Effects of Drought, Temperature, and Salinity on Symbiotic Nitrogen Fixation in Legumes, with Emphasis on Chickpea and Pigeonpea

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Abstract

*The legume-*Rhizobium *symbiosis is a highly integrated and, to a considerable degree, self-regulating process. It is particularly sensitive to such environmental influences as drought, salinity, and extremes of temperature, more so than rhizobia growing alone. Although the effect of these factors on the symbiosis has been studied mostly under laboratory conditions, a good understanding has been achieved of how the symbiosis responds to various forms of stress. All three stress factors impair the development of root hairs and the site of entry of rhizobia into the host, resulting in poor nodulation or even absence of nodules. Salinity and high temperature more greatly affect nodulated plants than nitrogen-fertilized plants. Since existing knowledge on the effects of stress factors on symbiotic nitrogen fixation has been derived mostly from studies with legumes other than chickpea or pigeonpea, detailed studies of these effects are needed for these two crops. In addition, because strains of rhizobia tolerant to these stress factors are either available or identifiable by laboratory screening procedures, efforts to identify individual chickpea and pigeonpea genotypes tolerant to these stress factors could help improve the overall tolerance of the symbiosis.*

Introduction

Nitrogen is both essential for plant growth and the element that most frequently limits crop production. Soil nitrogen is depleted by cropping, denitrification, leaching, and erosion; it is accreted by application of fertilizer nitrogen and manures, and by biological nitrogen fixation (BNF).

Knowledge of the BNF phenomenon is about a century old: BNF was recognized as a natural process that permitted legume growth on soils low in nitrogen. During the initial phase of knowledge acquisition, because sophisticated laboratory equipment was often lacking and fertilizer nitrogen not readily available, this subject did not attract much research effort. In the late 1950s and early 1960s, however, there was a strong surge of BNF research, particularly with regard to its biochemical aspects. Since the 1970s the field has expanded, as indicated by the frequency of international symposia and the increased number of books on BNF (Postgate 1971; Quispel 1974; Stewart 1975; Torrey and Clarkson 1975; Nutman 1976; Broughton et al. 1979; Bergersson 1980; Broughton 1981, 1982, 1983; Graham and Harris 1982), as well as reviews (Stewart 1973; Dazzo and Hubbell 1974; Dilworth 1974; Shanmugam and Valentine 1975; Child 1976; Skinner 1976; Winter and Burris 1976; Vance 1983).

Because the energy cost of synthesizing and transporting combined nitrogen as fertilizer is high and because increased use of fertilizer nitrogen may lead to pollution hazards, enhancing fixed nitrogen may

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become a viable proposition not only in marginal lands of the semi-arid tropics but also in good soils.

The profound influence of environment on symbiotic nitrogen fixation has been known for a long time. But little is known so far about the basic reaction underlying the variations in symbiotic nitrogen fixation in legumes, particularly chickpea and pigeonpea, as revealed under stress conditions. The results obtained so far with different legumes would seem to justify further research effort.

Since the estimates of nitrogen fixed by legumes in general are questioned (LaRue and Patterson 1981), we have refrained from estimating yield losses attributable to the effect of various stress factors on nitrogen fixation.

Drought

Few studies are available on the survival of rhizobia under drought stress. One study showed that, depending on species, between 50% and 99% of rhizobia died with cycles of wetting and drying (Pena-Cabriales and Alexander 1979). A 10 000-fold reduction was also observed in the *Rhizobium* of *Lotus* when the soil was kept dry for several months (Foulds 1971). It was found that soil conditions under which drying occurs affect the extent of decline (Bushby and Marshall 1977), and that type and amount of clay in the soil are important for protecting rhizobia (Osa-Afiana and Alexander 1982). A population of 1000 rhizobia g^{-1} dry soil or more can be counted for both chickpea and pigeonpea in the surface 5-cm Vertisol soil during the hot, dry summers of peninsular India (Table 1). Thus, despite a decline in population over this period, rhizobia of both chickpea and pigeonpea (cowpea group rhizobia) can still survive drought. With appropriate selection pressures, it should be possible to identify rhizobia that are better able to survive drought conditions.

