

Increasing yield potential of legume crops – similarities and contrasts with cereals.

Thomas R. Sinclair

Agronomy Department, University of Florida, P.O. Box 110965, Gainesville, FL 32611-0965. Email trsincl@ifas.ufl.edu

Abstract

Legumes offer special characteristics that make them highly desirable as critical components of cropping systems. The key advantage of legumes is their ability to form symbioses with specific bacteria to fix atmospheric nitrogen (N_2) into organic compounds for use by plants. As a result, soils on which legumes are grown do not normally need to be fertilized with N. Also, legumes tend to accumulate high concentrations of N in their tissues, which make the tissues valuable human food or animal feed. Many of the unique characteristics of legumes are associated with N_2 fixation capability and the accumulation of high N concentrations in their tissues. Nitrogen fixation in all legumes is very sensitive to residual nitrate in the soil. While genetic variation in this trait has been identified, there has as of yet been no successful incorporation of nitrate tolerance into commercial cultivars. Also, N_2 fixation in legumes that transport urides, e.g. soybean and cowpea, are extremely sensitive to soil drying. Recent studies with soybean have resulted in the identification of genotypes that have considerable N_2 fixation tolerance to soil drying. High energy flux is necessary to sustain high N_2 fixation rates and, consequently, legumes require phosphorus (P) in reasonably large amounts to sustain the energy flow pathways. Not surprisingly, several legume species have evolved especially effective methods to accumulate P from various P complexes in the soil that are unavailable to other species. Overall, legumes offer many traits that are highly desirable for improving many cropping systems.

Media Summary

Legumes have the capability of forming symbioses with specific bacteria to fix atmospheric nitrogen into organic compounds. Understanding of this system can lead to improved cropping systems in developed countries to minimize soil fertilization and in developing countries where fertilizer is not available.

Key Words

Nitrogen Fixation, Photosynthesis, Drought Stress, Phosphorus Accumulation, Soybean, Peanut

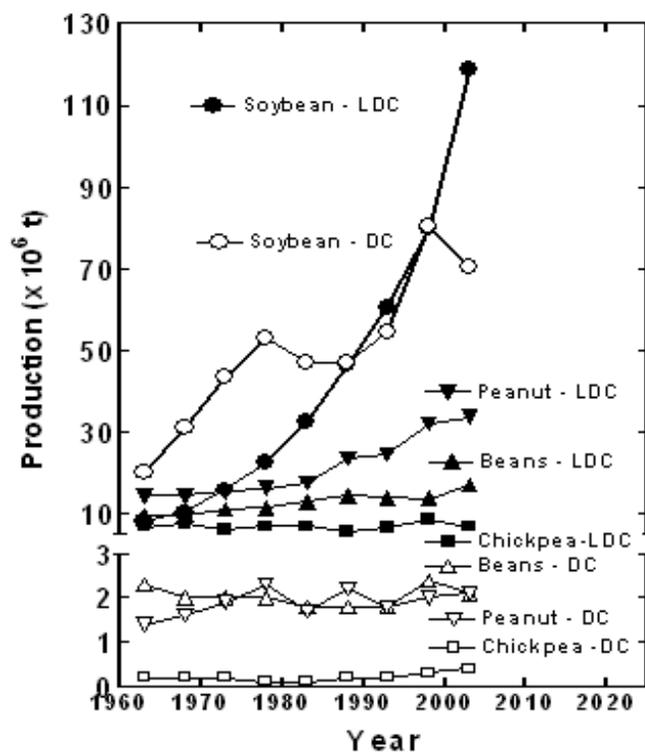
Introduction

Legumes have been recommended as a part of cropping systems since ancient history. The key benefit of legumes, of course, is their ability to form symbioses with specific bacteria that allow the fixation of atmospheric dinitrogen (N_2). The fixed N is used in legume plants to synthesize the essential amino acids, nucleic acids, and other nitrogenous compounds required to sustain and grow plant tissue. The ability of legumes to fix atmospheric N_2 can make these species especially valuable assets in cropping systems.

In roughly the past half century, however, the role of legumes in cropping systems has often diminished in favor of increasing emphasis on cereals. Rather than relying on N input to the cropping system from legumes, in many regions of the world N fertilizer is applied in increasing amounts in the production of cereals. Now, however, the continuous production of cereals with applications of large amounts of fertilizer is facing several constraints. From several perspectives, legumes can become critical components of alternate cropping systems, particularly if legume yields can be enhanced. In industrial countries, the abundant application of N fertilizers to fields has raised environmental concerns and regulations have been established to control nitrogenous compounds in ground water and water ways. In developing countries, the opposite concern exists in that lack of manufactured N fertilizer causes severe limitations on cereal yields. Nitrogen fertilizer availability in the developing countries is constrained by the cost of manufacture, distribution, and credit for farmers to purchase fertilizer.

Further, the high protein concentrations of legume tissues make them desirable as feed and food sources. The vegetative tissues of virtually all legumes are especially high in protein concentrations, which make them very desirable as forage for animals. Seeds of grain legumes are high in protein so that when digestive inhibitors in some species are inactivated, they are excellent foods. Human consumption of seeds of grain legumes is important world wide, including common beans (*Phaseolus* sp.), soybean (*Glycine max*), peas (*Pisum sativum*), peanut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*), and pigeon pea (*Cajanus cajan*).

