

Agronomic and symbiotic characteristics of chickpea, *Cicer arietinum* (L.), as influenced by *Rhizobium* inoculation and phosphorus fertilization under farming systems of Wolaita area, Ethiopia



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Acronyms

BNF	biological nitrogen fixation
CSA	Central Statistics Authority, Ethiopia
NDWPP	nodule dry weight per plant
NNPP	number of nodules per plant
NNPPM	number of nodules per plant at maturity
DAS	days after sowing
NSPP	nodule score per plant
NSPPM	nodule score per plant at maturity
NPPP	number of pods per plant
NoSPP	number of seeds per pod
NBPP	number of branches per plant
SDWPP	shoot dry weight per plant
SFWPP	shoot fresh weight per plant
TN	total nitrogen
AP	available phosphorus
FAOSTAT	Food and Agricultural Organization statistics
1000sw	thousand seeds weight
GY	grain yield
SNNPRS	Southern Nations, Nationalities and Peoples Regional State
MPN	most probable number
STN	straw total nitrogen
STP	straw total phosphorus

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Foreword

This experiment was conducted at Taba kebele of Damot Galle woreda located in Wolaita zone, Ethiopia from August 2012 till January 2013. The study was initiated and fully funded by the N2Africa project and executed as part of MSc thesis research for fulfilment of Masters of sciences in Agronomy/ plant sciences. N2AFRICA is a large scale research project focused on putting nitrogen fixation to work for smallholder farmers growing legume crops in Africa. The project came into action in 2010 and it is funded by the Bill & Melinda Gates Foundation and the Howard G. Buffet Foundation through a grant to Plant Production Systems chair group of Wageningen University in the Netherlands. It is led by Wageningen University, CIAT-TSBF and IITA along with many partners in 13 African countries, including five countries with which a new partnership was established recently. Ethiopia is one of these five countries. The aims of the project are to raise average grain legume yields by 954 kg ha⁻¹ in four crops i.e. groundnut, cowpea, soybean and common bean, increase average biological nitrogen fixation by 46 kg/ha, and increase household income by \$465, directly benefitting 225,000 households in eight sub-Saharan African countries. The first phase of the project ends in October this year, and based on the results obtained so far, the project seems to be in a good position to meet the set target.

In Ethiopia, partnerships have been established with various research and development organizations, among which Hawassa University. N2Africa considers the newly joining Ethiopia as a potential improvement area as far as productivity of grain legumes of smallholder farmers is concerned and it was within this setting that this experiment was conducted on farmers' field. The aim of the experiment was to assess the response of chickpea to the packages of N2Africa, being phosphorus fertilizers and *Rhizobium* inoculants, under farming conditions of Wolaita area, one of the high potential areas for Ethiopian chickpea production. Accordingly, the experiment was conducted with full financial support of the N2Africa project and technical assistance of the soil microbiology laboratory of the School of Plants Sciences, Hawassa University of Ethiopia. The results obtained are used in this MSc thesis report to be submitted to the Plant Production Systems group, Wageningen University.

Summary

Chickpea is the third most widely cultivated food grain legume in Ethiopia and it is known as a multifunctional crop that fits well in rotation with cereals like maize and tef. Ethiopia is the largest chickpea grower in Africa with a share of 39% of the total production in 2011. Despite its potential yield of more than 3 ton ha⁻¹, current chickpea productivity is only 1.6 ton ha⁻¹ in Ethiopia. In this regard, there is a need to improve the current productivity through improved technologies and agronomic practices. N2Africa, a large research project focused on putting biological nitrogen fixation to work for smallholder farmers growing legume crops in Africa, provides *Rhizobium* inoculants and P fertilizer technologies to farmers in several African countries and achieved satisfactory results so far. This experiment was conducted with the aim of evaluating the response of chickpea to inoculation, P fertilizer, and their combined application under farming conditions in the Wolaita area of Ethiopia, which is among the major food insecure and densely populated regions in the country.

For this purpose, *Mesorhizobium ciceri* strain CP 41 was obtained from the soil microbiology laboratory of the School of Plant and Horticultural Sciences of Hawassa University, Ethiopia and used along with a chickpea variety Natoli. Four treatments, including control, inoculants, P-fertilizer (TSP) and the combination of inoculants + P-fertilizer, were tested on twenty farms spread over two soil types. The experiment was conducted from September 2012 to January 2013 at Taba Kebele of Damot Gale woreda in Wolaita zone, where the majority of farmers grow chickpea at the end of the main rainy season. To measure the response of chickpea to the treatments, data were collected on various symbiotic, phenological, yield and yield related traits of chickpea and subjected to analysis of variance using statistical software GENSTAT 15.

Compared with the control, inoculation and P fertilizer significantly improved nodulation, growth and yield of chickpea, with a more prominent improvement with combined application of inoculation and P. Inoculation, P and their combined application increased grain yield of chickpea by 26%, 19% and 33% over the control respectively. Similarly, the total nitrogen content of the straw increased by 56% and 82% compared to control due to inoculation and P + inoculation treatments respectively. Positive effects the soil fertility treatments were also observed for the total N uptake of chickpea as eventually 28, 53, 42, and 65 kg N ha⁻¹ was recorded for the control, inoculation, P, and P + inoculation treatments respectively.

For total straw P content, the use of P and P + inoculation resulted in 41% and 28% more total P uptake.

On the other hand, the result of the most probable number test showed that the soils of this area were inhabited by a very small population of resident rhizobia ($< 10 \text{ gram}^{-1}$ of soil).

For almost all of the variables studied a large variation across farms was observed. On 85% of the farms, the soil fertility treatments gave higher grain yield compared to the control treatment and the yield range was $0.5\text{-}2.5 \text{ ton ha}^{-1}$, $0.5\text{-}2.8 \text{ ton ha}^{-1}$, $0.7\text{-}3.0 \text{ ton ha}^{-1}$ and $0.7\text{-}2.9 \text{ ton ha}^{-1}$ for the control, inoculation, P, and inoculation + P treatments respectively with their respective overall average yield of 1.6, 2, 1.9 and 2.1 ton ha^{-1} . The magnitude of yield response to the soil fertility treatments showed a negative but non-significant correlation with initial soil fertility of the farms. Although yields for all treatments were larger on black than on red soils, the magnitude of the yield response to the treatments was greater on the red than on the black soils. Generally, the observed improvement in chickpea performance can be attributed to the increased supply of nitrogen, through enhanced biological nitrogen fixation, and phosphorus, which were both present in low supply in the soils.

These positive effects of the soil fertility treatments will, if adopted by the local farmers, contribute to increasing chickpea productivity and household income. Given the small landholdings (on average 0.6 ha) of the households, the observed improvement may play a modest role in improving the livelihood of the smallholders in this area. The use of inoculation, P fertilization, and their combination on 25% of the land would increase the household income by 63, 33, and 63 USD respectively. However, the unavailability of P fertilizers and inoculants on the market in the country might greatly constrain the promotion of these technologies. Therefore, solutions need to be sought by working closely with private and governmental organizations and stimulate their interest to get involved in the business.

1. Introduction

The concern of soil fertility decline in Africa has been raised since many years, influencing top policy priorities to establish a range of initiatives with the aim of alleviating the problem (Elias and Scoones 1999). Mineral mining due to continuous cultivation, lack of inorganic fertilizer inputs, and limited application of organic inputs are some of the reasons for the depletion of soil fertility in developing countries. Large number of African farmers generally consider soil fertility decline as the major limitation to farming (Smaling et al. 1997). Poor soil fertility has resulted in low crop productivity and is one of the causes of food insecurity in the region. In Ethiopia, where the agricultural sector is dominated by smallholder farmers, the soil fertility situation is said to be desperate (Pound et al. 2005). The food insecurity problem is aggravated by human population growth, with decreasing farm sizes as a consequence. Therefore, there is a need to improve soil fertility and increase productivity thereby producing sufficient food for the expanding population. To achieve this, fertilizer use is proposed as a solution (Foth and Ellis 1997).

In African agriculture, the nitrogen requirement is by far the greatest of all major nutrients (Woldeyohannes et al. 2007) and projected to rise significantly (FAO 2012). However, in developing countries small-scale farmers often cannot afford costly inorganic fertilizer. The situation is further aggravated by poor infrastructure and lack of credit services. Therefore, there is a need to reduce the dependence on nitrogen fertilizer thereby minimizing environmental degradation while improving crop productivity. As stated by Shamseldin and Werner (2004), biological nitrogen fixation (BNF) can reduce the need for fertilizer. Each season, grain legumes fix about 15 - 210 kg N ha⁻¹ in Africa (Dakora and Keya 1997). More specifically, chickpea can fix up to 140 kg N ha⁻¹ in a growing season (Kumar and Abbo 2001) and its inclusion in crop rotations of cereal dominated farming systems can also reduce disease severity of non-legume crops through disrupting the disease cycle.

In soils where native rhizobial population densities are low, BNF can be enhanced by inoculation of legume seeds with an effective and persistent *Rhizobium* strain. In this regard, *Rhizobium* inoculation of chickpea seed may substitute costly N fertilizers in chickpea production (Kosgey 1994). Several studies demonstrated improved yields with inoculation: Hailemariam and Tsige (2003, cited by Yoseph 2011) reported a yield advantage of 10 to 50% for faba bean, field pea and lentil inoculated with *Rhizobium leguminosarum*.

Similarly, the same study showed that the inoculation of chickpea seed with an appropriate strain increased yields up to 38% over the control. The development of rhizobial inoculants is seen as the greatest success achieved in scientific research conducted on BNF (Giller and Cadisch 1995). Depending on the native *Rhizobium* population in the soil, inoculation is proven worthy as it is economically cheap, environmentally sound, and ecologically beneficial.

However, the process of nitrogen fixation is sensitive to P deficiency, which results in reduced nodule mass and low ureide production. P plays an important role in N cycling as adenosine triphosphate is required in large quantities for legumes to undergo N₂ fixation (Vance 2001; Sinclair and Vadez 2002). Unfortunately, low soil P availability is limiting crop production both at country level and in the Southern Nations, Nationalities and Peoples Region (SNNPR) of Ethiopia (Mamo and Haque 1987; Schulze et al. 2006). Particularly soils of many areas in Wolaita suffer from remarkable P deficiencies due to P fixation (Elias and Scoones 1999).

Ethiopia is the largest chickpea producer in Africa, with a share of about 39% of total chickpea production of the continent in 2011 (FAOSTAT 2012). In SNNPR more than 6000 ha of land were planted with chickpea in the year 2008. In the same year, Wolaita Zone stood third in the region as far as chickpea production area is concerned (Kassie et al. 2009). In Wolaita chickpea is one of the most important grain legumes produced by small scale farmers and it serves as a source of food and cash. Given its ability to grow on residual moisture, chickpea plays an important role in the farming system by fitting in the crop rotation. As a result, growing chickpea allows the farmers to produce extra crop each year. However, in spite of its potential to produce about 3 ton ha⁻¹, the average productivity of chickpea in Ethiopia for 2011 was only 1.6 ton ha⁻¹ (FAOSTAT 2012). In Wolaita, like in other parts of the country, productivity of chickpea and other crops is constrained by poor soil fertility. With the aim of improving crop productivity, the Ethiopian government has been providing and promoting various technologies and improved practices to the farmers including improved cultivars and fertilizer.

Also, given the potential of BNF, several effective strains of *Rhizobium* have been identified for chickpea. However, these strains were identified through on-station research and hardly tested on farmers' fields. Moreover, only limited efforts have been made to distribute the identified strains to farmers so that they could benefit from this technology.

The present study, therefore, was conducted with the following objectives:

- To determine the status of resident indigenous rhizobia in the soils of the study area
- To investigate the effect of *Rhizobium* inoculation, P fertilization and their combination on nodule development, plant growth, grain yield and residue production and quality of chickpea sown on farmers' fields in Wolaita area
- To investigate the effect of soil type on the potential benefit of *Rhizobium* inoculation and P fertilization on chickpea yield
- To understand the variation in response to the soil fertility treatments across farms

1.1. Chickpea

1.1.1. Origin, distribution and global production trend

Chickpea, *Cicer arietinum* (L.), one of the most important cool season crops, is believed to have originated in present-day south eastern Turkey and adjoining Syria where several of its natural species are found (Saxena and Singh 1987). The crop later spread to India, Europe and subsequently reached Africa, Latin and central American countries. Chickpea is a self-pollinated annual crop that can complete its life cycle in 90 to 180 days depending on the prevailing meteorological conditions. Chickpea can be grown under a wide range of agro-climatic conditions around the world (Singh and Diwakar 1995). Chickpea growing areas can be classified into the following four major geographical regions: Indian subcontinent; West Asia, North Africa, and southern Europe; Ethiopia and East Africa; and the Americas and Australia (Smithson 1985; Singh and Diwakar 1995).

Currently, chickpea is produced worldwide and it is the world's third most important food legume next to haricot bean and soybean (Namvar and Sharifi 2011). In 2011, chickpea was grown on about 13.2 million hectares of land across the world with an average productivity of 0.9 ton ha⁻¹. Globally, during 1994 – 2005, the annual chickpea production increased by 1.87% (Kassie et al. 2009). Chickpea is among the widely cultivated pulse crops by small scale farmers of the semi-arid tropics (Anbessa and Bejiga 2002). Generally, Desi and Kabuli types are the two major types of chickpea grown in the world with major differences in seed size, seed colour, surface and thickness of the seed coat. The Desi type is characterized by small seeds with angular appearance, sharp edges and varying colours but usually light brown. On the other hand, the Kabuli type produces large round seeds of white or pale cream or yellow colour.

1.1.2. Production and roles of chickpea in Ethiopia

In Ethiopia, chickpea is mainly grown in the central, northern and eastern highland areas of the country at an altitude of 1400-2300 m.a.s.l., where annual rainfall ranges between 700 and 2000 mm (Bejiga 1994; Anbessa and Bejiga 2002). During the 2010/11 cropping year, Ethiopia produced 322,839 ton of chickpea on 208,389 ha of land and stood sixth on the global ranking of chickpea producing countries (FAOSTAT 2012). The average productivity of Ethiopian chickpea in 2011 was 1.6 ton ha⁻¹ which is nearly half of the 3 ton ha⁻¹ that can be expected under prevailing conditions. In this year, chickpea contributed about 17% of

Ethiopia's total food legume production stood third after faba bean and haricot bean (Fig.1B) (FAOSTAT 2012).

In the same year, Ethiopia's share in African chickpea production was 39% (Fig.1A) (FAOSTAT 2012). Over the past years there has been an increasing trend in total area of production, the quantity of chickpea produced and productivity of chickpea in Ethiopia (Abate et al. 2011). During 1995-2005, Ethiopian chickpea planted area and chickpea production showed annual growth rate of 2.1% and 7.6% respectively (Kassie et al. 2009).

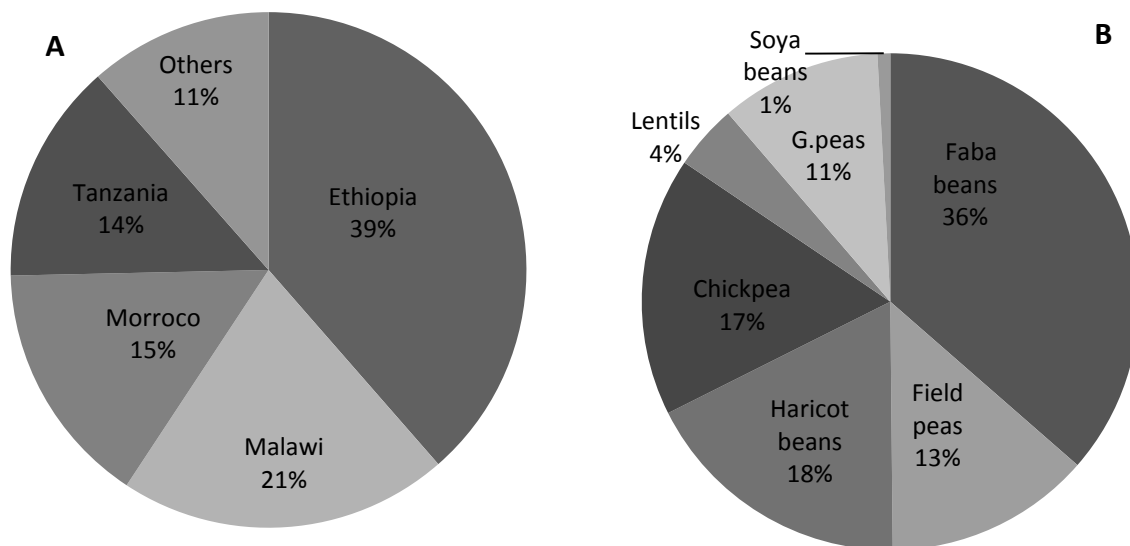


Figure 1: Major African chickpea producing countries with their share of total production in Africa during 2011 (A) and major pulse crops in Ethiopia with their share of total grain legume production in 2011 (B) (adapted from CSA and FAOSTAT, 2012)

The crop can be grown on different soil types as long as good drainage is ensured. However, to achieve optimum growth well drained black soils (usually Vertisols) are identified as the most suitable soil type (Kassie et al. 2009). In Ethiopia, chickpea is mostly grown on vertisols which have good water holding capacity. Ethiopian farmers essentially grow chickpea on residual moisture after the end of the main rainy season, which is usually in September - October. Of the two types of chickpea, traditionally the Desi types are more widely cultivated in Ethiopia (Kassie et al. 2009).

