A chapter from

Adaptation of Chickpea
in the West Asia and North Africa Region

Edited by

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5.5. Symbiotic Nitrogen Fixation by Chickpea in WANA and SAT

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Introduction

The ability of chickpea to fix atmospheric nitrogen lessens its dependence on soil N and reinforces its role in the cropping systems of WANA and SAT. However, the crop is often considered inferior in this regard to other legume crops grown in wheat-based rotations (Papastylianou 1987; Keatinge et al. 1988). Published estimates of N_2 fixation by chickpea in the WANA region range from 0 to 176 kg ha^{-1} per season, with the proportion of total N from fixation (P_{fix}) varying between 0 and 82%, depending on the method of measurement, cultivar, presence of appropriate rhizobia, and environmental variables (Rizk 1966; Papastylianou 1987; Keatinge et al. 1988; Beck et al. 1991). Nitrogen fixation has a positive effect on soil N_2 balance and growth of the subsequent crop (Evans 1982; Heichel 1987; Keatinge et al. 1988). Therefore, practices which increase N fixation will minimize the quantity of soil N utilized by the crop, and thereby increase yields in the subsequent non-legume crop.

The main strategies for improving biological nitrogen fixation (BNF) in chickpea are similar to those for most legumes. Because the process of N_2 fixation is photosynthate-driven, increasing chickpea yield is the simplest and generally the most successful strategy to improve BNF. Breeding for improved N_2 fixation is rarely done because it gets less priority than that for yield and resistance to biotic/abiotic stresses, and also because measuring N_2 fixation may be a difficult and expensive process. Optimizing the host-rhizobia association, by inoculating the chickpea plants with selected rhizobia, is therefore, the most common approach to improve N_2 fixation.

Techniques for measuring N_2 fixation are essential to any attempts to improve BNF, and the strengths and weaknesses of various techniques should be well understood before a BNF program is initiated. Reviews of some of these techniques have been published by Witty (1983), Chalk (1985), Danso (1988), Witty and Minchin (1988), Witty et al. (1988), Peoples et al. (1989), Herridge et al. (1992), and Beck et al. (1993).

Agronomic and Environmental Constraints

Winter-sown chickpea enjoys more favorable soil moisture and temperature conditions during late vegetative and reproductive growth periods than spring-sown chickpea in the area around the Mediterranean Sea (Wery et al. 1988; Saxena et al. 1990). In a series of trials to measure N_2 fixation in spring- and winter-sown chickpea under varying agroenvironments, the results showed that winter sowing improved P_{fix} at all locations (Beck et al. 1991), due to the improved conditions prevalent during the period of maximum fixation. In Syria, N_2 fixation levels in winter-sown chickpea were high (80–81%), whereas P_{fix} values in spring-sown chickpea, where drought limited growth as early as anthesis, were negligible (8–27%). Differences between N_2 fixed in spring- and winter-sown chickpea in Montpellier, France—which has a Mediterranean climate similar to WANA—were found to be smaller because of extended moisture availability through the later stages of plant growth. N_2 fixation reached a maximum of only 55% in Montpellier, where fixation was depressed by high levels of soil nitrate (Beck et al. 1991).

High to moderate levels of available soil N are known to suppress N_2 fixation in legumes, by inhibiting nodulation and interfering with

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the fixation process (Munns 1977; Streeter 1988). This factor is particularly important in experiments conducted at research stations, where soil fertility (N in particular) tends to be high.

Critical tolerance level and degree of suppression vary with legume species (Harper and Gibson 1984). The limited research on chickpea indicates that levels of NO_3-N below 10 ppm will not adversely affect N_2 fixation (Rawsthorne et al. 1985), but variation with cultivars is expected (Rupela and Johansen 1992a).

Chickpea also appears to be a fairly efficient scavenger of soil N, especially under conditions where sufficient rhizobia are not present for efficient symbiosis (Beck 1992). The practice of fertilizing chickpea with 20 kg N ha⁻¹ at sowing is widely recommended in SAT, probably because it sometimes helps to negate the adverse effect of high temperature on symbiosis (Rawsthorne et al. 1985).