An optimum supply of water, around 60-75% of the water-holding capacity, is considered essential for maximum plant growth. Because symbiosis is an ultrasensitive process, however, its water requirements should be considered separately from those of the individual plants. Pigeonpea grown in the unpredictable rainy season, for instance, may experience either shortage or excess of water during vegetative growth. When reproductive growth extends into the postrainy season, the crop primarily depends on stored moisture in the soil. Chickpea and pigeonpea grown in the postrainy season thus face a progressively increasing soil moisture deficit. Legume symbiosis can recover if exposed to short stress periods, but prolonged exposure may lead to permanent damage and shedding of nodules (Wilson 1931, cited by Lie 1981).

Table 1. Effect of depth on population (log 10 MPN g^{-1} soil) of chickpea and cowpea group rhizobia in a Vertisol and an Alfisol, ICRISAT Center.

Depth (cm)	Chickpea rhizobia[1]		Depth (cm)	Cowpea group[2] rhizobia in Alfisol
	Vertisol	Alfisol		
0 5	4.62	4.81	0 5	3.38
5 15	5.34	4.61	5 10	4.65
15-30	3.85	3.89	20 30	4.19
30 60	3.81	3.69	50 60	3.21
60-90	2.53	-[3]	100 110	3.37
90 120	2.13	-	150-160	3.30

1 Sampling done in May 1979 (ICRISAT 1980).

2. Sampling done in June 1978 (J.V.D.K. Kumar Rao and P.J. Dart, unpublished).

3 - = not determined

Dry soils inhibit normal root hair formation and, hence, infection by *Rhizobium*. With watering, the abnormal root hairs may resume growth. On the other hand, nodule development initiated under normal moisture conditions is set back by later dry conditions (Worrall and Roughley 1976).

Sprent (1971) carried out detailed studies on the effect of drought on detached soybean nodules. When water loss from nodules exceeded 20% of the initial nodule fresh weight, acetylene reduction activity (ARA) was irreversibly lost. Splitting of cell walls and rupturing of plasmodesmatal connections occurred in the bacteroid-containing zone. Under these conditions, the cytoplasmic structure in the vacuolated cells of the nodule cortex collapsed (Sprent 1972a). Reduced respiration rates and ARA due to drought stress were seen when nodules were assayed at high pO_2 or after slicing or homogenization (Pankhurst and Sprent 1975). The results suggested that the cortical collapse inhibited nodule activity by reducing the diffusion of O_2 into the bacteroid-containing zone. Results of studies on detached nodules may not be applicable to nodules still attached to the host plant, but they do provide some interesting insight into the system.

Unless drought stress caused wilting of the lower leaves of *Glycine max* and *Vicia faba*, ARA recovered rapidly after watering (Sprent 1972b);

moreover, irrigation after a period of drying increased nitrogen fixation more than ten-fold (Sprent 1976). Reduced ARA during drought may be due to reduced photosynthate supply: in *Glycine max*, for instance, it was observed that the percentage reduction of ARA and of net photosynthesis were similar (Huang et al 1975a). The recovery of ARA after rewatering, however, lagged behind that of photosynthesis (Huang et al. 1975b). A 3-day drought stress imposed on *Glycine max* caused a 40-80% reduction in the proportion of ^{14}C-labeled photosynthates subsequently supplied to the nodules (Silivus et al. 1977).

Similar studies on chickpea and pigeonpea are lacking. A substantial increase in nodulation and ARA is seen due to irrigation in chickpea (Fig. 1). Without irrigation, the maximum nodule growth was achieved by about 40 days. With irrigation at about 10-day intervals, nodules continued growing until about 65 days and ARA continued even up to 89 days. Similar responses to irrigation were seen in nodulation and ARA of pigeonpea grown in the postrainy season (Fig. 2).

A water budget drawn up for a pea plant and its nodules for a period of rapid vegetative growth of 9 days, between 21 and 30 days after sowing, in an environment of 18°C during the day and 12°C during the night, indicated a requirement of 10 ml water by the nodules during this period (Pate 1976). Of this, 9.7 ml was estimated to have been used for exporting 27.3 mg of fixed nitrogen to the plant, and 0.3 ml was consumed in nodule growth. Twenty percent of the 10 ml came from the phloem, 13% was absorbed from the nodule surface, and the remaining 67% was extracted laterally from the adjacent roots. During the same period, 140 ml water was transpired by the plant, which was 14 times more than the amount passing through the nodules (Pate 1976). Although this estimate may not be precise, it clearly indicates that under normal conditions roots supply a major portion of the water required by nodules.

