atmospheric nitrogen.

Initially, the proper *Rhizobium* species proliferates in the root zone of a temperate leguminous plant and becomes attracted and attached to the root hair. A poorly described chemotactic response attracts the rhizobia to the root surface. At the surface, the bacteria alter the growth of epidermal root hairs so that they are deformed. In some semi-tropical legumes such as peanut (*Arachis*), root hairs are not the primary invasive sites, but the alternative invasion process is not well described, although infection is reported at the site of lateral root emergence.

The generalized root hair infection process consists of several events leading to nodule formation. These have been summarized as follows: (a) recognition by the rhizobia of the legume, (b) attachment to the root hair, (c) curling of the root hair, (d) root hair infection by the bacteria, (e) formation

of an infection thread, (f) nodule initiation, and (g) transformation of the vegetative cells in the nodules to enlarged pleomorphic forms called bacteroids, which fix nitrogen. This sequence is especially characteristic of the fast growing rhizobia (Elkan, 1992). In some legumes, such as peanut, there are no prominent root hairs, and infection is initiated at the axial region of branching roots (R. W. Gibbons, unpubl. data, 1977).

Based on morphology, there are two kinds of nodules: determinative and indeterminative. In general, indeterminative nodules are formed by fast growing rhizobia and are characterized by a defined meristem during nodule growth. Determinative nodules arise from cortex tissue. Legumes nodulated by *Bradyrhizobium* form determinative nodules close to the endodermis, which is near the xylem poles in the root.

The formation of nodules on legumes is the result of a coordinated development involving many plant and bacterial genes. Studies of the nodulation (*nod*) genes of rhizobia have depended on the development of molecular genetic tools. Many of the genes involved in the nodulation process have been located and identified.

INOCULATION

Only a few years after Beijerinck's (1888) success in isolating and growing rhizobia in pure culture, Nobbe and Hiltner received a patent on a process for inoculating legumes or, alternatively, mixing the culture with some soil, which was then mixed with the legume seed (U.S. Letter Patent 570,813, 1895). Today, estimates are that the inoculum industry in the U.S. is worth about $15 m/annum. While this is a small market, many biotechnology companies have been emphasizing biological nitrogen fixation research. Using current technology and available strains, often it is difficult to demonstrate a response from inoculation in excess of the rate of nitrogen fixation resulting from the indigenous microflora.

THE PEANUT-RHIZOBIAL SYMBIOSIS

Domesticated peanuts belong to that large group of tropical legumes, including many common native weeds nodulated by *Bradyrhizobium* strains belonging to the so-called "cowpea miscellany." Thus, there is generally found an indigenous population of rhizobia potentially able to nodulate an introduced peanut crop. As a result, often no positive effects have been reported, under field conditions, when commercial inoculants were used with peanuts; analysis of the nodules shows that the inoculant often is not competitive with the indigenous rhizobia.

Peanuts and their microsymbionts are only moderately efficient in fixing and translocating atmospheric nitrogen. In grain legumes, as measured by ^{15}N isotope tracer, as much as 80% of the plant protoplasmic nitrogen comes from atmospheric N_2. In common beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.), considered rather poor fixers, only about 40% of the plant nitrogen is derived from atmospheric N_2. In peanuts, considered intermediately effective, about 55% of the plant nitrogen needs are delivered

from air via nitrogen fixation (Hardarson, 1993). Thus, in peanuts there is the potential of enhancing N_2 fixation to the level found in the more effective associations.

Information is generally lacking as to the reasons for the relatively low efficiency of atmospheric nitrogen fixation in peanuts, but it was reported by Osman *et al.* (1983) that differences in nodule weight, nodule number, and nitrogenase activity between peanut cultivars could be attributed to differences in leaf area and leaf weight between these cultivars. Furthermore, modest defoliation treatment resulted in decreases in nitrogen fixation traits. Since the photosynthetic energy supply depends mainly on leaf area (Fred and Wilson, 1934; Boote *et al.*, 1980), it is concluded that the availability of energy limits the extent of nitrogen fixation. However, the rate of nitrogen fixation in peanuts is controlled by more than just the availability of photosynthate energy. There are interactions between nitrogen availability and the partitioning of the photosynthate into various sinks, but the mechanisms of the control functions are unknown (Boote *et al.*, 1980).

