

Role of Symbiotic Nitrogen Fixation in the Improvement of Legume Productivity under Stressed Environments

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Abstract

The inclusion of a legume in a cropping system does not always ensure the attainment of optimal levels of symbiotic nitrogen fixation (SNF) in the field. Several environmental factors including drought, temperature, and soil nutrient status are known to dramatically affect the process at molecular/functional level and thus play a part in determining the actual amount of nitrogen fixed by a given legume in the field. This paper reviews the status of SNF in response to most significant environmental constraints, and focuses on specific cases of harnessing SNF by improving its tolerance to stress factors with the aim of enhancing system productivity. Several examples are discussed, including selecting legume crops tolerant to drought and salinity and/or allowing high biomass production and solubilization of phosphorus, identifying high nitrogen-fixing and nitrate-N tolerant genotypes and including them in relevant cropping systems, and making changes in agronomical management practices for the better integration of legumes in cropping systems. Finally, a general framework is discussed for agro-physiological contributions that can help overcome SNF limitation where this is caused by environmental constraints. The on-farm application of these knowledge-based SNF technologies will strengthen the role of N-fixing legumes in cropping systems.

Introduction

Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining the fertility of marginal lands and in the smallholder systems of the semi-arid tropics. It is anticipated that the importance of legumes and SNF will continue to expand with the increasing development of sustainable agricultural practices. The first step toward maximizing SNF technologies is to increase the land area under legumes and enhance their grain and fodder yields through overcoming environmental limitations of SNF and legume productivity.

Substantial qualitative information is available on the net benefits of SNF and its residual effects on grain, herbaceous, and tree legumes.

However, SNF by legumes is particularly sensitive to various environmental stresses such as drought, waterlogging, soil salinity or acidity, temperature, insect pests, diseases, and low phosphorus (P) and other nutrient limitations (see Zahram, 1999 for a review). Consequently, legume productivity can be greatly depressed if subjected to these environmental constraints. For instance, the sensitivity to drought and salt stress of both establishment and activity of the legume-*Rhizobium* symbiosis has long been recognized (Wilson, 1931; Bernstein and Ogata, 1966). Although drought and salinity effects on N₂ fixation have been extensively studied in several legume species, the physiological mechanisms involved in the inhibition are still poorly

understood. The N₂-fixing legume plants usually require more P than plants dependent on mineral N fertilizer. Nodule establishment and function are important sinks for P, and nodules usually have the highest P content in the plant. Therefore, P deficiency conditions result in reduced SNF potential and P fertilization will usually result in enhanced nodule number and mass, as well as greater N₂-fixation activity per plant.

This paper focuses on the agro-physiological constraints that limit SNF potential, and the agro-physio-genetic resilient traits associated with legume genetic tolerance as well as management options of abiotic stresses. Information provided about some of the candidate mechanisms will strengthen the knowledge base for initiating genetic manipulation and eventual gene transfer to enhance the productivity of legumes in the semi-arid environments. Moreover, such knowledge will facilitate development of appropriate management options for harnessing the benefits of increased SNF contributions in these systems.

Environmental stress affecting SNF processes

Most stress factors influence all physiological processes in plants as the stress develops (Table 1). They influence all aspects of nodulation and symbiotic N₂-fixation, in some cases reducing rhizobial survival and diversity in soil, in others essentially affecting nodulation and nitrogenase activity. It is often difficult to isolate the effects of the stress factors on the success of inoculation from their effects on symbiosis functioning and nitrogen-fixation. The most important stresses include abiotic factors such as drought and salinity, waterlogging, temperature, soil acidity, and inadequate mineral nutrition (Table 1), and biotic factors such as insect pests and diseases. A critical question with regard to N₂-fixation is whether the stresses first affect other physiological processes, which

then influence N₂-fixation, or whether the stress initially and directly affects N₂-fixation mechanisms. Physiological understanding of the most stress-sensitive steps is also essential for establishing strategies for crop improvement and adequate management practices to optimize legume N₂-fixation and increase its role in cropping systems. For instance, N₂-fixation has been found to be more sensitive to soil dehydration than leaf gas exchange (Sinclair *et al.*, 1987; Djekoun and Planchon, 1991), nitrate assimilation (Purcell and King, 1996) and dry matter accumulation (Sinclair *et al.*, 1987; Wery *et al.*, 1994). Similarly, several studies have shown that N₂ fixation was more sensitive to salt stress than plant growth (Delgado *et al.*, 1994; Serraj and Drevon, 1998).