In chickpea growing in the postrainy season on receding residual moisture, the top 15 cm of the soil dries up within 3 weeks (N.P. Saxena, ICRISAT, personal communication), but nodules remain tur-

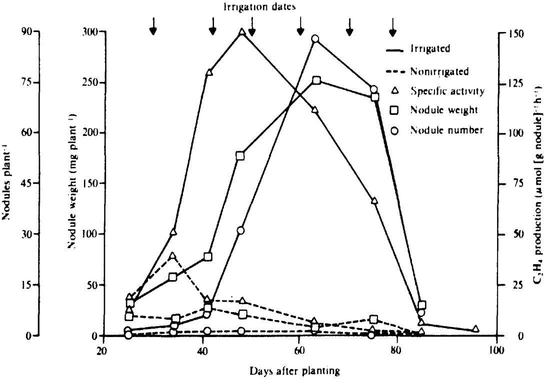

Figure 1. Nodulation and nitrogen fixation of cultivar K 850, with and without irrigation, in a Vertisol at ICRISAT Center, 1979/80 (ICRISAT Annual Report 1981, p. 93).

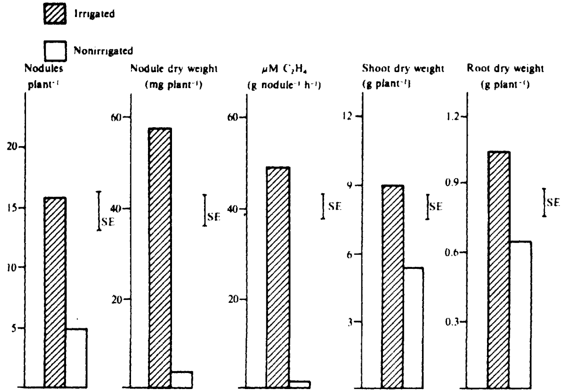

Figure 2. Effect of irrigation on nodulation, nitrogenase activity, and dry-matter production (at 90 days) of pigeonpea cultivar C 11 grown on a Vertisol, ICRISAT Center, postrainy season 1981/82. (Source: J.V.D.K. Kumar Rao and J.A. Thompson, unpublished).

gid and functional for more than 40 days after sowing (see Fig. 1). The water required for nodule functions is obviously coming from deeper layers. However, nodules may also be losing some water to the dry soil, instead of absorbing it. Since pigeonpea experiences unpredictable cycles of wetting and drying (Huda and Virmani 1987), dips and surges in nodule formation and functions during the growing season have been observed.

Chickpea in farmers' fields and in watersheds is generally not as well nodulated as in precision fields at ICRISAT Center, even though *Rhizobium* populations are similar. This indicates that lack of appropriate *Rhizobium* is not completely responsible for poor nodulation. The poor nodulation of legumes sown on residual moisture, compared to crops sown with irrigation, tempts us to say that the early processes of nodulation—such as infection by rhizobia, nodule initiation and formation, etc.—are rather more affected by moisture level than is nodule function. Rhizobia are known to survive various degrees of dessication expected in the soil, but we should concentrate on identifying plant genotypes that can form functional symbioses under drought conditions.

Temperature

The effect of temperature on nitrogen fixation was reviewed by Lie (1974, 1981) and Dart et al. (1976). Temperature can affect viability of rhizobia applied to seeds at sowing, growth and survival of saprophytic rhizobia in the soil, and the various symbiotic processes from recognition of rhizobia by legume roots to nodule function.

Rhizobia are susceptible to higher temperatures, particularly when conditions are moist rather than dry (Wilkins 1967). Survival of rhizobia on seeds of *Trifolium* spp, *Pisum sativum*, and *Medicago sativa* sown in moist soils was greatly reduced at 40° C. The degree of reduction depended on the size of the initial population the time of exposure and the strain of *Rhizobium* (Bowen and Kennedy 1959 cited in Bushby 1982) Although strains differ in their capacity to tolerate temperature the upper limit is close to 40°C (Bowen and Kennedy 1959 and Ishizawa 1953 cited in Bushby 1982) Soil temperatures reaching 40°C are not uncommon in chickpea and pigeonpea growing areas (ICRISAT 1978) but the tolerance limits of these rhizobia are not yet known However the rhizobia of chickpea and pigeonpea do seem to survive these temperature conditions (see Table 1)

The temperature of the rooting medium has a strong influence on root hair infection (Frings 1976 cited in Sutton 1983) and ARA (Waughman 1977) In *Trifolium* nodules it was observed that at 11 19°C the first senescent zones were detected 10 days after bacteroid differentiation while at 7°C there was still no appearance of the senescent zone after 20 days (Roughley 1970) The longer period and higher nitrogen fixation rates for chickpea grown in northern India (29° 10 N) than in southern India (17 32 N) (ICRISAT 1982) may partly be due to lower temperatures which can sometimes be 10 C