Soybean especially has had a rapid increase in production over the past 20 years (Fig. 1) due to the fact that the grain is high in both protein and oil concentrations. While increased production of soybean has occurred in developed countries (DC), the more dramatic increase has occurred in less developed countries (LDC), especially Brazil. Brazil will shortly become the global leader in soybean production. Other areas of the world have the potential to increase production to meet the ever increasing demand for vegetable protein and oil.



Source: FAOstat 2004

Figure 1. Total production by developed countries (DC) and less developed countries (LDC) plotted against year of production for various grain legumes.

To sustain legume production and stimulate expanded use of legumes in cropping system, there is a need to further increase legume yield both in terms of plant mass and total plant N accumulation. In this brief review, the topics to be considered for possible improvement in order to increase yields include plant development, carbon assimilation, nitrogen assimilation, and stress amelioration. Not surprisingly, most of this research with legumes has been done with soybean, so much of the discussion will focus on soybean.

Plant Development

The basic scheme of temperature-driven plant development in legumes is not unlike that in cereal species. That is, rates of germination and emergence, leaf emergence, and flowering are all quantitatively linked to the temperature to which the plants are subjected. Rates of development increase in an approximately linear manner until temperature becomes too high and results in inhibition of development. In addition, many legumes are especially sensitive to the daylength under which they develop. Soybean was one of the first species in which photoperiod sensitivity was discovered (Borthwick & Parker, 1938 or Garner & Allard, 1920). Soybean is a short-day species so that shortening daylength results in an accelerated rate to flowering.

The daylength response is so strong in soybean that cultivars are generally narrowly adapted to a specific latitudinal zone. Individual cultivars are classified by maturity group ranging from 00 to X with those cultivars classified as 00 being adapted to high latitudes and those as X being adapted to low latitudes (~20°). Until relatively recent times, soybean could not be grown commercially at very low latitudes because short daylengths triggered very early flowering and only small plants with very low yields were produced. However, long-juvenile (which actually is a botanical misnomer) genes were identified in soybean (Hartwig and Kiihl, 1979) and these genes result in delayed flowering even under short daylengths by slowing the overall development rate to flowering (Sinclair and Hinson, 1992). Long-juvenile genes have now been used extensively in breeding programs so that cultivars are available for production in low-latitude regions including tropical Australia. The incorporation of the long-juvenile genes into productive cultivars was crucial in the tremendous expansion of soybean production in recent years into northern Brazil reaching to the equator.

Little is known about the genetic regulation of development rate of plants. Some legumes are handicapped by especially slow production of leaves causing low crop growth during early stages of development. Pigeon pea, for example, has small leaves that are produced at a slow rate so that the time to achieve full radiation interception is substantially delayed as compared to other species (Muchow, 1985). Consequently, major increases in yield potential of pigeon pea and other relatively small-leaved species seem possible if the rate of leaf area development could be increased.

Increases in harvest index have been a major component of yield increases for cereals and a few grain legumes. Soybean, for example, commonly now has a harvest index of approximately 0.40 or greater. For a number of grain legumes, harvest indices can be substantially below this level. Pigeon pea, for example, may have a harvest index as low as 0.20 (Lawn and Troedson, 1990). Success with other crops in increasing harvest index indicates that such selections in breeding programs could be a practical approach to achieving rapid grain yield increases in some legumes.

Carbon Assimilation

Leaf photosynthetic potential of legumes seems to be as high as C3 cereals. Measurements of leaf photosynthesis rates of field-grown soybean have been reported over $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a number of observations near or above $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Sinclair, 1980; Lauer and Shibles, 1987). Leaf rates at or above $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ have also been reported for field-grown peanut (Bennett et al., 1993; Bell et al., 1994). The fact that virtually no data have been reported on the leaf photosynthetic activity of other grain legumes under field conditions may offer an important research opportunity for characterizing growth and yield potential in other legume species.

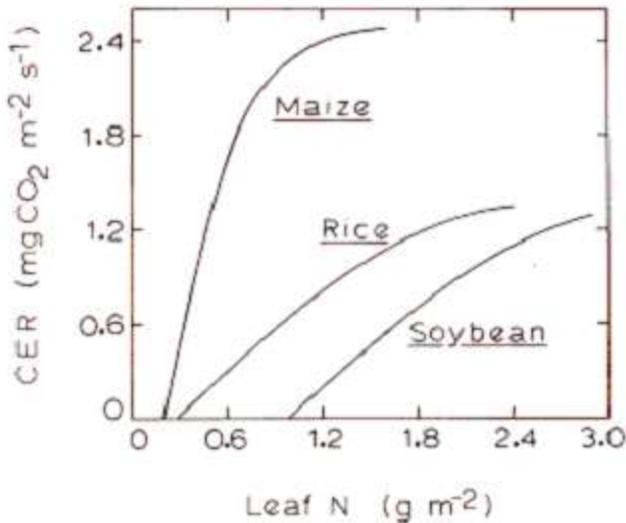


Figure 2. Summary graphs of carbon exchange rate (CER) as a function of leaf nitrogen content (Sinclair and Horie, 1989).