Factors Affecting Symbiotic Nitrogen Fixation in Peanuts

Various environmental factors such as temperature, moisture, acidity, and availability of chemical components such as calcium, phosphorus, and molybdenum all influence biological nitrogen fixation (FAO, 1984). The detrimental effects of soil acidity on legume-*Bradyrhizobium* symbiosis have been attributed to the direct effect of pH or H^+ concentration, indirect effects such as toxicities of aluminum and/or manganese, and deficiencies of calcium, phosphorus, and molybdenum (Lie, 1971; Munns, 1976; Andrew, 1978). An imbalance in the supply of essential elements also has been implicated (Robson, 1978). Since low pH usually reduces nodulation, this may be due to sensitivity of the infection process (Date, 1981) or reduced growth and reproduction of soil rhizobia (Mulder *et al.*, 1977; Rice *et al.*, 1977). The application of lime to reduce acidity and toxicities of aluminum and/or manganese and to increase the availability of calcium, magnesium, phosphorus, and molybdenum and of essential nutrients generally improves nodulation, N_2 fixation, and production of legumes in acid soils (Kamprath, 1978).

Improved yield and pod fill of peanuts have been related to the availability of calcium in the pegging zone (Cox *et al.*, 1982). In three greenhouse experiments, growth and symbiotic N_2 fixation of peanuts was evaluated using soil treated with aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$] and calcium oxide (CaO) to produce various levels of pH and Al content. Nodulation and N_2 fixation was inhibited when grown at pH 3.8 or 3.9 treated with 2 mEq $Al_2(SO_4)_3 \cdot 18H_2O$/100 g soil or its $(SO_4)_3$ equivalent of 960 ppm sulfuric acid (H_2SO_4). Shoot growth was also drastically reduced and root damage typical of Al toxicity was observed. As pH was increased by CaO treatments, shoot and root growth, nodulation and N_2 fixation improved. Maximum peanut root growth occurred at pH 7.3. Shoot growth, nodulation, and N_2 fixation of peanuts and cowpeas were best in the pH 5.9-6.3 range (Chong *et al.*, 1987).

Often it is difficult to determine if these effects on nodulation or fixation are due to influences on the microsymbiont, host cultivar or the interaction of both. In most cases, however, the peanut cultivar is more sensitive than the rhizobia and, thus, the environmental stress factor is alleviated without primary regard to subsequent nitrogen fixation. However, temperature and moisture extremes can affect inoculation success and survival of rhizobia in soil with high temperatures. Differences in optimum growth temperatures and temperature tolerances have been reported for peanut rhizobia (Elsaeid *et al.*, 1990; Kishinevsky *et al.*, 1992) so that it is desirable to test and select the proper rhizobia for temperature tolerance. This will help insure persistence in soil of the strain used. High temperature also limits fixation rates. However, there has been little work done to identify peanut rhizobia able to effectively fix at temperature extremes.

Assuming that biological nitrogen fixation can be increased in peanuts, several approaches have been considered. These include (a) selection and genetic manipulation of the *Bradyrhizobium* strain, (b) manipulation of the host genotype, (c) determining and exploiting a cultivar-by-strain interaction, and (d) improving inoculation technology.