Chickpea, a multi-functional crop, has an important role in the diet of the Ethiopian small-scale farmers' households and also serves as protein source for the rural poor who cannot afford to buy animal products. The crop also serves as a source of cash income and plays a major role in Ethiopia's foreign exchange earnings through export to Asia and Europe. Its straw is also used for animal feed and due to its capacity of biological nitrogen fixation,

chickpea can improve the soil fertility status (Pundir and Mengesha 1995). In spite of all these virtues and benefits, the productivity of this crop remains very low in Ethiopian agriculture.

Over the past 38 years, Ethiopian chickpea research has focused mainly on breeding and selection of improved cultivars with better yield and disease resistance. Through this approach, Ethiopian Agricultural Research Organization (EARO) released 11 improved chickpea varieties (6 Kabuli types and 5 Desi types) from 1974 to 2005 (Kassie et al. 2009). However, this did not result in the desired level of productivity as the average yield was still below 1.6 ton ha⁻¹ in 2011. Variety development can be seen as a component of a package through which crop yield can be improved and it has to be supported by appropriate agronomic management including optimum fertilizer rate, proper weeding, planting at a right time, inoculation, and disease and pest control measures. Therefore, one way of improving yield of leguminous crops is inoculation of their seeds with *Rhizobium* bacteria that has already shown remarkable result in other African countries (Woomer 2012).

1.2. Phosphorus and its importance in crop production

Next to nitrogen, phosphorus is the most important element for adequate grain production. The evolution of science, particularly in the past century, has clearly demonstrated the significance of phosphorus for all animal and plant life on the earth (Ryan et al. 2012). Rock phosphate is the global source of raw material for P fertilizer and it is wise to efficiently use this finite resource. Substantial amount of P is found in different parts of plants. Large quantities of P are found in seed and fruit, and it is considered essential for seed formation (Gidago et al. 2012). Especially in the early stages of plant development, adequate supply of P is required for development of the reproductive parts and P has a positive effect on root growth, early maturity, and reduced disease incidence. Despite the presence of a large P pool, Africa is suffering from shortage of available P, which remains a yield limiting factor. Also in the highlands of Ethiopia, available P is often a limiting element in crop production (Mamo and Haque 1987), with 70 to 75% of the agricultural soils deficient in P. Similarly, available P content of most soils in SNNPR of Ethiopia is low (Gidago et al. 2012).

Application of small amounts of P fertilizer (26 kg P ha^{-1}) dramatically increased nodulation, N accumulation, and seed yields of haricot bean grown on farmers' fields in northern Tanzania (Giller et al. 1998). From data obtained from multi location experiments, consistent yield response of faba bean to P fertilization was observed (Ghizaw et al. 1999). Likewise, application of 10 kg P ha^{-1} significantly improved grain yield and biological yield of haricot bean planted at Areka research station, SNNPR-Ethiopia (Gidago et al. 2012). The same study revealed that application of P enhanced physiological maturity and yield components, though not in a statistically significant way. Similarly, application of P fertilizer enhanced mid-season dry matter accumulation and tissue P accumulation of both Desi and Kabuli chickpea, but grain yield was increased only modestly for Desi chickpea, while yield of Kabuli chickpea was not affected by application of P (Walley et al. 2005)

1.3. Nitrogen and its role in crop production

Nitrogen is known to be an essential nutrient for plant growth and development (Werner and Newton 2005) due to its role in biochemical, physiological and morphological processes of plant production (Novoa and Loomis 1981).

Although this critically important element is abundant in the atmosphere, nitrogen is the most limiting element for crop growth worldwide. In sub-Saharan Africa both yield and quality of crops are highly constrained by low nitrogen availability. Application of mineral fertilizer, addition of organic material and enhancing biological N₂ fixation are the main ways of improving the nitrogen availability to the plants.

Application of nitrogen fertilizer is reported to have positive effects on morphological traits and yield of crops in general and chickpea in particular. The usage of 100 kg urea ha⁻¹, for example, resulted in the highest biomass production and grain yield of chickpea as compared to the control and lower rates of fertilizer on a silty loam soil in Iran. Compared to the control, addition of 100 kg urea ha⁻¹ improved grain yield of chickpea by 36% (Namvar et al. 2011). Similarly, application of 30 or 45 kg N ha⁻¹ significantly increased grain yield of Desi type chickpea by 221 kg ha⁻¹ relative to the 1.6 ton ha⁻¹ of control treatment when averaged across locations on clay loam soils of Saskatchewan, Canada (Walley et al. 2005). The same study revealed that nitrogen application increased shoot P accumulation of Desi type chickpea by 1.1 kg ha⁻¹.

1.4. Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a process by which N_2 in the atmosphere is reduced into a biologically useful, combined form of N-ammonia by living organisms (Hardy and Burns 1968; Giller 2001). The greatest proportion of N found on the earth is located in the atmosphere, as N_2 . Nevertheless, the majority of organisms cannot utilize this free and abundant, but highly stable source of N because they can only use N which is combined with other atoms into plant usable forms, such as ammonium, nitrates and ammonia (Giller 2001; Giller and Cadisch 1995). The process of making N_2 available constitutes a specialized and intricately evolved interaction of soil microbes (bacteria) and higher plants via the formation of nodules (Sessitsch et al. 2002). Nodules are formed on roots or, in some cases, stems (Tamimi and Timko 2003).

Each year, about 175 million ton of N is contributed by BNF globally, of which nearly 79% is accounted for by terrestrial fixation. Therefore, symbiotic nitrogen fixation is of great importance not only in the production of leguminous crops but also in the global nitrogen cycle (Ben Romdhane et al. 2008). The most important N_2 fixing agents in agricultural systems are the symbiotic associations between legumes and the microsymbiont rhizobia bacteria (Giller 2001).

Depending on the availability and effectiveness of the native rhizobia, one way of improving N_2 fixation in grain legumes is inoculation of the crop seeds with effective strains of rhizobia. Despite being mentioned by some as a promiscuous host (Rivas et al. 2007), there is consensus that both nodulation and growth of chickpea can be improved by inoculation (Giller 2001). The growth and yield of chickpea can be improved by inoculating seeds with competitive strains of rhizobia and this can be an economically feasible way of increasing productivity of chickpea (Ben Romdhane et al. 2008). Across 16 on-station trials inoculation of chickpea with *Rhizobium* increased grain yield by an average 342 kg ha^{-1} (Wani et al. 1995). Likewise, depending on cultivar, effectiveness of bacterial strain and environmental factors the association of chickpea and *Mesorhizobium Cicer* produced up to 176 kg N ha^{-1} annually in India (Rupela et al. 1987a).

1.5. Factors affecting Biological Nitrogen Fixation

The ability of symbiotic nitrogen fixing agents to fix N_2 is strongly influenced by the prevailing environmental conditions that can mainly be categorized as physical factors, chemical factors, and nutrient deficiencies (Giller 2001). In addition, *Rhizobium* species producing nodules in chickpea are specific only to this crop and inoculation with effective strains is advised in soils with no or weak bacterial presence (Rupela et al. 1987a; Somasegaran et al. 1988).

The survival of *Rhizobium* sp. in the soil is influenced by a combination of factors such as nitrogen and phosphorus availability, acidity, salinity, alkalinity, concentration of micronutrients, soil temperature, moisture, soil structure, and fertility status of the soil (Giller 2001; Slattery et al. 2001). These factors may influence the growth of microorganisms in the free-living state, the process of plant infection or nodule development, and the fixation of N_2 after the symbiosis has been established (Giller 2001). Depending on their type, rhizobia exhibit an optimum growth in a temperature range of 25 – 30°C (Kantar et al. 2010). However, some chickpea rhizobia have shown their maximum growth at 20°C (Rodrigues et al. 2006). Similarly, saline conditions may limit the symbiosis through affecting survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere, inhibiting the infection process, affecting root nodule function directly or indirectly by reducing plant growth and its other physiological processes (Singleton et al. 1982).

1.5.1. Soil Mineral Nitrogen

Depending on the accessible quantity present, the mineral nitrogen content of the soil can have both positive and negative effects on yield and growth response of chickpea to inoculation. Usually a higher mineral nitrogen content in the rhizosphere leads to poor N_2 fixation through inhibition of nodulation of chickpea (Namvar et al. 2011). On the other hand, small amounts of soil or fertilizer N often have a stimulatory effect on nodulation and N_2 fixation which is principally due to the positive effect of N on growth and plant establishment during the period between root emergence and the onset of active N_2 fixation (Giller and Cadisch 1995).

1.5.2. Soil Phosphorus

Usually, suboptimal availability of P leads to limited root growth, reduction of photosynthesis and translocation of sugars, thus affecting N fixation. Moreover, being a building block of a plant energy source, P is important in N cycling because adenosine triphosphate is required in large quantities by legumes to develop nodules and undergo the fixation process (Sessitsch et al. 2002). As N₂ fixation is an energy demanding process (Giller 2001), larger P quantities are needed by N₂ fixing plants than by mineral N supplied plants. In this regard, poor nodulation and poor plant vigour have been observed in beans grown in soils low in extractable P (Amijee and Giller 1998) while acute deficiency of phosphorus can even prevent nodulation by legumes (Giller 2001), showing the sensitivity of the process of N₂ fixation to P deficiency.

1.5.3. Competition between rhizobia

Indigenous rhizobia in soils can vary in population density and infectivity from place to place ranging from < 10 to 10^7 cells g⁻¹ of soil. Competition in case of rhizobia is mostly used to refer to the competition for nodule occupancy (Giller 2001), which is a complex and controversial area in the study of the legume-rhizobium symbiosis (Thies et al. 1992). However, competition between inoculated and indigenous rhizobia is most strongly influenced by the size of indigenous rhizobial populations whereas environmental factors did not play a major role other than affecting the size of the native rhizobial population. Decreasing nodule occupancy by inoculant strains was observed with increasing number of indigenous rhizobia for lima bean and cowpea (Thies et al. 1992).

1.5.4. Soil temperature, pH and salinity

Temperature plays an important role in the success of BNF due to its effect on survival and or growth rate of microorganisms. Both extremely high and low temperatures have either depressive or killing effect. Maximum soil temperatures in tropics regularly exceed 40 °C at 5cm and 50°C at 1cm depth (Hungria and Vargas 2000). Similarly, the soil pH greatly influences rhizobia content of soils and their ability to nodulate pulse crops (Slattery et al. 2004). Soil acidity reduces nitrogen fixation in legumes, particularly affecting *Rhizobium* survival in soil and reducing nodulation. Nodulation of soya bean and haricot bean was drastically reduced at pH of 4.5, whereas a pH of 5.2 resulted in good nodulation as well as satisfactory N₂ fixation (Hungria and Vargas 2000).

Moreover, production of grain legumes is severely reduced in salt affected soils mainly due to the impairing effect of both salinity and sodicity on the plant ability to form and maintain nitrogen fixing nodules (Rao et al. 2002). For chickpea, very small nodule dry mass was recorded for all the genotypes tested under highly saline soil (Rao et al. 2002). With increasing salinity, a sharp decrease in both nodule number and nodule biomass was observed for all chickpea genotypes tested.

1.5.5. Soil moisture status

The moisture content of the soil can have an effect on growth and survival of soil rhizobia as well as that of the plant itself. Drought can be considered to be among the most harmful abiotic constraints to BNF, mainly due to its effect on soil physical and biological characteristics (Kantar et al. 2010). Drought has a pronounced effect both on the number of rhizobia and N₂ fixation rates. N₂ fixation is more sensitive to reductions in soil water content than other physiological processes (Giller 2001). The effect of drought on the soil microbial community can be in two ways: either reducing the number of water filled pores and the thickness of water films around soil particles or increasing the salt concentration in the soil solution. On the other hand, also excess soil water negatively affects both the growth and survival of soil rhizobia and plants. In conditions of water logging, the occupation of all soil pores results in limited O₂ availability for both rhizobia and plant roots, thereby causing reduced respiration.

1.5.6. Agronomic management

Establishment of effective symbiosis between rhizobia and the host plant primarily requires optimal conditions that are necessary for growth of the host plants. In this regard, agronomic practices have a profound influence on both the soil and the crop under consideration. For example, the organic matter content of the soil is influenced by the agronomic management and has several positive influences on soil fertility, moisture holding capacity and microbial activity. Agronomic factors that influence BNF by affecting both the crop and the microbial activity in the rhizosphere include tillage practices, selection of effective or responsive crops, appropriate cropping systems, method of sowing, time of sowing, use of agrochemicals, use of *Rhizobium* cultures and its frequency, the way of handling the inoculants and the method of inoculation (Kantar et al. 2010).

2. Materials and Method

2.1. Description of the study area and the prevailing farming system

The experiment was conducted on twenty farmers' fields at Taba kebele¹ close to Boditi town of Wolaita zone, Southern Ethiopia, from September 2012 to January 2013. The study area is located in Damot Galle, one of the 12 districts of Wolaita zone, which is found at about 350 km south-west of Addis Ababa, and 135 km from Hawassa, capital of Southern Nations, Nationalities, and Peoples' Regional State (SNNPRS) of Ethiopia. The area is situated at 07° 00'N and 37° 54' E at an altitude of 1900 meters above sea level (masl).The soils are dominantly Vertisols and Nitisols with pH values of 5-6.

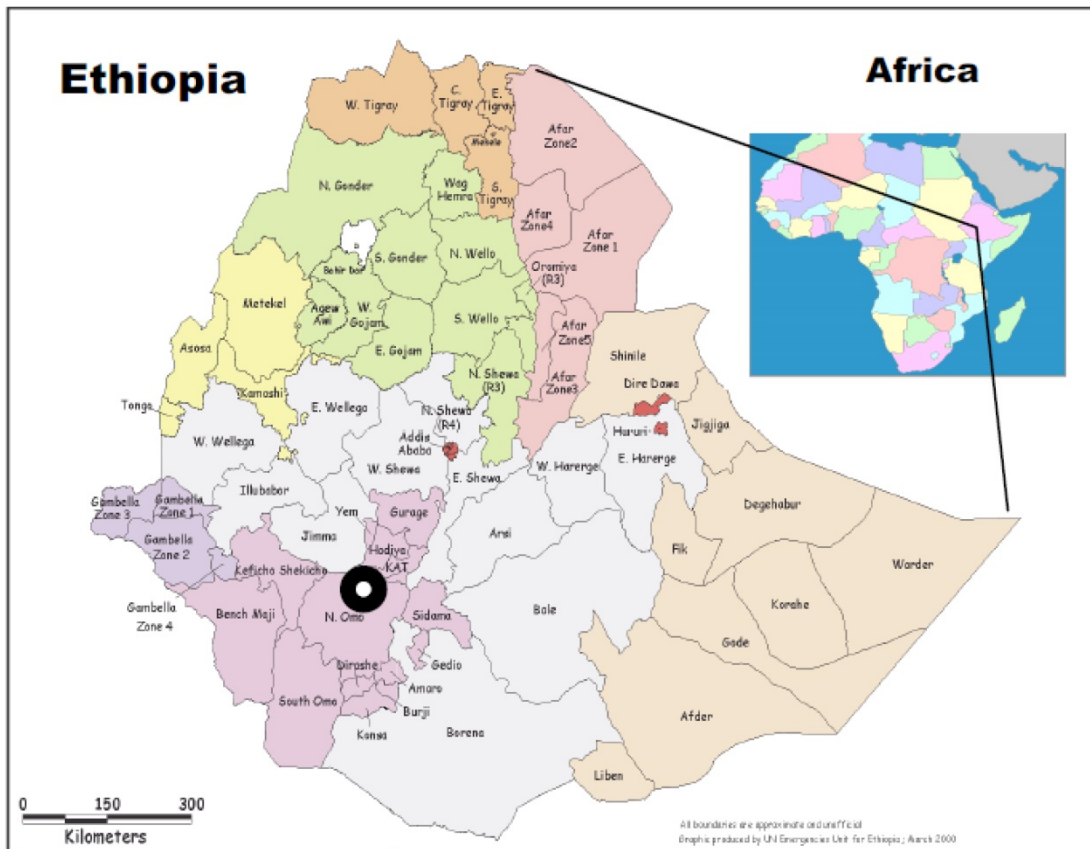


Figure 2: Map of the study area (Adapted from (Jufare 2008))

For the majority of the household in Wolaita agriculture is the main source of income with a significant supplement from off-farm activities. Agriculture is dominated by subsistence farming where limited usage of improved technologies and agricultural inputs significantly

¹ NB: Kebele is Amharic term for Peasant association and it is similar to a village.

limits productivity and per capita income. A very high population density of up to 746 persons per square kilometre has reduced the average land holding of the area to about 0.25-1 ha per household (Jufare 2008). The farming system is characterized by small-scale mixed production of crops and livestock (Eyasu 1998).

The area receives rainfall during a bimodal rainy season, which extends from March to September with April, July, August and September receiving peak amounts of rain. Average annual rainfall is 1200-1300 mm and the mean monthly temperature varies from 11 to 26°C. “Meher” is the main cropping season receiving rainfall from June to October whereas “Belg”, the second cropping season, lasts from February to June. Almost all cereal crop production of the area takes place during the Meher season. Cereals, root crops, pulses, and spices are the main crop categories produced in the area. Due to the bimodal rainfall pattern, resulting in a long growing season, multiple cropping practices such as intercropping, relay cropping and double cropping are common.

In spite of its relatively green coverage, the region experiences frequent food shortage and is known as the land of green famine (Elias and Scoones 1999). Currently, the Wolaita zone represents one of the major food deficit and famine prone areas in Ethiopia (Jufare 2008). Farmers consider soil fertility as the second most limiting factor for farming only next to shortage of draught oxen (Elias 1998). The soils in many areas of Wolaita have been depleted and degraded through continuous cultivation and the effects of leaching and erosion (Elias and Scoones 1999), so that soil fertility has been declining over time. In addition to lack of inorganic fertilizer, severe organic matter depletion, driven by competing uses for crop residues and manure, plays an important role in the decline of soil fertility. Compared to other regions of Ethiopia, the average livestock holding in this area of 1-2 heads per household is small. This has changed over time as two decades ago an average household used to keep 7 - 8 heads of cattle (Amede et al. 2001). Shortage of feed, conversion of grazing areas to cropland, and diseases are some of the reasons for the decline of livestock.