While legumes are capable of high leaf photosynthetic rates, these rates contrast with cereals in that they are achieved only with a much greater investment in leaf N content per unit leaf area than required in cereals. As illustrated in Fig.2, leaf N contents of soybean are shifted to about 0.7 g N m⁻² greater levels as compared to rice to achieve comparable photosynthetic rates.

The additional leaf N in soybean that seems to be needed to achieve photosynthetic rates comparable to C3 cereals is seemingly a disadvantage. The basis for this difference between cereals and legume has not

been explained. There appears to be no evidence that the photosynthetic biochemistry of legumes is less efficient than that of C3 cereals. One hypothesis is that the leaves of legumes simply store more protein

than cereals because of the higher relative abundance on N in legume plants as a result of N₂ fixation. Consistent with this hypothesis is the presence in many legumes leaves of a layer of paraveinal mesophyll cells (Lansing and Franceschi, 2000) that are rich in glycoproteins (Klauer et al., 1996). The paraveinal mesophyll cells (Fig. 3) have little photosynthetic capability and seem to function as storage and transport cells. The physiological role of the paraveinal mesophyll in legume leaves certainly deserves further attention as contributors to the carbon and N economy of these species.

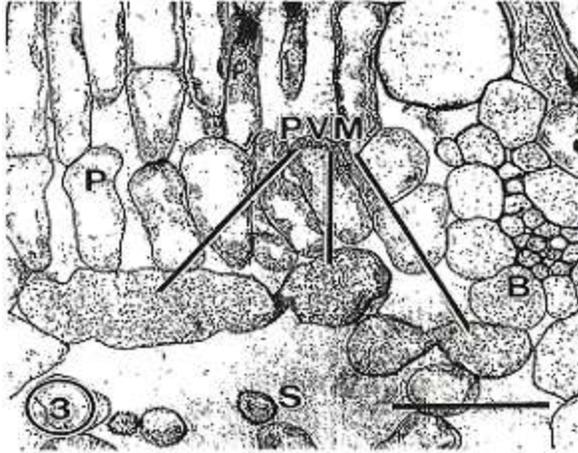


Figure 3. Cross-section of soybean leaf (Klauer et al., 1996). PVM = paraveinal mesophyll cells, P = palisade cell, B = bundle sheath cell, S = spongy mesophyll cell, bar = 50 μm .

The carbon assimilation capacity of the total crop can be effectively expressed by Radiation Use Efficiency (RUE, defined here as the ratio of mass accumulated to cumulative intercepted total solar radiation). Sinclair and Horie (1989) calculated the potential RUE of soybean based on leaf photosynthetic capacity as being about 1.2 g MJ^{-1} . This estimate was lower than that anticipated of C3 cereals (approximately 1.4 MJ^{-1}) as expected based on the difference in the higher energy content of the soybean mass.

In practice, there have been only a few reports of soybean RUE as high as the 1.2 g MJ^{-1} suggested by Sinclair and Horie (1989). Most experimental observations have reported RUE values in the range of 0.65 to 1.15 g MJ^{-1} with a median of approximately 0.9 g MJ^{-1} (Sinclair, 2004). Rochette et al. (1995) published detailed observations on soybean RUE and found that during the season when leaf area index was greater than 2.0 the value of RUE varied between 0.84 and 1.02 g MJ^{-1} . RUE values for other grain legumes have been in the same range as that found for soybean (Sinclair and Muchow, 1999).

Field observations of RUE may frequently be lower than theoretical values because RUE is sensitive to many stresses that decrease leaf photosynthesis rate. Consequently, water deficit or biotic pests that result in decreased leaf photosynthesis rate will result in decreased RUE. Probably, the most important environmental factor decreasing RUE is water deficit. Soil dehydration that results in loss of leaf photosynthetic activity will result directly in a decrease in RUE. Especially in legumes that may have shallower roots than cereals, the onset of decreases in RUE may occur early in the drying cycle.

Focused research to understand the commonly observed lower RUE than theoretically anticipated for legumes may lead to insights for increasing yields. Are there management or genetic options available to sustain high leaf photosynthetic rates of legume leaves, and consequently sustained high RUE?

Nitrogen Fixation

As discussed previously, the capability of legumes to undertake symbiotic N_2 fixation is a critical, distinguishing trait of legumes, and causes legumes to be a highly desirable component of many cropping systems. There are, however, several aspects of the N_2 fixation process that result in unique physiological considerations. One of these is the regulation of O_2 concentration in the nodule. Nitrogen fixation is catalyzed by nitrogenase, which is highly sensitive to oxygen and can be readily denatured when exposed to oxygen. Therefore, the first metabolic step leading to N_2 fixation is a paradox in that the reaction requires large amount of energy supported by oxidative respiration while the basic N_2 fixing enzyme is destroyed by the O_2 that is essential for respiration.

Nodules are well designed to meet the dual requirements of high O₂ supply rate for respiration within the nodule and maintenance of low O₂ concentration (Fig. 4). The internal volume of the nodule where fixation takes place is surrounded by a shell, two or three cells thick, which acts as an O₂ diffusion barrier. The O₂ permeability of the barrier is variable and adjusts to maintain a low O₂ concentration in the internal volume of the nodule (Serraj et al., 1999). Oxygen readily diffuses in the internal volume of nodules through a network of gas spaces. To facilitate diffusion of O₂ from the air spaces to the site of respiration within N₂ fixing cells, nodules of many species have O₂ transporters in these cells to which O₂ binds to facilitate diffusion. The transporter in soybean nodules is leghemoglobin, which gives the interior of healthy soybean nodules a pinkish/red color when they are cut open and the leghemoglobin is exposed to atmospheric oxygen concentrations.