Selection and Genetic Manipulation of the *Bradyrhizobium* Strain

There are well defined characteristics for useful inoculant strains of rhizobia (Brockwell *et al.*, 1982). These include (a) effective N_2 fixation over a range of environmental conditions, (b) competitive ability against other strains, (c) persistence in soil, (d) survival in seed pellets, (e) ability to survive in stored in oculum, (f) ability to colonize in soil, (g) ability to survive adverse environmental conditions, (h) stability during storage, and (i) nodule formation and N_2 fixation in the presence of soil nitrogen. These represent the major requirements to be considered in studies designed to optimize biological nitrogen fixation.

Peanuts form a symbiosis with many *Bradyrhizobium* strains of the "cowpea miscellany." Using DNA-DNA hybridization analysis, Ligon *et al.* (1981) found great diversity between 83 strains of these bradyrhizobia and thus concluded that peanuts are nodulated by a large group of unrelated slow growing rhizobia and, further, no subgrouping could be identified of bradyrhizobia preferentially nodulating peanuts. It has been reported many times, however, that peanuts are more selective as to effective nitrogen fixation with rhizobial strains. Some of the more promising strains have been isolated from other genera of legumes. A useful technique for isolating, identifying, and testing through single strain evaluation in plant tests conducted in the greenhouse has been reported (Elkan *et al.*, 1981). Specific host strain combinations thus identified test the nitrogen fixation potential of the symbiosis in the absence of the confounding influences of native soil rhizobia and under less complex environmental conditions. The results cannot always be extrapolated to field conditions. However, Elkan *et al.* (1981) determined nodulation and nitrogenase activity for 48 diverse peanut genotypes using rhizobial strains in single strain inoculations in a field site where the soil supported high populations of indigenous rhizobia. These

same rhizobia and genotypes had previously been evaluated in a greenhouse study (Elkan *et al.*, 1980). Variation in nodulation and nitrogenase activity for the single strain isolates in the presence of naturally occurring field populations indicated that the strains were competitive. Strains both less and more effective than the indigenous population were observed. Nitrogenase activity of the strains correlated with the previous greenhouse results. Alwi *et al.* (1989) tested 29 *Bradyrhizobium* strains against 16 cultivars and found that not only could superior strains be identified, but also that some of these strains were symbiotically effective with all cultivars tested while others ranged in degree of specificity. Traits useful for inoculants are high nitrogen fixation potential, competitiveness under field conditions, and effectivity with many potential peanut cultivars. In order to reduce testing required to identify specific host-strain combinations that maximize N_2 fixation, Alwi *et al.* (1989) developed a statistical design using numerical taxonomy of morphological characters to group peanut cultivars, eliminating the need to test individual cultivars. He was able to establish four groups of peanut cultivars and five groups of rhizobial strains and concluded that the amount of plant testing (Elkan *et al.*, 1981) could be reduced considerably by first classifying the symbionts into groups. A number of strains identified in these tests are now being used in commercial peanut inoculants.

Evidence is emerging that rhizobia do more than act as a nitrogen source for peanuts. One strain, *Bradyrhizobium* sp. NC92, increases yield under field conditions with several peanut cultivars (Nambiar *et al.*, 1984; ICRISAT, 1987). Yet, there is no evidence that this strain fixes more nitrogen than the control strains. In fact, it has been reported by Wilson *et al.* (1987) that use of a nonfixing mutant of NC92 still resulted in the yield increase. The mechanism of action, although under investigation, is unknown. Grimm (1987) presented evidence that specific *Bradyrhizobium* strains can affect the types of nitrogen translocation products produced in peanuts, thereby influencing the chemical composition of the seed. The data suggest that specific *Bradyrhizobium* strains can affect the composition and content of the free amino acids in peanut seed but do not significantly alter the free sugar or lipid fatty acid composition.