Farmers of Wolaita area normally divide their land into several parts and use each part for different purposes such as grazing, growing perennials like enset (false banana) and coffee, and growing maize, sweet potato and other tuber crops. The homestead plots typically contain crops such as enset, coffee, vegetables and also serves as seed bed to raise seedlings of trees and pepper. Usually farmers prefer to apply manure and other organic wastes to the fields in or closer to their homestead.

As a result, soils in the outer fields are less fertile and require mineral fertilizers to produce a reasonable yield. Maize, tef, potatoes, haricot bean, sorghum and pepper are crops mostly planted on the main/outer fields. Some plots are used for cut and carry fodder production for livestock feeding.

Damot Galle in general and Taba kebele in particular are the densest areas in the region. Unlike in previous times, the majority of farmers of this area are not food self-sufficient and they attribute this to the declining rainfall and delay in onset of rainfall, sharp increase of population size, and decreasing soil fertility. In this area, rain used to begin in December and its amount was enough to produce crops twice a year. Nowadays rainfall starts as late as early March and is received in a more erratic distribution. Consequently, the majority of the household heads are forced to flee to adjacent towns to find job opportunities and send some food to the family. Especially at the time of wheat harvest, which is around November, many farmers travel to the Arsi area, where they are employed in harvesting activities.

Taba is one of the leading kebeles in SNNRPS with regard to total area allocated to chickpea production and served as the centre for chickpea research in the region. Maize, sorghum, and tef are the main cereal crops whereas chickpea and haricot bean are the most widely cultivated pulse crops (Table1).

Table 1. List of annual crops and their cultivated area in the year 2011/12 at Taba, excluding perennials like enset and coffee (*Source*: Taba bureau of agriculture and development)

Crop	Area of cultivation (ha)
Maize	175.5
Potato	100
Haricot bean	81
Tef	80
Chickpea	37
Sweet potato	32
Pepper	25
Sorghum	10
Cassava	2
Abesha cabbage (local cabbage)	2
Boyya (root crop)	1.5
Total	546 ha

Many of the farmers in this site grow chickpea at least on part of their farm. In Taba kebele, chickpea is the fifth and second widely cultivated crop and food legume, respectively (Table 1). Chickpea's drought tolerance enables the farmers to prioritize it in rotation with cereals, thereby practicing efficient utilization of the already scarce land.

Chickpea is used in many ways such as the direct consumption of its green pod and dry beans, which are an important source of protein in the diet. Farmers also sell chickpea on the local markets where it can fetch a good price.

However, due to the need for cash to buy food and other items, farmers are often forced to sell most of the produced chickpea immediately after harvest, when prices are low. Also the straw of chickpea is used as high quality livestock feed, rich in crude protein. Another crucial reason why strengthening chickpea production in the area could be beneficial is related to its ability to grow on residual moisture and produce something in seasons with insufficient rainfall for more exigent crops, such as potato. Due to its lower input requirements than cereals, its multi-functionality and flexibility, chickpea is considered an important crop for smallholder, food-insecure farmers.

However, farmers often plant chickpea only on a small part of their land and leave the rest unplanted, mainly because of the lack of chickpea seed and fertilizer at the time of planting. Therefore, the provision of inputs at the time of planting would help in increasing productivity and enabling efficient use of the scarce land.

2.2. Farm Selection

Damot Gale as a district and Taba as a kebele are among the leading chickpea growing areas in SNNPRS, and were therefore chosen for this experiment. 20 farms located on two different soil types were selected. To make the selection, we visited the farms in the area well ahead of planting time to get insights on the farming system and soil differences. Farmers in the area classify their soil based on colour and there were two types of soil in the kebele, which was also confirmed by the Taba Office of agriculture and development. Farms found on the uplands were characterized by black soil and towards the valley area the soil was red. Ten farms, which frequently grow chickpea, were selected in each landscape position in consultation with the local development agent, Mr. Mulatu Chafo. Care was taken to include farmers from different wealth categories (poor, medium and rich). The Taba Office of agriculture and development classifies farmers on the bases of assets like herd size and land size. However, we were informed that rich farmers are hardly present in this site and poor farmers are much more numerous.

To represent this distribution, three farmers were picked from the medium wealth group and seven from the resource poor category for each landscape position and therefore soil type.

A clear discussion with the selected individuals on duties and responsibilities of the two parties (researchers and farmers) was conducted and they agreed to provide 100 m² area of land for the experiment.

2.3. Estimation of indigenous rhizobial population of the study soils

For the purpose of determining the presence of native and viable rhizobia cells in the soils of the study area, a test was conducted by implementing most probable number (MPN) method (Somasegaran and Hoben 1985). For this purpose, soils were sampled two days ahead of planting from the top 15 cm at five locations and bulked to form one composite sample per farm. The samples were brought to the laboratory and stored in a fridge at 4°C until the date of inoculation (see next paragraph).

Chickpea, cv Natoli, was used as an indicator plant. Seeds of uniform size and high viability were selected. The seeds were surface sterilized with ethanol [CH₃CH₂OH] for 5-8 minutes and then transferred to Sodium hypochlorite for 10 seconds. Petri dishes were prepared and their bottom covered with tissue paper, after which they were autoclaved and then used to pre-germinate the seeds. The number of seeds was intentionally larger than required just in case some failed to germinate. Accordingly, the required number of growth pouches (two for each plant where the bottom one served for nutrient and water supply) were prepared. The upper pouch was holed and cotton thread was inserted so that water and nutrients could be taken up from the bottom pouch. The pouches were filled with sand, covered tightly with aluminium foil and autoclaved at 121°C for three hours. The pre-germinated seeds were aseptically transferred into the growth pouches.

Meanwhile, any root or dirt material was removed from the soil samples being kept in the fridge. A tenfold serial dilution was prepared by adding 10 gram of soil into 90 ml of distilled water and sequentially diluting 1 in 10 to give a dilution series to 10⁻¹⁰. When all plants had germinated, the pouches were inoculated with each of the ten serial dilutions from the soil by using 1ml aliquots. The plants were frequently inspected and water and nutrients provided periodically. When the plants were about four weeks old, they were carefully uprooted and nodulated units were recorded. This was done for all the four replications and the non-inoculated control.

2.4. Experimental details of field experiment

2.4.1. Crop cultivar

The experiment was carried out with chickpea, cv Natoli. While selecting the variety, consideration was given to its market demand, farmers' interest, and availability of seeds. Accordingly, the improved chickpea variety Natoli was selected because it is among the five top yielding chickpea varieties used by farmers of the study area and has a market potential too.

2.4.2. Treatments and Layout

The experiment included four treatments that were applied on all farms. The four treatments were control (un-inoculated plot), *Rhizobium* inoculated plot (I), non-inoculated but phosphorus fertilized plot (P), and *Rhizobium* inoculated and phosphorus fertilized plot (I+P). For the P and I+P treatments, Triple Super Phosphate (TSP), with its composition of 0-46-0 for NPK respectively, was used at a rate of 100 kg TSP ha⁻¹ or 46 kg P ha⁻¹.

On each of the 20 selected farms, a 100 m² plot was divided in to four equal subplots with gross and net size of 20.25 m² and 17.55 m² respectively. A distance of 1 metre was left between each subplot to enable easy management and data collection. During planting, rows were kept 30 cm apart and 10 cm intra row spacing was used. Each plot consisted of 15 rows out of which the outer two rows were considered as border rows and each row consisted of 45 plants. Treatments were assigned randomly to the subplots. The experiment was not replicated on each farm, but farms were considered as replicates.

2.4.3. *Rhizobium* preparation and inoculation

Mesorhizobium ciceri strain CP 41 was obtained from the soil microbiology laboratory of the School of Plant and Horticultural Sciences, Hawassa University. This strain has been proven to enhance the nodulation capacity of chickpea seeds under wide ecological conditions and is considered the best strain of the laboratory as far as agronomic and yield performance of chickpea is concerned.

Strain multiplication was undertaken prior to planting in a sufficient amount at this laboratory and sterile peat was obtained from Legume Technology Ltd. in the UK. During both inoculum preparation and inoculation, all the necessary aseptic measures were given due attention. Accordingly, peat based inoculation was done at the recommended rate of 10g kg seed⁻¹.

Before planting, inoculation of the seeds was done on-farm using the peat as a carrier and sugar as adhesive material to stick the inoculum on the seeds. In order to maintain viability of the rhizobia, inoculation was done in the shade to avoid direct sun light. The inoculated seeds were kept in the shade for a few minutes to let them air dry before planting. In all farms, non-inoculated seeds were sown first and followed by the inoculated seeds to avoid cross contamination. Moreover, measure was taken to divide the labour into two so that one group planted the non-inoculated plots and the other group planted the inoculated plots.

2.5. Rainfall Data

With the aim of assessing the amount and variability of rainfall across the two landscape positions (upland and valley), two ordinary rain gauges were manufactured at the local metal workshop and used to record daily rainfall data.

The diameter of the rain collecting cylinder was 18 cm whereas the above ground height of the rain gauge was 1.5 m. The rain gauge was fixed to be 100% level. On each soil type, one household with an individual capable of writing and reading figures was selected. The rain gauges were fixed at a convenient position near to the houses, but care was taken to keep them away from tall objects. Accordingly, the daily rainfall was recorded for each soil type throughout the whole growing period.

2.6. Soil sampling and analysis

Before planting, soil samples were taken from each subplot to a depth of 0-30 cm using an auger. Samples from the four subplots of each farm were bulked together and a composite sample was prepared for physico-chemical analysis. Soils were air dried to a constant weight, grinded and mixed thoroughly and passed through a 2 mm sieve.

In the laboratory, the soils were analysed for particle size, total N, available P, organic carbon, pH, CEC, Ca, Mg, K, and Na. Standard procedures were followed for each parameter. CEC was determined using the ammonium distillation method whereas pH was measured with the potentiometric water extraction method. Available P was measured by the Olsen et al. (1954) method and for the determination of the total N, the Kjeldahl (1883) method was employed. Textural class and organic carbon content of the soil were determined using hydrometer and the wet combustion method of Walkley and Black (1934), respectively. Determination of exchangeable Na and K was carried out using ammonium acetate extraction and flame photometry after wet combustion.

For the determination of exchangeable Ca and Mg, ammonium acetate extraction was followed by flame atomic absorption spectrometry (AAS). The conductometric method was used for measuring electrical conductivity.

2.7. Plant tissue analysis

Doing the plant analysis for all the treatments on 20 farms was costly and a solution was found by randomly selecting three farms for each soil type and five plants per treatment from the middle rows. The plants were sampled at the time of harvest and separated into grain and straw.

The straw part was oven dried at 70°C for 48 hours, milled with mechanical miller and passed through a 1mm sieve. Subsequently, the plant samples were analysed for total N, P and ash content.

The straw total N uptake was first calculated as a product of the straw yield and its N content obtained from the lab analysis. Grain N content was assumed to be about twice of that of the straw (Kassa 2009). Hence, this grain N content was multiplied by the grain yield to give grain total N uptake. Consequently, total N uptake was calculated as a summation of straw and grain total N uptake.

2.8. Management practices

In the preceding season, five types of crops had been grown on the experimental fields of the twenty farms. Haricot bean, potato, maize, tef and sweet potato were grown on 55%, 15%, 15%, 10% and 5% of the fields respectively. Before planting, all fields were thoroughly ploughed and levelled by the owner of the land. Accordingly, planting started on September 6 and was completed within three consecutive days for 18 farms whereas two farms (farms 19 and 20) were planted on September 15. In all cases, there was frequent rainfall during the early plant stages, which guaranteed good initial plant establishment. All fields were weeded twice during the growing period. During our frequent field supervision, no severe disease symptoms were observed on the farms. However, on several farms there was occurrence of boll worm (*Helicoverpa*) during flowering and pod setting. As a control measure, Lambda-cyhalothrin chemical was sprayed for each plot. Nevertheless, yield loss due to this problem was inevitable as some pods were holed and devoid of seeds during harvesting. Harvesting started around mid-December and finished on January 19 as the farms differed with respect to time to maturity.

2.9. Observations and Data collection

2.9.1. Crop phenology

Days to emergence was recorded for each plot when more than 50% of the plants emerged. Around three weeks after planting, the total number of plants emerged was counted from the middle 13 rows to conduct a stand count at emergence. Similarly, days to flowering and maturity were recorded when more than 50% of the plants in each plot attained flowering and physiological maturity respectively. At the time of flowering, six plants were randomly selected from the 13 middle rows and their height from the ground to the tip measured using a ruler. From the six randomly uprooted sample plants at maturity (see 2.9.4), the number of branches (both primary and secondary) was counted and averaged to give number of branches plant⁻¹ (NBPP).

2.9.2. Nodulation

Nodulation assessment was conducted at three physiological stages. For all treatments, at 23 days after sowing (DAS), six plants were selected from the two border rows, 3 from each, and gently uprooted. The root was washed with tap water to remove the adhering soil.

The number of nodules per plant was counted and the values averaged to give the number of nodules per plant (NNPP). The same plants were used for nodule scoring per plant (NSPP) on 0-5 scale according to a protocol developed by Bala et al. (2010) where plants with nodule number of zero, <5, 5-10, 11-20, 21-50, >50 were scored 0,1,2,3,4 and 5 respectively. These scores refer to absent, rare, few, moderate, abundant and super nodulation of roots in ascending order. At 45 DAS, which is around flowering time, again six plants were randomly selected and uprooted from the interior rows and these plants were separated in to shoot and root and NNPP and NSPP were recorded. In addition, the nodules were severed from the roots, oven dried at 70°C for 48 hours and their dry weight recorded to give nodule dry weight per plant (NDWPP) at 45 DAS. At harvesting time, six plants were randomly marked, watered a day ahead, and gently uprooted the next day. The roots of these plants were washed with tap water and after removing all the adhering soil and debris, NNPP and NSPP were recorded.

2.9.3. Shoot weight

The shoot part of the plants that were sampled at 45 DAS were cleaned and weighed, and the values were averaged to constitute shoot fresh weight per plant. The same plants were oven

dried at 70°C for 48 hours to a constant weight and weighed again to determine dry shoot weight per plant.

2.9.4. Grain yield, yield related traits and total biomass production

Just before harvesting, the total number of plants was counted from the interior 13 rows of each plot to give stand count at harvest. During harvesting time, data was recorded for yield attributing parameters such as number of pods per plant, number of seeds per pod, and thousand seed weight for each plot. For this purpose, six plants were randomly selected from the interior 13 rows and dug up.

All of the plants, apart from the two external rows, were manually harvested and brought together on a plastic sheet for each plot and measured to give weight of total biomass per plot. Then the plants were threshed separately for each plot and the seeds blown to air to separate seeds from debris/husks. The grain was put in a cloth sack and weighed to give yield per plot. On the same date, seeds were sampled and taken to the laboratory to measure their moisture content using a seed moisture meter (Model HOH- Express He 50). To keep uniformity, the final grain yield of each plot was adjusted to 12% seed moisture content.

2.9.5. Data on household characteristics

After harvest, farmers were interviewed to get their opinion about the treatments as well as their household characteristics. For this purpose, a questionnaire or field evaluation book designed by the N2Africa project was used and farmers were interviewed at their homestead. Data was collected of farmers' household characteristics, previous management practices and their perception on fertility of their farm. The household level data were explored to explain the differences in treatment performance between farms.

2.10. Statistical analysis

After checking the compliance of the data with the assumptions of the statistical test, analysis of variance was conducted using a split plot design where soil type was applied to the whole plot and the four treatments were applied to the subplot. Each farm was considered as a block. A threshold P value of <0.05 was used to declare effects and interactions to be significant. When the effects were found to be significant ($P<0.05$), a LSD test ($\alpha = 0.05$) was used to study which means differed significantly and the comparison was made by employing Duncan multiple range test (DMRT). In addition, the relationship among the studied parameters was assessed using Pearson's simple correlation analysis.

3. Results

3.1. Soil characteristics of the study farms

3.1.1. Physico-chemical properties

The soil textural class of fourteen of the experimental fields was loam with average proportions of 24% clay, 40% silt and 36% sand. Of the remaining fields, soil texture was silt loam, clay and clay loam on two, one and three farms respectively (Appendix 1). This result is in agreement with that of Kassa (2009) who reported the soil texture of Taba kebele to be loam. The pH value of the soil ranged from 5.8 to 7.3. Of the twenty soil samples, pH values of soils from fourteen farms ranged between 6 and 7. Generally, the average pH value was 6.5 which is only slightly acidic and within the optimum range for crop production (Havlin et al. 1999), as well as for chickpea infective strains of rhizobia (Rodrigues et al. 2006).

According to Havlin et al. (1999), total nitrogen content (TN) of a soil can be classified as very low (<0.1%), low (0.1-0.15%), medium (0.15-0.25%), and high (>0.25%). According to this classification, the total nitrogen content of the soils from the study farms was found to be very low, low, and medium for 6, 12 and 2 farms respectively. The average total nitrogen for the twenty farms was 0.11%, which is within the bottom range of the low total nitrogen class. Indicative ranges of available phosphorus have been established by Olsen et al. (1954), including < 5 mg kg⁻¹ (very low), 5-15 mg kg⁻¹ (low), 15-25 mg kg⁻¹ (medium), and > 25 mg kg⁻¹ of soil (high). Based on this criterion, the available phosphorus content was found to be very low, low, medium and high for 1, 9, 6 and 4 farms respectively. As far as soil organic carbon (OC) is concerned, the value ranged from 0.55 to 1.62%, which is within the range of low organic carbon content (Landon 1991). The cation exchange capacity (CEC) is referred to be low (5-15 cmol kg⁻¹), medium (15-25 cmol kg⁻¹), or high (25-40 cmol kg⁻¹). For this parameter, soils of nineteen farms had a medium CEC and only one farm was characterized by low CEC.

