

Symbiotic Nitrogen Fixation

Prospects for Enhanced
Application in
Tropical Agriculture

Editor
Rachid Serraj



DFID Department for
International
Development



Oxford & IBH Publishing Co. Pvt. Ltd.
New Delhi

Improvement of Legume Productivity and Role of Symbiotic Nitrogen Fixation in Cropping Systems: Overcoming the Physiological and Agronomic Limitations

R. Serraj¹, J. Adu-Gyamfi², O.P. Rupela¹ and J.J. Drevon³*

¹International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
Patancheru 502324, Andhra Pradesh, India
²ICRISAT-Kano, Nigeria
³INRA-Montpellier, France

ABSTRACT

Nitrogen-fixing legumes can meet most of their N-needs through symbiotic nitrogen fixation (SNF). However, in most cases, inclusion of a legume in a cropping system does not ensure the attainment of such levels of SNF in the field. Several environmental factors including drought, temperature and soil nutrient status 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 chapter 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 the selection of 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 their inclusion in relevant cropping systems, and changes in agronomical management practices for better integration of legumes in cereal cropping systems. Finally, a general framework is discussed for agro-physiological contributions that can help overcome SNF limitation by environmental constraints. The on-farm application of these knowledge-based SNF technologies will strengthen the role of N₂-fixing legumes in cropping systems.

*Corresponding author, E-mail: r_serraj@cgiar.org

INTRODUCTION

Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining fertility of marginal lands and in 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 and growing concern and awareness about the environment. 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. 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 is one of four background documents that analyze the various component approaches to SNF, including legume genomics (Crouch et al., op cit), participatory approaches (Twomlow et al., op cit) and socioeconomic and policy issues (Shiferaw et al., op cit). The document 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 to deal with drought, soil salinity and acidity, nutritional stress, and temperature. 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 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 N_2 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 N_2 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 N_2 fixation is whether the stresses first affect other physiological processes, which then influence N_2 fixation, or whether the stress initially and directly affects N_2 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 N_2 fixation and increase its role in cropping systems. For instance, N_2 fixation has been found 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 N_2 fixation was more sensitive to salt stress than plant growth (Delgado et al. 1994, Serraj and Drevon 1998).

Rupela and Rao (1987) showed that legume-*Rhizobium* symbiosis is particularly sensitive to drought, salinity and extremes of temperature in chickpea and pigeonpea plants, much more so than rhizobia growing alone. All three stress factors were found to impair the development of root hairs and the site of entry of rhizobia into the host, resulting in poor or no nodulation. Salinity and high temperature affected nodulated plants more than they did for N-fertilized plants.

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

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 et al. 2001, Walsh 1995.
Temperature	328				Rennie and Kemp (1982), Day et al. (1978), Piha and Munns (1987b), Roughley et al. (1995), Rupela and Rao (1987)
Low		**	***	**	
High		**	***	**	
Carbon dioxide	66	*	***	**	Hardy and Havelka 1976, Hebeisen et al. 1997, Cabrerizo et al. 2001, Serraj et al. 1998b
Oxygen	221	–	**	***	Minchin 1997, Walsh et al. 1989, Walsh 1995
Drought (or water stress)	341	*	**	***	Serraj et al. 1999b, Sinclair et al. 1987, Williams and de Mallorca 1984
Salinity	149	*	**	***	Rupela and Rao 1987, Serraj et al. 1998a, Singleton et al. 1982, vanHoorn et al. 2001
Nitrate	655	*	***	***	Herridge et al. 1994, Streeter 1988, Walsh 1989
Soil acidity	498	**	**	**	Cooper 1988, Glenn et al. 1988, Hungria and Vargas 2000
Low phosphorus	136	–	**	***	Cassman et al. 1961, Israel and Rufty 1988, Vance 2001, Sinclair and Vadez 2002
Micronutrients	20	–	**	**	O'Hara et al. 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.

of N_2 fixation to abiotic and biotic stress factors. The need to assess genetic diversity of legumes in terms of SNF potential, in order to screen and utilize available legume germplasm for efficient SNF is an important 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 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 N_2 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 N_2 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. This conclusion from field studies was supported by detailed observations in glasshouse studies, which showed that the effects of soil dehydration on N_2 fixation, as measured by an acetylene reduction assay, occurred at much higher soil water contents than the effects on C accumulation (Serraj and Sinclair 1997).

The fact that N_2 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, Beck et al. (1991) concluded that even if the drought stress effects on N_2 fixation do not always directly affect grain yield, drought may result in a significant decrease in the total N balance. Loss of N_2 fixation under water deficits would then reduce the advantage of using legume crops in rotations, for green manuring and 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.

Chickpea

The vast majority of chickpea produced worldwide is grown by resource-poor farmers under rainfed conditions, usually planted after the main rainy season and grown on declining soil moisture. Terminal drought tolerance is, therefore, a primary constraint to chickpea productivity. Significant progress has been made in developing improved chickpea varieties of short duration that mature in 70-90 days in mild winter chickpea growing conditions, able to escape terminal drought (Kumar et al. 1996). Even extra-short duration (ESD) chickpea varieties, termed *super-early* have now been developed (Kumar and van Rheenen 2000). Development of these new varieties has expanded the options of including chickpea as a crop in many prevailing and evolving new production systems, such as the rice fallows of South Asia (Musa et al. 2001). This work needs, however, to be extended to include other key legume crops, with a specific focus on SNF, to overcome the soil fertility and environmental constraints.

Large and deep root systems have been characterized as important drought avoidance traits, useful in greater extraction of available soil moisture, and have been widely used for the genetic enhancement of chickpea under terminal drought (ICRISAT 1992). The routine application of molecular markers, combined with the use of adequate genetic populations offers a paradigm shift in the ability to study and manipulate root traits. The chickpea line ICC 4958 has the multiple drought avoidance traits of large root size, a rapid rate of root development and extraction of water, and a rapid seed development rate related to its large seed size. Recombinant inbred lines (RIL) of a chickpea cross (ICC 4958 x Annigeri) have also been phenotyped for root traits. Identification of QTLs for the large root system of ICC 4958 to develop a marker-assisted selection technique is currently in progress (Krishnamurthy et al. 2003, Kashiwagi et al. 2003, Chandra et al. 2003, in preparation).

As an amide producer, chickpea has been found relatively tolerant to drought in terms of N_2 fixation response, compared to ureide producers

(Sinclair and Serraj 1995). However, more work is needed to investigate the genetic variability of nodulation and SNF response to water deficits.

Pigeonpea

Important putative drought tolerance traits in pigeonpea include early vigor, leaf area maintenance, root and shoot growth rate and plasticity in development (Johansen 2001). Early growth vigor is an important factor in drought resistance as it permits establishment of a root system that is very effective in extracting water during later drought periods. This is considered the main reason for the better growth and yield of pigeonpea hybrids such as ICPH 8 and ICPH 9, compared to varieties from which they are derived, under both drought and well-watered conditions.

There are considerable differences in early growth vigor of different pigeonpea varieties (Johansen 2001). Early-maturing genotypes generally show more vigor than later-maturing ones, with hybrids showing most vigor, but there are exploitable differences in this trait within maturity groups. The recently developed extra-short duration (ESD) genotypes mature in less than 110 days, with yield potentials comparable to short- and medium-duration cultivars when grown under adequate moisture supply (Chauhan et al. 1992). However, ESD genotypes are poorly adapted to rainfed conditions because their shallow rooting behavior makes them susceptible to drought stress, particularly during flowering and pod filling, resulting in severe yield losses (Nam et al. 2001).

Despite the demonstrated ability of pigeonpea to grow in N-deficient soils without inputs of N-fertilizer (Kumar Rao et al. 1983), the quantification of the amounts of N_2 fixed has proved difficult (Peoples et al. 1989). However, the xylem ureide assay has been successfully used for the quantification of SNF capacity in pigeonpea (Peoples et al. 1989). The drought response of SNF in pigeonpea has previously received little investigation. However, there is indication that as a ureide producer, pigeonpea is likely to be essentially drought sensitive (Serraj et al., unpublished data). The genetic variability of nodulation and SNF under drought in the various maturity groups of pigeonpea needs to be further investigated.

Groundnut

ICRISAT has adopted a holistic approach in screening and selecting groundnut varieties with super performance at the two most critical stages of drought (mid- and end-season). Several such lines are now available for use in breeding programs. The physiological basis of genotypic response to drought in groundnut was identified as involving Harvest Index (HI), total amount of water transpired (T) and transpiration efficiency (TE). Genotypes derived from parental lines selected in field drought screening at ICRISAT showed superior yield performances because of higher TE and HI, while for

other cultivars, the dominant contribution to the yield was T and/or HI. The T and TE were estimated indirectly from SPAD-chlorophyll meter readings, specific leaf area (SLA) and specific leaf nitrogen (SLN).