Nitrogen fixation in chickpea seems to be more sensitive than grain production and N assimilation (which is mainly limited by availability) to high temperatures (Rawsthorne et al. 1985) and drought (Wery et al. 1988). In field studies with six chickpea cultivars in Syria (ICARDA 1992), drought stress depressed P_{fix} more than N uptake (Fig. 5.5.1). A line-source sprinkler was used over 2 seasons in these studies, where P_{fix} increased more rapidly than yield at lower moisture levels, indicating that fixation was severely limited at the lower end. Values for P_{fix} in different cultivars at lower moisture levels varied widely from 15 to 38%. Fixation efficiency reached an average maximum of 68% at about 5000 kg ha⁻¹ dry matter produced. Nitrogen uptake from soil remained constant until moisture became sufficient for maximum fixation, when soil N uptake increased (Fig. 5.5.1). The correlations between dry matter produced and N yields were high, with coefficients of 0.92 for total N and 0.90 for fixed N.

The high correlation between dry matter production and N yield indicates that N_2 fixation in chickpea, under conditions where adequate rhizobia are present, is yield-driven, and that environmental
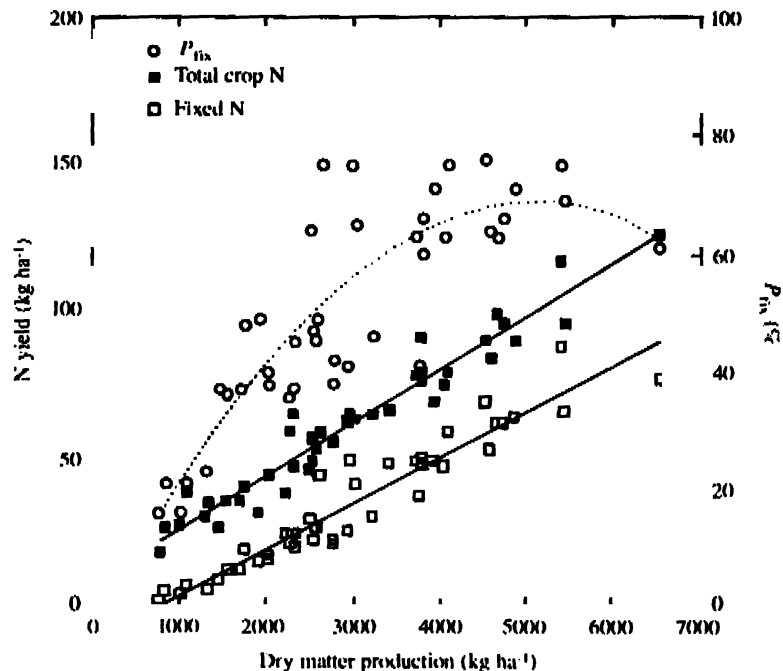

Figure 5.5.1. Relationship of dry matter production with N yield and source in chickpea cultivars, northern Syria, 1987–91.

constraints on plant yield will limit N_2 fixation. These results may partly explain why N fertilization improves yield of nonirrigated chickpea in low N soils, but does not affect yield in the irrigated crop (ICRISAT 1992).

Breeding for Improved N_2 Fixation

Agronomic and environmental considerations often limit the biomass yield of a legume crop and therefore the capacity of that crop to fix

N_2. Yield is also determined genetically, for example, low N yield may be a characteristic of some species. In studies over a range of environments and agronomic practices, N yield and N_2 fixation by chickpea were consistently less than for the other cool-season food legumes (Rennie and Dubetz 1986; Evans and Herridge 1987; Smith et al. 1987; Beck et al. 1991). Average yields were 100 kg N ha⁻¹ for chickpea, 196 kg N ha⁻¹ for lentil, 185 kg N ha⁻¹ for field pea, and 200 kg N ha⁻¹ for faba bean. These studies did not indicate that the inherent capacity of chickpea for nodulation and N_2 fixation was less than for the other species. It may be concluded, therefore, that increasing N yield of chickpea may result in increased N_2 fixation.