The two soil types showed differences in their initial content of major nutrients and characteristics, with the black soil somewhat more fertile than the red soil. The black soil contained significantly ($P=0.013$) higher TN (0.12 %) and highly significantly ($P<0.001$) higher OC (1.4 %) than the red soil with TN 0.091% and OC 0.96%. For P, CEC and pH values no significant difference was observed between the soil types.

Nonetheless, the black soil contained higher mean P (19 mg kg⁻¹) and CEC (17.9 cmol kg⁻¹) than the red soil whose mean P and CEC were 13 mg kg⁻¹ and 17.5 cmol kg⁻¹ respectively. Opposite to this, the pH was higher for the red soil (6.7) than for the black soil (6.4).

Generally, the soils of the experimental fields were deficient in total nitrogen content, available phosphorus, and organic carbon, whereas for almost all farms, the CEC was in the medium category (Appendix 1). Since these are the major elements of soil fertility, the result indicates poor fertility status of the soil that would in turn limit crops to achieve their potential productivity.

3.1.2. Native rhizobia populations

The MPN test revealed that the population of indigenous rhizobia of the soils in the study area was very low, ranging from none to <10 rhizobia cells gram⁻¹ of soil. In 50% of the soil samples tested, there was no viable and infective rhizobia recovered and in the remaining 50% of the samples, the rhizobia population was <10 rhizobia cell gram⁻¹ of soil (Table 2). This indicates that the population is not abundant enough to initiate optimum nodulation. In 75% of the farms on black soils, there was no measurable population of rhizobia, whereas on the red soil type, 25% of the farms had no measurable rhizobia.

Table 2. Most probable number test result of soil samples for some farms of the study area (n=8)

Soil type	Farm number	rhizobia population g ⁻¹ of soil
Black	2	none
Black	7	none
Black	19	none
Black	20	< 10
Red	4	< 10
Red	9	none
Red	13	< 10
Red	17	< 10

3.2. Rainfall data

212 and 214 mm of rainfall was received during the whole growing period (September to January) on black and red soils respectively (Appendix 2). In both cases, the highest amount of rain (100 mm) was recorded in the month of September. Generally, rainfall amount declined from planting time to harvest with less than 5mm in December (Appendix 2). Both the daily and cumulative rainfall remained almost similar for the two soil types (Fig. 3).

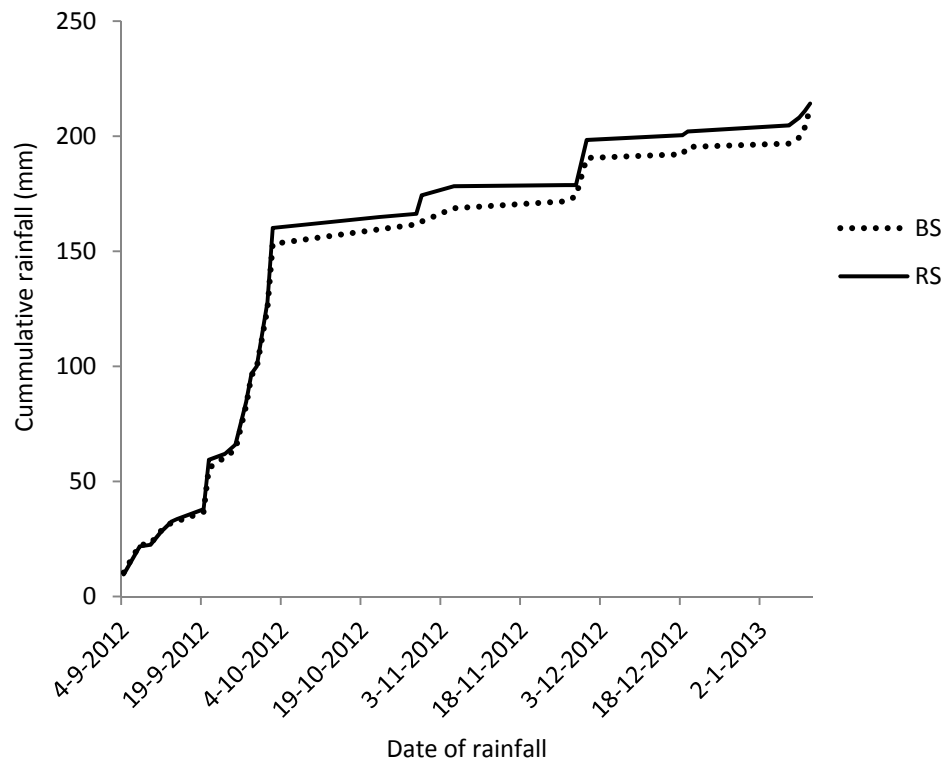


Figure 3: Cumulative rainfall of the two soil types for the growing period. BS = black soil, RS = red soil.

3.3. Effects of soil types on nodulation, growth, pod production and days to maturity of chickpea (*Cicer arietinum* L.)

The effect of soil type was studied for all the variables investigated in this study. However, significant effects of soil type were only observed for the six variables presented in Table 3.

At 23 DAS a higher mean nodule number plant⁻¹ was produced by plants grown on red soil (26) than plants grown on black soil (19) (Table 3). Similarly, nodule score plant⁻¹ at 23 DAS was significantly ($P=0.036$) higher for plants raised on red soil with mean value of 3.6 whereas mean NSPP was 3.1 for plants on black soil type. Chickpea plants grown on black soil were significantly ($P<0.001$) taller (45 cm) than those on red soil (38 cm) (Table 3). Likewise, a significant ($P=0.015$) effect of soil type was observed for number of branches plant⁻¹ (NBPP). A higher NBPP was recorded for plants on black soil (11) than on of red soil (9) (Table 3). In addition, a significantly ($P=0.018$) higher number of pods plant⁻¹ (NPPP) was produced by plants raised on black soil (56 pods) compared with plants on red soil (44 pods). The effect of soil type was also significant ($P=0.046$) for days to maturity with plants on red soil requiring 98 days, whereas 108 days were required for plants on black soil to reach maturity (Table 3).

Table 3. Effects of soil types on nodulation, growth, pod production and days to maturity of chickpea (*Cicer arietinum* L.) (n=40)

Soil type	NNPP at 23 DAS	NSPP at 23DAS	Plant height (cm)	NBPP	NPPP	Days to maturity
Black soil	19 ^a	3.1 ^a	46 ^b	11 ^b	56 ^b	108 ^b
Red soil	26 ^b	3.6 ^b	38 ^a	9 ^a	44 ^a	98 ^a

Notes: NNPP = nodule number per plant, DAS = days after sowing, NSPP = nodule score per plant, NBPP = number of branches per plant, NPPP = number of pods per plant. Means followed by the same letter(s) with in a column are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

3.4. Effects of *Rhizobium* inoculation and / or Phosphorus fertilization on nodulation of chickpea (*Cicer arietinum* L.)

3.4.1. Nodule number plant⁻¹

The assessment of nodule number plant⁻¹ (NNPP) was carried out at three different physiological stages of the crop and except for NNPP at 23DAS, a significant and positive effect of the soil fertility treatments was observed. At the early crop stages, nodule number plant⁻¹ of chickpea was improved only by combined application of inoculation and P fertilizer. In later stages, however, application of all the three soil fertility treatments resulted in increased NNPP. At all the three stages, maximum improvement of NNPP was achieved by the combined application of inoculation and P fertilizer. In addition, NNPP of chickpea was highest at 45 DAS and lowest at maturity (Fig. 4).

Similarly, a highly significant ($P < 0.001$) effect of the soil fertility treatments was observed for NNPP at 45 DAS. For this parameter, the maximum nodule number (48) was obtained from application of the I+P treatment which was 138% higher than the NNPP of the control treatment. Also at maturity the effect of the soil fertility treatments on NNPP remained highly significant ($P < 0.001$), with the maximum NNPP (13) obtained from the application of I+P (Fig. 4).

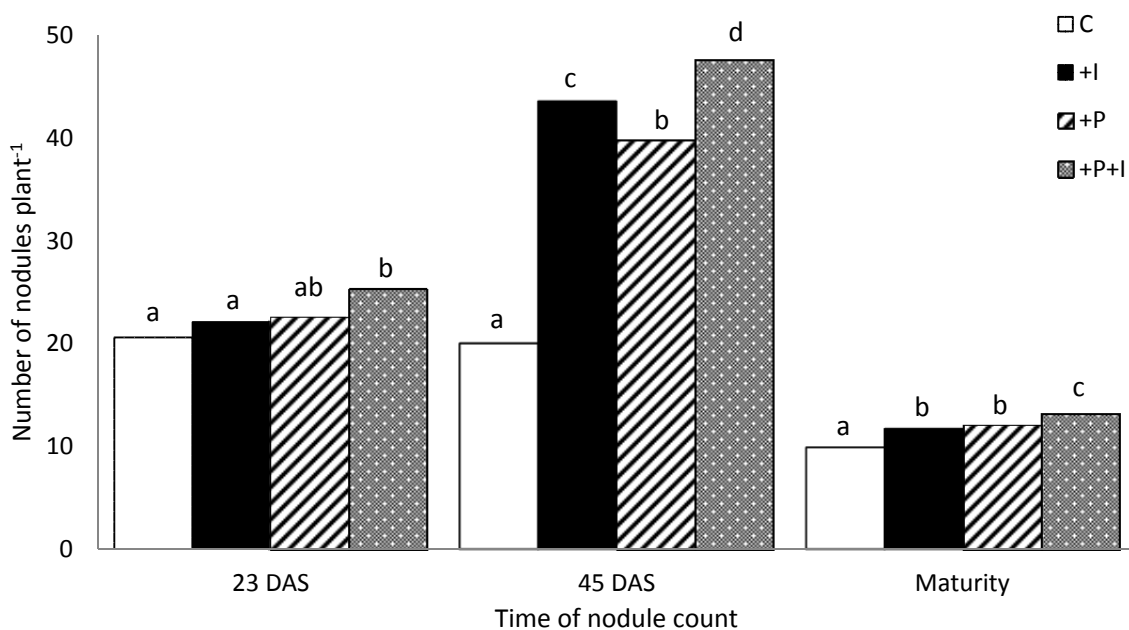


Figure 4: Nodule numbers plant⁻¹ at three physiological stages (DAS = days after sowing) for four treatments (C = control, +I = inoculation, +P = phosphorus fertilized, +I+P = both inoculation and phosphorus application). Treatments are significantly different if they have no letter in common $P=0.03$ at 23 DAS, $P<0.001$ at 45 DAS and maturity.

3.4.2. Nodule score plant⁻¹

Nodule score plant⁻¹ (NSPP) of chickpea followed an almost identical trend with NNPP and was enhanced by application of inoculation and/ or P fertilization. At 23 DAS NSPP was significantly ($P=0.026$) improved only by the combined application of inoculation and P, resulting in the maximum (3.6) NSPP. On the other hand, NSPP at 45 DAS was observed to be significantly ($P<0.001$) and positively enhanced by all the treatments, with a more pronounced effect when inoculation and P were applied in combination (Table 4). The effect of the treatments on NSPP remained highly significant ($P<.001$) at maturity. Accordingly, the highest (2.7) NSPP was recorded for plants that received the treatment I + P. There was no significant difference between inoculation and P treatments, but both gave higher NSPP than the control treatment (Table 4).

3.4.3. Nodule dry weight plant⁻¹ at 45 DAS, (NDWPP at 45DAS)

The application of inoculation and / or P significantly ($P<.001$) enhanced nodule dry weight plant⁻¹ of chickpea at 45 days after sowing. Although all the three treatments gave significantly higher NDWPP over the control treatment, the maximum value (78 mg plant⁻¹) was recorded from plants that received the I+P treatment (Table 4).

There was no significant difference between inoculation and P fertilization, but both gave higher NDWPP than the control (55 mg plant⁻¹) (Table 4).

Table 4. Effects of *Rhizobium* inoculation and / or phosphorus fertilization on nodulation of chickpea (*Cicer arietinum* L.) (n=20)

Treatments	NDWPP at 45 DAS mg/plant	NSPP at 23 DAS	NSPP at 45 DAS	NSPP at Maturity
Control	55 ^a	3.3 ^a	3.4 ^a	2.3 ^a
Inoculation	70 ^b	3.4 ^{ab}	4.3 ^c	2.6 ^b
Phosphorus	65 ^b	3.3 ^a	4.1 ^b	2.6 ^b
Inoculation + P	78 ^c	3.6 ^b	4.5 ^d	2.7 ^c

Notes: DAS = days after sowing, NDWPP = nodule dry weight per plant in milligram, NSPP = nodule score per plant. Means followed by the same letter(s) with in a column are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

3.5. Effects of *Rhizobium* inoculation and / or phosphorus fertilization on phenological and agronomic traits of chickpea (*Cicer arietinum* L.)

3.5.1. Shoot fresh weight plant⁻¹

Shoot development of chickpea was enhanced by P fertilization and *Rhizobium* inoculation with an even more pronounced improvement under combined application of inoculation and P (I+P). Though no statistically significant difference was observed between inoculation and P, and between I+P and P treatments, separate application of inoculation and P resulted in significantly higher SFWPP than the control treatment (Table 5). The maximum SFWPP (9.0 g plant⁻¹) was recorded from plants that received a treatment in which inoculation and P were combined.

3.5.2. Days to maturity

Days to maturity was significantly ($P < 0.001$) extended by the applied soil fertility treatments with the shortest maturity time observed from the control treatment (Table 5). Inoculation, both alone or in combination with P, resulted in the longest (104 days) growing period of chickpea. Likewise, a separate application of P also exerted a highly significant effect on days to maturity with mean maturity days of 103, which was significantly shorter than that of I+P and I treatments but longer than that of the control treatment (Table 5). Nevertheless, days to maturity did not change considerably with the fertility treatments, ranging only from 101 to 104 days.

3.5.3. Number of branches plant⁻¹

Number of branches plant⁻¹ (NBPP) of chickpea increased significantly ($P < .001$) due to the applied soil fertility treatments. The maximum NBPP (11) was produced by plants that received the I+P treatment whereas the plants in the control produced the lowest number of branches (8). Likewise, compared to the control higher NBPP was produced by plants that received a separate application of inoculation and P (Table 5). However, non-significant differences in NBPP between inoculated and P fertilized plants, as well as between I+P and P treated plants were observed (Table 5).

3.5.4. Plant height (cm)

The application of inoculation and / or phosphorus resulted in significantly ($P < .001$) taller chickpea plants over the control treatment.

Though all the treatment effects remained on par, plant height was significantly improved as compared to the control, with the combined application of inoculation and P resulting in the tallest plants (43 cm) (Table 5).

Table 5. Effects of *Rhizobium* inoculation and / or phosphorus fertilization on phenological and morphological characteristics of Chickpea (*Cicer arietinum* L.)(n = 20)

Treatments	SFWPP at 45 DAS (g/plant)	Days to maturity	NBPP	Plant height (cm)
Control	7.0 ^a	101 ^a	8 ^a	40 ^a
Inoculation	8.4 ^b	104 ^c	10 ^b	43 ^b
Phosphorus	8.5 ^{bc}	103 ^b	10 ^{bc}	42 ^b
Inoculation + P	9.0 ^c	104 ^c	11 ^c	43 ^b

Notes: SFWPP = shoot fresh weight per plant, DAS = days after sowing, NBPP = number of branches per plant. Means followed by the same letter(s) with in a column are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

3.6. Effects of *Rhizobium* inoculation and / or Phosphorus fertilization on yield and yield related traits of chickpea (*Cicer arietinum* L.)

3.6.1. Number of pods plant⁻¹ (NPPP) and number of seeds pod⁻¹ (NSPP)

Inoculation and/ or P significantly increased the number of pods plant⁻¹ compared to the control treatment. Accordingly, the lowest NPPP (45) was recorded in the control treatment whereas the highest (54) NPPP was recorded for plants receiving I+P treatment followed by 51 and 50 NPPP for the inoculation and P treatments respectively (Table 6).

Similarly, inoculation, either alone or in combination with P, significantly ($P=0.006$) increased number of seeds pod⁻¹ (NoSPP). Nonetheless, a separate application of P gave NoSPP which was statistically not different to that of the control. The maximum mean NoSPP (1.6) was recorded from the inoculated plants whereas the lowest mean (1.4) NoSPP was recorded from the plants in the control treatment (Table 6).

3.6.2. Total biomass production (ton ha⁻¹)

Analysis of variance indicated that total biomass of chickpea was significantly ($P<.001$) improved by inoculation and / or phosphorus fertilization treatments. Application of each of the fertility treatments had a positive and highly significant effect on total biomass of chickpea as compared to the control. Consequently, the maximum mean total biomass (4.5 ton ha⁻¹) resulted from application of the I+P treatment, which was followed by 4.3 ton ha⁻¹ from the inoculation treatment. The lowest mean total biomass (3.5 ton ha⁻¹) was recorded from the control treatment (Table 6). For this parameter, there were no statistically significant differences observed between inoculation and P treatments, and between sole P and I+P treatments.