More recently, dry-down experiments were carried out under controlled environment for the analysis of TE and stomatal regulation under water deficit, in relation to nodulation and plant N status. Evaluation of genetic variability in plant water use and leaf gas exchange responses to soil drying has been carried out in RILs selected for high water use efficiency (comparison of lines with high and low TE from previous selection experiments). The data confirmed the genotypic variation observed previously in the field in total amount of water transpired and TE. These data have also shown that TE in groundnut leaves is correlated with SLA, nodulation and N status (Serraj et al., unpublished). This currently ongoing work aims to confirm the link between TE and nodulation under drought and for the development of genetic linkage mapping. This will facilitate the characterization of QTLs and offer practical means for manipulating the underlying traits for water use efficiency in groundnut breeding programs.

Other Legume Species

The variability of N_2 fixation sensitivity to drought has been analyzed with several grain legumes including soybean, cowpea, black gram, chickpea, common bean, faba bean, lupine, pea, and peanut (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.

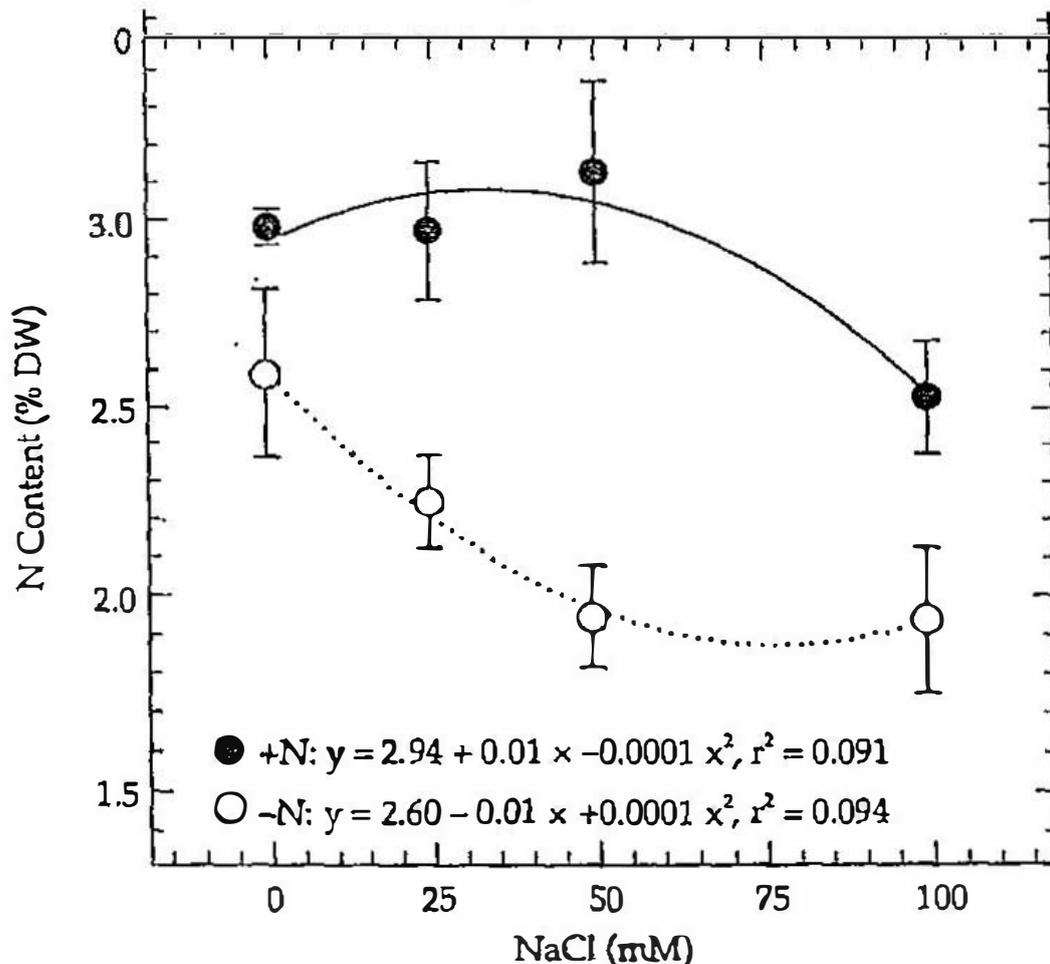
Serraj et al. (1999a) investigated the inhibition of N_2 fixation in soybean due to water deficits and showed that ureides (allantoin and allantoic acid) were involved in the sensitivity to drought. Consistent with this observation, variability in N_2 fixation sensitivity among legume species and cultivars 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 N_2 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 (*Dolichus 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 et al. (1990) observed significant differences among pigeonpea *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.



Source: Data of Serraj and Drevon 1998.

Figure 1. Effect of NaCl concentration on alfalfa (*Medicago sativa* L.) N content in presence (+N) or absence (-N) of 3 mM nitrate.

The effects of salinity on biomass and N accumulation in alfalfa were compared in plants fed with nitrate (NO_3) or dependent on N_2 fixation (Serraj and Drevon 1998). NaCl inhibited nitrogen accumulation in both NO_3 -fed plants and N_2 -fixing plants, which was seen as a decrease in N concentration (%N in plant biomass). The decrease was larger for N_2 -fixing plants than for NO_3 -fed plants (Fig. 1). The %N in NO_3 -fed plants was not affected by low concentrations of NaCl (up to 50 mM), whereas N_2 -fixing plants showed a significant decrease in %N with increasing levels of NaCl. Below 50 mM NaCl, %N was about 100% and 75% of that in the control plants for NO_3 -fed plants and N_2 -fixing plants, respectively. The absence of a significant effect of low NaCl concentrations on %N in NO_3 -fed plants supports previous reports showing inhibitory effects of NaCl on growth without any decrease in %N (Pessarakli and Zhou 1990, Cordovilla et al. 1995). This contrasts with the relatively large effect of NaCl on %N content of N_2 -fixing plants (Serraj and Drevon 1998). Therefore, N accumulation appears to be more salt sensitive in N_2 -fixing plants than in NO_3 -fed plants, demonstrating the higher sensitivity to salt stress in N_2 -fixing plants.

A recent study of four grain legumes including broadbean, chickpea, lentil and soybean confirmed the effects of soil salinity on crop yield, total nitrogen uptake and N_2 fixation (van Hoorn et al. 2001). The existence of inter- and intraspecific variability in the sensitivity of N_2 fixation to salinity has also been reported in legumes (Serraj et al. 1998a, 2001). The level of N_2 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 compared to 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 Murns 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 sensitivity of N_2 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.