It was noted that ARA of *Trifolium subterraneum* continued to increase for 3 days after transfer from 22° to 30°C and then decreased by 90% in the next 3 days (Pankhurst and Gibson 1973) This was accompanied by marked structural changes in the bacteroid-containing cells and by accelerated growth and branching of infection threads In contrast nodules formed by a particular strain TA 1 were fully stable at 30°C In similar experiments with *Trifolium subterraneum* *Trifolium pratense* and *Medicago sativa* reduced frequency was recorded of infection threads as well as reduced ARA on transferring plants from lower to higher root temperatures (Day and Dart 1970 cited in Dart 1977) Those observed differences in the reaction of infection threads could be due to different *Rhizobium* strains *Pisum sativum* nodules formed at 22°C lost 67% of their ARA and 17% of their leghaemoglobin after 2 days exposure to 30°C (Frings 1976 cited by Sutton 1983) Both plants and rhizobia grew satisfactorily at 30 C when supplied with combined nitrogen

With chickpeas it was found that daily cycles of 23 33 23 C or 23 36 23 C root temperature during the day resulted in decreased ARA and plant growth (Dart et al 1975) Two cycles did not affect plant growth significantly but five and ten cycles caused an 18 34% reduction in nitrogen fixation over the control when measured 14 days after the treatment When chickpea was grown continuously at four different temperature regimes with mean soil temperatures of 24 26 28 and 30 C during the day nodule mass ARA and plant growth were adversely affected with increasing temperature (Table 2) ARA decreased 51% at 26 C 79% at 28 C and 90% at 30 C over the ARA obtained at 24°C while the corresponding decrease in plant growth was 23 52 and 67% More ARA of chickpea nodules was observed when day night temperature was 22 10 C or 22 18 C than when it was 30 10 C or 30 18 C (Minchin et al 1980)

At higher temperatures photosynthesis is reduced drastically (Black 1973 Black et al 1978) and hence nitrogen fixation can be indirectly affected by reduced supply of photosynthates *Rhizobium* strains that can fix nitrogen under temperature stress conditions can be obtained (Ek Jander and Fahraeus 1971 Lie 1974) For chickpea it has been possible to identify strains which can fix nitrogen at about 30 C a temperature that the crop is expected to face in the conditions of peninsular India (ICRISAT 1978 ICRISAT 1984)

Table 2 Effect of soil temperature[1] (°C) on nodulation nitrogen fixation and plant growth of chickpea (ICRISAT 1983)

Temperature (°C)	ARA[2] (μM C_2H_4 pot h)	Nodules pot	Nodule dry mass (g pot)	Top dry wt (g pot)
25	22.7	1480	1.95	21.9
30	11.1	1580	1.55	16.9
32	4.7	1490	1.33	10.4
35	2.2	800	0.83	6.8
SE	±1.79	±152	±0.163	±0.74

1 Plastic pots containing a Vertisol with a high *Rhizobium* count were immersed in water baths of different temperatures for 8 h per day (0800 to 1600) beginning 6 days after sowing

2 ARA acetylene reduction activity

Salinity

Information on the effect of salinity and alkalinity on rhizobia is scanty, and it is particularly lacking for chickpea and pigeonpea. Most of the available studies have used broth cultures, and the initial broth pH can change over the growth period (Damirgi et al. 1967). Growth of a range of strains in broths of initial pH 8.0 to 10.0 has been reported (Graham and Parker 1964). Only one slow-growing strain could grow at pH 9.0, but all strains of *Rhizobium meliloti* (fast growers) grew at pH 9.5. None of the strains could grow at pH 10.0.

Salts of Na and Ca are known to be toxic to *Rhizobium* at high concentrations. Salt (generally NaCl) concentrations of more than 1% inhibit the growth of rhizobia. It was observed that growth rates of rhizobia of chickpea and cowpea were reduced at more than 1% of NaCl (Gandhi and Vyas 1969). *Rhizobium trifoli* and *Rhizobium leguminosarum* were found to be sensitive to 0.4% $NaHCO_3$ (Wilson 1931, cited by Bushby 1982). Also KCl was more inhibitory than NaCl at equivalent concentrations (45 mM) for four strains of *Rhizobium japonicum* (Upchurch and Elkan 1977). Non-gum-producing colony variants from these four strains were more sensitive to salt than large, gum-producing colonies.