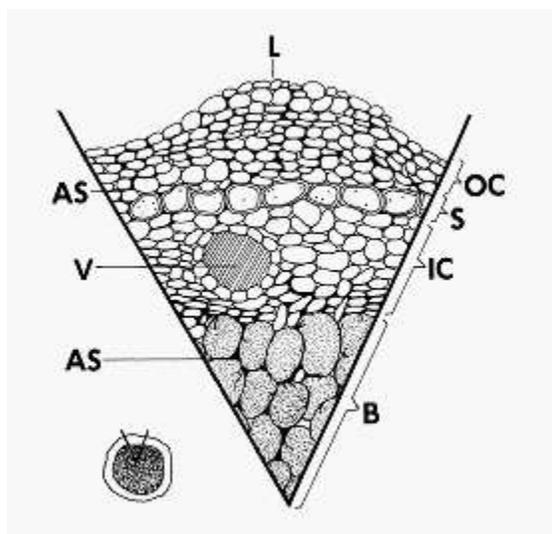


Figure 4. Cross-sectional drawing of nodule (bottom left) and the magnified slice of the nodule. The components of the nodule are L = lenticel, OC = outer cortex, s = sclerid layer, IC = inner cortex, B = bacteriodal volume, AS = air space, and V = vascular bundle.

Amides and/or ureides are the nitrogenous compounds synthesized in nodules from ammonia produced in the primary step of N₂ fixation. Based on a limited sample, those grain legumes that transport ureides seem to have a higher N₂ fixation rates than those that transport amides. Ureides are efficient for N transport since their C:N ratio is 1:1. However, the solubility of ureides is low (Sprent, 1980) and this fact may contribute to the requirement by ureide-transporting species of relatively high water fluxes out of nodules via the xylem (Serraj et al., 1999). Since the source of the water for xylem outflow is phloem flow into the nodule, interruption of phloem flow into nodules results in rapid decreases in N₂ fixation rates. In addition, feedback of nitrogenous compounds from the mother plant exerts a control on nodule activity (Serraj et al., 1999). The tight regulation of nodule activity by water and nitrogen flow results in a close balance between N supply and N requirements to support plant growth.

In cropping situations it would seemingly be beneficial to maximize N₂ fixation to increase yield and/or protein content of the plant products, and to increase the production of fixed N for the overall cropping system. Currently, there is little information about what may be the ultimate limit on N₂ fixation rate. The highest N₂ fixation rate for soybean appears to be about 0.6 g N m⁻² d⁻¹, which was reported for plants grown on a gravel-hydroponic culture in the field (Harper, 1971). One possibility is that N₂ fixation rate in soybean might simply be limited by the rate of formation of vegetative storage 'compartments' in which to deposit the fixed N. One interesting research approach to investigate maximizing N₂ fixation rate would be to undertake studies with legumes with large tubers (Tropical Legumes, 1979). These tubers might provide both energy reserves and storage capacity in experiments to investigate the limit for maximum N₂ fixation rate.

Another major limitation on N₂ fixation rate when legumes are rotated with a cereal in a cropping system is the large amounts of N fertilizer that may be applied to the cereal. High levels of residual nitrate in the soil will inhibit nodulation and N₂ fixation activity (Streeter, 1988). Delayed formation of nodules and initiation of N₂ fixation can result in a period after the soil nitrate is exhausted when there is little or no N₂ fixation. Consequently, there may be several weeks during critical stages of plant development when N supply within the plant is restricted and the legume suffers N deficiency symptoms and loss of yielding capability.

There has, therefore, been considerable interest in developing soybean cultivars with N₂ fixation that is tolerant of residual nitrate for use following heavily fertilized cereals in cropping rotations. A goal has been to identify genotypes that can develop nodules in the presence of nitrate so that N₂ fixation activity is initiated prior to or as soon as the soil nitrate is exhausted. Several soybean genotypes have been identified that have less sensitivity of N₂ fixation rates to nitrate than most soybean (Hardarson et al., 1984; Gibson and Harper, 1985; and Serraj et al., 1992). Soybean breeding programs to enhance N₂ fixation tolerance of nitrate by soybean (Betts and Herridge, 1987; Herridge and Rose, 1994; Raffin et al., 1995) have, however, appeared not to have yet led to the release of commercial varieties.

Another approach to enhancing nitrate tolerance was the identification of genotypes that form unusually large numbers of nodules in the absence of nitrate. Even though nitrate resulted in the same relative decrease in these 'super nodulating' lines as normal lines, the number of nodules on these lines under nitrate treatment was still high (Carroll et al., 1985; Gremaud and Harper, 1989). However, the retention of a reasonable nodule population when subjected to nitrate has failed to result in yield increase (Wu and Harper, 1991; Maloney and Oplinger, 1997).