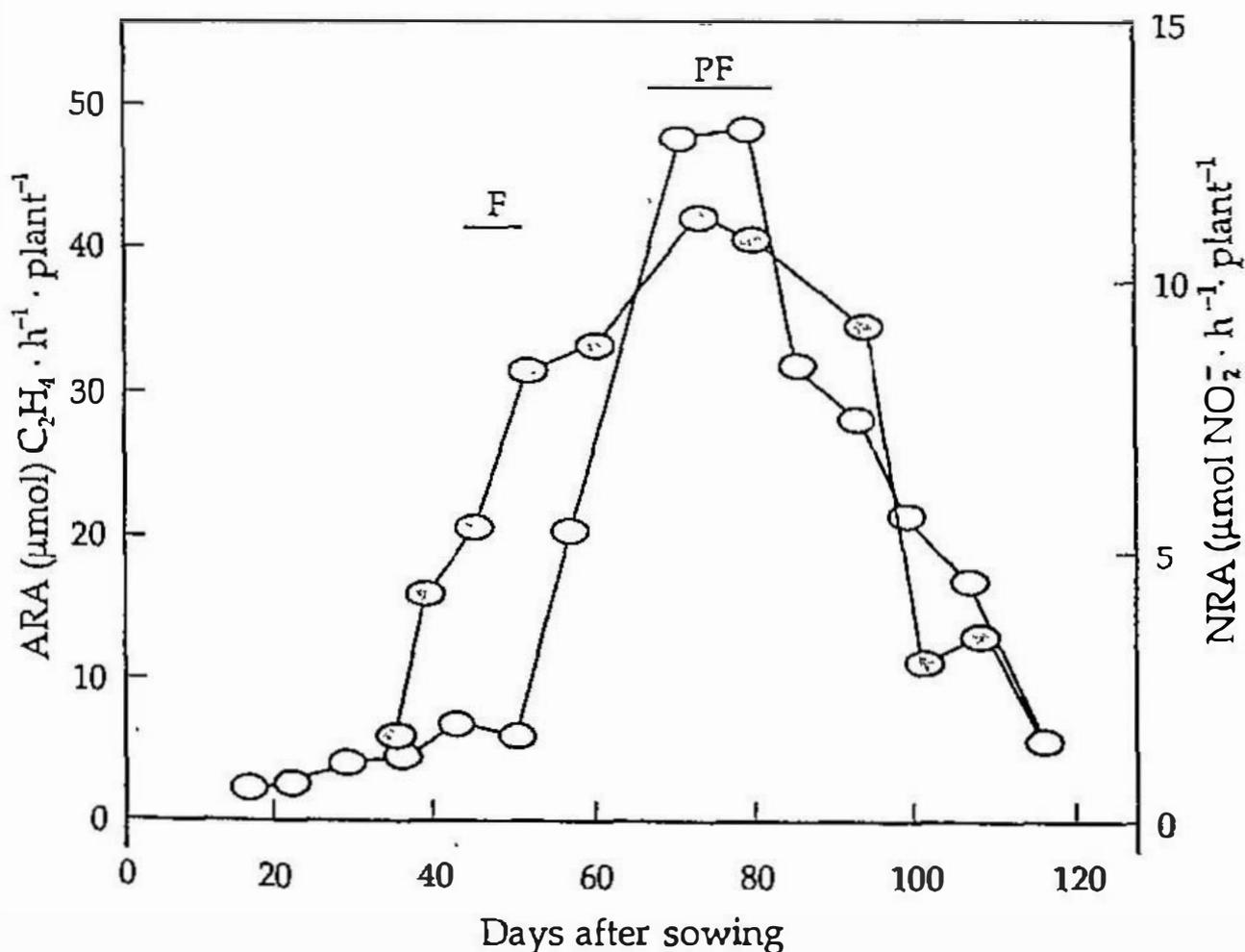
The legume-*Rhizobium* symbiosis imposes additional nutritional requirements apart from the minerals needed for plant growth as a whole. Nutrients that affect SNF include high NO_3^- , N, P, B, Zn, S, molybdenum (Mo) and cobalt (Co). Some of these (S, Co, Mo, Zn and Ni) have been extensively addressed 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 little fertilizer is applied by farmers, high soil 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 semi-arid tropical 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 sowing of a legume crop (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_2 fixation by combined nitrogen 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 nitrogen supply. Inhibition of N_2 fixation by NO_3 is common in all legumes, although it varies between 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_2 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 requires 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 either occur 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).



Source: From data of Serraj et al. 1993.

Figure 2. Pattern of foliar nitrate reductase activity (open circles) and acetylene reduction activity (filled circles) during the growth cycle of undeterminate soybeans grown in the field. F and PF indicate flowering and pod-filling stages, respectively.

Phosphorus

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 (Sinclair and Vadez 2002). 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.

There are two potential physiological approaches to improving plant growth and yield under low soil P availability (Clarkson 1985), namely (1) efficient uptake of external P, and (2) 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 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 like 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 nodulated root, 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 in part to the 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

There are more than 800 million ha of Oxisols and Ultisols in Latin America alone that have a pH less than 5.0. 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. In white clover nodulation is inhibited by Al, even at relatively high pH values (5.5-6.0). 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 the 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 nitrogen. 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 yield and more N₂ fixation (Rennie and Kemp 1982). Using eleven common bean cultivars representing a wide range of types, and grown in nitrogen-free medium in a controlled environment at two temperature regimes, these authors showed differences between cultivars in their ability to fix N₂ under the two temperature regimes.

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).

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 environment 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 tolerance to drought.

Oxygen Nodule Diffusion and Regulation of SNF

Because N₂ fixation has a high-energy demand, oxygen supply is highly critical in the regulation of nitrogenase activity and N₂ 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, O₂ must be maintained at an extremely low concentration in the infected cells to prevent inhibition of nitrogenase. This is made possible by the presence of leghemoglobin and the existence of a variable nodule O₂ permeability (Po) (Minchin 1997). The variable component of nodule Po 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 Po 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 Po (Serraj et al. 1994, Serraj and Sinclair 1996).

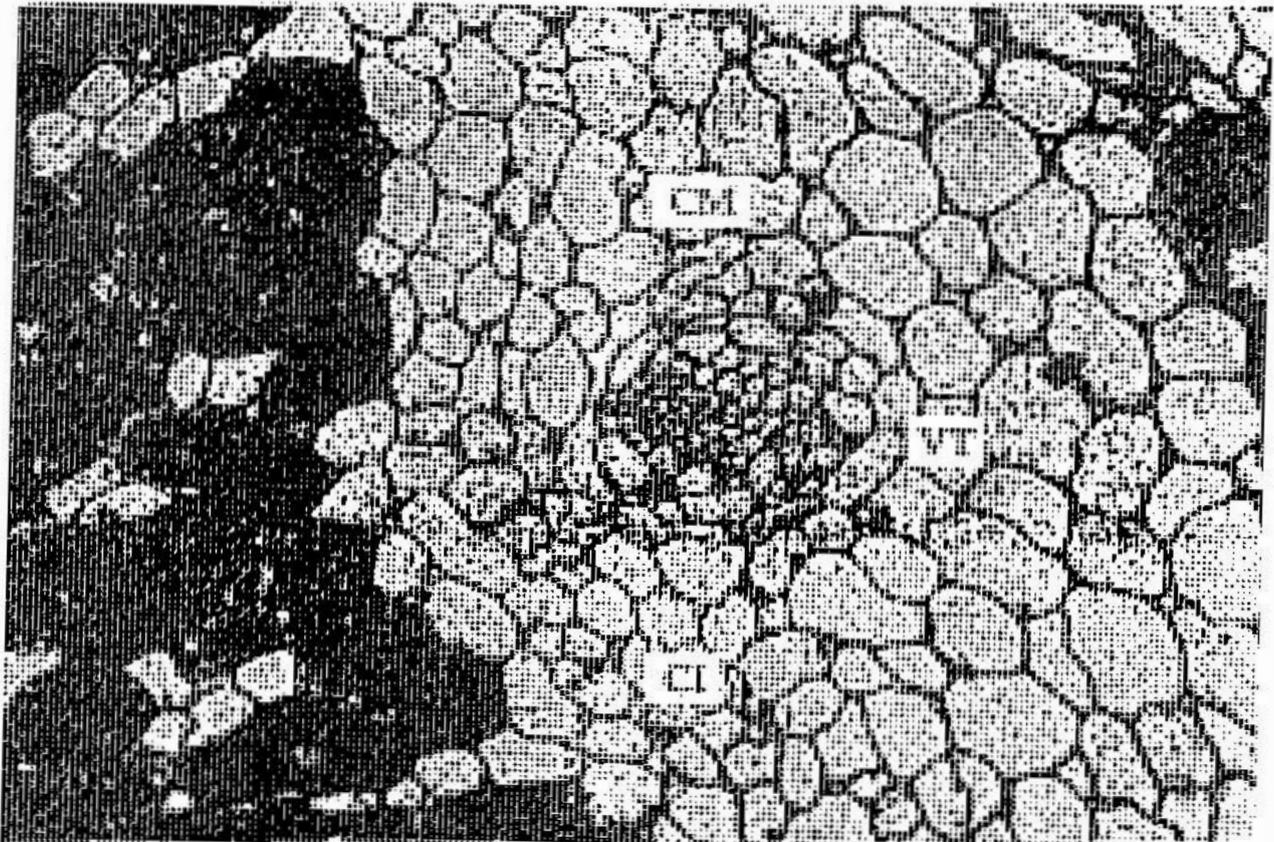
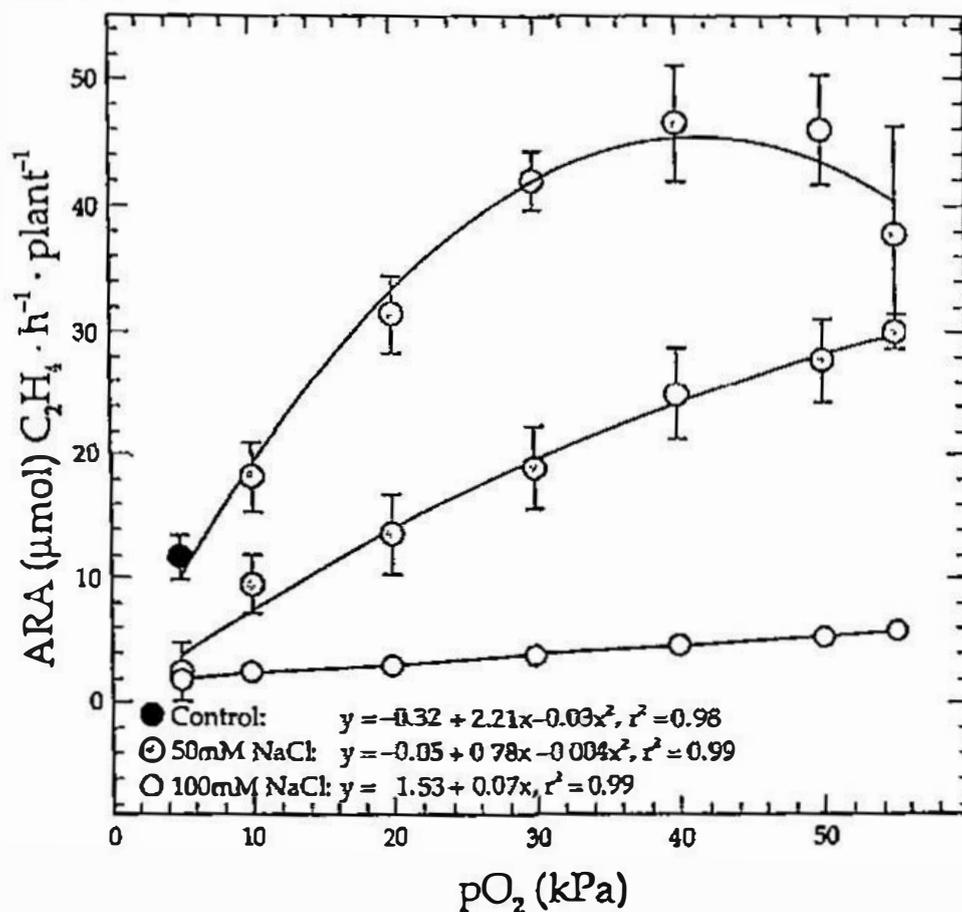