The existence of genetic variability in tolerance to most environmental stress factors has been shown in both legume host plants and their respective rhizobial strains (see review by Hungria and Vargas, 2000). This suggests the possibility of overcoming, at least partly, the environmental constraints limiting legume SNF potential. Success in decreasing the sensitivity of legumes to environmental stress would be achieved by focusing on both partners of the symbiosis, although it is generally agreed that *Rhizobium* strains are relatively more tolerant than the corresponding host plants (e.g., in the case of salt tolerance, Singleton *et al.*, 1982). Similarly, compared to host plants, rhizobial strains are quite resistant to soil desiccation, and can survive in water films surrounding soil particles (Williams and de Mallorca, 1984).

The current challenges are to understand the mechanisms responsible for stress sensitivity at the level of the whole plant and to improve the tolerance of N₂-fixation to abiotic and biotic stress factors. Assessing the genetic diversity of legumes in terms of SNF potential, in order to screen and utilize available legume germplasm for efficient SNF is an important

TABLE 1

Effects of major environmental constraints on symbiotic nitrogen fixation. Number of papers found in databases and effects of various constraints on legume-Rhizobium establishment and functioning processes.

Constraint	Number of References (Agricola, 1979-2003 and CAB Abstracts, 1973-2003) ^a	Processes affected			Key references and reviews
		<i>Rhizobium</i> survival	Nodule formation & growth	N ₂ -Fixation	
Light	265	–	* (b)	**	Hirsch and McFall-Ngai, 2000, Swaraj <i>et al.</i> , 2001; Walsh, 1995.
Temperature	328				
Low		**	***	**	Rennie and Kemp (1982); Day <i>et al.</i> , (1978); Piha and Munns (1987b); Roughley <i>et al.</i> , (1995)
High		**	***	**	
Carbon dioxide	66	*	***	**	Hardy and Havelka, 1976; Hebeisen <i>et al.</i> , 1997; Cabrerizo <i>et al.</i> , 2001; Serraj <i>et al.</i> , 1998b
Oxygen	221	–	**	***	Minchin 1997; Walsh <i>et al.</i> , 1989; Walsh, 1995
Drought (or water stress)	341	*	**	***	Serraj <i>et al.</i> , 1999b, Sinclair <i>et al.</i> , 1987, Williams and de Mallorca, 1984
Salinity	149	*	**	***	Serraj <i>et al.</i> , 1998a; Singleton <i>et al.</i> , 1982; vanHoorn <i>et al.</i> , 2001
Nitrate	655	*	***	***	Herridge <i>et al.</i> , 1994; Streeter, 1988; Walsh, 1989
Soil acidity	498	**	**	**	Cooper 1988; Glenn <i>et al.</i> , 1998; Hungria and Vargas, 2000
Low phosphorus	136	–	**	***	Cassman <i>et al.</i> , 1981; Israel and Rufy, 1988; Vance, 2001; Sinclair and Vadez, 2002
Micronutrients	20	–	**	**	O'Hara <i>et al.</i> , 1993; Ali and Mishra, 2000; Giller, 2001

(a) Search with key words 'legume' and 'Nitrogen fixation', combined with the various constraints.

(b) The (*) symbols indicate relative level of process sensitivity to the constraint.

first step. This may also offer a critical resource in physiological investigations and plant breeding efforts targeted at increasing SNF in relevant cropping systems.

Drought stress

Legume productivity in the semi-arid tropics (SAT) is largely limited by low soil moisture availability in addition to nutrient deficiencies. The relatively high sensitivity of nitrogen and biomass accumulation to soil dehydration under field conditions was demonstrated for soybean (*Glycine max* L.) grown on a soil with virtually no mineral N reserve (Sinclair *et al.*, 1987; Serraj and Sinclair, 1997). With essentially all N uptake resulting from N₂-fixation, a comparison of biomass accumulation and N accumulation rates offered an index of the relative sensitivity to water-deficit conditions under which the plants were grown. Sinclair *et al.* (1987) concluded from their study on soybean that N₂-fixation was more sensitive to drought than was carbon assimilation. In a similar study on 24 soybean lines, Serraj and Sinclair (1997) found that, in almost all the soybean cultivars tested N accumulation was more sensitive to soil dehydration than was biomass accumulation.

The fact that N₂-fixation is more sensitive to decreasing soil water content relative to leaf gas exchange is an important constraint on N accumulation and the yield potential of legumes subjected to soil drying (Sinclair *et al.* 1987; Wery *et al.*, 1994). For cool-season food legumes such as chickpea (*Cicer arietinum* L.), Beck *et al.*, (1991) concluded that even if the drought stress effects on N₂-fixation do not always directly affect grain yield, drought may result in a significant decrease in the total N balance. Loss of N₂-fixation under water deficits would then reduce the advantage of using legume crops in rotations, for green manuring, and for soil fertility improvement.