Nitrogen fixation in those species that transport ureides from the nodules to the shoot have been found to be extremely sensitive to soil water deficits (Sinclair and Serraj, 1995). Nitrogen fixation rates in the ureide-transporting species, e.g. soybean and cowpea, begins to decline as result of soil drying well in advance of any other physiological process. As discussed previously, this sensitivity may in part be a consequence of the need for high water flux to and from nodules to sustain ureide transport. In addition, there appears to be an especially strong negative feedback from the shoot to the roots (Serraj et al., 1999) involving elevated ureide concentrations that signal decreased N₂ fixation rates. Nitrogen fixation in amide-transporting species, on the other hand, does not appear to be any more sensitive to water deficits than other physiological responses.

The sensitivity of N₂ fixation to drying soil has proved to be greater in ureide-transporting legumes than any other physiological processes in these plants (Serraj et al., 1999). This sensitivity has been confirmed in field studies employing various water-deficit treatments on a sandy soil with no residual N (Sinclair et al., 1987). Hence, nitrogen accumulation reflected N₂ fixation and the results were expressed relative to the well-watered treatment. A plot of relative N accumulation vs. relative mass accumulation (Fig. 5) illustrated that in every water-deficit treatment the relative nitrogen accumulation was decreased to a greater extent than mass accumulation. Further, Purcell and King (1996) showed directly a loss of soybean yield as a result of N₂ fixation sensitivity to soil drying. In a field experiment, plots subjected to drought were either left with no added nitrate or nitrate was added. Those plots to which nitrate was added as a source of N during the drought had an 18% greater yield than the plots untreated with nitrate and dependent solely on N₂ fixation.

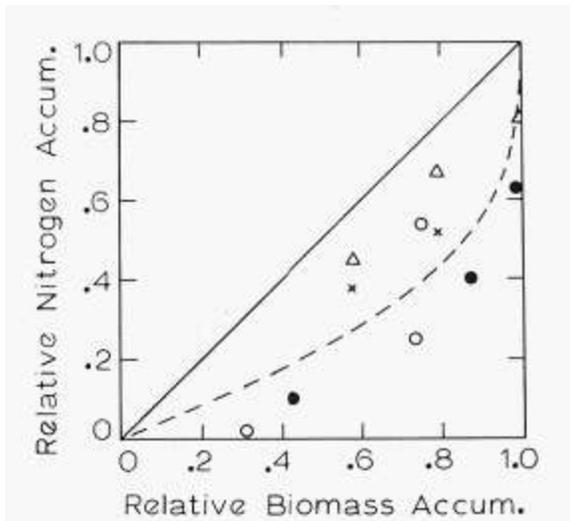


Figure 5. The relative N accumulation and mass accumulation of soybean cultivar Biloxi subjected to differing water-deficit treatments grown under field conditions (Sinclair et al., 1987).

We have undertaken a program to identify and breed for soybean genotypes with improved tolerance of N_2 fixation to soil drying. Early in this study, cultivar Jackson was identified as having substantial N_2 fixation tolerance to soil water deficit (Sall and Sinclair, 1991). In Jackson, the relative N_2 fixation rate was found to decrease with soil drying essentially in parallel with decreases in relative transpiration rate (Fig. 6). This response contrasted with other genotypes as exemplified by CNS (Fig. 6). A breeding program was subsequently initiated using Jackson as one parent. Progeny lines have now been yield tested at six locations in the southern U.S. under non-irrigated conditions and several lines had mean yields at least 5% greater than the commercial, check lines.

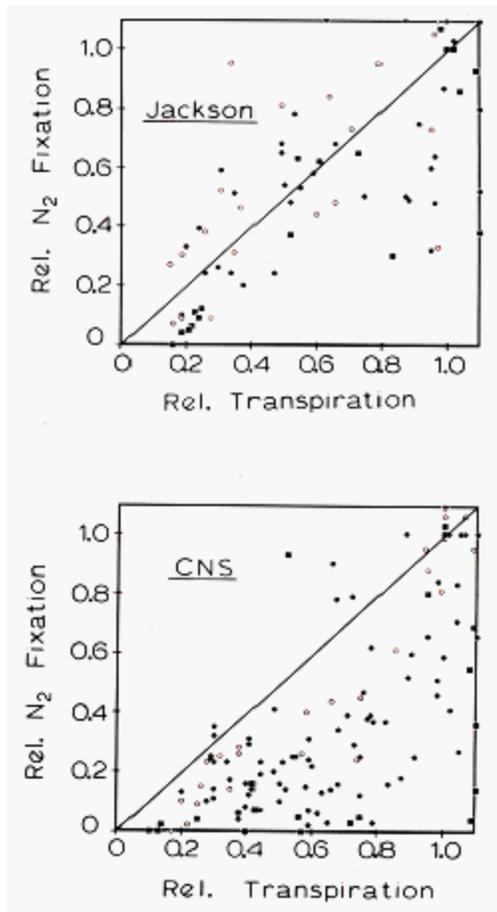


Figure 5. Relative N₂ fixation rate plotted as a function of relative transpiration of individual plants during a drying cycle for soybean cultivars Jackson and CNS (Sall and Sinclair, 1991).

In addition, a major effort was undertaken to identify additional soybean germplasm that would have greater tolerance of N₂ fixation to water deficit than Jackson. A three-stage screening program was developed in which over 3000 plant introduction lines were ultimately screened and eight lines were identified to have substantial N₂-fixation tolerance (Sinclair et al., 2000). In addition, these eight tolerant lines were found to exhibit little feedback inhibition of N₂ fixation rates when supplied with high levels of ureides (Sinclair et al., 2003). These tolerant lines are now being included in breeding efforts to develop drought tolerant soybean cultivars.