Figure 3. Light micrograph of transverse section of a soybean nodule, (CI, internal cortex; CM, middle cortex; VT, vascular trace).



Source: Serraj and Drevon 1998.

Figure 4. Effect of NaCl concentration and external oxygen pressure (pO_2) on acetylene reduction activity of alfalfa nodules.

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 mM NaCl was completely reversible by increasing pO_2 around the nodules (Fig. 4), 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 (Fig. 4). 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).

PHYSIOLOGICAL MECHANISMS OF SNF REGULATION UNDER STRESS

Drought

The effect of drought stress on N_2 fixation has usually been perceived as a one-dimensional physiological process acting on nitrogenase activity and involving exclusively one of three hypotheses: Oxygen limitation, feedback regulation by ureides, and carbon shortage. These hypotheses were recently considered together with water transport and nodule structure to be putative mechanisms affecting N_2 fixation in response to drought stress (Serraj et al. 1999b). There is growing evidence for interactions between water and N transport, C metabolism, nodule permeability to oxygen, and nodule growth and function. It was therefore concluded that the various hypotheses proposed so far for effects of drought on N_2 fixation should be integrated into a multi-dimensional model of physiological response to drought.

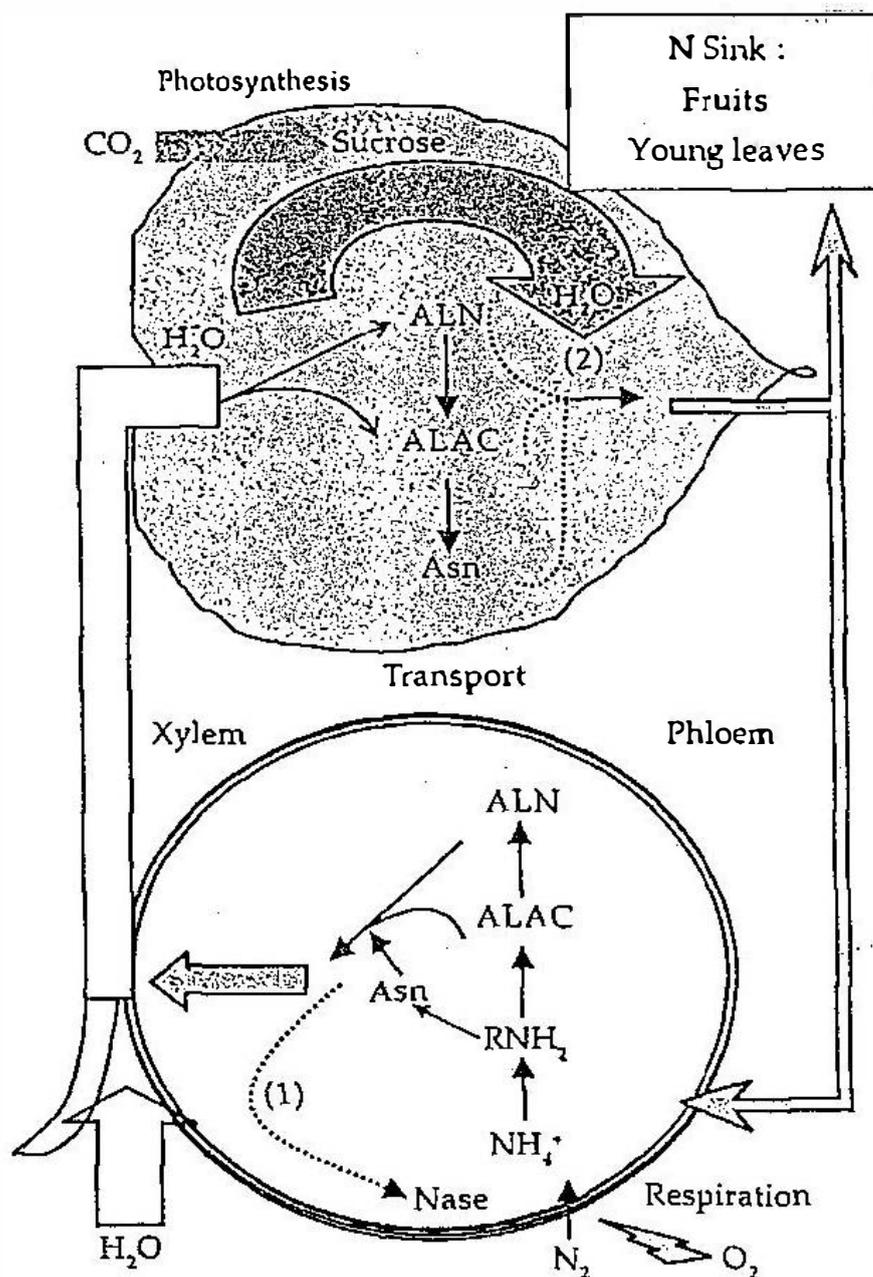
The mechanism by which ureide accumulation may trigger the inhibition of nitrogenase activity is still unclear. The low solubility of ureides (Sprent 1980) may be important in the association of high ureide concentrations with drought sensitivity. The response of N_2 fixation in soybean plants to various nitrogenous compounds further demonstrated the importance of a ureide-related regulation (Serraj et al. 1999a). Plants exposed to 10 mM allantoic acid had decreased ARA and P_o within 3 d of exposure, although plants exposed to 10 mM asparagine had a greater decrease in ARA and P_o that was initiated after only 2 d of exposure. The severity of the ureide-induced decrease in ARA was dependent on the ureide concentration in the nutrient solution and was partially reversible upon the removal of the ureides from the solution. These results indicated an important role of ureides in influencing nodule activity and P_o , but also that asparagine might have a more direct role in this regulation.

Overall, there is now abundant evidence that N feedback on nodule activity is important in the sensitivity of the N_2 fixation response to water deficit conditions (Fig. 5). The feedback in those legumes that export ureides from the nodules seems to be especially aggravated by the accumulation of ureides in the plant. However, the specific feedback signal compound or the mode of action of the feedback is not yet known.

The variation among legume species and cultivars in sensitivity of N_2 fixation to water deficit indicates that the tolerance trait found in some genotypes may be useful in breeding programs for N_2 fixation drought tolerance in legumes. Although the mechanisms of interaction between ureide metabolism and N_2 fixation response to drought are still unknown (Serraj et al. 1999b), measurements of ureide concentration in the petiole and or the xylem sap may be useful in screening large numbers of germplasm of ureide-producing legumes for drought tolerance. Purcell et al. (1998) used petiole ureide concentration measurement to screen a large number of soybean plant introductions, and they found a broad variation in ureide contents. Among the low-ureide producers, they isolated a few soybean lines that showed a substantial level of drought tolerance of N_2 fixation. This observation is important because it indicates that grain legumes can be selected for decreased sensitivity of N_2 fixation to soil drying in regions where drought is a recurring problem.