3.6.3. Grain yield (ton ha⁻¹)

Analysis of variance revealed a highly significant ($P<.001$) effect of the treatments on the grain yield of chickpea. The maximum mean grain yield (2.1 ton ha⁻¹) was obtained from the I+P treatment which, in fact, was statistically on par with that of the inoculation treatment (2.0 ton ha⁻¹) and slightly greater than the treatment with P only (Table 6).

3.6.4. Thousand Seeds weight (gram)

The weight of thousand seeds of chickpea responded positively and significantly ($P=0.002$) to the application of the fertility treatments with heavier seeds compared with the control.

The maximum mean weight of thousand seeds was recorded in the I+P treatment (308 g), whose effect was statistically on par with that of sole P (307 g) which, in turn, remained statistically similar to that of sole inoculation (294 g) (Table 6).

Table 6. Effects of *Rhizobium* inoculation and / or phosphorus fertilization on yield and yield related traits of Chickpea (*Cicer arietinum* L.)(n=20)

Treatments	NPPP	NSPP	Total biomass (ton ha ⁻¹)	Grain yield (ton ha ⁻¹)	1000 SW (g)
Control	45 ^a	1.4 ^a	3.5 ^a	1.6 ^a	285 ^a
Inoculation	51 ^{bc}	1.6 ^b	4.3 ^{bc}	2.0 ^{bc}	294 ^{ab}
Phosphorus	50 ^b	1.5 ^{ab}	4.0 ^b	1.9 ^b	307 ^{bc}
Inoculation + P	54 ^c	1.6 ^b	4.5 ^c	2.1 ^c	308 ^c

Notes: NPPP = number of pods per plant, NSPP = number of seeds per pod, 1000 SW= thousand seeds weight. Means followed by the same letter(s) with in a column are not significantly different at $P= 0.05$ (Duncan's Multiple Range Test).

3.7. Effects of *Rhizobium* inoculation and/ or phosphorus fertilization on straw total nitrogen (STN, %) and phosphorus content (STP, mg kg⁻¹) and total N uptake of chickpea

The application of all three fertility treatments significantly ($P < 0.001$) improved the total nitrogen content of chickpea straw over that of the control treatment. STN of the inoculation treatment (0.84%) remained statistically on par with the STN of both the P fertilization treatment (0.72%) and the I+P treatment (0.98%) (Table 7). Furthermore, inoculation, either alone or in combination with P increased the total N uptake compared with the control. The least total N uptake (28 kg ha⁻¹) was recorded from the control treatment whereas the maximum total N uptake (65 kg ha⁻¹) was recorded from the I+P treatment that was statistically alike with the total N uptake of the I treatment (53 kg ha⁻¹) (Table 7).

Similarly, also for total phosphorus content of chickpea straw the effect of the treatments was highly significant ($P < 0.001$) All the fertility treatments, except when only inoculation is used, were able to improve the STP over the control treatment. The highest STP (367 mg kg⁻¹) was recorded from plants receiving P fertilization, followed by that of I+P treatment (334 mg kg⁻¹), with which it was statistically on par (Table 7).

Table 7. Effects of *Rhizobium* inoculation and/or phosphorus fertilization on straw total nitrogen and phosphorus content, and total N uptake of chickpea (*Cicer arietinum* L.) (n=6)

Treatments	STN %	Total N uptake (kg ha ⁻¹)	STP (mg kg ⁻¹)
Control	0.54 ^a	28 ^a	261 ^a
Inoculation	0.84 ^{bc}	53 ^b	273 ^a
Phosphorus	0.72 ^b	42 ^{ab}	367 ^b
Inoculation + Phosphorus	0.98 ^c	65 ^b	334 ^b

Notes: STN % = straw total nitrogen %, STP = straw total phosphorus (mg kg⁻¹). Means followed by the same letter(s) with in a column are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

3.8. Interaction effects between soil fertility treatments and soil types on growth and performance of chickpea

Interaction effects of *Rhizobium* inoculation and/or P fertilization and the soil types were studied for all the variables investigated. However, the analysis of variance showed that only three variables among all were significantly influenced by the interaction of the soil fertility treatments and the soil types. Only these three variables (NNPP at 45 DAS, NSPP at 45 DAS and days to maturity) are presented in Table 8 and the below text.

3.8.1. Nodule number and score plant⁻¹ at 45 days after sowing (45 DAS)

There was a highly significant ($P < 0.001$) effect exerted on NNPP at 45 DAS by the interaction between the treatments and the two soil types. There was large variation observed among various combinations of factors for this variable. The number of nodules per plant ranged from 20 in black soil of the control treatment to 52 in red soil of the I+P treatment (Table 8). For control and P fertilization treatments, NNPP remained alike in both soil types. However, for inoculation and I+P treatments nodule numbers were higher in the red soil.

The interaction effect between the treatments and the soil types was also significant ($P = 0.003$) for nodule score plant⁻¹ studied at 45 days after sowing. In general, the highest (4.6) score was recorded for plants received I+P treatment on red soil whereas the lowest (3.4) score was recorded from control treatment under both soil types (Table 8).

3.8.2. Days to maturity

The interaction between soil types and the treatments had a significant ($P = 0.007$) effect on days to maturity of chickpea. With the inoculation and control treatments, chickpea had a shorted maturity period when grown on red soil, whereas no effect of soil type was observed for the sole P and I+P treatments. Of all treatment combinations, the shortest (96 days) maturity period was recorded for the control treatment on the red soil type. In contrast to this, application of I+P on the black soil resulted in the longest (109 days) growing period. Generally, the soil fertility treatments advanced the maturity of chickpea on the black soil whereas the treatments delayed maturity on the red soil (Table 8).

Table 8. Effects of interaction between soil fertility treatments and soil type on nodulation and days to maturity of chickpea (*Cicer arietinum* L)(n=20)

Treatments	Soil type	NNPP at 45DAS	NSPP at 45DAS	Days to maturity
Control	Black	20 ^a	3.4 ^a	110 ^d
	Red	20 ^a	3.4 ^a	96 ^a
Inoculation	Black	39 ^b	4.2 ^{bc}	105 ^c
	Red	48 ^{cd}	4.4 ^{cd}	98 ^{ab}
Phosphorus	Black	40 ^b	4.2 ^{bc}	108 ^{bcd}
	Red	39 ^b	4.1 ^b	99 ^{ab}
Inoc + P	Black	43 ^{bc}	4.3 ^{bc}	109 ^{cd}
	Red	52 ^d	4.6 ^d	99 ^{abc}

Notes: NNPP = nodule number per plant, DAS = days after sowing, NSPP = nodule score per plant. Means followed by the same letter(s) with in a column are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

3.9. Correlations among the symbiotic and agronomic variables of chickpea

Positive and significant correlation among related traits was found whereas the opposite was observed between divergent traits. Grain yield of chickpea was positively and significantly correlated with number of seeds pod⁻¹ ($r = 0.358^{***}$), number of pods plant⁻¹ ($r = 0.418^{***}$), number of nodules plant⁻¹ at 45 DAS ($r = 0.308^{**}$), days to maturity ($r = 0.322^{**}$), plant height ($r = 0.397^{***}$) and total biomass ($r = 0.855^{***}$) (Appendix 8). Similarly, positive and significant correlation was observed for total biomass production with NNPP at 45 DAS ($r = 0.273^*$), initial soil organic carbon ($r = 0.229^*$), initial soil available P ($r = 0.305^{**}$), plant height ($r = 0.351^{**}$), number of branches plant⁻¹ ($r = 0.342^{**}$) and shoot fresh weight at 45 DAS ($r = 0.253^*$) (Appendix 8). On the other hand, negative correlation ($r = -0.295^{**}$) was observed between the number of nodule plant⁻¹ at 23 DAS. Similarly, days to maturity was negatively correlated with NNPP at 23 DAS ($r = -0.306^{**}$), stand count at harvest ($r = -0.363^{***}$), shoot fresh weight plant⁻¹ at 45 DAS ($r = -0.554^{***}$), and shoot dry weight plant⁻¹ at 45 DAS ($r = -0.576^{***}$) (Appendix 8).

3.10. Across farm variability in response to the soil fertility treatments

3.10.1. Yield and total biomass production

Across the twenty farms, large variability in growth and stand of chickpea plants was observed for each treatment (Fig. 6). Nevertheless, a positive response to the soil fertility treatments was recorded on more than 85% of the farms, the least yield being recorded from the control treatment. Overall, the yield ranged from 0.5 ton ha⁻¹ on a control plot to 3.0 ton ha⁻¹ on a P treated plot (Fig. 5A). Regardless of the huge variability, on the majority of farms yield response was most pronounced under I+P treatment application. For all four treatments the lowest yield, including a weak response, was produced on farm 20 and this might be related to poorer plant establishment (16%, 14%, 17% and 22% plant emergence recorded for C, I, P and I+P treatments respectively) due to waterlogging, which also affected other crops on this farm.

Almost similar response to the soil fertility treatments was recorded for the total chickpea biomass. A positive response of the soil fertility treatments was recorded on more than 70% of the farms, in which the control treatment produced the lowest total biomass and the I+P treatment the highest total biomass. As such, the total biomass production ranged from 1.4 ton ha⁻¹ on a control plot to 7.1 ton ha⁻¹ on a plot that received the I+P treatment (Fig. 5B). For both yield and biomass, positive responses were observed across the range in control yields/biomass (Figs. 5A and 5B).

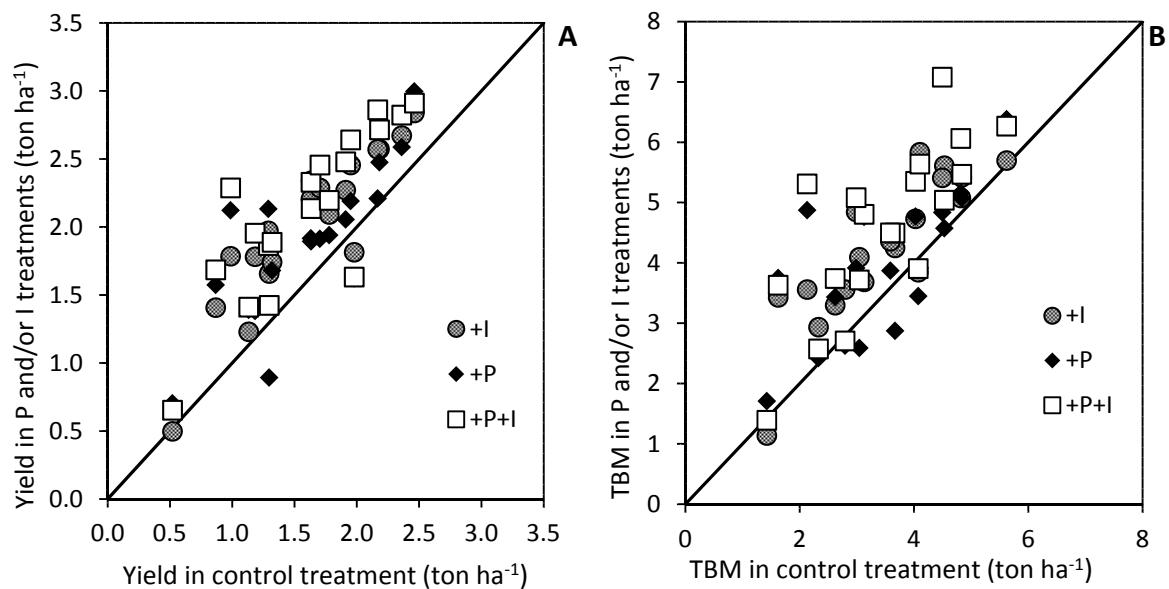


Figure 5: Response of chickpea grain yield (A) and total biomass (B) (ton ha⁻¹) to inoculation and/or P treatments. Notes: +I = inoculation, +P = phosphorus fertilized, I+P = both inoculated and P fertilized, TBM =total biomass.



Figure 6: Between farm difference of chickpea growth in response to the treatments

3.10.2. Nodule number plant⁻¹ and shoot fresh weight plant⁻¹ at 45 DAS

The trend of response and variability was almost alike for most of the variables studied. Here only NNPP at 45 DAS and SFWPP are presented, as they are clearly linked to yield and give an insight of the variability in almost all other parameters. Both nodule number plant⁻¹ (NNPP) at 45 DAS and SFWPP positively responded to the P and/or I treatments on almost all farms studied (Figs 7A and 7B). On the majority of farms, the lowest NNPP was recorded for the plants from the control treatment whereas the highest NNPP was counted from the plants receiving the I+P treatment. Overall, the NNPP ranged from 13 in the control treatment of farm 20 to 57 in the I+P treatment of farm 10.

The SFWPP response to the treatments also exhibited large across farm variability. For the control treatment SFWPP ranged from 2.6 g plant⁻¹ to 14.7 g plant⁻¹ on farm 17 and 9 respectively whereas for I+P treatment alone the SFWPP ranged from 4 g plant⁻¹ to 19 g plant⁻¹ on farm 4 and 9 respectively. Nonetheless, for the majority of the farms the lowest SFWPP was recorded on the control (Fig. 7B).

Similar to yield and biomass, positive responses were observed across the range in control NNPP and SFWPP (Figs 7A and 7B), with still moderate across farm variability in response to the treatments.

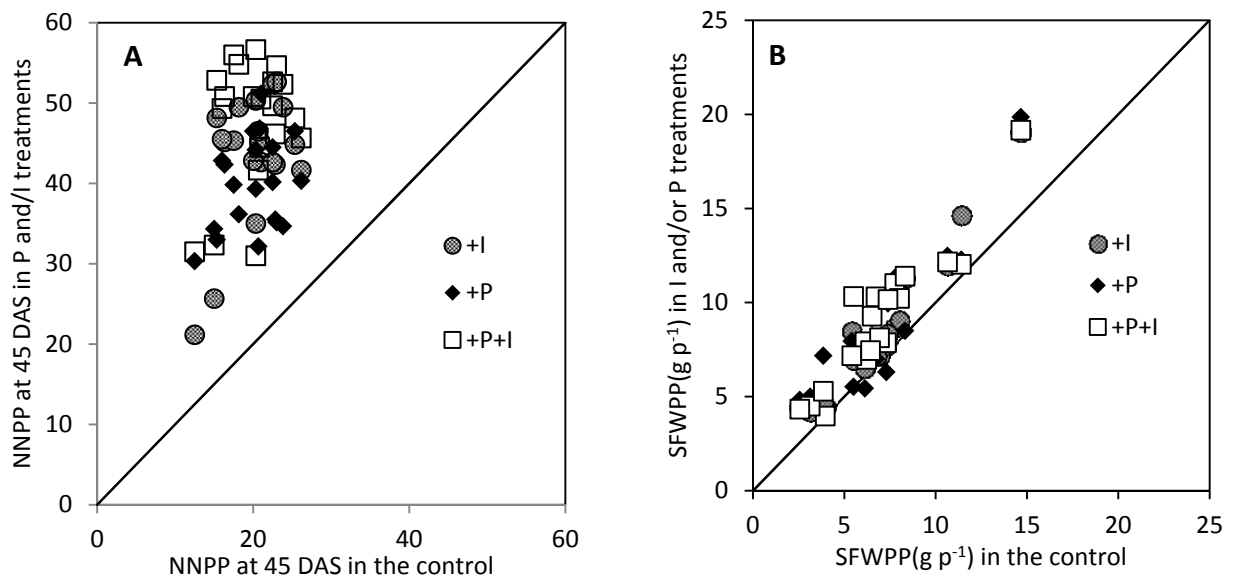


Figure 7: Response to inoculation and/or phosphorus fertilization of nodule number plant⁻¹ (A) and shoot fresh weight plant⁻¹ (g plant⁻¹) (B) at 45 days after sowing of chickpea. Notes: +I = inoculation, +P = phosphorus fertilized, +I+P = both inoculation and phosphorus added, DAS = days after sowing, NNPP = nodule numbers plant⁻¹, SFWPP = shoot fresh weight plant⁻¹.

3.11. Across farm variability in nodulation, biomass, yield and their response to the soil fertility treatments under the two soil types

3.11.1. Nodule number plant⁻¹

For both soil types, moderate across farm variability was exhibited for NNPP at 45 DAS. However, the variability appears to be smaller on the red soil than the black soil (Figs 8A and 8B).

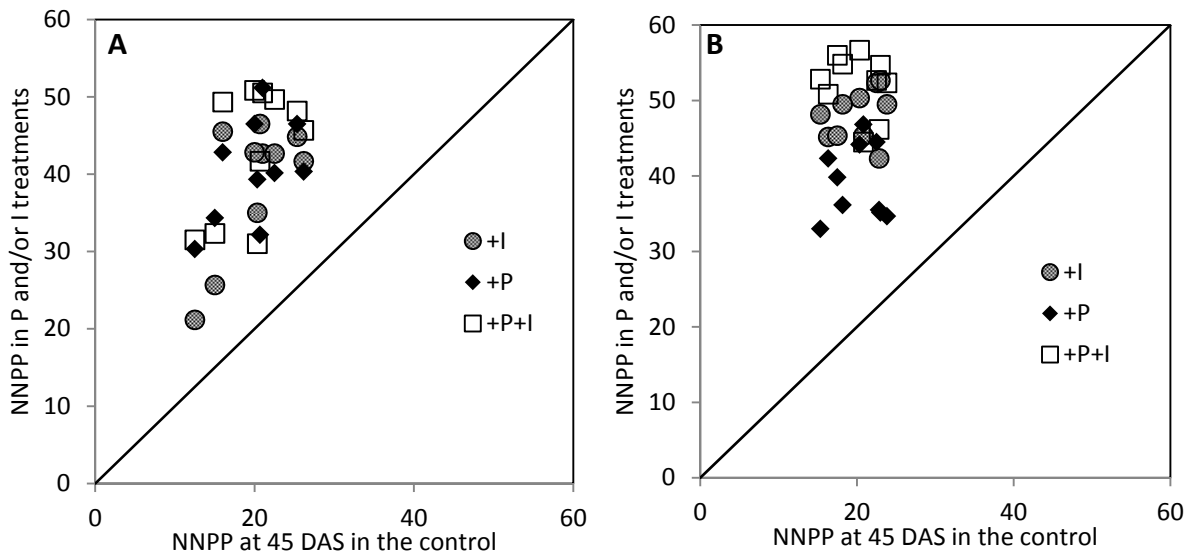


Figure 8: Nodule number plant⁻¹ at 45 days after sowing response of chickpea to inoculation and/or phosphorus fertilization on the black soil (A) and on the red soil (B). NNPP = nodule number plant⁻¹, DAS = days after sowing, I = inoculation, P = phosphorus fertilization, I+P = inoculation and phosphorus fertilization.