Although it is recognized that drought-tolerant varieties have evolved with different

traits, the traits most often specified are those of roots. A deeper root system with enhanced water uptake capacity is considered synonymous with drought avoidance in many crops (Gregory *et al.*, 1994). Thus, legumes with deep root systems are preferentially grown in climates with limited rainfall, where they can withstand prolonged periods of drought.

Substantial efforts have been devoted to selecting and breeding legumes tolerant to drought, but with very little success because of the complexity of the genes controlling drought. More important, the simulated drought environment in which screening is conducted is often not well-defined and therefore not reproducible. Accurate field phenotyping of mapping populations for traits associated with drought tolerance requires extra efforts in conceptualization, design, and management of phenotyping programs, to maximize the chances of identifying quantitative trait loci (QTL) that will be useful in future improvement of tolerance in the target crop and the target environments (Bidinger, 2001). Establishing screening conditions representative of the larger environment is difficult, involving major trade-offs between providing representative day length, vapor pressure, and temperature conditions.

The variability of N₂ fixation sensitivity to drought has been analyzed with several grain legumes including soybean, cowpea (*Vigna unguiculata* L.), black gram (*Vigna mungo* L.), chickpea, common bean (*Phaseolus vulgaris* L.), fababean (*Vicia faba* L.), lupin (*Lupinus albus* L.), pea (*Pisum sativum* L.), and peanut (*Arachis hypogaea* L.) (Sinclair and Serraj 1995). The results obtained from soybean and cowpea showed that the sensitivity of acetylene reduction activity (ARA) in these species to soil drying was greater than transpiration in nearly all cases. Surprisingly, all other grain legumes showed that ARA was less sensitive to water deficits than was transpiration during the water-deficit period (Sinclair and Serraj,

1995). The drought tolerance trait was associated with the biochemical form of N exported by the nodules, with ureide transporters being more sensitive than amide producers.

Serraj *et al.* (1999a) investigated the inhibition of N₂-fixation in soybean due to water deficits and showed that ureides (allantoin and allantoic acid) were involved in the sensitivity to drought. Consistent with this observation, variability in N₂-fixation sensitivity among legume species and cultivars to water deficit has been associated with the amount of ureides that they accumulate (Serraj *et al.*, 1999a). Further evidence of the importance of ureides in the sensitivity of N₂-fixation to water deficit is the substantial increase observed in ureide concentrations in soybean shoots and nodules upon soil drying (Purcell *et al.*, 1998; Serraj *et al.*, 1999b).

Other promising and under-utilized legume species include "arid legumes" such as mothbean (*Vigna aconitifolia*), tepary bean (*Phaseolus acutifolius*), clusterbean (*Psophocarpus tetragonolobus*), horsegram (*Dolichos biflorus* L.), Bambara groundnut (*Vigna subterranea*) and cowpea (*Vigna unguiculata* L.), which are all well-adapted to arid and semi-arid areas under very limited water resource conditions. However, despite their potential importance in sustainable agriculture in drylands, few studies have focused on the factors limiting the production and wider use of these legume species in the arid and semi-arid zones.

Salinity

It was reported that rhizobia can generally tolerate a higher level of salinity than the host legume (Singleton *et al.*, 1982). Fast-growing rhizobial strains are more salt-tolerant than slow-growing ones. Subbarao (1990) observed significant differences among pigeonpea (*Cajanus cajan*) *Rhizobium* strains in their ability to nodulate and fix nitrogen with a pigeonpea genotype under saline conditions, and further observed that nodule initiation was the most

salt-susceptible aspect of pigeonpea growth. Wild pigeonpea species (*Cajanus platycarpus* and *C. albicans*) have been reported to tolerate salinity up to 12 dS m⁻¹, compared to 6 dS m⁻¹ for cultivated species (ICPL 227). Mechanisms for salinity tolerance in pigeonpea involve exclusion of Na⁺ and Cl⁻ ions from the shoot and the maintenance of high K levels.

The effects of salinity on biomass and N accumulation in alfalfa were compared in plants fed with nitrate (NO₃) or dependent on N₂ fixation (Serraj and Drevon, 1998). NaCl inhibited N accumulation in both NO₃-fed plants and N₂-fixing plants, which was seen as a decrease in N concentration (%N in plant biomass). The decrease was larger for N₂-fixing plants than for NO₃-fed plants (Fig. 1). The %N in NO₃-fed plants was not affected by low concentrations of NaCl (up to 50 mM), whereas N₂-fixing plants showed a significant decrease in %N with increasing levels of NaCl. Below 50 mM NaCl, %N was about 100% of that in the control plants for NO₃-fed plants and 75% of %N in N₂-fixing plants. The absence of a significant effect of low NaCl concentrations on %N in NO₃ fed plants supports previous reports showing inhibitory effects of NaCl on growth without any decrease in %N (Pessaraki and Zhou, 1990; Cordovilla *et al.*, 1995). This contrasts with the relatively large effect of NaCl on %N content of N₂-fixing plants (Serraj and Drevon, 1998). Therefore, N accumulation appears to be more salt sensitive in N₂-fixing plants than in NO₃-fed plants, demonstrating the higher sensitivity to salt stress in N₂-fixing plants.