Plant breeders select for high yield within the constraints of local environments and crop yields largely determine the amount of N_2 that is fixed by the crop, particularly in low N soils (Hardarson et al. 1984; Kumar Rao and Dart 1987). Therefore, breeders who mostly work in low N soils will tend to select for material with good capacity for N_2 fixation. Breeding for symbiotic characteristics in chickpea is possible. Examples of possible strategies to exploit are nitrate tolerance (i.e., the ability of the plant to nodulate and fix N_2 in the presence of soil nitrate), the capacity to fix N_2 at low available moisture levels, and general nodulation capacity. Chickpea cultivars selected for drought tolerance seem to vary in their capacity for N_2 fixation under drought stress. Natural variation for nitrate tolerance (Rupela and Johansen 1995) and nodulation capacity (Rupela 1994) also exist in chickpea. It may be impossible to produce a legume that is dependent solely upon N_2 for growth and cannot use nitrate, but there is scope to improve P_{fix} in the presence of nitrate for chickpea.

Some argue that legumes should be able to use both atmospheric and soil N sources so that they can scavenge nitrate from the soil which would otherwise be lost through leaching and denitrification, while others argue that in many soils, nitrate is relatively stable over time and can be considered as a stable pool of N. Secondly, if deple-tion of soil nitrate was considered necessary, it would make more sense to use a cereal crop with a higher demand for N and greater economic return.

Because N yield and dry matter production are generally highly correlated (Mytton 1983), the following procedure could be followed to enhance N_2 fixation in chickpea:

1. Screen a large and diverse germplasm (500–1000 genotypes) of chickpea, inoculated with highly effective rhizobia, for production of dry matter under low N conditions (preferably in the field, but it could be done in a greenhouse).
2. Select superior genotypes (e.g., top 10%) for further evaluation. The second round of screening is ideally done in the field on low N-fertility soil, again with a mixture of highly effective rhizobia. Assessments should include measurements of grain yield and total N yield.
3. Compare elite genotypes over a range of edaphic (particularly soil N fertility) and environmental (including diverse rhizobial population) conditions for grain yield, N yield, and N_2 fixation, the latter using ¹⁵N methods.

Genotypes that are identified at this stage as superior in all three attributes and adapted to the soils and environments for which they are likely to be used would have immediate commercial application. High N_2-fixing genotypes that produce low grain yields or grain of low quality could be used as donor parents in a breeding program.

It is also important to remove the effect of crop duration on N yield. Increased N yield due to high growth and assimilation rates is more useful because it can be expressed in any environment; whereas increased crop N due to longer crop duration can only be expressed if the duration of the season in a particular environment or cropping system is sufficiently long. In commercial agriculture, individual crops must fit into cropping systems which are determined by seasonal

changes in temperature, moisture availability, radiation, availability of land and resources to grow and harvest the crop, marketing arrangements, etc. The optimum duration of any crop is therefore determined by several factors, the least important of which is N yield or N_2 fixation.

Inoculation

Local production or import of inoculants for farmers can only be justified if the legume benefits from inoculation are shown by increases in yield or in N_2 fixation in field trials and farmers' fields. It is essential to determine the need for inoculation before initiating any program on inoculant development, production, distribution, or use. Response to inoculation by legumes has been shown to be influenced mainly by cropping history (Brockwell et al. 1982), soil N availability (Somasegaran and Bohlool 1990), and most importantly, the indigenous population of rhizobia that nodulate the host (Thies et al. 1991). Various methods to determine the need for inoculation are described in detail by Beck et al. (1993).

The introduction of cold-tolerant, ascochyta blight resistant lines for winter sowing into new, drier production areas of WANA has been accompanied by nodulation deficiency in several areas (M Solh and S P S Beniwal, personal communication). In these new production areas, soils are less likely to contain adequate populations of the *Cicer*-specific rhizobia than traditional chickpea areas, and crops may show significant yield increases when seeds are inoculated with selected rhizobial strains. Extensive surveys of native rhizobia-nodulating chickpea have been recently conducted in Syria and Turkey, where symbiotic effectiveness and size of native populations were measured (Keatinge et al. 1995). It was found that even within the major chickpea-growing regions, many soils contained rhizobial populations either at very low levels or with low symbiotic effectiveness on the cultivars

tested. It has been suggested that this deficiency may be one reason for the generally low average chickpea yields from these areas.