Genetic improvement of nitrogen-fixing bacteria is a goal of bacteriologists and genetic techniques such as conjugal transfer, chromosome mobilization, and phage-mediated transduction have been developed for rhizobia. In fast growing *Rhizobium*, the nitrogen fixation genes (*nif*) and nodulation genes (*nod*) are closely linked and localized on large indigenous plasmids known as *Sym* plasmids. In slow growing *Bradyrhizobium*, linkage of *nif* and *nod* gene clusters are not yet established; thus most of the genetic constructs have been with the fast growing rhizobia (in altered host range, etc.). Even among the fast growers, increasing N_2 fixation is not easy. For example, Barbour and Elkan (1989) were able to make deletion mutants with lowered fixation rates but, when they added extra copies of *nif* genes, activity did not increase beyond the original level. The problem is that there are many regulatory genes, both plant genes and rhizobial genes, that signal development of nodules and trigger *nif* functions. These are not necessarily clustered with

nif and *nod* genes and, therefore, are not transferred in the constructs.

Manipulation of the Host-Genotype

Improvement of symbiotic nitrogen fixation has focused traditionally on *Rhizobium* strain selection; however, since an estimated 50% of the symbiosis is plant-controlled, manipulation of the host genotype can affect biological nitrogen fixation. The development of the symbiosis is dependent upon the exchange of low molecular weight signal molecules between the plant and rhizobia. Ample variability among peanut genotypes exists to accept effectively different rhizobial strains. There are generic differences so that a given *Bradyrhizobium* strain will be more effective on one cross-inoculation legume (for example, cowpea) than on peanut. Differential responses are expressed within peanuts so that virginia-type parents are generally superior to spanish and valencia types for symbiotic nitrogen fixation traits. Nonnodulating genotypes also exist with genetic control attributed to two recessive genes (Nigam *et al.*, 1982).

Significant variation in nodulation and nitrogen fixation among peanut genotypes in the field was first reported by Duggar (1935a,b) and similar results for nodulation and nitrogen fixation rates have been observed more recently (Burton, 1976; Nambiar and Dart, 1980; Wynne *et al.*, 1981). Generally, virginia-type cultivars are more heavily nodulated and fix more nitrogen than either spanish or valencia types, although there is considerable variation between cultivars within these types for nitrogen fixation traits (Wynne *et al.*, 1978, 1980; Nambiar and Dart, 1980).

Genetic estimates for traits related to N_2 fixation have been reported for several peanut populations. Nonnodulating genotypes were observed in F_2 progeny from two single crosses (Nigam *et al.*, 1982). Control of nodulation was found to be conditioned by duplicate recessive genes (Nigam *et al.*, 1982). Analysis of the F_1 generation of a diallel cross of 10 nodulating South American cultivars indicated specific combining ability was significant and accounted for more variability than general combining ability for nodule number and weight, specific nitrogenase activity, shoot weight, and total plant nitrogen (Isleib *et al.*, 1980). Maternal effects also were important for these traits. Among-family variance was generally significant for nodule number and weight, nitrogenase activity, and shoot weight but not specific nitrogenase activity when traits were measured on F_2-derived families evaluated in the F_5 and F_6 generations (Arrendell *et al.*, 1985). Selection for high and low nitrogenase activity or shoot weight effectively differentiated groups based on nitrogenase activity and shoot weight, respectively; however, the indirect effect of selection on traits used to classify a genotype with respect to botanical variety (e.g., growth habit) was unclear (Arrendell *et al.*, 1986).

These and other studies all indicate that there is ample variability among peanut cultivars and thus potential to increase biological nitrogen fixation in the peanut through conventional plant breeding methods. Differences in nodulation and N_2 fixation between virginia, spanish, and valencia peanuts were found to be heritable and controlled primarily by general combining

ability (Duggar, 1935a,b). The plant factors responsible for the variations observed have not been well determined. In one of the few studies reported, field experiments involving eight peanut cultivars representing the three botanical varieties were established to generate 12 growth analysis traits for each cultivar as described by Kvet *et al.* (1971) and to determine the effects of these plant traits on nodule number, nodule dry weight per plant, and nitrogen fixation (Wynne *et al.*, 1981, 1983). When the 12 growth analysis traits were analyzed using multiple regression and used as independent variables and regressed on the dependent variable, N_2 fixation, leaf area duration accounted for more than 70% of the variation in N_2 fixation. The only other trait accounting for a significant variability in N_2 fixation was leaf area index (Wynne *et al.*, 1981, 1983).