Salinity

The short-term response of N_2 fixation to salt showed a 2-phase inhibition of nitrogenase activity, i.e. a dramatic decrease in nodule ARA during the first hour of the treatment with NaCl, followed by a slower rate of decrease (Serraj et al. 1994, 1998a). Similarly, Munns (1993) proposed a biphasic model for plant growth response to salinity, the first effect of NaCl being osmotic and



Source: Serraj et al. 2001.

Figure 5. Hypothetical scheme of the relationship between legume nodule and leaf metabolism and possible origins for feedback regulation of nodule activity by N compounds.

the second being toxic ion accumulation (see also Kingsbury and Epstein 1986, Ortiz et al. 1994). Therefore, the initial effect of NaCl on nodule nitrogenase might be caused by a decrease in phloem sap supply to nodules, i.e. a water deficiency, because of an osmotic effect of NaCl at the whole plant level. Fortmeier and Schubert (1995) have suggested a similar mechanism for the inhibition of leaf growth that occurs within minutes after exposure to NaCl (Yeo et al. 1991, Ortiz et al. 1994). The hypothesis of salt inhibition of nodule activity is supported by the similarity of the short-term effects of NaCl and water deficit on nodule activity (Serraj et al. 1994, Serraj and Sinclair 1996).

The existence of inter- and intra-specific variability in the sensitivity of N_2 fixation to salinity has been recently confirmed in legumes (Serraj et al. 1998a). Salt limited nodule growth and nitrogenase activity in soybean, common bean and alfalfa (Serraj et al. 1998a). Exposure to NaCl also resulted in a significant decrease in plant biomass accumulation in common bean and soybean. In contrast, there was no significant effect on biomass accumulation of alfalfa. Although the exposure to salt induced an immediate decrease in nodule N_2 fixation for all symbioses, the rate of inhibition was faster in common bean than in soybean and alfalfa. This level of N_2 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. Common bean nodules accumulated higher NaCl levels than soybean and alfalfa (Serraj et al. 1998a), which confirmed previous evidence showing 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 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.

Mineral Nitrogen

The concept of N feedback regulation has been proposed as an alternative general mechanism for the inhibition of N_2 fixation by nitrate and other environmental factors. Silsbury et al. (1986) first suggested a feedback control of nodule activity mediated through the pool of soluble N in the plant. Parsons et al. (1993) developed this concept further by suggesting that nodule formation, nitrogenase activity and nodule permeability to oxygen might be controlled by the concentration of reduced N compounds entering the nodule through the phloem (Fig. 5).

No precise signal molecule for feedback on N_2 fixation under drought conditions has been found so far. However, several reports have shown that free amino acids such as alanine, GABA and proline accumulate markedly in drought-stressed plants and cells (Handa et al. 1983, Rhodes et al. 1986, Raggi 1994). Although the exact physiological significance of such accumulation remains unknown, it has generally been interpreted as an osmotic adjustment mechanism (Handa et al. 1983, Raggi 1994). Bacanamwo and Harper (1997) proposed that the changes in shoot asparagine level and/or products of its metabolism in the nodules might be involved in the feedback control of nodule activity. Baker et al. (1997) suggested that a similar mechanism in *Alnus glutinosa* would involve changes in xylem citrulline. Neo and Layzell (1997) proposed that changes in phloem sap glutamine content may trigger the inhibition of nodule metabolism and nitrogenase

activity. Finally, Vadez et al. (2000) showed that asparagine cannot be the only compound involved in the feedback inhibition of N_2 fixation in soybean, but ureides and asparagine are probably both involved, either directly by accumulation of products that fail to be exported from the nodules, or by feedback from the shoot due to an N-compound supply that exceeds shoot requirements.

P deficiency

Acute deficiency of P can prevent legume nodulation. Work at ICRISAT and elsewhere has shown that legumes like lupin, chickpea and pigeonpea have the ability to extract P from sparingly soluble P sources. Pigeonpea is better able to utilize P bound to the iron fraction of the soil (Fe-P) than chickpea, and soybean, and this explains why the crop responds less to added P than other crops in Alfisols, where Fe-P is high. The carboxylic anions (picidate, citrate, and malonate) exuded from pigeonpea roots have high P-solubilizing ability. Ishikawa et al. (2002) reported genotypic differences in the P-solubilizing ability of pigeonpea; thus the inclusion of some genotypes of pigeonpea in cropping systems could enhance the available P pool in soils.

Although the understanding at the physiological level has led to the current progress at the molecular level, more work is needed to assess the efficacy of the mechanism of rhizosphere acidification, and carboxylic anion exudation. The genetic manipulation of root exudates is hampered by numerous technical problems in collecting, analyzing and quantifying the exudates. Very little success (if any) has been achieved in measuring root exudates in the soil rhizosphere. In addition, there is lack of a simple, fast and inexpensive technique for the assessment of large numbers of genotypes of a segregation population without using the expensive spectrophotometer. The technique recently reported by Ishikawa et al. (2002) that uses a filter paper qualitative assay method to screen for the P solubilizing ability of pigeonpea will benefit both conventional and genetic engineering approaches to enhancing P use efficiency of legumes. Other aspects of SNF and P acquisition have been extensively discussed recently (Vance 2001, Sinclair and Vadez 2002).

ROLE OF SNF IN TROPICAL CROPPING SYSTEMS

Symbiotic nitrogen fixation accounts for a large proportion of the N currently utilized in agriculture and will be an increasingly important component in future crop productivity especially for sustainable agricultural systems, small-scale operations and marginal land utilization. It is imperative that we not only understand the contribution of this process to various agricultural systems, but we must also appreciate current limitations to SNF under field conditions. Reducing fertilizer use, while maintaining the native soil N

resource and enhancing crop N output is desirable from both environmental and economic perspectives. This may be possible by obtaining more N on the soil through SNF, reducing loss of N and by recycling of N captures in vegetation during the off-season.

Adu-Gyamfi et al. (1997a) have reviewed the dynamics and management of N in sorghum/pigeonpea intercropping systems in the SAT. Their review indicates that the effective management of indigenous soil N and N derived in situ through SNF has the potential to enhance the N nutrition and N use efficiency of crops and the total N output from a sorghum/pigeonpea intercropping. In a four-year study, where the proportion and amount of N derived from air were estimated by both the natural abundance and the relative ureide abundance methods, pigeonpea intercropped with sorghum derived 56-85% N from N_2 fixation, which was more than the %Nd_{fa} by sole crop pigeonpea (32-58%). The amount of N derived from fixation was higher in intercropping than in sole cropping. Data from Adu-Gyamfi et al. (1995) show that the method of fertilization had significant effect on %Nd_{fa}. Highest values were observed for split-banding (87%) compared to broadcasting (67%) in pigeonpea intercrop. In sole crop, the values were 26% for broadcast and 40% for split-band.

From the relative ureide abundance method, the proportion of ureides, amino acids and nitrate (NO_3-N) concentration in xylem sap at the different sampling times were estimated. Nitrate-N accounted for 50-80% of the composition of N solutes in xylem sap exudates in sole crop. The proportion of ureide in xylem sap exudates of sole crop pigeonpea decreased with increased N application. Significantly higher proportion of ureides and amino acids in xylem exudates were recorded for intercropped than for sole crop pigeonpea. Ureide concentration (mM) in the exudates was higher in intercrop pigeonpea than in sole crop; and there was a marked decrease in %Nd_{fa} by sole crop but not in intercropped pigeonpea as fertilizer rates increased.

Intercropped pigeonpea derived about 80% of the N in plant from the air at 65 days after sowing (DAS) compared to 60% for sole crop. The % Nd_{fa} value was higher in intercrop than in sole crop, but significantly lower in delayed than in basal treatments. The % Nd_{fa} significantly increased with DAS.

In a pigeonpea-millet-groundnut intercropping system, higher proportion and amount of N was derived from N_2 fixation compared to the other combinations. These results suggest that more efficient utilization of N can be achieved by appropriate combination of component crops. Intercropped pigeonpea fixed between 80-100 kg ha⁻¹. The intercropped pigeonpea had less opportunity to acquire N from fertilizer and soil compared to sole crop pigeonpea, probably because of the rapid depletion of N by the cereal companion crop, thereby increasing the dependency on SNF (Adu-Gyamfi et al. 1997b, Tobita et al. 1994).