A recent study of four grain legumes including broadbean (*Vicia faba* L.), chickpea, lentil (*Lens culinaris*) and soybean confirmed the effects of soil salinity on crop yield, total nitrogen uptake and nitrogen fixation (van Hoorn *et al.*, 2001). The existence of inter- and intraspecific variability in the sensitivity of N₂ fixation to salinity has also been reported in legumes (Serraj *et al.*, 1998a, 2001).

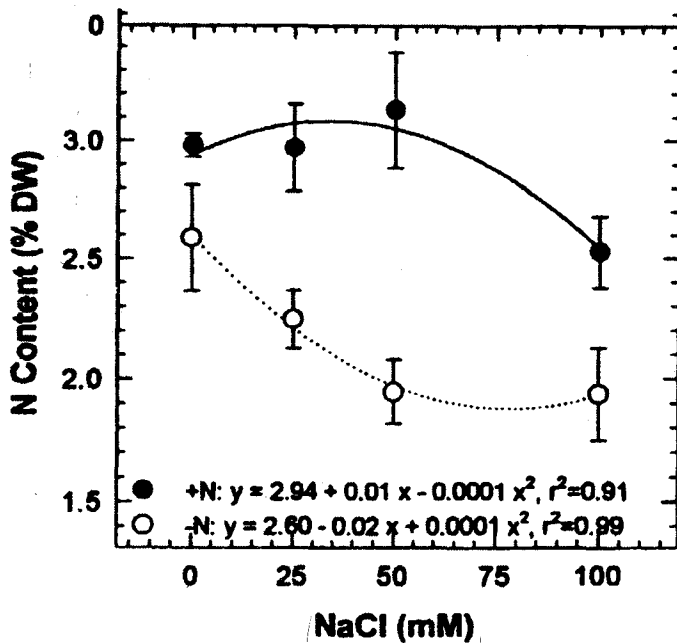


Figure 1. Effect of NaCl concentration on alfalfa (*Medicago sativa* L.) N content in presence (+N) or absence (-N) of 3 mM nitrate. (From data of Serraj and Drevon, 1998).

The level of N₂-fixation sensitivity to salt stress was associated with the level of salt accumulation in the nodules. Exposure to NaCl increased the Na⁺ and Cl⁻ content of all plant tissues and cultivars, although the content was higher in nodules than in shoot tissues. Nodules in common bean accumulated higher NaCl levels than those of soybean and alfalfa (Serraj *et al.*, 1998a), which confirmed previous evidence that salt tolerance in mesophytes was correlated with ion exclusion (Greenway and Munns, 1980). Furthermore, legume species and cultivars differ in ion distribution and especially the ratio of Na/K within plant organs (Ortiz *et al.*, 1994; Cordovilla *et al.*, 1995). However, little information is available on the effect of salt on ion distribution in legume nodules.

Overall, the existence of genetic variability among legume species and cultivars in the sen-

sitivity of N₂-fixation to salt may prove useful in further elucidating the mechanism of NaCl inhibition of SNF and in selection of optimal *Rhizobium*-legume symbioses for agricultural production in saline soils.

Availability of nutrients

In the less fertile rainfall deficient regions of the SAT, no improved cultivar has a reasonable chance of achieving substantial and sustainable yield in the farmer's field unless the critical constraints of soil fertility are addressed. Whereas a lot of effort has gone into breeding for disease-, pest- and drought-resistant crops, little attention has been devoted to identifying and exploiting physio-genetic systems that increase the uptake and utilization efficiencies of legume crops.

The legume-*Rhizobium* symbiosis imposes additional nutritional requirements apart from the minerals needed for plant growth

as a whole. Nutrients that affect SNF include high $\text{NO}_3\text{-N}$, P, B, Zn, S, molybdenum (Mo) and cobalt (Co). Some of these (S, Co, Mo, Zn and Ni) have been extensively addressed elsewhere (Giller, 2001). This paper focuses only on nitrate and phosphorus.