Cultivar-by-Strain Interaction

Nodulation and subsequent nitrogen fixation involves a series of interactions between the symbiotic partners, which requires coordinated and sequential expression of specific plant and bacterial genes.

Peanut roots, grown axenically, do not appear morphologically distinctive from other plant roots so that the ability of these plants to respond to microbial signals and then alter their metabolism to form nodules is not explained just by morphology. It has been conclusively demonstrated that genetic information from both symbionts controls nodulation and the host range of nodulation by a *Rhizobium* species (Barbour *et al.*, 1992). Metabolically, there are three types of nodules (often termed effective, inefficient, and ineffective). Effective nodules contain a high density of rhizobia which are actively fixing dinitrogen. Inefficient nodules may contain a similar density of the bacteria, but only a low level of fixed dinitrogen results from the symbiosis. Ineffective nodules result from a symbiosis with bacteria which are not able to carry out nitrogen fixation. Because the regulatory roles of the plant and bacterial genes in nitrogen fixation have not been generally elucidated, the reasons for differential nitrogen-fixing ability of nodules remains obscure. There is, however, a generally observed legume host-*Rhizobium* interaction that results in optimization of dinitrogen fixation. Such an interaction makes it possible to optimize dinitrogen fixation under field conditions in a cultivar through introduction of an effective *Rhizobium*. The identified plant and bacterial genes and their roles in the symbiosis have recently been extensively summarized and reviewed (Brewin *et al.*, 1992; Downie and Brewin, 1992; Elkan, 1992).

The most productive approach to optimizing nitrogen fixation then would seem to involve manipulating the peanut cultivar and *Bradyrhizobium* strain together as one system. Burton (1976) demonstrated a host x strain interaction even though peanut generally is considered to be promiscuous and effective with many cowpea miscellany *Bradyrhizobium* strains (Date and Haliday, 1980). Wynne *et al.* (1983) found significant host-strain interactions for nodule number and weight, nitrogenase activity, and plant color when six peanut genotypes and 10 *Bradyrhizobium* strains were used in a greenhouse

study. Several other greenhouse studies at North Carolina State University have shown cultivar-strain interactions (Wynne *et al.*, 1983). Nambiar and Dart (1980) reported significant pod yield increases with the peanut genotype Robut 33-1 and *Bradyrhizobium* NC92. These yield improvements were observed for several seasons and were demonstrated at several locations. Additional tests in Gujarat, India, and Cameroon have produced yield increases using the strain NC92.

SUMMARY

Improvement of symbiotic nitrogen fixation in peanut (*A. hypogaea*) is complicated because the genetic systems of both the plant and the *Bradyrhizobium* symbiont are involved. Nitrogen fixation is influenced by the host plant, *Bradyrhizobium* strain, environment, and interactions between these factors. Even though peanuts in the field are usually well nodulated by indigenous rhizobia, the symbiosis only supplies 55-60% of the nitrogen required for optimum growth (as compared to the more efficient grain legumes, such as faba beans, which derive nearly 80% of their nitrogen needs from atmospheric N_2). Thus, there is a need for optimizing nitrogen fixation in peanuts. Considerable genotypic variability for traits associated with nitrogen fixation potential have been observed in the field to allow breeding procedures for enhanced nodulation and nitrogen fixation. Similar variation has been found among *Bradyrhizobium* sp. isolates, making it desirable to select more efficient strains for the symbiont hosts. Since specific cultivar x *Bradyrhizobium* sp. interactions have been observed, co-manipulation of the cultivar and bacterium offers the best approach to optimizing biological nitrogen fixation in designated cultivars.

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