The highly specific rhizobial requirement of chickpea extends to strain-cultivar specificity for N_2 fixation (Beck 1992). This implies that limited effectiveness of naturalized rhizobial populations with newly introduced cultivars may restrict the genetic potential for dinitrogen fixation. Necessity for inoculation may therefore also exist where introduced cultivars—selected for high yields—cannot express their full capability for N_2 fixation in symbiosis with native rhizobial populations that have developed in adaptation with local landraces.

In trials conducted over 4 seasons (1987/88–1990/91) in northern Syria (seasonal rainfall of 300–500 mm), variations in N_2 fixation and yield of chickpea cultivars inoculated with selected *Rhizobium* strains were evaluated. The purpose was to establish base-line values for P_{fix} in recommended cultivars so that improvements through rhizobial strain selection and legume breeding could be quantified. Use of ^{15}N methodology and nonnodulating chickpea and barley as reference crops allowed accurate evaluation of N_2 fixation under a wide range of environmental conditions. Indigenous chickpea rhizobial populations based on the most probable number (MPN) estimations in the field soils were low to moderate, ranging from 9.1×10^1 to 4.2×10^3 rhizobia g^{-1} soil. Rhizobial strains were selected according to the N_2-fixing performance in aseptic hydroponic culture in greenhouse trials.

Inoculation had no general effect on crop dry matter yields at lower rainfall sites (Fig. 5.5.2). At 340 mm rainfall, however, cultivars began to show differential yield effects with rhizobial inoculation, ranging from no response to a 750 kg ha^{-1} increase. Under conditions of higher moisture (504 mm), the average inoculated cultivar yielded about 800 kg ha^{-1} more dry matter than when not inoculated (Fig. 5.5.2). Cultivar yields, which differed little at low rainfall, varied widely at high rainfall; yield response to inoculation varied from no response in cultivar ILC 5396 to 1.9 t ha^{-1} in ILC 482.
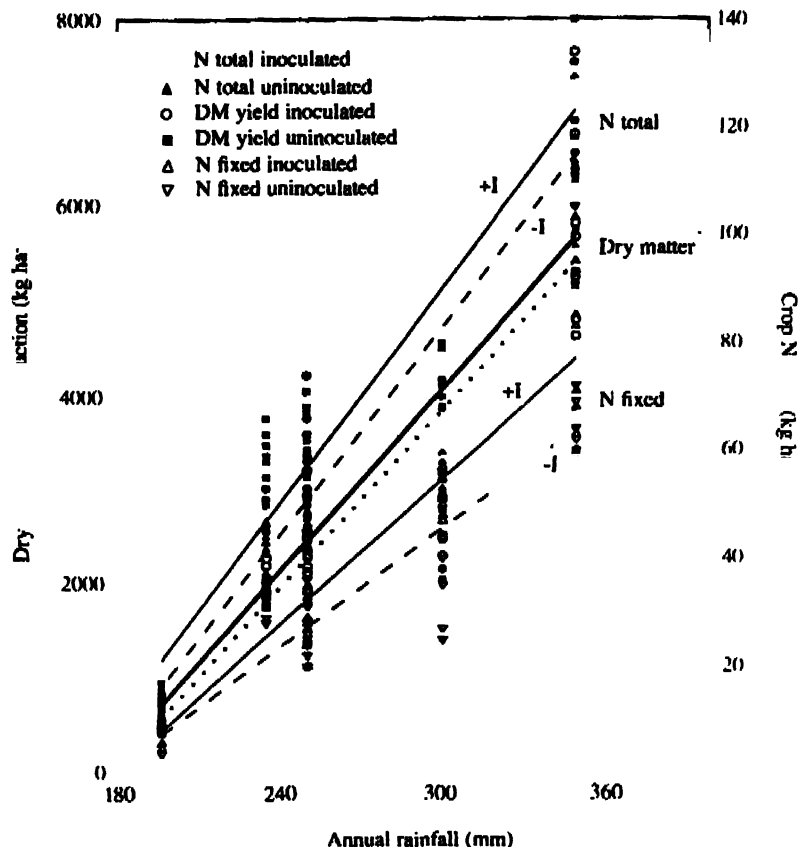
Figure 5.5.2. Effect of inoculation on dry matter production and N yield in chickpea, northern Syria, 1987–91.