Stress Amelioration

Water Deficit. The previous discussion highlighted the special limitation of soil drying on N₂ fixation activity of those legumes that transport ureides. Aside from N₂ fixation, growth and water loss by grain legumes varies with soil water content (Fig. 7) in the same pattern as observed for all crop species (Sinclair and Ludlow, 1986). That is, there is no influence on plant gas exchange until only 1/3 of the transpirable water remains in the soil. At this 1/3 point, the plant gas exchange rates decrease approximately linearly until the stomata are closed. For comparison, the response of N accumulation as a result of N₂ fixation is included in Fig. 6 illustrating that N₂ fixation is declining when as much as 2/3 of the transpirable water still remains in the soil.

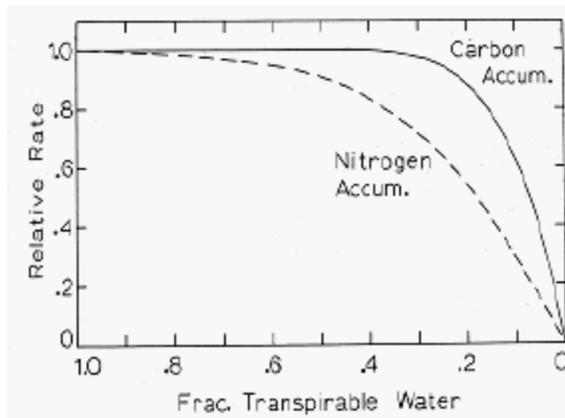


Figure 7. Soybean responses in plant gas exchange and nitrogen accumulation by N_2 fixation on drying soil, i.e. decreasing fractional transpirable soil water.

Once there is no transpirable water remaining in the soil, plants enter a survival phase in which they are no longer growing but they continue to extract soil water at a very slow rate to maintain plant water balance and avoid desiccation. Interestingly, there are very large differences among grain legumes in the ability to survive these severe stress conditions. Soybean has a very poor ability to survive severe stress while pigeon pea can survive long-term stress (Sinclair and Ludlow, 1986). However, Serraj and Sinclair (2002) argued that these differences in survival of severe drought are likely not to be particularly important in high-production agriculture. If the total amount of available water is so limited to cause crop survival to be a concern, then yield potential is likely to be severely restricted and little or no yield can be harvested from such a crop. On the other hand, in perennial forage legumes survival traits could be important to allow on-going production once precipitation returns.

A water-deficit response that has generated considerable interest in soybean is the slow-wilting phenotype. PI 416937 was identified as being able to maintain turgor with soil drying such that the plants of this genotype wilted several days later than other genotypes (Sloane et al., 1990). The basis of this unique phenotypic expression is speculated to be a result of either deeper rooting in the soil or a water-conservation strategy in which plants use water at a slightly lower rate than other lines. Depending on the environment through the growing season, such traits could be of considerable benefit in some dry seasons and result in little or no penalty in wet seasons. Research is underway to evaluate these hypotheses and to determine the impact of the slow-wilting traits on yield.

Phosphorous. Nitrogen limitations in cropping systems can be minimized by using legumes but often there remains a limitation on plant growth due to inadequate phosphorus. Certain legumes, however, appear to have the capacity to make a major contribution to improving the availability of P in rotational cropping systems. Several legume species have been identified that exhibit substantial superiority in recovering soil P that is unavailable to other species. For example, Ae et al. (1990) reported that pigeon pea thrived on an Alfisol soil with most of the P bound to iron while maize (*Zea mays*), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), and soybean died from P deficiency within one month after sowing. Ishikawa et al. (2002) found that roots of pigeon pea excreted large amounts of citric and piscidic acid and these contributed to the mobilization of P in the soil. They concluded, however, that this mechanism was inadequate to account for all of the P taken up by the plant. The root cell-walls of pigeon pea contain a small molecule that has P-solubilizing abilities (Ae and Shen, 2002) and likely contributes to the recovery of bound P from the soil. Peanut also exhibited the same P-solubilizing abilities, which was associated with survival on low P-available soil while maize, sorghum, and soybean died. Ohwaki and Hirata (1992) also reported that chickpea exuded more citric and malic acid than five other legumes indicating that this species may be another good candidate for use on soils with low P availability.

In addition to differences among species in recovering non-soluble P, there are genotypic differences within species. Lynch and Beebe (1995) compared 364 common bean genotypes under P-deficient

conditions and found substantial differences in growth. It was confirmed in a comparison of six contrasting cultivars of common bean that there were substantial differences in the ability to recover P from different forms of immobile P (Yan et al., 1996). Ishikawa et al. (2002) included four cultivars of pigeon pea in their study of P recovery from various sources and found distinct differences among genotypes, which depended on the form of immobilized P.

Temperature. Legume production has been extended over a wide range of climates from cold, northern latitudes, to hot, tropical regions. Much of the expansion in the range of adaptation has resulted from empirical selection of superior performance of individual genotypes for new climate zones. There have, however, been research programs targeted specifically at identifying genetic variation within legume species for adaptation to various temperature regimes.