Sanginga (2003) recently reported that promiscuous soybeans were used to develop sustainable cropping systems in the moist savannahs in West Africa, where N has been gradually depleted from soils, causing serious threats to food production. The actual amounts of N_2 fixed by soybeans and their residual N benefits to subsequent cereal crops varied between 38 and 126 kg N ha⁻¹. When only seeds of soybeans were removed from the plots, the net N accrual of soil nitrogen ranged between -8 and +47 kg N ha⁻¹ depending on the soybean cultivar (Sanginga 2003). Residual N values of 10-24 kg N ha⁻¹ were also obtained in a soybean-maize rotation. These authors also demonstrated that the relative increase in maize N was smaller than the relative increase in dry-matter yield, which indicates that the increased maize yields following soybeans were not entirely due to the carry-over of N from soybean residues, but to other rotational effects as well. It was therefore concluded that the N benefit of grain legumes to non-legumes is generally small compared to the level of N fertilizer use in more intensive cereal production systems but is significant in the context of the low amounts of input in subsistence farming.

CONCLUSIONS: A FRAMEWORK FOR AGRO-PHYSIOLOGICAL CONTRIBUTIONS IN OVERCOMING SNF LIMITATION BY ENVIRONMENTAL CONSTRAINTS

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.

It is common knowledge that drought-tolerant varieties in general have low yield potential. Therefore, the degree of drought tolerance incorporated needs to be matched with the magnitude of drought in the target environment. 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.

In conclusion, several interventions should be considered for the agro-physiological optimization of SNF in cropping systems:

- On-farm participatory screening and integration of already identified stress-adapted legume varieties in various crop and crop-livestock systems
- Catalyzing farmer-to-farmer seed diffusion of selected improved legumes tolerant to various environmental stresses, permitting strategic seed reserve development at household level

- Documenting the nodulation status and inventory of current legume varieties tolerant to biotic and abiotic stresses in a target agroecological environment
- Field studies to establish heritabilities of root morphological traits associated with nutrient and water uptake by legumes
- Field studies to evaluate the efficiency of utilization of fixed N by legumes and subsequent crops
- The genetic improvement of legume tolerance to stress, with additional effort for understanding the physiological limitations of SNF at the levels of host plant and rhizobial strains, and their interaction
- Physiological and genetic dissection of traits involved in abiotic stress tolerance among legume species exporting amides or ureides
- Using the information on the genetic variability in P acquisition and nitrate tolerance by legumes to improve P efficiency and develop nitrate-tolerant legume crops
- Identification and evaluation of land management options to increased SNF contribution in cropping systems based on land capability and agro-ecological potential
- On-farm participatory evaluation and adaptation of improved management options for increased SNF contributions in farming systems

REFERENCES

- Adu-Gyamfi JJ, Fujita K and Ogata S. 1989. Phosphorus absorption and utilization efficiency of pigeon pea (*Cajanus cajan* (L.) Millsp.) in relation to dry matter production and dinitrogen fixation. *Plant and Soil* 119:315-324.
- Adu-Gyamfi JJ, Katayama K, Ito O, Devi, G and Rao TP. 1995. Nitrogen fixation in pigeonpea-based intercropping on Alfisols of the semi-arid tropics. In *Nitrogen fixation: Fundamentals and applications* (Tikhonovich IA, Provorov NA, Romanov VI and Newton WE, eds.). Dordrecht, The Netherlands: Kluwer Academic Publishers, 822 pp.
- Adu-Gyamfi JJ, Ito O, Yoneyama T and Katayama K. 1997a. Nitrogen management and biological nitrogen fixation in sorghum/pigeonpea intercropping on Alfisols of the semi-arid tropics. *Soil Science and Plant Nutrition* 43:1061-1066.
- Adu-Gyamfi JJ, Ito O, Yoneyama T, Devi G and Katayama K. 1997b. Timing of N fertilization on N₂ fixation, N recovery and soil profile nitrate dynamics on sorghum/pigeonpea intercrops on Alfisols of the semi-arid tropics. *Nutrient Cycling in Agroecosystems* 48:197-208.
- Ae N, Arihara J, Okada K, Yoshihara T and Johansen C. 1990. Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian subcontinent. *Science* 248:477-480.
- Ali M and Mishra JP. 2000. Nutrient management in pulses and pulse-based cropping systems. *Fertilizer News* 45:57-69.
- Allen Jr LH. 1994. Carbon dioxide increase: Direct impacts on crops and indirect effects mediated through anticipated climatic changes. Pages 425-459 in *Physiology and*

- determination of crop yield (Boote KJ, Bennett JM, Sinclair TR and Paulsen GM, eds.). ASA, CSSA, and SSSA, Madison, WI, USA.
- Bacanamwo M and Harper JE. 1997. The feedback mechanism of nitrate inhibition of nitrogenase activity in soybean may involve asparagine and/or products of its metabolism. *Physiologica Plantarum*, 100:371-377.
- Baker A, Hill GF and Parsons R. 1997. Evidence for N feedback regulation of nitrogen fixation in *Alnus glutinosa* L. *Journal of Experimental Botany* 48:67-73.
- Beck D, Wery J, Saxena MC and Ayadi A. 1991. Dinitrogen fixation and nitrogen balance in cold season food legumes. *Agronomy Journal* 83:334-341.
- Bernstein L and Ogata G. 1966. Effects of salinity on nodulation, nitrogen fixation and growth of soybean and alfalfa. *Agronomy Journal* 58:201-203.
- Bidinger FR. 2001. Field screening for drought tolerance - principles and illustrations. Pages 109-124 in *International Workshop on Field screening for Drought Tolerance in Rice* (Saxena NP, ed.). Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics.
- Cabrerizo PM, González EM, Aparicio-Tejo PM and Arrese-Igor C. 2001. Continuous CO₂ enrichment leads to increased nodule biomass, carbon availability to nodules and activity of carbon-metabolising enzymes, but does not enhance specific nitrogen fixation in pea. *Physiol. Plant.* 113:33-10.
- Cassman KG, Whitney AS and Fox RL. 1981. Phosphorus requirements of soybean and cowpea as affected by mode of N nutrition. *Agronomy Journal* 73 17-22.
- Chauhan YS, Saxena NP and Johansen C. 1992. Abiotic factors limiting chickpea and pigeonpea production. Pages 111-123 in *Proceedings of the National Symposium on New Frontiers in Pulses Research and Development, 10-12 November 1989* (Sachan JN, ed.). Kanpur, India: Directorate of Pulses Research.
- Clarkson DT. 1985. Factors affecting mineral nutrient acquisition by plants. *Annual Review Plant Physiology* 36:77-115.
- Cooper JE. 1988. Nodulation of legumes by rhizobia in acid soils. Pages 57-61 in *Developments in soil science 18* (Vancura V and Kunc F, eds.). Amsterdam, The Netherlands: Elsevier Science Publishers.
- Cordovilla MP, Ocaña A, Ligeró F and Lluch C. 1995. Growth and macronutrient contents of faba bean plants: effects of salinity and nitrate nutrition. *Journal of Plant Nutrition* 18:1611-1628.
- Day JM, Roughley RJ, Eaglesham ARJ, Dye M and White SP. 1978. Effect of high soil temperatures on nodulation of cowpea, *Vigna unguiculata*. *Annals of Applied Biology* 88:476-481.
- Delgado MJ, Ligeró F and Lluch C. 1994. Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. *Soil Biology and Biochemistry* 26:371-376.
- Diaz del Castillo L, Hunt S and Layzell DB. 1994. The role of oxygen in the regulation of nitrogenase activity in drought-stressed soybean nodules. *Plant Physiology* 106:949-955.
- Djekoun A and Planchon D. 1991. Water status effect on dinitrogen fixation and photosynthesis in soybean. *Agronomy Journal* 83:316-322.
- Drevon JJ, Derensard C, Irekti H, Payre H, Roy G and Serraj R. 1995. La salinité abaisse la conductance des nodosités à la diffusion de l'oxygène. Pages 73-84 in *Facteurs limitant la fixation symbiotique de l'azote dans le bassin méditerranéen* (Drevon JJ, ed.). INRA (Les Colloques no 77), Paris.
- Föhse D, Claassen N and Jungk A. 1988. Phosphorus efficiency of plants. I. External and internal P requirement and P uptake efficiency of different plant species. *Plant Soil* 110:101-109.
- Fortmeier R and Schubert S. 1995. Salt tolerance of maize (*Zea mays* L.): the role of sodium exclusion. *Plant Cell Environment* 18:1041-1047.