shows average soil N uptake (the distance between total N and fixed N curves) increasing from 20 kg ha⁻¹ to nearly 50 kg ha⁻¹ over the range of dry matter produced in the trials. In contrast, the efficiency of N_2 fixation has clearly increased at higher yield levels as a result of rhizobial inoculation (Fig. 5.5.4). In inoculated cultivars, P_{fix} increases with dry matter production, reaching a maximum of 80% at the highest yield levels. Increased fixation efficiency with yield results in a high proportion of fixation-derived N in the plant and a low, relatively constant fraction of soil-derived N (Fig. 5.5.4).
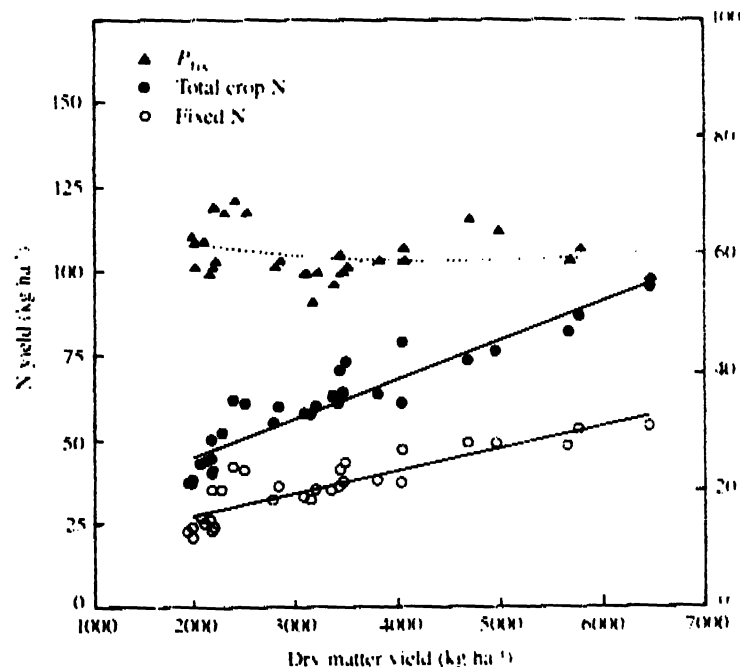
In uninoculated cultivars, P_{fix} remains relatively constant at about 60% between 2000 and 7000 kg ha⁻¹ dry matter production (Fig. 5.5.3). The effect of this constant proportion of fixed- to soil-derived N in the plant is that with increasing dry matter (and N) production, the quantities of soil N taken up by the crop increase. Figure 5.5.3

Figure 5.5.3. Nitrogen yield and source in uninoculated chickpea cultivars, northern Syria, 1988–90.
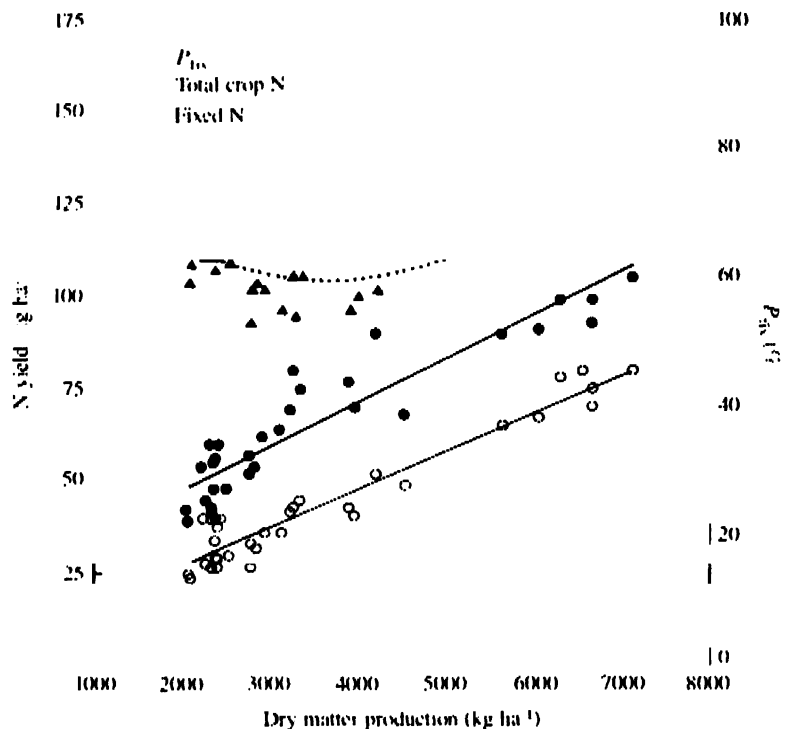
Figure 5.5.4. Nitrogen yield and source in inoculated chickpea cultivars, northern Syria, 1988–90.