Nitrate

Although the problem of high N is usually highlighted as affecting SNF, in the SAT environments where the soils are low in organic matter (less than 1%) and farmers apply little fertilizer, high soil nitrate (NO_3) may not be a critical factor. However, high soil NO_3 could be a limiting factor in the high input rice–legume–wheat systems. Mineralization of organic matter and nitrification can also result in increased NO_3 concentration in tropical soils. In many SAT soils, there is a flush of mineralization of organic matter in the surface soil layers at the start of the rainy season, due to drying and wetting cycles that accelerate mineralization of the labile fraction of soil organic matter, resulting in a flush of mineral N in the top soil layers (Wani *et al.*, 1997). Surveys of farmers' fields in South Asia showed the occurrence of high levels of soil mineral N before a legume crop was sown (up to 70 ppm in soil surface), which can prevent nodulation and N_2 -fixation (Wani *et al.*, 1997). Indeed, high levels of soil mineral N (30 ppm) at sowing reduced nodulation of chickpea by at least 14% and the proportion of fixed N by 63%. In the case of pigeonpea, suppression of N_2 -fixation was recorded at 43 ppm soil N, and in cowpea at 66 ppm. A direct negative relationship was also observed between soil N levels and nitrogenase activity (Wani *et al.*, 1997).

The inhibition of nodulation and N fixation by combined N prevents optimal exploitation of both pathways of legume N nutrition (SNF and nitrate assimilation). This inhibition results from complex events occurring at different stages of nodule development (Streeter, 1988) and depends upon many factors such as plant

genotype, *Rhizobium* strain, and form and concentration of combined N supply. Inhibition of N_2 -fixation by NO_3 is common in all legumes, although it varies among legume species and cultivars (Piha and Munns, 1987a; Serraj *et al.*, 1992; Herridge *et al.*, 1994). Most studies to enhance N_2 -fixation capacity of legumes in the presence of high levels of nitrate (NO_3 -tolerance) have focused on the host plant. This is entirely justified by the results showing limited variation in N fixation in rhizobial strains under high NO_3 conditions (McNeil, 1982).

In most grain legumes and cropping situations, SNF alone is not capable of ensuring total N requirement for optimal growth and productivity, which require a certain level of complementary N delivered through absorption and assimilation of mineral N. In this case, the two principal enzymatic activities responsible for N assimilation, i.e., nitrate reductase activity and nitrogenase activity, could occur either successively or simultaneously during plant development, depending on the level of available soil N. For instance, Serraj *et al.* (1993) showed that both activities varied in parallel (Fig. 2), which indicated that the two modes of N nutrition could be complementary. The similar patterns of nitrate reductase and nitrogenase activities during the growth cycle of soybean agreed with previous results in lucerne (Wery *et al.*, 1986). However, other reports (Harper, 1974; Obaton *et al.*, 1982) showed that both N nutrition pathways were successive during the growth cycle. These contrasting conclusions are likely to be related to the variability of NO_3 level in the medium (Serraj *et al.*, 1993).

Phosphorus

The N_2 -fixing legume plants usually require more P than plants dependent on mineral N fertilizer. Nodule establishment and function are important sinks for P, and nodules usually have the highest P content in the plant (Sinclair and Vadez, 2002). Therefore, P deficiency conditions result in reduced SNF potential and

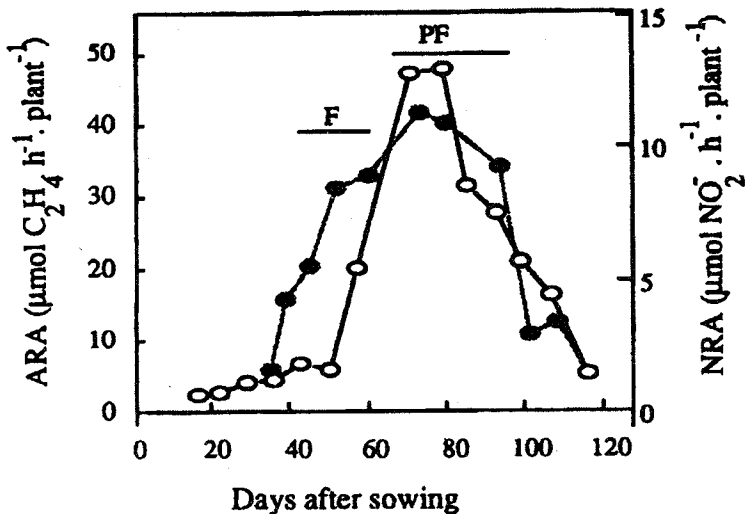


Figure 2. Pattern of foliar nitrate reductase activity (open circles) and acetylene reduction activity (filled circles) during the growth cycle of indeterminate soybeans grown in the field. (From data of Serraj *et al.*, 1993.) F and PF indicate Flowering and pod-filling stages, respectively.