3.11.2. Yield and biomass

Moderate to large across farm variability in yield and biomass production of the treatments occurred under both soil types. Nonetheless, both soil types resulted in positive response to the soil treatments. For both yield and biomass, the variability was smaller on the red soil (Figs 9C and 9D) than on the black soil (Figs 9A and 9B).

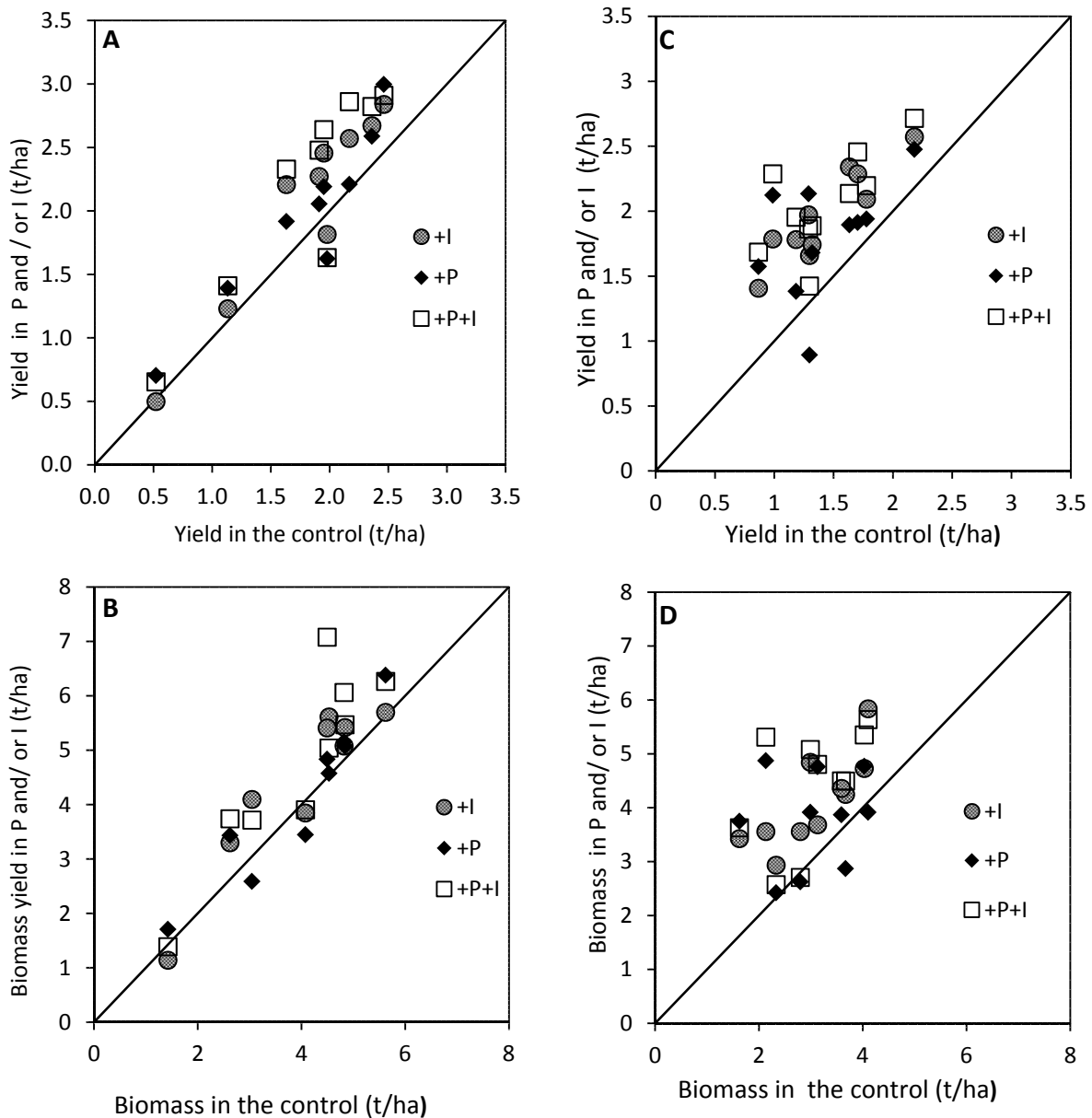


Figure 9: Yield and biomass response of chickpea to inoculation and/ or P fertilization on the black soil (A&B) and the red soil (C&D). +I = inoculation, +P = phosphorus fertilized, and P+I = both P fertilization and inoculation.

3.11.3. Yield response to the soil fertility treatments of the two soil types

Although positive response to the soil fertility treatments was recorded on both soil types, larger magnitude of response to the applied soil fertility treatments was recorded on the red than the black soil. Application on the red soil of I, P, and I+P treatments resulted in 0.27 ton ha⁻¹, 0.21 ton ha⁻¹ and 0.2 ton ha⁻¹ more average grain yield than their application on the black soil respectively (Fig 10).

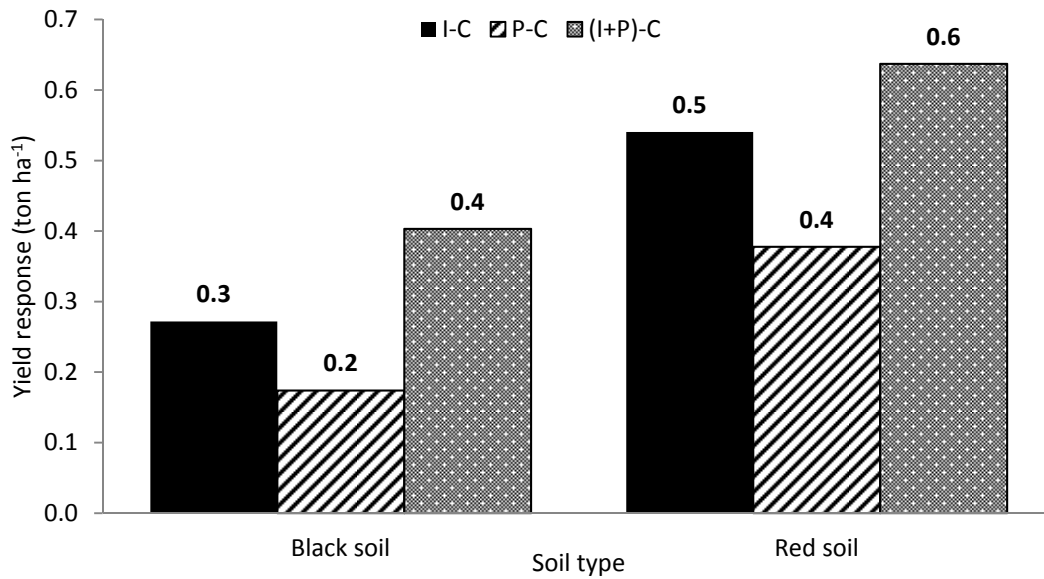


Figure 10: Yield response to inoculation and/ or phosphorus fertilization of chickpea on black and red soil types. I-C = yield response due to inoculation (I), P-C = yield response due to phosphorus fertilization (P), (I+P)-C = yield response due to application of both inoculation and phosphorus fertilization (I+P), C = yield in the control treatment.

3.12. The effect of initial soil fertility on yield and magnitude of yield response to the soil fertility treatments

A highly significant and positive correlation was found only between the available P content of the soil and yield in +I ($r = 0.874$) and I+P ($r = 0.882$) treatments (Appendix 9). Nonetheless, for the control and I+P treatments yield was observed to increase with increase in OC%, TN%, and Av. P content of the soil (Fig. 11). The yield increment seems to be more pronounced under the control treatment compared to the I+P treatment (Fig. 11).

For the magnitude of the yield response (I-C, I-P, and (I+P)-C) to the soil fertility treatments, significant correlations were found between OC and I-C ($r = -0.58$) and P-C ($r = -0.357$) (Appendix 9). Nonetheless, except for TN and (I+P) - C, negative correlations were observed between initial soil fertility and yield response to the applied soil fertility treatments (Appendix 9 and Fig. 12).

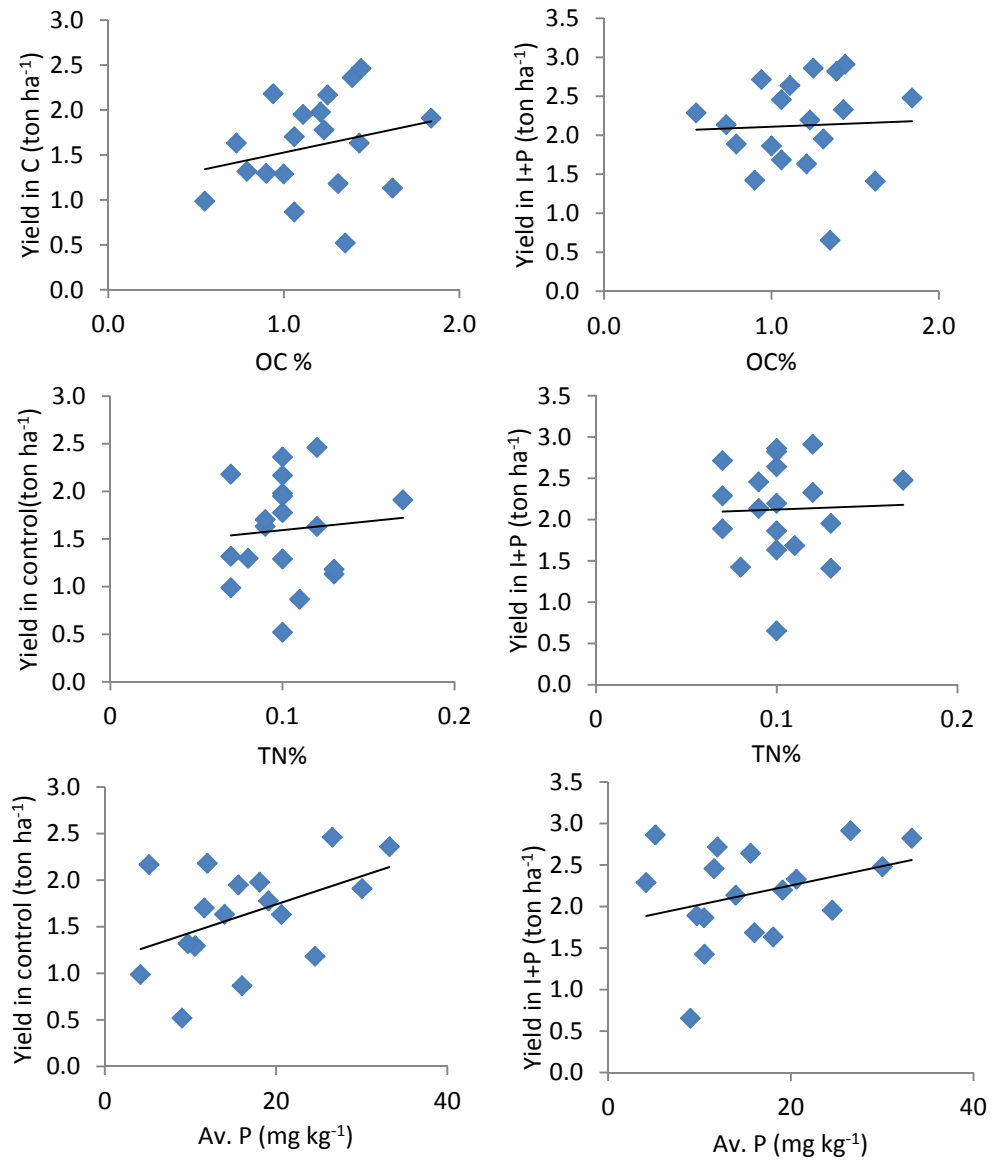


Figure 11: Scatter plots of chickpea yield in control (left) and I+P (right) treatments against initial soil fertility variables, with regression line plotted. Notes: OC = organic carbon content, TN = total nitrogen content and Av. P = available phosphorus, C = control, and I+P = inoculation + phosphorus.

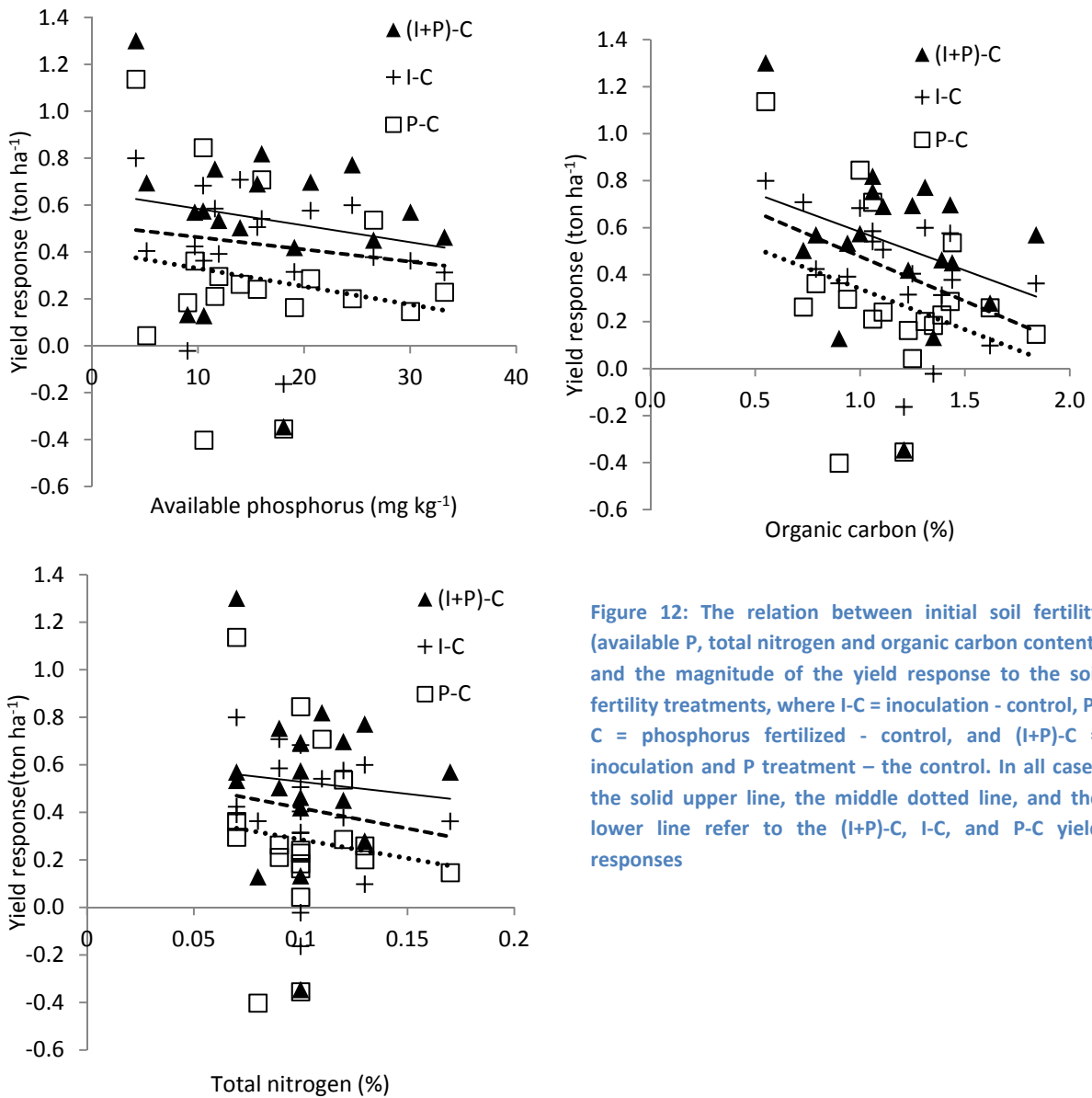


Figure 12: The relation between initial soil fertility (available P, total nitrogen and organic carbon content) and the magnitude of the yield response to the soil fertility treatments, where I-C = inoculation - control, P-C = phosphorus fertilized - control, and (I+P)-C = inoculation and P treatment – the control. In all cases the solid upper line, the middle dotted line, and the lower line refer to the (I+P)-C, I-C, and P-C yield responses

3.13. Household characteristics

Family size ranged from 3 to 8 with average family size of 6 per household. In addition, the livestock holdings of the households ranged from 0.13 to 3.74 tropical livestock unit (TLU) per household with an average of 1.9 TLU per household. This is consistent with the previous report of Amede et al. (2001) that indicated the average livestock holding of households in Wolaita area to be 1-2 heads. The average landholding of the households in the study area was 0.6 ha, with a range from 0.25 to 2 hectares. Per capita landholding of the households also ranged from 0.06 – 0.25 ha with the average of 0.11ha (Appendix 3).