- Giller KE. 2001. Nitrogen fixation in tropical cropping systems, Wallingford, Oxon; New York: CAB International Publishers, 423 pp.
- Glenn AR, Reeve WC, Tiwari RP and Dilworth MJ. 1998. Rhizobial genes essential for acid tolerance. Pages 491-492 in *Biological nitrogen fixation for the 21st century: Proceedings of the 11th International Congress on Nitrogen Fixation, Institut Pasteur, Paris, France, July 20-25, 1997*, (Elmerich C, Kondorosi A and Newton WE, eds.) Dordrecht, Boston: Kluwer Academic Publishers.
- Greenway H and Munns R. 1980. Mechanism of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* 31:149-190.
- Gregory PJ, Saxena NP, Arihara J and Ito O. 1994. Root form and function in relation to crop productivity in cool season food legumes. page 809-820, in *Expanding the production and use of cool season food legumes* (Muehlbauer FJ and Kaiser WJ, eds.). The Netherlands: Kluwer Academic Publishers.
- Gunawardena SFBN, Danso SKA and Zapata F. 1992. Phosphorus requirements and nitrogen accumulation by three mungbean (*Vigna radiata* (L.) Welzek) cultivars. *Plant and Soil* 147:267-274.
- Gunawardena SFBN, Danso SKA and Zapata F. 1993. Phosphorus requirement and sources of nitrogen in three soybean (*Glycine max*) genotypes: Bragg, nts 382 and Chippewa. *Plant and Soil* 151:1-9.
- Handa S, Bressan RA, Handa AK, Carpita NC, and Hasegawa PM. 1983. Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiology* 73:834-843.
- Hardy RWF and Havelka UD. 1976. Photosynthate as a major factor limiting nitrogen fixation by field-grown legumes with emphasis on soybeans. Pages 421-439 in *Symbiotic nitrogen fixation in plants* (Nutman PS, ed). International Biology Programme, Vol. 7. Cambridge, MA, USA: Cambridge University Press.
- Harper JE. 1974. Soil and symbiotic nitrogen requirements for optimum soybean production. *Crop Science* 14:255-260.
- Hebeisen T, Lüscher A, Zanetti S, Fischer BU, Hartwig UA, Frehner M, Hendrey GR, Blum H and Nösberger J. 1997. Growth response of *Trifolium repens* L. and *Lolium perenne* L. as monocultures and bi-species mixture to free air CO₂ enrichment and management. *Global Change Biology* 3:149-160.
- Herridge DF, Rupela OP, Serraj R and Beck DP. 1994. Screening techniques and improved biological nitrogen fixation in cool-season food legumes. *Euphytica* 73:95-108.
- Hinsinger P. 1998. How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Advances Agronomy* 64:225-265.
- Hirsch AM and McFall-Ngai MJ. 2000. Fundamental concepts in symbiotic interactions: light and dark, day and night, squid and legume. *Journal of Plant Growth Regulation* 19:113-130.
- Hungria M and Vargas MAT. 2000. Environmental factors affecting N₂ fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Research* 65:151-164.
- ICRISAT. 1992. ICC 4958: a drought resistant chickpea. *Plant Material Description No. 33*. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics.
- IPCC. Third assessment report, 2001 <http://www.ipcc.ch/pub/un/syrenng/spm.pdf>).
- Ishikawa S, Adu-Gyamfi JJ, Nakamura T, Yoshihara T and Wagatsuma T. 2002. Genotypic variability in phosphorus solubilizing activity of root exudates by pigeonpea grown in low-nutrient environments. *Plant Soil* 245:71-81.
- Israel DW and Ruffy Jr TW. 1988. Influence of phosphorus nutrition on phosphorus and nitrogen utilization efficiencies and associated physiological responses in soybean. *Crop Science* 28:954-960.
- Jakobsen L. 1985. The role of phosphorus in nitrogen fixation by young pea plants (*Pisum sativum*). *Physiologica Plantarum* 64:190-196.

- Johansen C. 2001. An overview of prospects for genetic enhancement of drought resistance in pigeonpea. Pages 143-150 in Management of agricultural drought-Agronomic and genetic options. Saxena NP, ed.), New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.
- Kashiwagi JW, Krishnamurthy L, Crouch JH and Serraj R. 2003. Variability of early root growth and contribution to seed yield in chickpea (*Cicer arietinum* L.). Crop Science (Submitted).
- Kingsbury RW and Epstein E. 1986. Salt sensitivity in wheat. A case for specific ion toxicity. Plant Physiology 80:651-654.
- Krishnamurthy L, Kashiwagi JW, Crouch JH and Serraj R. 2003. Phenotypic variation in root traits and the scope for its use in molecular breeding for terminal drought tolerance in chickpea (*Cicer arietinum* L.). Field Crops Research (Submitted).
- Kumar J, Sethi SC, Johansen C, Kelley TG, Rahman MM and van Rheenen HA. 1996. Potential of short-duration varieties in chickpea. Indian Journal of Dryland Agricultural Research 11:26-29.
- Kumar J and van Rheenen HA. 2000. A major gene for time of flowering in chickpea. Journal of Heredity 91:67-68.
- Kumar Rao JVDK, Dart PJ and Sastry PVSS. 1983. Residual effect of pigeonpea (*Cajanus cajan*) on yield and nitrogen response of maize. Experimental Agriculture 19:131-141.
- McNeil DL. 1982. Variation in ability of *Rhizobium japonicum* strains to nodulate soybeans and maintain fixation in the presence of nitrate. Applied and Environmental Microbiology 44:647-652.
- Minchin FR. 1997. Regulation of oxygen diffusion in legume nodules. Soil Biology and Biochemistry 29:881-888.
- Munns R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant, Cell and Environment 16:15-24.
- Musa AM, Harris D, Johansen C and Kumar J. 2001. Short duration chickpea to replace fallow after aman rice: the role of on-farm seed priming in the High Barind Tract of Bangladesh. Experimental Agriculture 37:509-521.
- Nam NH, Chauhan YS and Johansen C. 2001. Effects of timing of drought stress on extra-short duration pigeonpea. Journal of Agricultural Science (Cambridge) 136:179-189.
- Neo HH and Layzell DB. 1997. Phloem glutamine and the regulation of O₂ diffusion in legume nodules. Plant Physiology 113:259-267.
- Obaton M, Miquel M, Robin P, Conejero G, Domenach AM and Bardin R. 1982. Influence du déficit hydrique sur l'activité nitratre réductase et nitrégénase chez le soja (*Glycine max* L. Merr. cv. Hodgson). Comptes Rendus de L'Académie des Sciences Paris 294:1007-1012.
- O'Hara GW, Hartzook A, Bell RW and Loneragan JF. 1993. Differences between *Bradyrhizobium* strains NC92 and TAL1000 in their nodulation and nitrogen fixation with peanut in iron deficient soil. Plant Soil 156: 333-336.
- Ohwaki Y and Hirata H. 1992. Differences in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid level in roots. Soil Science and Plant Nutrition 38:235-243
- Ortiz A, Martinez V and Cerdá A. 1994. Effects of osmotic shock and calcium on growth and solute composition in *Phaseolus vulgaris* plants. Physiologica Plantarum 91:408-476.
- Parsons R, Stanforth A, Raven JA and Sprent JI. 1993. Nodule growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. Plant, Cell and Environment 16:125-136.
- Peoples MB, Hebb DM, Gibson AH and Herridge DF. 1989. Development of the xylem ureide assay for the measurement of nitrogen fixation by pigeonpea (*Cajanus cajan* (L.) Millsp.). Journal of Experimental Botany 40:535-542.
- Pessaraki M and Zhou M. 1990. Effect of salt stress on nitrogen fixation by different cultivars of green beans. Journal of Plant Nutrition 13:611-629.