In peanut, cold night temperatures of less than 15 C resulted in lost leaf photosynthetic activity during the following day (Sinclair et al., 1994; Bell et al., 1994). Bell et al. (1994) studied eight genotypes and found significant differences in the influence of cold night temperature. While the threshold for the negative influence of temperature did not seem to vary among genotypes, some genotypes (OAC Ruby and OAC Garroy) had smaller decreases in photosynthetic rates at lower temperatures than the other genotypes. These results indicated that selection in legumes for photosynthetic tolerance of cold temperatures is possible.

In cowpea, high night temperatures (>30 C) resulted in flower abscission (Warrag and Hall, 1984). The high night temperature was shown to result in indehiscence of anthers and low pollen viability (Warrag and Hall, 1983). A screen of 58 cultivars resulted in the identification of three cultivars that had high pod-set under high night temperatures (Warrag and Hall, 1983). A pedigree breeding program was undertaken and eventually a heat-tolerant commercial cultivar was released (Ehlers et al., 2000).

Perspective

Recent, dramatic expansions in global soybean production are likely to continue for the foreseeable future. Protein demand will increase as human diets improve and/or shift to greater amounts of meat, virtually necessitating an increase in the production of all grain legumes. This increasing demand will result in a number of opportunities and challenges. The opportunities are to use innovative approaches to fully integrate grain legume production into cropping systems. What approaches take full advantage of the ability of legumes to symbiotically fix N₂? One suggestion for developing countries, for example, is the development of dual-purpose grain legume cultivars with high N₂ fixation capability to produce a modest grain crop in the growth year for immediate economic benefit to the grower and to generate also a reasonable amount of high N-content plant residue to be incorporated into the soil for use by subsequent cereal crops (CIAR-TSBFI Working Group on BNF, 2004). Also, full exploitation of some grain legumes to recover P from the soil could be a very important asset in many cropping situations. The challenges of fully exploiting legumes are likely to be focused on their sensitivity to a number of environmental stresses including photoperiod, temperature, and especially water deficits. Continued success in increasing the productivity of grain legumes and their use in cropping systems will likely be well rewarded with improved economic and environmental viability of crop production over many areas of the globe.

References

Ae N, Arihara J, Okada K, Yoshihara T, Johansen C (1990) Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. *Science* 248,477-480.

Ae N, Shen RF (2002) Root cell-wall properties are proposed to contribute to phosphorus (P) mobilization by ground nut and pigeonpea. *Plant Soil* 245,95-103.

Bell MJ, Gillespie TJ, Roy RC, Michaels TE, Tollenaar M (1994) Peanut leaf photosynthetic activity in cool field environments. *Crop Sci.* 34,1023-1029.

- Bennett JM, Sinclair TR, Ma L, Boote KJ (1993) Single leaf carbon exchange and canopy radiation use efficiency of four peanut cultivars. *Peanut Sci.* 20,1-5.
- Betts JH, Herridge DF (1987) Isolation of soybean lines capable of nodulation and nitrogen fixation under high levels of nitrate supply. *Crop Sci.* 27:1156-1161.
- CIAT-TSBFI Working Group in BNF. (2004) Biological nitrogen fixation: A key input for integrated soil fertility management in the tropics. In 'Symbiotic Nitrogen Fixation: Prospects for Enhanced Application (Ed. R. Serraj) pp. 113-143 (Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi).
- Carroll BJ, McNeil DL, Gresshoff PM (1985) A supernodulation and nitrate-tolerant symbiotic (NTS) soybean mutant. *Plant Physiol.* 78,34-40.
- Ehlers JD, Hall AE, Patel PN, Roberts PA, Matthews WC (2000) Registration of 'California Blackeye 27' cowpea. *Crop Sci.* 40,854-855.
- Gibson AH, Harper JE (1985) Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum*. *Crop Sci.* 25,497-501.
- Gremaud MF, Harper JE (1989) Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. *Plant Physiol.* 89,169-173.
- Hardarson G, Zapata F, Danso SKA (1984) Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars. *Plant Soil* 82,397-405.
- Harper JE (1971) Seasonal nutrient uptake and accumulation patterns in soybeans. *Crop Sci.* 11,347-350.
- Hartwig EE, Kiihl RAS (1979) Identification and utilization of a delayed flowering character in soybean for short-day conditions. *Field Crops Res.* 2,145-151.
- Herridge DF, Rose IA (1994) Heritability and repeatability of enhanced N₂ fixation in early and late inbreeding generations of soybean. *Crop Sci.* 34,360-367.
- Ishikawa S, Adu-Gyamfi JJ, Nakamura T, Yoshihara T, Watanabe T, Wagatsuma T. (2002) Genotypic variability in phosphorus solubilizing activity of root exudates by pigeonpea grown in low-nutrient environments. *Plant Soil* 245,71-81.
- Klauer SF, Franceschi VR, Ku MSB, Zhang D (1996) Identification and localization of vegetative storage proteins in legume leaves. *Am. J. Botany* 83,1-10.
- Lansing AJ, Franceschi VR (2000) The paraveinal mesophyll: a specialized path for intermediary transfer of assimilates in legume leaves. *Aust. J. Plant Physiol.* 27,757-767.
- Lauer MJ, Shibles R (1987) Soybean leaf photosynthetic response to changing sink demand. *Crop Sci.* 27,1197-1201.
- Lawn RJ, Troedson RJ (1990) Pigeonpea: Physiology of yield formation. In 'The Pigeon Pea' (Ed. YL Nene, SD Hall, VK Sheila) pp. 179-208 (CAB International, Wallingford, UK).
- Lynch JP, Beebe SE (1995) Adaptation of beans (*Phaseolus vulgaris* L.) to low phosphorus availability. *HortScience* 30,1165-1171.
- Maloney TS, Oplinger ES (1997) Yield and nitrogen recovery from field-grown supernodulating soybean. *J. Prod. Agric.* 10,418-424.