The statistical analysis showed that both the initial fertility of the soil, except for P, and yield of the farms were not significantly affected by the livestock herd size. However, positive relation was observed between livestock herd size and OC and TN. This might have resulted from the small herd sizes across the board. Lack of significant effect of the livestock herd size on initial fertility and yield of the plots could also be attributed to the competing uses for crop residues and manure as livestock feed and fuel respectively. In conditions when crop residues are used as livestock feed and fuel instead of soil amendments, more livestock leads to larger decrease in soil fertility serving as driver of the competing use of the crop residue. The landholding of the households was negatively and highly significantly correlated to Av. P of the soil ($r = -0.189$) and P-C yield response (-0.292) (Appendix 9). Also, positive and significant ($r = 0.49$) correlations were recorded between TLU and Av.P of the soil. Although statistically not significant, yield of the control treatment and per capita landholding of the households were also positively correlated (Appendix 9).

4. Discussion

In these experiments, positive effects of inoculation and/or P fertilization on nodulation, growth and yield of chickpea were found.

4.1. Nodulation

As expected from the well-established knowledge on the phenomenon of symbiosis between legumes and rhizobia, the inoculation treatments (I and I+P) resulted in increased nodulation of chickpea that in turn, led to enhanced growth and yield through increased supply of nitrogen. Unlike at 23 DAS where only the I+P treatment gave higher nodules than the control, nodulation of chickpea was improved by all the three soil fertility treatments at 45 DAS and maturity, with the most pronounced improvement at 45 DAS. Of the three stages, minimum nodulation was recorded at maturity which can be attributed to nodule degradation (Shukla and Yadav 1982).

Inoculation did not significantly improve nodulation of chickpea at 23 DAS. This could be due to the relatively long time required for BNF to begin, which, usually takes place between 2-5 weeks after planting (Werner and Newton 2005). In contrast, inoculation improved nodulation significantly at 45 DAS. Similar results were reported by Kassa (2009), who indicated that in the same location, inoculation of chickpea seed improved NNPP by 60% over that of the control. Moreover, a positive and significant response of legume nodulation to inoculation was also reported by others (Otieno et al. 2009; Namvar and Sharifi 2011; Verma et al. 2013) in Kenya, Iran and India respectively. Similar to the effect of inoculation, P application did not improve nodulation of chickpea in the early stage of plant growth, i.e. at 23 DAS. This could be related to the fact that P dissolution takes about 3-6 weeks. Nonetheless, the application of P resulted in a highly significant improvement of nodulation in the later stages. This is in agreement with the findings of a previous study in which on farm application of 46 kg P ha⁻¹ increased nodule numbers and NDWPP of chickpea by 157% and 114% over the control treatment respectively (Kassa 2009). Such positive effects of P on legume nodulation have also been reported by several other studies conducted on chickpea, haricot bean and faba bean in Ethiopia, Tanzania and Pakistan (Yoseph 2011; Amijee and Giller 1998; Shukla and Yadav 1982; Ali et al. 2004; Ghizaw et al. 1999). From an experiment that included five levels of P, Idris et al. (1989) also reported that phosphorus application significantly improved both number and dry weight of nodule in chickpea. Also for soybean, the alleviation of phosphorus deficiency resulted in highly significant increase in

nodule mass, nodule number, and nodule dry weight plant⁻¹ (Israel (1987). Generally, the observed improvement is explained by the positive effect of P on root growth, nodule initiation and functioning.

Nodulation was significantly affected by soil type only at 23 DAS, when the red soil had higher NNPP and NSPP than chickpea on the black soil. This might have resulted from the relatively higher resident rhizobia found in the red soil compared with the black soil (Table 2). The result of interaction between the treatments and the soil types showed that inoculation, either alone or in combination with P, resulted in higher nodulation when applied on red soil compared to the black soil. In this experiment nodulation was assessed with two methods, i.e. counting all the nodules, resulting in NNPP and visually scoring the nodules, resulting in NSPP. Though both methods assess the number of nodules per plant, the latter employs a 1-5 scale, which eases the work as counting all nodules is not required. Unlike NNPP, the method also takes into account the distribution of nodules. However, the lack of accuracy and the need for adjustment for each crop and location can be mentioned as disadvantages of NSPP. For small sample size using NNPP might enable accurate measurement and show all the differences existing between treatments. In this study, both methods were used and mostly found to follow the same trend for all the treatments.

The MPN test result showed that the majority of the soils in the study area are inhabited by very small populations of rhizobia. Similarly, Rupela et al. (1987b) found fields with low native rhizobial populations in regions where chickpea had been grown for centuries. As *Rhizobium* concentrations of <10² g⁻¹ of soil are insufficient to establish effective symbiosis and initiate nodulation (Rupela et al. (1987b), BNF in the soils of the study area was severely constrained. Absence of viable native rhizobia in these soils might have resulted in the observed positive response of chickpea plants to inoculation. Thus, artificial inoculation is strongly recommended to improve the productivity of chickpea in this area.

4.2. Morphological and phenological traits of chickpea

Both separate and combined application of inoculation and P significantly improved shoot fresh weight plant⁻¹ (SFWPP), number of branches plant⁻¹ (NBPP) and plant height of chickpea. The role of inoculation in enhancing nitrogen fixation and thereby increasing N uptake by plants contributed to improved vegetative growth of chickpea.

On the other hand, the poor shoot weight recorded from the control treatment might be due to the low population of native rhizobia that ranged from none to <10 cells gram⁻¹ of soil.

This result might be explained further by the below optimum available P content of these soils (Appendix 1). Such positive response of chickpea shoot development to inoculation and P fertilization was also reported elsewhere (Elkoca et al. 2007; Verma et al. 2013).

Application of the soil fertility treatments extended the growing period of chickpea compared with the control. The longer maturity periods might have been caused by the promoted vegetative growth due to enhanced supply of N and P. Eventually, late maturing crops were observed to give higher yield compared to the early maturing ones. A similar effect was reported earlier where inoculation delayed maturity time of chickpea (Gan et al. 2009b) and white bean (Buttery et al. 1987). Also the delaying effect of P and K fertilizer was reported for white bean (Buttery et al. 1987). Of the two soil types, plants growing on black soils took longest to mature. The higher water holding capacity of the black soil might have favoured vegetative growth of the plants due to sufficient water availability even at a later stage. Similarly, Gan et al. (2009b) has reported that maturity of chickpea varied from 91 to 136 days mainly due to differences in soil environment.

With respect to the morphological traits, our findings are in accordance with those of Ahmed et al. (2010) and Dutta and Bandyopadhyay (2009) confirming positive and significant effects of inoculation and P on number of branches and plant height of chickpea respectively. Also numerous other studies (Ghizaw et al. 1999; Elkoca et al. 2007; Togay et al. 2008; Ahmed et al. 2010; Namvar and Sharifi 2011; Yoseph 2011) reported enhanced morphological growth due to inoculation and/or P due to the increased supply of nitrogen, through BNF, and direct application of phosphorus. Again, the taller plants and higher number of branches on the black soil compared to the red soil might have resulted from the higher moisture holding capacity of the former

4.3. Yield and yield-related traits of chickpea

Positive effects of the soil fertility treatments were also observed for NPPP and NoSPP of chickpea. The results of the analysis of variance showed that NPPP was significantly superior in all the three treatments compared to the control whereas NoSPP was improved by the application of inoculation and I+P treatments only. Our results are in accordance with the findings of several other studies (Kassa 2009; Ahmed et al. 2010; Yoseph 2011; Namvar and Sharifi 2011; Tellawi et al. 1986; Togay et al. 2008; Dutta and Bandyopadhyay 2009) and explained by the increased supply of nitrogen through BNF and the direct supplementation of phosphorus that in turn play important roles in enhanced growth and assimilate accumulation,

thereby improving the reproductive performance of the plants. NPPP was higher on the black soil than on the red soil, which might be due to higher moisture availability in the black soil during pod setting and flowering of the plants.

The total biomass of chickpea is of great importance in farm level nutrient cycling due its role as either animal feed or organic matter to be returned to the soil. In this study, total biomass of chickpea was highly significantly improved by the soil fertility treatments due to the positive effects of nitrogen and phosphorus on vegetative growth, and the synergy of these two nutrients on plant growth. This result is in agreement with some previous findings reported on chickpea (Ahmed et al. 2010; Ali et al. 2004; Elkoca et al. 2007). Moreover, from an experiment conducted under similar condition, Kassa (2009) reported that inoculated chickpea produced 22% more straw than the control. Also, the positive effect of P on total biomass was in agreement with earlier studies (Kassa 2009; Gidago et al. 2012; Idris et al. 1989; Togay et al. 2008), as well as the strongest effect by the combined application of inoculation and P (Ali et al. 2004).

Inoculation of chickpea seed significantly improves its grain yield (Rokhzadi and Toashih 2011; Togay et al. 2008; Ahmed et al. 2010; Namvar and Sharifi 2011; Verma et al. 2013). In this study, the soil fertility treatments resulted in a significantly higher yield compared to the control (1.6 ton ha⁻¹), with the highest (2.1 ton ha⁻¹) grain yield recorded in the I+P treatment. Similarly, inoculation of two different strains increased chickpea yield in the same study area by 45% and 32% compared to the control (Kassa 2009). Also, Gan et al. (2009a) reported that inoculated chickpea produced a 37% higher seed yield over the control, which was similar to that obtained from a 112 kg N ha⁻¹ fertilized plot. In our study, application of 46 kg ha⁻¹ of P fertilizer has also resulted in 19% higher chickpea grain yield over the control. Similar effect of P on grain yield of legumes was found by previous researchers (Idris et al. 1989; Dutta and Bandyopadhyay 2009; Kassa 2009) and attributed to the role of P in the meristematic activity of plant tissue, and in the synthesis of other growth components from carbohydrates (Ahmed and Badr 2009). The effect was even more pronounced when inoculation and P were applied in combination resulting in a yield advantage of 33% over the control. This might have resulted from the positive effects of P on the process of N₂ fixation where the increased supply of N through inoculation result in enhanced plant growth that eventually leads to P deficiency.

Thus, application of P again improves the growth of the plants further. In line with this, Fatima et al. (2007) reported that combined application of inoculation and P increased cowpea grain yield by 63% over that of the control. The observed yield improvements are due to the increased N and P availability and are in line with the improvements observed for the yield related traits discussed above. In general, the observed improvement in chickpea grain yield due to the soil fertility treatments is attributed to the enhanced supply of nitrogen and P elements whose role in both vegetative growth and grain filling are of paramount importance.

Unlike most of the parameters studied in this experiment, thousand seeds weight of chickpea was not significantly increased by inoculation. Similar result was reported by Elkoca et al. (2007) and Kassa (2009) who found no significant effect of inoculation on thousand seeds weight of chickpea. However, contrary to these, some studies found a positive response of seed weight to inoculation of annual legumes (Namvar and Sharifi 2011; Ali et al. 2004; Yoseph 2011). Nonetheless, P, either alone or in combination with inoculation, resulted in a significantly larger weight of thousand seeds of chickpea, which is in good agreement with the findings of some other studies (Ali et al. 2004; Yoseph 2011). The reason that the maximum 1000 seeds weight was recorded in the I+P treatment could be due to enhanced growth and development of plants that resulted from phosphorus supply and its positive effect on nitrogen fixation. The resulting increased N availability might have promoted the supply of assimilates to seed thereby enabling them to gain more weight.

4.4. Straw quality and total N uptake

Positive effects of inoculation and P fertilization were also recorded for total N and total P content of chickpea straw. As such, separate application of inoculation resulted in 56% higher STN% compared to the control, which is clearly related to the enhanced availability of nitrogen through fixation of atmospheric N₂. This result is in good agreement with the findings of Gan et al. (2010) who reported that straw of inoculated chickpea gave 1.3 mg g⁻¹ higher N concentration than the non- inoculated crop. In line with this result, enhanced straw N concentration of chickpea due to inoculation was also reported by some other studies (Kassa 2009; Zaidi et al. 2003; Rokhzadi and Toashih 2011; Alagawadi and Gaur 1988).

Total N uptake of legumes can serve as good indication of N₂ fixation. The statistical analysis of variance showed that the total N uptake was increased by application of the soil fertility treatments. Inoculation, either alone or in combination with P, significantly improved

the total N uptake with yet a more pronounced improvement under the combined application of inoculation and P. Given the very low native rhizobial populations of the study soil, this improvement in total N uptake due to inoculation could be due to the positive role of inoculation on the process of N₂ fixation.

Likewise, the separate application of P was also able to result in significant improvement of STN content of chickpea over the control. A similar positive effect of P on TN concentration of chickpea straw was reported (Kassa 2009). Moreover, this finding is supported by the result of some other authors (Fatima et al. 2007; Zaidi et al. 2003; Rokhzadi and Toashih 2011).

However, the effects were even more pronounced when the I+P treatment was applied, resulting in the highest STN of about 0.98%, exceeding the control treatment by 82%. This result is in agreement with the findings of Fatima et al. (2007) who reported the maximum N concentration in soybean straw under the combined application of inoculation and P. The resulting highest N shoot uptake was attributed to the increased supply of P having a positive role in N₂ fixation by rhizobia, thereby enhancing nitrogen uptake by the plant shoots. The observed positive effect of P, either alone or in combination with inoculation, on STP content of chickpea might have resulted from increased availability of P in the soil and its subsequent vital role in physiological and developmental processes of plants and its favourable effect on growth and nutrient uptake. Generally, the results of this study are in line with the previous conclusions that in addition to its role in host plant growth, phosphorus has specific roles in nodule initiation, growth, and functioning (Israel 1987).

4.5. Across farm variability

For all treatments and variables, a consistent pattern of high variability was observed across farms. Nonetheless, on the majority of farms, more than 85% in case of yield, positive effects of the soil fertility treatments were recorded. In line with Tittonell et al. (2010), the soil test result showed wide differences between farms for initial soil fertility. However, soil fertility was poor across all farms. The magnitude of response to the soil fertility treatments was also different across the farms with better yield response observed on relatively less fertile soils. Although the red soil contained relatively higher number of native rhizobia than the black soil, for all treatments including the control higher yield and yield related traits were recorded on the black soil than the red soil which could be due to the better holding capacity of the black soil.

Contrary, the magnitude of response to the treatments was higher on the red soil than the black soil. Such positive but variable on-farm yield response to inoculation and P treatments was also reported for cowpea, groundnut, and soybean in Ghana (Bressers 2012).

Mostly regional or national recommendations are made based on on-station trials where management and inputs are optimized. In the present study's area, there were few replicated on-farm trials. Usually, experiments are conducted only on a single farm but meant to represent all farms located in the kebele. Given the huge across farm variability observed in response to the soil fertility treatments, it is important to test technology packages on several farms thereby identifying how often and to which extent do the technologies perform. Nonetheless, according to the results of this study especially farmers with low fertile soil seem to benefit from application of P and I. In this regard, the information obtained from the current study fills an important knowledge gap.

4.6. Cost benefit analysis

As indicated above, the average landholding of the households in the area is about 0.6 ha and farmers usually plant chickpea on small part of their land. Assuming chickpea is at least planted on a quarter of their land, the use of these technologies will enable each household to get a yield increment of 60 kg, 45 kg and 75 kg per household from inoculation, P fertilization, and P+I treatments respectively. Converting this into monetary value and the cost of these inputs considered, the net benefit that each household would get by using these technologies would be 63, 33, and 63 USD for using inoculation, P fertilization, and P+I treatments respectively with no difference in return due to inoculation and P+I treatments. While making the analysis, the costs of P fertilizer, inoculants and chickpea grain were assumed to be 100 USD ha⁻¹, 8 USD ha⁻¹ and 1 USD kg⁻¹ respectively. As such, a yield improvement of 1 kg, 15 kg and 16 kg is required to compensate the cost of inputs for inoculation, P fertilization, and I+P treatments respectively, using the application rates as in the experiment. There seem to be equal benefit obtained from the use of inoculation and I+P treatments. Given the high cost of P fertilizer, the use of inoculation appears to be a priority for smallholder farmers growing chickpea in this area. Likewise, it can be inferred that inoculation is a means to increase the economic benefits of P fertilizer use.

The vast majority of the households in Taba area are resource poor farmers who have been experiencing shortage of food fuelled by the low agricultural input usage on the already degraded land. At the same time, in spite of using low yielding local varieties and lack of

fertilizer input, these farmers have been practising crop rotation through inclusion of food grain legumes in cereal dominated farming. Given the small landholdings, the observed yield improvement seems to be small. However, the observed yield improvements due to the treatments can still somehow increase the harvest of each household which can be either used for consumption or sold and serve as cash income. On the other hand, the increased total biomass production can increase the fertility status of the soil for the next cereal crop, if left on the farm or serve as good source of livestock feed.

5. Conclusions

In this study the response of chickpea to inoculation and/or P fertilization was evaluated on farmers' fields in Taba kebele of Wolaita area. The results of the soil test clearly showed that regardless of the fact that in Taba chickpea has been cultivated for a long time, the soils of the area are inhabited by small populations of rhizobia. Furthermore, soils of all the study farms were deficient in major plant nutrients. As was expected in such soils, artificial inoculation of chickpea seed improved the growth and yield of the crop. For all treatments, including the control, larger yields and higher values for yield related traits were recorded on the black soil than on the red soil. On the other hand, the response to the soil fertility treatments was larger on the red soil than the black soil.

Both nodulation and phenological traits of chickpea were improved by inoculation and P fertilization, consequently resulting in improved yield and yield related traits. For all plant variables studied, the improvement was more pronounced for the combined application of phosphorus and inoculation than for their separate application. This underlines the positive effect of P on biological nitrogen fixation. For all the variables studied, moderate to large across farm variability was exhibited, with relatively smaller variability on the red soil. Assuming farmers would sow chickpea on a quarter of their cropland (average 0.6 ha), households would get an average extra income of 63, 33, and 63 USD by applying inoculation, P fertilization, and P+I treatments respectively.