In most cultivars tested, inoculation did not increase the amount of crop N per unit dry matter produced. The proportion of crop N derived from fixation was, however, often increased by inoculation. The effect of this improvement—that can be detected only with N_2 fixation measurement techniques such as those incorporating ^{15}N—is improved soil fertility. Although the effects of inoculation on yield are limited, the quantities of soil N preserved could be significant in a systems context. Farmers, however, will not adopt inoculant technol-

ogy if they do not get as a result of applying the technology, increased yields of the legume or of the subsequent cereal crop.

The interaction between strains and cultivars for N_2 fixation efficiency, in addition to a similar interaction for competition and nodule formation, complicates the approach to wide-scale inoculation of chickpea cultivars, especially where new improved cultivars are being released on a regular basis. Two strategies may be used to increase N fixed by the chickpea crop. Selection of cultivars for high N_2 fixation with a broad range of rhizobia reduces the need for inoculation with specific strains. This, however, may fail where native strains are absent or ineffective. Alternatively, mixtures of highly effective strains may be used as inoculants. This works with some cultivars, but is dependent on strain-cultivar interaction for competitiveness in nodule formation, and on the successful use of inoculant technology by farmers.

Even where inoculation can increase yields, its effectiveness is heavily dependent on the quality of the inoculant and the way the product is applied. Experience has shown that successful transfer of inoculant technology to farmers for improvement of BNF is difficult at best (Thompson 1991). *Rhizobium* inoculants are biological products and therefore susceptible to major problems with manufacturing (quality control), distribution (loss of viability during transport and distribution), and extension (Roughley 1988). Distribution of poor quality inoculants is not uncommon, and is generally followed quickly by farmer disinterest in inoculation.

Contribution of N_2 Fixation to Cropping Systems

Results from legume-based rotation experiments in rainfed cropping areas of many countries have been published in recent years (e.g., Evans and Taylor 1987; Evans et al. 1989). These experiments reflect the growing concern of scientists and farmers in those areas about declining levels of N fertility in the soils and reduced production of

cereal grain and protein. In all the trials where wheat followed grain legumes, its yield was higher than when it was continuously cropped, irrespective of the species of legume (Herridge et al. 1992).

In a long-term two-course rotational trial in Syria, soil N levels were measured after 6 years of rotation. Total soil N in the surface 40 cm of the chickpea-wheat rotation that did not receive any fertilizer N, did not differ significantly from that in the continuous wheat and fallow-wheat rotation (H Harris and A Matar, unpublished data). Soil organic carbon levels in the three rotational treatments also did not differ (0.9–1.0%), but incubation measurements of the N mineralization potential (Matar et al. 1991) showed large differences between rotational treatments. Mineralization potentials of soils in continuous wheat with 75 mg N kg^{-1} soil and fallow-wheat with 61 mg N kg^{-1} soil, were similar. In the chickpea-wheat soil, however, mineralization potential was 118 mg N kg^{-1} soil, indicating an increased capacity to supply plant-available N from the total N pool. These data are supported by studies at 40 northern Syrian sites under different crop rotations, where mineralization potential measurements gave the best indication of N uptake in wheat under legume-cereal rotations (Matar et al. 1989).

Potential improvements in chickpea N_2 fixation are therefore important to system productivity and sustainability. Research to improve chickpea N_2 fixation will ultimately have impact beyond increased chickpea yields, increasingly so in view of the present trends toward continuous cereal production and coincident soil fertility degradation.

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