P fertilization will usually result in enhanced nodule number and mass, as well as greater N₂-fixation activity per plant.

There are two potential physiological approaches to improving plant growth and yield under low soil P availability (Clarkson 1985), namely (i) efficient uptake of external P, and (ii) efficient utilization of internal P. The first approach involves plant-soil interactions such as modification of soil exploration by roots, improved interactions with soil microorganisms such as mycorrhizal fungi, and rhizosphere modification to increase P availability (Ohwaki and Hirata, 1992; Hinsinger, 1998). The second approach involves efficient partitioning and subsequent utilization of P within the plant, resulting in more biomass being produced and more N₂ fixed per unit of P taken up (Föhse *et al.*, 1988).

The P requirements for N₂ fixation have been investigated in various legume crops such as cowpea (Cassman *et al.*, 1981), pea (Jakobsen, 1985), soybean (Israel and Rufty, 1988)

and *Acacia mangium* (Ribet and Drevon, 1996). These studies show that P requirements are generally higher for N₂-fixation than for shoot growth and mineral N assimilation, since nodules are an additional strong sink for P. Furthermore, P requirement for N₂ fixation has been shown to vary among genotypes in pigeonpea (Adu-Gyamfi *et al.*, 1989) and mungbean (Gunawardena *et al.*, 1992) or *Casuarina-Frankia* symbioses (Sanginga *et al.*, 1989). Differences in N₂-fixation related to the efficiency of utilization of P were also found among soybean genotypes (Gunawardena *et al.*, 1993) and *Acacia mangium* populations (Vadez *et al.*, 1995). According to Cassman *et al.*, (1981), efficient P utilization in N₂-fixing symbioses may be closely related to an adequate P partitioning between shoot and nodule, and between root and nodules.

Long duration legumes having indeterminate growth and low HI have been identified as critical to improving P and N sustainability in smallholder and subsistence agriculture in

Africa (Snapp, 1998). Intercropping of pigeonpea with cereals is also a proven management scheme for increasing available P. In India, more than 90% of pigeonpea production is in intercropping. And this success is due to exudation of piscidic acid from pigeonpea roots, which enhances the availability of phosphate from iron-phosphate (Ae *et al.*, 1990). Mycorrhizal inoculation of pigeonpea was observed to further improve P uptake in this study. Thus, in addition to providing an immediate source of dietary N, incorporation of pigeonpea residues after seed harvest make P and N more available to a subsequent crop.

Soil acidity

Acid soils pose a major challenge to sustainable agriculture, and particularly to the establishment of N₂-fixing symbioses. SNF can be seriously reduced in such soils, due to the effects of high H⁺ concentration, toxic levels of Al and Mn, and induced deficiencies of Ca, P, and Mo. Soil acidity limits rhizobial growth and survival in the soils, as well as root nodule development. Growth in acidified culture media has proved useful for selecting strains with an ability to colonize the rhizosphere and nodulate their host plant in acid soils (Cooper, 1988). Acidity affects several steps in the development of the symbiosis, including the exchange of molecular signals between the legume and the microsymbiont (Hungria and Vargas, 2000). Therefore, nodule formation in many legumes is delayed or inhibited by low pH, lack of calcium, and the presence of dissolved Al. Mechanisms governing competition between rhizobial strains for nodule formation under acid conditions are poorly understood and the genetic basis of acid tolerance in rhizobia has yet to be elucidated. Liming is effective in overcoming soil acidity and aluminium toxicity. Selection of rhizobial inoculant strains that are genetically stable under acid soil conditions is also essential, but this is impaired by a lack of knowledge of tolerance in the microsymbiont.

Large variations in tolerance of acidity factors are found both within and between *Rhizobium* species. Fast-growing rhizobia are generally considered more acid-sensitive than *Bradyrhizobium*, but low pH-tolerant strains exist in many species. Variations in acid tolerance within species of root nodule bacteria imply a genetic basis to low pH tolerance and studies of acid-sensitive mutants suggested that a large number of genes and regulatory systems could be involved (Glenn *et al.*, 1998).

In the extensive agriculture of the developing world, it was recommended that the first phase of reclaiming acid soils low in P and N should be the use of a legume cover crop supported by liming and conservative P application (von Uexküll and Mutert, 1995). Incorporation of the legume residue will also result in higher soil organic matter content and increased P and N availability (Vance, 2001).