- Muchow RC (1985) Canopy development in grain legumes grown under different soil water regimes in a semi-arid tropical environment. *Field Crops Res.* 11,99-109.
- Ohwaki Y, Hirata H. (1992) Difference in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid level in roots. *Soil Sci. Plant Nutr.* 38,235-243.
- Purcell LC, King CA (1996) Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. *J. Plant Nutrition* 19,969–993.
- Raffin A, Roumet P, Obaton M (1995) Tolerance of nitrogen fixation to nitrate in soybean: A progeny (tolerant x non-tolerant) evaluation. *Eur. J. Agon.* 4,143-149.
- Rochette P, Desjardins RL, Pattey E, Lessard R (1995) Crop net carbon dioxide exchange rate and radiation use efficiency in soybean. *Agronomy J.* 87,22-28.
- Sall K, Sinclair TR (1991) Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. *Plant Soil* 133,31–37.
- Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions. *Plant Cell Envir.* 25,333-341.
- Serraj R, Drevon JJ, Obaton M, Vidal A (1992) Variation in nitrate tolerance of nitrogen fixation in soybean (*Glycine max*)-*Bradyrhizobium* symbiosis. *J. Plant Physiol.* 140,366-371.
- Serraj R, Sinclair TR, Purcell LC (1999) Symbiotic N₂ fixation response to drought. *J. Exp. Botany* 50,143-155.
- Sinclair, TR (1980) Leaf CER from post-flowering to senescence of field-grown soybean cultivars. *Crop Sci.* 20,196-200.
- Sinclair, TR (2004) Improved carbon and nitrogen assimilation for increased yield. In 'Soybeans: Improvement, Production, and Uses (Ed. J. Specht, R. Boerma) (Am. Soc. Agronomy, Madison, WI).
- Sinclair TR, Bennett JM, Drake GM (1994) Cool night temperature and peanut leaf photosynthetic activity. *Proc. Soil Crop Sci. Soc. Florida* 53,74-76.
- Sinclair TR, deWit CT (1975) Photosynthate and nitrogen requirements for seed production by various crops. *Science* 189,565-567.
- Sinclair TR, Hinson K (1992) Soybean flowering response to long-juvenile trait. *Crop Sci.* 32,1242-1248.
- Sinclair TR, Horie T (1989) Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. *Crop Sci.* 29,90-98.
- Sinclair TR, Ludlow MM (1986) Influence of soil water supply on the plant water balance of four tropical grain legumes. *Aust. J. Plant Physiol.* 13:329-341.
- Sinclair TR, Muchow RC (1999) Radiation use efficiency *Adv. Agronomy* 65,215-265.
- Sinclair, TR, Muchow RC, Bennett JM, Hammond, LC (1987) Relative sensitivity of nitrogen and biomass accumulation of drought in field-grown soybean. *Agron. J.* 79,986-991.
- Sinclair TR, Purcell LC, Sneller, CH (2004) Crop transformation and the challenge to increase yield potential. *Trends in Plant Sci.* 9:70-75.

- Sinclair TR, Purcell LC, Vadez V, Serraj R, King CA, Nelson R. (2000) Identification of soybean genotypes with N₂ fixation tolerance to water deficits. *Crop Sci.* 40,1803-1809.
- Sinclair TR, Vadez V, Chenu K. (2003) Ureide accumulations in response to Mn nutrition by eight soybean genotypes with N₂ fixation tolerance to soil drying. *Crop Sci.* 43,592-597.
- Sloane RL, Patterson RP, Carter TE Jr. (1990) Field drought tolerance of a soybean plant introduction. *Crop Sci.* 30,118-123.
- Sprent JL. (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 N₂ fixation by nitrate. *Crit. Rev. Plant Sci.* 7:1-23.
- Tropical Legumes: Resources for the Future. (1979) National Academy of Sciences, Washington, D.C.
- Warrag MOA, Hall AE (1983) Reproductive responses of cowpea to heat stress: Genotypic differences in tolerance to heat at flowering. *Crop Sci.* 23,1088-1092.
- Warrag MOA, Hall AE (1984) Reproductive response of cowpea (*Vigna unguiculata* (L.) Walp) to heat stress. II. Responses to night air temperature. *Field Crops Res.* 8,17-33.
- Wu S, Harper JE. (1991) Dinitrogen fixation potential and yield of hypernodulating soybean mutants: A field evaluation. *Crop Sci.* 31,1233-1240.
- Yan X, Lynch JP, Beebe SE (1996) Utilization of phosphorus substrates by contrasting common bean genotypes. *Crop Sci.* 36,936-941.