However, most of the farmers in this area cannot afford P fertilizers, so credit facilities or direct supply of fertilizer would be necessary. Generally, depending on the content of native rhizobia of a soil and the availability of elite strain, *Rhizobium* inoculation of chickpea seed may either reduce or substitute the need for costly N fertilizers for smallholder farmers. Although there are limited number of inoculant producing companies in the country, the on market unavailability of this product may hinder the likely absorption of the technology. Moreover, the small landholding of the households might negatively affect the adoption of these technologies as the improvement might be considered insignificant by the farmers.

6. Implications of the results for policy and research

The results of this study suggest that increasing chickpea productivity on smallholder farms is possible and that the use of *Rhizobium* inoculants is an appealing strategy to achieve this. However, the experiment only included one strain of bacteria and a single variety of Desi type chickpea. Basically, there is huge variability among the regions of Ethiopia for rainfall, soil type, and other soil characteristics. Hence, though positive and appealing yield and growth improvement were observed with the strain and chickpea variety used, I would suggest that more strains (domestic or imported) and more varieties (including Kabuli type) would be tested in order to ascertain the best alternative. Based on visual assessment, the performance of chickpea plants in the control treatment was better than the plants sown by the owners of the farms, which could be due to the differences in time and method of sowing. Most of the farmers normally broadcast chickpea and, compared with the row planted plots, the stand in the broadcasted fields was poor. Moreover, most of the farmers in the area were sowing chickpea late September whereas for this experiment plating was done early September. Given the observed shortage of rainfall towards maturity, once the land is available it is strongly advisable to sow chickpea as early as possible starting from mid-August. This study was conducted only in the southern region of Ethiopia, whose share of Ethiopian chickpea production is only about 2%. Therefore, regarding promotion of technologies related to chickpea, priority should be given to farmers in Amhara and Oromia regions of Ethiopia whose chickpea share is about 61% and 32% of the country respectively. In addition, it is important to note that P fertilizers, except the commonly known DAP, are not available on market across the country except what is imported for research purpose by agricultural research organizations. Moreover, the very limited numbers of inoculant producing companies in the country need to be further strengthen in order to ensure active involvement of private companies as well as governmental organizations. In addition, attempts should be made in creating farmers awareness concerning the potential benefits of these technologies and this needs to be followed by establishing a link between the actual production and input supply to the farmers.

7. References

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8. Appendices

Appendix 1. Physico-chemical properties of soils from the twenty studied farms

Farm No.	Soil colour	pH _{H2O} 1:2.5	OC (%)	TN (%)	Av. P (mg/Kg)	CEC & Exchangeable Bases (cmol(+)/kg Soil)					EC (mS/cm)	Texture			Soil class
						CEC	Ca	Mg	Na	K		% Clay	% Silt	% Sand	
1	Black	5.8	1.5	0.17	12	20	11.6	6.6	0.22	0.82	0.08	25	50	25	Silt- loam
2	Black	7.2	1.8	0.17	30	23	39.9	9.3	0.18	2.45	0.18	23	50	27	Loam
3	Black	5.9	1.4	0.12	21	0.1	18.4	10.9	2.62	0.26	0.84	24	50	26	Loam
4	Red	6.4	0.9	0.07	12	17	16	6.3	0.18	0.86	0.07	25	34	41	Loam
5	Black	6.7	1.6	0.13	-	22	9.4	3.3	0.1	2.8	0.29	24	44	31	Loam
6	Red	6.8	0.9	0.08	11	15	10.5	3.2	0.16	1.16	0.06	23	32	45	Loam
7	Black	6.3	1.2	0.1	18	12	14.6	4.8	0.24	1.02	0.08	18	46	36	Loam
8	Red	7.1	1	0.1	11	20	9.1	6	0.14	2.55	0.15	20	34	46	Loam
9	Red	7.2	1.3	0.13	25	19	12.7	3.6	0.12	3.51	0.16	20	35	45	Loam
10	Red	7.3	1.1	0.11	16	17	12.8	4.8	0.14	3.23	0.11	35	37	29	Clay- loam
11	Red	6.5	0.7	0.09	14	18	12.5	6.8	0.17	1.32	0.08	43	34	24	clay
12	Red	6.6	0.8	0.07	10	16	14	4.5	0.14	0.91	0.06	28	30	42	Clay loam
13	Red	6.4	1.2	0.1	19	18	9.1	7.1	0.19	1.27	0.15	24	37	38	Loam
14	Black	6.0	1.4	0.1	33	17	9.2	5.4	0.21	0.86	0.08	19	50	31	Loam
15	Red	6.1	1.1	0.09	12	19	11.3	5.9	0.19	1.08	0.1	24	36	40	Loam
16	Black	6.2	1.1	0.1	16	17	10.4	3.3	0.24	0.87	0.11	16	38	46	Loam
17	Red	6.6	0.6	0.07	4	15	10.8	5.5	0.17	1.26	0.04	37	26	37	Clay- loam
18	Black	6.4	1.4	0.12	27	18	2.3	12.8	0.14	1.58	0.07	19	45	36	Loam
19	Black	6.5	1.3	0.1	5	16	7.1	4.5	0.18	1.49	0.12	19	42	38	Loam
20	Black	6.7	1.4	0.1	9	16	12.7	4.9	0.36	1.16	0.09	17	50	33	Silt- loam
	RS	6.7	0.96	0.09	13	17	11.9	5.4	0.2	1.7	0.1	28	34	39	Clay loam
Average	BS	6.4	1.4	0.12	19	18	13.6	6.6	0.4	1.3	0.2	20	47	33	Loam
	OA	6.5	1.2	0.11	16	17	12.7	6.0	0.30	1.52	0.40	24	40	36	Loam

Notes: OC = organic carbon, TN = total nitrogen, Av. P = available phosphorus, EC = electrical conductivity, RS = red soil, BS = black soil, OA = over all. NB: The result for Av. P of farm 5 was found to be an outlier and excluded.

Appendix 2. Rainfall data for the whole growing period of the two soil types

Date	Amount of rainfall(mm) recorded	
	Black soil	Red soil
Sept. 4/2012	10.4	9.6
Sept. 7/2012	11.9	12.2
Sept. 9/2012	0.5	0.7
Sept. 11/2013	5.6	5.6
Sept. 13/2012	3.6	4.6
Sept. 14/2012	0.6	1.1
Sept. 19/2012	4.0	4.2
Sept. 20/2012	19.1	21.5
Sept.23/2012	4.7	2.6
Sept. 25/2012	3.3	4.0
Sept. 27/2012	19.1	18.6
Sept. 28/2012	13.2	12.3
Sept. 29/2012	3.5	3.2
Sum(Sept.)	100	100
Oct. 1/2012	25.7	28.0
Oct. 2/2012	28.0	32.2
Oct. 22/2012	6.3	4.8
Oct. 29/2012	2.2	1.4
Oct. 30/2012	1.3	8.1
Sum(Oct.)	63.4	74.4
Nov. 5/2012	5.8	3.9
Nov. 26/2012	3.0	0.5
Nov. 28/2012	2.2	0.0
Nov. 30/2012	16.7	19.6
Sum (Nov.)	27.6	24
Dec. 18/2012	1.6	2.1
Dec.19/2012	3.2	1.6
Sum(Dec.)	4.8	3.7
Jan. 7/2013	1.4	2.7
Jan. 9/2013	2.7	3.5
Jan. 10/2013	4.4	2.8
Jan. 11/2013	8.5	3.2
Sum(Jan)	17	12.2
Total	212.3	214.3

Appendix 3. Household characteristics of the farms under the present study

Farm No	Family size	Land holding (ha)	Per capita landholding (ha)	Cattle	Sheep/goat	Poultry	Donkey	TLU
1	6	0.5	0.08	1	0	7	1	0.97
2	7	0.8	0.11	3	0	0	0	2.1
3	5	0.5	0.10	3	2	0	0	2.3
4	7	0.6	0.09	2	0	5	0	1.45
5	4	0.6	0.16	2	0	0	0	1.4
6	5	0.5	0.10	4	0	0	0	2.8
7	6	1.0	0.17	2	1	9	0	1.59
8	6	0.5	0.08	2	1	3	0	1.53
9	8	2.0	0.25	4	0	2	1	3.02
10	5	0.3	0.05	2	3	0	0	1.7
11	7	0.5	0.07	3	0	0	0	2.1
12	7	0.5	0.07	1	0	0	0	0.7
13	6	0.4	0.06	2	0	0	0	1.4
14	6	0.8	0.13	5	0	4	1	3.74
15	6	0.5	0.08	2	2	0	0	1.6
16	6	0.8	0.13	4	0	0	1	3
17	7	0.5	0.07	1	2	0	0	0.9
18	3	0.5	0.17	4	4	10	1	3.5
19	4	0.3	0.06	0	1	3	0	0.13
20	5	1.0	0.20	3	1	0	0	2.2
Mean	5.8	0.6	0.11	2.5	0.85	2.15	0.25	1.9

Notes: TLU = tropical livestock unit, where 1 TLU = 0.7 cattle, = 0.2 pigs, 0.1 sheep/goat, and 0.01 = poultry.

Appendix 4. F values for chickpea nodulation traits

Source of variation	DF	NNPP at 23 DAS	NNPP at 45 DAS	NNPPM	NDWPP at 45 DAS	NSPP at 23 DAS	NSPP at 45 DAS	NSPPM
Soil type	1	890*	372.67***	35.113	0.3	5.1681*	0.23472	0.2
Error a	18	159.69	84.75	57.163	1191.5	1.0004	0.14444	0.59178
Treatments	3	78.34*	2991.35***	38.193***	1886.1	0.5194*	4.2912***	0.61733***
Soil type *Trt	3	12.66	157.09	0.909	114.8***	0.0949	0.25231**	0.00933
Error b	54	24.35	17.1	2.046	137.7	0.1565	0.04774	0.03444

Note: *** = significant at 0.1%, ** = significant at 1% and, * = significant at 5%. DF = degrees of freedom, NNPP = number of nodule plant⁻¹, DAS = days after sowing, NNPPM = number of nodule plant⁻¹ at maturity, NDWPP = nodule dry weight plant⁻¹, NSPP = nodule score plant⁻¹, NSPPM = nodule score plant⁻¹ at maturity.

Appendix 5. F values for morphological and phenological traits of chickpea

Source of variation	DF	SFWPP at 45 DAS	Days to maturity	NBPP	Plant height
Soil type	1	43.5199	2268.45*	72.178*	1156.467***
Error a	18	43.7288	496.033	10.034	73.842
Treatments	3	15.3695***	75.517***	27.786***	29.228***
Soil type *Treatment	3	1.8452	8.45**	0.541	1.893
Error b	54	0.9912	1.9	1.805	3.492

Note: *** = significant at 0.1%, ** = significant at 1% and, * = significant at 5%. DF = degrees of freedom, SFWPP = shoot fresh weight plant⁻¹, NBPP = number of branches plant⁻¹.

Appendix 6. F values for yield and yield related traits of chickpea

Source of variation	DF	NPPP	NSPP	TBM (ton ha ⁻¹)	Grain yield (ton ha ⁻¹)	1000 SW (g)
Soil type	1	2814.47 [*]	0.49568	2.3033	0.25385	5837.1
Error a	18	416.5	0.14096	5.2254	1.23023	3517.3
Treatments	3	294.75 ^{***}	0.10738 ^{**}	3.9199 ^{***}	0.96371 ^{***}	2436.1 ^{**}
Soil type *Trt	3	16.65	0.0406	0.4303	0.07741	446
Error b	54	27.39	0.02337	0.3211	0.03936	435.3

Note: *** = significant at 0.1%, ** = significant at 1% and, * = significant at 5%. DF = degrees of freedom, NPPP = number of pods plant⁻¹, NSPP = number of seeds pod⁻¹, total biomass.

Appendix 7. F values for total nitrogen and phosphorus in chickpea straw

Source of variation	DF	STN (%)	STP(mg kg ⁻¹)
Soil type	1	0.07707	13151
Error a	18	0.01258	39048
Treatments	3	0.20036***	15037***
Soil type * Treatment	3	0.00581	728
Error b	54	0.01797	1190

Note: *** = significant at 0.1%, DF = degrees of freedom, STN = straw total nitrogen, STP = straw total phosphorus.

Appendix 8. Pearson's correlation coefficients among the variables (n=80)

	NSPPM	NSPP at 23 DAS	NSPP At 45 DAS	NNPP At 23 DAS	NNPP At 45 DAS	NNPPM	SFWPP at 45 DAS	Pl ht (cm)	NBPP	NPPP
NSPPM	1									
NSPP at 23 DAS	0.14	1								
NSPP At 45 DAS	0.293**	0.26*	1							
NNPP At 23 DAS	0.102	0.956***	0.244*	1						
NNPP At 45 DAS	0.33**	0.299**	0.96***	0.28*	1					
NNPPM	0.959***	0.127	0.349**	0.078	0.38***	1				
SFWPP at 45 DAS	0.262*	0.243*	0.311**	0.261*	0.349**	0.29*	1			
Pl ht (cm)	0.26*	-0.278*	-0.099	-0.337	-0.123	0.238*	-0.228*	1		
NBPP	0.253*	-0.086	0.198	-0.16	0.18	0.284*	0.247*	0.578***	1	
NPPP	0.388***	-0.285*	0.035	-0.37***	0.05	0.404***	-0.05	0.779***	0.63***	1
NoSPP	0.356***	-0.042	-0.022	-0.123	0.025	0.338**	-0.146	0.602***	0.392***	0.584***
DM	0.209	-0.278*	-0.071	-0.306**	-0.056	0.186	-0.554***	0.697***	0.247*	0.589***
TBM (ton ha ⁻¹)	0.453***	0.069	0.279*	0.047	0.273*	0.44***	0.253*	0.351**	0.342**	0.346**
Yield (ton ha ⁻¹)	0.53***	0.032	0.282*	0.017	0.308**	0.518***	0.044	0.397***	0.217	0.418***
1000SW (g)	0.202	0.054	0.253*	0.048	0.203	0.204	0.023	0.175	0.241*	0.216

Notes: ***, ** and * = significant at 0.001, 0.01 and 0.05 respectively. DAS = days after sowing, NSPPM = nodule score plant⁻¹ at maturity, NSPP = nodule score plant⁻¹, Pl ht = plant height, SFWPP = shoot fresh weight plant⁻¹, TBM = total biomass, NoSPP = number of seeds pod⁻¹, NPPP = number of pods plant⁻¹, NNPP = number of nodules plant⁻¹, NNPPM = number of nodules plant⁻¹ at maturity, NBPP = number of branches plant⁻¹, DM = days to maturity, 1000SW = thousand seeds weight.

Appendix 8. Pearson's correlation coefficients among the variables (*Continued*)

	NoSPP	DM	TBM (ton ha ⁻¹)	Yield (ton ha ⁻¹)	1000SW(g)
NSPP	1				
DM	0.507 ^{***}	1			
TBM (ton ha ⁻¹)	0.182	0.11	1		
Yield (ton ha ⁻¹)	0.358 ^{***}	0.322 ^{**}	0.855 ^{***}	1	
1000SW(g)	-0.138	0.144	0.251 [*]	0.113	1

Notes: ^{***}, ^{**} and ^{*} = significant at 0.001, 0.01 and 0.05 respectively. NoSPP = number of seeds pod⁻¹, DM = days to maturity, TBM = total biomass, 1000SW = thousand seeds weight.

Appendix 9. Pearson's correlation coefficients of soil fertility variables, yield of the treatments, yield response and household characteristics (n=20)

	OC%	TN	Av. P	Yield in (C)	Yield in (+I)	Yield in (+P)	Yield in (I+P)	(+I)-C	(+P)-C	(I+P)-C	TLU	PCLH	Landholding
OC%	1												
TN	0.841	1											
Av. P	0.02*	-0.08	1										
Yield in (C)	0.178	-0.025	0.301	1									
Yield in (+I)	0.06	-0.059	0.874***	0.888***	1								
Yield in (+P)	0.02	-0.08	0.086	0.74***	0.874***	1							
Yield in (I+P)	0.122	0.005	0.882***	0.781***	0.921***	0.882***	1						
(+I)-C	-0.58**	-0.24	0.126	-0.272	0.32	0.126	0.17	1					
(+P)-C	-0.357*	-0.147	0.311	-0.228	0.046	0.311	0.107	0.572*	1				
(I+P)-C	-0.236	0.021	0.192	-0.231	0.087	0.192	0.32	-0.359	0.471*	1			
TLU	0.449	0.38	0.49*	0.171	0.227	0.006	0.154	-0.074	-0.128	-0.119	1		
PCLH	0.553	0.443	-0.174	0.084	-0.052	-0.174	-0.117	-0.434	-0.277	0.471	0.615*	1	
Landholding	0.35	0.239	-0.189***	0.042	-0.078	-0.189	-0.148	-0.359	-0.292***	-0.148	0.449	0.861	1

Notes: ***, ** and * = significant at 0.001, 0.01 and 0.05 respectively. OC = soil organic carbon, TN = soil total nitrogen, Av. P = soil available phosphorus, Yield in C= yield in control treatment, +I = yield in inoculated treatment, +P = yield in phosphorus fertilized treatment, (I+P) = yield in inoculated and phosphorus fertilized treatment, (I-C) = yield of inoculated treatment – yield of the control treatment, (P-C) = yield of phosphorus fertilized treatment – yield of the control treatment, (I+P)-C = yield of inoculated and P fertilized treatment – yield of the control treatment, TLU, tropical livestock unit, PCLH = per capita land holding.