Temperature

It has been well documented that both low and high temperature extremes can prevent nodulation, or if nodulation occurs, can inhibit SNF. Rennie and Kemp (1982) studied effects of temperature on nodulation and acetylene reduction in *P. vulgaris*, and showed that both processes occurred at temperatures as low as 10 °C. At the lower temperatures, the cold adaptability of the plant for early root growth determined its ability to nodulate and fix N. At higher temperatures, plant growth stage was a determining factor. Inoculation with *Rhizobium phaseoli* at more advanced growth stages decreased the time of nodulation at all temperature treatments, but resulted in higher yields and more N₂-fixation (Rennie and Kemp, 1982).

Day *et al.*, (1978) reported that in northern Nigeria, rhizobial populations of only 4–40 cells g⁻¹ soil were found at the surface (5 cm soil depth), while up to 10⁴ cell g⁻¹ soils were found at a depth of 20–25 cm below the soil surface, indicating the sensitivity

of rhizobia to high temperatures. Tropical legumes have adapted to a wide range of temperatures and there is a wide variability in the ability of different legumes to adapt to different temperatures. In chickpea, N₂-fixation seems to be more sensitive to high temperature stress than seed production and N assimilation (Rawsthorne *et al.*, 1985). Piha and Munns (1987b) have earlier reported the existence of inter- and intra specific genetic variability in SNF sensitivity to high temperature. Soybean genotypes showed a higher level of genetic variability in their SNF response to high temperature compared to common bean genotypes (Piha and Munns, 1987b).

Atmospheric carbon dioxide

The increase in atmospheric CO₂ concentration associated with global climate change is now well-documented (Allen, 1994), and these increases are expected to be even more dramatic in the future (IPCC Third assessment report 2001 <http://www.ipcc.ch/pub/un/syrenq/spm.pdf>).

Increased CO₂ concentrations stimulate plant photosynthesis rates, but increases in overall plant growth in the natural environment in response to increased CO₂ are less certain. Plant growth is often limited by factors other than potential photosynthetic rate, of which lack of water and N availability are the two most common (Seligman and Sinclair, 1995). It has been hypothesized that legumes might particularly benefit from increased atmospheric CO₂ because their capability of establishing symbioses with N₂-fixing bacteria allows them to minimize natural N limitations to growth (Hebeisen *et al.*, 1997). Legumes have, indeed, been shown to be highly responsive to increased CO₂ under well-watered conditions (Hebeisen *et al.*, 1997; Serraj *et al.*, 1998b). However, the physiological basis of this effect and its relationship with SNF are still unresolved. An early report by Hardy

and Havelka (1976) showed that short-term CO₂ enrichment resulted in a significant stimulation of ARA in field-grown soybean. However, the long-term CO₂ effect promoted nodule growth, but not nodule-specific activity. Similarly, Cabrerizo *et al.* (2001) recently confirmed that continuous CO₂ enrichment led to increased nodule biomass and carbon availability to nodules but did not enhance specific N₂-fixation in pea.

An important consideration, however, is that N₂-fixation in some legumes is highly sensitive to soil drying (see section on Drought). Because global environmental changes associated with increased atmospheric CO₂ are likely to include variable weather conditions, including more frequent and severe episodes of drought, there is the possibility that the importance of the N₂-fixation advantage of legumes in response to CO₂ might be neutralized or completely lost under these circumstances.

Serraj *et al.* (1998b) showed that exposure of soybean plants to increased CO₂ combined with water-deficit treatments resulted in water conservation under both well-watered and drought treatments. It was also discovered that the N₂-fixation activity response to soil drying was greatly altered by increased CO₂. Consistent with earlier observations, N₂ fixation under ambient CO₂ was very sensitive to soil drying and decreased in response to soil drying before the other measured processes (Sinclair *et al.*, 1987; Sinclair and Serraj, 1995). In sharp contrast, N₂-fixation became highly tolerant to soil drying under the 700 μmol CO₂ mol⁻¹ treatment. Only in the final stage of soil drying when the drought stress was quite severe did N₂-fixation under the 700 μmol CO₂ mol⁻¹ finally decrease. These results indicated that the advantage of legumes under global climate change is even greater than anticipated because of the induced increase in N₂-fixation from tolerance to drought.