It was suggested that salts affect the host rather than the *Rhizobium* (Wilson 1970). However, the processes involving the interaction between the two are likely to be more sensitive to salinity and alkalinity. Roots of *Medicago sativa* growing with 0.2% $NaHCO_3$, for instance, were devoid of root hairs and mucilaginous layers and the formation of infection threads was prevented (Lakshmi Kumari et al. 1974). However, under the same salt concentrations, the growth of rhizobia was optimum. In soybean, high salinity caused shrinkage of root hairs and, hence, failure of nodulation (Tu 1981). Sodium chloride at 120 mM concentration inhibited nodulation of soybean. Plants depending on symbiotic nitrogen were more affected by salts than those depending on mineral nitrogen. In contrast, both nitrate-fed and symbiotic plants of *Medicago sativa* were relatively unaffected by salt (Bernstein and Ogata 1966). Nodulated mungbean was more affected by salinity than cowpea (Balasubramanian and Sinha 1976), and in *Vicia faba*, the reduced number of nodules per plant due to salinity was compensated in part by increased nodule size (Yousef and Sprent 1983).

Symbiotic susceptibility to salt stress also varies from salt to salt. In lucerne 0.7% NaCl completely suppressed nodule formation. However, nodules were formed even with up to 0.75% of KCl and $MgCl_2$ (Singh et al. 1972).

Growth of chickpea in sand culture in the greenhouse was depressed by NaCl at only 20 mM, unless mineral nitrogen was provided. Without NaCl, the performance of 22 *Rhizobium* strains was comparable to strains with an ammonium nitrate treatment; with addition of 75 mM NaCl, however, only one strain was significantly better than the control (Lauter et al. 1981). *Rhizobium* strains could grow with even up to 120 mM of NaCl, indicating that the symbiotic processes from root hair infection onward are more sensitive than from rhizobia alone.

Almost all the studies on nodulation and nitrogen fixation have been done with defined salts under laboratory conditions. Field situations would be different and more difficult to study. The laboratory studies provide a basic understanding of the system, and thus need to be done. Besides differences between legume species in tolerance to salt stress, there can be genotypic variability within a species. Five lentil cultivars tested at different salt concentrations indicated genotypic differences (Rai 1983). Differences between genotypes of pigeonpea do exist, but there is a need to identify genotypes tolerant to salinity levels experienced in the field (Y.S. Chauhan, ICRISAT, personal communication). A recently concluded study at ICRISAT Center indicated genotypic differences between pigeonpea lines and their associated rhizobia for tolerance to different salt concentrations (Subba Rao 1984).

Rhizobium strains obtained from salt-affected soils may be more tolerant to salinity and better able to establish a symbiosis with the host. Chickpea strain IC-53 (ex 161a), isolated from a saline field at ICRISAT Center, produced greater shoot weight than *Rhizobium* strains from normal fields, when compared in pots containing saline soil. This strain produced 63% more grain yield than the control treatment in field trials in a saline soil in Sudan (Ibrahim and Salih 1980). Similar observations were made for *Rhizobium* of *Sesbania* isolated from salt-affected soils (Bhardwaj 1972).

Conclusion and Future Research Needs

Most of our knowledge on the effects of stress factors on BNF comes from studies on legumes other than chickpea and pigeonpea. Hence, more studies on how these two crop plants react to the stress factors are required. *Rhizobium* strains growing as saprophytes in the soil can tolerate stress environments much better than the host legumes and the symbiosis. Also, *Rhizobium* strains tolerant to some of these stress factors have been isolated; they can also be identified more easily than tolerant host plants. Plants depending on symbiotic nitrogen are more prone to the adverse effects of drought, soil temperature, high pH, and salts than are plants fertilized with nitrogen. This suggests that identification of genotypes tolerant to these stress factors should be a first step in overcoming the adverse environmental effects. All the stress factors result in absence or distortion of root hairs, the site where rhizobia enter the host prior to establishment of the symbiosis. Hence, in selecting legume genotypes better able to tolerate stress factors, the ability to form normal root hairs should be a major consideration.

A study as to why, and at which stage, the symbiosis breaks down under environmental stress is important from a practical point of view, and it may provide more information about the process itself. An important contribution may be expected from a comparative study between genotypes that differ in their capacity to establish a symbiosis under stress conditions.

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