- Piha MI and Munns DN 1987a. Nitrogen fixation capacity of field-grown bean compared to other grain legumes. *Agronomy Journal* 79:690-696.
- Piha MI and Munns DN 1987b. Sensitivity of common bean (*Phaseolus vulgaris* L.) symbiosis to high temperature. *Plant Soil* 98:183-194.
- Purcell LC and Sinclair TR. 1994. An osmotic hypothesis for the regulation of oxygen permeability in soybean nodules. *Plant, Cell and Environment* 17:837-843.
- Purcell LC and King CA. 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. *Journal of Plant Nutrition* 19:969-993.
- Purcell LC, Serraj R, deSilva M, Sinclair TR and Bona S. 1998. Ureide concentration of field-grown soybean in response to drought and the relationship to nitrogen fixation. *Journal of Plant Nutrition* 21:949-966.
- Raggi V. 1994. Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. *Physiologica Plantarum* 91:427-434.
- Rawsthorne S, Hadley P, Surrunerfield RJ and Roberts EH. 1985. Effects of supplemental nitrate and thermal regime on the nitrogen nutrition of chickpea (*Cicer arietinum* L.). II. Symbiotic development and nitrogen assimilation. *Plant Soil* 83:279-293.
- Rennie RJ and Kemp GA. 1982. Dinitrogen fixation in *Phaseolus vulgaris* at low temperatures: interaction of temperature, growth stage, and time of inoculation. *Canadian Journal of Botany* 60:1423-1427.
- Rhodes D, Handa S and Bressan RA. 1986. Metabolic changes associated with adaptation of plant cells to water stress. *Plant Physiology* 82:890-903.
- Ribet J and Drevon JJ. 1996. The phosphorus requirement of N₂-fixing and urea-fed *Acacia mangium*. *New Phytology* 132:383-390.
- Roughley RJ, Simanungkalit RDM, Gemell LG, Hartley EJ and Cain P. 1995. Growth and survival of root-nodule bacteria in legume inoculants stored at high temperatures. *Soil Biology and Biochemistry* 27:707-712.
- Rupela OP and Rao JVDK. 1987. Effects of drought, temperature, and salinity on symbiotic nitrogen fixation in legumes, with emphasis on chickpea and pigeonpea. Pages 123-131 in *Adaptation of chickpea and pigeonpea to abiotic stress: Proceedings of the Consultants' Workshop held at ICRISAT, India, 19-21 December 1984*. Patancheru, Andhra Pradesh: International Crops Research Institute for the Semi-Arid Tropics.
- Sanginga N, Danso SKA and Bowen GD. 1989. Nodulation and growth response of *Allocastrum* and *Casuarina* species to phosphorus fertilization. *Plant Soil* 118:125-132.
- Sanginga N. 2003. Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. *Plant Soil* 252:25-39.
- Seligman NG and Sinclair TR. 1995. Global environment change and simulated forage quality of wheat. II. Water and nitrogen stress. *Field Crops Research* 40:29-37.
- Serraj R, Drevon JJ, Vidal A and Obaton M. 1992. Variation in nitrate tolerance of nitrogen fixation in the soybean (*Glycine max.* L.)-*Bradyrhizobium* symbiosis. *Journal of Plant Physiology* 140:366-371.
- Serraj R, Obaton M and Vidal A. 1993. Nitrogen fixation and nitrate assimilation of determinate, semi-determinate and indeterminate soybeans (*Glycine max* L.). *Journal of Agronomy and Crop Science* 171:36-45.
- Serraj R, Roy G and Drevon JJ. 1994. Salt stress induces a decrease in the oxygen uptake of soybean nodules and their permeability to oxygen diffusion. *Physiologica Plantarum* 91:161-168.
- Serraj R and Sinclair TR. 1996. Inhibition of nitrogenase activity and nodule oxygen permeability by water deficit. *Journal of Experimental Botany* 47:1067-1073.
- Serraj R and Sinclair TR. 1997. Variation among soybean cultivars in dinitrogen fixation response to drought. *Agronomy Journal* 89:963-969.
- Serraj R and Drevon JJ. 1998. Effects of salinity and nitrogen source on growth and nitrogen fixation in alfalfa. *Journal of Plant Nutrition* 21:1805-1818.

- Serraj R, Vasquez-Diaz H and Drevon JJ. 1998a. Effects of salt stress on nitrogen fixation, oxygen diffusion and ion distribution in soybean, common bean and alfalfa. *Journal of Plant Nutrition* 21:475-488.
- Serraj R, Allen HL and Sinclair TR. 1998b. Soybean nodulation and N_2 fixation response to drought under carbon dioxide enrichment. *Plant, Cell and Environment* 21:491-500.
- Serraj R, Vadez V, Denison RF and Sinclair TR. 1999a. Involvement of ureides in nitrogen fixation inhibition in soybean. *Plant Physiology* 119:289-296.
- Serraj R, Purcell LC and Sinclair TR. 1999b. Inhibition of N_2 fixation by drought. *Journal of Experimental Botany* 50:143-155.
- Serraj R, Vasquez-Diaz H, Hernandez G and Drevon JJ. 2001. Genotypic difference in response of nitrogenase activity (C_2H_2 Reduction) to salinity and oxygen in common bean. *Agronomie* 21:645-65.
- Silisbury JH, Catchpole DW and Wallace W. 1986. Effects of nitrate and ammonium on nitrogenase (C_2H_2 reduction) activity of swards of subterranean clover, *Trifolium subterraneum* L. *Australian Journal of Plant Physiology* 13:257-273.
- Sinclair TR and Serraj R. 1995. Dinitrogen fixation sensitivity to drought among grain legume species. *Nature* 378:344.
- Sinclair TR, Muchow RC, Bennett JM and Hammond LC. 1987. Relative sensitivity of nitrogen and biomass accumulation to drought in field-grown soybean. *Agronomy Journal* 79:986-991.
- Sinclair TR and Vadez V. 2002. Physiological traits for crop yield improvement in low N and P environments. *Plant and Soil* 245:1-15.
- Singleton PW, El-Swaifi, SA and Bohlool BB. 1982. Effect of salinity on Rhizobium growth and survival. *Applied and Environmental Microbiology* 44:884-890.
- Snapp SS. 1998. Phosphorus and sustainability of sub-Saharan African smallholder farms. Pages 71-83 in *Phosphorus in Plant Biology: Regulatory Roles in Molecular, Cellular, Organismic and Ecosystem Processes* (Lynch JP and Deikman J, eds). American Society of Plant Physiology, Rockville, MD, USA.
- Sprent JI. 1980. Root nodule anatomy, type of export product and evolutionary origin in some leguminosae. *Plant, Cell and Environment* 3:35-43.
- Streeter J. 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. *Critical Reviews in Plant Sciences* 7:1-23.
- Subbarao GV, Johansen C, Jana MK and Rao JVDKK. 1990. Comparative salinity tolerance of symbiotically dependent and nitrogen-fed pigeonpea (*Cajanus cajan*) and its wild relative *Alyosia platycarpa*. *Biology and Fertility of Soils* 10:11-16.
- Swaraj K, Sheokand S, Fernandez-Pascual MM and de Felipe M.R. 2001. Dark-induced changes in legume nodule functioning. *Australian Journal of Plant Physiology* 28:429-438.
- Tobita S, Ito O, Matsunaga R, Rao TP, Rego TJ, Johansen C and Yoneyama T. 1994. Field evaluation of nitrogen fixation and use of nitrogen fertilizer by sorghum/pigeonpea. *Biology and Fertility of Soils* 17:241-248.
- Vadez V, Lim G, Durand P and Diem HG. 1995. Comparative growth and symbiotic performance of four *Acacia mangium* provenances from Papua New Guinea in response to the supply of phosphorus at various concentrations. *Biology and Fertility of Soils* 19:60-64.
- Vadez V, Sinclair TR and Serraj R. 2000. Asparagine and ureide accumulation in nodules and shoots as feedback inhibitors of N_2 fixation in soybean. *Physiologia Plantarum* 110:215-223.
- Vance CP. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. *Plant nutrition in a world of declining renewable resources*. *Plant Physiology* 127:390-397.
- VanHoom JW, Katerji N, Hamdy A and Mastrorilli M. 2001. Effect of salinity on yield and nitrogen uptake of four grain legumes and on biological nitrogen contribution from the soil. *Agricultural Water Management* 51:87-98.

- von Uexkull HR and Mutert E. 1995. Global extent, development and economic impact of acid soils. *Plant Soil* 171:1-15.
- Walsh KB. 1995. Physiology of the legume nodule and its response to stress. *Soil Biology and Biochemistry* 2:637-655.
- Walsh KB, Canny NJ and Layzell DB. 1989. Vascular transport and soybean nodule function. II. A role for phloem supply in product export. *Plant, Cell and Environment* 12:713-723.
- Waru SP, Rupela OP and Lee KK. 1997. Soil mineral nitrogen concentration and its influence on biological nitrogen fixation of grain legumes. In *Extending nitrogen fixation research to farmers' fields: Proceedings of an International Workshop, 20-24 August 1996, ICRISAT, India* (Rupela OP, Johansen C and Herridge DF, eds.). Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.
- Wery J, Turc O and Salsac L. 1986. Relationship between growth, nitrogen fixation and assimilation in a legume (*Medicago sativa* L.). *Plant Soil* 96:17-29.
- Wery J, Silim SN, Knight EJ, Malhotra RS and Cousin R. 1994. Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes. *Euphytica* 73:73-83.
- Williams PM and deMallorca SM. 1984. Effects of osmotically induced leaf moisture stress on nodulation and nitrogenase activity of *Glycine max*. *Plant Soil* 80: 267-283.
- Wilson JK. 1931. The shedding of nodules by beans. *Agronomy Journal* 23:670-674.
- Witty JF, Skot L and Revsbeck NP. 1987. Direct evidence for changes in the resistance of legume root nodules to O₂ diffusion. *Journal Experimental Botany* 38:1129-1140.
- Yeo AR, Lee KS, Izard P, Boursier PJ and Flowers TJ. 1991. Short and long-term effects of salinity on leaf growth in rice (*Oryza sativa* L.). *Journal of Experimental Botany* 42:881-889.