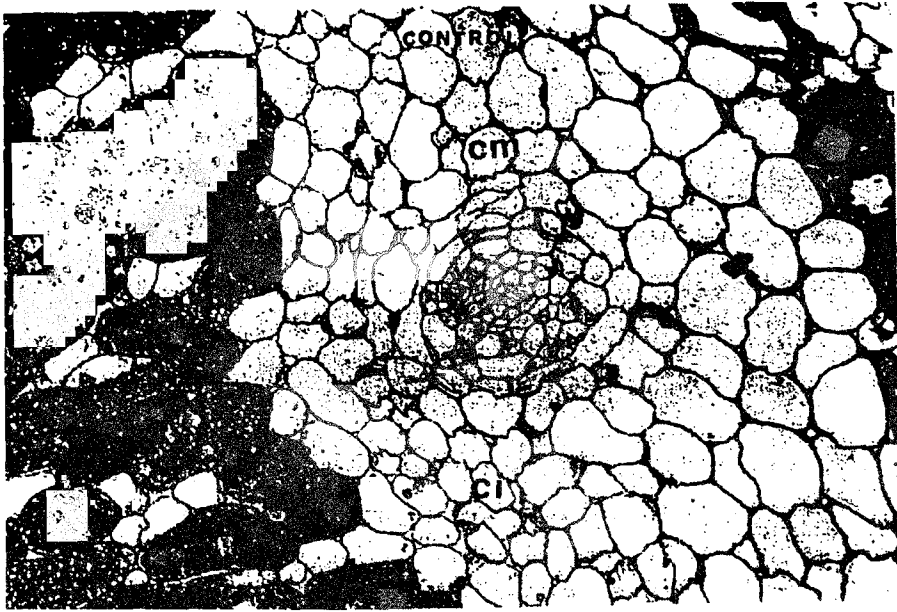


Figure 3. Light micrograph of transverse section of a soybean nodule, (ci, internal cortex; cm, middle cortex; VT, vascular trace).

Oxygen nodule diffusion and regulation of SNF

Because N_2 -fixation has a high-energy demand, oxygen supply is highly critical in the regulation of nitrogenase activity and N_2 -fixation (see review by Minchin, 1997). The respiration rates in the nodules must be very high to provide sufficient ATP and reducing capacity. At the same time, O_2 must be maintained at an extremely low concentration in the bacteroidal zone to prevent inhibition of nitrogenase. This is made possible by the presence of leghaemoglobin and the existence of a variable nodule O_2 permeability (P_o) (Minchin, 1997). The variable component of nodule P_o involves changes in the distribution of air spaces within the nodule internal cortex (Fig. 3), resulting from an occlusion of intercellular space and/or changes in the volume of some of the cells (Walsh *et al.*, 1989). An osmotic model of regulation of P_o in the nodule cortex in response to environmental factors has been proposed by several authors (Witty *et al.*, 1987;

Purcell and Sinclair, 1994). Both salinity and drought stress, possibly mediated by decreases in phloem flow to the nodules, have been found to affect nodule P_o (Serraj *et al.*, 1994; Serraj and Sinclair, 1996).

Serraj and Drevon (1998) showed that the responses of alfalfa nodules to rhizosphere external oxygen pressure (pO_2) varied significantly with the NaCl concentrations in the culture medium. Importantly, the inhibition of nodule ARA by 50 μ M NaCl was completely reversible by increasing pO_2 around the nodules (Serraj and Drevon, 1998), which indicated that an oxygen limitation within the nodules caused by NaCl may have inhibited respiration and nitrogenase activity. When a rapid stress was imposed on soybean plants by adding polyethylene glycol (PEG) in the solution around the roots, it was observed that the decrease in respiration that followed the PEG treatment resulted in a decrease in P_o (as calculated from respiration rates) (Serraj and Sinclair, 1996). Importantly, the PEG-induced

decline in the first hours after treatment was reversible by increasing pO_2 around the nodules, which indicated that an O_2 limitation within the nodules inhibited respiration and nitrogenase activity within the first hours following the PEG treatment. This interpretation agrees with the model of nodule O_2 regulation proposed by Drevon *et al.* (1995), suggesting that nodule P_o is controlled by a mechanism of contraction/expansion of osmocontractile cells in the nodular internal cortex.

In contrast to the ability of increased pO_2 to reverse the decline in N_2 -fixation rates in the presence of moderate stresses, nodules exposed to high NaCl concentration did not have ARA stimulated by pO_2 (Serraj and Drevon, 1998). These results indicate that nitrogenase activity under this severe stress was constrained by factors other than pO_2 . A similar conclusion has been made in the case of drought stress, showing that damage in nodule activity after exposure to severe water deficits was not reversible (Diaz del Castillo *et al.*, 1994; Serraj and Sinclair, 1996).

Conclusion

The extreme sensitivity of SNF to environmental and agronomic stress results in a significant decrease of N accumulation in legume crops exposed to these stress factors. Consequently, legume yields are seriously limited under these conditions and most of their potential benefit in the cropping systems is reduced. Research should therefore focus on the physiological basis of resilience traits so that systematic efforts might be made to incorporate characters in new varieties. Precise identification of traits is important both in conventional trait-based breeding and in identifying the genetic markers related to the trait.

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